a93359

## 03-P001 Short-Term Delivery of Fibrin-Bound VEGF Protein in Osteogenic Grafts ensures both Increased Vascularization and Efficient Bone Formation

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Spontaneous vascularization of large osteogenic constructs based on bone marrow-derived mesenchymal stem cells (BMSC) is too slow for progenitor survival in vivo. We found that sustained over-expression of vascular endothelial growth factor-A (VEGF) by genetically modified human BMSC effectively improved osteogenic graft vascularization, but also impaired bone formation through excessive osteoclast recruitment. Here we hypothesized that short-term delivery of VEGF protein, immobilized in fibrin gels, may improve graft vascularization without impairing bone formation.

Recombinant VEGF was engineered with a transglutaminase substrate sequence (TG-VEGF) allowing covalent cross-linking into fibrin hydrogels. Human BMSC were embedded in the fibrin gels and seeded on apatite granules. Bone formation and vascularization were assessed 1, 4 and 8 weeks after orthotopic implantation in nude rats. Retrovirally transduced BMSC stably expressing VEGF were used as control.

At all times, constructs containing fibrin-bound TG-VEGF with naive BMSC or VEGF-expressing BMSC displayed increased vascularization compared to the controls with naive BMSC only. After 4 weeks fibrin gels were completely degraded in all conditions. However, while bone formation at 8 weeks was severely impaired with VEGF-expressing BMSC as expected, fibrin-bound recombinant TG-VEGF allowed the formation of bone tissue as efficiently as naive BMSC alone. Interestingly, TG-VEGF improved the bone formation kinetics, as TG-VEGF constructs contained more bone than even naïve controls after 4 weeks.

In conclusion, VEGF effects on promoting vascularization and bone resorption can be uncoupled by short-term delivery of recombinant VEGF protein, providing an attractive and clinically applicable strategy to ensure both robust vascularization and bone formation.

a93275

## 03-P002 Calcium Releasing Pro-angiogenic PLA Nanofibers. An Angiogenic Potential Study for Bone Healing

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The major challenge for biomaterials in bone regeneration is a good integration with the host tissue, in which a proper vasculatization is crucial. Calcium phosphate (CP) materials have gain importance in bone regeneration since it has been proved that they stimulate the formation of bone. However, little is known about their angiogenic potential. Recent findings in our group suggest that $Ca^{2+}$ have a role in angiogenesis[1,2,3]. In this study we developed different $Ca^{2+}$ releasing scaffolds by combining different sol-gel CP degradable nanoparticles (containing only Ca and P) with electrospun polylactic acid (PLA) nanofibers. Scaffolds were seeded with human mesenchymal stem cells (hMSCs) and cultured in both regular (RM) and osteogenic (OM) media. Cell proliferation, Alkaline Phosphatase (ALP) activity, VEGF synthesis and L-lactate release were assessed. Angiogenesis was examined *in vitro* by HUVEC tube formation and *in vivo* by using the chick choriallantoic membrane (CAM) angiogenic model. Scaffolds showed a long term (up to 20 days) $Ca^{2+}$ release in both culture media. The presence of the particles in the scaffolds enhanced hMSCs adhesion and increased their proliferation as well as the ALP activity in OM. hMSCs substantially increased their production of L-lactate and VEGF when seeded on the scaffolds containing the particles in RM. However, this increase was minimized when cultured in OM. HUVEC showed an enhancement in tube formation when cultured in the conditioned media obtained from culturing the hMSCs on the scaffolds. This time, no differences were found between the scaffolds with or without particles. Finally, the CAM assay showed a significant increase in the formation of new blood vessels for the scaffolds containing the particles. Their angiogenic response was similar to a VEGF loaded PLA fibers used as a positive control. We demonstrate that the presence of the $Ca^{2+}$ releasing particles enhanced several angiogenic parameters. However, some of these parameters were significantly reduced in OM due to the osteogenic differentiation of hMSCs.

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a91022

## 03-P003 Bioactive hybrid scaffolds impregnated with heparin for promoting bone angiogenesis

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Chitosan and hydroxyapatite have been extensively exploited as important components of scaffolds designed for a number of medical applications, including bone repair and regeneration. Due to their excellent biocompatibility and biodegradability, they have been widely studied for this application [1]. However, the formation of blood vessels during bone regeneration still represents a major challenge and deficient neovascularization leads to improper healing [2].

Heparin is a glycosaminoglycan that binds with several angiogenic modulators. Recently, it has drawn wide attention for its capacity to promote angiogenesis [3].

In the current work, we have fabricated a hybrid scaffold for bone healing applications utilising three components. The scaffold was based on chitosan, which forms interconnected porous structures, and hydroxyapatite, which increases bioactivity. Additionally, we impregnated heparin to obtain a proangiogenic effect. This system promoted and enhanced vascularization in our *in vivo* studies.

Scaffolds were synthesized using freeze-gelation method and subsequently characterized by various analytical techniques and biological tests. SEM images showed the composite's interconnected microporosity, which is necessary for blood vessels to interconnect. FTIR and Raman spectroscopies confirmed the chemical interaction of chitosan, hydroxyapatite, and heparin within the composite. A preliminary evaluation of cytotoxicity was performed with osteoblasts through Alamar Blue assay, which confirmed that synthesized material is non-toxic. Finally, a Chick Chorioallantoic Membrane (CAM) assay was used to observe the potential of the heparinized scaffolds to enhance angiogenesis. A strong angiogenic response was observed in heparin-loaded scaffolds confirming the potential of bioactive hybrid scaffolds impregnated with heparin for bone regeneration applications.

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a91361

## 03-P004 Short-term delivery of fibrin-bound VEGF protein in osteogenic grafts: increased vascularization coupled to efficient bone formation

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Upon implantation in vivo, a major challenge for large-size bone grafts based on bone marrow-derived mesenchymal stem cells (BMSC) is the maintenance of cell viability in the core of the scaffold, which critically depends on the rapid invasion of the construct by the host blood vessels. Over-expression of vascular endothelial growth factor-A (VEGF) by genetically modified human BMSC was shown to improve vascularization in osteogenic grafts, but also to impair bone formation through excessive osteoclast recruitment. This suggested that prolonged exposure to high doses of VEGF had a detrimental effect on bone formation. Here we hypothesized that short-term controlled delivery of VEGF protein may improve graft vascularization without impairing bone formation.

Recombinant VEGF was engineered with the transglutaminase substrate sequence (TG-VEGF) to allow covalent cross-linking into fibrin hydrogels and release by enzymatic cleavage in vivo. Osteogenic grafts were prepared with human BMSC and hydroxyapatite granules in a fibrin hydrogel alone or in combination with 1 µg/ml TG-VEGF. VEGF-expressing BMSC were used as control. Bone formation and vascularization were determined histologically 1, 4 and 8 weeks after ectopic implantation in nude mice and orthotopic implantation in rat calvaria.

At every time point, constructs with fibrin-bound TG-VEGF and VEGF-expressing BMSC displayed significantly increased vascularization compared to the controls with naïve BMSC only. After 4 weeks, fibrin was almost completely degraded and initial formation of dense collagenous matrix could be observed in the constructs containing BMSC only or in combination with TG-VEGF. After 8 weeks, bone formation was severely impaired with VEGF-expressing BMSC as expected, while fibrin-bound TG-VEGF allowed the formation of bone tissue as efficiently as by naive BMSC alone, preventing excessive osteoclast recruitment. In a critical-size bone defect in rat calvaria, grafts with TG-VEGF displayed increased vascularization and a significantly higher coverage of the defect compared to the ones containing VEGF-expressing BMSC, both after 4 and 8 weeks.

These data suggest that VEGF effects on promoting vascularization and bone resorption can be uncoupled by short-term delivery of VEGF protein, providing an attractive and clinically applicable strategy to ensure both rapid vascularization and efficient bone formation, while avoiding the safety concerns related to genetic modification of progenitors.

a90272

## 03-P005 Pro-angiogenic Thermosensitive Injectable Hydrogels for Bone Regeneration

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Hydrogels being similar to extracellular matrix (ECM) of tissues have very attractive features mitigating surgical treatments, and improving sustainable delivery of drugs and growth factors. Thermosensitive polymer solutions show excellent phase transition capacity from sol to gel when cooled or heated. Once low temperature processed polymer solutions are injected into the body, gelation formation occurs in-situ at tissue defects under the stimuli of body heat. In this project, we investigate thermosensitive chitosan matrixed hydrogel compositions in which hydroxyapatite and heparin are impregnated for bone repair and regeneration. Having similar properties to collagen, chitosan has a unique natural polysaccharide structure possessing good biocompatibility, biodegradability combined with the gelation capability makes it an ideal material to be used in tailor-making scaffolds for Tissue Engineering applications. Although chitosan-based thermoresponsive hydrogels have been clinically used for cartilage regeneration, there is limited research on their use in bone regeneration. The chitosan hydrogels with the impregnation of hydroxyapatite increase bioactivity and mechanical strength, and heparin inclusion induces vascularisation, ultimately helping bone regeneration. In this study, chitosan-based gels have been prepared with the varying amount of hydroxyapatite and heparin. These homogeneous composite gels have presented irreversible gelation behavior nearly at the body temperature accordingly the rheology tests ($T_{initial\ gelation}$ = ~31˚C). The initial gelation time of the solutions have been determined as ~ 10 min via test tube invert method. Injection performance of prepared solutions have been tested through the syringes with a range of scaled needles. The finest needle size in which the solutions could have a proper flow was a 21-gauge needle. The chemical structural properties of gels formed at 37 ˚ C have been studied by Infrared and Raman spectroscopies. Morphological properties have also been fully evaluated. Biological properties to assess their biocompatibility and vascularisation potential have also been carried out. Results obtained to date are excellent confirming the great potential of thermosensitive gels to induce bone vascularisation.

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a92693

## 03-P006 Development of a Compromised Maxillofacial Wound Healing Model for Bone Tissue Engineering

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**Objectives:** Defects in the maxillofacial skeleton pose a considerable challenge for reconstruction, especially in cases presenting compromised wound beds. Numerous tissue engineering strategies have been proposed to facilitate maxillofacial bone repair, and they have been investigated in a variety of pre-clinical animal models. However, the animal models typically employ an optimal wound bed that does not fully reflect the complexities underlying the clinical need. Accordingly, the objective of this study was to develop a pre-clinical model of compromised maxillofacial wound healing to approximate the complexities of the clinical scenario.

**Methods:** Twenty male New Zealand White rabbits were separated into irradiated (n=10; 36Gy fractioned over 6 doses of 6Gy each) and non-irradiated (n=10) groups. Dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) was performed on each rabbit at study initiation, prior to surgery, and at study conclusion. A 10-mm circular defect was created in the mandible and an overlaying molar crown was removed in all rabbits, with the surgery occurring 4 weeks after completion of radiation in the irradiated group. Four weeks after surgery, the mandibular defect and surrounding soft tissue was collected and analyzed via micro-CT, H&E histology, and immunohistochemistry.

**Results:** Decreased bone healing was observed in the irradiated group relative to the non-irradiated group, as reflected in micro-CT and histological analysis. Differences between groups were observed through immunohistochemistry analysis for growth factor expression (VEGF, BMP-2 and TGF-beta1). Ongoing DCE-MRI data analysis seeks to characterize differences in perfusion parameters between groups.

**Conclusions:** The data collectively support the development of a novel pre-clinical model of compromised wound healing. The new model presents the ability to perform pre-clinical investigation of tissue engineering approaches for maxillofacial bone healing in a clinically relevant environment.

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a92642

## 03-P007 Study on bone formation in *Ano5*-knockout mice

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Gnathodiaphyseal dysplasia (GDD; OMIM#166260) is a rare skeletal disorder with autosomal dominant inherit pattern. It is characterized by lesions of jawbones, thickening cortical diaphysis of tubular bones and frequent fractures as a results of minimal injury. We found previously that the silence of Ano5 gene lead to increased mineral nodule formation in differentiating MC3T3-E1 osteoblast precursors in vitro and findings suggests that ANO5 plays a role in osteoblast differentiation. However, the pathological effects of ANO5 deficiency on GDD in vivo has not been elucidated completely. Now we generated a *Ano5*- konckout mouse modal with CRISPR/Cas 9 method. The expression of *Ano5* in bone tissue decreased significantly and some clinical features of human GDD have been replicated. Meanwhile, the mouse calvarial osteoblast (mCOBs) cultures was performed and the expression of osteoblast-related genes as well as bone matrix formation assays were investigated by quantitative PCR and alizarin red staining. The results showed that *Osteocalcin, Col1a1*, *Runx2*, *Osterix*, *Osteopontin* and *Rankl* highly elevated and mineralization enhanced drastically in $Ano5^{KO/KO}$ mCOB. The data are consistent with the achievements we observed before in vitro. We believe this new mouse model can contribute to he research into the pathogenesis of skeletal abnormalities in GDD and provide the clues to develop the therapeutic approaches for GDD.

a90547

## 03-P008 A Biomimetic 3D Scaffold for Long Bone Repair

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It's a big challenge to cure long bone defects for bone tissue engineering in clinical therapy. In our study, we designed a biomimetic 3D scaffold with PLGA porous scaffold as inner layer and PLGA/HA nanofibers spiraled outside for long bone repair. Rabbit radius defects model with critical defect size of 15 mm was used to evaluate bone repair effects of the 3D scaffolds we designed. According to the implantation, rabbits are divided into five groups: (1) blank control without any implantation (control), (2) PLGA porous scaffolds (porous), (3) PLGA porous and nanofibers composite scaffolds (composite), (4) porous scaffolds seeded with rabbit bone marrow derived mesenchymal stem cells (porous/MSCs), and (5) composite scaffolds seeded with MSCs (composite/MSCs). The SEM results showed that the porous layer of PLGA had high porosity with pore size of 450 $\mu$m, and the diameter of PLGA nanofibers ranged from 150 nm to 270 nm. At week 4, 8, 12, and 16, it can be seen from X-Ray images that porous/MSCs group showed the best bone regeneration potential following with composite group. The micro-CT results showed the morphology and cross-section images of regenerated bones in each group, which indicated the bone regeneration and re-union. H&E and immunofluorescence staining results showed that more micro blood vessels appeared in composite group and porous/MSCs groups after implanted 4 weeks, which indicated the importance of new blood vessels for initial bone regeneration. SEM images of new bone at week 4 showed collagen formation and calcium deposition in composite group. The mechanical test of regenerated bones from each group showed that porous/MSCs group has similar mechanical property with normal bones. Taken together, we think MSCs could enhance the angiogenesis at the early stage of bone defection and PLGA porous scaffolds seeded with MSCs is one potential candidate for bone regeneration.

a91764

## 03-P009 *Utilizing a scaffold-based local chemotherapy approach for the treatment of Osteosarcoma in an orthotopic humanized mouse model*

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Osteosarcoma (OS) is the most frequent malignant bone tumor, affecting mainly children and adolescents, with a 5-year survival rate for recurrent OS at less than 25%. Systemically administered chemotherapy together with surgery is the current gold standard of treatment, yet the non-specificity of the cytotoxic agents cause severe side effects whilst reducing life quality. To avoid exposure of cytotoxic agents to healthy tissues we have developed melt-electrospun poly( ε -caprolactone) (mPCL)-scaffolds loaded with different dosages of doxorubicin (DOX), the most commonly used drug to treat OS, to exhibit a sustained-release profile after local implantation. An orthotopic humanized tissue engineered bone (ohTEB) was generated at the femur of NSG-mice to create a human niche for OS development. After 6 weeks, primary osteosarcoma was developed by injecting human SAOS2 tumour cells into ohTEB. After 5 weeks in-vivo development of OS we created a biopsy-like defect and implanted the scaffold-based drug delivery system (DDS) into the tumor. Our results showed that in contrast to the intravenous control, high-performance liquid chromatography did not detect DOX in the blood of mice receiving the DOX loaded scaffolds while haematological analysis revealed no signs of myeloablation. Preliminary histological evaluation showed no typical side effects like cardiomyopathy and renal fibrosis as commonly seen during intravenous application of DOX.We anticipate that ongoing histological analysis will show local tumor cell death and decreased tumor load and will be presented at the TERMIS Conference. Altogether, we aim to show that mPCL-based DDS could be implanted during diagnostic biopsy to locally treat potential OS lesions before definite diagnosis, without causing any common side effects.

a90372

## 03-P010 Time-lapsed *In Vivo* Micro-CT Imaging Allows Longitudinal Assessment of Biomaterials in a Mouse Femur Defect Model

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The *in vivo* characterization of novel biomaterials for treatment of critical size bone defects is essential prior to clinical application. However, preclinical studies were often not able to reliably capture the bone healing potential of biomaterials due to limitations in study design: (I) cross-sectional setup, (II) assessment not considering defect sub-volumes, (III) assessment not specific to healing phases. Here, we present a longitudinal *in vivo* approach based on time-lapsed imaging for profound characterization of biomaterials using porous collagen scaffolds and bone morphogenetic protein (BMP-2) as trial materials in a mouse femur defect model. Female C57BL/6J mice received a 2mm femur osteotomy (MouseExFix, RISystem, Davos, Switzerland). Collagen scaffolds (d=2mm, h=2mm; ILS, Saint Priest, France) ± BMP-2 (2.5µg/scaffold; PeproTech, London, UK) were inserted in the defects (n=8/group). The defect area was scanned weekly (week 0-6; vivaCT 40, Scanco Medical, Brüttisellen, Switzerland). Scans were registered consecutively and morphometric indices computed (threshold: 395mg HA/cm$^3$) in the defect center (DC) and periphery (DP). To assess mineralization progression, a second threshold (645mg HA/cm$^3$) was applied. Statistics: 2-way-ANOVA ($p$<0.05). In the BMP-2 group, a significant 38x increase in bone formation (week 2) indicated progression from the inflammation to repair phase with maximum osseous callus volumes by week 3 (DC+DP). Subsequently, decreasing callus dimensions and increasing resorptive activities indicated onset of the remodeling phase. Direct comparison showed the BMP-2 group to have ≥130% (week2-6) more bone in the defect than controls. In the DC sub-volume, BMP-2 application led to a significantly larger callus fraction of highly mineralized bone compared to controls ($BV_{641/394}$ week5: 79±3 vs. 71±6%) indicating advanced callus maturation. By week 6, cortical bridging occurred more frequently in BMP-2 treated animals compared to controls (100 vs. 13%). Using time-lapsed *in vivo* micro-CT, we were able to longitudinally assess the influence of biomaterials (collagen ± BMP-2) on callus progression during fracture healing. Supplementing this approach with controlled mechanical loading will allow to assess the bone healing potential of biomaterials under conditions relevant for clinical applications.

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a92102

## 03-P011 Zonal constructs for cartilage repair; from *in vitro* development to long-term implantation in an equine model

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Hydrogels are promising for cartilage repair, but currently unable to reproduce the native tissue's mechanical properties. Despite encouraging *in vitro* results, *in vivo* hydrogels often fail to meet the biological and mechanical requirements needed to induce native-like hyaline cartilage. In this context, we developed composite constructs with zonal cell distribution based on a thiol-ene cross-linkable hyaluronic acid/poly(glycidol) hybrid hydrogel and a 3D-printed poly(e-caprolactone) (PCL) osteochondral anchor, and tested the effects of the zonal configuration on cartilage repair in a long-term equine model.

**M&M:** P(AGE/G)-HA-SH hydrogel was cast in a layer-wise fashion on a custom-developed 3D-printed PCL osteochondral scaffold that mimicked the architecture of the subchondral bone plate and provided PCL reinforcement for the hydrogel, as well as allowing press-fit fixation of the implant. The chondral portion of the constructs was fabricated by subsequent casting of hydrogel layers seeded with equine chondrocytes and chondroprogenitor cells respectively on the osteochondral anchor.

The chondrogenic potential of these zonal cell-laden constructs was evaluated *in vitro* and *in vivo* in a short-term (4 weeks) and a long-term study (6 months). The latter with direct comparison of zonal vs non-zonal constructs in 8 adult Shetland ponies (one defect per stifle joint).

**R:** The printed anchor facilitated surgical handling of the construct and allowed good integration of the construct *in vivo*. The PCL-reinforced layered hydrogels showed collagen II production and chondrogenic differentiation *in vitro*. After 4 weeks *in vivo,* hydrogels were still present and showed early signs of chondrogenic differentiation. Six months after implantation, only hydrogel fragments were detected; defects were filled with a mixture of fibrocartilaginous tissue, and new bone infiltrated the anchor. Stiffness of the repair tissue in the zonal group was 120 ± 17 kPa, higher than in the non-zonal group (94 ± 14 kPa). Histological scoring analysis of the repair tissue did not show significant differences between zonal and non-zonal constructs.

**C:** Constructs with a zonal composition were successfully biofabricated and implanted in a large animal equine model. The biomaterial persisted partly up to 6 months. Stiffness of the zonal constructs was increased compared to the non-zonal group, despite no clear histological differences, which may be too subtle to be detected by current scoring methods.

a93226

## 03-P012 Molecular and histological characterization of nasal chondrocyte-based cartilage grafts for the treatment of kissing lesions in the knee

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**Introduction** Articular cartilage "kissing" lesions in the knee are untreatable lesions that lead to osteoarthritis. They are characterized by two defects in direct contact to each other. The aim of this study was to characterize and evaluate the quality of engineered cartilage grafts prior to implantation in the defect induced in the sheep animal model. Grafts were produced using sheep nasal chondrocytes obrained from the nasal biopsy and cultured on the collagen-based scaffold for two days (N-CAM) or two weeks (N-TEC). Assessment of the N-CAM and N-TEC quality was performed using histological and molecular methods.

**Methods** Sheep nasal septum biopsy was dissected into small pieces and digested in collagenase solution. Chondrocytes were expanded in monolayer for 13 days, seeded on a collagen scaffold (Chondro-Gide membrane) and cultured in chondrogenic medium for 2 days for N-CAM grafts or 2 weeks for N-TEC grafts. Manufactured grafts were fixed and embedded in paraffin for histological analysis. Grafts were stained using HE, safranin O, picrosirius red and immunostained against collagen type I, II and aggrecan. Total RNA from cartilage grafts was isolated using TRIzol reagent and reverse transcribed to cDNA to perform RT-qPCR and quantify gene expression of collagen I, II and aggrecan.

**Results** Histologically, the N-TEC grafts contained higher amount of glycosaminoglycans (GAG) accompanied with higher content of collagen type II and aggrecan, compared to N-CAM grafts. Collagen type I level was low in both N-CAM and N-TEC grafts. qPCR analysis confirmed higher collagen type II and aggrecan expression in N-TEC grafts.

**Conclusion** Two types of engineered grafts shows different expression of GAG and extracellular matrix cartilage specific proteins which can impact the treatment of articular cartilage kissing lesions. N-CAM is immature graft with little or no extracellular matrix while N-TEC is mature graft with extracellular matrix containing cartilage specific proteins. The regenerative potential of these grafts will be evaluated after implantation.

**Keywords:** nasal chondrocytes, tissue engineering, cartilage, kissing lesions

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a90006

## 03-P013 Reconstruction of osteochondral defects using a microenvironment created from autologous endothelial progenitor cells and PLGA scaffolds in rabbit model

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**Objective:** Repairing articular cartilage is clinically challenging. Previous study demonstrated that a degradable PLGA created a temporary space for neo-tissue development and facilitated the regeneration process of cartilage defects [1]. Endothelial progenitor cells (EPC) provide a high capacity for regeneration and vasculogenesis in different tissues. The applications for EPC have been reported for cardiovascular diseases and bone regeneration [2]. We investigated a simple and clinically feasible cell-based therapeutic approach using a poly(lactide-co-glycolide) (PLGA) scaffold seeded with endothelial progenitor cells (EPC) to repair a full-thickness osteochondral defect in rabbits. **Methods:** EPC obtained by purifying a small amount of peripheral blood from rabbits were seeded into a biocompatible PLGA scaffold, namely, EPC-PLGA, and implanted into the osteochondral defect in the medial femoral condyle. Rabbits were randomized into three groups: the empty defect group, the PLGA-only group or the EPC-PLGA group. The defect sites were evaluated 4 and 12 weeks after implantation. **Results:** At the end of testing, only the EPC-PLGA group showed the development of new cartilage tissue with a hyaline articular surface. At week 4, the EPC-PLGA group showed a greater amount of synthesized glycosaminoglycan (GAG) content, and a higher degree of osteochondral angiogenesis in repaired tissues. At week 12, the EPC-PLGA group showed more hyaline cartilage regeneration, and greater GAG and collagen type II content. Moreover, the EPC-PLGA group showed significantly higher bone volume per tissue volume and trabecular thickness. **Conclusion:**The present EPC-PLGA system generates a suitable in situ microenvironment for osteochondral regeneration without the supplement of exogenous growth factors.

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a90828

## 03-P014 Bone Healing in the Axolotl – A novel Surgical Approach to Femur Osteotomies

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**Introduction**

Axolotls have the great ability to regenerate the complete limb after amputation. This model of whole limb regeneration is widely used in regenerative research. However, the bone healing capacities of axolotls remain still relatively poorly understood. In most previous studies, healing was analyzed in lower limb where out of two bones one was fractured and the other served as fracture fixation (e.g. ulna/radius and tibia/fibula). Here, we present for the first time a surgical technique that allows to stabilize the femur of the axolotls with a customized fracture fixator plate after osteotomy.

**Methods**

Surgery on 12 axolotls (1.5-2 years old) was performed after anesthesia using 0,3% benzocaine solution, in which the animals were placed. Similar to rodents, a longitudinal skin incision on the anterolateral side of the femur was created, followed by a blunt preparation to the bone periosteum. In 6 animals each, either a stiff or flexible fixator plate was used to allow analyzing bone healing under different mechanical constrains (RISystems, Davos, Switzerland). A 0.7mm gap was created in the midshaft of the bone. The skin was stitched over the plates. Post-surgery the animals received pain medication given into the water. Healing outcome was evaluated 3 weeks after osteotomy.

**Results**

The size of these axolotl femurs were comparable to mice femurs even though the midshaft of the bone was more narrow. The muscles were colorless, transparent and difficult in handling compared to mice or rat. The bone itself seemed to be softer compared to mice which was noticeable when drilling and inserting the screws. All animals recovered well and moved their legs normally during swimming. One drop out had to be documented after a week. After 3 weeks, the µCT evaluation demonstrated a bone structure of cancellous bone metaphyseal and diaphyseal but showed no signs of healing.

**Discussion**

Axolotls have a higher regenerative capacity for whole limb regeneration where it takes roughly 5-6 months for full limb regeneration in animals of this age and size. However, the healing of a bone osteotomy was not detected within 3 weeks, independent of fracture fixation. Mice with a similarly sized osteotomy completely bridge after 3 weeks of healing. The here demonstrated surgery for osteotomy fixation opens the possibility of controlled bone healing experiments in axolotls while ensuring the comparability to healing in mammals (mice).

a90512

## 03-P015 PLGA Microspheres: In-vivo Evaluation for Osteomyelitic Treatment

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The prophetic medicine is getting more attention in the present days among the researchers. The synergistic effects of *Nigella sativa* when combined with conventional drugs have been reported in many scientific articles in treating diseases. In this current study, PLGA microspheres loaded with *N. sativa* and gentamicin were fabricated intended to treat osteomyelitis with the ability to give a sustained release characteristic for both active pharmaceutical ingredients. The emulsion evaporation method was used to fabricate 54 formulations of microspheres with the encapsulation efficiency was the main evaluation to be analysed, other than size distribution, external morphology and zeta potential. Ten formulations with the best encapsulation efficiency were selected for the release profile study utilizing the compressed and powdered microspheres. After 4 weeks of sustained release study, one formulation was chosen from *N. sativa* and gentamicin group before being evaluated in animal model. 16 rabbits were divided into 4 groups and each of them were inoculated with *Staphylococcus aureus* to initiate the infectious condition mimicking osteomyelitis. Three of the groups were given treatment using the microspheres and no treatment for another group (control group). X-rays images and blood counts indicated the microspheres were able to eradicate the infection after 6 weeks prior to the inoculation. The result was further supported by the post-surgery microbial study showing the ability of the microspheres to treat the osteomyelitic condition in rabbits. This promising findings open a new potential approach in treating osteomyelitis and to be further improved as an alternative treatment.

a91222

## 03-P016 In vivo evaluation of 3D-printed and tissue engineered bone grafts for alveolar cleft osteoplasty

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One of the most common hereditary craniofacial anomalies in humans are cleft lips, cleft alveolar bone with or without cleft palate. Clinically, the augmentation of the persisting alveolar bone defect, called alveolar cleft osteoplasty, is performed by using autologous bone grafts. The accompanying disadvantages are leading to an intensive search for alternatives. Objective of the present study was the in vivo application of 3D-printed and tissue-engineered bone grafts and their evaluation regarding the potential to promote osseous defect healing.Scaffolds were designed according to the particular defect geometry and produced by 3D-printing of a calcium phosphate cement paste (Innotere GmbH, GER) under mild conditions. Two different pore designs could be achieved by 60° (scaffold 1) and 30° (scaffold 2) rotation of the strand orientation of consecutive layers during the printing process. After hardening the scaffolds were colonized with undifferentiated murine mesenchymal stromal cells (MSC). Artificial bone defects with a diameter of 3.3 mm were created surgically in the palate of 80 adult Lewis rats. Five experimental groups were examined: scaffold 1 without cells, scaffold 1 with MSC, scaffold 2 without cells and scaffold 2 with MSC. In a control group, the defect remained empty. After 6 and 12 weeks, the remaining defect width and percentage of bone formation were quantified histologically.The clinical application of all bone grafts was easy and their fitting very good. 78 of 80 animals completed the study. After 12 weeks, the remaining defect width measuredbetween 2.26 ± 0.41 mm (control) and 2.76 ± 0.25 mm (scaffold 1 with MSC). Bone formation was ongoing up to 12 weeks. This was significant for the control group (6 weeks: 13.2 ± 5.2%; 12 weeks: 22.5 ± 6.3%) and scaffold1 without cells (6weeks: 8.2 ± 4.5%; 12 weeks: 19.0 ± 6.7%). Scaffold 2 exposed a significantly reduced bone formation comparing to control and scaffold 1. An additional MSC-colonization did not enhance defect healing irrespective of the scaffold geometry.3D-printing of calcium phosphate cement paste is suitable for building scaffolds, which are fitting exactly to an artificial alveolar defect. The pore geometry influences bone formation significantly and a 60° strand rotation shall be preferred. The creation of a sufficient 3D-printed and tissue-engineered bone graft for alveolar cleft osteoplasty could preserve patients from donor site morbidity.

a92520

## 03-P017 Establishment of osteomyelitis model in rat induced Methicilin-resistant Staphylococcus aureus

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**Introduction.** The bone defect problems due to traumatic injury are steadily increasing. The bone implant market is quickly developing, implant quality is gradually improving and more biomaterials are implanted every year. But, this is an invasive surgery with an increased risk of infections. Nowdays, the incidence of osteomyelitis is 1–2% in the United States and is more prevalent in developing countries with mortality rate as high as 2%. Staphylococcus aureus is the predominant causative agent of osteomyelitis, it usually invade osteoblasts, leading to pervasive inflammation, necrosis and bone destruction at the infection site. Furthermore, increasing numbers of osteomyelitis cases are caused by multi-drug resistant bacterial strains such as methicillin-resistant Staphylococcus aureus and possess even more formidable clinical challenges. A better understanding of the complex pathogenesis of osteomyelitis and definition of osteomyelits are required for the development of effective strategies to combat osteomyelitis. thus, To develop the more effective therapy, we have established a calvaria defect model induced osteomyelitis by MRSA.

**Materials and methods.** It was carried out by using the 36 male Sprague-Dawley(SD) rats. Rat model of osteomyelitis was made by direct inoculation of MRSA into calvaria bone defect. To examine the relationship between the inoculation dose of the bacteria and the progression of the osteomyelitis, the inoculated lesions were assessed for changes in histological, bacteriological, clinical and haematological parameters at 4week after infection. Serial dilutions of the bacteria [$6{\cdot}10^{0}$ to $6{\cdot}10^{5}$ colony-forming units (CFU)/10 ul(G0~G5)] suspended in TSB or saline alone were inoculated.

**Results.** S. aureus infection was confirmed by the above parameters. control group did not develop osteomyelitis. By combining the clinical, haematological, bacteriological and histological scroe collected during the experimental follow-up, we were able to differentiate between the control and the infected condition and development of significant signs of osteomyelitis required an inoculum of at least $6{\cdot}10^{3}$ CFU/5μl.

**Conclusion.** this results suggest that an inoculum $6{\cdot}10^{3}$ CFU/5μl induces the development of osteomyelitis with clear infective destruction in the calvaria, and that our model may be applied to the identification of virulence factors in studies of posttraumatic osteomyelitis.

**Keywords.** Animal model, calvaria bone defect, osteomyelitis

a93296

## 03-P018 Early application of the bisphosphonates after radiation hadn't prevention the occurrence of radioactive osteonecrosis in the mandible

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Mandibular osteoradionecrosis (osteoradionecrosis, ORN) often result in the maxillofacial tissue and organ defects, serious impact on cancer patients quality of life, the pathological changes of irreversible, the diversity of clinical manifestations and treatment of intractable, shows the importance of the study of the pathogenesis and prevention. On the basis of the previous study that established a large animal model of mandibular ORN in miniature pigs, preliminary discussed the pathogenesis and autologous bone marrow mesenchymal stem cell transplantation showed successful treatment and prevention, We used the simulation method of clinical tumor radiation therapy to illuminate of miniature pigs with 25Gy. In the early stage after the illuminate, the bisphosphonates, 800mg/day,for 4 weeks, had been used to inhibit osteoclast in the experimental group and the placebo in control group. Through different point bone tissue pathology, osteogenesis, Osteoclast and blood vessel related gene and protein expression differences, as well as the jaw observation of the animal model, We did not observe the prevention of radioactive osteonecrosis in the mandible by the bisphosphonates in early stage after radiation.

a92958

## 03-P019 The effect of transcutaneous $CO_2$ application on distraction osteogenesis of rabbit tibia

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**INTRODUCTION:**
Distraction osteogenesis has been widely used in the treatment of various structural bone deformities. However, prolonged healing time remains a major problem. We previously demonstrated that transcutaneous application of $CO_2$ by means of a novel hydrogel accelerates fracture repair in rats [1]. In this study, we investigated the effect of the $CO_2$ application on distraction osteogenesis of rabbit tibia.
**METHODS:**
Animal model A distraction osteogenesis of rabbit tibia model (n=15) was created as previously described [2]. After 7-day latency period, the distraction was continued at 1 mm/day for 10 days. From the next day of the operation, 20-minute transcutaneous application of $CO_2$ by means of a hydrogel on operated leg was performed five times a week for 45 days (7-day latency period, 10-day distraction period and 4-week consolidation period) in the $CO_2$ group (n = 7). Sham treatment with air application was done in the control group (n = 8).
Radiographic assessment Radiographs of the distracted tibia on the anteroposterior views were taken just after the distraction period and every week of consolidation period. Bone density of the distracted area was quantified by analyzing pixels with the Image J program to be quantitatively compared between the two groups.
Histological assessment After 4-week consolidation period, all distracted tibias were harvested and used for histological assessment to investigate the difference in bone regeneration process between two groups. Sagittal sections were stained with hematoxylin and eosin.
**RESULTS:**
Radiographic assessment Pixel values for the distraction gap area indicated that the $CO_2$ group showed significantly higher pixel values than the control group at 2 and 4 weeks of consolidation period ($p < 0.05$).
Histological assessment At 4 weeks of consolidation period, $CO_2$ group histologically showed greater volume of trabeculae bone than control group.
**DISCUSSION:** In the current study, transcutaneous application of $CO_2$ accelerated bone regeneration in distraction osteogenesis model of rabbit tibia. Previous study has suggested that $CO_2$ may enhance bone fracture healing with the promotion of angiogenesis, blood flow, and endochondral ossification [1]. It is possible that similar mechanisms may work in distraction osteogenesis.
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a91238

## 03-P020 Different production technology of blood derived products influences origin of extracellular vesicles

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Extracellular vesicles (EVs) have been increasingly recognized as central players in intercellular communication. Depending on their cargo, they can influence various biological functions in the recipient cells and have been implicated in the progression of various diseases. EVs are present in most physiological fluids, including plasma and synovial fluid. In this study, we investigated the presence of EVs within two autologous blood products, platelet rich plasma (PRP) and hypACT serum, in order to gain understanding of the mechanisms of action of these products. PRP, in particular, is frequently clinically applied to support the treatment of osteoarthritis, but its specific mechanism of action remains to be elucidated.
PRP and hypACT serum were produced from whole blood derived from the same donor using standardized, but distinct isolation protocols. EDTA or citrate was used in PRP samples as anticoagulants. The abundance and average size of EVs in these blood products were determined by flow cytometry and nanoparticle tracking analysis (hypACT serum: 4600 EVs/µl ± 717, 156 nm ± 0.72; PRP (EDTA): 3000 EVs/µl ± 314, 149 nm ± 5.9; PRP (citrate): 6900 EVs/µl ± 4909, 158 nm ± 13.04). The cellular origin of the EVs was determined using flow cytometry with CD41 as platelet marker and CD235a as red blood cell marker, while lactadherin (LA) served as marker for phosphatidylserine exposing EVs.
The majority of EVs in hypACT serum originated from platelets ($LA^+/CD41^+$). Interestingly, the two anticoagulants had a strong impact on the cellular origin of EVs present in PRP. EVs from PRP samples including EDTA as an anticoagulant descended mainly from red blood cells ($LA^+/CD235a^+$) whereas EVs from citrate treated PRP samples mainly originated from platelets ($LA^+/CD41^+$). Next, we isolated EVs from the respective blood products by ultracentrifugation and verified successful isolation by staining with EV-specific markers. These isolated EVs will be further used to investigate their role in cell culture models of inflammation and osteoarthritis. In conclusion, our data reveal differences between PRP and hypACT serum both with respect to EV counts and cellular origin, which may have implications for the therapeutic application of these blood products. In addition, our results demonstrated that anticoagulants have a strong impact on the cellular origin of EVs and subsequently on their biological functions.

a92033

## 03-P021 Combination of Endogenous Stem Cell Mobilizer and Osteoinductive Nanofibrous Scaffolds for *In Situ* Bone Tissue Engineering

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For bone tissue engineering, both exogenous cells and scaffolds are conventionally required. Despite promising results, clinical adoption of this strategy has been limited due to various drawbacks such as low cell survival rate, extensive cell expansion steps, and the possibility of immune-rejection. To overcome these limitations, the self-regenerative capacity of the host is exploited by mobilizing endogenous stem cells from bone marrow to the injured site. Systemic injection of substance P (SP) induces mobilization of host bone marrow-derived mesenchymal stem cells to the injury site and enhances bone tissue regeneration in a critical-sized bone defect model. To provide an appropriate environment for endogenous stem cells to survive and differentiate into osteogenic lineage cells, electrospun nanofibrous polycaprolactone scaffolds are functionalized with hydroxyapatite (HA) particles via a polydopamine coating. HA-functionalized, highly osteoinductive scaffolds are implanted in critical-sized calvarial defect sites. The combination of the highly osteoinductive scaffolds and SP treatment enhances *in situ* bone tissue regeneration in calvarial bone defects. In our study, this *in situ* bone regeneration strategy combining recruitment of endogenous mesenchymal stem cells from the host bone marrow to injury sites and implantation of a HA-functionalized osteoinductive cell-free scaffold system can provide an effective stem cell therapeutic platform in regenerative medicine.

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a90865

## 03-P022 Programming adult human mesenchymal stromal cells towards stable chondrogenesis following developmental cues

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It is generally accepted that adult human bone marrow-derived mesenchymal stromal cells (hMSCs) are default committed towards osteogenesis, typically forming hypertrophic cartilage that undergoes endochondral ossification upon implantation. Since embryonic mesenchyme is also competent to generate stable cartilage, it is questioned whether a correspondence exists between mesenchymal progenitor compartments during development and in adulthood. Here we tested whether forcing specific early events of articular cartilage development can program hMSCs towards stable chondrogenesis. Inspired by recent findings that spatial restriction of Bone Morphogenetic Protein (BMP) signaling guides embryonic progenitors towards articular cartilage formation[1], we hypothesized that selective inhibition of BMP drives also the phenotypic stability of adult hMSCs-derived chondrocytes. Two BMP type-I receptor inhibitors were screened in a microfluidic platform[2] for their time- and dose-dependent effect on chondrogenesis. We demonstrate that transient blockade of both ALK2 and ALK3 receptors, while permissive to hMSC cartilage formation, is sufficient to maintain a stable chondrocyte phenotype, resulting in a gene profile coherent with articular cartilage development and function. Even upon compound removal hMSCs were no longer competent to undergo hypertrophy in vitro and endochondral ossification in vivo, indicating the onset of a constitutive change. We also postulate the role of ALK2 and ALK3 inhibition in triggering a protective mechanism against vascularization, as demonstrated by increased Chondromodulin and LIF expression, eventually favouring the maintenance of stable and avascular cartilage tissue. Our findings demonstrate that adult hMSCs effectively share properties of embryonic mesenchyme not only in the formation of transient but also of stable cartilage. This opens potential pharmacological strategies to articular cartilage regeneration and broadly indicates the relevance of developmentally inspired protocols to control the fate of adult progenitor systems.

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a90872

## 03-P023 The Effect of Betulinic Acid on Human Mesenchymal Stem Cell Proliferation and Osteogenic Differentiation

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Osteoporosis is a major health problem of elderly caused by an imbalance between bone formation and bone resorption. Current treatments such as bisphosphonates or strontium ranelate usually cause several side effects including severe bone fractures and vein thrombosis. Betulinic acid (BetA) is a pentacyclic triterpenoid found in *Sambucus williamsii* Hance (Elderberry). Recent studies showed that BetA had anti-osteoclastogenic effects and also induced calcium deposition in mice osteoblasts. In this work the effects of betulinic acid on cell proliferation and osteogenic differentiation were investigated in human mesenchymal stem cell (UE7T-13, JCRB no. 1154). Cell proliferations were measured by alamarBlue assay. The result showed that BetA at the concentration of 0-20 μM had no effect on cell viability of UE7T-13 after 3 days and 7 days exposure. Alkaline phosphatase (ALP) activity assay showed that BetA at the concentration of 10 and 20 mM significantly increased ALP activity at day 3 and day 7 ($n=3$, $p<0.05$). Calcium accretion analysis with calcium colorimetric assay (*o*-cresolphthalein-based assay) and Alizarin Red staining showed that BetA at 10 and 20 mM induced more calcium deposition at day 10 and 14. This suggested that betulinic acid can induce osteogenic differentiation in hMSC by enhancing ALP activity and increasing calcium deposition. Further studies on the associated pathways are under investigations.

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a91183

## 03-P024 Electrospun nanofibrous scaffolds enhance paracrine function of mesenchymal stem cells (MSCs) for cartilage regeneration

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MSCs release a plethora of biologically active factors that elicit biological responses that could regulate the local immune response and mediate in overall tissue regeneration. A potentially useful MSC-based strategy is the exploitation of the paracrine factors of MSCs towards a cell-free therapy in cartilage tissue engineering. An area of active interest is how paracrine factor production and release by MSCs can be modulated towards a specific regenerative application.

Pre-conditioning of MSCs to various microenvironmental cues has been shown to significantly change the repertoire of the MSC secretome. To date, the functional impact of MSC secretome generated from nanofibrous scaffold on MSC chondrogenesis have yet to be explored. In this study, we investigated the effect of distinct nano-patterned fibrous scaffolds on the MSC secretome repertoire and the consequence of these differential paracrine effects on MSC and chondrocyte chondrogenesis.

Human MSCs were cultured under normal tissue culture plate, with aligned and random electrospun polyester nanofibrous scaffolds to generate differential conditioned media.

Subsequently, each of the differential conditioned media was normalized to cell count and applied to MSC or chondrocyte 3D-pellet culture undergoing chondrogenic differentiation.

Conditioned media derived from both nano-patterned fibrous scaffolds showed significant increased induction of chondrogenesis, compared to that from tissue culture plate alone. Our results suggest that MSCs pre-conditioned on nanofibrous scaffolds have enhanced the chondrogenic potential, indicating an alteration of the secretome profile. This work demonstrated the potential of producing a tailored-made MSC secretome for cartilage tissue regeneration.

a92089

## 03-P025 Incorporation of cannabidiol-loaded microspheres into gelatin/nano-hydroxyapatit promote bone regeneration in rat radial bone defects

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Critical-sized bone defects constitute a major health issue in orthopedics which usually cause mal/nonunions. Recruitment of mesenchymal stem cells (MSCs) to the injury site and their differentiation into the desired cell lineage are of great importance in bone regeneration. An ideal structure which provides these demands for bone regeneration has not been introduced yet.The current study is aimed to develop a novel scaffold by incorporation of cannabidiol (CBD) into porous osteoconductive scaffold to induce the migration of MSCs towards the defect site and enhance bone regeneration. We fabricated Gelatin/nano-hydroxyapatite (G/nHAp) scaffold to deliver cannabidiol (CBD)-loaded poly (lactic-co-glycolic acid)(PLGA) microspheres to critical size radial bone defects in a rat model. The fabricated scaffolds were characterized by X-ray diffraction (XRD), scanning electron microscopy (SEM) and analyzed for porosity and degradation rate. The release profile of CBD from PLGA microsphere and CBD-PLGA-G/nHAp scaffold was analyzed by fluorescence spectroscopy. The effect of CBD delivery on cellular behaviors of migration, viability and osteogenic differentiation were subsequently evaluated under in vitro conditions. For animal study, the defects were randomly filled with CBD-free G/nHAp, CBD-PLGA-G/nHAp, autograft, and a group was left empty without any treatment.
Physical characteristics of fabricated scaffolds confirmed uniform distribution of CBD-microsphere within G/nHAp scaffold. Scanning electron microscopy (SEM) images and MTT assay demonstrated the attachment and viability of MSCs in the presence of CBD, respectively. *In vitro* migration assay showed that CBD considerably increased the migration of MSCs. The qRT-PCR results showed the expression of osteogenic markers up-regulated in the presence of CBD. Histological and immunohistochemical findings confirmed new bone formation and defect reconstruction at 4 and 12 weeks post-implantation in CBD-PLGA-G/nHAp. Immuno-fluorescent analysis revealed enhanced migration of MSCs into the defect areas in CBD-containing group *in vivo*. Therefore, it is concluded that CBD improved bone healing and demonstrate the critical role of MSCs migration in bone regeneration process.

a92733

## 03-P026 *In vitro* beneficial potential of human Adipose-derived Stromal Cell secretome for osteoarthritis

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Background – Adipose-derived Stromal Cells (ASCs) possess a strong anti-inflammatory potential, which is mediated by a wide array of released bioactive factors. The use of conditioned medium (CM) instead of cells presents substantial advantages especially in terms of handling and safety. We have been studying the potential of hASC secretome in contrasting osteoarthritis (OA), an inflammatory disease characterized by hypertrophic differentiation of chondrocytes (CHs) and extracellular-matrix-degrading proteases production.
Materials & Methods – CM was collected from hASCs derived from subcutaneous adipose tissue, cultured for 72 hours in starving conditions. The CM was then concentrated trough Amicon Ultra-15 Centrifugal Filter Unit (Merck-Millipore) of about 46 ± 10-folds (n=26). Hypertrophy was induced *in vitro* in human primary articular chondrocytes with 10ng/ml TNFα. CM (ratio 5:1, hASCs:hCHs) effect on cell proliferation (up to 9 days) and on gene and protein expression of MMPs and other hypertrophic markers (24 and 72 hours) were evaluated.
Results – hCH proliferation was prompted (+40%) by a 9-day-treatment with 10 ng/ml TNFα, suggesting the induction of a hypertrophic growth status. Despite the lack of CM effect on CH proliferation, the conditioned medium treatment reverted the TNFα-induced significant increase in *MMP3* and *MMP13* expression (-50% and -30%, respectively), after 24 hours. The reduction in MMP13 protein expression was also evident after both 24 and 72 hours. Moreover, hASC conditioned medium inhibited TNFα-mediated osteocalcin release. The effect of CM on other OA and hypertrophic markers, in TNFα-inflamed CHs, is currently under investigation.
Conclusions - Our data reinforce the idea that ASC secretome might be considered a promising source of factors for future therapeutic applications in the OA treatment. The analysis of CM sub-components (EVs and soluble factors) and the comparison of ASC secretome with CM from cells lacking this therapeutic potential, might allow us to reveal the factors responsible for secretome beneficial action.

a92825

## 03-P027 Osteogenic potential of tauroursodeoxycholic acid (TUDCA) as an alternative to rhBMP - 2 in a mouse spinal fusion model

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The non-union rate following lumbar spinal fusion is potentially as high as 48%. To support efficient bone regeneration, recombinant human bone morphogenetic protein-2 (rhBMP-2) is commonly used as it is regarded as the most potent bone inducing molecule. However recently, there have been increasing concerns on the use of rhMBMP-2 such as serious complications including seroma and heterotopic ossification and the low quality of bone at the center of fusion mass. Thus, many studies were conducted to find and to develop a potential alternative to rhBMP-2. In this study, we investigated the osteogenic potential of tauroursodeoxycholic acid (TUDCA) in mouse fusion model and compared its effects with rhBMP- 2. Twenty-four mice underwent bilateral posterolateral lumbar spinal fusion bone formation at L4-L5. Collagen sponge infused with either saline, TUDCA, or rhBMP-2 were implanted at the fusion area. Two (2) and four (4) weeks post-implantation, bone formation and tissue regeneration were evaluated via micro-computed tomography and histological analysis.
Compared to TUDCA treated group, the rhBMP-2 treatment produced higher amount of bone fusion formation after 2 weeks but also showed higher resorption of the centralized bone after 4 weeks. Interestingly, TUDCA treated group developed higher trabecular thickness compared to rhBMP-2 after 4 weeks. Moreover, TUDCA treatment showed distinct angiogenic activity in human umbilical vein endothelial cells (HUVEC) as confirmed by in vitro tube formation assay. Our findings suggest that TUDCA is comparable to rhBMP-2 in supporting bone regeneration and spinal bone formationfusion by increasing trabecular thickness and promoting angiogenesis. Finally, our results indicate that TUDCA can be utilized as a potential alternative to rhBMP-2.

a90609

## 03-P028 Injectable Depot/Release-Controlled Lovastatin Nanoparticles Enhance Alveolar Bone Repair in Rabbits

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**Objectives:** Statins, in appropriate dosages, stimulate bone formation *in vitro* and *in vivo*, via inducing osteogenesis and angiogenesis. Hence, a release-controlled delivery strategy is desirable to enhance safety and therapeutic effects. In this work, stable, biocompatible, biodegradable and release-controlled core-shell nanoparticles loaded with Lovastatin were formulated, characterized and evaluated for the non-invasive and dose-responsive accelerated repair of alveolar bone defects in rabbits after a single injection/application. **Methods:** Colloidal suspensions of multilayered nanoparticles composed of a liposomal core and a bi-polyelectrolyte shell were prepared via modified thin-film hydration/layer-by-layer self-assembly techniques. Formulation parameters were optimized to obtain a reproducible delivery system for Lovastatin. The resulting reservoir nanoparticles were characterized for size, surface charge, morphology, loading capacity and release kinetics over an extended period of 21 days. Cytotoxicity was assayed with murine pre-osteoblasts and viability was determined by colorimetry. Critical-sized alveolar defects were created in the maxilla of 40 New Zealand White rabbits (control/experimental groups), to further investigate dose-responsiveness of the distinctive formulations. Biocompatibility of unloaded/loaded nanoparticles was also examined.
**Results:** The modified self-assembly deposition technique succeeded in building a spherical, monodisperse and stable hybrid nanoparticulate (<100nm) drug delivery system, with high entrappment efficiency, for the sustained linear release of statins. All rabbits showed no toxic health effects, immune responses and/or change in organ functions. The system localizes the effect of the released bioactive load within the site of injection with no significant tissue distress. Histomorphometry revealed 90% mature bone-fill (trabecular) in the defects treated with Lovastatin nanoparticles within 30 days. **Conclusions:** The formulated depot core-shell nanoparticles prolonged the bioactive release and localized residence time of dose-responsive Lovastatin in rabbits. Significant formation of dense *de novo* alveolar bone resulted in 4 weeks. Our pre-clinical results suggest the therapeutic potential of release-controlled Lovastatin for non-invasive alveolar bone defect restoration.

a92139

## 03-P029 Local administration of nuclear factor of activated T cells (NFAT) c1 inhibitor to suppress early resorption and inflammation induced by bone morphogenetic protein-2

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Nuclear factor of activated T cells (NFAT)-c1 is known as a key regulator in osteoclast differentiation and immune response. As follow-up to our previous study showing the anti-resorptive activity of VIVIT, a peptide type NFATc1 inhibitor, using absorbable collagen sponge (ACS), the current study aimed to investigate the effective concentration range of local VIVIT that suppresses early excessive osteoclast activation and inflammation induced by high-dose recombinant human bone morphogenetic protein (rhBMP)-2 and concomitantly enhances bone healing in a rat critical-sized calvaria defect model. High-dose rhBMP-2 (40 mg/defect) alone significantly increased *in vivo* osteoclast activation and expression of the inflammatory cytokines interleukin-1b and transforming necrosis factor-a on the scaffold at 7 days after surgery. However, rhBMP-2 had no direct effect on osteoclast activation *in vitro*. Osteoclast activation by rhBMP-2 was significantly suppressed by combined treatment with VIVIT at concentrations of 75 and 150 mM, but not at 15 mM, whereas suppression of inflammation occurred at all doses of VIVIT. Micro computed tomography at 4 and 8 weeks after implantation revealed that the combination of rhBMP-2 and VIVIT at 75 mM VIVIT led to a greater bone fraction at the initial defect area, compared with rhBMP-2 alone. These findings revealed that local administration of VIVIT at certain concentrations has multiple positive effects to weaken early excessive osteoimmunological responses and enhance bone healing after rhBMP-2 administration. VIVIT has possible potential to expand the therapeutic area of high-dose rhBMP-2 therapy to inflammatory bone loss.

a92492

## 03-P030 Effect of Mesenchymal Stromal Cell Recruiting Chemokine CCL25 on Healthy and OA Cartilage

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**Objective:** We have recently reported that the thymus expressed chemokine CCL25 is a potent cell attractant for mesenchymal stromal cells, and therefore a promising candidate for *in situ* tissue engineering. Moreover, we have established a GMP-compliant injectable implant of hyaluronic acid and CCL25-releasing PLGA particles to recruit endogeneous cells for cartilage repair. However, the influence of CCL25 on cartilage is still unknown. Here we report the effect of CCL25 on *in vitro* tissue-engineered healthy and osteoarthritic (OA) cartilage, and also on *in vivo* OA progression in the *Dunkin-Hartley* Guinea Pig (DHGP) model of spontaneous OA. **Methods:** Porcine chondrocytes were 3D-cultured in a micromass model that has been proven to mimic key-aspects of human cartilage and OA alterations upon stimulation with TNF-α. After 2 weeks, chondrocytes were stimulated with 0.05-500 nmol/L CCL25 alone or together with TNF-α for 1 week. The effect of CCL25 was evaluated by life/dead and Safranin O staining, histomorphometrical analysis of glycosaminoglycans (GAGs), and GeneChip analysis. Moreover, hyaluronic acid alone, 70-7000 pg CCL25 alone, or a combination thereof was intra-articularly injected (5x, weeks 1-5) in 12 months old DHGPs, and after 6 months the effect on OA progression was evaluated by H&E and GAG staining, and subsequent cartilage scoring. **Results:** We found no CCL25 stimulated cell death in *in vitro* tissue-engineered healthy or OA cartilage. However, 500 nmol/L CCL25 led to a significant reduction of GAGs. Global expression profiling revealed a stable cartilage marker gene expression when using 5 nmol/L CCL25, and a significantly changed expression of collagens like *COL2A1*, GAGs, matrix metallopeptidases like *MMP1* and *MMP13* in the 500 nmol/L group. Gene Ontology analysis showed a significantly enrichment of cartilage related terms like cartilage development only in the 500 nmol/L CCL25 group. In the DHGP model of spontaneous OA, 12 months old animals showed clear signs of OA. Moreover, based on GAG staining and subsequent cartilage scoring, intra-articular injection of 700 pg CCL25 in combination with hyaluronic acid could delay OA progression. **Conclusion:** Here we report that CCL25 has a dose-dependent effect on *in vitro* tissue-engineered healthy and OA cartilage, and show the influence of this chemokine on OA progression *in vivo*. We believe that, in appropriate concentration, CCL25 is a promising candidate for *in situ* tissue engineering.

a91425

## 03-P031 *In vitro* and *in vivo* study of the safety and efficiency of a TGF β1-loaded hyaluronan-based scaffold for cartilage tissue engineering

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**Introduction**: Growth factors (GF) such as TGF β1 play a crucial role in initiating and maintaining chondrogenesis. However little is known about their optimal dose and release profile for cartilage tissue engineering. GFs are potent molecules that can trigger unwanted side effects when released from the scaffold in an uncontrolled manner. Our aim is to develop a TGF β1-loaded scaffold and ensure the safety and efficiency of the obtained tissue-engineered product.
**Material and Methods**: Hyaluronan-Transglutaminase gels (HA-TG) were obtained by modifying high molecular weight hyaluronan with two different fibrinogen-derived peptides, which can be crosslinked by the transglutaminase FXIII. Firstly, to avoid a burst release of TGF β1, we covalently grafted heparin to HA-TG using the same chemistry (HA-Hep-TG) and validated the sustained release with ELISA. Secondly, a dose screening to find the optimal concentration of GF was performed *in vitro* by loading various amounts of TGF β1 in HA-Hep-TG gels, and subsequently looking at matrix deposition by encapsulated cells after 21 days of culture. The cells used were human chondroprogenitor cells (hCCs), mixed with the gel precursors prior to crosslinking. Thirdly, we tested the selected doses *in vivo* by implanting the scaffolds subcutaneously in nude mice for 6 and 12 weeks. Side effects of GF release such as swelling at the site of implantation and vessel ingrowth were monitored. Matrix production was assessed by histology.
**Results**: HA-Hep TG efficiently retained TGF β1 in the scaffold since only 50% of the initial loaded amount was released from the gels after 2 weeks compared to 90% retention in the absence of heparin. *In vivo*, absence of heparin led to more severe side effects such as hematomas and swelling at the site of implantation, compared to scaffolds with crosslinked heparin. Photoacoutic measurements suggested no vessel ingrowth. Histological stainings did not show major differences between the loading strategies and the TGF doses, contrary to what was observed *in vitro*, highlighting the limitations of *in vitro* studies to predict the effect of bioactive molecules *in vivo.*
**Discussion**: HA-Hep-TG shows high potential due to cartilaginous matrix deposition and limited side effects *in vivo* when using crosslinked heparin to sustain the release. Ongoing long-term studies in mice and a pilot experiment in the sheep will give more information regarding the safety and efficacy of HA-Hep-TG loaded with TGF β1.

a91048

## 03-P032 Screening of Osteogenic-Enhancing Short Peptides from BMPs for Biomimetic Material Applications

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The long-term efficacy of bone implants critically depends on the regeneration efficacy of the disturbed tissue surrounding the implant. Late regeneration can burden patient with cost and time, and can decline QOL of the patient.
Peptides are one of most promised bio-compatible materials that could be chosen for surface modification, since it could eliminate both risks of animal-derived infections and interspecific immune response found in other large proteins and antibodies. There had been many researches that reports cell adhesion peptides, such as RGD peptides, found from extracellular matrixes (ECM) to be introduced for medical device surface function.
To solve the regeneration of bone tissue, we focused on cytokines that have a role of cell adhesion, proliferation, differentiation and maturation. Thus we hypothesized that peptides included in cytokines have ability that enhance bone regeneration. And we searched the sequence of cytokines that are related to bone regeneration comparing with several species.
Previous study, we have screened several cell-adhesion peptides [1] using peptide array method utilizing SPOT-synthesis technique [4]. In this study, we applied this method for proliferation and differentiation of mesenchymal stem cells (MSCs) and osteoblast cells (OBCs).

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a92905

## 03-P033 Induction of bone formation *in vivo* using nanoclay gel and bone morphogenic protein: an optimisation study in a murine subcutaneous implant model

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Introduction: For almost three decades, bone morphogenic protein-2 (BMP-2) has been demonstrated to be a key regulatory molecule able to induce bone formation. BMP-2 is licensed for clinical use in spinal arthrodesis and open tibial fractures. However, dose-dependent side effects, including osteolysis, swelling and unwanted heterotopic ossification have hindered BMP-2 usage in recent practice. Clay nanoparticle gels (Laponite) have recently been shown to localise BMP-2 and induce bone at physiological doses[1]. In the current study, we have investigated the optimum concentrations and dosage of Laponite and BMP-2 required to induce ectopic bone using a murine subcutaneous implant model.

Methods: Laponite combined with BMP-2 on an absorbable collagen sponge (ACS) was implanted in a murine subcutaneous model. The following Laponite concentrations 0%, 1.4%, 2.1% and 2.8% and BMP-2 doses 0, 0.05, 0.5, and 5 $\mu$g/ml (n=6 for each treatment combination) were examined. Micro-CT scanning was performed at 2, 4, 6 and 8 weeks to quantify the volume of induced ectopic bone.

Results: The highest concentration of Laponite (2.8%) was observed to induce significantly higher volumes of ectopic bone, compared to 0% Laponite ($p<0.005$), 1.4% ($p<0.005$) and 2.1% ($p<0.01$) at 5ug/ml dose of BMP-2. The mean volumes of induced bone were 0, 0.131, 0.249 and 1.141 $mm^3$ for 0%, 1.4%, 2.1% and 2.8% Laponite respectively. 2-way ANOVA test demonstrated at week 4, Laponite concentration and BMP-2 dose significantly affected the volume of ectopic bone formed ($p<0.01$ and $p<0.01$ respectively). The effect of Laponite concentration on bone induction remained significant at 8 weeks ($p<0.02$), whereas the effect of BMP-2 dose was not significant at this extended time point.

Conclusion: The current study demonstrates that Laponite augments the osteoinductive action of BMP-2. Significantly enhanced bone formation was observed at the highest nanoclay concentration examined, with Laponite exerting an osteoinductive factor across the study timeframe in comparison to BMP-2. This study has significant implications for the application of nanoclays for skeletal growth factor delivery, such as BMP-2, at physiological concentrations, and opportunities therein for improved safe, efficacious and cost-effective strategies for bone augmentation in orthopaedics.

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a93353

## 03-P034 CXCL12, TGFbeta3 and ATP released by injured cartilage stimulate migration of synovial fluid and bone marrow-derived MSCs to the injury site

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Introduction:Although considered to be a tissue of poor reparative capacity, many recent studies in human and animals show that intrinsic repair of arcaticular cartilage can occur. The mechanisms of this repair are unknown. Mesenchymal stem cells (MSCs) can migrate to the site of cartilage injury; we investigated the mechanisms by which MSCs in the synovial fluid and the bone marrow (SF- and BM-MSCs) respond to traumatic cartilage injury.
Methods: SF and BM-MSCs were isolated from bovine metacarpophalangeal joints. To collect molecules released by traumatic injury of cartilage, finely chopped articular cartilage with serum-free media (DMEM+0.1%BSA) for 24h, at 37. This "injury-conditioned medium" (ICM) was collected at the end of the incubation. MSC migration was studied using classical migration assays (Transwell, scratch and exclusion zone assays).
Results and Discussion: MSCs demonstrated significant migration ($p<0.001$) to freshly injured articular cartilage compared to non-injured cartilage. ICM significantly stimulated ($p<0.0001$) both SF- and BM-MSC migration in scratch assays which involve creating a scratch/injury across the cell monolayer. Both SF-and BM-MSC migration was significantly ($p<0.0001$) inhibited by treating ICM with neutralising antibodies to CXCL12 and TGF$\beta$3 and the CXCR4 inhibitor, AMD3100. However, ICM stimulated BM-MSC migration but not that of SF-MSC in cell exclusion zone assays (no scratch/injury' is required in this assay ). Migration was only seen in this assay when an ICM plus an injury was created (by scraping off cells) close to the exclusion zone. Cell injury (by scraping of cells) released metabolised ATP which was rapidly metabolized (189µM at 30min and 24µM at 4h after injury). Addition of stable nucleotides, ATP$\gamma$S or UTP$\gamma$S (250µm) with ICM significantly stimulated ($p<0.0001$)..SF-MSC migration was also be completely inhibited by addition of the P2 purine receptor antagonist suramin. Therefore, on injury of articular cartilage, TGF$\beta$3 and CXCL12 were released. Release of ATP was essential to prime/activate the SF-MSCs but not BM-MSCs, while TGF$\beta$3 and CXCL12 stimulated cell homing of both SF-and BM-MSCs.
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a92922

## 03-P035 *Escherichia coli*-derived BMP-2-incorporated β -TCP granules induces bone regeneration in femoral defect in rabbits

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**Purpose:** The purpose of this study was to investigate whether *Escherichia coli*-derived BMP-2 (E-BMP-2) incorporated beta tricalcium phosphate (β-TCP) granules could induce successful bone regeneration of critical-sized femoral segmental defects in rabbits.

**Methods:** Bone defects of 20 mm stabilized with external fixators were created in the femurs of rabbits, which were divided into BMP-2 group and control group. In BMP-2 group, E-BMP-2 incorporated β-TCP granules were implanted to the defect area. In control group, β-TCP granules alone were implanted. At week 12 and 24 after surgery, radiographs of the femurs were taken, and histological examination and biomechanical assessment of the defect area were performed. For quantification of bone regeneration, μ-CT imaging analysis was performed at week 24.

**Results:** Bone regeneration in all samples of BMP-2 group was confirmed by radiographic and histologic findings whereas no bone regeneration was shown in control group. No fracture occurred in the newly-formed bone after removing external fixator at 12 weeks in BMP-2 group. At week 24, tissue mineral density, the percentage of bone volume out of total volume, and volumetric bone mineral density of the callus were greater in BMP-2 group than in control group. In BMP-2 group, the ultimate stress, extrinsic stiffness, and failure energy measurements for the femurs were significantly greater at week 24 than those at week 12.

**Conclusion:** E-BMP-2 incorporated β-TCP granules can be a charming option for bone regeneration of long bone defects.

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a91363

## 03-P036 Exosomes derived from mesenchymal stem cells as a possible therapy for osteoarthritis

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**Introduction**

Osteoarthritis is a pathological condition that affects a large part of the elderly population in the world. The main cause seems to be the establishment of an inflammatory process, that brings to the degradation of the articular cartilage, degeneration of ligaments and thickening of the subchondral bone. In recent years, human mesenchymal stem cells (hMSCs) are emerging as promising cell therapy candidate for the treatment of this clinical condition. Many studies demonstrate that MSCs attend to tissue repair through secretion of trophic factors or extracellular vesicles. We developed a "donor-to-patient" closed, scalable and automated system for aseptic therapeutic cell manufacturing using a xeno-free medium. We validated the potential therapeutics benefits of secreted factors, conditioned medium and exosomes isolated from MSC culture in this innovative culture system, for cartilage repair.

**Methods**

We isolated hMSCs from iliac crest marrow aspirates of healthy donors and human articular chondrocytes (HACs) from cartilage biopsies, after informed consent. MSCs-derived exosomes or secretome were given to HAC cultured under both physiological and inflammatory conditions, to evaluate their role in cartilage homeostasis maintenance.

**Results**

In a damaged tissue, the initial inflammatory response plays a key role triggering tissue repair and homeostasis, but can be detrimental in the long term, causing fibrosis. We observed that under inflammatory condition HAC are able to internalize and recruit more MSC-derived exosomes, compare the control chondrocytes. We will focus on the characterization of MSC conditioned media and exosomes and we will investigate their effects in maintenance of cartilage commitment and in the activation of different regeneration pathways (IL6, IL8, COX2 and PGE-2). The effect of MSCs-derived exosomes could be protective for the articular cartilage and we will evaluate in vitro and in vivo if they may be a possible therapy for osteoarthritis.

**Conclusions**

Our study suggests that MSC exosome may exert protective effects in degenerative joint conditions and provide support for further studies of this innovative approach in joint disease.

a93011

## 03-P037 Directed *in situ* regeneration of cartilage tissue by bio-functionalised polymer implants for traumatic and early osteoarthritis lesions

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**Objectives:** We are developing a biomimetic implant which is bio-functionalised in a similar conformational and biochemical context to articular cartilage, to promote tissue regeneration *in situ.* The implant is designed to be used with microfracture to attract, retain mesenchymal stem cells (MSCs), their differentiation and chondrogenesis.

**Materials and Methods**: The biofunctionalization can be applied to various biomaterials. And electrospun poly-L-lactic acid (PLLA) random-fibre mats were used as exemplar scaffolds. Surface charge of the scaffolds was modified by plasma polymerisation, followed by binding of heparin and pmol amounts of chondrogenic and stem-cell homing factors. The bio-functionalised scaffold was assessed *in vitro* for its ability to support long-term cell viability and chondrogenesis by bone-marrow MSCs. Cell viability was assessed with resazurin dye and extracellular matrix (ECM) formation determined by glycosaminoglycan measurement. *In vivo* activity was assessed by implantation into surgically created full-thickness chondral lesions with subchondral bone micro-fracture, in the medial femoral condyles of sheep.

**Results and conclusions:** *In vitro,* functionalisation of the PLLA scaffold with a combination of TGF$\beta$3 and CXCL12 promoted MSC attachment and cell viability within the construct was maintained in the absence of added chondrogenic factors or serum for at least 5-6 weeks *in vitro*. The MSCs underwent chondrogenesis and produced significantly more ECM ($P \leq 0.05$) than non-functionalised or partially functionalised scaffolds. *In vivo*, implantation of TGF$\beta$3 and CXCL12 functionalised implants showed biological efficacy with regeneration of neocartilage with hyaline features (determined histologically) beginning within 4 weeks with the bio-functionalised implants but not with empty defects or control non-functionalised implants.

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a90254

## 03-P038 Therapeutic Potential of Bromodomain Inhibitors on TNF α -Impaired Bone Regeneration

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Excess inflammation impairs bone regeneration, causing a failure of fracture healing and progression to non-union, a devastating complication associated with patients' reduced quality of life and heavy socio-economic burden. Impaired bone regeneration is partially caused by a highly-elevated level of tumor necrosis factor-α (TNF-α). TNF-α inhibits the formation of osteoblasts, leading to drastically reduced new bone formation. Current anti-TNFα therapeutics shows limited clinical benefits; hence, the demand of new therapeutics to modulate TNFα-induced pathological effects and enhance new bone formation is very high. Previously, we have discovered two bromodomain inhibitors, N-methylpyrrolidone (NMP) and N, N-dimethylacetamide (DMA), which have the potential to reduce inflammation and favorably influence the bone remodeling cycle. Therefore, the purpose of this study is to investigate the potential of NMP/DMA as therapeutics for TNFα -compromised bone regeneration.

Through analyzing osteoblastic-specific early-, late-stage genetic markers and critical transcription factor, Runx2, we showed that NMP/DMA recovered TNFα-inhibited osteoblastogenesis. The analysis of the matrix mineralization in primary bone-marrow-derived mesenchymal stem cells (BM-MSCs) allowed us to further demonstrate that NMP/DMA helped MSCs to overcome suppressing effects of TNF-α during the early stage of osteoblastic differentiation when stem cells underwent lineage specification. Furthermore, we showed that the mechanism of action (MOA) of NMP/DMA is to suppress TNFα-activated ERK signaling. For a potential clinical translation by a local delivery of bromodomain inhibitors, we designed and then produced a biomimetic tricalcium phosphate-based porous scaffold by additive manufacturing.

In conclusion, we showed that NMP/DMA counteract suppressing effects of TNF-α on osteoblastogenesis via an ERK-dependent mechanism and demonstrated the feasibility of additive-manufactured scaffolds for a local delivery. Our finding provides a proof-of-concept for using bromodomain inhibitors, particularly NMP/DMA, to treat TNFα-impaired bone regeneration and a rationale for designing personalized drug delivery scaffolds for optimized therapeutic effects.

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a92665

## 03-P039 Oral administration of an egg yolk-derived peptide promotes fracture healing in a mouse model

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We recently found an egg yolk-derived peptide that induced proliferation of osteoblasts and chondrocytes and production of insulin-like growth factor-1 in the liver. The bioactive peptide was identified from proteolytic products of chicken egg yolk and generated by peptide engineering approaches. When orally administered, it is processed into smaller peptides and absorbed in the intestine. Here we report that oral administration of the egg yolk-derived bioactive peptide promotes fracture healing in a mouse model. We created fractures by a transverse osteotomy in tibias of 8-week-old male C57BL/6J mice. Beginning on postoperative day (POD) 1, we orally administered the peptide (10 mg/kg/day, daily) to the mice for up to 14 postoperative days. Three-dimensional micro-computed tomography (3D-$\mu$CT) scanning revealed that the callus volume was significantly higher in the peptide-administered group than in the vehicle-administered group. Histomorphometric data suggested that the peptide administration accelerated the fracture healing process; three-point bending test further revealed a trend towards higher mechanical strength of fractured sites in the peptide-administered group than in the vehicle-administered group. When compared with the vehicle administration, the increase in callus volume by the peptide administration was comparable to that by intermittent administration of recombinant parathyroid hormone (1-34) (50 $\mu$g/kg/day, daily). In addition, 3-week administration of the peptide recovered the impaired callus formation of ovariectomized mice to the control level. We then examined effects of long-term administration of the peptide on callus formation for up to 56 postoperative days. 3D-$\mu$CT analysis demonstrated that the callus volume was significantly higher in the peptide-administered group than in the vehicle-administered group on POD 11, 14, and 28. Lastly, we found that the peptide enhanced osteoblast differentiation of the pre-osteoblastic cell line MC3T3-E1 at 100 nM within 14 days of the culture compared with control. These data suggest that the oral administration of the egg yolk-derived bioactive peptide, which is a novel candidate for a bone anabolic agent, can be a facile strategy for managing fracture healing as well as bone quality itself.

a93517

## 03-P040 Synthetic intrinsically-disordered-peptides for bone tissue engineering

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**INTRODUCTION:** Vertebrate biomineralisation is closely associated with intrinsically disordered proteins (IDPs). Furthermore IDPs are involved not only in biomineralisation, but are also involved in inflammatory responses in bone and teeth [1]. The proline-rich regions of these proteins have remained remarkably well-conserved through evolution, suggesting that they may have functional importance. In previous studies, artificial consensus peptides based on these conserved domains were synthesized. The designed peptide contains a polyproline sequence of 25AA length, further comprising a systematic variation of non-proline residues [2,3]. **METHODS:** Human primary osteoblasts (hOBs) were seeded in cell culture plates. When confluent, hOBs growth media was supplemented with the different IDPs at a final concentration of 2.5 $\mu$M. As positive control, Emdogain™ (EMD), a purified extract of proteins from enamel matrix was added to growth media. As well, cells without supplementation were used as negative control (C). Cytotoxicity of the IDPs was evaluated after 1 and 3 days. Gene expression of osteogenic markers was evaluated after 1, 3, 7 and 14 d using qPCR. Alkaline phosphatase (ALP) activity and calcium deposition were analysed after 21 d. Additionally, a wound healing *in vitro* assay was performed. Minimum two out of four hOBs donors (3-4 replicates each) were used for the experiments. **RESULTS:** As expected, no cytotoxic effect of the IDPs was observed. Cells treated with P2, upregulated the expression of the osteogenic markers collagen-I$\alpha$1 and alkaline phosphatase at earlier time points than control- and EMD-supplemented groups. Both, cells treated with P2 and EMD, increased calcium deposition although P2 effect was not statistically significant. EMD osteogenic effect did not involve ALP expression or activity upregulation. Cells treated with P2 showed the smallest open wound area after 24 h.

**DISCUSSION & CONCLUSIONS:** Results show that synthetic IDPs are able to influence hOBs biomineralization. The earlier upregulation of osteogenic markers together with the decreased wound area induced by P2 indicate that it has potential to induce bone regeneration. Bone-related proteins, cytokines and chemokines released to culture media are already being analysed to confirm these results. IDPs might be a reliable option to develop new biomaterials and tissue engineering strategies.

a91294

## 03-P041 The Effect of *Cissus quadrangularis* Shoot Extracts on Osteogenic Differentiation of Human Mesenchymal Stem Cells

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*Cissus quadrangularis* (CQ) is a traditional therapeutic herb used for bone fracture healing and hemorrhoid. In this study, CQ shoot was sequentially extracted into five fractions based on the polarity of solvents. The extracts were then treated on human bone marrow derived mesenchymal stem cells (hMSC) isolated from femur head and hMSC line UE7T-13 (JCRB no. 1154). The cytotoxicity was analyzed using alamarBlue® assay after 72 hrs. All extracts at the concentration between 0-250 µg/ml did not alter hMSC metabolic activity. The effect of CQ extracts on hMSC osteogenic differentiation was that CQ hexane extract enhanced hMSC osteogenic differentiation by increasing alkaline phosphatase (ALP) activity and calcium deposition ($P<0.05$, n=3). Interestingly, without significant alteration in ALP activity at the 7 day time point, ethyl acetate (EtOAC) extract increased calcium deposition in hMSC in a higher extent than hexane extract. Based on the total flavonoid assay, the CQ ethyl acetate contained triple amount of flavonoid compared to the CQ hexane extract. In addition, we performed single extraction using ethyl acetate and the extract was further analyzed for its components and osteogenic induction potentials. Thin layer chromatography showed that the new CQ EtOAC has a combination of chemical compositions between CQ hexane and CQ EtOAC in previous sequential extracts. The extract was subsequently isolated into 9 sub-fractions using column chromatography. Chemical composition analysis with HPLC and NMR along with *in vitro* analysis in hMSC osteogenic differentiation are being investigated for each sub-fractions.

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a92334

## 03-P042 *Role of glucocorticoids during Chondrogenic commitment in mesenchymal stromal stem cells*

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Stem cell based therapy represents a promising approach for regenerative medicine, although the translation of the *in vitro* studies into clinical applications is still complicated by the difficulty in obtaining a high yield of differentiation within specific and homogenous lineages.

Mesenchymal stromal cells (MSCs) are one of the most responsive type of stem cells used for differentiation. However, the yield of commitment is a variable process influenced by a number of factors and the propensity for MSCs to undergo terminal hypertrophy. It is known that during chondrogenesis glucocorticoids(GCs) are required for differentiation of MSCs *in vitro*; however, the exact role of GCs in this process is not known. GCs can inhibit NF-κB activation by direct interaction when bound to the GC receptor in its monomeric form (transrepression). Alternatively, when forming a GC receptor dimer, the complex enters the nucleus and activates gene transcription (transactivation).

Here, we aim to investigate the role of GCs on chondrogenic differentiation of hMSCs in the presence or absence of dexamethasone (DEX) and a GC agonist: (+)-ZK 216348 (ZK216348), a drug used for the treatment of inflammation that inhibits NF-κB by way of transrepression but does not activate the transactivation pathway. Thus, the relative role of transactivation and transrepression should become clearer.

**Methods and Results**

We induced chondrogenesis in hMSCs (n=3, obtained with full ethic consent) using 10 ng of TGFβ for 21 days in the presence or absence of DEX (100nM) and ZK216348. The gene expression analysis was performed after 14 and 21 days. GAG and Histology using Safranin-O were analyzed after 21 Days.

Within this preliminary study, GC treatment by ZK216348 downregulated gene expression of matrix components Col2, ACAN and ColX and decreased cartilage matrix-sulfated proteoglycans and GAG production. Interestingly, chondrogenic commitment in the absence of conventional DEX used at 100 nM showed an increase compared to hMSC pellets in the presence of DEX.

**Conclusions**

The use of GC treatment during chondrogenesis strongly influences stem cell fate. Our results suggest that ZK216348 massively counteracts differentiation, controlling NF-κB pathway activation by preventing nuclear translocation and subsequent gene expression activation for NF-κB activated genes involved in chondrogenesis. Our results provide new insights for understanding the molecular mechanisms behind MSCs commitment *in vitro*.

a92585

## 03-P043 Screening of small molecules applied in traditional Chinese medicine: towards biological treatment of osteoarthritis

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Osteoarthritis (OA) is the most prevalent degenerative joint disorder and it affects millions of patients globally. There is currently no effective cure or preventative treatment available. Pharmacologic therapy for OA shows efficacy in pain relief but is frequently associated with adverse effects, desiring a transition from pharmacological treatment to biotherapies.

In this study, the anabolic and anti-inflammatory effects of 40 Traditional Chinese Medicine (TCM) compounds were assessed biochemically (GAG/DNA) using human OA chondrocytes. The most chondrogenically active compounds were assessed transcriptionally (real-time PCR) and histologically in an inflammatory model. Cartilage pellets were generated in chondrogenic medium followed by 3 days of inflammatory induction with cytokines (IL-1$\beta$/TNF-$\alpha$). The inflamed tissues were exposed to different types and concentrations of TCM compounds.

After induction of inflammation, the GAG/DNA ratio and anabolic gene expression were significantly decreased, while inflammatory and catabolic markers were up-regulated. From the 40 compounds tested, some of them showed significantly enhanced anabolic effects. The most chondrogenically active compounds were tested in the inflammatory model, whereby the GAG/DNA ratio was restored by 5-hydroxymethylfurfural treatment, and Safranin O staining confirmed the accumulation of cartilaginous matrix. The expression of the anabolic marker gene COL2A1 was higher in 5-hydroxymethylfurfural treated compared to untreated pellets, while the hypertrophy marker COL10 was completely suppressed.

In conclusion, 5-hydroxymethylfurfural increased cartilage matrix production of human osteoarthritic chondrocytes towards a healthier phenotype and showed regenerative effects. A local drug delivery system for the bioactive compound is envisioned and optimization of the release mechanisms of the loaded compound(s) and their efficacy in terms of cartilage repair will be tested.

a92879

## 03-P044 poly-UDCA (ursodeoxycholic acid) enhances the osteogenic differentiation of human mesenchymal stem cells and bone regeneration

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Ursodeoxycholic acid (UDCA) is a bile acid and known that inhibit apoptosis and decrease ROS level in cells. The decreasing of apoptosis and ROS level is important to induce the osteogenic differentiation of mesenchymal stem cells, effectively. However, there is no research about the effect of UDCA which is known as anti-apoptosis and anti-ROS reagent on the differentiation of mesenchymal stem cells. In addition, it is reported that polymerization of prodrug improves the drug stability and long-term release. Therefore, we investigated the adipogenic and osteogenic differentiation of human adipose-derived mesenchymal stem cells (hASCs) with the treatment of polymeric UDCA nanoparticles (poly-UDCA) and the capacity of differentiation was measured by histological staining, qPCR, and western blotting. UDCA was released from the poly-UDCA when it is exposed to $H_2O$ or $H_2O_2$. At 7 and 14 days after treatment, it was found that the poly-UDCA enhanced delivery of UDCA into cells and the osteogenic differentiation of hASCs compared with UDCA. However, poly-UDCA decreased the adipogenic differentiation. Moreover, we investigated *in vivo* experiment using poly-UDCA. Bone regeneration by poly-UDCA was observed using drill-hole defect rat model and estimated by micro-CT and histological analysis. Consistent with *in vitro* experiments, poly-UDCA improved the bone defect compared with the UDCA. Based on these results, poly-UDCA could be a new drug for strong bone regeneration by decreasing the adipogenic differentiation and increasing the osteogenic differentiation of mesenchymal stem cells.

a93355

## 03-P045 Cell Membrane Tethered BMP-9 Enhances Proliferation and Induces Osteoblastic Differentiation in Bone Mesenchymal Stem Cells

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The cell membrane of first passage rat bone marrow mesenchymal stem cells (MSC) was labeled with 100 $\mu$M of a heterobifunctional maliemide/N-hydroxysuccinimide polyethylene glycol linker (MW 3000). The heterobifunctional linker was capped with BMP-9 (also known as GDF-2) by exposing linker labeled cells for 30 minutes to 1 ng/ml (low) or 3 ng/ml (high) BMP-9. In osteogenic media, tethered BMP-9 (low) enhanced MSC proliferation and osteoblastic differentiation compared to MSCs cultured in both osteogenic media alone or osteogenic media supplemented regularly with 1 ng/ml of BMP-9. Increasing the concentration of tethered BMP-9 (high), had a negative impact on cell proliferation. Cultures were monitored for 7 days. Osteoblastic differentiation was monitored using alkaline phosphatase (ALP) activity, Alizarin Red staining, and o-cresolphthalen complexone quantification of plate calcium. Cell proliferation was assessed with PicoGreeen and alamarBlue.

a92725

## 03-P046 Osteochondral Repair Using Decellularized Man-Made Hyaline-Like Cartilage Graft

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The key challenge of lower-limb-joint osteochondral regeneration lies in restoration of the avascular articular cartilage on top of the self-repairable subchondral bones. A quality articular cartilage engraftment is validated by the graft's hyaline cartilaginous phenotype and genuine microstructural architecture. In this tissue engineering study, we endeavored to repair traumatic osteochondral lesions in rabbit knee models using a novel man-made hyaline-like cartilage xenograft that was produced by 3D cultured porcine chondrocytes in vitro - free of non-cartilaginous constituent. Comparative trials were conducted in parallel among samples with or without graft decellularization, with or without additional installation of an integrated scaffold as subchondral bone substitute. Here, we show sound osteochondral regeneration of both cartilage and subchondral bones from cartilage engraftment within 100 days after implantation including the recoveries in form and function with correct osteochondral composition, structure and phenotype. The decellularized grafts without engineered subchondral bone plugs exceled other counterparts, which opens this engrafting system to enjoy all superiorities and conveniences brought up by decellularization process in terms of logistic simplicity, immune biocompatibility and micro-architectural genuineness.

a91174

## 03-P047 Engineering Bone Tissue with Spider Silk: Effects of calcium on osteogenic differentiation

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Matrices made of the recombinant spider silk protein 4RepCT have previously been shown to support cell culture of primary mammalian cells. Especially the recombinant silk protein with an incorporated RGD motif from fibronectin enhances cell attachment and support cell growth[1]. Previous studies have shown that it is possible to incorporate calcium during the formation of silk, which might be beneficial to induce osteoblast differentiation from mesenchymal stem cells as well as mineralization of the scaffold.

AIM: The aim of the present study was to evaluate the effect of incorporated calcium on the proliferation and osteogenic differentiation of mesenchymal stem cells assembled together with recombinant spider silk protein.

METHODS: Alamar blue viability assay was used to determine viability and metabolic activity before and during differentiation. Osteogenic differentiation and mineralization was evaluated by alkaline phosphatase activity, Alizarin Red S staining, and immunofluorescence staining of osteocalcin in cryosections.

RESULTS: The results show that hMSCs can be efficiently incorporated into silk fibers. The hMSCs proliferated in the presence of $Ca^{2+}$, up to a concentration of 8.5 mM, to a similar degree as in calcium-free controls. Moreover, positive alkaline phosphatase activity confirmed the differentiation of hMSCs into osteoblasts after osteogenic conditions was induced. Also, findings of increased osteocalcin expression within the fibers after differentiation suggest osteogenic maturation. After 21 days of osteogenic differentiation, calcium deposits were detected by Alizarin Red S, indicating bone formation.

CONSLUSION: Together, the results suggest that incorporation of calcium into silk fibers can enhance osteogenic differentiation of hMSCs as well as mineralization.

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### ACKNOWLEDGMENTS

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a90990

## 03-P048 Self-assembled 3D-printed Janus scaffolds for the regeneration of the osteochondral interface

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Tissue engineering of the osteochondral interface represents a challenge due to the coexistence of both chondrocytes and osteoblast, and the different chemical and mechanical properties that the ECM of bone and cartilage present. Although an extensive library of stratified scaffolds can be found on the literature for this matter, co-culture and self-organization of the cells within these materials remains still elusive.

Additive manufacturing (AM) represents a simple, fast and reliable technique for the fabrication of complex structures and has been exploited on TE of osteochondral defects with single cell populations leading to rather isotropic tissues. Self-assembling of polymer blends is a well know phenomena that leads to the formation of long-range ordered structures. This phenomenon is particularly exploited on 2D systems, but the controlled development of such well-ordered structures on 3D systems is still challenging.

In an attempt to combine both the rapid fabrication of complex 3D structures and the development of well-ordered internal structures, we have developed a controlled system based on poly (ε-caprolactone) (PCL) and poly (D,L-lactide) (PDLLA) blends that readily self-assemble under AM processing conditions. The fabrication of 3D structures based on different ratios of PCL:PDLLA blends resulted in scaffolds with surface and internal ordered structures and, thus, also a spatially controlled chemistry. The system proved flexible and small variations on the polymer ratio results on the development of different structures such as lamellae or cylindrical motifs.

Of particular interest is the formation of Janus scaffolds with ordered fibres presenting a side-by-side phase-separation of PCL and PDLLA. These materials, when functionalized with peptide sequences representative of osteocalcin (a bone gamma-carboxyglutamic acid-containing protein) and chondroadherin (a cartilage matrix protein), drive the selective attachment of co-cultures of chondrocytes and osteoblasts. Thus, PCL-osteocalcin selectively drives the attachment of osteoblast and PLA-chondroadherin the attachment of chondrocytes. The selective attachment of cells is exploited to form an osteochondral interface that mimics the native scenario where these two cell types come together to form a continuous but gradient tissue. These materials can be further exploited for the regeneration of the osteochondral interfaces among other tissue interface types.

a91266

## 03-P049 Silk Elastin-like Co-Recombinamers bioactive hydrogel embedded with mature chondrocytes as injectable scaffolds for cartilage regeneration in an *Ex Vivo* culture platform

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Articular cartilage is central to the proper functioning of synovial joints. It's is becoming one of the most important target for tissue engineering because of the high need for self-repair and regeneration. Elastin like Recombinamers (ELRs) play a key role as a novel biomaterial-based regenerative therapy. ELRs are able to self-assemble into different structures, of which hydrogels are the most promising for tissue regeneration [1]; moreover according to the high percentage of elastin present in the native chondral matrix, ELR-based hydrogels are likely to be similar to hyaline cartilage. Recombinant DNA techniques were used for the synthesis of an amphiphilic Silk Elastin-Like Co-Recombinamers (SELbcRs) based on Elastin-Like, Silk domains and RGD cell-adhesion sequence; which were expressed and bioproduced in *E. coli*. Furthermore, the jellification properties of this system were improved by a special treatment based on supramolecular self-assembly to form nanofibrillar matrices *in situ* obtaining the best conditions for the chondrocytes embedded in the hydrogel. TEM and Circular Dichroism confirm the capacity to form an ECM fibrillary structure upon injection; moreover, rheological analysis of the injectable hydrogel showed adequate modulus for cartilage repair. *In vitro* studies have shown a great cytocompatibility of SELbcRs hydrogel; furthermore, biochemical and histological analysis of collagen and glycosaminoglycans (GAGs) revealed the cells' capacity to develop their own ECM. In order to monitor the healing progress and to refine and reduce the number of animal studies performed in the field of cartilage repair, an *ex vivo* study was performed. A standardized and representative *Ex vivo* model [2] has shown better proliferation of chondrocyes and higher amounts of GAGs. The IHC staining of collagens revealed clear production of collagen type II (Hyaline carilage) by chondrocyes, collagen type I (fibrocartilage) was not detected. To conclude, SELbcRs hydrogels have shown to provide an ideal environment for mature chondrocytes to achieve an optimal osteochondral tissue repair. The analysis recorded for the *ex vivo* study supported the importance of a native environment for the production of hyaline cartilage.

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a90801

## 03-P050 COMPARATIVE STUDY OF C OMPOSITE MATERIAL FOR HYALINE CARTILAGE REGENERATION: DATA OF IN VIVO STUDY

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Reconstitution of authentic hyaline cartilage (HC) structure after damage is a complex task due to weak regenerative potential of the tissue, its histological structure and propensity to form fibrous tissue in damaged area, ets. There are many offers on biotech market (Zimmer, Inc., Thoratec Corporation), and new products approved by EMA and FDA (Carticel from Genzyme), whereas data of some studies[1] do not demonstrate significant advantages of new methods, thus suggesting the urgency of HC restoration problem.

To restore HC we propose a combination of 120÷150 μm electrospun matrices (EPS) from nylon 6 or PLGA and photopolymerized blend (PhPB). The PhPB containing gelatin and chondroitin-4-sulfate both modified by glycidyl methacrylate[2] and Darocur 2959 was gelled by 365 nm LED light illumination and have no toxicity against human chondrocytes. The PhPB-wetted EPS (2-3 discs) were placed, covered with PhPB and photopolymerized layer by layer in a defect (diameter 4 mm, 1÷1,5 mm depth) produced by low speed milling cutter in intercondylar groove of rabbits femur. Biocompatibility of the implants was evaluated at 1, 3 and 6 months after surgery. Defect after milling, defect filled with ChondoGide fixed with fibrin glue, defect filled with ChondoGide fixed with PhPB were used as a control. Totally 29 rabbits were operated. After explantation and general description combined with observed microscopy of the implants, femoral heads were fixed in 6% formaldehyde and then decalcified in 20% EDTA. Cryosections were stained with hematoxylin-eosin or von Kossa stains. All materials demonstrate good contacts with surrounding tissue, even the holes were almost filled with novel tissue already after 1 month. Nevertheless in contrast to all controls EPS sheets combined with PhPB demonstrate no any signs of inflammation, very good integration with surrounding HC expressed in the intensive infiltration of the implant with cells, with formation of the isogenic groups and columnar structures of chondrocytes, unified capsule and absence of the areas of hypertrophic cells. Nylon 6 layer is maintained during the whole observation time, while the layer from the PLGA degrades just a month after implantation. Implantation of the materials in knee-joins of sheep is planned.

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a91229

## 03-P051 A Comparative Study of Osteoconductivity by the Combination of Biphasic Calcium Phosphate Alloplast and Resorbable Barrier Membrane on Rat Calvarial Defect Model

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The bone regenerative medicine is an important technology to maintain the quality of life in the ageing era. The grafting materials and barrier membranes are the essential components to achieve the efficient bone regeneration. In this study, we compared two different kinds of commercial products of biphasic calcium phosphate (BCP) alloplasts and resorbable barrier membranes on rat calvarial defect model. All animal experiments were performed in Sang-Hyun An with Daegu Gyoungbuk Medical Innovation Foundation Animal Care and Use Committee guidelines. Four experimental groups with the combinations of two alloplasts and two barrier membranes were evaluated against three control groups. The osteoconductivity on 6 weeks and 12 weeks after implantation was analyzed by micro-CT imaging system and histology. The expression of collagen type1 alpha1 and osteopontin at defect site was observed to compare a degree of bone regeneration by immunohistochemistry. This study describes an effectivity of commercially available BCP alloplast and barrier membrane in bone regeneration, and suggests an efficient combination of them.

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a93141

## 03-P052 Development of cryopreserved cell-laden scaffold using a cell printing system supplemented with low-temperature processing method

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Department of Biomechatronic Engineering

One of the specific issues of ideal wound healing scaffold should be prepared in short period. Because rapid healing could prevent the possibility of infection, scar and irreversible loss of damaged tissue. Premade and preserved scaffold is one of the solution for this issue. Here, we propose a cryopreserved cell-laden scaffold with hollow structure. We used coaxial nozzle for dispensing cell and cell preservation solution in the core region, and gelatin methacrylate(GelMA) in the shell region for structure support and rapid gelation. To obtain optimum processing conditions, various low temperatures from -5° C to -30° C were applied to the working stage. In addition, the rapid temperature change during processing was a critically important parameter affecting initial cell viability. To reduce this effect, we used various cooling temperature before the cell-laden solution was printed onto low-temperature plate. We successfully fabricated various 3D cell-laden structures, and the in-situ cell viabilities for osteoblast-like cell laden scaffolds were 85%. These results suggest that this new cryopreserved scaffold has great potential for long-term preservation and rapid transplantation for various tissues.

a90666

## 03-P053 Application of 3D Bioprinter to Evaluate Tumor-Osteocyte Interactions

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Bone is a frequent site of metastasis from breast cancer. To understand the potential role of osteocytes in bone metastasis, we investigated tumor-osteocyte interactions using two cell lines derived from the MDA-MB-231 breast cancer cells, primary breast cancer cells, and MLO-A5/MLO-Y4 osteocyte cells. We also employed 3D bio-printing to generate a physiologically relevant bone-tumor microenvironment.

When three-dimensional (3D) tumor spheroids were grown with osteocyte spheroids, tumor spheroids fused with osteocyte spheroids and shrank. This size reduction was also observed when tumor spheroids were exposed to conditioned medium isolated from osteocyte cells. To evaluate differential responses of tumor cells in the bone microenvironment, we generated a tumor-osteocyte hybrid construct using 3D bio-printing. The construct consisted of fluorescently-labeled tumor spheroids on one needle and MLO-A5 spheroids on the neighboring needle. After 48 h, the construct was imaged using confocal microscopy. Profiling the average fluorescence along the construct showed that TMD migratory tumor cells spread onto neighboring MLO-A5 spheroids more quickly than BMD metastasized tumor cells. Mass spectrometry-based analysis predicted that several bone matrix proteins (e.g., collagen, biglycan) in conditioned medium could be responsible for tumor shrinkage. The osteocyte-driven shrinkage was mimicked by type I collagen, the most abundant organic component in bone, but not by hydroxyapatite, a major inorganic component in bone. RNA and protein expression analysis revealed that tumor-osteocyte interactions downregulated Snail, a transcription factor involved in epithelial-to-mesenchymal transition (EMT). An agarose bead assay showed that bone matrix proteins act as a tumor attractant.

Collectively, the study herein demonstrates that osteocytes attract and compact migratory breast cancer cells through bone matrix proteins, suppress tumor migration, by Snail downregulation, and promote subsequent metastatic colonization. Use of 3D bio-printer allowed to precisely control the bone-tumor microenvironment. The result on tumor-osteocyte signaling might contribute to developing novel therapies to prevent bone metastasis associated with breast cancer.

a91160

## 03-P054 Mechanical Stiffness of 3D Bioprinted hMSCs-laden Scaffolds Influences Cell Mineralization, Proliferation and Differentiation in a Static Bone Bioreactor

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There has been increasing interests in understanding how mechanical properties of 3D extracellular matrix influence stem cell fate. The objective of this study is to investigate the influence of mechanical stiffness of 3D bioprinted cell-laden scaffolds on cell mineralization, proliferation and differentiation for bone tissue engineering. Human mesenchymal stromal cells (hMSCs) were mixed with 0.8% and 1.8% (w/v) alginate/gelatin composite hydrogels at a concentration of 5 million cells/ml to prepare the bioinks. 3D scaffolds were bioprinted by the extrusion of the bioink using the INKREDIBLE$^+$ cell bioprinter in a lattice-rod model. Mechanical properties of the cell-laden scaffolds were characterized by measurement of compressive modulus at day 7 of cell culture. In-situ bone-like tissue formation within the static bioreactors was assessed by weekly micro-computed tomography scans for 6 weeks in osteogenic media. DNA content, alkaline phosphatase (ALP) activity and osteogenic related gene expression were evaluated for 1, 7, 14, 28 and 42 days. The results showed that higher alginate concentration resulted in higher compressive modulus: 0.66 ± 0.09 kPa for 0.8%alg and 5.39 ± 1.2kPa for 1.8%alg scaffolds. In both groups, mineralized tissue volume increased over time and 0.8%alg scaffolds showed significantly more mineralized tissue formation (43.5±7.1mm$^3$) than 1.8%alg scaffolds (22.6±6.0mm$^3$) at day 42. DNA content decreased after day 7, but was significantly higher for 0.8%alg compared to 1.8%alg scaffolds at day 14, 28 and 42. The 0.8%alg scaffolds exhibited a significantly enhanced ALP activity compared to 1.8%alg scaffolds at day 7 and 28. Similarly, ALP and OC gene expression levels were significantly higher in the 0.8%alg scaffolds compared to the 1.8%alg scaffolds at day 14 and 28, respectively. Furthermore, 0.8%alg exhibited higher dentin matrix acidic phosphoprotein 1 mRNA expression than 1.8%alg scaffolds at day 28 and 42. Based on histology and immunohistochemistry, cells in 0.8%alg exhibited an osteocyte-like phenotype and had more collagen I and osteocalcin expression than 1.8%alg scaffolds at day 42. Finally, the less stiff 0.8%alg scaffolds exhibited more hydroxyapatite-like mineralization as well as better cell proliferation and ALP activity, altogether demonstrating the strong potential of 3D bioprinting for applications in bone tissue engineering. JZ gratefully acknowledges financial support from Chinese Scholarship Council.

a91298

## 03-P055 Converging of melt electrowriting and extrusion-based bioprinting for cartilage tissue regeneration

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Extrusion-based bioprinting enables hierarchical printing of cell-encapsulated hydrogels, whereas melt electrowriting (MEW) enables fabrication of (sub)-micrometer-scale fibres [1]. These techniques are already implemented in a two-step approach, i.e. fabrication of the fibre scaffold and then crosslinking a cell laden hydrogel inside. Reinforcement of gelatin methacryloyl (gelMA) was shown with a box-structured micro fibre scaffold obtained by MEW, which resulted in composite constructs with compressive properties similar to those of articular cartilage and a beneficial environment for cartilage matrix formation [2]. However, by using this two-step approach it was not possible to achieve functional cartilage constructs with adequate mechanical integrity and zonal organization of cartilage. Therefore, we combine these two techniques in a single-step manufacturing process, to fabricate constructs that further mimic the complex hierarchical structure of articular cartilage.

Polycaprolactone (PCL) MEW boxes of 400 x 400 µm, and 10% gelMA containing Equine derived Mesenchymal Stromal Cells (MSCs) (density=$20*10^6$/ml), were converged printed in a 1-step approach using a Bioarchitect bioprinter (3DDiscovery,regenHU) after which the constructs were cultured for 4 weeks. Viability, Metabolic activity, chondrogenic differentiation, and compressive properties were measured using a LIVE/DEAD assay, Alamar Blue assay, GAG/DNA assay, and dynamical mechanical analysis, respectively.

High resolution (10µm), multilayered zonal constructs were successfully fabricated in a single-step manufacturing process. Viability, metabolic activity, and chondrogenic differentiation of MSCs were not compromised by the fabrication process that required high voltages to generate the MEW micro fibre scaffolds. The compressive peak- and equilibrium modulus (from 19.85 ± 7.51 kPa (gel alone), to 246.84 ± 66.42 kPa (converged, reinforced gel), and from 11.90±4.09 kPa (gel alone) to 53.02±8.73 kPa (converged, reinforced gel), respectively, show that the reinforcing effect of MEW is still present after converged printing.

This single-step manufacturing approach results in more control over construct architecture, allowing for the creation of scaffolds that closely resemble the native tissue composition and architecture. These are the first steps in moving towards larger constructs that could possibly replace or repair large defects in joints.

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[2] Visser J. et al. 2015

a90340

## 03-P056 3D Bioprinting Spatial Gradients of VEGF to Enhance Vascularization for Bone Tissue Engineering

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### Introduction

Despite the tremendous potential of tissue engineering for regenerating large bone defects, relatively few new therapies have reached the clinic. One of the major challenges in bone tissue engineering is vascularization, especially when considering the treatment of larger defects. The overall aim of this study was to use 3D bioprinting to develop constructs that can (1) promote rapid vascularization *in vivo*, and following this, to (2) promote osteogenic differentiation of host progenitor cells that are recruited into the implant.

### Methods

Bioinks were prepared by dissolving RGD $\gamma$ -irradiated alginate (3.5% w/v), methylcellulose (1:2% w/w) and nano hydroxyapaptite (nHA) (1:1% w/w) in $\alpha$MEM. The bioink was mixed with 60mM CaSO4 at a v/v ratio of 4:3 using a dual syringe approach and crosslinked for 30 mins. The bioink was printed alongside a polycaprolactone network to generate 3 experimental groups: [1] No VEGF; [2] Homogenous: a bioink loaded with 100ng/mL VEGF throughout; [3] Gradient: a bioink loaded with 400ng/ml VEGF in the centre of the implant. VEGF release was measured using an ELISA. Constructs were implanted subcutaneously in BALB/c nude mice for 2 & 4 weeks (n=8). µCT was performed on each sample. Constructs were then embedded, sectioned, and stained with H&E/Alizarin Red. Vessel quantitative analysis was performed on H&E stained sections.

### Results

The addition of methylcellulose and nHA significantly increased the release of VEGF, and allowed for sustained release of VEGF over 10 days in vitro. At 2 weeks, the Gradient group had significantly more vessels present within the construct than the other two groups. All three groups had significantly more vessels at 4 weeks compared to 2 weeks. At 4 weeks, the Gradient group again had significantly more vessels present than the other two groups (H&E). At 4 weeks there was significantly more mineral deposition in the Gradient group than the Homogenous group (µCT & Alizarin Red).

### Discussion

Results from this study demonstrate that biomimetic hydrogels with distinct gradients of VEGF significantly increased the number of perfused vessels, and this correlated with increased mineralization compared to homogenous VEFG loaded constructs. Together, we have developed a cell-free construct that will promote rapid vascularization, and following this promote osteogenic differentiation of host progenitor cells that are recruited into the implant.

### Acknowledgements

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a92351

## 03-P057 Biphase 3D Printing Scaffold coated with IL4 for Osteochondral Defect Regeneration

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Tissue engineering emerges as a promising approach for osteochondral defect repair. Recently, 3D printing technologies have been increasingly and widely used for the preparation of tissue engineering scaffolds. In this study, a novel bi-phasic scaffold is designed by integrating GelMA (top phase) and polycaprolactone-hydroxyapatite (PCL-HA) composite (down phase). Using a digital light processing (DLP)-based 3D printing technology, the GelMA phase is fabricated as a radially-oriented scaffold with good biocompatibility and porosity; it has been verified to be able to recruit cells. On the other hand, the PCL-HA composite was developed through extrusion printing to create the down phase to provide the appropriate mechanical support for newly formed bones (compressive modulus ~73Mpa). Additionally, GelMA was utilized as a carrier for the sustained-release of IL4 (~7 days ) in order to promote scaffold-to-tissue integration and macrophage polarization toward the M2-type, essential for the repair after tissue injury. The bi-phasic 3D printing scaffold can support cell attachment, proliferation, and differentiation *in vitro* . Results also illustrated that the IL4-coated scaffold is superior to the non-coated and the control group *in vivo;* therefore, these novel bi-phasic 3D printing scaffolds hold a great potential for osteochondral defect regeneration in the clinic.

a91542

## 03-P058 Bioprinting of Fibrocartilage: Combining Photocrosslinkable Gelatin and Chondrocyte Micro-Aggregates

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For the engineering of fibrocartilage, a bottom-up strategy can be applied where high quality microtissues are used as modular units for the fabrication of a larger scale construct. The goal of this study is to generate 3D micro-aggregates with an extracellular matrix (ECM) similar to the avascular inner part of the meniscus. Articular chondrocytes (AC) are a potential cell source for meniscus engineering since dedifferentiation of chondrocytes leads to a fibrochondrocyte phenotype. For the directed assembly of these fibrocartilage aggregates by 3D bioprinting, a printable hydrogel, such as a modified photocrosslinkable gelatin (gel-MOD), can be used as a bioink. This study evaluated the effect of gel-MOD encapsulation on the viability and phenotype of the chondrocyte aggregates.

Porcine AC were enzymatically isolated from the articular cartilage of the femur, and were expanded in monolayer culture. For the generation of 3D micro-aggregates, a high throughput agarose micro-well system, with 2865 pores of 200 µm diameter, was used. After seeding 0.5 x $10^6$ cells per well, uniform AC aggregates of approximately 175 cells were obtained and cultivated at 5% $O_2$ up to day 21 in chondrogenic medium containing TGF-$\beta$1. For the encapsulation in gelatin methacrylamide, aggregates were mixed in a 10, 15 or 20 w/v% gel-MOD solution (PBM Group-UGent) with 2 mol% Irgacure 2959 or 20 mol% VA-086 as photo-initiator. Chondrocyte aggregates were bioprinted with a 3D Discovery Instrument (RegenHU). The viability of the micro-aggregates was evaluated by live/dead staining. The morphology, proliferation and ECM were evaluated by (immuno)histochemistry.

After 3 days of culture, compact circular aggregates were formed and were viable up to day 21 (diameter =138 µm). Histological evaluation showed that the ECM consisted of sulphated glycosaminoglycans (GAG), collagen I, collagen II and aggrecan. AC aggregates encapsulated in gel-MOD remained viable. In the 10 w/v% gel-MOD, cells were able to grow out into the hydrogel after 7 days of encapsulation. Encapsulated AC aggregates were positively stained for GAG, collagen I and collagen II.

This study demonstrated that AC micro-aggregates express a desirable phenotype and can be used as modular building blocks for the engineering of a larger scale fibrocartilage construct. Furthermore, gel-MOD supports viability and phenotype of the aggregates and seems a suitable bioink for the 3D-bioprinting of a fibrocartilage construct.

a90059

## 03-P059 Development of smart NanoBioInk for BioPrinting of Artificial Bone using iPS Cell Technology ….. *A STEP TOWARDS SUSTAINABILITY*

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VETERINARY COLLEGE SHIMOGA

Current research findings reported that iPS cells are capable of differentiation into the constituent cells of a wide range of tissues and organs. However, organs are more complex because of their three-dimensional (3D) structure. Small livers have been reported (Takebe *et al.,* 2013) but there are as yet no reports of large 3D, functional organs of human size. This is an area that requires a combination of iPS cell technologies with 3D printers, biomaterials, and other technologies. Soft materials that are biocompatible may lack the mechanical properties necessary for orthopedic applications. To resolve these principle issues, we devised a strategy using newer BioInk that combines the mechanical advantages of biodegradable polymers with the biological functions of natural biomaterial scaffolds which achieves the correct strength requirements while enhancing the regeneration of healthy bone tissue. In the present study, we have developed fully biocompatible and native dECM with novel technology (Dhoolappa *et al.,* 2016) with promising renewable and affordable raw material for the development of BioINK for 3D BioPrinting of bone plates using iPS Cell technology for Bone Engineering. BioInk for post-traumatic osteoregeneration are a new class of composites, biologically active fracture healing materials, consisting of several fundamental building blocks including dECM, nano-hydroxyapatite, self-assembling amphiphilic peptides, iPSCs and calcium alginate microbeads, nanoporous silicon enclosures and bio-adhesive.**References:** Takebe *et al., 2013,* Vascularized and functional human liver from an iPSC-derived organ bud transplant. *Nature* 499 (7459):481-4.

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a90501

## 03-P060 Biomimetic design strategy of 3D bioprinted tissue construct for craniofacial bone reconstruction

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Three-dimensional (3D) bioprinting is emerging as a promising enabling technology for tissue engineering applications. In this study, we developed a workflow to take 3D medical images and utilized them for biomimetic bone construct design, bioprinting, and *in vivo* validation. Medical imaging and 3D bioprinting strategy converged to allow the fabrication of a structure with complex shape and inner architecture based on patient anatomy and biomimicry. Bone constructs were made of a composition of poly( ε -caprolactone) and b-tricalcium phosphate (PCL/TCP) and fabricated on the Integrated Tissue-Organ Printing (ITOP) system [1]. We examined bone regeneration in a critically sized defect at 4 and 8 weeks with the printed bone constructs. The results showed the increased bone density and volume and new bone formation and maturation with time. We demonstrated that the printed bone construts were able to organize into mature tissues of their specific characteristics *in vitro* and *in vivo*. We validated the concept that patient-specific anatomy could be translated to 3D bioprinting strategy through medical imaging and image processing software with strong clinical relevance.

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a90981

## 03-P061 Nano cellulose, and its clinically approved parallel bacterial nano cellulose, as bioink for 3D bioprinting of cartilage

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Nano cellulose has been proven suitable for 3D bioprinting of cartilage. The ink has good printability, prints with high fidelity and creates a suitable environment for proliferation of chondrocytes. Bacterial nano cellulose (BNC) is FDA-approved for use in humans and therefore development of a BNC-based ink for 3D bioprinting would be a step towards clinical use of 3D bioprinting with cellulose-based inks.

BNC nanofibrils secreted by *G. xylinus* were disentangled by Aqueous Counter Collision and bioink was prepared to a final composition of 2.5% BNC. Gridded structures (6 x 6 x 1.2 mm) were printed with both nano cellulose- and BNC-based bioink, mixed with human chondrocytes and printed in an extrusion 3D bioprinter. The constructs were immediately implanted in a subcutaneous pocket in nude mice in consecutive experiments.

After 30 and 60 days, the implants were harvested. Paraffin sections were stained with Alcian blue and van Gieson and examined morphologically. The number of chondrocytes per $mm^2$ of the section area was counted.

Both bioinks showed good printability and good sheer-thinning behavior. The constructs retained their 3D structure and were mechanically stable over time. Cartilage formation consisted of gradually increasing glucosaminoglucan (GAG) deposition and increasing number of GAG-positive cells. An abundance of GAG-positive clusters of cells were seen as a sign of proliferation of chondrocytes. FISH confirmed that the chondrocytes were of human origin.

After 30 and 60 days, the number of cells in constructs printed with BNC were 32.8 ± 13.8 (mean ± SD) and 85.6 ± 30.0 cells per $mm^2$ ($p$ = 0.002), respectively. At the same time intervals, in constructs printed with nano cellulose, the number of cells were 63.5 ± 86.2 and 269.6 ± 78.8 cells per $mm^2$ (p = 0.001), respectively.

Both nano cellulose and BNC are suitable for 3D bioprinting due to the ability to create an ink with high printability, high fidelity and a suitable environment for chondrocytes. The development of BNC-based bioink is a contribution towards clinical application of cellulose-based inks for 3D bioprinting.

a91069

## 03-P062 The physicochemical properties and cell behavior of biodegradable 3D-printed calcium silicate scaffolds

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Tissue engineering strategies have been tackled in leveraging three-dimensional (3D) printing technology for engineered bone tissue over past few years. Several studies showed that composite scaffolds composed of polymers and inorganic materials through 3D printing process provided substantially osteogenic activity and cellular benefits. Among the variety of biocompatible polymers, polycaprolactone (PCL) have been approved by the FDA for clinical use with low toxicity when fed to metabolic pathways and the low melting point makes it a high performance printable ink in a lab environment. However, the flaws of PCL still lack in its suitable degradability and hydrophilicity properties. Bioceramics based on calcium silicate (CS) materials have drawn much attention owing to their high biological activity, which has excellent osteogenic differentiation both *in vitro* and *in vivo*. Herein, this study proposed a facile route for fabricating the ideal porous 3D scaffolds with tailored degradability and osteogenic activity by introducing CS into PCL. As the results, a great amount of precipitated bone-like apatite grew on the surface of scaffolds after immersion in the simulated body fluid. The *in vitro* degradation experiments shown that the weight of CS/PCL scaffolds lost 49% within three months. In addition, the results indicated that the addition of CS significantly improved the wettability on the surface of PCL. This also directly further affects cell attachment and proliferation. Moreover, the CS/PCL scaffolds offered higher levels of osteogenic- and angiogenesis-related gene expression of Wharton's Jelly mesenchymal stem cells. These results suggest that the CS/PCL composites may have a potential application for bone tissue engineering.

a91124

## 03-P063 The cell-laden hydrogel/ dopamine-inspired calcium silicate complex hierarchical porous scaffold fabricated by 3D bioprinting

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The 3D bioprinting has been popularly used in the bone tissue engineering, as many of the biomaterials for this field of study can be prepared for and produced from this additive manufacturing technique. We strategized a method of producing the polydopamine-modified calcium silicate (PDACS) scaffold with Wharton's jelly mesenchymal stem cells (WJMSCs) incorporated with human umbilical vein endothelial cells (HUVEC)-laden hydrogel. The PDACS/hydrogel 3D scaffold yielded Young's modulus of the 3D scaffolds as high as 75 MPa. The vascular morphogenesis and cellular behaviors regulated by our hybrid scaffolds were also intricately considered. Furthermore, the HUVEC in the bioink indicated higher levels of angiogenic biomarkers and showed potential for the formation of complex vascular networks. Higher levels of bone tissue regeneration marker were also observed in our bioscaffold. Such a hybrid of synthetic materials with cell constituents not only enhances osteogenesis but also stimulates vessel network development in angiogenesis, presenting the fact that 3D printing can be further applied in improving bone tissue regeneration in numerous aspects. We believe that this method may serve as a useful and effective approach for the regeneration of defective complex hard tissues in deep bone structures.

a91844

## 03-P064 The fabrication of strontium-doped calcium silicate 3D scaffold for bone regeneration

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Strontium (Sr) plays an important role in bone regeneration by stimulating bone formation and inhibiting bone resorption. Bone scaffolds should possess excellent biocompatibility and osteoinductive for the clinical application of bone tissue engineering. A 3D-printing technique was applied to prepare scaffolds with controlled microstructure. In the previous study, we developed the fast setting calcium silicate that not only exhibited good biocompatibility but also promoted the osteoconductivity of various cells. To meet this need, a 3D printed Sr-calcium silicate (Sr-CS) scaffold for bone defect repair has been developed in our group. In this study, we fabricated Sr-CS scaffold aiming at facilitating the regeneration of osteoporotic bone. Regarding in vitro bioactivity, the scaffolds with different Sr concentration were immersed in simulated body fluid that was cover abundant apatite spherulites after immersion for 7 days. In addition, the biological synergism is proved *in vivo* by implanting in dorsal subcutaneous tissues of rats. The pronounced hard tissue formation four weeks after the operation is presented based on histomorphometric and micro-computed tomographic analyses. The functional biomaterials releasing Sr ion is considered to be a promising therapeutic platform enhancing both angiogenesis and osteoclastogenesis in bone tissue engineering. In conclusion, our results are paving the way for using Sr in biomaterials designed for osteoporosis patients.

a91860

## 03-P065 A combinational 3D printed gradient construct of polycaprolactone and hyaluronic acid for osteochondral tissue engineering

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Temporomandibular joint (TMJ) disorders can be characterized by pain of decreased mandibular function. In patients with debilitating, end-stage disorders, TMJ reconstruction using autologous or alloplastic implants are often required for symptomatic relief and improved function. Traditional monophasic tissue engineered scaffolds do not provide adequate environmental cues to develop an osteochondral interface. We have combined two commonly used 3D printing methods, fused deposition modelling (FDM) and direct ink writing (DIW) into a single platform. Our combinational 3D printer allows for the precise fabrication of a patient-specific tissue engineered condyle for regenerative TMJ implants. To fabricate an osteochondral region within the implant, natural materials such as devitalized cartilage (DVC), demineralized bone (DBM), hydroxyapatite (HAp), and pentanoate-functionalized hyaluronic acid (PHA) were composited with polycaprolactone (PCL) to create an osteo- or chondroinductive environment. FDM extrusion of PCL/DBM, PCL/HAp, or PCL/DBM/HAp compose the bone region, and DIW extrusion of PHA/DVC or PHA/HAp/DVC compose the cartilage region of the condylar implant. To couple the softer PHA composites to the stiffer PCL composites, four integration methods were developed and tested for interfacial adhesion. Additionally, we have developed a unique fabrication paradigm that harnesses capillary action within our regenerative constructs to homogenously distribute cells and prevent cell necrosis. Wharton's Jelly Cells (WJCs) were seeded onto the gradient multiphase constructs and allowed to culture for 6 weeks. Results were analyzed after 2, 4, and 6 weeks using micro-computed tomography (μCT) to assess and quantify mineralization. Osteocalcin expression was tracked during the experiment and correlated to WJC differentiation within the constructs. Histology was used to assess bone or cartilage regeneration. Safranin-O/Fast Green staining was used for glycosaminoglycans (GAGs) within the cartilage region, and Alizarin Red staining was used to observe calcium deposition within the bone region. At the end of the 6-week study, we observed increased mineralization in our constructs compared to the control, and observed increased GAG staining in our constructs compared to the control.

a92076

## 03-P066 Fabrication of a chondrogenic-active hydrogel for *in situ* 3D bioprinting

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The application of bioscaffolds for tissue regeneration requires the proper diffusion of growth factors and nutrients through the material to be effective over a long period. The direct functionalization of hydrogels with slow-release microspheres will enhance the production of the generated tissues. The aim of this study is to produce a chondrogenic-active hydrogel to induce self-differentiation of stem cells.Chondrogenic Alginate-Polycaprolactone Spheres (CAPS), containing BMP6 and TGFb3, were incorporated into Gelatin Methacrylate (GelMa) and Hyaluronic Acid (HA) hydrogel in combination with human adipose derived stem cells (hADSCs), and 3D printed through Core/Shell strategy (1,2). The Bioscaffolds were then maintained for three weeks in basal cell culture media. The release profile of chondrogenic growth factors from CAPS was analysed by ELISA test. The chondrogenic capacity of CAPS was analysed qualitatively by immunostaining and quantitatively by GAG content and qRT-PCR analyses, to verify the expression of Collagen I, II, Sox 9, glycosaminoglycans, and compared to no-CAPS GelMa-HA standard chondrogenic culture.The release profile of BMP6 and TGFb3 loaded into CAPS showed an initial burst release of the chondrogenic factors within 8 hours of 3D printing, followed by a slow gradual release of the remaining factors until 21 days post printing. Furthermore, GelMa-HA functionalized with CAPS efficiently induced chondrogenic differentiation of 3D printed hADSCs.The use of Chondrogenic Alginate-Polycaprolactone Spheres is straightforward for the clinical applicability of *in situ* 3D Bioprinting, as the chondrogenic-active hydrogel could produce hyaline-like cartilage in vivo without being dependent on external stimuli.

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a92087

## 03-P067 Integration of hydrogel-based cartilage constructs on ceramic subchondral bone substitutes combining multiple 3D printing technologies

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Cell-laden hydrogels are promising candidates to aid in filling and regeneration of chondral defects. However, their utilization in larger osteochondral (OC) defects is hindered by limited integration with the subchondral bone substitute due to mechanical mismatch. Also, restoration of function, the integration between chondral grafts and the surrounding native tissue is an additional challenge. In this study, we developed a multi-material OC graft with a reinforced cartilage-bone interface by combining 3D printed bone cement with a cartilage-mimetic cell-laden hydrogel, which was anchored via a melt electrospun microfiber mesh.

A low-temperature setting printable Calcium Phosphate paste (PCaP) consisting of α-tricalciumphosphate (α-TCP), nano-hydroxyapatite and biodegradable, crosslinkable poloxamer[1] was prepared to print a porous bone substitute with a non-macroporous layer mimicking the subchondral region. The interfacial layer was either unmodified or was plotted (3D Discovery, regenHu) directly onto a polycaprolactone mesh (PCL, 0-90° laydown) produced by melt electrospinning writing (MEW, custom-made device). Finally, gelatin methacryloyl (gelMA) loaded with a co-culture of chondrocytes and mesenchymal stromal cells (MSC)[2] was infused to fill the space between the PCL microfibers.

The PCL meshes were efficiently embedded in the PCaP upon setting of the cement, and protruded into the cartilage region (confirmed by SEM micrographs), acting as a mechanical reinforcement, and increasing sevenfold the interfacial strength of the anchored gelMA hydrogels. For the bone compartment, the compressive modulus and ultimate strength were in the range of cancellous bone. MSCs were able to proliferate on the PCaP and underwent osteogenic differentiation. The whole OC construct was implanted in an equine *ex vivo* OC defect model[3] in a bioreactor, preserving the viability of the bone and cartilage component, to assess cartilage synthesis in the hydrogel and feasibility of surgical implantation.

By employing different technologies, an engineered OC plug with enhanced interfacial strength between the hydrogel-based cartilage layer and the bone substitute was successfully generated. The principle needs further testing, but is promising for the fabrication of OC constructs in orthopaedic regenerative medicine.

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a92380

## 03-P068 Osteoinductive composite implant made by stereolithography for orbital floor fracture repair

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### INTRODUCTION

Orbital floor (OF) fractures are commonly treated by implanting either bioinert titanium or polyethylene implants, or by autologous grafts. As alternative, we introduce a personalized implant made of poly(trimethylene carbonate) loaded with hydroxyapatite (PTMC-HA). The implant is produced using stereolithography (SLA) based on patient CT scan. It is degradable and osteoinductive. In this preclinical study, we validated the workflow for the production of personalized PTMC-HA implants and assessed their performance (*i.e.* implant stability, orbit position, bone formation) compared to standardized titanium implants in a repair OF defect sheep model.

### METHODS

Implants fabrication was done using SLA of photo-crosslinkable PTMC mixed with HA [1-3]. Preclinical study: (sheep n=12, ethic number 34_2016) was conducted by first scanning the OF bone of each sheep in order to design and to fabricate patient specific implants (PSI) made of PTMC-HA. The fabricated PSI was implanted after creating OF defect. Bone formation and defect healing was compared to manually shaped titanium mesh using time-laps X-ray analyses, histology (Giemsa-Eosin staining) and sequential fluorochrome staining over 3-months. Additionally, the osteoinductive property of the biomaterials was assessed by intramuscular implantation (IM).

### RESULTS AND DISCUSSION

In this study, we showed that the composite PTMC-HA allowed for ectopic bone formation after IM implantation, without requiring any biotherapeutics. In addition, we could repair OF defect on sheep using SLA-fabricated PTMC-HA with a good shape fidelity (compared to the virtual implant) and a better bone integration compared to the titanium mesh.

### CONCLUSIONS

This study opens the field of patient-specific implants made of degradable and osteoinductive scaffolds fabricated using additive manufacturing to replace advantageously autologous bone and titanium implants.

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### ACKNOWLEDGEMENTS

NSFC-DG-RTD Joint Scheme (Project No. 51361130034) and the European Union's 7th Framework Program under grant agreement n° NMP3-SL-2013-604517.

a90348

## 03-P069 3D printing of composite scaffolds functionalized with ECM components for bone defect repair

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### Introduction

Polycapralactone (PCL) is the most commonly used thermopolymer in 3D printing of implantable scaffolds, however alone PCL provides little in the way of instructive cues to enhance regeneration. The overall aim of this study was to develop implants for bone defect repair that are not only mechanically functional at the time of implantation, but will also provide stem cells that home from the bone marrow and/or surrounding tissues into the implant with appropriate biochemical and biophysical cues to enable their differentiation into osteoblasts. This will be achieved by developing composite filaments of PCL and bone extracellular matrix (ECM), and then printing porous scaffolds with mechanical properties comparable with porous cancellous bone.

### Methods

Decellularised bone ECM was generated by blasting bovine bone fragments with water to remove all marrow. The fragments were then placed in a series of detergent and nuclease washes to remove all DNA. Following the washes, the fragments were rinsed with PBS, lyophilized, and cryomilled to form a powder. The bone ECM (30% w/w) was added to the PCL to generate a composite thermopolymer. Scaffolds of Ø 4mm by 4mm high with a fiber spacing of 1.2mm were printed with PCL and PCL+Bone ECM. The mechanical properties of the scaffolds was assessed by performing unconfined compression tests. Scaffolds were seeded with 1x106 porcine MSCs and allowed to adhere for 2 hours before the addition of growth medium ($\alpha$MEM + 10% FBS + 5% Penstrep). Scaffolds were cultured in growth medium for up to 21 days.

### Results

The PCL+Bone ECM scaffolds were significantly stiffer than the PCL alone scaffolds. Increased cell attachment was observed on the PCL+Bone ECM scaffolds throughout culture. The MSCs seeded on the PCL+Bone ECM group started to deposit mineral as early as 7 days into culture, with increased mineral deposition observed over the next 14 days (Calcium & µCT). The PCL group had no mineral deposition.

### Discussion

The addition of the decellularised ECM within the PCL significantly increases the mechanical properties of the scaffolds, aided in cell attachment, and lead to enhanced osteogenesis of MSCs without the use of exogenous growth factors. Taken together, we have developed a scaffold that is not only mechanically functional but also provides appropriate biochemical and biophysical cues to enable the osteogenic differentiation of MSCs in vitro.

### Acknowledgements

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a92063

## 03-P070 Clinical Relevant Bioinks for Enhanced Cartilaginous Matrix Deposition

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Damaged cartilage tissue greatly reduces the quality of life and current treatments to regenerate or replace cartilage are highly invasive and often have poor clinical outcome. Cell based therapies like autologous chondrocyte implantation (ACI) become more and more common and accepted by regulatory authorities. Our objective is to advance these treatments to the next level, by using unmodified, FDA-compliant polymers, primary chondrocytes, and 3D bioprinting to create cell-laden cartilage tissue grafts. To accomplish this, a novel bioink based on alginate, gellan and hyaluronic acid (HA) was developed. Every component adds valuable properties, which results in an ink that 1) can be easily crosslinked (alginate), 2) has shear thinning and fast shear recovery properties for excellent printability and structure fidelity (gellan) and 3) contains biological, native extracellular matrix component of cartilage (HA) to improve the quality of the tissue produced.

Passaged primary bovine chondrocytes were mixed in the bioink to create cell-laden, bioprinted small discs (d=4mm, h=2mm) that were cultured *in vitro* for up to 8 weeks. TGF$\beta$1 was either supplemented constantly in the media or loaded in the bioink before printing. Tissue grafts were analyzed by fluorescent live/dead assay, compression tests and immunohistochemical stainings.

Cells in bioprinted tissue grafts, cultured in TGF$\beta$1-supplemented media, remain highly viable (91% ± 0.7) and proliferate. After 8 weeks of *in vitro* culture immunohistochemical analysis reveal a very dense, homogeneous collagen II deposition throughout the whole scaffold, with only faint traces of collagen I. This results in a significant improvement of the compressive modulus from 46 kPa ± 8.6 at day 1 to 302 kPa ± 76 at 8 weeks (n=3, p=0.01).
In addition, we demonstrated successful TGF$\beta$1 loading of bioprinted tissue grafts and further studies will aim at direct implantation to eliminate the need for pre-culture in TGF$\beta$1-supplemented media.

In conclusion, we introduced a novel, biomimetic bioink with FDA-approved materials, which stimulates production of cartilaginous extracellular matrix in bioprinted tissue grafts and could be used for clinical applications in the maxillofacial and orthopedic field.

a91419

## 03-P071 3D Differentiation of human Adipose-derived stem cells/hTERT in Methacrylate Gelatin Hydrogels with Different Stiffness

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Methacrylamide-modified gelatin (Gel-MOD) hydrogels represent an attractive source for biofabrication of three-dimensional (3D) tissue-engineered constructs, as they have tunable mechanical properties, are compatible with different types of cells and resemble elements found in natural cell-matrix environments. It has been demonstrated that Gel-MOD properties can be altered depending on the degree of methacrylation. In our study we investigated how 5%, 7.5% and 10% (m/V) Gel-MOD hydrogels (degree of methacrylation 63%) affect proliferation and differentiation of human adipose-derived stem cells/hTERT (hASC/hTERT) after their encapsulation in microspheroid form and their 3 or 5-week exposure to osteogenic or chondrogenic differentiation medium, respectively. Employing confocal microscopy we observed that all experimental conditions supported hASC/hTERT viability during the entire testing period. Morphological evaluation and gene expression analysis of selected genes *SOX9*, *ACAN* and *COL2A1* proved that compared to the 3D control (undifferentiated) sample, chondrogenic differentiation of hASC/hTERT was successfully achieved in all three different formulations of Gel-MOD and was most prominent in 5% gel. Interestingly, when selected osteogenic genes (*RUNX2*, *BGLAP*, *ALPL*, *COL1A1*) were analyzed in 3D control or osteogenically differentiated samples and were compared to the expression obtained from hASC/hTERT after their monolayer expansion (prior encapsulation – day 0), their expression in 3D increased to a similar extent in both conditions. This could suggest that Gel-MOD alone (without any induction medium) is able to shift the behavior of of hASC/hTERT towards osteogenic lineage. The acquired preliminary data indicate that Gel-MOD shows very promising potential in the field of osteo-chondral tissue engineering.

a93200

## 03-P072 Reconstruction of Large bone defect in sheep with customized 3D printed calcium phosphate scaffolds

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The reconstruction of large bone defects resulting from severe trauma or resection of tumors remains a challenge for orthopedic and plastic surgeons. Today, a vascularized bone is taken from the patient and grafted into the defect. However, this transplantation adds morbidity and requires extensive micro surgery to adapt to both the vasculature and the skeleton. We propose an alternative approach consisting of manufacturing patient specific biomaterial scaffolds through 3D printing. This study aims to demonstrate the feasibility of regenerating large bone defects with 3D printed anatomically accurate biomaterial scaffolds in sheep.The left posterior limb was scanned by computed tomography (CT), and a contrast agent was injected in order to visualize the metatarsus and the vasculature. CT data were imported into medical imaging software and converted to STL files. The metatarsus bone of sheep was 3D printed with PLA filament from the CT scans. It allowed surgical planning with the placement of an osteosynthesis plate and screws. A cutting guide was also designed and 3D printed in order to create a segmental defect of 35 mm in the metatarsus. A specific biomaterial was produced by 3D printing using a calcium phosphate /pluronic paste that hardens into a porous scaffold. Three groups were considered: empty defects, and defects filled with either a customized biomaterial scaffold, or the biomaterial scaffold with a vascular pedicle running through it. After surgery, bone regeneration and vascularization were followed by CT at 30, 60 and 90 days. Sheep were euthanized and a vasculature contrast agent (Microfil) was injected into the femoral artery. Metatarsus were dissected, and analysed by microCT and histology study.The scaffold had interconnected porosity to favor bone regeneration. CT scans indicated that the empty defect remained non-bridged after 3 months. A limited bone healing was observed with the 3D scaffold. The vascular pedicle going through the scaffold was functional without thrombosis. The vasculature favored bone regeneration of the critical size metatarsus defect.

This pre-clinical study demonstrated the feasibility of 3D printing patient specific biomaterial scaffolds for regeneration of large bone defects resulting from severe trauma or resection of tumor.

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a93221

## 03-P073 3D Bioprinted hydrogel model incorporating β -tricalcium phosphate for calcified cartilage tissue engineering

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One promising tissue engineering approach for osteochondral tissue is the use of cell-encapsulated three-dimensional hydrogel scaffolds to fill the defect. Osteochondral tissue engineering strategies are categorized into monophasic, biphasic and triphasic models. When mono- and biphasic models are well documented, the triphasic models is still largely a novel approach. In the triphasic model, the most important role plays calcified cartilage which is a narrow zone between the articular cartilage and the underlying bone. The objective of this research was to fabricate calcified cartilage tissue from MSCs-laden in alginate mixed with gelatine methacrylamide (GelMA) hydrogels filled with ceramics nanoparticles in form of ß-tricalcium phosphate (TCP). We demonstrate how to fabricate 3D biomimetic precursor solution undergo physically and chemically crosslinking process with high cell density ($10^7$cells $ml^{-1}$) resulted in high viability (>80-85%) and high printing resolution (~100 µm) through two-coaxial needles system. After bioprinting the scaffolds were cultured in chondrogenic media to evaluate the differentiating effects through 21 days. Qualitatively and quantitatively material's characterization data make our approach a valid candidate for advanced engineering of calcified tissue.

a91251

## 03-P074 Fabricating an Extracellular Matrix Analogue Using Natural Polymers for Cartilage Tissue Engineering Applications

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Osteoarthritis is the most common degenerative disease of the joint. Current treatment options prove inadequate at restoring full joint function due to the complexity of the implicated cartilage tissue. This avascular connective tissue consists of a specialised extracellular matrix (ECM) with a nanofibre-based hierarchical ultrastructure that imparts remarkable biomechanical properties to the tissue; essential for protecting the articular surfaces of load-bearing joints. The advent of smart biomaterials in the field of tissue engineering offers hope to alleviate the shortcomings in current treatments as they can better recapitulate the natural structure of cartilage ECM. We have demonstrated previously that blends of cellulose and silk can stimulate human bone marrow stem cell (BMSC) chondrogenesis without biochemical induction[1].

An electrospinning technique has been employed to fabricate nanofibrous materials that can mimic the *in vivo* ECM using these blends. Cellulose and silk natural polymers were used to fabricate nonwoven neat and composite nanofibres. Scanning electron microscopy was used to characterise the materials. Fibre diameters could be tuned through adjustments in electrospinning flow rate and voltage. Further, environmental temperature and humidity influenced resulting fibre diameters and beading. Fourier transform infrared spectroscopy confirmed the absence of solvent following regeneration of the polymer materials. Neat cellulose, neat silk and composite cellulose:silk in 50:50 and 75:25 mass ratios were fabricated to produce nanofibrous 3D scaffolds, and fibre diameters could be systematically tuned in the range of 150 to 60 nanometres. All materials demonstrated biocompatibility when cultured *in vitro* with BMSCs over a 14 day study period.

Accordingly, the biocompatible neat and composite natural polymer nanofibres show promise for tissue engineering applications. Work is currently underway to explore the inductive capability of this configuration in driving stem cell differentiation, specifically chondrogenic gene expression[1].

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a91750

## 03-P075 Bioceramics Bilayer Scaffolds for Osteochondral Defect Repair

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Osteochondral defects are common in the young and active population, it usually caused by trauma or disease such as osteochondritis dissecans, osteonecrosis and traumatic injury. There is no gold standard has been established for treating osteochondral injury. In recent years, the potentiality of treating osteochondral defects with tissue engineering approaches became more and more popular. As those reporting unmet medical needs and limitations, a biomimicry, low cost and one-step approach need to be developed. Three-dimensional (3D) printing techniques can be used for the fabrication of customized, versatility constructs for tissue repair. To meet this need, a 3D printed bilayer polyurethane (PU)-based bioceramics scaffold for osteochondral defect repair has been developed in our group. To investigate the potential of the 3D printed bilayer PU-based bioceramics scaffold for regeneration of osteochondral tissue, the scaffolds were analysis with cell viability, degradation rate, mechanical properties, Alcian blue staining, Alizarin red staining, and ALP analysis. The contact angle gradually decreased with the percentage of bioceramics content increasing. And it also showed good cytocompatibility by SEM and fluorescent images. Regarding in vitro bioactivity, the different content bioceramics scaffolds were soaked in simulated body fluid, the scaffold surface was cover abundant apatite spherulites after immersion for 28 days. Furthermore, in vitro alkaline phosphatase activity assay evidently indicated that cells cultured in bioceramics scaffolds showed significantly greater osteogenesis ability after 1 and 2 weeks. In summary, the PU-based bioceramics 3D printed-scaffolds might have potential in osteochondral tissue engineering.

a90476

## 03-P076 The Chondrogenic Potential of Nanocellulose-Alginate in Combination with Nasoseptal Chondrocytes for Tissue Engineering Purposes

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### Background

Contemporary reconstruction of cartilage defects relies on autologous tissue or synthetic implants. These options are limited by donor site morbidity and infection/extrusion respectively. Worldwide research attempts over three decades to engineer cartilage has focused on combining synthetic scaffolds with non-specific stem cells, without successful clinical translation. Our strategy involves a natural biomaterial in combination with tissue specific cells, to replicate native tissue micro- and macro-architecture.

### Methods

Primary human nasal progenitor cells (NP) were isolated by fibronectin adhesion assay, while non-adherent cells were cultured as differentiated nasal chondrocytes (DNC) and the original cell population containing both subpopulations, cartilage derived cells (CDC) served as a control. DNC cells were mixed in sterilised nanocellulose with 2.5% alginate (NA) or alginate (AG) on its own to confirm which biomaterial encourages chondrogenesis as alginate is known to have chondrogenic properties. DNC and NP with the different biomaterials were cultured for 14, and 21 days followed by gene expression analysis, viability studies using Live/Dead assay, and histological analysis.

### Results

Live/Dead assay results of cells embedded within both biomaterials demonstrated to have a high viability indicating the biomaterials used are biocompatible. qPCR data comparing CDC, DNC, and NP in NC or AG have shown NC to enhance chondrogenic potential of all cell subpopulations in comparison to AG most significantly seen in collagen type II at 14 days (CDC 15.7-fold, $p<0.001$; DNC 166-fold, $p<0.001$; PC 22.5-fold, $p<0.001$). Overall the highest expression of chondrogenic marker was observed in the CDC population embedded in NC than the AG counterpart as indicated by the up-regulation of matrix genes at 14 days (aggrecan ~2.3-fold, $p<0.01$; SOX9 ~2.4-fold, $p<0.05$; collagen type II ~15.7-fold, $p<0.001$) and 21 days (collagen type II ~6.9-fold, $p<0.001$). These results are further confirmed at the protein level by histological staining including alcian blue, safranin O, and toluidine blue staining, indicating enhanced GAG synthesis after 21 days in culture in comparison to CDC in AG and DNC and NP in NC. CDCs are shown to form cell clusters surrounded by territorial matrix recapitulating a similar distribution as in native cartilage tissue. These results indicate that NC is a promising biomaterial for cartilage tissue engineering.

a92440

## 03-P077 Decellularized Hyaline Cartilage powder and Autologous adipose-derived stromal vascular fraction (SVF) for Articular cartilage regeneration

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Currently, many cell therapy agents such as stem cells and autologous chondrocytes for regenerating knee cartilage have been developed. Although it has been proven to be a safe and effective treatment for injured knee joints, these cell therapies have a limit of two operations because they require about 4 weeks of incubation to harvest the tissues of the patient and obtain sufficient cells. In addition, it is difficult to regenerate hyaline articular cartilage because of the disadvantages such as unstable adhesion and uneven distribution of cartilage defect due to gravity in transplanted cells. In this study, adipose-derived hyaline cartilage powder (ECM), a major component of cartilage, was used to induce differentiation of hyaline cartilage rather than fibrocartilage. Identify effective hyaline cartilage regeneration at the site. Therefore, we expect that the 3D bio-printing technique and the patient-tailored porous scaffold made of self-adipose-derived SVF / decellularized hyaline cartilage powder for hyaline cartilage regeneration will be the treatment of Osteoarthritis (OA).

A 3D bio-printed patient-tailored porous scaffold made from fibrin glue encapsulated Adipose-derived SVF / Decellularized hyaline cartilage powder proved to be effective for the regeneration of hyaline cartilage. Decellularized hyaline cartilage can support the differentiation of adipose-derived SVF into hyaline cartilage, and it is expected to solve problems such as cell adhesion and loss of existing cell therapy drugs by applying 3D printing.

a93446

## 03-P078 Advanced capability of BMP-2-loaded mesoporous calcium silicate scaffolds for bone regeneration

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The calcium silicate-based materials were broadly used as bone grafts with good osteoconductivity due to the slow degradation rate and biocompatibility, but lacking osteoinductive limited the application in dental surgery. And it has been proved that applying the mesoporous calcium silicate nanoparticles shown a great potential as controlled drug-delivery system. The aim of this study was to investigate a novel osteoinductive scaffold by loading bone morphogenetic protein-2 (BMP-2) to mesoporous calcium silicate (MesoCS) and fabricate as 3D-printed scaffolds by fused deposition modeling combined with polycaprolactone (PCL). The MesoCS/BMP-2 scaffold showed the similar pattern to CS scaffold in releasing of calcium and silicon ions in simulated body fluid (SBF) soaking analysis for 7 days, but the BMP-2 was continued released from MesoCS/BMP-2 scaffold more than CS scaffold significantly from 48 hours to 7 days. The adhesion and proliferation behaviors of human dental pulp cells (hDPCs) cultured on MesoCS/BMP-2 scaffold were also significantly better than scaffolds without BMP-2 or mesoporous, as well as the results of the test on alkaline phosphatase (ALP) activity. The results support that the novel 3D-printed MesoCS scaffold performed well as BMP-2 delivery system and would be an osteoinductive biomaterial in hard tissue regeneration.

a92587

## 03-P079 3D Printed Bioinspired Scaffold Architecture for Meniscus Regeneration

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The meniscus is a complex cartilaginous structure located within the knee joint and plays a vital role in knee joint functionality associated with load distribution and shock absorption. Knees are subjected to high forces; up to twenty-four times body weight during high impact activities with the meniscus bearing up to 75% of these joint loads, which predisposes them susceptible to tears. However, the meniscus has very poor healing capabilities, with meniscectomy being the current primary treatment for meniscus tears. It has been well established that removal of the meniscus inevitably results in bone on bone contact, leading to the early onset of degenerative osteoarthritis. Therefore, there is a pressing need to develop novel approaches for meniscus regeneration to restore the functionality of the native meniscus. To date, tissue engineered scaffolds typically lack the complex architecture of the native tissue and are yet to yield long-term reliable results. Having characterised bovine meniscus tissue to elucidate the role of glycosaminoglycan content and collagen orientation on the mechanical properties and its regional variation, the aim of this study was to design a 3D caffold with an architecture replicating the tissue organisation of the native meniscus. To achieve this goal, bioprinting technology was utilised to produce high-resolution polycaprolactone (PCL) scaffolds with a complex tailored internal 3D microstructure. Scaffolds were characterised by mechanical testing, micro-computed tomography (μCT), scanning electron microscopy (SEM) and cell viability to demonstrate their biomimicry to the native tissue. The scaffolds produced displayed an inhomogeneous internal microstructure with anisotropic mechanical properties similar to the native tissue. Fibroblast cells seeded on the scaffolds exhibited good cellular growth, proliferation, and cellular orientation along the fiber direction. This study demonstrates that this bioinspired approach for developing meniscal scaffolds shows a promising potential for meniscus tissue engineering applications.

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a93127

## 03-P080 3D Hybrid Multilayer Scaffolds for Cartilage Tissue Engineering

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Articular cartilage diseases seriously affect the quality of lives of patients worldwide. According to statistics, about 20% of people suffers from some form of arthritis. In this study, we manufactured bionic cartilage scaffolds with complex internal porous structure and different structure per layer by three-dimensional (3D) printing technology to mimic the mechanical properties of natural cartilage, and used the cell-encapsulating hydrogels to improve the potential of cartilage differentiation and cartilage tissue maturation. Furthermore, this study combined a digital light process (DLP) 3D printing technology and extrusion printing system to develop a new process to fabricate customized hybrid multilayer scaffolds with cell-encapsulating hydrogels for cartilage tissue engineering.

a91087

## 03-P081 3D printing of high strength hydrogel scaffolds for bone/cartilage repair

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The emerging 3D bioprinting technique that is strongly dependent on the development of bioinks offers a promising opportunity to customize personalized bioscaffolds for precision and individualized therapy of bone/cartilage defects. Hydrogels are one sort of attractive sca ff olding materials due to their resemblance to extracellular matrices. Although much progress has been made in designing and fabricating high strength hydrogels, very few of them have been extended to the treatment of bone defects. In this study, we successfully developed a series of high strength hydrogels as the bioinks for 3d printed bone/cartilage regenerative scaffolds. Then, both in vitro and in vivo experiments demonstrated these high strength hydrogels significantly accelerated regeneration of cartilage or bone. Therefore, this works offer new possibilities to develop numerous bioinks for 3D-printing of desired bioscaffolds to realize individualized repair of degenerated load-bearing tissues

a91059

## 03-P082 Pure titanium custom implant for the mandibular continuity defect

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Purpose: The purpose of this study was to compare the daily food intake rate and the rate of screw loosening between 2 groups of rabbits with mandibular continuity defects: custom implant (CI) group and 5-hole mini-plate group.

Materials and method: Two types of cylindrical implants were printed, and their physical strength was compared. In this study using rabbits, 1 group (n = 5) received a CI for the reconstruction of a mandibular continuity defect (CI group) and the other group (n = 5) received a 5-hole mini-plate without a bone graft (reconstruction plate [RP] group). After reconstruction, the daily food intake rate and the rate of screw loosening were examined postoperatively. Histologic examination in the CI group was performed 3 months after the operation.

Results: The design that mimicked the mandible showed greater physical strength. The amount of time required to achieve 50% recovery was shorter in the CI group than in the RP group (P = .011). The total number of loosened screws in the CI group was lower than that in the RP group at 3 months postoperatively (P = .008). New bone formation in the porous CI was evident in the CI group.

Conclusions: Rabbits with mandibular continuity defects treated with CIs for reconstruction showed faster recovery of the daily food intake rate and fewer loosened screws than those treated with a 5-hole mini-plate without bone graft.

a92158

## 03-P083 A Novel Method for Determining Cellular Morphology Within Living Cartilage Using Confocal Microscopy

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**Introduction:** We recently reported the use of incremental compressive loading to generate tissue constructs with high modulus and cartilage-like histology [1]. Our overall aim is to improve efficacy of construct growth, accelerating clinical translation. As a first step, we aimed to establish a method for staining, visualisation and quantification of cell morphology within living tissue under non-loaded conditions as a prelude to subsequently determining cell deformation behaviour under compressive load. In order to be able to determine effect(s) of incremental compressive loading on cell deformation in living cartilage, selected chondrocytes must be imaged multiple times without significant signal loss. Our aim in the present study was to develop an imaging system to reflect these capabilities and compare image acquisition in the confocal microscope using a traditional galvo scanner and a high speed resonant scanner to potentially retain signal.

**Method:** Hoechst 33342 and CellMask ™ Green plasma membrane stains were applied to cartilage discs from bovine knee joints to visualise cell nuclei and plasma membranes respectively. Specimens were placed in a novel compression device designed and built for precise strain application to mounted samples for imaging in the confocal microscope. Image acquisition was conducted on non-loaded samples using both galvo and resonant scanning. Three sequential stacks of 2D high resolution images of the individual chondrocytes were compiled and compared with respect to signal loss and calculated cell volume, using Nikon "Elements" software.

**Results:** Nuclei and plasma membranes were specifically stained and highly resolved. Maximum intensity projection and mean cell volume measurements obtained using traditional galvo scanning were reduced by 23% and 15 ± 4.1% (average ± SD) respectively on repeated scanning compared to 8% and 5±1.2% for images obtained using the resonant scanner.

**Conclusion:** The method described here is the first to be applied to living cartilage. The results showed that resonant scanning significantly improved retention of signal on repeated scans of a single chondrocyte. The methodology will next be used in experiments to determine cell deformation under compression in order to optimise construct maturation in the longer term.

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a90970

## 03-P084 Reverse Engineering Methods to Study Osteochondral Regulatory Networks

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Chondrocyte differentiation involves a genetic switch from a proliferative cell state towards a hypertrophic cell state. The control of this process is tuned precisely by a complex network of signalling molecules and is essential for bone formation during development. Dysregulation of articular chondrocytes in pathogenic circumstances can lead to a recovery of the articular chondrocytes' ability to switch towards hypertrophy. Understanding these regulatory mechanisms is of primordial importance for the identification of pathogenic factors as potential therapeutic targets in degenerative disease as well as for the development of tissue engineered (TE) constructs for osteochondral regeneration. To facilitate the understanding of this kind of complex biological systems computational models are developed. Here we propose to use two different reverse engineering approaches to unravel the complexity of the regulation of chondrocyte differentiation by using high throughput expression data. On one hand, network inference methods served to build a consensus gene regulatory network based on gene expression profiles in order to validate a previously developed literature derived topology. The consensus network topology arises from the processing of various published micro-array data sets with multiple inference algorithms using a consensus approach. Inference showed that the literature-derived model performs better in uncovering regulatory interactions, compared to the consensus network topology as golden standard, than a network that was created using the STRING database. On the other hand, we used a genetic optimisation algorithm to discover the parameters enabling the network output to fit known expression profiles. This led to the construction of an ensemble of reverse engineered additive models enabling the identification of key molecular factors implied in the stability of the state of the chondrocyte. Indeed, analysing the dynamics of this system and its behaviour under perturbations such as *in silico* knockout or over-activation might highlight key factors to act on to trigger or modulate chondrocyte differentiation. Currently, this model is used to design experimental strategies favouring robust osteochondral ossification in the context of bone tissue engineering.

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a91552

## 03-P085 Biomechanical Response of Regenerated Intervertebral Disc using Genipin and Platelet-Rich Plasma Therapies: Experimental and Computational Studies

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Degeneration of the Intervertebral disc (IVD), regardless of the phenotypes, risk factors and initiating mechanisms is directly linked to acute and chronic low back pain, the leading cause of disability worldwide. Regenerative therapeutic techniques are hence of great interest to both research and clinical communities and meanwhile assessment of the effect of disc degeneration/regeneration on the biomechanical response is essential [1]. Therefore, this study aimed to characterize the mechanical properties of the intact, degenerated, and regenerated (using Genipin and Platelet-Rich Plasma (PRP) therapies) IVDs. For this purpose, 4 different disc groups, namely intact (I), Trypsin-Treated Degenerated (D), Genipin-Treated Regenerated (GR), and PRP–Treated Regenerated (GPRP) were used for the ex-vivo experiments, such as 8 specimens were included in each group. For this investigation, IVDs were harvested from 6-month-old pigs and degenerated artificially using trypsin injection. In regenerated groups, the IVDs were further injected with either Genepin or PRP injection and then incubated in an organ culture system with application of diurnal loadings for 7 days. After incubation, the creep and impact tests were performed, and moreover the biochemical properties of IVD, i.e., GAG content within IVD matrix, were measured. To identify the specimen-specific material properties of IVDs in different groups, a validated inverse poroelastic finite element (FE) methodology [2] was combined with the results of both creep and impact tests. The FE model results were in good agreement with the experiments for all disc groups. The comparative statistical analyses showed that the elastic modulus and hydraulic permeability decreased significantly for the degenerated disc group. The results revealed that both Genipin and PRP can significantly recover the mechanical properties of denatured discs. Both Genipin and PRP therapy increased the GAG content of degenerated IVDs but could not recover it to the intact level. However, PRP-Treated discs showed slightly better condition than Genipin in terms of recovering the GAG content. It is concluded that both Genipin and PRP therapy can be proposed as potential therapeutic techniques in clinic. Further studies are needed to evaluate the effect of these treatments using in-vivo studies.

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a90469

## 03-P086 Frictional drag dissipation promotes chondrogenesis in mechanically stimulated cell-seeded scaffolds

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The function of the chondrocytes in articular cartilage is influenced, among other factors, by a set of fluid and solid related physical cues such as interstitial fluid pressure and transmitted stress/strain. Accordingly, optimal cells biosynthesis depends not only on characteristics of the biomaterials but also on the pattern of mechanical stimulation. In view of the complex interplay of all these parameters, considerable chondrogenic variations have been observed in different combinations of cell-scaffold constructs and mechanical stimuli. In order to allow a more straightforward correlation between an applied mechanical stimulus and an observed tissue differentiation, mechanical hysteresis has been recently proposed as an overarching mechanobiological variable. Interestingly, healthy cartilage can dissipate a considerable part of the input energy due to its rich viscoelastic nature. The objective of this study is, therefore, to investigate the role of frictional drag dissipation on human chondro-progenitor cells biophysical response. For this purpose, dissipative scaffolds with similar degree of stiffness and dissipation level to articular cartilage were developed. We accomplished this by coherent design of permeability and stiffness of the network to have same level of dissipation in a standard cyclic compression. Biocompatibility of the scaffolds was confirmed by cells proliferation and viability for a period of 10 days. Seeded scaffolds were pre-cultured for 3 days in proliferation medium followed by an incubation in differentiation medium and supplemental mechanical stimulation for 4 days (2 hrs/day). The results showed a higher expression of chondrogenic markers for stimulated groups of dissipative scaffolds compared to their free swelling control group. While better cells penetration and proliferation were associated to higher permeability, maximal mRNA level for Col2a1, Acan and Sox9 after stimulation was observed for scaffolds having a lower permeability. In fact, by reducing the permeability, frictional drag dominates the mechanism of energy dissipation under compressive loading. Indeed, cyclic compression of visco-porous scaffolds leads to spatiotemporal fluid flow and cells deformation which might trigger different signalling pathways. However, chondrocytes favour the mechanical cues which mimics more similarly *in vivo* cartilage microenvironment (poor permeability, higher fluid pressure and frictional drag dissipation upon loading).

a90853

## 03-P087 Biomechanical Evaluation of Decellularized Cartilage Scaffold for Tissue Engineering Using Two Different Protocols

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Numerous studies have investigated the possibility of using synthetic and natural scaffolds combined with chondrocytes or mesenchymal stem cells for cartilage tissue engineering. Recent investigation showed scaffolds derived from decellularized cartilage matrices are able to provide a highly organized structure. The objective of this study was to investigate the biomechanical response of two decellularization techniques using both in-vitro and finite element (FE) modeling. Cartilage specimens were harvested from the femoral condyles of mature bovine within 4 h after death. Thirty Specimens were punched (D=5 mm, h= 7mm) and equally assigned into 3 groups (i.e., (1) control (C), Decellularized using (2) Triton X-100 (TX), and (3) Sodium Dodecyl Sulfate (SDS)). C group did not receive any forms of treatments. TX and SDS groups were decellularized according to the instructions in literature and all steps were conducted under continuous shaking [1]. All 30 specimens were mounted in a chamber filled with PBS and underwent a stress-relaxation test using a mechanical testing apparatus (Zwick/Roell, Ulm-Germany). A validated inverse poroelastic FE methodology [2] in conjunction with in-vitro experiments were further used to find the mechanical properties. The results of the FE models were well fitted with the in-vitro experiments (Error of 6.89 (±4.08)%). Compared to C group the Elastic modulus significantly decreased for SDS, however no significant difference was observed in TX group. Permeability increased for both TX and SDS group which enhance the fluid flow capability. It is therefore concluded that TX Decellularization may be a suitable candidate as a scaffold for cartilage tissue engineering.

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a91394

## 03-P088 Numerical feasibility study of an adaptive torsional testing setup to analyse mechanical properties for future tissue engineering applications

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Torsion is an important loading scenario, which is omnipresent in all kinds of tissue in the human body. But in the biomechanical characterization of tissue engineering scaffolds torsion is an underrepresented topic. For this reason, the following study shows a numerical evaluation of a torsional testing setup, which is designed for the testing of a broad spectrum of samples from long bone to small scaffolds. In order to analyze the “worst case” of the upper end of the sample range, horse bone was used to validate the setup dimensioning. To set up the model, equine bone was experimentally investigated with special focus on cancellous bone, because of the lack of material parameters in literature.

43 cubic samples were tested for the determination of the Young`s modulus and Poisson’s ratio of equine cancellous bone. Samples with 12mm edge length were obtained by embedding of an equine proximal humerus in polyurethane and then cutting orthogonal cubes from it by using a bandsaw. Non-destructive compressive testing of the samples in all three orientations was performed on a uniaxial loading device and additional optical observation was used for the determination of the Possion’s ratio. For the numerical analysis of the new testing setup all components were designed using the CAD software Autodesk Inventor. The setup was planned as an expansion to the uniaxial loading machine. A FE analysis including a convergence study with local mesh refinement was performed using the software ANSYS. The Young’s modulus of the equine cancellous bone cubes was determined to a mean ± SD of 106±45 MPa. The orientation dependent means were only deviating by max. 14% from the total mean. For this small difference it was concluded that the anisotropy of equine cancellous bone will not be accounted in the mathematical models. The obtained mean value for the Poisson’s ratio, which was also used in the model, was 0.15±0.08. All resulting stresses (von Mises) for the mechanical setup components remained below the yield strength, which resulted in a safety factor of at least 2.

This analysis of equine cancellous bone as well as the torsional testing setup using numerical engineering tools shows that the setup is able to successfully transmit up to 135 Nm of torque towards a horse bone. In consequence, the critical conditions of the setup are mathematically validated and in a next step the experimental verification for long bones as well as for small scaffolds must be performed.

a91490

## 03-P089 Nanovibrational stimulation (Nanokicking) for 3D osteogenesis in biphasic scaffolds; compositing of freeze dried collagen sponge-hydroxyapatite in hydrogels for bone tissue engineering

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Nanovibrational stimulation(nanokicking) promotes osteoblastogenesis of human mesenchymal stem cells (MSCs) in 2D and 3D(1). We are developing biphasic scaffolds compositing collagen hydrogels and freeze dried collagen sponges to allow 3D culture within nanokick bioreactor aimed at clinical application.

To study the gel phase, Stro1 selected human MSCs seeded in 1.8 mg/ml rat tail collagen type I hydrogels containing 0%,35%,58%,70% dry weight hydroxyapatite (HA) were prepared. The mean elastic moduli of the HA-gels were 180,194.6,204.8,182.8 Pa as measured by rheology. At 1000 Hz frequency of nanovibrational stimulation, gel displacement amplitudes were consistency measured by interferometry at ~90 nm. Alamar blue and live-dead stains showed that the HA gels and nanovibration had no effect on cell viability. After 9 days of stimulation, a trend of osteogenic gene up-regulation (RUNX2,osteonectin,osterix) was observed. After 7 days of stimulation, western blotting showed phosphoRUNX2 vs total RUNX2 up-regulation in nanovibrated MSC scaffolds. A metabolomic study after 1 week of nanostimulation demonstrated involvement of lipid metabolism (energy), predicted activation of ERK1/2 pathway and inflammatory metabolites highly suggestive that the nanovibration enhanced osteogenesis through natural bone healing pathways.

To study the biphasic scaffold, 5%freeze dried collagen sponges were produced and integrated into MSC seeded gels.The average elastic modulus of dry sponges were 137.3 MPa (SD=71.6) measured by compression test and SEM showed the average pore size was 227.7 mm (SD 72.9). Interferometry showed good fidelity of nanovibrational stimulation for the biphasic scaffold. After 7 days of stimulation,gels were allowed to contract onto sponges; this is to use the stimulated cells natural contraction to stiffen the construct. At day 9,microscopy showed MSCs migrating from the gel into the sponge.

Nanovibrational stimulation in HA-hydrogels is safe for cells and, with nanovibrational stimulation, promotes 3D osteoblastogenesis. Biphasic collagen scaffolds allowed nanovibrational force transmission and improved composite handleability for clinical use. Biological effects of nanovibrational stimulation in biphasic scaffolds will be presented.

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a92490

## 03-P090 Osteogenesis of embryonic stem cells by nanokicking

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*In vitro* differentiation of embryonic stem cells (ESCs) to the osteoblast lineage has been well established, typically using culture medium supplements (such as ascorbic acid, dexamethasone etc) to promote osteogenesis[1]. However, stimulation by nanoscale mechanotransduction using the 'Nanokick' bioreactor has been previously shown to promote osteogenesis of human mesenchymal stem cells[2]. 'Nanokicking' can generate reproducible osteoblasts without the need for expensive growth factors and supplements, allowing easier scale-up for commercial applications.

We show the application of nanovibrations promotes osteogenesis of embryonic stem cells without the need for traditional osteogenic medium supplements. Our results show nanokicking causes mineralisation and expression of osteogenic markers RUNX2 and osteocalcin in mESCs, at levels similar to calvarial osteoblasts (used as control). Interestingly nanokicking caused ESCs to form more numerous bone nodules with distinct morphology compared to those produced by osteogenic medium. The combination of nanokicking with standard osteogenic supplements also caused a synergistic increase in alkaline phosphatase activity, higher than either method alone.

Nanokicking also promotes chemotactic behaviour, a key function of osteoblasts during bone turnover and repair. As studied by an adherent chemotaxis model, osteoblast response to PDGF-BB (a known osteoblast chemoattractant[3]) is increased following Nanokick stimulation, revealing the influence of nanoscale mechanotransduction on the development of migratory behaviour of osteoblasts.

Nanovibrational osteogenesis provides a platform for inexpensive, reproducible osteoblast culture. The technique is scalable for regenerative medicine applications, using stem cell derived osteoblasts instead of patient derived cells and therefore reducing batch variability.

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a91327

## 03-P091 Maintaining zonal chondrocyte phenotype and overcoming chondrocytes de-differentiation in dynamic microcarrier culture

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The zonal property of articular cartilage endows the tissue with biphasic mechanical properties to withstand shearing force and compressional loading. Current treatments for articular cartilage damage are not able to efficiently repair the injured tissue with zonal organisation and functionality. Size-based sorting of freshly isolated chondrocytes from full thickness cartilage tissue using a spiral microfluidic chip was shown to efficiently separate and enrich superficial, middle and deep zone chondrocytes. However, zonal chondrocytes tend to lose phenotypic property during 2D expansion. This study aims to develop an expansion strategy that could maintain the zonal phenotype during chondrocyte propagation and enable zonal chondrocytes separation after expansion. Porcine articular cartilage chondrocytes were expanded on microcarrier in dynamic condition using spinner flask. Dynamic microcarrier expansion was able to support chondrocytes proliferation comparable to tissue culture plate (TCP) expansion. F-actin distribution analysis indicates similar chondrocyte morphology on microcarrier to that in mature articular cartilage. Cell size of the microcarrier expanded chondrocytes is tightly maintained, with average size similar to the freshly sorted chondrocytes, unlike drastically enlarged TCP expanded chondrocytes. The microcarrier expanded chondrocytes are then subjected to microfluidic separation for zonal chondrocytes subpopulations. Phenotypic characteristic of the separated zonal chondrocytes are confirmed via PRG4, aggrecan, Type II and IX Collagen gene expression level and further evaluated after *in vitro* cartilage reformation in 3D hydrogel, by gene expression, matrix formation and mechanical properties. The results demonstrated a propagation and separation protocol for zonal chondrocytes that is applicable to stratified zonal chondrocyte implantation for clinical repair of critical size articular cartilage defect.

a92207

## 03-P092 Buccal fat pad as a potential source of stem cells for bone regeneration: an *in vitro* study

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Adipose tissues hold great promise in bone tissue engineering since they are available in large quantities as a waste material. The buccal fat pad (BFP) is a specialized mass of adipose tissue that can be easily obtained via the oral cavity without injury to the external body surface. Another advantage of BFP over subcutaneous fat is that its size appears to be similar among different people, independent of body weight and fat distribution. However, limited studies have been conducted on the osteogenic capability of stem cells derived from BFP (BFPSCs).

In this study, the BFPSCs were characterized for their osteogenic differentiation potential especially in contact with a synthetic scaffold in a perfusion bioreactor. The features of BFPSCs were compared with bone marrow-derived stem cells (BMSCs) as a well known cell source for bone tissue engineering.

Comparing BFPSCs with BMSCs indicated similar morphology, but faster proliferation rate of BMSCs. Moreover, when properly induced for two weeks, BFPSCs resembled BMSCs in the production of bone-specific markers, such as alkaline phosphatase, collagen, bone morphogenic protein (BMP), Runx2, and osteocalcin. Both cell types attached nicely to the pores of a gelatin-coated $\beta$-Tricalcium phosphate scaffolds. More osteogenic differentiation potential was observed for both cells under dynamic culture in a perfusion bioreactor compared with static culture. The highest collagen content and BMP production were observed in BFPSCs cultured in the bioreactor for two weeks. These results define BFP as a new, rich, and accessible source of stem cells for tissue engineering purposes.

a90841

## 03-P093 A Novel Dynamic in Vitro 2.5D Cell Culture Model for Bone Tissue Engineering

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### Introduction

Physiological fluid shear stress (FSS) in bone canaliculi in vivo ranges from 0.8 to 3 Pa. This FSS level has been widely used in 2D in vitro studies in bone tissue engineering, using a parallel-plate flow chamber (PFC). However, during a dynamic 3D culture in vitro, FSS inside a tissue engineered construct is typically less than 0.3 Pa; and cellular response at this FSS range is little understood. In this study, we created a novel 2.5D in vitro cell culture model to study the behavior of cells inside a scaffold-like micro-environment. Cellular behavior of mesenchymal stem cells (MSCs) in the 2.5D model were studied and compared to conventional 2D PFC model. Finite element analysis (FEA) was used to simulate the fluid flow around the 3D cell shape reconstructed from the in vitro experiment.

### Experimental

Human bone marrow derived MSCs were seeded in the borosilicate glass capillary tube or glass bottom PFC and incubated overnight for attachment. Osteogenic medium was then perfused through the tube or PFC at respective 0.4 or 2 ml/min for 7 days (d7per), inducing ~0.2 Pa FSS to both bioreactors. Cells after attachment (d0) or under static culture for 7 days (d7sta) were also studied. Live dead cell assay and phalloidin actin stain were used. CellProfiler was used to characterize cell viability, size, aspect ratio, orientation and actin arrangement. 3D cell shape was obtained from confocal microscope and advanced imaging analysis method. FEA COMSOL Multiphysics was used to study the fluid flow around the 3D cell shape.

### Results and Discussion

The fractions of viable cells inside the 2.5D and 2D model were greater than 80% and 70%, respectively. In both models, compared to d0, d7sta MSCs became more elongated whereas d7per cells were bigger and more polygonal. D7per cells in 2.5D model orientated perpendicular to the flow. Actin filaments were more bundled in the 2D model than those in 2.5D model in d7sta. There was a significant difference in 3D cell shape between 2.5D and 2D model, leading to different flow field around the cells. In this study, cells in the 2.5D model showed a different behavior compared to conventional 2D in vitro model at a less-known FSS level. FEA showed that the fluid flow at the cellular level was different in the two models. This study can benefit tissue engineers using perfusion bioreactors to understand how the fluid flow conditions affect cells locally.

### Acknowledgements

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a91015

## 03-P094 A novel electron emission-based cell culture device promotes cell proliferation and differentiation of pre-osteoblastic MC3T3-E1cells

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This is the first report to demonstrate the effect of our novel electron emission-based cell culture device developed by SHARP CORPORATION on the proliferation and differentiation of cell lines such as osteoblast MC3T3-E1. The device has an electron emission element with the feature of having a heterogeneous semi-conductive layer made of insulating materials and conductive nanoparticles, being able to emit electrons stably in the atmosphere[1]. Electrons in the atmosphere generate various radicals and negative ions by the reaction with gas molecules, which can induce variety of biochemical reactions in our cell culturing system. Electron energy can be modulated by element drive voltage and kind of reaction products can be controlled by purpose without specific gas such as He, $CO_2$, and $N_2$. In addition, because the applied voltage is relatively low (about 20V), our device doesn't make large strong space field nor induce extensive ionization or dissociation of gas molecule in comparison with analogous art such as discharge, or plasma irradiation. In this study, we used this new electron emission-based cell culture device to demonstrate that our system can affect cell proliferation and differentiation of pre-osteoblastic MC3T3-E1 cells. Electron emission stimulation (EES) was directly provided to a culture medium containing plated cells for a few minutes, then the number of living cells and mRNA levels of osteosis-related genes were evaluated after treatment with EES for a few days. The number of EES-exposed cells increased about 20% on an average, compared with that observed with the unexposed control cells. We also found that EES could up-regulate the mRNA levels of osteogenic specific genes such as COL1, Cbfa1, and OCN. These results suggest that our new electron emission-based cell culture device, which provides relatively weak stimulus, could promote cell proliferation and differentiation probably through combination of electrical stimulation and reaction products generated by electron emission, and would be expected to application to regenerative medicine, such as bone regeneration.

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a91060

## 03-P095 Co-culture of Umbilical Cord derived Mesenchymal Stem Cells (UCMSCs) and Bone Marrow derived Mesenchymal Stem Cells (BMMSCs) /Chondrocytes improved the Osteogenic/Chondrogenic differentiation

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**Introduction:** UCMSCs are believed to have intermediate features with embryonic stem cells and adult stem cells. Thus, UCMSCs are probably more primordial, so some environmental condition may influenced to differentiation. With this in mind, the objective of this study was to assess whether co-culture of UCMSCs and BMMSCs/chondrocytes influences the osteogenesis/chondrogenesis.

**Materials and methods**: The umbilical stromal tissue were cut into about 5-mm sections. Then, due to obtain UCMSCs, these were incubated and the second passage cells were applied for the experiments. A collagen sponge was used as a scaffold. The specimens, which 1 × 10 5 of UCMSCs were seeded and were treated single culture, were used as control. The co-culture was carried out in direct and indirect type. Control were induced osteogenic/chondrogenic differentiation. Specimens co-cultured with BMMSCs were induced osteogenic differentiation. Specimens co-cultured with chondrocytes were induced chondrogenic differentiation. After 5 weeks, each specimen were evaluated histologically. In addition, UCMSCs which were labeled and used for direct co-culture were observed with a fluorescence microscope.

**Results:** In the osteogenic differentiation, the specimens were stained by alizarin red staining, but no obvious osteoblast-like cells were observed in the indirect and direct co-culture. On the other hand, controls were not stained by alizarin red.In chondrogenic differentiation, cartilage-like tissues were observed both in indirect and direct co-culture, also in controls. UCMSCs which were labeled were scarce in cartilage-like tissues of direct co-cultured specimens.

**Discussion:** The above results lead us to consider that UCMSCs were more easily differentiate into chondrocytes. The histological assessments suggest that UCMSCs were enhanced chondrogenesis with chondrocytes, and were improved osteogenesis with BMMSCs. UCMSCs were poor in cartilage-like tissues of direct co-cultured specimens. Thus the need for investigate a co-culture method suitable for the purpose was considered.

**Conclusions:** The utility of co-culture of UCMSCs and BMMSCs in osteogenic differentiation induction could be expected.

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a92095

## 03-P096 Identifying metabolic alterations during chondrogenic differentiation of periosteal cells cultured in spheroids

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The use of cartilage intermediate templates via endochondral ossification is a promising tissue engineering (TE) strategy for the healing of large defects. Recently, the use of 3D cell microaggregates has been introduced, as this format mimics the in vivo environment allowing cell-cell and cell-extracellular matrix interactions. Considering the role of metabolism as a key regulator of stem cell fate and the inherent capability of metabolomics to provide a high resolution and quantitative view of metabolic networks, this study aims to find critical biomarkers indicative of a functional cartilage intermediate TE construct.

In this study, we conducted LC-MS (liquid chromatography-mass spectrometry) tracer analysis experiments to gain mechanistic insight during chondrogenic differentiation of spheroids of hPDCs (human periosteum derived stem cells). $^{13}C$ labeled glucose, glutamine but also serine and aspartate have been used, as these metabolites showed significant differences between the time points of interest in a prior exometabolomics study. Cell suspensions were drop seeded in 24 wells containing molded microwells at the bottom, resulting in the formation of spheroids containing 250 cells each.Twice a week 50% of the medium was refreshed. Samples were analyzed at day 0, day 14 and day 21. These time points capture the transition of hPDCs-derived chondrocyte phenotypes from proliferating to prehypertrophic and finally hypertrophic state, based on gene expression of specific markers. Total DNA content was used to normalize the data.

Our tracer analysis results showed progressive $^{13}C$ glucose enrichment in palmitate from 0% at day 0 to 8% at day 14 and 22% at day 21, suggesting activation of fatty acid synthesis. Furthermore, we observed $^{13}C$ glutamine enrichment in proline from 0 % at day 0 to 20 % at day 14 and 42,5 % at day 21 and a similar trend of $^{13}C$ glutamine contribution to hydroxyproline (from 0 % at day 0 to 35 % at day 14 and 38 % at day 21).These data indicate that glutamine contributes progressively more to proline biosynthesis, an important amino acid for collagen production. In agreement, we observed significant production of GAG (glucosaminoglycans) rich extracellular matrix (ECM) after Day 7. These observations suggest that the consecutive stages of chondrogenic differentiation of hPDCs are characterized by metabolic adaptations.

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a91328

## 03-P097 Nondestructive Raman Spectroscopy-Based Quality Controls for Tissue Engineered Cartilage

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INTRODUCTION

We are manufacturing engineered cartilage grafts to treat articular cartilage defects using autologous nasal chondrocytes in an ongoing phase II clinical trial. Current methods to assess the quality of native and engineered tissues are destructive, eliminating the possibility to continuously monitor graft quality. Additionally, these methods only allow for the assessment of a small amount of sample, which may not be representative of the overall quality. Raman spectroscopy, a method that measures chemical compositions, has the potential to nondestructively and more comprehensively characterize tissue quality from the initial biopsy and throughout the cartilage tissue engineering process.

METHODS

Nasal cartilage biopsies were measured with Raman spectroscopy. Nasal chondrocytes were isolated from the biopsies, expanded, seeded onto 3D collagen scaffolds, and cultured for up to 2 weeks in chondrogenic conditions. Raman spectra of the engineered cartilage were measured after 0, 1, and 2 weeks of maturation.

RESULTS

Raman spectra peaks and ranges were associated with DNA, proteins, and lipids. The intensity of the Raman spectral peak associated with glycosaminoglycans (1061 $cm^{-1}$) generally increased with culture time and related to the amounts of glycosaminoglycans determined by destructive biochemical methods. Principal component analysis of the spectral fingerprint regions (Raman shift of 600-1800 $cm^{-1}$) revealed differences between high and low quality native tissue biopsies. Moreover, Raman fingerprint spectra from high quality engineered cartilage were more similar to native tissue than from low quality grafts.

DISCUSSION & CONCLUSIONS

Raman spectroscopy has the potential to nondestructively assess the quality of native cartilage biopsies and of engineered cartilage during the process of maturation. Establishing Raman spectroscopy-based quality controls for the manufacturing of grafts for clinical applications would ensure product quality and facilitate regulatory compliance.

a91847

## 03-P098 Characterization and application of size-based spiral microchannel sorted zonal chondrocytes for articular cartilage regeneration

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Current approaches for articular cartilage repair to restore their hierarchically organized architecture, and thus the full mechanical function and durability, by implanting zonal chondrocyte subpopulations in multilayer construct has not yet been realized due to the lack of efficient and specific zonal chondrocyte isolation protocols to yield adequate viable zonal cells. This study aims at developing a highly efficient zonal chondrocyte separation approach based on cell size for the stratified zonal chondrocyte implantation. Full thickness chondrocytes derived from porcine femoral condyle cartilage was subjected to serial spiral microchannel sorting to segregate zonal chondrocytes based on cell size. The size-sorted cells show zone-specific characteristics in RT-PCR analysis of PRG4, aggrecan, Type II and IX collagen. Both freshly sorted and two-passage expanded zonal chondrocytes form cartilage tissue in three-dimensional hydrogel *in vitro* bearing respective zonal characteristics, indicated by RT-PCR, histology, quantification of the extracellular matrix proteins, and mechanical compression test. Lastly, when delivered in a bi-layered stratified hydrogel construct, the sorted zonal chondrocytes facilitate better cartilage repair with mechanically enhanced cartilage tissue in rat chondral defect model, in comparison to conventional chondrocytes implantation. Our study demonstrates that, using a spiral microchannel device, the superficial, middle and deep zone chondrocytes can be separated and enriched from full thickness cartilage in a high-throughput, un-labelled manner based on cell size. This study provides an effective approach to obtain zonal chondrocytes in large number, and demonstrate the translational potential of stratified zonal chondrocyte implantation for clinical repair of critical size cartilage defects.

a90353

## 03-P099 Development study for quality assessment of cell therapy including scaffold

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Recently, there has been a rapid increase in the development of a complex product, which is a cell therapy agent containing biocompatible scaffold, by utilizing tissue engineering and advanced biotechnology. This study aimed to effectively separate and culture chondrocytes using abandoned cartilage tissue during nasal surgery for the treatment of nasal congestion related nasal septum. Then, this study aimed to conduct a small scale manufacturing with chondrocyte and scaffolds, characterize the complex products, and develop the scientific evaluation methods for stability, potency, and safety as cell therapeutic agents.Chondrocytes were effectively isolated from the discarded cartilage tissue during the operation of rhinitis. We established laboratory-level protocols for cell isolation, culture, and mass production and verified the cell production protocol with the quality evaluation analysis (endotoxin, mycoplasma, aseptic test, etc.). For the small scale manufacturing of chondrocyte therapeutic agent containing the scaffold, we blended the chondrocyte with the clinically applicable collagen and producted the complex cell therapeutic agents. Then, as a part of the in vitro performance, the survival and death of the cells were investigated for quality (stability) test of the product. Stability of the cells was assessed by staining and survival analysis. As a result, it was confirmed that the cells were uniformly distributed within the supporter and maintained a high survival rate of about 60% within 24 hours. For the potency of the complex agent, the characteristics of chondrocytes were evaluated by immunostaining for chondrocyte-specific extracellular matrix proteins (SOX9, Aggrecan, Col2A). As a result, the expression level of extracellular matrix proteins is over 90%. For the safety of the complex agent, the microbiological safety of the cells in the scaffold was partially confirmed through the mycoplasma negativity test (PCR) of the cells combined with the scaffold. This suggests the standard of quality evaluation of products after the practical small-scale manufacture of complex cell therapy agents, the future new industry development drive. It can be applied to preemptive standardization including complex cell treatment agent. It would be possible to contribute to the establishment of the safety of medicines through standardization.

a91044

## 03-P100 Identification of altered gene expression in the expansion culture of polydactyly-derived chondrocytes

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Purpose: Efficacy of autologous chondrocyte sheet transplantation in the treatment of cartilage defects associated with osteoarthritis (OA) was studied in eight patients with OA of the knee. Successful regeneration of articular cartilage was verified in all cases without any serious adverse events. To establish an alternative cell source for chondrocyte sheets, we have investigated the use of allogeneic chondrocytes obtained from the cartilaginous tissue within surgical remains of juvenile polydactyly patients. Polydactyly-derived chondrocytes (PD cells) have excellent proliferation ability and can be expanded for more than 12 passages. However, applicability of expanded PD cells for chondrocyte sheets has not yet been verified. In this study, we conducted a comparative microarray analysis between PD cells at an early passage that are currently used in our second clinical study and their expanded cells to predict the effect of expansion culture on the quality of PD cells.

Methods: Experiments were performed under the approval and guidance of the Clinical Research Review Committee of Tokai University School of Medicine. Informed consents were obtained in all cases. Cartilaginous tissue from juvenile polydactyly patients (n = 3; age = 8-15 mo; avg = 11.7 mo) were minced and digested in collagenase, and the isolated cells were seeded to culture dishes and cryopreserved when subconfluent as Passage 1 (P1) cells. P1 cells were then seeded and expanded on culture dishes and subsequently passaged when subconfluent. RNAs extracted from P2 cells and P6 cells from each patient were analyzed by Agilent SurePrint G3 Human GE v3 8x60K Microarray to map RNA expressions. Agilent GeneSpring was used to analyze RNAs with changes in signal intensity of greater than 2 or 5 fold (paired *t*-test, $P < 0.05$).

Results: RNA array data showed significant differences (fold change>2) in gene expression through expansion culture in 1% of all probes. We identified 7 genes of increased expression and 18 genes of decreased expression in P6 cells by more than 5 fold.

Discussion: Expansion culture of PD cells caused changes in gene expression including known transcription factors. The identified genes could be involved in property alteration of expanded PD cells and might affect the in vivo efficacy of chondrocyte sheets created from PD cells. These genes may be useful as indicators of cell source quality when expansion culture is applied to PD cells.

a91945

## 03-P101 Best fraction of isolated pig immature alveolar osteoblasts for use in periodontal bone defect regeneration therapy

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**Introduction:** Establishment of human osteoblast cultures that retain bone-forming capacity is one of the prerequisite for successful bone regeneration therapy. Osteoblasts harvested from adult's exhibit limited growth, thus the use of immature osteoblasts that can expand ex vivo should greatly facilitate bone regeneration therapy. We have previously reported that immature human osteoblasts can be isolated from human alveolar bone (HAOBs). In this study we isolated immature osteoblast from alveolar bone of micro-mini pig (PAOB) and determined best fraction in terms of growth and expansion of cells and gene expression ability to be used in developing a preclinical study for establishing transplantation protocol.

**Method:** Alveolar bone of micro-mini pig was sequentially digested using 2% collagenase solution to isolate immature alveolar osteoblast. 12 population fractions were obtained with the first 3 populations being discarded. The remaining 9 fractions were then incubated with MF Start medium and on reaching ~80% confluence were passaged and these cells were maintained as PAOB. Each fraction was analyzed for osteogenic differentiation ability by treatment with osteogenic differentiation medium for an incubation period of 10days. Mineralization ability and osteogenic differentiation ability was investigated by alizarin red and alkaline phosphatase activity. Analysis of mRNA expression of osteogenic differentiation marker genes such as osterix, osteocalcin, RUNX2, osteopontin and Type I collagen was subsequently done by performing real time Q-PCR.

**Result:** PAOB was successfully isolated from pig alveolar bone which exhibited matrix mineralization, alkaline phosphatase activity and expressed osteogenic marker genes such as osterix, osteocalcin, osteopontin, RUNX2 and Type 1 collagen after treatment with osteogenic differentiation medium. All fractions obtained by collagenase digestion showed similar osteogenic capability with no marked differences in mineralization ability, alkaline phosphatase activity and osteogenic gene expression.

**Conclusion:** PAOB isolated from pig alveolar bone showed osteogenic capability and thus are useful source for cell transplantation in a pig experimental model as a pre-clinical study for periodontal bone defect regenerative therapy.

a93074

## 03-P102 Down-Regulation of Transglutaminase 2 Stimulates Redifferentiation of Dedifferentiated Chondrocytes through Enhancing Glucose Metabolism

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Expansion of chondrocytes for repair of articular cartilage can lead to dedifferentiation, making it difficult to obtain a sufficient quantity of chondrocytes. Although previous studies have suggested that culture in a three-dimensional environment induces redifferentiation of dedifferentiated chondrocytes, its underlying mechanisms are still poorly understood in terms of metabolism compared with a two-dimensional environment. In this study, we demonstrate that attenuation of transglutaminase 2 (TG2), a multifunctional enzyme, stimulates redifferentiation of dedifferentiated chondrocytes. Fibroblast-like morphological changes increased as TG2 expression increased in passage-dependent manner. When dedifferentiated chondrocytes were cultured in a pellet culture system, TG2 expression was reduced and glycolytic enzyme expression up-regulated. Previous studies demonstrated that TG2 influences energy metabolism, and impaired glycolytic metabolism causes chondrocyte dedifferentiation. Interestingly, TG2 knockdown improved chondrogenic gene expression, glycolytic enzyme expression, and lactate production in a monolayer culture system. Taken together, down-regulation of TG2 is involved in redifferentiaton of dedifferentiated chondrocytes through enhancing glucose metabolism.

a92005

## 03-P103 Microarray Gene Expression Analysis of Polydactyly-Derived Chondrocyte Sheets and Adult Chondrocyte Sheets

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Objective: We previously evaluated in a clinical study the safety of autologous chondrocyte sheets for the treatment of cartilage defects associated with osteoarthritis (OA) and are currently conducting a second clinical study to evaluate the safety of allogeneic chondrocyte sheets. As an alternative to autologous chondrocytes, we have used allogeneic chondrocytes obtained from surgical remains of juvenile polydactyly patients to fabricate polydactyly-derived chondrocyte sheets (PD sheets). Through xenogeneic transplantation, we previously demonstrated that the efficacy of PD sheets was comparable to, if not better than, that of adult chondrocyte sheets created from the surgical remains of total knee arthroplasty (TKA sheets). In this study, we performed a microarray gene expression analysis to compare PD sheets and TKA sheets.

Methods: All experiments were conducted with the approval of the Tokai University Ethics Committee and patients' informed consent. Chondrocytes were isolated from cartilage tissue obtained from three polydactyly and six TKA patients. PD sheets were created as previously described (Maehara et al., 2017). TKA sheets were also created as previously described (Kokubo et al., 2016). Following fabrication, total RNA was extracted and then analyzed using Agilent SurePrint G3 Human GE v3 8x60K Microarray. Agilent GeneSpring software was used for gene level normalization, and differentially expressed genes (*t*-test; $q<0.05$;>2-fold change) were identified. Hierarchical clustering analysis was performed using R. Functional enrichment for GO functional annotations, KEGG pathways, and InterPro protein domains was performed using the STRING database.

Results: 1681 probes were identified as differentially expressed. 845 probes were upregulated and 836 downregulated. GO analysis identified cellular component organization and mitotic cell cycle under Biological Process, macromolecular complex binding and extracellular matrix binding under Molecular Function, and chromosomal region under Cellular Component among the highly enriched. Focal adhesion and cell cycle was highly enriched among KEGG pathways.

Discussion: GO analysis confirmed the juvenile nature of PD sheets, as genes associated with cell proliferation and cytoskeletal rearrangement were highly enriched. CILP2, COMP, DKK1, TIMP2, and TIMP3 were also highly expressed in PD sheets and are known to be important in cartilage homeostasis. Further analysis may reveal the advantages of PD sheets.

a91801

## 03-P104 Challenges of Commercializing Autologous Chondrocyte Sheets Used in Regenerative Medicine

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Purpose: Cell-based products require various manufacturing related materials such as animal-derived enzymes and cell culture media. Such raw materials pose various risks including microbiological contamination, and to reduce such risks, it is important to use GMP-grade, not research-grade, materials for commercialization. Recently, some industries began offering products tailored to regenerative medicine with traceability documents including certificates of analyses and origins. To replace a conventionally-used enzyme reagent with a GMP-grade product, in this study we evaluated a GMP-grade enzyme in our routine process of producing adult chondrocyte sheets. Furthermore, after fabrication of adult chondrocyte sheets, we also investigated different media for transportation purposes.

Methods: All experiments were conducted under the approval of the Clinical Research Committee at Tokai University School of Medicine, and with patients' informed consent. Cartilage tissue was obtained from surgical remains of patients who underwent total knee arthroplasty (TKA). The tissue was minced and digested by a conventional enzyme or a GMP-grade enzyme. Isolated chondrocytes were then seeded to temperature-responsive cell cultureware (UpCell®, CellSeed Inc.). TKA sheets fabricated by those enzymes were evaluated for the number of viable cells and viability. Cell surface markers were also examined by flow cytometric analysis. To determine the optimum transport medium, TKA sheets were cultured for several days in candidates of transport media or basic culture medium. TKA sheets were again evaluated for the number of viable cells.

Results: The number of viable cells was slightly higher in TKA sheets derived from the conventional enzyme than those from the GMP-grade enzyme. On the other hand, cell viability was greater than 90% in both cases. No differences in cell surface markers were identified. Significant differences were observed for the number of viable cells for TKA sheets evaluated in different transport media.

Discussion: For clinical applications in commercialization, we confirmed that the GMP-grade enzyme can be used safely and effectively to isolate chondrocytes from cartilage tissue. The optimal candidates of transport media were narrowed down according to the number of viable cells under various conditions. We will finalize the optimal transport media and conditions using sheets created in our cell processing center.

a90922

## 03-P105 Bone Tissue Engineering using a Novel Multi-Layered Cell Sheet Technology

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**Introduction:** There is a major need for bone tissue engineering. In the UK alone, osteoporotic fractures are predicted to cost £2.2+ billion by 2020 [1]. This project aims to investigate the potential of using multi-layered cell sheet (MLCS) technology and stem cell therapy to improve the efficacy of bone tissue engineering. MLCS technology is a highly promising approach to utilise intact stem cell sheets (that retain maximum cell numbers with cell-cell junctions and extracellular matrix including some growth factors) for use as a 3D engineered tissue graft for clinical treatment.

**Methods:** Human dental pulp stem cells (DPSCs) were characterised, then cultured for 1-3 weeks at different densities in temperature-responsive dishes to form monolayer cell sheets. MLCS (3-6 layers) were assembled using various stacking techniques before further culture in osteogenic medium and subsequent analysis for cell viability, morphology and osteogenic differentiation using fluorescence microscopy, SEM, histology and immunohistochemistry.

**Results:** 1) Live/dead labelling revealed good cell viability is maintained within MLCS after 3-6 weeks of *in vitro* culture. 2) Cells within the monolayers maintained their osteogenic phenotype which was confirmed by alkaline phosphatase (ALP) staining and ALP specific activity. 3) Increasing cell density and culture duration in osteogenic medium enhanced the thickness of the monolayers, which consequently increased assembled MLCS thickness. MLCS structure was confirmed by SEM and histology; the test group formed thicker tissues and stained stronger positive for collagen type I and calcium deposition.

**Conclusion:** These results demonstrated the potential of using osteogenic monolayer cell sheets to assemble MLCS *in vitro*. These are predicted to match or comparable to the performance of autologous tissue grafts, which makes a successful cell-based therapy for bone tissue engineering applications more beneficial.

**References:**

[1] Burge, R.T. et al. Journal of Medical Economics. 4(1-4), 51-62, 2001.

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a93071

## 03-P106 Glycosaminoglycan biomimicry enhances human mesenchymal stem cell repair of osteochondral defects

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Mesenchymal stem cells (MSCs) offer significant potential to patients suffering articular cartilage damage. However, their low number in bone marrow necessitates further culture-expansion prior to therapeutic use. Such practices often result in a loss of stem cell characteristics and poor outcomes for patients. Endogenous FGF2 is critical to the expansion and naivety of such isolated cells. We show that endogenous signaling through an FGF2-mediated pathway can be sustained through culture supplementation with heparan sugars with increased affinity for FGF2 (termed HS8). HS8 helps create a gradient of FGF2 that provides signals to more distant MSCs, and, when these cells were serially-passaged under HS8 supplementation ($MSC^{HS8}$), a 2-3-fold increase in the number of population doublings was achieved compared with control conditions ($MSC^{control}$). Moreover, despite an increase in the number of divisions, $MSC^{HS8}$ cultures showed no reduction in telomere length, colony-forming efficiency, biomarker expression or tri-lineage potential. Notably, $MSC^{HS8}$ cultures had increased STRO-1 expression (~ 60%) compared to $MSC^{control}$. Therapeutically, $MSC^{HS8}$ in a fibrin gel applied to osteochondral defects in the femoral trochlear groove of NIH nude rats resulted in ~60% of rat defects achieving normal or nearly normal ICRS I scores and > 70% of defects with high ICRS II, and O'Driscoll scores. In comparison, treatment with $MSC^{control}$ in a fibrin gel resulted in ~50% of defects having high ICRS II and O'Driscoll scores. Interestingly, defects left untreated and those treated with fibrin gel alone had no (0%) defects with high scores. Similar improvements in osteochondral repair following treatment with $MSC^{HS8}$ compared with $MSC^{control}$ was also observed in micropigs models, with $MSC^{HS8}$ showing significant increases in ICRS I, ICRS II and O'Driscoll scores. Magnetic Resonance Imaging confirmed that $MSC^{HS8}$-treated micropig defects had reduced osteochondral lesioning and Instron testing showed enhanced biomechanical properties compared with $MSC^{control}$. These data highlight the critical role that heparan glycosaminoglycans play in the growth factor-mediated bioprocessing of stem cells. Furthermore, this study strongly advocates for the further development of affinity-isolated sugars capable of mimicking cellular microenvironments as a strategy for clinical regeneration.

a91555

## 03-P107 The role of exogenous haematopoietic progenitor cells in osteochondral repair following microfracture in a large animal model

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University of Cambridge

**Introduction** Cartilage defects are a key cause of Osteoarthritis. These present a particular clinical challenge as hyaline cartilage has a poor self-repair capacity. Microfracture is a commonly performed surgical procedure to promote articular cartilage healing by permitting bone marrow derived mesenchymal stem and progenitor cells into the chondral defect site. However, fibrocartilage is often produced. One goal of improving the efficacy of microfracture is to modify the repair tissue produced and the use of autologous peripheral blood cells (PBC) to successfully augment microfracture has also been described. However, in the clinical studies described, the PBC were derived using G-CSF mobilisation and apheresis and recent studies in small animals has suggested that G-CSF alone may have a direct effect on cartilage repair, indicating that these factors may be playing a significant role in the therapeutic response.

The aim of this study was to use G-CSF stimulation and apheresis derived autologous PBC in an ovine model of cartilage defects treated with microfracture to characterise the cell types mobilised during G-CSF treatment and to analyse the effects of G-CSF on cartilage healing.

**Methods** 8mm cartilage defects were created in the medial femoral condyle and treated with micro fracture, under regulatory approval, in forty adult female sheep. Following G-CSF stimulation, PBC were collected using apheresis and the CD34 values measured by flow cytometry. PBC plus hyaluronic acid were injected intra-particularly in 20 sheep and animals sacrificed at 2,4,8 and 26w post surgery. Weight bearing on the operated limb was measured monthly. Post-mortem cartilage defects were scored macroscopically using the ICRS grading system and histologically using the modified O'Driscoll score.

**Results** At 6months, animals that had received PBC/HA had a significantly higher ICRS macroscopic scoring and modified O'Driscoll score than control animals. When the cellular responses to G-CSF before and after apheresis were evaluated, the data suggested that the response to G-CSF (as measured by increase in white blood cells relative to before G-CSF administration) was correlated to cartilage defect healing, rather than any apheretic product (e.g. monocyte number, CD34+ number).

**Discussion** In this large animal model, the use of G-CSF promoted healing of a cartilage defect treated by micro fracture, rather than the use of PBC, suggesting that G-CSF could be used therapeutically.

a92308

## 03-P108 Endochondral Bone Tissue Engineering Using Chondrogenically Differentiated Human Induced Pluripotent Stem Cells

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**Introduction**

There has been great interest in the use of induced pluripotent stem cells (iPSCs) in bone regenerative strategies for nonunion and bone defects. In the present study, we investigated whether implantation of chondrogenically differentiated iPSC-derived mesenchymal stem cells (MSCs) could lead to successful bone regeneration of bone defects in nude mice.

**Methods**

Human iPSCs (201B7 and 454E2; Riken BRC) were used. After generation of undifferentiated iPSCs into MSCs, chondrogenic differentiation was performed by three-dimensional pellet culture. Twenty-eight male BALB/cAJcl-nu/nu mice were used. A 2-mm defect was created at the middle-third of the radius of each mouse with scissors. One or two chodrogenically differentiated iPSC-derived MSC pellets were placed in the defect. For control animals, saline was implanted as a sham treatment. For radiographic assessment of bone regeneration, micro-computed tomography ($\mu$-CT) imaging analysis was performed at week 8 after transplantation. For assessment of gene expression at bone defect sites, animals from each group were euthanized at weeks 2. The newly generated tissues were harvested, and total RNA was extracted. Expression of human and mouse vascular endothelial growth factor (VEGF) and osteocalcin (OC) was analyzed by reverse transcription-polymerase chain reaction (RT-PCR).

**Results**

For the rate of bone regeneration assessed by $\mu$-CT at week 8, 11 out of 11 (100%) radius in the 201B7 cells derived-pellet transplantation group achieved bone healing, and 7 out of 10 (70%) radius in the 454E2 cells derived-pellet transplantation group achieved bone healing. 2 out of 11 radius (18%) achieved bone healing in the control group. The bone regeneration rates in the treated groups were significantly higher than that in control group ($p < 0.05$). RT-PCR revealed positive expression of human VEGF, mouse VEGF, and mouse OC at the grafted bone defect sites, whereas expression of human OC was not detected at the defect sites.

**Discussion**

Recently, there have been increasing interest in regenerating bone through a cartilage-mediated process similar to endochondral bone ossification. Our results showed for the first time that chondrogenically differentiated iPSC-derived MSCs were able to repair bone defects through endochondoral bone ossification. Our findings provide insights on the development of an effective technology for bone regeneration using iPSCs.

a92602

## 03-P109 Repair of focal articular cartilage defect with human iPSC-derived cartilage in a mini-pig model treated with immunosuppressor

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Articular cartilage locates in the end of bone and provides lubrication to diarthrodial joints. Articular cartilage has limited capacity for repair, and its damage seldom heals, eventually leading to osteoarthritis. Focal defects in articular cartilage are typically treated with cell transplantation, however, there is a need for better sources of chondrocytes or cartilage for the transplant. Using human induced pluripotent stem cells (iPSCs), we have established protocols for the generation of stable cartilage *in vitro*. In this study, we investigated the efficacy of hiPSC-derived cartilage for the repair of focal articular cartilage defects in mini-pigs as a large animal transplantation model.

We used 6-10 months-old male mini-pigs weighing 20.6–30.5 kg. We created defects of 2 mm diameter and 1.5 mm depth using a biopsy punch at the femoral groove and transplanted hiPSC-derived cartilaginous particles into the defects. The mini-pigs were treated with a combination of immunosuppressive drugs that included Tacrolimus, Mycophenolate mofetil and Prednisolone, and sacrificed one month later for histological analysis.

Immunohistochemistry showed that chondrocytes in the cartilage filling the defect expressed human VIMENTIN, suggesting that the progeny of hiPSC-derived cartilaginous particles survived and repaired the defects. Histological analysis showed that the particles began to integrate with the host articular cartilage at the sides and subchondral bones at the bottom. Tumor formation was not observed.

These results suggest that hiPSC-derived cartilage can be a potentially viable cell source for the treatment of focal articular cartilage defects. This study represents an important step toward translating hiPSC-derived cartilage to the clinical setting.

a91311

## 03-P110 Osteoclastic Initiation of Osteogenic Differentiation and Bone Formation

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While most research on cell therapy for bone regeneration is focused on (progenitor) cells that can form bone (e.g. mesenchymal stromal cells, MSCs), we have actively explored the role of inflammatory cells and their successors in the initiation of bone formation. In that perspective, we cultured macrophage-derived osteoclasts on surfaces with different roughness and used conditioned medium thereof to evaluate effects on the osteogenic differentiation of MSCs. Alternatively, we prepared osteoclast-based constructs and evaluated their bone forming capacity using an ectopic implantation model in mice.

The culture of osteoclast on surfaces with different roughness showed to result in distinct differences in osteoclast characteristics, including osteoclast size, number, and nuclearity, with increasing surface roughness leading to the formation of smaller osteoclasts, lower osteoclast density, and less nuclei per osteoclast. Further, differential osteogenic differentiation effects on MSCs were observed upon their culture in conditioned medium showing significantly increased mineralization for conditioned medium derived from osteoclasts cultured on relatively smooth surfaces. A clear correlation between osteoclast nuclearity and the osteogenic differentiation capacity of conditioned medium was found.

Osteoclast-based constructs were prepared by inducing osteoclastogenesis in macrophage cultures on BioOss, while BMP-2 loaded and cell-free constructs served as positive and negative controls, respectively. Upon ectopic implantation in subcutaneous pockets for 6 weeks, the observed incidence of bone formation for osteoclast-based constructs was 100% (11 out of 11 constructs). Similarly, positive controls showed a 100% incidence of bone formation. Negative controls did not show bone formation. In addition to bone formation, both osteoclast-based constructs and positive controls showed osteoclastic activity by positive histological staining for tartrate-resistant acid phosphatase (TRAP).

With these results, we here demonstrate that osteoclasts are key players in the initiation of osteogenic differentiation and bone formation. As such, this knowledge will propel translational efforts to improve bone formation using osteoclasts as key cellular elements.

a93187

## 03-P111 Translation of Remote Control Regenerative Technologies for Bone Repair

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The role of mechanotransduction in normal bone homeostasis and repair is understood to facilitate effective osteogenesis of mesenchymal stem cells (MSCs) *in vitro*. Mechanotransduction has been integrated into a multitude of *in vitro* bone tissue engineering strategies and provides an effective means of controlling cell behaviour towards therapeutic outcomes. However, the delivery of mechanical stimuli to MSC populations post implantation poses a significant translational hurdle. To tackle this, we have developed a novel bio-magnetic strategy, MICA, where magnetic nanoparticles (MNPs) are used to remotely deliver mechanical stimuli to the cell-surface mechanoreceptor, TREK-1, resulting in activation and downstream signalling via an external magnetic array. In this way, we can deliver functional mechanical stimuli to therapeutic cell populations *in vivo* following injection. Here, we describe the preclinical translation of MICA for the treatment of bone injury in an ovine model.

Methodology involved the delivery of autologous STRO-4 positive MSCs to a bone injury in the femoral condyle of sheep. Implanted cells were stimulated for 13 weeks by custom built magnetic array, bone growth assessed by $\mu$CT and validated histologically. We describe the development of a magnetic array capable of *in vivo* MNP manipulation and subsequent osteogenesis *in vitro*. We further demonstrate that viability of MICA-activated MSCs *in vivo* is unaffected 48hrs post delivery. We present evidence to support early accelerated repair and preliminary enhanced bone growth in MICA-activated defects within individuals compared to internal controls. The variability in donor responses to MICA-activation *in vitro* revealed that donors with poor osteogenic potential were most improved by MICA. We present a clear relationship between responders to MICA *in vitro* and *in vivo*. This offers exciting clinical applications for cell-based therapies as a practical *in vivo* source of dynamic loading in real-time.

a90720

## 03-P113 THE EFFECT OF STICOPHUS CHLORONOTUS AQUEOUS EXTRACT On HUMAN OSTEOARTHRITIS ARTICULAR CHONDROCYTES in THREE-DIMENSIONAL (3D) COLLAGEN TYPE 1 hydrogel in vitro

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**Background**: Autologous chondrocyte-seeded scaffolds has proved to be one of the most promising alternative therapies for articular cartilage defects. However, the chondrocytes are derived from avascular tissues that have specific nutritional requirements and therefore risk of dedifferentiation during in vitro expansion. Sticophus chloronotus, a marine sea cucumber invertebrate is rich in *n*-3 PUFAs and phenolic compound, which has potential in the regeneration of the cartilage. **Methods**: Human osteoarthritis articular chondrocytes (HOAC) were isolated from the knee joint cartilage of patients underwent total knee replacement surgery. Human osteoarthritis articular chondrocytes were encapsulated in collagen type 1 hydrogel and culture in basic medium with 0, 0.1 and 0.2% of sticophus chloronotus aqueous extract (SCAE). The chondrocytes in three dimensional (3D) culture were evaluated by means cell morphology and proliferation, quantitative phenotypic expression of collagen type 1, 11, aggrecan core protein and sox-9. H&E, toluidine blue stainning and sGAG production was analyzed after 7 days in culture. **Results**: HOAC cultured in 3D with various SCAE concentration appeared an almost polygonal morphology maintaining their chondrocytes characteristic. SCAE supplementation promoted chondrocytes proliferation and the ability of the cells to express gene encoding type I collagen decreased, whereas its ability to express type II collagen, aggrecan and sox-9 increased as compared to control. The cartilaginous matrices were positively stained toluidine blue concomitant with higher sulfated glycosaminoglycan (sGAG) accumulation in SCAE supplemented culture medium. **Conclusions:** This study showed that SCAE may beneficial for in vitro development of 3D chondrocytes culture for use in cartilage tissue engineering therapies.

a92163

## 03-P114 Quantitating the expression of the surface marker tissue non-specific alkaline phosphatase (TNAP) on human dental pulp stromal cells

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**Introduction**: Tissue non-specific alkaline phosphatase (TNAP) is a cell surface marker that has been used to identify and select for mesenchymal stromal cells (MSCs), including MSCs from human dental pulp (hDPSCs) with a high mineralising potential. It is therefore potentially of use as a biomarker in cell capture technologies that use marker-recognition to enrich MSC populations for use in bone regeneration and repair. Previous work has shown that the percentage of hDPSCs expressing TNAP increases with cell seeding density [1], however, it is not known whether the actual number of TNAP molecules varies at the cell surface. This is important information when designing "smart" surfaces for cell capture based on TNAP recognition and binding. Our aim therefore was to determine the total number of TNAP molecules present on hDPSC surfaces across a range of seeding densities.

**Method**: hDPSCs obtained with informed consent from 3 different donors were cultured at passages from 3-7 in basal medium at five increasing seeding densities for 1 week, after which % number of cells expressing TNAP in each of the cultures was determined using flow cytometry. Antibodies used were TNAP-APC and mouse IgG1 APC with 7ADD used as a viability dye (all Biolegend, USA). The average number of TNAP molecules per cell in the cultures was measured using a *p*-nitrophenyl phosphate assay including human TNAP protein (Sino Biological, China) as a protein standard. The percentage of TNAP+ cells within each of the cell populations was then combined with the average number of TNAP molecules per cell for that population, to provide the number of TNAP molecules per TNAP+ cell.

**Results**: TNAP expression increased with seeding density in hDPSCs as previously described [1]. However, assuming all TNAP molecules were active, the total number of TNAP molecules present per cell remained constant at 30,410 ± 3090 TNAP molecules per TNAP+ cell. This result was consistent for cells from each of the three donors.

**Conclusion**: The number of TNAP molecules present on TNAP+ cells remains relatively constant independent of cell seeding density. These data will now be used when developing capture surfaces for TNAP+ cells for use in cell based therapies for bone regeneration and repair.

References: [1] M.Tomlinson, C. Dennis, X.Yang, and J. Kirkham, "Tissue non-specific alkaline phosphatase production by human dental pulp stromal cells is enhanced by high density cell culture," *Cell Tissue Res.*, 2015.

a91518

## 03-P115 Bone marrow harvesting technique influences functional heterogeneity of mesenchymal stem/stromal cells and cartilage regeneration

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**Introduction:** The cartilage regeneration capacity of bone marrow (BM) derived mesenchymal stem/stromal cells (MSCs) remains unpredictable due to MSC heterogeneity. We hypothesized that the method and site of harvest of BM may highly influence MSC heterogeneity because MSCs have distinct localization within bone marrow.

**Methods:** To address these questions, MSCs obtained from the femur of eight donors undergoing total hip replacement by two different harvest methods: BM aspiration and BM collection by bone rasping (rasped BM) were evaluated for chondrogenic ability and immunophenotype by flow cytometry. The anatomical localization of different MSC subsets in BM was verified by immunohistochemistry.

**Results:** We showed that cells cultured from the rasped BM were significantly more chondrogenic than the donor matched aspirate whereas no notable difference in their osteogenic or adipogenic differentiation potential was observed. We then assessed whether distinct immunophenotypically defined MSC subsets in aspirate and rasped BM were responsible for the different chondrogenic differentiation capacity. Cells directly isolated from rasp contained a higher percentage of CD45-CD271+CD56+ MSCs (average 7.2 fold) compared to BM aspirates. The presence of this subset in the harvested BM strongly correlated with culture's chondrogenic differentiation ability, indicating that CD45-CD271+CD56+ MSCs are enriched in chondroprogenitors. In addition, different anatomical localizations of these MSC subsets in BM were analyzed. CD271+CD56+ MSCs were localized in the bone-lining regions whereas CD271+CD56- MSCs were found in the perivascular regions. Since iliac crest remains a frequent site of BM harvest for musculoskeletal regeneration, we also compared the spatial distribution of these subsets in trabaculae of femoral head and iliac crest and found CD271+CD56+ bone-lining MSCs in both tissues.

**Conclusion:** In conclusion, we show that for cartilage repair applications, the method and site of harvest of BM is of major importance in determining the number of chondroprogenitors and hence the efficiency of cartilage formation.

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a92091

## 03-P116 3D versus 2D culture: Tissue-like culture conditions enable the detection of individualized cartilage-specific differentiation possibilities

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Injuries of joints based on sports trauma or osteoarthritis result in damaged or lost cartilage. However, self-repair of this joint tissue is very limited. Several repair strategies have emerged but with limited success. Recent therapeutic approaches use cartilage cells or artificial tissues to help the body rebuilding the lost tissue. Using these cell-based therapies, autologous cells are best tolerated but sometimes fail in forming functional cartilage. The aim of the study was to capture and visualize individual differentiation capacities of chondrocytes derived from different donors with regard to a possible personal regeneration capacity using a cell-based therapy.

The differentiation potential of chondrocytes propagated in monolayer culture (2D) was analyzed in a scaffold-free three-dimensional (3D) tissue model. 3D chondrocyte constructs were cultured for four weeks in a basic medium (MEM alpha medium/HAM's F12) containing human serum & basic medium enriched with TGF-$\beta$2 and/or Vitamin C. Analysis were done using histology (Safranin O-Fast Green), immunohistology (expression of collagen type I, collagen type II, and S100) and quantitative RT-PCR (collagen type I and type II, proteoglycan 4, s100B, and cartilage oligomeric matrix protein (COMP)) in monolayer-cultured cells and microtissue cryosections.

In 2D culture, cells from all donors showed an almost identical de-differentiated profile. In contrast, the differentiation state of 3D microtissues revealed clear differences with respect to individual donors. Analyses at the protein and mRNA level showed high variations regarding cartilage-typical matrix components (e.g. proteoglycans, collagen type II) and intracellular proteins (e.g. S100). Interestingly, only donor chondrocytes with a basic tendency to re-differentiate in a 3D environment were able to increase this tissue-specific maturation when exposed to L-ascorbic acid and/or TGF-$\beta$2.

Comparing the phenotype of isolated chondrocytes from different donors in vitro revealed an individual cartilage-specific differentiation capacity. This personalized cell behaviour was not detectable until the monolayer cells were given the opportunity to rearrange in a 3D cell assembly. Therefore, cells in 2D culture may not be a suitable basis to discriminate responders from non-responders with respect to a personalized cell-based therapy to treat cartilage defects.

Martin F, Lehmann M, Sack U, Anderer U. Exp Biol Med, 242: 1746–1756, 2017. *Featured Article*.

a92980

## 03-P117 Inferior In Vivo Osteogenesis and Superior Angiogeneis of Human Adipose-Derived Stromal Cells Compared with Bone Marrow - Derived Stromal Cells Cultured in Xeno - Free Conditions

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Adipose tissue-derived stromal cells (ATSC) may be an alternative to bone marrow - derived stromal cells (BMSC) for tissue repair due to the accessibility, high cell yield, and rapid *in vitro* expansion of ATSC. For bone repair therapeutics, their osteo-induction capacity *in vivo* in comparison to BMSC must be proven. The aim of this study was to compare the osteogenesis of human xenofree - expanded ATSC and BMSC *in vitro* and in a mouse model of bone formation.

*In vitro* osteogenic differentiation was assessed by osteogenic gene expression, intra - and extra – cellular ALP, and calcium deposition. Human MSC from the same donors were implanted with biphasic calcium phosphate biomaterials in subcutis pockets for 8 weeks in nude mice. Implant groups were: BMSC, ATSC, BMSC and ATSC mixed together in different ratios, as well as MSC primed with either standard osteogenic supplements (250$\mu$M ascorbic acid, 10 mM$\beta$ - glycerolphosphate, and 10 nM dexamethasone) or 50 ng/ml recombinant bone morphogenetic protein 4 prior to implantation. Bone formation was quantified on Masson trichrome stained sections, while neovascularization was observed by immunohistochemistry. *In situ* hybridization, together with qRT-PCR, assessed human versus host cell presence in the implants.

*In vitro* results confirm osteogenic gene expression and differentiation potentials of ATSC. Despite this, when MSC of passage 2 were implanted *in vivo*, ATSC failed to form ectopic bone, in stark contrast to the abundant bone and bone marrow formed by BMSC. Interestingly, neovascularization was significantly enhanced *in vivo* by transplantation of ATSC compared with BMSC. In addition, osteogenic priming with standard osteogenic supplements or BMP-4 prior to implantation did impart minor osteogenesis to ATSC. Finally, less ATSC engrafted compared with BMSC.

In the content of bone regeneration, the advantages of ATSC over BMSC, may be negated by their lack of osteogenesis and prerequisite for osteogenic differentiation prior to transplantation. Nevertheless, ATSC do still benefit from ease of harvesting, high cell yield, and improved neovascularization over BMSC and may therefore be advantageous for alternative cell therapy applications.

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a90763

## 03-P118 Microfluidic label-free selection of MSC subpopulation during culture expansion extends the chondrogenic potential *in vitro* and the Mechanism about the Performance of the Selected MSCs

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<p class="MsoNormal" style="text-align:justify;text-justify:inter-ideograph; line-height:150%">Mesenchymal stem cells (MSCs) have been shown as potential candidates for cell-based therapies for a diverse range of tissue regenerative applications. Therapeutic use of MSCs usually requires culture expansion, which increases the heterogeneity of MSCs *in vitro*, thus affecting the potency of the MSCs for more specific indications. The capacity for identifying and isolating special subsets of MSCs for treatment of specific diseases therefore holds great clinical significance. An important therapeutic application of MSC is for the regeneration of cartilage tissue. Here we utilize a spiral micro-channel device to separate culture expanded MSCs into five subgroups according to cell size, and study their proliferation and chondrogenesis at early, middle and late passages. Results show that in all passages, the medium-size subpopulation (cell size of 17-21 $\mu$m), compared to other subpopulations, displays significantly higher proliferation rate and chondrogenic capacity in terms of cartilage extracellular matrix formation. Also, the small cell subpopulation (average cell size of 11-12 $\mu$m) shows lower viability, and large cell subpopulation (average cell size 23-25 $\mu$m) expresses higher level of senescence-associated $\beta$-galactosidase. Finally, we show that repeated microfluidic exclusion of MSCs larger than 21 $\mu$m and smaller than 17 $\mu$m at every passage during continuous culture expansion resulted in selected MSCs with faster proliferation and better chondrogenic potential as compared to MSC derived from conventional expansion approach. The protein and gene array are using to identify the potential pathway for the MSC chondrogenesis enhancement after the physical marker selection.

a91283

## 03-P119 Nasal chondrocytes are potential autologous cell-transplant candidates for treating degenerative disc disease

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Low back pain affects more than 600 million people globally, and intervertebral disc (IVD) degeneration is the most common cause of specific low back pain. Degenerative disc disease is evoked by an imbalance of the anabolic and catabolic processes in resident cells leading to loss of extracellular matrix. Treatments dedicated to restoring IVD homeostasis are greatly in demand, one of which is cell therapy. In recent years, nasal chondrocytes (NCs), have gained a reputation for cartilage tissue regeneration. To assess whether NCs can be considered as an autologous cell source for cell therapy of disc degeneration, we compared the response of NCs to those of mesenchymal stromal cells (MSCs) and articular chondrocytes (ACs), two cells sources presently used in phase two clinical trials for the treatment of DDD.

To implement conditions mimicking the milieu of degenerated IVD *in vitro,* cells were cultured in low glucose media (1gr/L) in hypoxia (2% $O_2$) for 28 days. The media was additionally adjusted to pH 6.8 and/or supplemented with proinflammatory cytokines TNF$\alpha$, IL1$\beta$, and IL6 as is found in the degenerated disc. Furthermore, cells underwent chondrogenic instructional priming due to the addition of TGF$\beta$1 for the first seven days of culture.

Analysis of histology, immunohistochemistry, biochemistry and quantitative rtPCR demonstrated that even though MSCs could be chondrogenicaly primed, harsh environmental conditions and donor variability play a crucial role in their successful survival and chondrogenesis. ACs were sensitive to priming in that they could accumulate glycosaminoglycans (GAG), however, lack the ability to synthesize Collagen type II (Col2). Commendably, primed NCs produce ECM rich in both GAG and Col2 in all nonacidic conditions. Even though an acidic environment reduces its GAG production the same as ACs, the supplementation of the inflammatory cytokines affect the NCs to a lower extent compared to ACs.

Our data indicate that NCs are more resident to harsher environments than ACs or MSCs upon chondrogenic priming. These findings encourage the assessment that employing NCs in a cell therapy treatment of degenerative disc disease could promote new matrix production in the disc, which could inhibit or delay further disc height loss if not even lead to disc height gain.

a91292

## 03-P120 Interchange in culture supplementation from human serum to platelet-rich plasma achieves both proliferation and matrix turnover of chondrocytes

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**Introduction:**

Articular cartilage of synovial joints lacks endogenous repair and regeneration during a traumatic injury which results in a cartilage defect. Cartilage defects are treated by a tissue engineering approach known as the matrix-assisted autologous chondrocyte implantation (MACI). Autologous chondrocytes are usually cultured in the presence of autologous human serum (HS) for growth medium supplementation, but the resulting matrix production is fibrocartilage than hyaline-like cartilage. To address this issue, we tested alternatives such as serum derivative of platelet-rich fibrin (SPRF) and platelet-rich plasma (PRP) as a replacement to HS for culturing MACI constructs.

**Methods:**

Collagen hydrogels were embedded with dedifferentiated OA chondrocytes by initial 2D expansion at passage 0 from OA patients undergoing total knee replacement. Study design consists two parts; Part A (non-interchange) was conducted in a fashion where constructs were cultured in growth medium supplemented with 10% HS or 10% SPRF or 10% PRP for 7 and 14 days. For Part B (interchange) constructs were cultured under 10% HS or 10% SPRF for 7 days and interchanged to 10% PRP from 7-14 days under normoxic/hypoxic conditions. Constructs were evaluated at 7 and 14 days for cell proliferation, DNA quantification, glycosaminoglycan content, matrix turnover gene expression by RT-qPCR, cell secreted anabolic (TGF-b, PDGF-BB, IGF-1, BMP-2) and catabolic cytokines (IL-6, IL-1b) by quantitative ELISA.

**Results:**

Non-interchange study results demonstrate an increase in cell proliferation under both HS and SPRF conditions, but PRP inhibited proliferation from day 0. Glycosaminoglycan content was accumulated under all conditions. Matrix turnover gene expression COL2A1, SOX9 was enhanced but HS and SPRF led to an increase in dedifferentiation markers COL1A1. ELISA results indicate a correlation of IL-6 being less secreted when COL2A1 gene expression is increased followed by more secretion of TGF-b, PDGF-BB, BMP-2 at day 14 under 10% PRP conditions. Interchange study demonstrated that an interchange to PRP inhibits COL1A1 reversing dedifferentiation from 7-14 days followed by an increase in SOX9 expression together.

**Discussion:**

Our study indicates that culture media with HS or SPRF selects the chondrocyte for proliferation, but an interchange to PRP could achieve matrix turnover by reversing dedifferentiation. Our in vitro culture protocol could be clinical relevant particularly for MACI procedures.

a92206

## 03-P121 Chondrogenic Stimulation in Mesenchymal Stem Cells Using Scaffold -based Sustained Release of Platelet-rich Plasma

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Mimicking the native structure of cartilage tissue by engineered scaffolds seems a promising approach in cartilage tissue regeneration. Injectable hydrogels, which can simultaneously embed cell and growth factors could provide accelerated regeneration process. The purpose of this study is to fabricate a scaffold- based sustained release PRP, as a source of growth factors, embedded in an alginate(Alg)/polyvinyl alcohol(PVA) composite injectable hydrogel for the articular cartilage regeneration.

PRP extraction protocol was set up successfully and PRP embedded alginate sulphate(AlgS) microbeads were prepared. The compressive modulus, microstructure, swelling ratio and biodegradation properties of the composite hydrogels were well manipulated by varying the weight ratio of Alg and PVA. Cytotoxicity and cell proliferation of the optimized hydrogel with and without PRP were evaluated by MTT assay of human adipose-derived mesenchymal stem cell (hADMSC). Then, the effect of PRP sustained release on hADMSC proliferation and differentiation were evaluated after 14 days. Samples were stained by alcian blue, siryus red and type Ⅰ and Ⅱ collagen antibody.

The total concentration of hydrogel was optimized on 4% (w/v) and the hydrogel components ratio was optimized around 3:1 Alg:PVA. Adding PVA to Alg resulted to a mild increase of swelling ratio. The proliferation of hADMSC cells was significantly increased by PRP sustained releasing from the AlgS beads in comparison to Alg beads and free PRP. Collagen and glycosaminoglycan (GAG) contents indicated that the hydrogel system favored cartilaginous extracellular matrix (ECM) deposition. Furthermore, the controlled release system could differentiate hADMSC to the chondrocyte phenotype and enhance chondrogenesis, which was evaluated in terms of their protein and gene expression.

As summery, considering the cartilage tissue structure and characteristics, the Alg:PVA composite hydrogel showed the optimized mechanical properties. Incorporation the encapsulated PRP as a growth factors depot, significantly resulted to increase the regeneration of the hADMSC cells; so could accelerate articular cartilage defects regeneration. These features support that Alg/PVA hydrogels have a potential as an in situ forming cell delivery carrier scaffold in cartilage tissue engineering.

a92883

## 03-P122 Temporal Effect of Pulsed Electro-Magnetic Field on Mesenchymal Stem Cell Chondrogenesis

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Mesenchymal stem cells (MSCs), a promising cell source for cartilage repair have been manipulated by use of biomaterials and/or through physical stimulation to enhance and direct MSC chondrogenesis. Pulse electromagnetic field (PEMF) exposure have been shown to evoke mechanotransduction signaling cascades in progenitor cells and have been implicated in the modulation of mesenchymal stem cell (MSC) chondrogenesis. In this study, using an in-house designed PEMF delivery system producing precise magnetic field of low intensity and frequency, we characterized the degree of chondrogenesis induced by PEMF exposure by varying the pulse intensity, duration and the dosage. We demonstrated that MSCs undergoing chondrogenic induction are preferentially responsive to a well-defined window of PEMF stimulation at an amplitude of 2mT for 10mins duration given once at the initial induction stage of chondrogenesis. In contrast, repeated PEMF exposure diminished the chondrogenic outcome. Chondrogenesis is known to be modulated by calcium signaling cascades of specific temporal sensitivity. With the co-administration of EGTA or antagonists to the mechanosensitive cation-permeable channels, Transient receptor potential (TRP) channels, we show that PEMF transduced calcium influx was responsible for PEMF induced initiation of MSC chondrogenesis. On the other hand, preventing calcium entry during repeated PEMF exposure precludes PEMF induced inhibition. This study highlights the intricacies of calcium homeostasis during chondrogenesis and the constraints that are placed on PEMF-based therapeutic strategies aimed at promoting MSC chondrogenesis. The demonstrated efficacy of our optimized PEMF regimens has clear clinical implications for future regenerative strategies for cartilage.

a93280

## 03-P124 Dynamic Culture of Mesenchymal Stem Cell on Gelatin Microsphere Enhance Chondrogenic Properties

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Mesenchymal stem cells (MSCs) cultured on 2D environment results a loss of the mitotic ability and colony forming unit efficiency. Thus, 3D environment, which mimics the *in vivo* condition, is more desirable. In this study, the stemness properties, multilineage differentiation and proliferation activity of bone marrow-derived mesenchymal stem cells (BMSCs) was investigated by culturing cells on gelatin microspheres (GM as 3D culture) and tissue culture plate (2D culture). Furthermore, the efficiency of in-vitro chondrogenesis of BMSCs on GM in static and dynamic culture conditions was also evaluated. Expanded BMSCs were cultured in BioLevitator™ both in static and dynamic culture with F12: DMEM (1:1) + 10% FBS (FD) and chondrogenic induction medium (CIM). It was shown that BMSCs proliferated better on GM compared to tissue culture plate during culture with 2 fold increased. GM also remarkably preserved the four stemness genes of BMSCs with mean fold changes of 2.3, 2.8, 2.2, and 13.1 for Oct4, Sox2, Nanog, and Rex1. GM also potentially enhance the efficiency of in-vitro chondrogenesis of BMSCs especially in the dynamic culture with higher cell proliferation in FD (0.41 ± 0.20) and CIM (0.33 ± 0.13) compared to static culture. However, no significant difference was shown between this 2 medium in the dynamic culture. Furthermore, BMSCs cultured with CIM in dynamic culture showed better protein expression (sGAG:2.33 ± 1.03 & type II collagen staining) compared to static culture. These results suggest that culturing BMSCs on GM in dynamic culture condition can be a suitable culture platform for the cells in cartilage tissue engineering.

a91902

## 03-P125 Porcine Nasoseptal Chondrocytes in Silk-Based Scaffolds for *In Vitro* Cartilage Tissue Engineering

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Cartilage tissue engineering holds promise in regenerating autologous cartilage tissue for an ever-pressing tissue replacement market need. To ensure the safety and efficacy of tissue engineered therapies, there is a need to test biomaterials *in vitro* first. The porcine model for chondrocytes has been shown to have clinical relevance in the testing of biomaterials for cartilage tissue engineering. However, current methods to obtain chondrocytes from the porcine model tend to be limited to articular or auricular regions, with few studies demonstrating the use of porcine Nasoseptal Chondrocytes (pNCs). This is especially important for *in vitro* testing of nasal cartilage tissue engineered therapies. This study demonstrates the potential of using primary pNCs, in both monolayer and 3D scaffold cell systems. pNCs isolated from the nasal septum cartilage were seeded onto either a 24-well cell culture plate, or lyophilized silk scaffolds. LIVE/DEAD assay, Safranin O and Alcian Blue staining were performed to evaluate cell viability and ECM production at various time points to characterize the proliferative ability and phenotype of the cells. This study demonstrated the potential of using pNCs as a viable cell source for *in vitro* testing of biomaterials used for cartilage tissue engineering. Further evaluation is required to quantify the amount of ECM production, and to compare it with articular and auricular cartilages to demonstrate the differences between the different cartilage phenotypes.

a93426

## 03-P126 Identification of micro-RNA associated with the chondrogenic ability of human bone marrow mesenchymal stem cells (BMSCs)

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Articular cartilage has the poor repair potential due to the tissue characteristics, avascularity, rich extracellular matrix, and poor cellularity. The cell therapy with BMSCs is a promising option to promote the cartilage repair, because of their differentiation ability[1]. However, the culture condition in vitro as well as donor individuality would vary the ability of BMSCs[2]. We hypothesized that some micro RNAs may regulate the chondrogenic ability, and tried to identify them.

We analyzed the BMSCs isolated from 30 donors. We could repeat the quantitative outcomes that were previously reported for the effects of bFGF on the chondrogenic ability of the BMSCs during the expansion phase[3]. We observed the chondrogenic ability with chondrogenic pellet culture system[4] and obtained independent quantitative data for each donor, including the responsiveness for bFGF.

The RNA profile of each donor, generated before BMSC differentiation, was analyzed comprehensively using an RNA microarray system (EXIQON Inc. miRCURY LNA miRNA array). We selected some candidate micro RNAs as markers of chondrogenic ability of BMSCs. These markers were verified by quantitative PCR, and miR708-5p was found to suppress chondrogenic differentiation. However, the variation in miR708-5p expression between donors was large, suggesting that other factors could also be involved in regulating the chondrogenic ability of BMSCs.

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a93479

## 03-P127 Hesperidin improves antioxidant capacity in hydrogen peroxide-stimulated chondrocytes

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Oxidative stress is involved in the initiation and progression of osteoarthritis (OA). An increase in the reactive oxygen species (ROS) level has been found in the OA knee, which can cause extracellular matrix (ECM) degradation, induce chondrocyte apoptosis, and amplify the inflammatory response in cartilage. Hesperidin is known to have antioxidant capacity and may be used to prevent OA progresses. Accordingly, we proposed that the hesperidin may have therapeutic potential to chondrocyte. In this study, primary chondrocytes were isolated from human cartilage tissues. The does-dependent cytotoxicity and chondroprotective effect of hesperidin on chondrocytes were first evaluated. After that, cells were stimulated by hydrogen peroxide and subsequently supplied with hesperidin. Results showed that high-dose of hesperidin has a deleterious effect to cells. While hydrogen peroxide-stimulation resulted in an inflammatory status to chondrocytes, hesperidin supplement partially restored the antioxidant capacity as well as the phenotype to stimulated cells. In conclusion, hesperidin has the potential to ameliorate the anti-oxidative enzymes in OA chondrocytes.

a92990

## 03-P128 Chondrogenic and osteogenic priming of umbilical cord blood MSC induces bone formation *in vivo*: a comparative study with bone marrow MSC in subcutis sites in nude mice

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Human umbilical cord blood mesenchymal stromal cells (UBMSC) are an attractive possible alternative to bone marrow MSCs (BMSC) for bone regeneration since they show similar characteristics to BMSC, avoid the invasive BM harvesting procedure, and can be banked at birth for later use. For clinical relevance it is essential to demonstrate their regeneration capacity. The aim of this study was to investigate the bone formation potential of human CBMSC in comparison to BMSCs.

CBMSC and BMSC, each from 3 different human donors were either cultured in basal media or differentiated towards osteogenic (by either using standard osteogenic supplements: 10 mM $\beta$-glycerolphosphate, 250 $\mu$M ascorbic acid and 10nM dexamethasone, or by the addition of BMP-4) or chondrogenic (10 ng/mL TGF-b3, 50 mg/ mL ascorbic acid, 4.7 mg/mL linoleic acid, 100 nM dexamethasone and 1x insulin-transferrinselenium) lineages for 6 days. Phenotypic analysis was conducted by using flow cytometry and tri-lineage potential was also investigated. Multiplex arrays measured cytokine secretion, while RT-PCR assessed gene expression. After priming, 2 million cells were mixed with 50 mg biphasic calcium phosphate (BCP) particles and allowed to attach for 1 hour prior to implantation in subcutis sites in nude mice for 8 weeks. Bone tissue in excised samples was identified by using the Masson Trichrome staining method.

Phenotypic profiles were similar for both CBMSC and BMSC, similarly to tri-linage potential, except for the superior tendency of CBMSC for chodrogenic differentiation. Intracellular alkaline phosphatase, and osteocalcin secretion were significantly higher in BMSC compared to UCMSC, while Interleukin (IL)-6, IL-8, and monocyte chemoattractant protein-1 was elevated in CBMSC compared with BMSC. Unprimed CBMSC did not induce any bone formation (bone formed in 0/6 implants), in stark contrast to the unprimed BMSCs which formed abundant bone in 10/10 implants. Both BMP-4 and chondrogenic priming imparted bone forming potential to CBMSC, while priming of BMSC actually inhibited bone formation.

To the best of our knowledge, this is the first study to show the osteoinductive potential imparted to CBMSC as a consequence of chondrogenic priming. This may be an important avenue for the use of CBMSC for bone regeneration in the clinic.

a92760

## 03-P129 Photofunctionalized titanium guided bone regeneration (GBR) around dental implants

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**Introduction**: Ultraviolet(UV) irradiation on titanium surfaces improves its osteointegration capability because of cleaning hydrocarbon, acquiring superhydrophilicity, and converting positively charged surface.The improvement has been advocated as UV-mediated photofunctionalization for titanium implants. Dental implant therapy to the atrophied jaws still has challenging aspects. The aim of this study is to evaluate photofunctionalized titanium guided bone regeneration in bone deficient sites of the jaws. **Material and Methods:** This study was a retrospective, single-center study conducted at Yokohama City University Hospital from 2006 to 2017. Dental implants placed in the bone augmented site with particulate cancellous bone and marrow (PCBM) using titanium mesh were evaluated. All implants were loaded using a submerged approach and observed for one-year and more after functional loading was started. Photofunctionalization was performed immediately prior to implantation with UV light for 15 minutes by a chairside device.Success rate, treatment period by prosthesis setting were analyzed. **Results:** One hundred thirty-eight implants in thirty-five patients/thirty-eight sites were included. Seventy-two untreated implants were placed in the bone augmented site (staged approach group) and sixty-six photofunctionalized implants were simultaneously placed (simultaneous group). The overall success rate was 89.1%. The success rate of untreated implants was 86.1%, while that of photofunctionalized implant was 92.4%. No significant difference between those success rates was seen. The mean treatment period of simultaneous group was 8.9 months, and that of staged approach group was 14.9 months. The treatment period of simultaneous group was significantly shorter than that of staged approach group. **Discussion:** Photofunctionalized implants placed with simultaneous approach using titanium mesh and PCBM could shorten the treatment period without reducing success rate to staged approach, suggesting photofunctionalized implant guided bone regeneration in the bone deficient sites is promising treatment procedure to improve quality of life in edentulous patients with bone defect of the jaws.

a92588

## 03-P130 Bone-targeting chitosan-PLGA nanoparticles for alendronate delivery

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BACKGROUND & AIMS:

Osteoporosis is a major health burden. Current therapeutic treatments have disadvantages such as systemic side effect and low bioavailability. Bone-targeting drug delivery system are designed to improve the therapeutic effect of drugs and minimize the potential toxic side effects. We fabricated a novel nanocarrier for bone-targeting alendronate delivery using glycol chitosan (GC)-poly(lactide-co-glycolide) (PLGA) and PLGA-alendronate conjugates.

MATERIALS & METHODS:

PLGA is a biocompatible, biodegradable and nontoxic material widely utilized to obtain nano- and microparticle drug delivery systems and has received worldwide marketing approvals. Glycol chitosan is a linear polysaccharide with potential for bone targeting because of positive charge. Alendronate sodium, a commonly used bisphosphonate drug for osteoporosis therapy, is also reported as bone-targeting ligand due to its strong affinity to hydroxyapatite mineral of the bone. Nanoparticles made of GC-PLGA and PLGA-a were prepared using nanoprecipitation method. The size of the nanoparticles was determined by dynamic light scattering (DLS) measurement. The morphology of the GC-PLGA/PLGA-alendronate nanoparticles was examined by transmission electron microscopy (TEM). Drug release profile and cytotoxicity were evaluated in vitro. Bone-targeting potential was assessed by hydroxyapatite binding assay and cellular uptake assay.

RESULTS:

The conjugation of GC-PLGA and PLGA-alendronate was confirmed by Fourier-transform Infrared Spectroscopy (FTIR) and nuclear magnetic resonance (NMR) analysis. The prepared nanoparticles are highly aqueous dispersible with an average size of 219 nm. In vitro tests demonstrated good biocompatibility and sustained release of alendronate. Hydroxyapaptite affinity test and cellular uptake assay comfirmed the bone-targeting potential.

CONCLUSION:

These results demonstrated that the GC-PLGA nanoparticles may be a potential drug delivery system for the treatment of osteoporosis.

a93286

## 03-P131 FORMATION OF HUMAN ARTICULAR CARTILAGE FROM IN VITRO TO IN VIVO SHORT-TERM ECTOPIC IMPLANTATION MODEL: *Biochemical and Biomechanical Analyses*

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### Abstract

This study aimed to evaluate the formation of tissue engineered cartilage (TEC) using cells derived from osteoarthritic patients. The cultured cells were transfected with SRY(Sex Determining Region Y)-Box9 (SOX9) gene and seeded on poly(lactic-*co*-glycolic acid) (PLGA) with and without fibrin. The research was performed through in vitro and in vivo experiment settings using an ectopic implantation model. Upon research approval, the TECs were cultured for three weeks in vitro and implanted subcutaneously at the dorsum of athymic mice for four weeks. All TECs were subjected to sulphated glycosaminoglycan (sGAG) and biomechanical analyses. For in vitro TECs, the wet-weight of transfected TECs was higher than the non-transfected TECs. Normalized by TECs dry-weight, the relative sGAG content for non-transfected TECs was higher than the transfected TECs. For in vivo TECs, the sGAG content of all TECs was comparable and showed no significant difference at 2 and 4 weeks post-implantation. Compressive strain (MPa) of in vivo TECs, determined by static compression mechanical test also showed no significant difference between the transfected and the non-transfected TECs. Previous evaluations using gross, histology, immunohistochemistry and gene expression analyses indicated that the TECs exhibited minimal tissue formation in vitro but formed quality cartilaginous tissue in vivo. However, these present findings showed mixed results, and so they raise some important questions for further investigations. There may be more than SOX9 needed to restore the chondrogenic properties of osteoarthritic cells. This work also invites further studies on guiding principles for hyaline-like cartilage growth and in sustaining TECs properties.

**Keywords**: articular cartilage; chondrocytes; osteoarthritis; SOX9 overexpression; gene transfer; PLGA; fibrin; tissue engineered construct; ectopic implantation model

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a91716

## 03-P132 Involvement of Estrogen-regulated MicroRNAs in Osteogenesis of Bone Marrow Mesenchymal Stem Cells

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MicroRNAs (miRNAs) have been widely demonstrated to interact with multiple cellular signaling pathways and to participate in a wide range of physiological processes. Estradiol-17$\beta$ (E2) is the most potent and prevalent endogenous estrogen that plays a vital role in promoting bone formation and reducing bone resorption. To date, little is known about the regulation of miRNAs in E2-induced osteogenic differentiation. In the present study, the primary bone marrow mesenchymal stem cells from rats (rBMSCs) were isolated and incubated with E2, followed by miRNA profiling. The microarray showed that 29 miRNAs were differentially expressed in response to E2 stimulation. Further verification by real-time reverse transcriptase-polymerase chain reaction (qRT-PCR) revealed that E2 enhanced the expression of let-7b and miR-25, but suppressed the miR-30b expression. Moreover, functional analysis confirmed that miR-30b negatively regulated the E2-induced osteogenic differentiation. These data suggest an important role of miRNAs in osteogenic differentiation.

a91240

## 03-P133 Genetic modification of human bone marrow aspirates via delivery of rAAV vectors coated on pNaSS-grafted poly( ε -caprolactone) scaffolds

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**Introduction:** Gene transfer using recombinant adeno-associated viral (rAAV) vectors is a powerful tool to enhance cartilage repair. Here, we tested the ability of various poly(sodium styrene sulfonate) grafted poly( ε -caprolactone) scaffolds to deliver rAAV to human bone marrow aspirates, a potential source of reparative cells for cartilage repair. **Methods:** The scaffolds were fabricated using a spin-coating method. The following various grafted scaffold films with poly(sodium styrene sulfonate) (pNaSS)conditions were tested: no coating, low coating (1.11 x 10-6 mol/g pNaSS), and high coating (1.30 x 10-5 mol/g pNaSS). rAAV-RFP carries the Discosoma sp. red fluorescent protein gene (RFP) and rAAV-lacZ the E. coli β-galactosidase (β-gal) gene (lacZ), both controlled by the CMV-IE promoter/enhancer. Vectors were labeled with Cy3 as described. Immobilization of rAAV on the scaffolds was performed by 0.002% poly-L-lysine. rAAV release from the scaffolds was measured by the AAV Titration ELISA. Bone marrow aspirates were obtained from the distal femurs of donors undergoing total knee arthroplasty. Aspirates (150 µl) were placed on rAAV-coated scaffolds with a mixture of fibrinogen /thrombin (Baxter). The constructs were maintained in DMEM, 10% FBS,100 U/ml penicillin at 37°C for up to 14 days. Transgene expression was monitored by detection of live fluorescence and by X-Gal staining. **Results:** Successful immobilization of rAAV on the scaffolds was confirmed by detection of a fluorescent signal from Cy3-labeled vectors compared with unlabeled vectors. No clear difference were noted regarding the ability of the three types of scaffolds to incorporate rAAV. Remarkably, the various rAAVcoated scaffolds were capable of releasing the vectors over time, especially the low and highly coated pNaSS constructs. Effective scaffold mediated rAAV gene transfer was achieved in human bone marrow aspirates over time as evidenced by detection of a strong fluorescent signal upon rAAVRFP delivery versus control conditions. Similar results were observed when the scaffolds were coated with rAAV-lacZ, revealing intense lacZ expression via X-gal staining compared with control treatments. **Conclusions:** Effective modification of human bone marrow aspirates can be achieved by delivery of rAAV vectors from pNaSS-grafted PCL scaffolds to treat cartilage defects.

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a91746

## 03-P134 Formation of In Vitro Cartilage Construct using SOX9 and TERT Genes Transfected Chondrocytes Seeded in Poly(Lactic-co-Glycolic Acid) (PLGA) with and without Fibrin

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Scaffold, listed as one of tissue engineering principle, is crucial for cell proliferation and extracellular matrix (ECM) secretion. The three-dimensional (3D) scaffold helps tissue formation by mimicking the living organism's microenvironment. In this study we tested the potential of the PLGA and fibrin combination scaffolds on the growth of chondrocytes transfected with SRY (Sex Determining Region Y)-Box 9 (SOX9) and Telomerase Reverse Transcriptase (TERT) genes. Upon animal ethical approval (IIUM/IACUC/Approval/2015/[5]/[24]), chondrocytes harvested from rabbits were transfected with SOX9 and/ or TERT genes via lipofection. The post-transfected chondrocytes were seeded and cultured in 3D PLGA and/or PLGA/fibrin at week-1, -2, and -3. Standard histology including Haematoxylin & Eosin (H&E), Alcian Blue (AB), Safranin O (SO), Toluidine Blue (TB), immunohistochemistry against collagen type-II and collagen type-I, 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenytetrazolium Bromide (MTT) assay and glycosaminoglycan (GAG) analyses were conducted on the chondrocyte-scaffold constructs. All chondrocyte groups seeded in both scaffolds exhibited positive cartilage-phenotype histologically. However, the chondrocytes are not homogenously distributed. They can be seen at the outer surface of scaffolds especially for PLGA group despite a slight increased in the cell numbers as demonstrated by MTT and histology images. The presence of GAG content was confirmed alongside positive AB, SO and TB staining. The presence of collagen type-II and -I was indicated by brown precipitation. The co-expression of the two proteins suggested that the newly formed cartilages are still developing. Although all groups indicated positive cartilage formation, this study showed that chondrocytes seeded in PLGA/fibrin showed better findings especially SOX9-transfected and SOX9/TERT-transfected chondrocytes.

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a91417

## 03-P135 Biological Effects of FGF-2 and IGF-I Co-Overexpression in Human Bone Marrow-Derived Mesenchymal Stem Cells via rAAV Vector Administration

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Introduction:

Gene-based modification of bone marrow-derived mesenchymal stem cells (MSCs)is an attractive approach to treat articular cartilage defects. Here, we tested the benefits of co-delivering rAAV-FGF-2 and rAAV-IGF-I in human MSCs as a tool to enhance the cartilage repair.

Methods:

rAAV-lacZ carries the E. coli $\beta$ -galactosidase (lacZ) gene, rAAV-hFGF-2 a human FGF-2 sequence, and rAAV-hIGF-I a human IGF-I sequence, all controlled by the CMV-IE promoter/enhancer. Bone marrow aspirates were obtained from the distal femurs of donors undergoing total knee arthroplasty, washed, and MSCs we prepared as previously described.Cells were (co)transduced with rAAV (FGF-2 + IGF-I: 40$\mu$ l each vector; lacZ: 40$\mu$l l vector) and kept in chondrogenic medium for up to 21 days. Histological and immunohistochemical analyses were performed on paraffin-embedded sections of the constructs (5$\mu$m) (toluidine blue staining; anti-FGF-2, anti-IGF-I, and anti-type-II/-I/-X collagen immunostaining). The proteoglycan contents were monitored by binding to dimethylmethylene blue dye and the DNA contents by Hoechst 33258 assay. Total RNA was extracted and reverse transcription carried out for cDNA amplification via real-time RT-PCR with GAPDH as control for normalization. Each condition was performed in duplicate in three independent experiments. A t-test was employed with $p \leq 0.05$ considered statistically significant.

Results and Conclusion

Successful overexpression of FGF-2 and IGF-I co-delivered via rAAV was observed in hMSC aggregates compared with the control (lacZ). Chondrogenic differentiation was evidenced in the cells cotransduced with FGF-2 and IGF-I ( toluidine blue staining and type-II collagen immunostaining)relative to the control treatment, Remarkably, immunoreactivity to type-I and -X collagen was less intense with the two FGF-2 and IGF-I candidate vectors. The real-time RT-PCR analysis revealing enhanced chondrogenic differentiation with FGF-2 and IGF-I versus control treatment (3-fold difference in COL2A1 expression) and reduced hypertrophic differentiation (up to 2- and 2.5 -fold difference in COL1A1 and COL10A1 expression), probably due to increased SOX9 levels (3-fold difference). Conclusion: Significant, sustained co-overexpression of FGF-2 with IGF-I in hMSC aggregates upon co-transduction via rAAV is capable of stimulating the chondrogenic activities to treat cartilage defects.

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a91497

## 03-P136 rAAV SOX9 Gene Transfer Stimulates the Chondrogenic Differentiation Activities in Human Peripheral Blood Aspirates

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Introduction

Implantation of genetically modified peripheral blood aspirates may be a promising approach to treat focal cartilage lesions. Here, we explored the effects of rAAV- SOX9 in human peripheral blood aspirates, allowing to durably enhance the chondrogenic differentiation activities in the samples.

Methods

rAAV-lacZ carries the E. coli beta-galactosidase (lacZ) gene and rAAV-FLAG-hsox9 a human FLAG-tagged sox9 sequence, both controlled by the CMV-IE promoter/enhancer. Peripheral blood was collected in the presence of hirudin from human donors were immediately transduced with rAAV (40µl) or let untreated and kept in chondrogenic medium for up to 21 days. Histological and immunohistochemical analyses were performed on paraffin-embedded sections of the constructs (5 $\mu$m) (toluidine blue staining; anti-SOX9 and anti-type-II/-I/-X collagen immunostaining). The proteoglycan contents in the aspirates were monitored by binding to dimethylmethylene blue dye and the DNA contents by Hoechst 33258 assay. Total RNA was extracted and reverse transcription carried out for cDNA amplification via real-time RT-PCR with GAPDH as control for normalization. Each condition was performed in duplicate in three independent experiments. A t-test was employed with $p \leq 0.05$ considered statistically significant.

Results and Conclusion

Transgene (SOX9) expression was observed in rAAV SOX9-treated aspirates relative to control conditions. Enhanced chondrogenic differentiation was achieved in the aspirates transduced with rAAV SOX9 after 21 days as noted by stronger toluidine blue staining and type-II collagen immunostaining. Of further interest, immunoreactivity to type-I and -X collagen was less intense when rAAV SOX9 was provided to the aspirates. The real-time RT-PCR analysis showing enhanced chondrogenic differentiation with SOX9 relative to the control treatments (up to 2- and 1.7-fold higher COL2A and ACN expression, respectively; $p \leq 0.001$) and reduced hypertrophic differentiation (up to 2.7-fold lower COL1A1 and COL10A1 expression, respectively; $p \leq 0.001$), probably resulting from increased levels of SOX9 expression (up to 5-fold difference; $p \leq 0.001$). Conclusion: These *in vitro* results show the potential of targeting human peripheral blood aspirates via therapeutic rAAV-sox9 transduction as a novel, convenient tool to treat articular cartilage defects.

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a90173

## 03-P137 *In Vitro and In Vivo* Evaluation of Plasmid DNA-Loaded Polycaprolactone/Hyaluronic Acid Hybrid Microspheres for Bone Regeneration

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Although bone is a tissue with superior regenerative capacity, massive bone defects created by trauma, tumor resection, or infection are a significant challenge in reconstructive surgery. The needs for microspheres made of synthetic biodegradable polymers have gradually increased with the developments of bioactive delivery systems, tissue engineering, and regenerative medicine. Poly( ε -caprolactone) (PCL) is one of the most attractive polymers, since it has excellent mechanical properties, flexibility, and easy processability as well as good biocompatibility and slow biodegradation rate. However, PCL itself has limited cell affinity and stem cell differentiation potential, which would restrict its wider clinical applications for bone regeneration. One of the promising ways of solving these limitations is to hybridize PCL with cell-compatible polymers such as hyaluronic acid (HA). The growth factors releasing from the gene-transfected cells is allowed many advantages including the minimal denaturation during storage or device formulation processes, low cost compared to growth factor itself, and the localized release of growth factors for maximal effectiveness. However, the long-term production of the growth factor from the gene-transfected cells can be prohibited in the body, because of the cell migration and apoptosis. In this study, plasmid pDNA (pDNA; BMP-2 encoding) complex-loaded PCL/HA hybrid microspheres were fabricated as a bone filler material for effective bone regeneration. The prepared pDNA complex-loaded PCL/HA microspheres were seeded with human bone marrow stem cells (BMSCs) as a model cell to demonstrate the feasibility of utilizing these microspheres for bone regeneration. For effective transfection of pDNA into stem cells, the pDNA was condensed by polyethyleneimine-polyethylene glycol copolymer (PEI-PEG) as a nonviral vector. The pDNA complex was effectively loaded into the PCL/HA microspheres and was continuously released from the microspheres for more than 6 months. From the *in vitro* cell culture using BMSCs, the pDNA complex-loaded PCL/HA microspheres showed the effective osteogenesis of BMSCs. From the *in vivo* animal study using a skull defect model of SD rats, the pDNA-loaded microspheres showed faster bone regeneration behavior than those without pDNA loading. From the results, we recognized that pDNA complex-loaded PCL/HA microspheres can be applicable for bone tissue engineering applications.

a91808

## 03-P138 Development of A Bioactive Meniscus Implant Using 3D Bioprinting

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3D Bioprinting utilizes the layer-by-layer method to create tissue-like structures. One of its applications is to fabricate tissue scaffolds to regenerate joints and ligaments. Some competitors are very active in this area and have been making progress. For instance, Cartiva launched the first synthetic cartilage implant approved by FDA.

Meniscus is a crescent-shaped fibrocartilage and functions as a natural shock absorber between the femur and tibia. Meniscal tearing is one of the most common knee injuries. A damaged meniscus can be replaced with a meniscal transplant using tissues from other parts of the body or from cadavers. The procedure has a low success rate and carries significant risks due to poor performance or tissue rejection. The geometry of meniscus varies from patient to patient. The matrix & cellular composition has regional variations, which makes it challenging to fabricate artificial meniscus using traditional approaches. The 3D Bioprinting is a very promising solution to resolve these issues due to its customized features.

In this study, MRI scan intact meniscus & convert into 3D images. 3D print high strength scaffolds are prepared using FDA cleared or approved biomaterials such as hydrogel, collagen and polymeric materials (PCL, PLLA). And then some biomechanical tests (dynamic compression and coefficient of friction) of 3D printed scaffold vs. cadaver meniscus should be done. Biological assessment also need to be carried out (cell survival and matrix deposition).

a93232

## 03-P139 Iron oxide nanoparticles as a tool for MRI tracking of nasal chondrocytes

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Superparamagnetic iron oxide nanoparticles (SPION) can be used as contrast agents for tracking and visualization of cells and tissues with magnetic resonance imaging (MRI). In this study, we focused on two types of nanoparticles, D-mannose coated and PEG coated iron oxide nanoparticles. These nanoparticles showed low cytotoxicity properties and were detectable with MRI in previous studies. Since nasal chondrocytes (NC) are used in clinical study for treatment of articular cartilage defects, we wanted to examine the possibility of NC labelling with iron oxide nanoparticles and to assess their effect on chondrocyte viability and differentiation.

Sheep nasal septum cartilage biopsies were dissected and digested in collagenase solution to obtain NC. Isolated chondrocytes were expanded in expansion medium until passage 2 and incubated with different concentrations of D-mannose $\gamma$-$Fe_2O_3$ and $Fe_3O_4$&$SiO_2$-PEG nanoparticles for 1.5, 24, 48 and 72 h. Nanoparticle cytotoxicity was determined by Crystal violet staining method and Live Dead assay. In order to test chondrocytes differentiation potential in the presence of PEG and D-mannose coated nanoparticles, three dimensional pellet culture was established. Pellets were collected after 1, 7 and 14 days. For histological evaluation, pellets were stained with Safranin O and Prussian blue, and immunostained against collagen type II. Expression of collagen type I, II and aggrecan was determined by qPCR. To confirm detection of nanoparticles with MRI, T2 and T2* weighted sequences were obtained.

Viability assays showed no significant reduction in chondrocyte viability after the treatment with D-mannose-coated and PEG-coated nanoparticles. Chondrocytes treated with D-mannose nanoparticles had higher proliferation rate than control cells or cells treated with PEG-coated nanoparticles. Histological staining and qPCR for chondrogenic markers confirmed secreted extracellular matrix in treated chondrocytes as in control cells. Prussian blue staining and MRI T2 and T2* maps confirmed presence of D-manose nanoparticles in pellets after 7 and 14 days.

D-mannose coated iron oxide nanoparticles do not reduce viability and proliferation of NC. Their redifferentiation capacity also remained unaffected. Labeling efficiency of NC with D-mannose nanoparticles make them a potential agent for chondrocyte tracking after graft implantation.

a90359

## 03-P140 Kartogenin-Incorporated Multifunctional Hyaluronic Acid Coated Ultra-Small Ceria Nanoparticles Used for Osteoarthritis Treatment

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**Purpose:** Increased oxidative stress and inflammatory reaction play an important role in the pathomechanism of osteoarthritis (OA). Ceria nanoparticles possess strong antioxidant and anti-inflammatory properties through facile cyclic oxidation states that switch between $Ce^{3+}$ and $Ce^{4+}$. Kartogenin (KGN) is a small molecule chondrogenesis inducing agent that significantly promotes chondrocyte differentiation of human MSCs and enhance cartilage repair. In this study, we designed a small molecule drug (KGN) controlled-release system based on hyaluronan-mediated biomineralization multifuctional ultra-small ceria nanoparticles.The purpose of this study is to verify its therapeutic effect in the treatment of osteoarthritis.

**Methods and Materials:** We used biomineralization method to prepare kartogenin-incorporated multifunctional hyaluronic acid coated ultra-small ceria nanoparticles (KGN-HA-USCNPs). Incorporation efficiency and in vitro release behavior of KGN were tested. DLS, TEM, XRD, FT-IR, UV-vis absorbance spectra, TGA analysis, XPS and CD spectra measurement were used for the characterization study of the KGN-HA-USCNPs. Rat articular chondrocytes were used to test its biological effects, such as: cytotoxicity, uperoxide dismutase mimetic activity, pro-inflammatory activity, chondrocytes uptake and sub-cellular localization. Rat bone mesenchymal stem cells (BMSCs) were used to test the in vitro chondrogenic differentiation ability of KGN-HA-USCNPs. The in vivo therapeutic effects of KGN-HA-USCNPs were investigated using a surgically-induced OA model in rats.

**Results:** The nano-materials had stable structure and KGN release behavior. In vitro experiments showed high superoxide dismutase mimetic and anti-inflammatory activity with good biocompatibility. In vitro BMSCs chondrogenic differentiation was evaluated by KGN-HA-USCNPs. All rats treated with HA-USCNPs or KGN by intra-articular injection showed much less degenerative changes than untreated control, while, rats treated with KGN-HA-USCNPs showed even better therapeutic effects than HA-USCNPs or KGN.

**Conclusion:** KGN-HA-USCNPs can be useful as an intra-articular drug complex to treat OA.

a92776

## 03-P141 Therapeutic ion loaded mesoporous nanoparticles for treating biofilms in chronic bone and skin wounds

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Biofilm related infections are a leading cause of both implant failure and wound necrosis. Preventing or removing established infections is essential to the successful treatment of wounds in bone and skin. Ions such as silver, copper and cerium have been shown as reliable treatments against a range of microorganisms. Antimicrobial agents are not effective unless coupled with an intelligent delivery system. Mesoporous nanoparticles are one such delivery system that can act as a platform for delivering therapeutic ions to treat bone and skin wounds. This project evaluates silver, cerium, and copper containing mesoporous nanoparticles for their antimicrobial effectiveness and the interplay of mixed ion delivery on a bone biofilm model.

Mesoporous therapeutic glasses (MTGs) and ordered mesoporous carbons (OMCs) were manufactured using sol-gel, spray drying and nanocasting then loaded with Ag+, Cu2+ or Ce3+. ICP was used to confirm ion loading. Samples were tested against standard and bone biofilm models. Fibroblast toxicity studies were conducted. The interplay of ions was also investigated by combining ions in concentration gradients to determine any synergistic effect. SEM, PrestoBlue, optical density measurements and crystal violet stain were used to assess biofilm viability. Single and multi-species bacteria biofilms were used to test samples using *S.aureus*, *P.aeruginosa* and *MRSA.*

Both MTGs and OMCs successfully released ions. Silver nanoparticles were highly effective at preventing biofilm formation yet unable to disrupt an existing biofilm. Copper was equally effective against established and forming biofilms but was less effective than silver at preventing biofilm formation. Cerium was found to aggregate bacteria and prevented bacteria from leaving established biofilms at higher concentrations, an effect which was amplified when combined with silver.

Successful delivery of therapeutic ions was tested against bone biofilm models and Ag/Ce ions were found to work synergistically against bacteria. This allows the mixing of nanoparticles loaded with different ions to provide customised payloads depending on the situation. Treating chronic infected wounds with these therapeutic containing mesoporous nanoparticles could rapidly reduce healing time by preventing or treating bacterial infections.

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a91475

## 03-P142 New Generation Spermine Based Polyurethane-Urea Derived High Strength Osteoconductive Scaffold

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The advent of oesteogenic biomaterials has increased over autografts and allografts to avoid additional second site operation and clinical complexities. In this study, we have prepared 2D rod like nano hydroxyapatite (nHA) decorated on graphene oxide (GO) sheet by hydrothermal precipitation reaction. The prepared nanohybrid (GO-nHA) fillers were incorporated into the novel synthesized Spermine based thermoplastic polyurethane-urea (PUU) matrices by in situ technique followed by salt leaching to fabricate the porous scaffolds. The prepared nanohybrid scaffold has been characterized using FTIR, HRTEM, SEM techniques. Hybrid scaffold (GO-nHA filled) displayed superior physico-mechanical properties compare to pristine scaffold. Osteoconductivity of the nanocomposites were evaluated by apatite layer formation on its surface, after immersion into simulated body fluid. Cytotoxicity study using osteoblast cell like MG63 cell line revealed positive cell viability and increased proliferation over a period of 12 days of culture. Furthermore, qRT-PCR showed positive expression of collagen type I and osteocalcin which indicated the maturation and biomineralization of osteoblasts. The favorable cell environment attributed to the increased hydrophilicity of scaffold, as assessed by the contact angle measurement. The incorporation of low percentage of GO into the scaffold was shown to improve mechanical properties, surface wettability and cell viability and cell proliferation drastically. Furthermore, *in vivo* study confirmed the promoted osteogenesis of PUU/GO-nHA scaffold *in vivo* without any cytotoxicity. Based on mechanical, *in vitro* and *in vivo* study these polymer composites exhibited potential orthopedic applications.

Keywords: polyurethane-urea, spermine, mechanical properties, cytotoxicity, bone regeneration, in vivo study

a92471

## 03-P143 Hydrothermally treated bioactive porous Ti6Al4V for accelerated femoral defects healing in rabbit

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**INTRODUCTION** Titanium and its alloys have been used widely in the field of orthopaedics owing to their biocompatibility, corrosion resistance and mechanical properties. Post-implanted failure is associated with challenges including micromotion, fibrosis, aseptic loosening and osteolysis originating from stress-shielding.An augmented mechanical interlock through osteogenesis and bone ingrowth along with an inbuilt mechanism to prevent bacterial infections may overcome early implantation failure.Introduction of macro-porosity reduces modulus as well as stress shielding, facilitate osseous tissue ingrowth but also increases bacterial adhesion. Nano-topography modulates cellular adhesion, proliferation and osteogenesis thereby promotes tissue turnover on implant surface.

**MATERIALS AND METHODS**

Present study demonstrates one-pot synthesis of titanate nanostructures on Ti6Al4V through simple hydrothermal treatment using aqueous calcium hydroxide and sodium tripolyphosphate (1:0.78, 200 ° C, 2 h). Microstructure and surface topography of the samples were analysed by SEM and AFM. Treated samples were assessed for cellular activity, osteogenic differentiation using hMSCs. Further bactericidal effects on treated surface was also evaluated. After treatment, porous Ti6Al4V (75% porosity, 11.4% pore interconnectivity, 178 µm average pore size, pore size distribution 66.3 to 507.2 µm) were implanted in rabbit femoral defects to assess the healing efficacy against empty defects.

**RESULTS AND DISCUSSION** Wire-like nano-features were developed on porous Ti6Al4V samples along with hydroxyapatite deposition on the surface simultaneously. RT-PCR studies revealed osteogenic differentiation of adipose derived stem cells. Furthermore, bacterial cells were perforated on pointed tips of nanowires. Implantation of treated porous Ti6Al4V in rabbit femoral defects exhibited accelerated bone healing compared to that of untreated samples as estimated through quantitative Micro-CT analysis.

**CONCLUSION** Therefore, porous Ti6Al4V implants with wire-like nano-features could be a better choice for accelerated healing of skeletal defects without using bactericidal aids.

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a92806

## 03-P144 Development of Chondrocyte-seeded magnetic gelatin nanocarrier for cartilage repair

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Osteoarthritis is a commonly occurring joint disorder characterized by degenerative changes in the articular cartilage. Due to the lack of a neural, vascular and lymphatic network, articular cartilage has a limited self healing potential after injury or degeneration, and the healing and repair of injured cartilage remains a difficult clinical problem. The goal of this study was to investigate the potentials of magnetic-guiding nanocarrier combined with chondrocytes to the osteoarthritis treatment. Amphiphilic gelatin-iron oxide has been prepared into magnetic-guiding nanocarrier for the fundamental characteristics and properties studies. The effects of the magnetic-guiding nanocarrier on the biological function of chondrocytes were analyzed. The preliminary results showed that the magnetic amphiphilic gelatin nanocapsules were synthesized using hexanoic anhydride-grafted gelatin and superparamagnetic iron oxide nanoparticles using double emulsification. The MTT assay showed that magnetic amphiphilic gelatin nanocapsules have insignificant cytotoxicity and are biocompatible to chondrocytes. Furthermore, magnetic amphiphilic gelatin nanocapsules could lead to the success in the cell guidance and cell retention under the application of an external magnetic field. The biochemical analysis demonstrated that introduction of magnetic amphiphilic gelatin nanocapsules with magnetic field would not did not perturb the characteristic phenotype and biochemical activities of chondrocytes. In addition, the gene expression levels of collagen type I were decreased in chondrocytes cultured with magnetic amphiphilic gelatin nanocapsules. Based on the above results, magnetic amphiphilic gelatin nanocapsules could maintain the physiological function of the performance of chondrocytes in vitro. It demonstrated that the applications of the magnetic amphiphilic gelatin nanocapsules in cartilage tissue engineering have great potential for further development.

a93397

## 03-P145 Antibacterial 3D printed scaffolds for bone tissue engineering

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A major source of morbidity in orthopaedic surgery is the implant-associated infection. The main focus of this study is the development of bifunctional 3D scaffolds from polycaprolactone (PCL) with incorporated silver nanoparticles (Ag-NPs) as an antibacterial component aiming to (i) promote the osteogenic response of the scaffold and (ii) provide an antibacterial protection by preventing biofilm formation. We aim to evaluate the cellular responses of pre-osteoblasts cultured on these bi-functional scaffolds in terms of cell adhesion, growth and osteogenic differentiation potential, as well as their antibacterial effect.

A new injection printing head was developed for a low-cost, open-source Fused Filament Fabrication device, turning it into a powerful printing unit, facilitating the integration of nanoparticles into printable biopolymers. Nanocomposite 3D scaffolds with a pore size of 100 $\mu$m were fabricated using PCL as matrix and Ag-NPs as impregnated material at a concentration of 30 ppm. Scaffolds were visualized by means of scanning electron microscopy. Ag-NPs were produced by an environmentally friendly method based on plant extracts. Antibacterial effect was assessed by means of a broth micro-dilution method and visualized by SEM. Pre-osteblastic MC3T3-E1 cells are used to investigate cell adhesion onto the scaffold, viability and growth, and osteogenic differentiation by means of alkaline phosphatase activity, collagen and calcium produced in the extracellular matrix. Additionally, we evaluate the antibacterial potential of the Ag-NPs incorporated in scaffolds by SEM.

Antibacterial polycaprolactone-based 3D scaffolds are printed in dimensions of 7 mm x 7 mm x 2 mm, consisting of a PCL matrix, in which Ag-NPs with diameters of 20-50 nm are incorporated. Kinetic investigations show a MIC of the Ag-NPs on E.coli at 15 $\mu$g/ml, and a bacteriocidal effect observed by SEM at 75 $\mu$g/ml after 5 h in culture. Scaffolds loaded with pre-osteoblastic cells show a strong cell adhesion after 2 days in culture. Cell morphology data are confirmed by the visualization of the cytoskeleton by means of confocal fluorescence microscopy. Cell proliferation increases after 7 and 14 days in culture. Ongoing osteogenic differentiation experiments indicate an elevation of the osteogenic markers such as the alkaline phosphatase activity, collagen secretion and calcium biomineralization in the ECM.

a92279

## 03-P146 Deciphering the Interactions of Magnetic Iron-doped Hydroxyapatite Nanoparticles with Bone Cells

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Strong coupling between nanotechnology and cell/molecular biology leaded to a breakthrough in medicine in the last decade due to the exiting opportunities in designing and developing a tailored approach in response to different disease. Magnetic nanoparticles (NPs) have attracted the attention of scientific community for biological and medical purposes as promising materials in drug or gene delivery, DNA/biomolecules separation, hypothermal treatment of tumours, contrast agents for imaging, and recently in tissue engineering and theranostic applications. Here novel biomimetic, fully biodegradable and cytocompatible NPs fabricated by doping hydroxyapatite (HA) with Fe ions (FeHA), avoiding the presence of magnetic secondary phases and coating, were biologically tested. A live monitoring of intracellular fate of FeHA NPs and the biodistribution with a pilot study in vivo were deeply investigated. FeHA NPs were prepared by a neutralization process using $FeCl_2$ and $FeCl_3$ as a source of $Fe^{2+}$ and $Fe^{3+}$ doping ions[1]; HA NPs and two commercial fluidMag NPs (Chemicell) were used as control groups. Different kinds of bone cells were cultured with 100 $\mu$g/ml NPs up to 72 hours. The molecular pathways of cellular response (apoptosis/necrosis, ROS production and autophagy) to NPs were investigated. Moreover the mechanism of internalization by Caveolae and Clathrin-mediated endocytosis were studied. In a pilot *in vivo* experiment the biodistribution of different concentrations of NPs was evaluated. Moreover, FeHA NPs seem to act as modulator of autophagy pathway. The data obtained on the cellular uptake of FeHA NPs lay the basis to clarify the intracellular fate of the FeHA NPs and open brilliant prospective for their use as innovative tools for nanomedicine. FeHA NPs could be injected and guided to a desired body site by an external magnetic field, avoiding any toxicity. Moreover, FeHA NPs could be easily functionalized with several biomolecules or drugs to direct cell fate in medical applications[2].

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a91524

## 03-P147 Bioinspired seeding of biomaterials using stem cell micro-aggregates improves cartilage formation in a growth factor-free manner

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Cartilage is naturally formed via mesenchymal condensation of progenitor cells, which drives chondrogenic differentiation. Yet cell-laden biomaterials do not take advantage of this developmental phenomenon, but instead attempt to force individual stem cells to undergo chondrogenesis. We here report a novel biomimetic approach that improves the chondrogenic capacity of engineered tissues by seeding biomaterials with microaggregated stem cells, which recapitulates native cartilage formation. Microaggregates of mesenchymal stem cells were produced in a monodisperse and high throughput manner using a microwell platform, which was developed using soft lithography and negative replica molding. Collagen hydrogels of 250 microliter were laden with 10^6 cells per ml in either single cell (golden standard) or microaggregated formulation. Comparing gene and protein expression revealed that microaggregation reduced the expression of stemness markers (CD73 and CD90), while increasing chondrogenic markers (SOX9, ACAN, and COL2) as compared to single cells. Moreover, smaller microaggregates of 50 or 100 cells were surprisingly more effective in inducing chondrogenesis than larger microaggregates of 250 and 500 cells. Importantly, biomaterials containing microaggregates formed large quantities of cartilage even without the supplementation of chondrogenic growth factors. Indeed, immunohistochemical analysis revealed high levels of SOX9 nuclear translocation microaggregates, specially for the smaller stem cell microaggregates, even under growth factor-free conditions. Subcutaneous implantation confirmed that microaggregated implants – without supplemented growth factors – formed significant amounts of cartilage formation after a single week. In comparison, single cells implants required TGFB supplementation to produce cartilage, which only became detectable three weeks post implantation. Strikingly, after having formed cartilage in vivo, the microaggregates rapidly dispersed throughout the biomaterial effectively becoming a single cell tissue, which is a key feature of mature cartilage. Thus, we revealed that biomaterials laden with microaggregated stem cells boost the implant's chondrogenic capacity by recapitulating the natural cartilage formation process, both in vitro and in vivo, as compared to the current single cell standard.

a91406

## 03-P148 Design of a 3D Organotypic Model to Study Monocyte Extravasation to the Osteoarthritic Joint using a Combined Computational/Experimental Approach

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Inflammation plays a crucial role in osteoarthritis (OA). In OA joints, an increased number of monocytes extravasates to the synovium, driven by chemokine gradients. Microfluidics allows generating 3D models recapitulating in vitro the in vivo conditions typical of specific biological processes. We exploited this feature to engineer a 3D organotypic model of the joint to investigate monocyte extravasation.

We designed a multi-compartment chip recapitulating different districts: synovium, postcapillary venules, synovial cavity, and cartilage. Synovium and cartilage were modeled as gel compartments flanked by a channel hosting synovial fluid. The synovium compartment enclosed a channel, mimicking a synovial postcapillary venule, for endothelial cell and monocyte injection. Trapezoidal posts were used to confine gel and maximize the surface available for cells to cross the endothelial wall. Computational fluid dynamic (CFD) modeling was applied to optimize the endothelial channel, determining shear stress distribution, and the gel compartments, evaluating the kinetic of chemokine gradient formation. The endothelial channel was designed 200 $\mu$m-wide and 100 µm-high, suitable to obtain a physiological 0.1 Pa shear stress using a 30 µL/h flow rate. Gel compartments were designed 400 µm-wide, suitable to obtain a stable chemokine gradient in 30 min, as experimentally confirmed monitoring FITC-dextran diffusion from the synovial fluid channel to the synovium compartment. Fibrin-embedded synovial fibroblasts and chondrocytes from OA patients were injected in the respective compartments, testing several culture conditions to select cell and fibrin densities able to limit cell outgrowth while promoting cell viability. To resemble the synovial venule, an endothelial monolayer was generated culturing endothelial cells in the 200-µm wide channel. To assess monolayer integrity, we verified the ability to block the diffusion of FITC-dextran across the endothelial wall in both directions, accordingly with CFD simulations. Finally, we validated the newly developed model by injecting monocytes in the endothelial channel and quantifying their extravasation in response to known concentrations of MCP-1. We designed and developed the first microfluidic model recapitulating the spatial organization of articular joint. This platform will be used in the next future to study monocyte extravasation and test potential chemokine-targeting drugs to counteract monocyte recruitment.

a91568

## 03-P149 A Human Bone/Bone-Marrow-on-a-Chip Approach for in vitro Toxicity Testing of Wear and Corrosion Products from Metallic Implants

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3D microfluidic cell culture systems (organ-on-a-chip) allow for studying the human (patho-) physiology in the organ context. Trabecular microarchitecture of cancellous bone provides not only mechanical stability of bone, but also a microenvironment for reticular connective tissue and vital hematopoiesis in the bone marrow (BM). In joint arthroplasty, one major clinical problem is aseptic peri-implant osteolysis due to local exposure to metallic particles and ions. Previously, we found that the *in vitro* osteogenic capacity of mesenchymal stromal cells (MSCs) is impaired by *in vivo* exposure to cobalt and chromium ions in the BM. For further studies we aim to establish a human bone/BM organ-on-a-chip system to (i) benchmark it to human BM specimens in regard to bone tissue homeostasis and cellular composition and (ii) to validate the system for predicting metal-ion-induced adverse biological effects.

Primary cells (MSCs, osteoblasts and mononuclear cells) from healthy BM were isolated. Human cancellous bone was decellularized, cylindrical scaffolds were prepared, MSCs and osteoblasts were seeded and matrix mineralization was induced for five days under static conditions. Subsequently, the scaffolds were transferred to the 2-Organ-Chip (TissUse GmbH, Berlin, Germany) and BM mononuclear cells were seeded before osteoclastogenesis was induced by growth factor supplementation under dynamic culture conditions (70 $\mu$l/min) for eight days. Subsequently, the scaffold was dynamically cultivated for 21 days without further growth factor supplementation.

Lactatdehydrogenase (LDH) quantification revealed cellular integrity over the whole experimental period. Quantification of soluble bone turnover factors indicated active bone metabolism. Two-photon excitation microscopy confirmed the presence of active osteoblasts, osteoclasts and the formation of reticular fibers. Flow cytometry indicated stable T-cell and monocyte populations as well as detectable levels of hematopoietic precursor cells and B-cells. For toxicity testing, treatment with clinically relevant metal ion concentrations revealed a significantly higher LDH release compared to untreated controls. Interestingly, for the same concentrations, this effect could not be observed in a 2D MSC monoculture assay.

Developing a reliable *in vitro* system for predicting adverse effects induced by implant wear in the BM is our main focus for improving preclinical routines to keep patient safety on the highest level.

a93251

## 03-P150 Development and Assessment of Microfluidic Platform for 3D Bone Cell Culture and Drug Evaluation

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This paper presents a novel bone tissue engineering system that combines a microfluidic bioreactor with a microscale-porosity, polymerized High Internal Phase Emulsion (polyHIPE)-based scaffold. Bone regeneration is a complex physiological process that involves synergistic chemical and mechanical stimulation. Our system combines for the first time high precision manufacturing of porous, biocompatible PolyHIPE scaffolds via laser photo-polymerisation with a microfluidic bioreactor that can control the mechanical and chemical stimulation. It permits monitoring of cell viability and differentiation over long time periods, enables computational studies, and provides a platform to evaluate drugs used to treat bone disorders.

The mold for the scaffold and microfluidic chamber were created via laser photocuring of polyethylene glycol diacrylate. Scaffolds were produced by casting 2-ethylhexyl acrylate/isobornyl acrylate blend polyHIPEs and curing under UV light.Human embryonic stem cell-derived mesenchymal progenitors were cultured on scaffolds for up to three weeks in both static, continuous and intermittent dynamic conditions. MTT, resazurin reduction and DNA assays assessed cell viability and number, and alkaline phosphatase activity (ALP), Alizarin Red S (ARS, calcium), and Direct red 80 (DR80 collagen) staining measured osteogenic differentiation. Effects of anabolic bone drugs (Lactoferrin, Icariin, and Menaquinone-4) on bone cells were tested under intermittent flow and compared to the static condition to assess the chips suitability.

Viability assays confirmed cell proliferation in the chip.Normalized ALP activity and ARS and DR80 staining indicated the effect of different flow regimes on osteogenesis, with increased osteogenic differentiation and bone-matrix deposition was observed under intermittent flow. These results permit a better understanding of the mechanical and biochemical cues associated with osteogenesis and demonstrate the potential of a more advanced *in-vitro* system to aid drug development.

We successfully created a microfluidic bioreactor platform that precise geometry of the chamber and scaffold enables the computational study of fluid flow. Short and long-term studies of hES-MPs showed significant positive results for osteogenesis with intermittent flow. Therefore, long-term studies of the cell lines treated with therapeutic reagents will provide more accurate, cheaper, and faster methods to investigate drug production process *in-vitro*.

a92359

## 03-P151 A 3D mechanically stimulated articular joint-on-chip for osteoarthritis modeling

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### Introduction

No pharmacological therapies exist to reverse pathological joint conditions found for instance in Osteoarthritis (OA, the most common joint disease) and patients are treated with palliative strategies. This is mainly due to the lack of valid pre-clinical models. Indeed OA is a complex and multi-factorial disease mechanism [1], involving the whole joint structure (bone, cartilage and interzone), synovium and immune system. Animal models lack similarity with human conditions, while standard in vitro tools are too simplistic to mimic the cellular and tissue complexity of the disease. Here, we describe an in vitro microfluidic device aimed at modeling the human joint in health and disease.

### Methods

Building on our recent microfluidic techniques [2,3], we developed a 3D microsystem to host cartilage microtissues in contact with bone microtissues to form a joint-on-chip model. The generated microjoint is cultured within an innovative microfluidic device enabling its controlled mechanical stimulation to induce OA traits. For the bone side human bone-marrow mesenchymal cells (BM-MSCs) were embedded in functionalized poly-ethyleneglycol (PEG)-based hydrogels; for cartilage side human chondrocytes were embedded in the same matrix. We conducted immunofluorescence (IF) and RT-PCR analyses on separated and composite constructs to assess the effective development of a bone-cartilage interface.

### Results

Between 7 and 14 DIV the bone side of the construct successfully maintained or increased expression of standard bone-related genes (COL1A, BSP, OCN, SP7); presence of collagen I and IBSP was also evident from IF analyses. Presence of calcein-positive mineralization increased during culture as shown by live calcein imaging and fixed IF stainings. Human chondrocytes developed viable cartilaginous constructs exhibiting aggrecan formation. Current experiments are aimed at characterizing the bone/cartilage interface and at driving OA traits by applying finely-tuned detrimental mechanical stimulation.

### Conclusion

Results obtained from microtissue characterization demonstrate the potential of the strategy to successfully develop a microfluidic model of the human joint (joint-on-chip) with possibility to induce OA traits by mechanical stimulation.

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a91400

## 03-P152 Microfluidic Vascularized Bone Tissue Models with Hydroxyapatite-Incorporated Extracellular Matrix

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The microvascular system has been promoted as an efficient three-dimensional (3D) in vitro model for studying complex biological phenomena in bone tissues. Current in vitro systems of bone vasculature fail to manipulate the 3D vessel formation with in vivo bone microenvironments. Here, we report a new platform of incorporating hydroxyapatite (HA) on microfluidic chip for manipulating bone angiogenesis in a mineralized matrix. Besides the stiffness of HA-extracellular matrix, HA particle itself has an excellent property in adsorbing and releasing growth factors from its nanocrystal surface. Therefore, the bone angiogenesis can be sensitively controlled by tuning the HA concentration.

a93227

## 03-P153 Heparinized alpha-tricalcium phosphate porous scaffolds for bone regeneration with/without bFGF modification in rat and canine model

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Bone defects attributed to severe periodontitis, trauma, and injury are frequently encountered in the fields of oral implant and orthopedics. Since autogenous bone grafting has several disadvantages including the second surgery and the limited supply of bone, artificial bone grafts are promising alternatives. Tricalcium phosphate (TCP) is a bioresorbable bone substitute used for oral implants and in orthopedics, but the osteoinduction activity for bone-regeneration is not satisfactory even when the porous structure was optimized. Then we developed a novel method for its surface modification with basic fibroblastgrowth factor (bFGF). Because chemical immobilization of bFGF would lead to its denaturation and inactivation, the modification process was as follows: alpha-TCP surface was modified with a cationic peptides by a biological reaction manner; heparin was reacted on it with an electrostatic manner; bFGF was bound to the heparin. The bone regeneration was proved in rat and canine bone defect models.

Immobilization of cationic peptide, heparin, and bFGF onto alpha-TCP was evaluated quantitatively using radioisotope experiments, which showed that introduced cationic peptide greatly improved the heparin immobilization and that the immobilized heparin greatly improved the "activity" of immobilized bFGF. Histological evaluation were performed two, four and eight weeks after implantation. Histological evaluation two weeks after implantation revealed that the porous alpha-TCP had degraded and bone had formed on the surface of alpha-TCP particles in the bFGF group. At eight weeks, continuous cortical bone with a Haversian structure covered the top of bone defects in the bFGF group. This seems to be caused by the stabilization effect of heparin against bFGF.

In addition, surprisingly, the heparinized alfa-TCP (without bFGF immobilization) induced great bone formation than that of intact alpha-TCP. Based on the histoanalysis, heparin was only conserved at the interface of bone and granules, indicating the heparin molecules can be a starting point of the new bone formation. A detailed mechanism is still unknown, but the results indicate that the heparinization of alpha-TCP might be a usable technique to enhance the bone forming capability of alpha-TCP.

a92638

## 03-P154 OHA- COL II biomimetic gel combined with autologous concentrated bone marrow cell for cartilage defect repair of minipig

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Articular cartilage is an avascular, aneural and alymphatic structure, thus, it has a limited self-repair potential. Cartilage tissue engineering is a promising approach for cartilage defect repair, however, there are still a lot of problems exist in this technology, such as: the program is complicated, poor mechanical properties of repaired tissue, bad integration with host tissue and fibrocartilaginous degeneration. Thus, it is of great significance to exoplore a more convenient repair strategy with better repair effect.

Thus, to simplify the operation sequence while increase the repair effect of cartilage defect, we proposed a new one-step strategy to repair the cartilage defect by oxidized hyaluronic acid (OHA)- type II collagen (COL II) biomimetic gel combined with autologous concentrated bone marrow cell. Hyaluronic acid and type II collagen are both normal composition of articular cartilage, thus, them were choiced to construct the biomimetic gel. Then, the gel was mixture with autologous concentrated bone marrow cell, and it was injected into cartilage defect by one-step method. Under the unique microenvironment of articular cavity and surrounding hyaluronic acid- type II collagen matrix, the implanted cells would differentiate into chondrocytes, and effectively repair the chondral defects. After that, we used animal experiments to test the effectiveness and safety of this repair strategy. Concentrated bone marrow cell was extracted at the beginning of the surgery, and mixed with pre-prepared type II collagen gel. Then, 8 milimeter diameter full-thickness cartilage defect in the femoral trochlea of Guizhou minipig was created. Next, oxidized hyaluronic acid was added, followed by cartilage defect injection, the gel was cross-linked by Schiff's base reaction. At 1, 3, and 6 months postoperatively, the regenerated tissue was evaluated by magnetic resonance imaging (MRI), macro- and microscopic observation, and histological analysis, respectively.

In conclusion, oxidized hyaluronic acid-type II collagen combined with concentrated bone marrow cell can be a promising option in the treatment of large full thickness chondral defects, the repair tissue was hyaline cartilage. Embedded concentrated bone marrow cell has self-renewal and chondrogenic differentiation capacity that participated in cartilage defect repair. Our data confirmed the feasibility and effectiveness of this one-step repair strategy, and implied its potential in clinical application.

a92058

## 03-P155 SmartCaP: A Next Generation Pro-angiogenic Fibrin based Bone Void Filler

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**Introduction**: Autografts are still the standard choice of treatment for bone defects, however; their use is limited by disadvantages such as limited availability, donor-site morbidity and the risk of infection[1]. There exists an unmet clinical need for novel bone graft substitutes that can eliminate the use of autografts. We utilized the technology behind a fibrin based dermal scaffold called Smart Matrix® (currently under clinical trials and manufactured in our labs) and developed a novel, off-the-shelf pro-angiogenic biomaterial called SmartCaP, to act as a bone void filler.

**Methods:** SmartCaP comprises mainly fibrin in the form of a fibrous sheet, coated with intermediary precursor bone mineral phases. It is not yet possible to disclose further information about the biomaterial because patent protection is being sought. SmartCaP was characterised using SEM, EDX, FTIR, Micro-CT and von kossa histological staining. Angiogenesis was tested using *ex ovo* CAM assays. MC3T3-E1 mouse pre-osteoblasts and human MSCs were seeded onto SmartCaP under osteogenic and non-osteogenic conditions over 28 days. Cell viability, proliferation and osteogenic differentiation was assessed using standard assays.

**Results:** SEM analysis revealed that SmartCaP is a homogeneously porous structure comprising of a wide pore size range. EDX, FTIR, Micro-CT and von kossa staining confirmed the deposition of calcium and phosphate precursor phases within the biomaterial. CAM assays showed that SmartCaP is pro-angiogenic and supported the infiltration and growth of blood vessels. SmartCaP supports the growth and proliferation of mouse and human MSCs as well as their osteogenic differentiation, which is more marked under osteogenic conditions.

**Conclusion:** The data suggests that this novel biomaterial is osteogenic, osteoconductive and biodegradable. Therefore, it can act as a template to regenerate bone naturally over time. Due to the physical nature of SmartCaP, it can be easily manipulated by the surgeons in the clinic. The future work is to test the potential of SmartCaP as a bone void filler in an ovine model of cancellous bone defects in the next few months. If successful, SmartCaP will be commercialised through our existing spin-off company called Smart Matrix Ltd. The use of such a biomaterial would eliminate the need for a second surgery to harvest autografts, significantly reducing costs and surgery times.

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a92250

## 03-P156 Periodic architectures for bone scaffolds: an evaluation of mechanical properties and cell response

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BACKGROUND:

The design of scaffolds to support the restoration of bone defects must be optimized to support cell proliferation (e.g. by adjusting porosity and pore size) and to meet the mechanical stresses to which bone is physiologically subject (e.g. compression). One starting point for the design of biomimetic extracellular support structures is triply-periodic-minimal surfaces (TPMS), with zero mean curvature on their surfaces, mimicking human trabecular bone [1].

METHODS:

The models for a selection of scaffold architectures (Gyroid, Diamond, Schwarz P) were generated with a C++ program and the open-source computational geometry library (CGAL), where the input is an implicit function that approximates a TPMS. Scaffolds were manufactured with a GESIM BioPlotter using polycaprolactone (PCL). Micro-computed tomography (µCT) was used to evaluate porosity, pore interconnectivity, etc., of the printed scaffolds. Tetrahedralized µCT reconstructions were fed into a Finite Element Model (FEM) compression simulation (a first-order polynomial linear elasticity code written in C++). The effective Young's modulus was calculated from the displacement output of the simulation. To validate the simulation, unconfined compression testing was performed on a uniaxial system. To evaluate the migration of human adipose-derived mesenchymal stem cells (AdMSCs) along the length of the scaffolds with different (Gyroid, Diamond, Schwarz P) architectures, a customized in vitro setup was developed. At 10, 20, and 30 days, nuclei and actin were fluorescently stained and imaged with confocal laser scanning microscopy. Extent of cell migration was measured using ImageJ.

RESULTS:

A linear regression of Young's modulus from simulation versus from compression testing indicate that the simulation is a reasonable predictor of the measurements. Preliminary cell culture observations suggest that the rate of cell migration along the scaffolds differs significantly between different architectures.

CONCLUSIONS:

This study successfully established a workflow for computer-aided design and engineering of scaffolds with TPMS architecture. Future work will include a simulation of cell propagation to predict the optimal internal architecture to fulfill the contrasting mechanical and biological constraints of bone scaffolds

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a92022

## 03-P157 Decellularized-demineralized osteochondral allografts testing on rabbit model. Preliminary report

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**Introduction** Chondral injuries are common following a knee trauma. There are numerous studies in which different ways of regenerating articular cartilage are tested, but without imposing results.

**Methods** From three freshly sacrificed rabbits the distal femurs were harvested and demineralised in 0,6M HCl (Chem-Lab, Belgium). Thirty pieces of osteochondral grafts were cutted with a 3.7 mm biopsy punch and decellularised in 0.5% SDS (Sigma, UK). To obtain mesenchymal stem cells (MSC), bone marrow has been collected from a 4 monts old rabbits, the nucleated cells were separated with HiSep LSM 1077 (HiMedia, India) and cultivated with HiMeso+10%FBS (HiMedia, India) at 37 ° C with 5%$CO_2$. The chondrocytes were isolated from cartilage harvested from a 5 months old rabbit, through digestion with 0,6% collagenase (Sigma. UK). The citotoxicity of decellularized-demineralized osteochondral allografts (ODDG) was evaluated by MTT assay (Sigma, USA) with MSC and chondrocytes (ISO 10993-5). The results were read in a spectrophotometer (TECAN) with a wavelength of 570 nm. The population test also was made with MSC and chodrocytes. The samples were examinated histologicaly at 8th and 20th day. To six 5 months old rabbits, in the medial condyle were transplanted perforated ODDG enriched with autologous MSC ($1x10^7$ per $1cm^3$). The rabitts were sacrificated at 6 and 12 weeks. The femoral condyles were harvested for histological examination.

**Results** At MTT assay in both cases cell viability was more than 80%. The population test showed an increase of grafts cellularization in both cases from 8th to 20th day. After rabbits sacrification, the defects were partially or totally covered with regenerated tissue and the histological examination with hematoxylin-eosin, shows the presence of a rich cellularity in the regenerated cartilage but with partial restoration of the normal morphological structure.

**Conclusions** The preparation of the ODDG with HCl and SDS had their undesirable effects on the grafts strength during preparation, despite that at MTT assay with MSC and chondrocytes, cell viability was more than 80% and the population test showed a constant multiplication of cells within the graft. The ODDG seems to have potential for regeneration of osteochondral defects and further tests are necessary to fully confirm the graft properties.

**Key words:** osteocondral graft, demineralization, decellularization, cartilage defect.

a90028

## 03-P158 New Freeze-Dried Bone Regenerative Material consisting of Octacalcium Phosphate Collagen and Teriparatide

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Octacalcium phosphate (OCP) and collagen (col) composite (OCPcol) demonstrated superior bone regeneration properties [1, 2], and its commercialization appears to be forthcoming through clinical trials [3]. As a practical medical material for new combination products, we developed a freeze-dried composite with OCPcol and teriparatide (TPTD) (OCPcolTPTDf), and investigated its bone regenerative properties, whereas it was recently reported that OCPcol with the single local administration of TPTD enhances bone regeneration [4]. OCPcol was prepared by mixing sieved granules of OCP and atelocollagen for medical use and a disk of this mixture was molded. Then, OCPcolTPTDf was prepared by impregnation of the OCPcol disc with 1.0 μg or 0.1 μg of TPTD solution (OCPcolTPTDf 1.0 and OCPcolTPTDf 0.1, respectively) followed by lyophilization. *In vitro* release profiles of TPTD from OCPcolTPTDf were determined using an enzyme-linked immunosorbent assay. After the creation of a rodent critical-sized calvarial defect, OCPcolTPTDf or OCPcol was implanted into the defect. Five defects in each group were fixed 12 weeks after implantation. The retention–release profiles of TPTD from OCPcolTPTDf and $\beta$-TCPcolTPTDf supported a higher degree of retention of TPTD. Radiographic, histologic, and histomorphometric examinations indicated that most of the defects in the OCPcolTPTDf groups were filled with newly formed bone. Additionally, the OCPcolTPTDf groups showed significantly enhanced bone regeneration compared with the OCPcol group. These results suggested that OCPcolTPTDf achieved sufficient bone regeneration if implanted into the rodent critical-sized bone defects, and this newly developed bone regenerative composite could be a practical medical material.

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Acknowledgments

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a92100

## 03-P159 Biomimetic microporous poly(lactide-co-trimethylene carbonate) scaffolds for human bone marrow stem cells

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Poly(lactide-co-trimethylene carbonate) poly(LA-co-TMC) lacks suitable functional groups and biological cues to enhance cell-material interactions. We have previously developed a strategy for the synthesis of polyester chains bearing pendant thiol-protected groups[1]. Such polymers thanks to the ubiquity of thiol groups in the biological environment and the pliability of thiol chemistry could be further modified and their potential use for biomedical applications was demonstrated. We present here the copolymerization with L-lactide and the subsequent reactions to produce an aliphatic copolyester bearing pyridyl disulfide groups. Materials were obtained by blending this functionalized copolymer with poly(LA-co-TMC) and editable porous scaffolds were subsequently produced by salt leaching technique. A short integrin-recognition sequence, the arginine-glycine-aspartic acid-cysteine peptide (RGDC) was covalently linked to the scaffold surface by disulfide exchange reaction in aqueous medium between pyridyl disulfide groups along the polymeric chains and the thiol group of the cysteine unit. RGD peptide is one of the most physiologically ubiquitous binding motifs, which is found in many natural adhesive proteins such as fibronectin, vitronectin and laminin. The intracellular cytoskeleton is linked by cellular integrins to the ECM via RGD. The amount of functionalization/pyridyl disulfide groups were used to control densities and orientations of RGDC peptides. UV spectroscopy was used to follow the reaction and estimate the concentration of the RGDC attached. The scaffolds were characterized by thermal and mechanical methods. Moreover, human bone marrow stem cells were seeded onto the scaffolds and the experimental evidence suggests the RGDC functionalized composites definitely warrant potential as scaffolds for bone grafts.

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a92479

## 03-P160 Multi-layered mineralized graphene oxide-collagen micro-scaffolds for bone tissue engineering: A biomimetic approach

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Bone tissue engineering is a potential strategy for repairing large bone defects. However, development of scaffolds with excellent mechanical properties, appropriate osteoconductivity and osteoinductivity properties still remains challenging. Therefore, there is a motivation to develop new biomimetic scaffolds with similar properties to bone tissue in molecular, structural and biological compatibility. In the present study, we fabricated a micron-sized and multi-layered scaffold through the covalent crosslinking of graphene oxide (GO) and collagen (Col). Then, bone-like apatite (Ap) were coated on each layer of the scaffolds respectively, using biomimetic mineralization method of immersing in simulated body fluids (SBFs). The resulting multi-layered GO-Col-Ap micro-scaffolds showed higher elastic modulus, compared to one-layered GO-Col-Ap micro-scaffolds and two-layered GO-Col-Ap micro-scaffolds. Furthermore, better cell adhesion, proliferation, and osteogenic differentiation properties in vitro for BMSCs were observed in multi-layered GO-Col-Ap group. Finally, the multi-layered GO-Col-Ap micro-scaffolds exhibited excellent bone regeneration effects in repairing rat calvarial defects. This study provides new insights for applying biomimetic scaffolds for tissue engineering and regenerative medicine.

a92147

## 03-P161 The use of double-layer collagen I scaffold combined with microfracture for the repair of cartilage defects

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For the lack of the blood supply, cartilage has a poor ability of self-repair which makes it remains a challenge for the treatment of cartilage defect. Microfracture is a widely used clinical treatment for cartilage injury by establish a communication of the cartilage defect with the subchondral bone marrow compartment. However, this microfracture surgery has limitations such as forming of fibrous cartilage and short of cell retention. This study aims to promote the cartilage regeneration by combination of microfracture and a novel double-layer collagen I scaffold with one porous layer and one compact layer which bio-mimic the normal cartilage and subchondral structure. Vivo and in vitro studies showed that the scaffold was with good biocompatibility.We examined the safety and efficacy of the combination of microfractures and the scaffold implantation in porcine model with femoral condylar cartilage defects in the knee. At 3 months and 8 months post operation, animals were sacrificed. Macroscopic, histological and MRI evaluations showed that the group with combination of microfracture and scaffold implantation (M+S) was with better defect filling and hyaline cartilage forming. What's more, mechanical also showed the newborn tissue in the M+S group had better compressive elastic modulus.

Combination of microfracture and double-layer collagen I scaffold implantation can be exploited to improve the repair of cartilage defect.

a92818

## 03-P162 Influence of bioactive calcium silicate/PCL 3D-printed scaffolds coated with biomimetic extracellular matrices for bone tissue engineering

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In current, the available orthopedic biomaterials are extremely biocompatible but lack specific biological characteristics that allow further interaction between the biomaterial and tissues in the clinical. Cell-derived extracellular matrix (ECM)-coated scaffolds have received considerable interest for hard tissue regeneration due to their ability to promote cellular behavior and decrease inflammatory responses. Hence, we delves into the design and fabrication of 3D-printed scaffold that is made out of calcium silicate (CS) and PCL that coated with a decellularized extracellular matrix (dECM) from MG63, thus generating a promising bone tissue engineering strategy that revolves around the concept of enhancing osteogenesis by creating an osteoinductive microenvironment through the incorporation of osteogenic-promoting MG63 dECM. We performed decellularization on MG63 seeded on CS/PCL scaffolds to obtain a dECM-coated bioscaffold and further studied the biological performance of these hybrid scaffolds. The *in vivo and in vitro* results indicated that the dECM-coated CS/PCL scaffolds exhibited excellent biocompatibility and effectively enhanced cellular adhesion, proliferation, and differentiation of human mesenchymal stem cells by increasing the expression of osteogenic-related genes. They also presented anti-inflammatory characteristics by showing a decrease in the expression of TNF-$\alpha$ and IL-1. Overall, our study presented a valuable technique for producing promising 3D scaffold that augment bone tissue regeneration effects in numerous that augment various aspects of bone tissue regeneration effects.

a92854

## 03-P163 Biodegradable mesoporous magnesium–calcium silicate/ polycaprolactone/ polybutylene succinate composite scaffolds for bone tissue engineering

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The properties of bone tissue engineering scaffold, including biocompatibility, highly interconnected porosity, and mechanical integrity are critical to promote cell adhesion, proliferation, and osteoinduction. In this study, we propose new composite scaffolds consisting of mesoporous magnesium–calcium silicate (MCS), polycaprolactone (PCL), and polybutylene succinate (PBS) manufactured by a rapid prototyping technique to provide a micro/macro porous structure.

The characteristics, in vitro bioactivity of MCS/PCL/PBS scaffold, as well as the cellular responses of mouse fibroblast-like cells (L929 cells) and human mesenchymal stem cells (MSCs) to the composite were investigated. Our results showed that the MCS/PCL/PBS scaffold possesses a large surface area, high porosity and hydrophilicity, resulting in improved degradation and biocompatibility compared to the PCL scaffold. In addition, results of a cytotoxicity test according to ISO 10993-12 and ISO 10993-5 clearly suggested that scaffold composed MCS, PCL, and PBS were non-toxic to cells. Moreover, the MCS/PCL/PBS scaffold provided a high surface area for cell adhesion and induced osteogenic differentiation of MSCs according to the component ratio of the scaffold. However, further studies are needed to confirm the optimal composition ratio for obtaining good mechanical properties and osteogenic differentiation control. The characteristics and higher biological efficacy of the MCS/PCL/PBS scaffold suggest that it is a promising candidate for use as a scaffold in cell-based bone regeneration.

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a90608

## 03-P164 Using Leukocyte and Platelet-Rich Fibrin During Periodontally Accelerated Osteogenic Orthodontics Reduces Edema and Pain

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**Objectives:** Periodontally Accelerated Osteogenic Orthodontics (PAOO) is a somewhat new surgical procedure which allows faster tooth movement via combining orthodontic forces with corticotomy and grafting of alveolar bone plates. Platelet Concentrates are autologous blood extracts obtained through centrifugation of whole blood. L-PRF is a relatively-new 3-D autogenous/autologous platelet concentrate (and biomaterial: slowly-/strongly-polymerized fibrin- gel; rich in growth factors/lymphocytes) derived via simple/rapid whole/venous blood centrifugation. L-PRF possess desirable hard- and soft-tissue healing properties and has been receiving utmost attention recently, mainly due to simplicity and user-friendliness. Further, evidence of pain-inhibitory and anti-inflammatory potential is growing. This clinical study explores the feasibility, intra- and post-operative effects of using L-PRF in PAOO in terms of post-operative pain, inflammation, infection and post-orthodontic stability. **Methods:** A prospective observational study involving a cohort of 11 patients receiving a Wilcko's modified PAOO technique with L-PRF (incorporated into the graft and as covering membrane) was performed with informed consent. Post-surgical pain, inflammation and infection were recorded for 10 days post-operatively, while the overall orthodontic treatment and post-treatment stability were followed up to 2 years. **Results:** Accelerated wound healing with no signs of infection or adverse reactions was evident. Post-surgical pain was either "mild" (45.5%) or "moderate" (54.5%). Immediate post-surgical inflammation was either "mild" (89.9%) or "moderate" (9.1%). Resolution began on day 4 where most patients experienced either "mild" or no inflammation (72.7% and 9.1%, respectively). Complete resolution was achieved in all patients by day 8. The average orthodontic treatment time was 9.3 months. All cases were deemed stable for 2 years. **Conclusions:** L-PRF is associated with early bone formation; accelerated soft-tissue healing; and reduced postsurgical pain/discomfort and edema. It is simple and safe to use in PAOO. L-PRF did not interfere with tooth movement and/or post-orthodontic stability; thus alleviating need for analgesics or anti-inflammatory medications. L-PRF preparation protocols require vigorous revision for standardization.

a91133

## 03-P165 Biosilica mineralized functional bone graft

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The growth factor carriers currently used in bone grafting are those to which BMPs are bound by a simple dipping method. Therethrough, it is difficult in regulating the release rate of BMPs, and a large amount of them is released in the early stages of transplantation, resulting in side effects such as edema and ectopic bone formation. Therefore, there is a need for a bone graft carrier that can continuously release growth factors while minimizing initial rapid release during bone regeneration after transplantation. Here, we prepared a bone graft carrier capable of sustained release of BMP2 by silica mineralization. Silica has attracted attention as a biomolecule carrier because of a large surface area and easy surface functionalization. Silica-mineralized bone grafting material was made by immersing bone grafts to which silica forming peptides were attached to a silicic acid solution for varying periods to induce silica deposition. The silica surface increased the bound amount of BMP2 and avoided the initial burst release of it with a sustained behavior as compared to a bone graft without silica mineralization. The release rate was additionally controlled by introducing the biopolymer to produce a silica-polymer composite. In the rat calvarial defect model, BMP2 loaded silica-mineralized bone graft showed better bone regeneration than the bare counterpart.

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a91074

## 03-P167 Bioactive magnesium-calcium silicate/chitosan coatedTi-6Al-4V scaffolds with improved bioactivity for hard tissue regeneration

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The 3D-printed metallic cellular scaffold is one of the best choices for bone tissue scaffold as a replacement of human body parts, especially titanium has the advantages of excellent mechanical properties, biocompatibility and corrosion resistance. Past studies have also confirmed that porous titanium implants can enhance the stability of implants in vivo. However, such porous titanium scaffolds cannot promote bone tissue growth and differentiation autonomously and effectively because titanium itself belongs to bio-inert and hydrophobicity material, which is unfavorable to the environment in which the cells grow. In this study, we choose chitosan and calcium silicate as the composite materials for surface modification. We use Ti6Al4V ELI powder as raw material to fabricate the scaffolds by using selective laser melting technology. After that, different properties of chitosan and calcium silicate were modified on the 3D printed porous titanium scaffold, then we analyzed Mechanical property, Biocompatibility assessment, Surface characterization. Follow-up and human mesenchymal stem cells (hMSCs) biocompatibility and bone differentiation test. Observation of the above test data hope that the implant has sufficient mechanical strength, but also have good bone consolidation results, to facilitate future clinical applications.

a91181

## 03-P168 A novel 3D printing composite scaffold with phytoestrogenic osteopomotive puerarin for potential bone regeneration

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**Background:** Phytoestrogen puerarin was found to be able to promote osteogenesis. In this study, 0.5% (by weight) of puerarin was incorporated into the PLGA/TCP scaffold (PT) by 3D priting to form a composite PTP for promoting osteogenesis. **Method:** The porosity of the composite scaffolds was tested by micro CT. High performance liquid chromatography was used to detect the puerarin *in vitro* release from PTP. MC 3T3-E1 Cells were used to test the osteogenic effect of the scaffold. **Results:** The porosity of PTP is 74.9%, and it has different pores (400~500 μm for macro pores or 5-50 μm for micro pores). Puerarin was rapidly released at the first 10 days and then kept steadily during 10 to 59 days, after that it released a little faster than before. Furthermore, PTP scaffolds appeared a better biocompatibility than PT, and its degradation medium could promote maturation of mineralization of MC3T3-E1 through regulating osteoblastic differentiation markers (Runx2, Osterix, OPN, OC and BSP) mRNA expression and stimulate ALP activity. **Conclusion:** Accordingly, puerarin was able to incorporated into the degradable PLGA/TCP scaffolds, slowly released from the scaffolds during degradation and exerted osteogenic effects *in vitro*. The *in vivo* study is ongoing. As a long-time clinical used drug, puerarin-incorporated scaffold has highly translational potential for the future application in bone regeneration.

**Acknowledgement:** This work was supported by Sino-Swiss collaborative project from Ministry of Science and Technology (2015DFG32200) and the Swiss National Science Foundation under the SSSTC program (156362), and Shenzhen Science and Technology Innovation Committee (GJHZ20150316143827260 and JCYJ20160229195640212 ).

a91215

## 03-P169 Innovative Bioceramics-based Nanocomposites towards Tissue Regeneration

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Bioceramics, natural and synthetic, have been developed to repair, reconstruct, and substitute diseased parts of the body and to promote tissue healing as an alternative to metallic implants. Applications embrace hip, knee, and ligament repair and replacement, maxillofacial reconstruction and augmentation, spinal fusion, bone filler, and repair of periodontal diseases.[1] Hierarchically structured nanocomposites comprising levels from the macroscopic tissue arrangement down to the molecular arrangement of proteins, have a great potential in tissue engineering and regenerative medicine. The nanoscale of the materials can provide a dramatic improvement in the mechanical performance and in the transduction of the mechanical stimuli to the cellular level.[2] Innovative bioceramics-biopolymer nanocomposites are able to bolster scientific efforts for tissue regeneration because of the excellent combination of bioactivity and osteoconductivity of the bioceramics, and the flexibility and shape controllability of the biopolymers. Furthermore, the incorporation of different ions (e.g. strontium, zinc, magnesium, manganese, silicon) into the nanocomposites showed an ionic released during bone graft resorption, and hence an influence in bone health, while strengthening the mechanical properties of the implants.[3] Besides, minerals and traces of metal elements may provide physicochemical modifications in the produced materials, which can accelerate bone formation and resorption *in vivo*.[3] In this study, we aim to evaluate calcium phosphates-based nanocomposites doped with different ions, namely related to their structural and mechanical properties, as well as cell proliferation and osteogenesis, towards tissue regeneration.

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a91788

## 03-P170 Design And Fabrication Of A Composite Scaffold For Treatment Of The Load Bearing Bone Defects

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Large bone injuries heal poorly even with surgical intervention, and their treatment has remained a significant clinical challenge with enormous impacts on medical expenditure and the patient's quality of life. The gold standard material for treatment of large bone defects is autologous bone graft harvested primarily from the patient`s iliac crest, rib or fibula. However, the success of bone grafting is severely limited by the availability of donor bone, donor site morbidity, risk of infection and geometric mismatch between harvested bone and the defect site. Driven by the pressing medical need to develop better approaches, recent research has focused on the development of synthetic bone substitutes, known as scaffolds, from a range of biocompatible polymeric and ceramic materials. Despite important advances in this field, however, currently available scaffolds still suffer from poor ability to withstand skeletal loads and to actively induce bone formation. To overcome this issue, a new design model based on the concept of a composite scaffold system composed of three different parts is proposed. In this approach by utilising finite element analyses, a cage shaped structure made from polycaprolactone is designed to enable a highly porous ceramic scaffold to withstand severe mechanical loading in relevant physiological range.

a91687

## 03-P171 Evaluation of Cartilage Regeneration in Gellan gum/agar blended Hydrogel with Improved Injectability

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GG (gellan gum) is biocompatible material which mimics ECM and could continously deliver stem cells. However, it has a limitation which makes it imposible to be extruded from a needle. In this study, various wt% mixture of agar was combined with GG, and the scaffolds were evaluated from the rheological parameters. Cell proliferation was measured by MTT assay and cartilage cells were photographed by confocal microscopy. Based on the experimental data, it can be found that the gellan gum / agar ratio with the best extrusion and cell proliferation. We anticipate that this hydrogel could increase the clinical application of GG material.

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a90325

## 03-P172 Using mesenchymal stem cells to treat inflammatory chondrocytes in vitro: conditioned medium is better than a co-culture system

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Osteoarthritis (OA), one of the most common joint disease, affects more than 80% of the population aged 70 or over. Mesenchymal stem cells (MSCs) show multi-potent differentiation and self-renewal capability, and, after exposure to an inflammatory environment, also exhibit immunosuppressive properties. In this study, we have used a model of lipopolysaccharide (LPS)-stimulated chondrocytes to evaluate MSC anti-inflammatory efficacy. The anti-inflammatory mechanism was tested in three models, (i) MSC-chondrocyte indirect contact system (Model I), (ii) MSC-chondrocyte direct contact system (Model II), and (iii) MSC-conditioned medium (MSC-CM) system (Model III). In all 3 models, chondrocytes were incubated with LPS for 4 h, then co-cultured with MSCs in the continued presence of LPS for a further 24 or 72 h in models I and II, or incubated with MSC-CM in the absence of LPS for a further 24 or 72 h. The results showed that MSCs reduced chondrocyte inflammation through both paracrine secretion (Model I) and cell-to-cell contact (Model II). The direct cell contact co-culture system showed reduced expression of inflammation-associated genes than the indirect cell contact co-culture system at 24 h, but increased expression of TNF-$\alpha$, IL-1$\beta$, and IL-6 at 72 h. In contrast, the MSC-CM system showed similar anti-inflammatory effect at 24 h, but no increase in expression of pro-inflammatory cytokines at 72 h. The expression of inflammation-related genes (TNF-$\alpha$, IL-1$\beta$, IL-6) and free radical-related genes (iNOS) was reduced and cell numbers increased. In conclusion, MSC-CM showed potential for MSC-based therapy for OA.

a90426

## 03-P173 Chondroprogenitor Cells as Source for Cartilage Repair Using a Biomimetic 3D Repair Model *in vitro*

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**Purpose:** Degeneration conditions like Osteoarthritis (OA) are a major cause of physical disability. This agerelated disease accompanied by chronic pain and inflammation leads most patients to undergo joint replacement surgery. The standard care surgery usually aims to slow down degeneration instead of improving tissue restoration. Regenerative medicine and tissue engineering technologies have become the new hope to ameliorate the joint functional healing using stem and progenitor cells. Characterization of their differentiation and immunosuppressant potential could hold a decisive cornerstone in the road to success in articular cartilage tissue engineering.

**Methods:** Human primary bone marrow hematopoietic stem cells (BM-MSC) and cartilage chondroprogenitors (CP) were isolated from the orthopedic biorepository at Houston Methodist Hospital Orthopedics and Sports Medicine Department. Cellular and molecular characteristics, including immunosuppression capacity, were assessed in vitro in 2D model and a 3D model represented by a biomimetic collagen scaffold functionalized with chondroitin sulfate.

**Results:** The results of the project to date describe an in-depth cellular characterization of human CPs from articular cartilage. CPs show a higher colony formation efficiency to BM-MSCs. CPs also maintain the immunosuppression potential expressing the main characteristic genes. Most of the results are significantly increased when the cells are cultured on the biomimetic scaffold.

**Conclusions:** These results show phenotypic characteristics of stem and progenitor cells that could become tools to identify cell sources for potential cartilage repair strategies. They also provide the scientific community with a 3D *in vitro* model that closes the huge gap between the classic 2D model and *in vivo* studies with cells.

a91227

## 03-P174 Fabrication of vascularized bone grafts using bone beads

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Bone regenerative medicine using osteogenic cells and/or osteoconductive materials has been expected as an effective approach for treatment of bone defects caused by trauma and diseases. Although implanted grafts gradually integrate with host tissues and accelerate bone healing, there is a limitation of therapeutic outcomes particularly in large bone defects. To extend this approach to large defect, prevascularization of grafts may be critical because vasculatures deliver not only oxygen and nutrients but also osteogenic cells for bone formation and subsequent remodeling. In this study, we propose an approach for fabrication of vascularized bone grafts composed of osteoblasts, bone matrix, and vasculatures. A unique aspect of our approach is that we first prepare collagen-rich cell aggregates, named bone beads, through spontaneous shrinkage of cell-embedded hydrogel drop by cell attractive forces. Thus, mesenchymal stem cells were encapsulated in 2-μl collagen microgels, which spontaneously contracted from 2-mm diameter to less than 500-$\mu$m during 24 hours of culture. Interestingly, cells in bone beads showed better osteogenic differentiation, including osteogenic marker expressions and bone matrix secretion, compared with conventional spheroid culture after 2 weeks of culture in osteogenic differentiation culture medium. To fabricate vascularized bone grafts, the bone beads were covered with vascular endothelial cells and then a few hundred of bone beads enveloped with endothelial cells were stacked into a culture chamber. During 2 days of culture, endothelial cells connected one another and formed luminal structures between each bone beads. Fabricated vascularized bone grafts were transplanted into 4-mm-diameter calvarium defects of nude mice and evaluated bone regeneration efficiency by micro computed tomography. Five weeks after the transplantation, vascularized bone grafts were repaired 83% of bone defects, which is significantly greater than other approaches such as transplantation of beta tricalcium phosphate powder. This can be a fundamental technique for engineering vascularized bone grafts.

a91058

## 03-P175 Regeneration of Hyaline Cartilage from Mesenchymal Stem Cells Using Interpenetrating Polymer Network Gels of Polysaccharides and Peptides

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[Background] In tissue engineering for cartilage disorders such as osteoarthritis and cartilage damages using autologous chondrocytes and mesenchymal stem cells (MSCs), it is important to regenerate articular cartilages as original hyaline cartilages. Our research group has developed the interpenetrating polymer network (IPN) gels consisting of chemical cross-linking network between carboxymethyl chitosan (CHI) and polyethylene glycol (PEG) and physical network of self-assembling peptides, RADA16 (Ac-$(RADA)_4$-$CONH_2$)[1]. CHI/PEG network will contribute mechanical strength of gels and RADA16 network will upregulate cellular functions by mimicking extracellular matrix. It has been demonstrated that primary chondrocytes were embedded in IPN gels without cell damage and culture in IPN gels increased glycosaminoglycan (GAG) secretion in vitro and in vivo. In this study, chondrogenic differentiation behaviors of human bone marrow-derived MSCs in CHI/PEG/RADA16 IPN gels were investigated.

[Methods] MSCs were embedded in IPN gels at $1\times10^7$ cells/ml and cultured in chondrogenic differentiation medium and normal medium up to 30 days. Frozen sectional specimens were prepared and observed using optical microscope after alcian blue and H&E staining. The expression of chondrocyte specific genes were evaluated by using real-time reverse-transcription PCR (RT-PCR). Amount of GAG and collagen were quantified by 1,9-dimethyl-methylene blue (DMMB) assay and hydroxyproline assay, respectively.

[Results and Discussion] After chondrogenic differentiation for 30 days, hyaline cartilage-like morphology with lacuna were observed in IPN gels, in contrast to fibrous cartilage-like morphology of spheroid culture. The expression of hyaline cartilage marker gene, *COL2A1* of MSCs in IPN gel was much higher than that of spheroid culture. The expression of fibrous cartilage marker gene, *COL1A1* and hypertrophic cartilage marker gene, *COLXA1* of MSCs in IPN gel were lower than those of spheroid culture. These results indicated that culture of MSC in CHI/PEG/RADA16 IPN gels promoted selective differentiation to hyaline cartilage compared with spheroid culture.

[Conclusion] CHI/PEG/RADA IPN gels are potential scaffolds for cartilage tissue engineering using MSCs.

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a91469

## 03-P176 Organized chitosan scaffold processed the human synovium-derived stem cells for cartilage tissue engineering: an *in vitro* study based on microfluidic technology

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**INTRODUCTION:** Degenerative osteoarthritis is very common in aging citizens and high economic cost is a concerned public burden. New treatment strategy such as cartilage tissue engineering is a potential effective therapy for degenerative osteoarthritis. Current scaffolds still possess several problems such as toxic degradation products, inflammatory reactions to materials and poor cell distribution[1]. Since chitosan has a similar structure of glycosaminoglycans which is the major components of extracellular matrix of hyaline cartilage, we consider to use chitosan for fabricating new scaffold by our microfluidic technology for providing a relative suitable environment for chondrogenesis [2] . Because of highly inherited chondrogenesis possibility, our study used human synovium-derived stem cells, seeded in organized chitosan scaffold, and analyzed the *in vitro* chondrogenesis efficiency.

**METHODS:** In this study, microfluidic flow-focusing technology was used to prepare a chitosan scaffold with a highly organized hexagonal structure. The biomechanical properties of the chitosan scaffold were further evaluated. Human synovium-derived stem cells were harvested from the knee joint during total knee arthroplasty. The cells were expanded, seeded into the chitosan scaffolds and cultured in the chondrogenic medium. Then the cell toxicity(LDH), cell viability(WST-1), extracellular matrix assay(GAG), DNA quantification, gene expression(real time PCR) analysis were further analyzed for evaluating the efficiency of chondrogenesis .

**RESULTS:** Histological analysis revealed that seeded cells expanded as cell pellets, maintained chondrogenic phenotype and secreted much extracellular matrix (ECM). Real time PCR revealed the differentiated chondrocytes can highly express aggrecan, SOX9 and collagen type II. The positive staining of Alcian blue revealed that much extracellular matrix was produced. This demonstrated the chitosan scaffold possessed potential in development of tissue engineered cartilage.

**DISCUSSION & CONCLUSIONS:** In this study, our new chitosan scaffold had high efficacy for cell growth, and the differentiated chondrocytes within this scaffold maintained functional phenotype with adequate ECM production. In summary, this highly organized chitosan scaffold provided a biocompatible environment for enhancing chondrogenic differentiation of human synovium-derived stem cells in this *in intro* study.

a90660

## 03-P177 Differentiation Potential and Proliferating Effect of Three-Dimensional Hydrogel Scaffolds on Mesenchymal Stem Cells

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All cells in tissues consist of a complex three-dimensional (3D) structure and are connected with neighboring cells with extracellular matrix. In this study, to investigate the enhancing properties of 3D culture system in equine adipose tissue-derived mesenchymal stem cells (eAD-MSCs), we performed encapsulating cells and determined changes in gene expression levels of eAD-MSCs. eAD-MSCs were seeded at normal plate ($1\times10^5$ cells/well, condition 1), normal plate with hydrogel ($1\times10^5$ cells/well, condition 2), transwell membrane ($0.4\times10^5$ cells/well, condition 3) and transwell membrane with hydrogel ($0.4\times10^5$ cells/well, condition 4) of 6-well culture plate. We observed that the expression levels of Ki-67, PCNA, OCT4, and SOX2 were significantly ($p < 0.05$) increased in conditions including hydrogel (condition 2 and 4) compared to those in conditions 1 and 3. In addition, expression levels of Ki-67, PCNA, and SOX2 were significantly ($p < 0.05$) higher in condition 4 compared to those in condition 2. Also, eAD-MSCs under all conditions were well differentiated into osteocytes in osteognic differentiation medium. Among them, expression level of osteocalcin was significantly ($p < 0.05$) increased in conditions including hydrogel (conditions 2 and 4) compared to those in conditions 1 and 3. These results suggest that 3D culture of eAD-MSCs through hydrogel scaffolds method can enhance the expression of proliferating and osteogenic factors. Also, surrounding culture condition is found to be better than plating culture condition including hydrogel. Therefore, these 3D culture systems can be used to enhance the efficiency of tissue engineering in eAD-MSCs compared to the traditional cell culture system.

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a91146

## 03-P178 Induction of autophagy with rapamycin enhances chondrogenesis of human mesenchymal stem cells

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**INTRODUCTION:** Cartilage lesions due to injury heal poorly, leading to early onset of osteoarthritis, chronic joint pain and disability. Mesenchymal stem cells (MSCs) in combination with polymeric carriers can be used to induce differentiation of the cells towards chondrogenesis. Autophagy is a cellular mechanism, required for differentiation of adult stem cells. Influencing autophagy during differentiation of MSC to chondrocytes may alter cell fate. Therefore, we investigated the effect of rapamycin, an autophagy activator, during differentiation of MSC in 3D microtissues to chondrocytes.

**METHODS:** Cartilaginous microtissues were prepared by mixing droplets of human MSCs in chitosan (sChi) with droplets of oxidized alginate (oxAlg). Microtissues were induced in chondrocyte differentiation media in presence and absence of rapamycin for 10, 14 and 21 days. Changes in gene and protein expression for collagen type II and X and autophagy markers Atg5 and LC3 were investigated by real-time-PCR and immunofluorescence.

**RESULTS:** Upon chondroinduction, down regulation of key autophagy gene Atg5 and Beclin1 was observed in the non-stimulated group. We observed an increased expression of collagen 2 and hypertrophic marker collagen X starting at day 10 with highest expression on day 21. Rapamycin treated group showed increased Atg5 gene expression with the highest peak at day 14. Treated microtissues exhibited better cell morphology and viability with high expression of Atg5 and punctated immunostaining pattern for LC3 confirming accumulation of autophagosomes.

**CONCLUSIONS:** Our study demonstrates that autophagy plays an important role in chondrodifferentiation of MSC. Administration of rapamycin could serve as novel therapeutic approach for treating cartilage injuries for future clinical applications.

a91704

## 03-P179 One-pot Synthesis of Injectable and Biodegradable Hydrogels with Interpenetration Polymer Network for Tissue Engineering Application

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[Background] In articular cartilage tissue engineering, it is essential that chondro-specific extracellular matrix (ECM) synthesis and accumulation of glycosaminoglycan (GAG) and type II collagen are efficiently promoted in cellular scaffolds. In previous study, we have fabricated the interpenetration polymer network (IPN) gels consisting of physical cross-linking networks of self-assembling peptides, RADA16 (Ac-$(RADA)_4$-$CONH_2$) and chemical cross-linking network of chitosan (CH) and polyethylene glycol (PEG) as a across-linker. RADA16 network upregulates cellular differentiation functions by mimicking ECM network structure and chemical cross-linking network contribute mechanical strength of gels. In this study, injectable and biodegradable hydrogels with IPN structure was prepared through the use of triblock copolymer (PEG-PLA-PEG) of PEG and poly(d,l-lactide) (PLA) as a cross-linker, and chondrocyte functions in CH/PEG-PLA-PEG/RADA16 gels were investigated.

[Method] To prepare CH/PEG-PLA-PEG/RADA16 IPN gel (volume: 50 $\mu$L), after NHS-functioned triblock polymer solution was added to CH solution suspended bovine chondrocyte (P1) of $5.0\times10^5$ cells, RADA16 solution was added immediately. Buffer ion concentration of hydrogels was adjusted to 150 mM by the addition of moderately concentrated PBS solution. Amount of GAG and collagen in medium and gel were quantified by 1,9-dimethyl-methylene blue (DMMB) assay and hydroxyproline assay, respectively. The expression of chondrocyte specific genes were evaluated by RT-PCR.

[Results and Discussion] Under cell culture condition, the degradation rate of CH/PEG-PLA-PEG/RADA16 was promoted compared to that in the absence of PLA unit in cross-linker. Interestingly, we confirmed the enhanced GAG and collagen synthesis when the CH/PEG-PLA-PEG/RADA16 hydrogels were used as degradable scaffolds. In addition, the expression of chondrogenic gene, *acan*, *col2a1*, were the most increased in the degradable IPN hydrogels, which was further enhanced in the coexistence of growth factor. These results indicated the existence of correlation between the degradation of scaffolds and enhanced cellular functions.

[Conclusion] CH/PEG-PLA-PEG/RADA16 injectable and biodegradable IPN hydrogels are promising scaffolds for cartilage tissue engineering.

a92830

## 03-P180 Gelatin Double Network Hydrogels for Cartilage Tissue Engineering

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Gelatin, a product of partially denatured and hydrolysed collagens, is an attractive biomaterial for tissue engineering applications due to its biocompatibility, and capability to promote cell adhesion, proliferation and extracellular matrix synthesis. Non-functionalized gelatin can be covalently crosslinked by visible light and photoinitiators $[Ru(bpy)_3]^{2+}$ (Ru) and sodium persulfate (SPS), resulting in hydrogels that can withstand high compressive stress and strain, and making this system a promising candidate for cartilage tissue engineering. Gelatin hydrogels crosslinked with this system are, however, softer than native cartilage and do not maintain structural integrity after long incubation at 37°C. To overcome such problems, we incorporated covalently crosslinked hyaluronic acid methacrylate (HAMA) or ionically crosslinked alginate (Alg) networks with unmodified gelatin hydrogels to form double-network hydrogels that are both biocompatible and mechanically robust. 9.5%-0.5% wt/vol gelatin-HAMA hydrogels were covalently crosslinked by Ru and SPS with blue light, and 10%-2% wt/vol gelatin-Alg hydrogels were covalently crosslinked by Ru-SPS and blue light and subsequently ionically crosslinked by $CaCl_2$. Primary human articular chondrocytes were encapsulated in the hydrogels and cultured for 14 days. Both gelatin-Alg and gelatin-HAMA hydrogels were structurally stable over 2 weeks of incubation. Notably, gelatin- Alg hydrogels exhibited compressive modulus of 120 kPa, and could withstand up to 2.8 MPa in compressive stress at 93% strain before failure. Encapsulated cells remained at high viability in both hydrogels groups after 2 weeks. Significant increase in gene expression levels of *ACAN* and *COL2A1* were observed in both groups, whereas *COL1A1* expression only increased in gelatin-HAMA. Our results indicate that while the incorporation of either HAMA or alginate to gelatin can produce stable hydrogels that allow for cell encapsulation at high viability, gelatin-Alg hydrogels exhibited superior mechanical properties and gene expression for cartilage tissue engineering.

a92136

## 03-P181 Mechanical Property Controlled Collagen Hydrogel for Bone Tissue Engineering

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Collagen (Col) hydrogels have been extensively used to mimic the extracellular matrix (ECM) for three dimensional (3D) tissue engineering applications because of their excellent biocompatibility and biodegradability. However, poor mechanical properties of collagen hydrogel cause substantial shrinkage during 3D cell culture. To this end, we used lyophilized polysaccharide nanofibers (NFs) to reduce shrinkage of Col hydrogels. We fabricated Col/NFs hydrogel made of Col and NFs for 3D culture system. Rheological properties of Col/NFs were measured using rheometer. Lyophilized NFs were stained with FITC and distributions of NFs in Col hydrogel were observed with confocal microscopy. Viability and proliferation of human mesenchymal stem cell (hMSCs), cultured within the hydrogel, were assessed using live & dead assay and CCK-8 assay, respectively. Osteogenic differentiation of hMSCs cultured in the hydrogel were confirmed using von kossa staining. Shrinkage rates of Col/NFs hydrogels after hMSCs culture were measured for 15 days. Viscosity and elastic modulus of Col/NFs hydrogels were increased with increase of NFs concentration. hMSCs cultured within Col/NFs hydrogel slowly proliferated compared with hMSCs cultured within Col hydrogel. hMSCs cultured within Col hydrogels and Col/NFs hydrogels showed more than 95% viabilities. Addition of polysaccharide nanofibers in Col hydrogel did not affect biological characteristics and osteogenic differentiation potentials of hMSCs cultured within the hydrogel. Shrinkages of Col/NFs hydrogels during hMSC culture were reduced by polysaccharide nanofibers. Shrinkage controlled Col/NFs hydrogels can be used bone tissue engineering.

a93175

## 03-P182 Injectable Double Network Hydrogels Towards Cartilage Repair

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The current surgical procedures for cartilage injuries and conventional cell transplantation technologies provide insufficient long-term benefits and fall short of the achieving high quality cartilage regeneration. The challenges of using the current state-of-the-art matrices for cell therapy, specifically with mesenchymal stem cells (MSCs), include controlling maintenance, differentiation and proliferation of the cells within the matrix microenvironment. In addition, injecting the cells and material with minimal cytotoxicity is a challenge, and mechanically protecting the implant during the *in vivo* transition period needs to be addressed. We developed an ultrahigh-strength and tough hydrogel that is (i) biodegradable, (ii) injectable, and (iii) can support the encapsulation and culture of viable MSCs. The hydrogel is a double network (DN) which combines a tightly covalently crosslinked PEGylated fibrinogen (PF) and hyaluronic acid (HA) with guest-host (GH) interactions. For optimizing the formulation, we combined 2% GH component in 8 mg/ml PF. The mechanical properties, including toughness and viscosity, were controlled by adding different ratios of polyethylene glycol diacrylate (PEG-DA). The biocompatibility of these biomaterial formulations was tested with MSCs encapsulated into the hydrogels and cultured for three weeks. We specifically demonstrated the capability of the DN hydrogel to support optimal survival conditions for cells using viability and differentiation assays at different time points during this culture. Based on our results, we conclude that this new DN hydrogel has the potential to be used as an injectable cell carrier for repairing focal cartilage injuries using cell therapy.

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a91376

## 03-P183 Preventing infection while enhancing bone regeneration: development of a biofunctional electrospun membrane

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Preventing deep bone infections and enhancing bone regeneration reduces the risk of post-surgical complications. Surgeons currently employ nanoscale hydroxyapatite (nHA) pastes to stimulate bone regrowth, however, this has no innate antimicrobial properties. Deep bone infections are very challenging to treat due to the difficulty of achieving a suitable antibiotic concentration in the affected area; preventing a biofilm formation immediately after surgery is currently the best treatment option. Silver has long been used to successfully treat bacterial and fungal infections, but, it can present associated risks, being sometimes involved in the delay of tissue healing. Therefore, the aim of this research is to design an innovative bifunctional membrane with both intrinsic osteogenic and antimicrobial capabilities. For this, silver-substituted nHA will be incorporated into electrospun membranes to be tested against bacterial and mammalian cells.

Silver substituted nHA was produced using a modified rapid mixing wet precipitation method at 2, 5, 10 mol% silver. The nHA was added to a PCL solution for electrospinning over SLM formed metallic templates. Clinical isolates of *E.coli* and *S.aureus* were collected and tested against the membrane, using contact and diffusion tests (N=2, n=4). MSCs and fibroblast 3T3 cells were both used to study non-contact toxicity (N=4, n=3). Rat MSCs were used to measure contact toxicity and osteogenicity of membranes using confocal imaging, PrestoBlue®, DNA and ALP measurements (N=2, n=3).

SEM, TEM and EDS identified silver within the HA fibres. Diffusion and contact bacteria studies demonstrated reduced bacterial presence. Mammalian toxicity was observed for the highest silver content materials (10%) using rat MSCs, although no toxicity was found with fibroblasts. In contact toxicity, an increase in MSC activity was observed over the 21 day culture. MSCs cultured on samples containing nHA produced increased alkaline phosphatase levels, although no impact of the silver concentration on differentiation was observed.

This innovative silver nHA membranes significantly reduced *E. coli* and *S. aureus* bacterial populations while maintaining cytocompatibility with mammalian cells and enhancing the differentiation of MSCs into osteoblasts. This study demonstrated that silver nHA containing membranes have the potential to act as antimicrobials while stimulating bone tissue regeneration.

a91632

## 03-P184 Essentail culture environments to regenerate chondron based cartilage

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**Introduction:** In the native articular cartilage tissue, chondrocytes are surrounded by a pericullar matrix (PCM) and together form the chondron. PCM is rich in collagen II, proteoglycans and collagen VI as a main marker for PCM, and it is easily destroyed in dissection and in vitro culture. One hallmark of functional cartilage generated by tissue engineering approach is to rebuild chondron cell population in the tissue. The aim of this study is to define essential culture environment which can facilitate chondron formation.

**Methods:** Bovine chondrocytes from knee of 18-month-old cows and rat mesenchymal stromal cells (MSC) were extracted by established enzymatically protocols. Three culture conditions were compared; 2D versus 3D culture; basal medium versus chondrogenic medium culture; mono-chondrocyte culture versus co-culture with MSC at the ratio of 50:50. For 3D culture, $5x10^3$ chondrocytes were seeded in agarose hydrogel. The culture was up to 21 days. Biochemical analysis was conducted including GAG analysis, collagen type II and VI immunostaining.

**Result and Discussion:** 3D culture maintained the chondrocytes in round shape and expressed higher chondrogenic markers regardless of type of culture medium. 2D culture led to fibroblast morphology and collagen VI were not detected even with MSC co-culture, nor 3D culture by 7 day culture. GAG production was 22.8 pg/cell in basal; 26.0 pg/cell in chondrogenic media in 3D culture but 10.0 pg/cell in basal and 15.5 pg/cell in chondrogenic media in 2D culture. The addition of 50% MSCs increased the GAG production to 28 pg/cell in basal and 32.0 pg/cell in chondrogenic media in 3D culture and16.4 pg/cell in basal and 18.6 pg/cell in chondrogenic media in 2D culture. Collagen II expressed in all type of culture with highest sraining in 3D culture with 50% MSC addition. 3D culture by 21 day, intensive collagen VI has been detected regardless of type of culture medium. The 3D co-culture with 50% MSCs enhanced the collagen VI production; the expression intensity was 38.8 in basal and 39.6 in chondrogenic media in comparison to 37.24 and 35.31 without MSC group. Thus, 3D culture and addition of MSC play most important roles in chondron population regeneration.

a92434

## 03-P185 Vascularized bone tissue engineered system comprising autologous growth differentiation factors and mesenchymal stem cells

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Reconstruction of large bone defects still remaining a major clinical orthopedic challenge, since repair of a bone defect is not only the process of new bone formation, but also the formation of new blood vessels - angiogenesis [1]. Bone morphogenetic proteins (BMPs) and vascular endothelial growth factor (VEGF) are involved on cell differentiation and bone vascularization to construct viable osseous tissue [2, 3]. Therefore, herein is hypothesized that the synergistic effect of autologous BMP-2 and VEGF parallel bound over a single nanofibrous substrate (NFMs) can lead to a successful osteogenic and angiogenic differentiation of mesenchymal stem cells. To achieve such ambitious goal, an engineered system was developed comprising BMP-2 and VEGF antibodies immobilized over the same structure in side-by-side fashion, trying to recreate the vasculature of a bone tissue. Furthermore, immobilized antibodies were capable of selectively immobilize the respective growth factor from a biological fluid (i.e. platelet lysate). The osteogenic and angiogenic potential of this engineered biofunctionalized system was assessed by culturing human bone marrow mesenchymal stem cells (hBM-MSCs) during 21 days without exogenous induction. Bare NFMs cultured with hBM-MSCs under basal and standard osteogenic or angiogenic differentiation media were used as negative and positive controls, respectively. Biological data demonstrate that the engineered biofunctional NFM comprising BMP-2 is able to promote osteogenesis, whereas the VEGF is able to promote angiogenesis of hBM-MSCs. Moreover, the vascularized bone tissue engineered system proposed is able to promote spatial angiogenesis and osteogenesis of hBM-MSCs, targeting an effective vascularized bone tissue engineering approach.

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a93458

## 03-P186 Cell-Cell Interactions Enhance Cartilage Formation in 3D Gradient Hydrogels that Mimics Tissue Zonal Organization

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Cartilage tissue is characterized by zonal organization with gradual transitions of biochemical and mechanical cues from superficial to deep zones. Previous tissue engineering strategies mostly employ scaffold with homogeneous cues, which fail to mimic the zonal organization of native cartilage. We have recently developed hydrogels as 3D cell niche with continuous gradient of biochemical and mechanical cues, which induce zonal-specific response of chondrocytes to form cartilage that mimics articular cartilage organization. While the role of cell-ECM interactions have been studied extensively, how cell-cell interactions across different zones influence cartilage zonal development remains unknown. The goal of this study is to examine the role of cell-cell interactions in modulating cartilage formation in 3D using gradient hydrogels. When encapsulated in PEG/CS gradient hydrogels, chondrocyte exhibit zonal-specific responses in cell proliferation as well as cartilage ECM deposition. In contrast, when each zone is cultured separately, the extent of zonal-response of chondrocytes were much lower than intact gradient hydrogel group. Co-culturing of hydrogels cut into 5 zones in the same well led to comparable cellular response across all zones. We further evaluated the memory of cell-cell interactions by first priming chondrocytes in intact gradient hydrogels for one week, then divided into separate zones and cultured separately. Priming enabled memory of cell-cell interactions and restored the zonal response. Together, our results highlight that paracrine signaling influences how cells are sensing their environmental cues in forming cartilage zonal tissue. Such gradient hydrogels could facilitate employing heterotypic cell interactions to enhance regeneration of cartilage with biomimetic zonal organizations.

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a93462

## 03-P187 A Novel Method to Fabricate 3D Gradient Hydrogels with Clinically Relevant Dimensions for Cartilage Repair

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Osteochondral defects due to severe osteoarthritis remain a challenge for regenerative treatment, as native cartilage tissue is characterized with zonal organizations from hyaline cartilage to subchondral bone, with mechanical and biochemical cues transition in a gradient manner. However, current clinical treatments and tissue engineering therapies have failed to capture this phenotype and mimicking zonal organization is important for regenerating cartilage with biomimetic structures and functions. We have previously reported that stiffness gradient hydrogels can induce zonal-specific chondrogenesis of human mesenchymal stem cells (hMSCs) that mimic native organization of articular cartilage. The goal of this study is to demonstrate a novel method for fabricating gradient hydrogels at clinically relevant dimensions to regenerate cartilage that mimics native zonal organization. Specifically, we designed a dual-syringe based method that allows fabrication of customized sizing of hydrogels (from -mm to -cm) with mechanical (stiffness) and biochemical (chondroitin sulfate) gradient cues that support homogeneous cell encapsulation with high viablity, proliferation and extracellular matrix deposition in 3D over time. Compressive mechanical testing and sGAG assay confirmed successful formation of mechanical and biochemical gradients within hydrogels. Increasing hydrogel stiffness led to enhanced cartilage gene expression (aggrecan and type II collagen), increased cell proliferation, as well as markedly enhanced collagen deposition. The trend of resulting ECM deposition and cell morphology mimics the superficial to deep zones of native articular cartilage. The platform reported here offers an enabling technology for engineering tissues with zonal organizations and may offer a new form of cell-mediated scaffold repair of osteochondral defect.

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a90231

## 03-P188 Time-response mechanical properties of in-vitro matured articular cartilages using growth factors

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During the transition from infant to adulthood, articular cartilages in synovial joints undergo a process of maturation that enables the unstructured cartilage tissue of newborns to develop the appropriate biomechanical characteristics for normal function. One key reason for the sub-optimal performance of transplanted tissue for the treatment of focal joint defects is the inability of repair cartilage to adopt a mature, and therefore, durable phenotype. Growth factors have been shown to accelerate *in-vitro* maturation of articular cartilage such that the biochemical content, surface roughness and equilibrium mechanical properties match those of native mature cartilage, therefore providing a powerful and rapid approach to tissue engineer cartilage tissue. In this work, we further confirm the ability of fibroblast growth factor-2 (FGF2) and transforming growth factor beta-1 (TGFb1) on the time-dependent nanoscale mechanical properties (complex dynamic modulus and phase angle) of *in-vitro* matured cartilage using a novel rheometer based technique.Because of the complex structure, cartilage mechanical properties are closely described by the poro-elasticity model where the material response depends on the frequency of the applied stimulus. Rheology was employed to test the mechanical properties of cartilages during maturation at different frequencies simulating the range of solicitations applied on our joints in daily life. G' (storage modulus) and G" (loss modulus) along with the corresponding phase angle (d) and dynamic modulus G*, were determined at frequency ranging from 10Hz to 0.01Hz at different loads.We found that in-vitro maturation of cartilage replicates the changes characteristic of in-vivo maturation. The GAG content of surface and middle zones of immature, in-vivo matured and in-vitro matured cartilages was also determined and positively correlated to the mechanical properties of the samples and their change during maturation.

a91492

## 03-P189 Micro Channel Networks Improved the Cells Migration and Survive in Deep Zone of Collagen Scaffolds

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This paper reports the strategy for the fabrication of collagen-hydroxyapatite composite scaffolds using a 3D printing technique to achieve such a controlled architecture. The *in vitro* performance of the scaffold in terms of cells' viability, migration and proliferation behavior using human mesenchymal stem cells (hMSC) is reported. The purpose of this paper is to study the effect of micro-channels on the cell migration, proliferation and differentiation.

The collagen-hydroxyapatite composite scaffolds were fabricated by using an indirect 3D print technique. The process involves fabrication of a sacrificial negative mould, casting of the collagen/HA dispersion, remove the negative mould and dehydration process. The micro-structure of the resultant scaffolds have been characterised by micro-CT, SEM and FIB examinations, associated mechanical properties were investigated by dynamic mechanical analysis (DMA). The cell viability/proliferation was evaluated in vitro using human mesenchymal stem cells (hMSCs).

It was demonstrated that scaffold featured predefined internal microchannels and interconnected pores network. The matrix have a porosity of 92%, with pore sizes distributed in the range of 100~300 micro meter. The dynamic mechanical properties and biodegradation profile are adjustable by crosslinking treatment. The *in vitro* evaluation revealed that the micro-channels could be well preserved during cell culture of 8 weeks *in vitro*. The hMSCc cell can migrate into the scaffold, preferably through the micro-channels to proliferate and differentiate. A combination of histological examination (include Alizarin red and Alcian blue, and ALP staining) revealed bone and cartilage-like tissue formed after 8 weeks culture.

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a91514

## 03-P190 In vitro co-culture model of human osteocytes and osteoclasts in collagen gels modified with biomimetically mineralized collagen

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Osteocytes represent the majority of bone cells and have been postulated to orchestrate bone homeostasis by regulating both bone-forming osteoblasts and bone-resorbing osteoclasts. Due to their location deeply embedded into rigid bone mineral matrix detailed analysis of osteocyte function is challenging and suitable in vitro models are rare. Most studies involve murine osteocyte cell lines. The aim of the present study was to establish an in vitro co-culture model comprising primary human osteocytes, isolated from human trabecular bone and human osteoclasts differentiated from human peripheral blood mononuclear cells (PBMC). Isolation of primary human osteocytes is difficult due to the location of the cells inside the bone matrix. We optimized the isolation protocol of sequential collagenase and EDTA digestion steps by combining digestion with resting steps which increased yield of osteocyte cells. Isolated osteocytes showed dendritic morphology, positive staining for Dentin Matrix Protein 1 (DMP-1) and gene expression of osteocyte markers podoplanin/E11, DMP-1, sclerostin and osteocalcin. A suitable matrix for in vitro cultivation of osteocytes was developed by blending of collagen gel with biomimetically mineralized collagen prepared by synchronous fibrillation and mineralization. Resulting gels were resistant against cell-mediated shrinkage which is frequently observed in pure collagen gels. Human osteocytes embedded into the gels retained their osteocytic morphology and gene expression pattern. Different approaches were tested to include human osteoclasts into the 3D model which involved the development of a suitable co-culture medium. Stable 3D co-cultures of osteoclasts and osteocytes were obtained by transferring pre-differentiated multinucleated osteoclasts onto the surface of osteocyte-containing collagen gels. Establishment of a triple culture including also human osteoblasts will be the aim of further studies.Collagen gels blended with biomimetically mineralized collagen are suitable for in vitro co-cultivation of osteocytes and osteoclasts to study their interactions. This work was financially supported by the Deutsche Forschungsgemeinschaft (DFG) (BE 5139/3-1).

a92115

## 03-P191 Tissue engineered bone – from stem cells to osteocytes

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Bone tissue engineering (BTE) aims at recreating the bone microenvironment *in vitro* by providing stimulating factors promoting osteogenesis. One of the challenges is to induce the deposition of bone-like tissue comprised of both cellular (osteoblasts/osteocytes) and extracellular matrix (ECM, organic/inorganic material, i.e. collagen/hydroxyapatite) components. Especially osteocytes, which have important regulatory roles, have been challenging to study as current cell lines do not sufficiently represent the phenotypic features, and primary cells rapidly differentiate to osteoblasts upon isolation. We used dynamic culture conditions, employing fluid-induced wall shear stress, to stimulate bone tissue formation *in vitro* which differentiated human bone marrow stromal cells (hBMSCs) into osteoblasts and further into osteocytes.

Porous silk fibroin scaffolds were seeded with hBMSCs. Constructs were cultured in osteogenic medium under static or dynamic conditions in spinner flask bioreactors for 7 weeks. The constructs were analyzed using a large set of microscopic tools (µCT, SEM, 3D FIB/SEM, TEM, fluorescence), combined with FTIR. Sections were immunohistochemically stained for osteocyte markers (DMP-1, podoplanin, sclerostin).

Dynamically cultured constructs showed increased collagen and mineral deposition whereas statically cultured constructs failed to deposit ECM. Osteocyte markers could be seen in constructs cultured under dynamic conditions, however not in the static cultures. These findings were supported by µCT images in which mineralization could only be seen under dynamic culture conditions. 3D FIB/SEM images showed osteocyte-like networks and elongated cell processes surrounded by a collagen matrix in dynamically cultured constructs. The mineral was characterized as carbonated hydroxyapatite by FTIR. TEM images showed that the mineral was embedded within the collagen matrix.

Only under dynamic culture conditions, hBMSCs differentiated into osteocytes that were embedded within the collagen matrix. These results set the ground for the use of our 3D *in vitro* system to study the collagen deposition and mineralization process in high resolution and cryogenic conditions. Furthermore, our system allows investigating osteocyte formation *in vitro* - both cell differentiation and their embedding into their matrix. This model can advance current humanized 3D *in vitro* BTE systems to resemble natural bone more closely.

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a90762

## 03-P192 A composite oxygen-generating system for bone regeneration

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Clinically, insufficient blood perfusion after fracture and the resultant tissue hypoxia can lead to delayed bone healing or even nonunion. As a crucial molecule to life, oxygen is required in several cellular processes, such as aerobic respiration, enzyme activation, cell differentiation, collagen synthesis, that are involved in fracture healing. Therefore, a major hurdle in promoting bone regeneration is to provide sufficient oxygen to support the survival and function of the newly formed tissue. In this work, a composite oxygen-generating system consisting of bone morphogenetic protein (BMP)-2- conjugated gelatin sponge Spongostan™ and calcium peroxide ($CaO_2$)/manganese dioxide ($MnO_2$)-encapsulated poly latic-co-glycotic acid (PLGA) is developed as an implantable scaffold for improving local oxygenation and enhancing fracture healing. Once exposed to water, solid $CaO_2$ particles can generate hydrogen peroxide ($H_2O_2$), which can be further converted into oxygen under the catalysis of $MnO_2$. The oxygen release profile of the composite and variations in $H_2O_2$ concentration and pH value during oxygen generation are characterized. According to our in vitro results, the as-prepared composite scaffold can effectively relieve cellular hypoxia and promote the osteodifferentiation of mesenchymal stem cells and bone mineralization. A rat model of femoral defect will be employed to evaluate the capability of the as-prepared oxygen-generating composite graft in promoting bone healing in the future.

a91624

## 03-P193 Biomimetic matrix-based cues for re-differentiation of chondrocytes

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The native extracellular matrix (ECM) of cartilage tissue could modulate cell-cell and cell-matrix interactions of chondrocytes to maintain their phenotypes and cartilage tissue homeostasis. However, due to the intrinsic nature of cartilage tissue, its regeneration potential is limited and cells presented within cartilage tissues undergo dedifferentiation during *in vitro* expansion. Here, we aim to develop a biomimetic matrix to support in vitro expansion of chondrocytes while maintain their phenotypes by employing various biomimetic matrix-based cues, such as matrix rigidity, surface charge density, surface roughness, and internal pore structures. Our findings reveal that synthetic matrix could support initial attachment, their round cell morphology, and native extracellular matrix productions. Such a biomimetic matrix that is easy to synthesize and cost-effective can offer an ideal tool to promote the outcomes of cell-based therapies.

a93515

## 03-P194 Creation of a 3D Bone Tissue Model to Study Bone Loss in Microgravity

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INTRODUCTION: Osteoporosis is a major health burden affecting millions worldwide. The extreme environment on the International Space Station (ISS) offers a unique model of accelerated bone loss (3 months in microgravity cause the same level of bone loss as that observed over about 5 years on the ground). We aim at engineering a veritable *in vitro* 3D human bone tissue model to study mechanisms of osteopenia in microgravity with the ultimate objective of improving health both in space and on the ground.

METHODS: We validated our α-TCP scaffold [1], by seeding on a disc human mesenchymal stem cells (hMSCs) (osteoblasts progenitors, OB) and monocytes (osteoclasts progenitors, OT) and assessing: viability, cytoskeletal structure, differentiation (RUNX2, BSPII, OPN, ALP, OPG, RANKL, TRAP, Cathepsin K, MMP-9), and baseline effluent content (alkaline phosphatase, Cathepsin K, MMP-9, RANKL, TRAP, OPG). Uniform 200-250μm diameter paraffin microspheres were used as porogen to generate by paraffin leaching (heating at 120C followed by Histoclear washes) 3D porous scaffold which were seeded with pre-differentiated OB and monocytes, cultured 7 to 14 days in a 50:50 osteogenic and osteoclastogenic media mix in static culture and within a rotatory cell culture system (RCCS TM-4; Synthecon Inc., Houston, TX, USA) to simulate microgravity.

RESULTS: Cells adhered, were viable, and proliferated well onto α-TCP scaffolds. Quantitative RT-PCR evidenced OB/OT differentiation. The porous, interconnected scaffolds could be seeded with pre-differentiated OB and monocytes, that successfully differentiated in the course of the test. Culturing in the RCCS system enhanced both OB and OT differentiation.

DISCUSSION: We achieved an engineered *in vitro* model of bone that could be used to model the response to microgravity. The scaffold supported osteogenesis and osteoclastogenesis and is amenable to generate 3D constructs that fit in our previously developed bioreactor [2,3] which is a closed system, gravity-independent for its operation, that could be deployed on the ISS. In simulated microgravity, activity of both OB and OT increased as reported by animal studies *in vivo* and on cells in monolayer culture on the ISS. Our *in vitro* model can already be considered viable for deployment on the ISS.

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a90213

## 03-P195 One Strike Loading Organ Culture Model to Investigate the Early Stage Degenerative Disc Disease Condition

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**INTRODUCTION**:

The underlying pathophysiology of Intervertebral disc (IVD) degeneration, especially in the early stage, is not clearly understood yet. High impact mechanical injury plays a major role in the initiation of IVD degeneration; reproducible in vitro models to elucidate disease mechanisms and identify therapeutic targets are an unmet need. The present study used an organ culture model to investigate the catabolic effect of one strike loading on whole IVDs.

**METHODS:**

Bovine caudal IVDs were cultured under one of 2 loading conditions: 1) Control group under physiological sinusoidal dynamic loading (0.02-0.2 MPa, 0.2 Hz, 2 hours/day); 2) Model group with one additional single axial strike, at displacement of 50%-disc height in 1 second. After 1 and 7 days, disc dynamic compressive stiffness was measured. The gene expression in the nucleus pulposus (NP) and annulus fibrosus (AF) tissue was analysed. The glycosaminoglycans (GAG) release into culture medium were quantified. The tissue structure was assessed using Safranin O/Fast Green staining.

**RESULTS:**

The disc tissue softened on day1 after one strike loading, indicated by lower dynamic compressive stiffness compared to the control group ($n=3$, $p<0.05$). On day1 after one strike load, the gene expressions of MMP1, MMP3, ADAMTS4, ADAMTS5, and IL-6 were up-regulated in NP but not AF of the model group compared to the control group ($n=8$, $p<0.01$). On day7 after one strike load, the gene expressions of MMP1 and ADAMTS5 in AF but not in NP of the model group were up-regulated ($n=14$, $p<0.05$). One strike loading induced higher GAG release into the culture medium ($n=3$, $p<0.05$). More disorganized tissue in the NP and increased distance between the AF lamellae was observed in the model group.

**DISCUSSION:**

One-strike degenerative disc model was developed to investigate the early changes in the degenerative process. NP was found to be first affected after exposure to the axial force. The response within the AF appeared one week later. After injury, the MMP and ADAMTS families were up-regulated in both NP and AF, which may be the initiators of disc degeneration processes. Increased GAG release into the culture medium in combination with the histological observations indicated break down of extracellular matrix after one-strike loading. This early-onset disc degeneration model has the potential for investigation of the degeneration mechanism and screening of new therapies for early intervention of degenerative disc disease.

a91243

## 03-P196 Effect of orientation on osteochondral plugs in a biotribological test system

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**Introduction**

Autologous osteochondral transplantation utilizing autografts is a long-standing treatment method for small-to-medium defects occurring in knee and ankle joints. Alignment of the autografts onto the defect site remains a pivotal aspect of reinstating joint biomechanical and biotribological properties towards successful autograft integration. This study aimed to elucidate the effects of bilateral orientation of bovine osteochondral plugs in an established biotribological test system.

**Methods**

For the in vitro study bovine osteochondral plugs from 8 different animals were punched out from the medial condyle and marked for their alignment within the host tissue. Osteochondral plugs were then tested in a cartilage-on-cartilage setting using a developed biotribological test system. The grafts were either aligned (uni) or rotated 90° (cross), simulating wrong orientations that occur during a surgical procedure. Tests were performed for a cycle of 2 hours with alternating 10 minutes stimulation and 10 minutes pause, followed by analyzation of biomechanical and molecular biological parameters.

**Results**

Based on the characterization of the surface cracks appeared in the uni as well as cross orientated osteochondral plugs with no differences in coefficient of friction values. Gene expression analysis showed a significantly higher expression of COL2A1 and ACAN in both treated groups compared to a control group, but no differences between aligned or 90 ° -rotated grafts. Also, the metabolic activity showed no difference between the orientations, but a significant decrease compared to the control group. The release of sulfated glycosaminoglycans during biotribological tests showed slight differences between the two treated groups. Histological analysis revealed different staining intensity in plugs from different points of isolation from the used medial cartilage.

**Discussion**

Using osteochondral plugs in a cartilage-on-cartilage biotribologicial system can be very challenging regarding sample size and surface flatness. In our study we only observed significant differences between tested and non-tested osteochondral plugs, but not between uni- and cross-orientated ones. We could infer from the methods deployed that the orientation of cartilage surfaces against each other does not make a difference.

a91595

## 03-P197 Optimising sintering process of porous Apatite-Wollastonite scaffolds to replicate bone structure

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Osteoarthritis is a musculoskeletal condition that affects the smooth cartilage lining of the joint in around 10 million people in the UK, and drug companies aim to find the most effective test with minimal costs to trial their developed medicine for patients. We propose innovative techniques to create an in vitro osteochondral model for initial drug testing. The progression of Osteoarthritis is generated by producing in vitro bone and cartilage tissue. This is a prelude to developing processes for the production and maintenance of in-vitro of bone-cartilage models.

The bone element of this model was produced by developing porous scaffolds using the glass ceramic (Apatite-Wollastonite) using Thermally Induced Phase Separation (TIPS) technique. The structure of scaffolds is vital in generating the final tissue. We generated 3D scaffolds of different patterns and porosities by exploiting different sintering temperatures. Apatite-Wollastonite (AW), provides a bioactive surface for cell attachment, the scaffolds were produced with interconnecting pores throughout which encourages cell adhesion and proliferation. Our results show that AW provides an encouraging platform for bone tissue engineering applications. To optimize the scaffold porosity, mechanically capabilities and bioactivity, different sintering temperatures were explored and optimised. All different temperatures have profound effects on the pore size, shrinkage of the scaffold, crystal growth, and necking between the AW particles, which also affects mechanical stability as well as bioactivity.

Our experimental results show the pore sizes decrease when the temperature increases, this is especially prevalent after 1250. There are minimal pores visible at 1260 and the scaffold is dense with excessive shrinkage from its initial structure. Pore size and distribution is relatively similar at 1240, 1245 and 1250, with varying crystal growths, this can cause differences in bioactivity and cell adhesion as well as mechanical stability. The fine line between mechanical stability, bioactivity and pore distribution is one that is explored thoroughly and developed an extensive method for sintering to acquire the best AW porous scaffolds for bone formation. We are in the process of 3D printing cells and growing tissue on these scaffolds, the results will be included in the final paper and presentation.

a92191

## 03-P198 Bacteria for regenerative medicine

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Use of materials is attractive in stem cell cultures as surface area can be scaled up using 3D printing or fibrous hydrogels. Materials can be tuned to deliver specific, and even dynamic, biological cues to stem cells. However, they are still very limited as stem cells are regulated by a complex biological milieu.

We present a novel approach using simple cells, bacteria, as a substrate to influence mesenchymal stem cells in a facile and temporal manner. *Lactococcus lactis* spontaneously develops biofilms on a variety of surfaces (e.g polymers, metals) and can be genetically modified to produce a variety of probiotic and efficacious proteins. Here we show that controlled expression of fibronectin fragments [1, 2] supports growth and temporal regulation of secreted bone morphogenetic protein 2 drives osteogenesis in an on-demand manner.

The fibronectin fragment allows mammalian cell adhesion through integrins and cells show the same behaviour as when seeded over fibronectin coated surfaces. Moreover, the direct contact of the mammalian cells with the bacteria allows for instantaneous delivery of the growth factor bone morphogenetic protein 2, a known and potent osteogenic inducer. The combination of these proteins has displayed stem cell differentiation in the short, mid and long term that is equivalent to that of 100 ng/mL of exogenous addition of bone morphogenetic protein 2; creating a new paradigm in surface engineering for regenerative medicine. The system also has the potential to express a variety of proteins in order to tackle a number of different therapeutic problems.

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a92711

## 03-P199 Assessment of the effects of follistatin on key processes in bone formation and in vivo defect repair

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Follistatin (FST) is a protein able to bind to activin A, neutralizing its inhibitory effects on bone formation. There are two FST variants (FST288 and FST315) with major differences in their ability to bind to cell surface proteoglycans, which may influence their effect in bone repair. We investigate the effect of FST in critical processes for bone repair, such as cell recruitment, osteogenesis and vascularization, and ultimately its use for bone tissue engineering (TE) by loading both variants in an *in situ* gelling formulation made by alginate and recombinant collagen-based peptide microspheres and implanted in a calvarial defect model.

FST was added at a dosage range (25-175 ng/ml) to study its effect on: migration of human MSCs and ECs, vascularization using tube-formation and spheroid-sprouting assays upon ECs, and osteogenic differentiation, for which MSCs and pre-osteoblasts were cultured in osteogenic differentiation medium with FST. The release profile of both FST variants from the alginate-based hydrogel was studied over 4 weeks. For *in vivo* studies FST315 and FST288 were loaded in the formulation and implanted in a calvarial defect model. Rats were scanned biweekly for 10 weeks. Samples were processed for histological evaluation. Linear mixed model, Student's T-test and two-way analysis of variance were applied.

FST stimulated MSC migration at all doses tested compared to controls ($P<0.01$) and EC migration at 70 ng/ml ($P<0.05$). FST promoted tube-formation and sprouting at 28 ng/ml ($P<0.05$). FST did not enhance osteogenic differentiation of MSCs, but increased pre-osteoblast mineralization at all tested doses ($P<0.01$). Most of the loaded FST315 was released over 4 weeks, contrary to FST288, which was mostly retained in the formulation ($P=0.0003$). However, none of the FST variants improved bone repair in calvarial defects compared to control.

FST has chemoattractant effects upon MSCs and ECs and promotes essential steps in the formation and expansion of the vasculature. FST stimulates committed osteoblasts mineralization, suggesting that its use to enhance mineralization is sensitive to which differentiation stage the osteoprogenitor cells are. Timing of secretion of FST during bone repair seems crucial and its use in a late release alginate-based system did not improve bone repair. We have shown that FST enhances crucial processes needed for bone repair. More studies are needed to investigate the optimal FST carrier for bone TE applications.

a92505

## 03-P200 In Vitro Comparison of Clinically Applied Biomaterials for Autologous Chondrocyte Implantation (ACI)

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**OBJECTIVE:** In ACI treatment there is no consensus on biomaterials used with respect to their optimal cell/matrix interactions. The aim of this study was to perform a comparative analysis of two clinically applied biomaterials in terms of: (1) Cell viability; (2) chondrogenic potential; (3) bio-adhesion and (4) lateral integration.

**METHODS:** Monolayer expanded (P2-3) human chondrocytes (n=3) were embedded in a porous type 1/3 collagen scaffold (CS) and an albumin-hyaluronic acid-based hydrogel (AH) (TETEC AG, Reutlingen, Germany). Cells were cultured in chondropermissive medium supplemented with and w/o IL-10 [100pg/ml] and BMP-2 [250ng/ml]. Gene expression of chondrogenic markers (COL1A1, COL2A1, COL10A1, ACAN, SOX9, TGF-b1) was detected via RT-qPCR and TGF-b1 release was quantified by ELISA. Biosynthesis of matrix compounds was analysed histologically (toluidine blue, type 1/2 collagen) and by DMMB assay. Morphological changes and cell viability were investigated by confocal laser-microscopy following phalloidin staining, and Live/Dead staining, respectively. In addition, cell invasion from surrounding native cartilage and adhesion of the material to adjacent cartilage were recorded by microscopy and a push-out test, respectively.

**RESULTS:** The changes of mRNA expression and GAG production indicated marked chondrogenic re-differentiation in both biomaterials. Shift of COL1/2 ratio towards COL2A1 was more pronounced in AH. In this context, chondrocytes cultured in AH showed a more spherical morphology and less microfilament expression compared to CS. BMP-2 and IL-10 significantly increased COL2A1, SOX9 and ACAN expression, which was paralleled by enhanced staining of GAGs and type 2 collagen in histological sections of CS and AH. None of the biomaterials showed mRNA expression of COL10A1. Better interfacial integration and enhanced cell invasion was observed in CS compared to the AH. Push-out test showed higher bonding strength to native cartilage for the CS.

**DISCUSSION:** A trend for more chondrocyte-like phenotype was identified in the AH-based hydrogel, while collagen scaffolds showed an advantage in terms of cellular invasion and interfacial adhesion. Both biomaterials demonstrated favorable chondrogenic properties in vitro with no superiority of one compared to the other with a significant increase upon addition of BMP-2 and IL-10.

a90350

## 03-P202 Development of hydrogels derived from demineralised and decellularised human bone extracellular matrix

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Decellularised tissue has been noted as a promising biological material for tissue regeneration. Its central feature is the elimination of the cellular components from the native tissues while still retaining the complex compositions and architecture of extracellular matrix (ECM) [1]. While decellularised ECM hydrogels from bovine and porcine bone tissues have been used previously in the field of tissue regeneration [2], to date, no studies have sourced or evaluated decellularised matrices from human bone. The objective of this study was to evaluate the hypothesis that physiochemical and biological properties of ECM hydrogel derived from demineralised and decellularised human bone matrix could be controlled through modulation of bone powder size and digestion time. We collected trabecular bone from human femoral head samples following hip arthroplasty and prepared demineralised human bone powders at various sizes (45-250 $\mu$m, 250-1000 $\mu$m, and 1000-2000 $\mu$m). After decellularisation, the powders were treated in pepsin solution for 3, 5, and 7 days. The concentration of proteins and gelation strength significantly increased as bone powder size decreased and digestion time increased. Evaluation of cultured human bone marrow-derived stromal cells on the ECM hydrogel derived from 45-250 $\mu$m bone powder size and treated for 7 days exhibited the most cell proliferation and lineage differentiation. In summary, a defined digestion time and powder size are crucial factors for generating demineralised and decellularised human bone ECM hydrogels that simultaneously have a high concentration of proteins and the ability to produce a hydrogel easily injected into bone defects of any shape. Taken together, these two properties make hydrogels derived from demineralised and decellularised human bone ECM a potent material to be used in localized bone regeneration.

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a91565

## 03-P203 Vitreous Humor as an Extracellular Matrix Hydrogel for Cartilage Tissue Engineering Applications

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Decellularisation of tissues, utilising their inherent biochemical cues, poses exciting tissue engineering (TE) opportunities. However, removing DNA from cartilage (dCart) requires harsh treatments due to its dense structure, causing loss of bioactivity and limiting its application as a cartilaginous extracellular matrix (ECM)(1). The aim of this study was to investigate if intact and un-modified vitreous humor (VH), a highly hydrated tissue closely resembling the glycosaminoglycan (GAG) and collagen composition of cartilage(2,3), can be applied as a novel cell-instructive ECM hydrogel for cartilage TE. DNA removal, retention of ECM components, cytocompatibility and chondrogenic differentiation were evaluated.

Equine VH was extracted followed by GAG (DMMB), total protein (PierceTM) and DNA (CyQUANT) quantifications and histological examinations (picrosirius red/alcian blue, collagen-I/II). Human mesenchymal stromal cells (hMSCs) and U937 cells were cultured in the presence of VH to study cytotoxicity and inflammation (ISO10993: cell-metabolism, -attachment and -morphology). VH hydrogels were seeded with human articular chondrocytes (hACs, 0.25-0.75x$10^6$ cells/50µl VH) and cultured (TGF-$\beta$) with standard pellet controls (0.25x$10^6$ hACs)(4) for 3w to study chondrogenesis. Live/dead®, metabolic activity (alamarblue®), GAG and DNA were quantified and matrix deposition was visualised using immunohistochemistry (Safranin-O, collagen-I/II).

VH was successfully extracted, exhibiting negligible amounts of DNA (0.4±0.4µg/mg dw) compared to reported dCart (23.4±33.3µg DNA/mg dw), and notable, preservation of ECM components (156±90µg GAG, 68±21µg protein). MSCs and U937 cultured with VH displayed neither a cytotoxic nor inflammatory response, and notably enhanced MSC proliferation. Interestingly, encapsulated hACs self-assembled the VH hydrogel into spheroids, displaying uniform distribution of GAGs and collagen type II, with significantly greater GAG/DNA (0.75x$10^6$ cells: 21.5 ± 5.2 g/g, 0.25x$10^6$ cells: 14.1 ± 2.5 g/g) compared to pellet controls (7.0 ± 1.3 g/g). This study demonstrated that VH can be favourably extracted and used for cell encapsulation, posing as a permissive source to fabricate cartilaginous ECM hydrogels for TE applications.

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a91741

## 03-P204 Superior Calvarial Bone Regeneration using Pentenoate-Functionalized Hyaluronic Acid Hydrogels with Devitalized Tendon Particles and application to Traumatic Brain Injury

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Traumatic brain injury (TBI) is a life-threatening condition defined by internal brain herniation. Severe TBI is commonly treated by a two-stage surgical intervention, where decompressive craniectomy is first conducted to remove a large portion of calvarial bone and allow unimpeded brain swelling. In the second surgery, spaced weeks to months after the first, cranioplasty is performed to restore the cranial bone. Hydrogels with paste-like precursor solutions for surgical placement may potentially revolutionize TBI treatment by permitting a single-stage surgical intervention, capable of being implanted with the initial surgery, remaining pliable during brain swelling, and tuned to regenerate calvarial bone after brain swelling has subsided. The current study evaluated the use of photocrosslinkable pentenoate-functionalized hyaluronic acid (PHA) and non-crosslinking hyaluronic acid (HA) hydrogels encapsulating naturally derived tissue particles of demineralized bone matrix (DBM), devitalized cartilage (DVC), devitalized meniscus (DVM), or devitalized tendon (DVT) for bone regeneration in critical-size rat calvarial defects. All hydrogel precursors exhibited a yield stress for placement and addition of particles increased the average material compressive modulus. The HA-DBM (4-30%), PHA (4%), and PHA-DVT (4-30%) groups had 5 ($p < 0.0001$), 3.1, and 3.2 ($p < 0.05$) times greater regenerated bone volume compared to the sham (untreated defect) group, respectively. *In vitro* cell studies suggested that the PHA-DVT (4-10%) group would have the most desirable performance for bone regeneration and future application to TBI. Overall, hydrogels containing DVT particles outperformed other materials in terms of bone regeneration *in vivo* and calcium deposition *in vitro*. Hydrogels containing DVT will be further evaluated in future rat TBI studies.

a91297

## 03-P205 Fractionated human adipose tissue as a native biomaterial for the generation of a bone organ by endochondral ossification

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Many steps are required to generate bone through endochondral ossification with adipose mesenchymal stromal cells (ASC), from cell isolation to *in vitro*, monolayer expansion, seeding into scaffolds and cartilaginous differentiation. Moreover, monolayer expansion and passaging of ASC strongly decreases their differentiation potential. Here, we proposed a novel approach based on adipose tissue as scaffold with cell expansion, matrix formation and chondrogenic differentiation directly inside native adipose tissue. Human liposuctions were fractionated and cultured for 3 weeks. The resulting constructs, named Adiscaf, were compared to constructs generated with a standard approach, i.e. collagen sponges seeded with monolayer-expanded ASC. After 4 weeks of chondrocytic differentiation, Adiscaf contained cartilage tissue, characterized by glycosaminoglycans and collagen type II. After 2 additional weeks of hypertrophic differentiation, Adiscaf showed upregulation of hypertrophic markers at the gene expression and protein levels. After 8 weeks of *in vivo* implantation, Adiscaf resulted in ectopic bone tissue formation, including bone marrow elements. Adiscaf showed superior *in vitro* differentiation and *in vivo* performance as compared to the standard paradigm involving isolation and monolayer expansion of ASC. We showed that our new paradigm exploit the physiological niche of adipose tissue in order to better maintain the functionality of cells during their *in vitro* expansion. This study demonstrates that adult human adipose tissue used as a native construct can generate a bone organ by endochondral ossification, introducing a novel concept in the generation of osteogenic grafts for bone repair.

a93004

## 03-P206 Thermally Responsive and Tissue Adhesive Hydrogel for Osteochondral Tissue Regeneration

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In this study, we investigated the feasibility of incorporating chondroitin sulfate (CS), a sulfated glycosaminoglycan, into a network of (*N*-isopropylacrylamide) (PNiPAAm)-based thermogelling macromer that contains pendant epoxy groups for crosslinking, and hydrolysable lactone ring for excretability. Two different modifications of CS were synthesized separately, one with adipic acid dihydrazide (ADH), and the other with N-hydroxysuccinimide (NHS), and were combined to form a stable CS network via hydrazide-NHS ester reaction. Unreacted ADH groups on the CS network could then covalently react with PNiPAAm via hydrazide-epoxy reaction, thus forming a non-shrinking, thermally responsive hydrogel at 37 ° C. In addition, excess NHS groups in the bulk hydrogel provided tissue adhesive properties. Both swelling and mechanical properties of the hydrogel were varied by changing the degree of CS modification, as well as the ratio between PNiPAAm and modified CS chains. Leachable products were shown to be cytocompatible regardless of the total amount of CS in the hydrogel, and modification degrees of CS chains. Altogether, this work establishes an injectable, *in-situ* crosslinking hydrogel system derived from biological and synthetic components that has potential application in osteochondral tissue regeneration.

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a92620

## 03-P207 Effect of Capacitively Coupled Electrical Stimulation Over Growth Plates of Rat Chondroepiphysis Explants

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The growth plate is a cartilaginous layer present from the gestation period until the end of puberty where it ossifies joining the diaphysis with epiphysis. During this growth period several endocrine, autocrine, and paracrine processes are presented within the growth plate. The molecular regulation within this cartilaginous tissue is carried out by chondrocytes, cells synthetize the characteristic molecules of the epiphyseal plate. A disruption in chondrocytes functions may lead to different pathologies that affect normal long bone development. Due to biophysical stimuli have directly influence in cell deformation, nutrient concentration gradients and stimulation of molecular synthesis, this work presents a novel framework to assess histomorphometrically the effect of external electric fields over chondroepiphysis explants. Bones were stimulated with 3.5 and 7 mV/cm, and for each of these electric fields, two exposure times were tested for 30 days (30 minutes and 1 hour). Results evidenced that electric fields increased the pre-hypertrophic and hypertrophic zones compared with controls. In addition, an electric field of 3.5 mV/cm applied for 1 hr maintain stable the growth plate zones. It was also evidenced that electric fields stimulated chondrocytes to maintain stable the columnar cell density and its orientation during culture. Additionally, it was shown that electrical stimulation induces chondrocytes to enter in a pre-hypertrophy state in the center of the chondroepiphysis. These findings allow to enhance the *in vitro* procedures either inducing changes in growth plate morphology or stimulating chondrocyte molecular synthesis to modify the morphophysiology of chondrocytes.

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### Conflict of Interest:

The authors declare that they have no conflict of interest.

a92239

## 03-P208 Enhanced wettability and improved mineralization on zwitterionic polymer brush grafted titanium surfaces

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Titanium and its alloys are the most widely used implant materials in medical applications because of their unique mechanical properties and corrosion resistance. The use of titanium as an implant material usually goes hand in hand with aging, dental disorders, accidents and sport injuries worldwide. Mechanical properties such as Young's modulus, fatigue strength and corrosion resistance of titanium is similar to the bone but also modification of titanium surfaces is crucial for interaction of cell at the Ti-bone interfaces. Surface properties of titanium such as wettability, protein adhesion, surface potential and topography had effect on the Ti-bone interactions. Although titanium implants is biocompatible, implantation of titanium implants may cause inflammation response. This kind of inflammation response has adverse effects on the patients and reduce the patients' comfort. Thus it's very important to examine the titanium surface properties because of their maximum tissue-implant compatibility. Thanks to its extremely high wettability and mineral inducing character, zwitterionic biocompatible phosphorylcholine was chosen as grafting agent on Ti substrates.

The surface-attachable initiator, were synthesized by w-undecylenyl alcohol, 2-bromoisobutyryl and trichlorosilane. The water-soluble ATRP initiator, oligomeric methoxy poly(ethylene glycol) 2-bromoisobutyrate (OEGBr), was synthesized by methoxyoligo(ethylene glycol and 2-bromoisobutyrl bromide. Surface initiator was attached onto titanium coupons with –OH functional groups and polymerization process were carried out.

Cu(I)Br, bpy, MPC was with OEGBr initiator was mixed together and was added into three-necked flat bottom flask which is containing titanium coupons. Reaction was carried for about 24h. at 20° C.

The wettability properties of titanium substrates were found to be enhanced after p(MPC) grafting. Water contact angle decrease was statistically significant respect to the untreated titanium surface ($p<0.05$). The most effective parameters were grafting density and brush layer thickness. The deposition of calcium apatite was also increased and both analysed by visual and analytical measurements. p(MPC) brush grafting on Ti surfaces enhanced the both wettability and apatite deposition *in vitro* and this approach can be applied to any bone-contacting Ti implants with various geometries.

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a90711

## 03-P209 Preclinical Studies of Alveolar Bone Formation Promoted by MicroRNA-200c and a New MicroRNA-200a Inhibitor in Sockets after Tooth Extraction

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Our previous studies have revealed that plasmid DNA encoding miR-200c and our new miR-200a inhibitor (PMIS-miR-200a) promote osteogenic differentiation and bone regeneration in vitro and in vivo. The present project tests the safety and efficacy of the application of plasmid miR-200c and PMIS-miR-200a for the promotion of alveolar bone formation in sockets after tooth extraction using a canine model. **Methods:** Collagen sponges were loaded with different dosages of plasmid DNA encoding miR-200c and PMIS-miR-200a and were implanted into the distal sockets of mandibular premolars in 1 year-old Beagle dogs. Empty sockets and collagen sponges loaded with empty vectors served as control therapies. All animals were euthanized 4 weeks post-operatively. Bone healing response in all treated sockets was analyzed with μCT and histomorphometry. The expression of systemic inflammatory biomarkers and liver toxicity was assessed weekly. **Results:** No clinical signs of inflammation at surgical sites were observed during the experimental period. Quantitatively, bone volume in the sockets treated with miR-200c and PMIS-miR-200a was significantly higher as compared to sites that received the sponge alone or the sponge loaded with empty vectors. There was no increase in the expression of proinflammatory biomarkers and indicators of liver toxicity. **Conclusion:** Plasmid encoding miR-200c and PMIS-miR-200a safely and effectively promoted bone formation in the sockets after tooth extraction in a preclinical animal model, which is indicative of the potential of this therapeutic strategy for oral and craniofacial bone regeneration.

a92277

## 03-P210 Engineered microenvironments for efficient regeneration of bone critical-size defects

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Material-based strategies seek to engineer synthetic microenvironments that recapitulate the characteristics of physiological extracellular matrices for applications in regenerative therapies, including bone repair and regeneration. In our group, we have developed a new technology using materials that promote fibronectin assembly to recruit and present growth factors (GF) in combination with the integrin binding domain of FN during bone tissue healing.

Fibrillar conformation of FN adsorbed on poly (ethyl acrylate) (PEA) favours the simultaneous availability of the GF binding domain (FNIII12-14) next to the integrin binding region (FNIII9-10), compared to standard polymers such as poly(methyl acrylate) (PMA), a material with similar chemistry to PEA, but where FN adopts a globular conformation. The crosstalk between integrins and GF receptors improves the osteogenic differentiation of mesenchymal stem cells (using BMP-2) and the vasculogenic response of human endothelial cells (using VEGF). The potential of this system as recruiter of GFs was investigated in a critical-size bone segmental defect in a mouse model. The synergistic integrin-GF signalling, induced by fibrillar FN, promoted bone formation and enhanced vascularisation *in vivo* with ultra-low doses of GFs compared to current advanced technologies[1,2]. Furthermore, we optimized the system for its potential use in translational research, seeking to address the clinical need of using biocompatible and biodegradable material implants. This allowed us to apply the technology to material systems with different geometries, including allogenic bone chips that were coated with a thin layer of plasma-polymerized PEA, which recruits and efficiently presents GF during healing of critical size defects. This technology, based on growth factor functionalised coatings, provides a new strategy to efficiently reduce the GF doses administrated in bone regenerative therapies and has been recently used to treat successfully a first veterinary patient.

a92630

## 03-P211 Early Weight-Bearing Improves Cartilage Repair in an *In vitro M*odel of Microfracture Comparison with Two Mechanical Loading on Fibrin Gel Scaffolds Containing Bone Marrow Mesenchymal Stem Cells

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**Background:** Postoperatively clinical failures and functional decline of microfracture are seen, because of lesser the mechanical property as well as the integration with the surrounding tissue. In rehabilitation after microfracture, it remains unclear how the loading regimens affect the new fibrocartilaginous tissue formation.

**Purpose:** To assess the influence of different mechanical loading regimens simulating weight bearing or passive motion exercises on an in vitro mimic of microfracture repair based on fibrin gel scaffolds containing mesenchymal stem cells (MSCs).

**Methods:** Bone marrow MSCs from the trabecular bone of juvenile bovine was seeded into fibrin gel scaffolds and filled to cylindrical core in bovine hyaline cartilage constructs. These were divided into 3 groups and applied by the different loading (free swelling, dynamic compressive loading, rotating shear loading) in chondrogenic medium with 10 ng/ml TGF-$\beta$3. On day 7, 14 and 21, the integration strength between outer chondral plug and the central core was measured with electro-force mechanical tester. After testing, central core samples were collected and analyzed by real-time RT-PCR, glycosaminoglycan and PicoGreen assay, and histology.

**Results:** Integration strengths between host tissue and the fibrin gel containing BM-MSCs group significantly increased for compressive loading regimes compared to control (P=0.007). Expression of chondrogenesis-associated genes (SOX9, collagen type II, and COL2/COL1) of the compressive loading group was significantly higher at day 21 compared with the free swelling group (P=0.0032, P <0.0001 and P = 0.0308). However, passive motion group was not significantly different and furthermore, the catabolic maker (MMP-3, ADAMTS 5) was significantly up-regulated in this group (P= 0.0234 and P <0.0001) at day21 compare with control.

**Conclusion:** Dynamic compressive loading on an in vitro mimic of microfracture has not only positively affected the generation of more hyaline cartilage, but also yielded higher integration strength.Taken together,early load bearing after microfracture could be beneficial in promoting the formation of more hyaline-like cartilage repair tissue and ROM exercise by CPM without weight bearing yield negatively affect to new formation tissue in knee joint during rehabilitation after surgery.

a92847

## 03-P212 Joint-preserving regenerative therapy using injectable rhFGF 2 for patients with early stage of osteonecrosis of the femoral head

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**Background:** For many years, minimally invasive joint-preserving regenerative therapy has been desired for the early stages of osteonecrosis of the femoral head (ONFH). The purpose of this study was to evaluate the safety and clinical outcomes of a single local administration of recombinant human fibroblast factor (rhFGF)-2-impregnated gelatin hydrogel for the precollapse stage of ONFH.

**Methods:** Ten patients with femoral heads up to precollapse stage 2 underwent a single local administration of 800-μg rhFGF-2-impregnated gelatin hydrogel and were followed up. Primary outcomes included adverse events and complications. Secondary outcomes included changes in Harris Hip Scores, visual analog scale pain scores, UCLA activity rating scores, radiological changes as determined via radiographs, computed tomography scans, and magnetic resonance imaging of the hip joint.

**Results:** All Adverse events were recovered without problem. The surgery was performed with a minimally invasive technique-based core decompression (1 cm of skin incision), and walking was allowed from the day after surgery. Mean clinical scores improved significantly after four years compared with before surgery (before vs. after: VAS for pain, 21.2 vs. 5.3 mm; UCLA activity score 5.5 vs. 6.6; HHS, 81.0 vs. 98.4 points, respectively). There was only one case of femoral head collapse, and it had the greatest necrosis volume fraction and was considered to be in the early collapse stage at the time of operation. The other nine cases did not involve ONFH stage progression, and collapse was prevented. CT images and recent MRI 4 years postoperatively confirmed bone regeneration and reduction of the necrotic area.

**Conclusions:** Clinical application of rhFGF-2-impregnated gelatin hydrogel for patients with precollapse stage of ONFH was feasible and safe.

a90163

## 03-P213 Osteoconductive microarchitecture realized by additive manufacturing

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The current gold standard bone substitute is still autologous bone, despite the fact that its harvest demands for a second operation site, causes additional pain, discomfort, potential destruction of the grafting site, and is limited in supply. Since newly developed clinical approaches like transplantation of cells are invasive and costly, and osteoinduction by bone morphogenetic proteins is expensive and is associated with mild to severe side effects, the optimization of osteoconduction appears as promising option to realize bone substitute based bone tissue engineering.

In the 90ties of the last century, the holy grail of pore size for scaffolds in bone tissue engineering was set between 0.3 and 0.5 mm. These values appeared reasonable since they fall in line with the diameter of osteons. More recently, 2 papers showed that pores even bigger than 1.2 mm perform equally well (von Doernberg, von Rechenberg et al. 2006, de Wild, Zimmermann et al. 2016). Therefore, the optimal microarchitecture for bone tissue engineering scaffolds in terms of pore size, constrictions, rod thickness, or rod distance is still unknown.

Additive manufacturing appears as an ideal tool to study those diverse microarchitecture options since it can generate scaffolds where size and location of pores and connections between pores can be tested. For the production of our test scaffolds, we use the lithography-based additive manufacturing machine CeraFab 7500 from Lithoz (Vienna, Austria). As in vivo test model, we used a calvarial defect model in rabbits. Histomorphometry revealed that all generatively produced structures were well osseointegrated into the surrounding bone. The histomorphometric analysis, based solely on the middle section, showed that bone formation was significantly increased with pores between 0.7-1.2 mm in diameter. Scaffolds with pores of 1.5 and 1.7 mm perform significantly worse. Therefore, pore diameters in osteoconductive bone substitutes should be 1.0-1.2 mm and thus much bigger than previously suggested.

In essence, osteoconductive microarchitectures of bone substitutes can be realized by lithography based additive manufacturing and this methodology appears as a promising tool for the production of osteoconductive, personalized bone tissue engineering scaffolds to be used in cranio-maxillofacial surgery, dentistry, and orthopedics.

a92369

## 03-P214 A polycaprolactone/fish collagen biocomposite supplemented with fish hydroxyapatite for hard tissue regeneration

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The tissue engineering emerges as a prospective technology that integrates different strategies to restore, maintain and enhance tissue function. Especially, natural materials such as collagen and gelatin are similarities with the extracellular matrix and chemical versatility. Moreover, natural materials provide good cell attachment and avoid the inflammatory or immunological reaction and cytotoxicity. However, the occurrence of zooanthroponosis has resulted in anxiety amongst users of medical products from land animal origins. Furthermore, the land animals like pig and cow have difficulty applying to medical products, due to religious barriers. Fortunately, since there have been no reports about zooanthroponosis in marine organism. In the present study, we isolated collagen and hydroxyapatite from marine teleost and designed scaffolds consisting of polycaprolactone (PCL)/fish collagen/fish hydroxyapatite fabricated by three axis plotting system for bone regeneration. The obtained collagen and hydroxyapatite have been characterized. Moreover, the effect of these biocomposites on various physical characteristics and cellular activities using MC3T3-E1 pre-osteoblasts to assess cell proliferation and mineralization. The results show that the cells were more rapidly differentiation and calcium deposition on PCL/fish collagen/fish hydroxyapatite composite compared to the PCL composite. Based on the results, the PCL/fish collagen/fish hydroxyapatite warrants further investigation of potential biomedical engineering field due to enhancement of osteogenesis.

a90295

## 03-P215 Dynamic cell-specific expression pattern and role of IGFBP4 during chondrogenesis

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Chondrogenesis results from a complex equilibrium between chondrocyte proliferation and differentiation. We previously found that the expression of IGFBP4 was higher in early chondrogenic differentiation stage than that in late stage, but its function is not known. In the current study, we employed the RCJ3.1C5.18 chondrogenic cell line, which differentiates spontaneously to growth plate chondrocytes in vitro. We demonstrated that in the early stage (1day), the number of cells in S phase was significantly higher than that in the late stage. Over 10 days of culture, cells sequentially acquired expressions of chondrogenic differentiation markers (1, 4 days) and terminal differentiation markers (7 days), while in the late stage (10 days), the percentage of apoptotic cells increased, suggesting that cells sequentially underwent proliferating chondrocytes, terminally mature chondrocytes and apoptosis. We also evaluated the IGFBPs expression pattern during chondrogenesis of RCJ cells. IGFBP2, 4, 6 mRNA expressions were consistent with Sox9 and Col II, while the expressions of IGFBP1, 3, 5 were related with that of Runx2 and MMP13 during different stages of differentiation. IGFBP4 knockdown impaired chondrogenic differentiation of RCJ cells, whereas IGFBP4 overexpression restored the chondrogenic potency of RCJ cells in the early stage (4 days), as evidenced by the increased expression of SOX9 and Col II. Furthermore, overexpression of IGFBP4 in RCJ cells at the terminal differentiation stage (7, 10 days) inhibited cell proliferation and promoted apoptosis. To investigate the underlying mechanism, we used a Phospho Explorer antibody microarray covering nearly all known signaling pathways. We found that factors or proteins showing substantial changes were enriched in MAPK and PI3k-Akt signaling pathways, which were well known to be related to chondrocyte proliferation and differentiation. These results highlight the potential use of IGFBP4 in regulating chondrocyte proliferation and differentiation, which might be related to the chondrogenic differentiation process.

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a90175

## 03-P216 Effect of Pulsed Ultrasound on Sol-Gel Transition Hydrogel for Bone Regeneration

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Ultrasound is described as an acoustic pressure wave which can transfer mechanical energy into tissues and cause beneficial biochemical events at the cellular level. It has been proven systemically by huge efforts using a variety of animal models over several decades. The use of ultrasound to promote bone regeneration was approved by the FDA for human application. Researchers believed that the stimulatory effect of ultrasound on bone healing is caused by the promotion of osteogenesis and mineral deposition, etc. at the site receiving stimulation. In this study, we prepared *in situ* gelling alginate (ALG)/hyaluronic acid (HA) hydrogels with a controllable gelation rate using $CaSO_4$ as a crosslinking agent and $Na_2HPO_4$ as a crosslinking retardation agent. The ALG/HA hydrogels with different ALG/HA ratios [ALG/HA (w/w): 10/0, 9/1, 8/2, 7/3, and 6/4] were prepared. Residence stability of the ALG/HA hydrogels slightly reduced with increasing HA ratio in the hydrogel. Compressive modulus of the hydrogels also gradually decreased with increasing HA ratio, but the difference was not significant, except ALG/HA (6/4). The compressive modulus of the ALG/HA (6/4) was low and could not use as a bone regeneration sample. We investigated the bone regeneration potential of the ALG/HA hydrogels with different ALG/HA ratios combined with pulsed ultrasound stimulation. The extent of bone regeneration by the ALG/HA hydrogels with ultrasound stimulation was evaluated using a skull defect model of SD rats. The animals were divided into two groups: ultrasound stimulation and non-stimulation groups. Each group was divided into 4 groups according to the ALG/HA rations [ALG/HA (w/w): 10/0, 9/1, 8/2, 7/3] hydrogels. Bone regeneration behavior was analyzed by micro-CT and Masson's trichrome staining at 4 and 8 weeks after surgery. The animal study result showed that the ultrasound stimulation groups regenerated more effectively bone regeneration than the groups without ultrasound stimulation. Particularly, ALG/HA (7/3) hydrogel group with ultrasound stimulation was found to be very effective in bone regeneration. From the results, we could suggest that the ALG/HA hydrogel with ultrasound stimulation seems to be a promising strategy for the enhanced bone regeneration.

a92929

## 03-P217 The effect of topical cutaneous $CO_2$ application on bone healing in a rat femoral defect model

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**Purpose:** The purpose of this study was to investigate the effect of topical cutaneous $CO_2$ application on bone healing using a rat femoral defect model.

**Methods:** We created a femoral defect immobilized with an external fixator in rat. The affected limb was shaved and $CO_2$ absorption enhancing hydrogel was applied. A polyethylene bag was used to seal the body surface and filled with 100% $CO_2$ for 20 minutes. This treatment was performed five days a week. Control animals received a sham treatment in which the $CO_2$ was replaced with air. Radiographic, histological, biomechanical, and μ-CT assessments were performed to evaluate bone healing.

**Results:** At week 4, radiographic assessments showed that the bone healing rate was significantly higher in $CO_2$ group than in control group ($p < 0.05$). At week 2, the degree of bone healing as scored using the Allen grading system in histological assessment was significantly greater in $CO_2$ group than in control group ($p < 0.05$). The ultimate stress, extrinsic stiffness, and failure energy were significantly greater in $CO_2$ group than in control group ($p < 0.05$). The volumetric bone mineral density of the callus evaluated with μ-CT was significantly greater in $CO_2$ group than in control group ($p < 0.05$).

**Conclusions:** Topical cutaneous $CO_2$ application can be a novel and useful treatment for accelerating bone healing in bone defects caused by severe trauma, nonunion, infection or tumor resection.

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a91686

## 03-P218 Coccoliths-Based Bone Graft Substitutes for Bone Tissue Regeneration

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Various marine organisms including coral and pearl oyster produce biominerals composed of calcium, carbonates, phosphates, silica, sulfates, and so on. Among them, a type of phytoplankton called coccolithophorid is covered with small plates of calcium carbonate (coccoliths). *Emiliania huxleyi*, which is the most abundant species of coccolithophorids, can supply coccoliths with low production cost due to plentiful biomass resource and simple refinement process. Coccoliths might be used as a potential graft material for bone tissue engineering due to its unique morphology and composition. For effective bone regeneration, bone graft materials should be degraded with an appropriate rate and replaced by newly formed bone. Although synthetic bone substitute could overcome several limitations of biological bone grafts, it has extremely low degradation rate in the body. In this work, we utilized coccoliths as novel bone substitutes. Coccoliths were rapidly degraded in physiological conditions because they are mostly composed of calcium carbonate. Through chemical treatment, we modified coccoliths to control their decomposition rate *in vivo* and to have similar composition to bone. Thus, coccoliths could be suggested as a novel bone graft material with good biocompatibility, osteogenic effect, and biosolubility.

a92542

## 03-P219 Micro- and nano-scale spectroscopic markers of tissue engineered bone nodules

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Bone tissue is highly textured composite of submicrometric inorganic particles embedded in organic matrices. In vitro bone engineered tissue from stem cells differentiation was successfully achieved and reported. Unfortunately, with heterogeneous cell population (*i.e.* uncommitted stem cells, pre-osteoblast, osteoblast and osteocyte) in addition to composite textured matrix, current standard biology techniques fail to provide a reliable chemical composition and a maturation state of the engineered bone tissue. Infrared (IR) spectroscopy is a direct probe of molecular vibrations in a tissue. IR spectra of bone compounds are currently used to specifically identify proteins, lipids, polysaccharides and minerals in both normal and pathological conditions as osteoporosis. In the present study, we combined microscopy and spectroscopy in order to carry out bone engineered tissue chemical analysis on micro and nanoscales. After four weeks of culture on bio-inspired phosphocalcique material, human derived stem cells started to form nodules ( $\approx$ 0.4 $mm^2$ in area). Examination of nodules histological sections revealed a cohesive tissue with continuous cell layers at the surface of the nodule with randomly distributed cells embedded within newly formed collagen and proteoglycans. Infrared micro-spectroscopy, at scale of 40-80 µm in transmission and reflexion modes, showed heterogeneities in the nodule chemical composition as a function of the analysed region. The major absorption features were attributed to amides bands from proteins (collagen and b-proteins), and characteristic bands from phospholipids, and phosphate bands from both organic and mineral origins. Infrared analysis performed by nanoIR, at the scale of 50-100 nm, highlighted heterogeneities in cell- and collagen-rich regions within the nodule. Indeed, the presence of proteins with various conformations confirmed the heterogeneity in the organization of collagen fibril structures. These findings shed the light of the potential application of spectroscopy as a tool for deciphering the chemical signature of *in vitro* generated bony tissues.

a92645

## 03-P220 Cartilage administrated biodegradable hydrogel containing basic fibroblast growth factor as an alternative for mastoid obliteration in an animal model

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Objective

The purpose of this study was to develope a graft material for mastoid cavity obliteration using cartilage in combination with biodegradable hydrogel containing basic fibroblast growth factor (bFGF) as in an animal model.

Method

Guinea pigs were used in this study. A skin incision was made posterior to the auricle. A surgical drill was used to expose the mastoid cavity and shave the bone cortex of this cavity. Auricular cartilage with perichondrium was harvested, and administrated biodegradable hydrogel containing bFGF. Each graft was implanted to the mastoid cavity. The procedure was performed in the same manner on both ears. Animals were divided into three comparison groups, control group and two experimental groups: In control group (group I), cartilage was not processed. In experimental group II (group II), cartilage was diced into 0.5 mm * 0.5 mm - 1 mm * 1 mm pieces using scissors. In experimental group III (group III), cartilage was implanted with a vascularized perichondrium flap. Ten weeks after implantation, guinea pigs were sacrificed. The grafts were evaluated grossly and histologically.

Result

The results showed that the mastoid cavities were filled with cartilaginous tissue, consisting of organized connective tissue along with chondrocytes incorporated into the adjacent cartilage. The cartilage with a vascularized perichondrium flap showed a tendency to grow significantly and obliterate the mastoid cavity.

Conclusion

The results of our experimental study suggest that cartilage with a vascularized perichondrium flap administrated biodegradable hydrogel containing bFGF would appear to be a better graft material for obliteration of mastoid cavities.

a92463

## 03-P221 Biodegradable Polyurethane Elastomers with Osteogenic Chain Extender as Hard Tissue Supports

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Bone is a hard tissue with limited regeneration capacity. The irregular size and geometry of bone defects necessitate the need of scaffold based approaches in bone tissue engineering. Polyurethanes (PUs) have increasing applications in hard tissue and musculoskeletal regeneration with their engineered bulk and surface properties. PUs posses the advantage of condensation polymerization in which, physicochemical, segmental properties and biodegradation period can be tailored via selection of suitable monomers and chain extenders. In the design of PU based hard tissue supports scaffolds, osteogenic chain extenders and osteogenic agents play critical role. In this study, we aimed to synthesize long term biodegradable PUs with superior elastomeric and osteogenic properties to serve as hard tissue support. In this manner, two-shot condensation polymerization was optimized with 1,6-hexamethylene diisocyanate (HDI), polycaprolactone diol (PCL-diol) monomers and $\beta$-glycerol phosphate chain extender. Prepolymer with molar ratio of 1.98:1.00 (HDI:PCL-diol) had number av. molecular weight 64.650, weight av. molecular weight 117.743 and heterogeneity index of 1.821 according to GPC analysis. The chemical, thermal, viscoelastic and biodegradation properties of $\beta$-glycerol phosphate chain extended PUs were characterized. Presence of phosphate groups decreased the hard segment crystallization and accelerated the biodegradation properties of PUs in hydrolytic, oxidative and enzymatic conditions. These biodegradable PU elastomers had promising outcome for hard tissue engineering scaffolds.

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a93428

## 03-P222 Alginate/ Gelatin/ nano-Silica hydrogel microcapsules for bone tissue engineering applications

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Hydrogel microcapsules have diverse applications in bone tissue engineering due to their hydrophilic nature and desirable structure for cell growth which allows for excellent nutrient and oxygen transfer. In this study 1 wt% alginate (Alg)/ 1.25 wt% gelatin (gel)/ 1 wt% nano-silica ($nSiO_2$) hydrogel microcapsules containing MG-63 cells with density of 2E6 cells per ml of gel were prepared in calcium chloride bath (0.1M) using electrostatic droplet generation method with voltage of 8 Kv. Alg/ gel/ $nSiO_2$ hydrogels were characterized by mechanical strength, swelling and biodegradation analysis and compared with control hydrogels (Alg/ gel). The presence of nano-silica in alg/ gel hydrogels led to controlled swelling and biodegradability behavior. Mechanical strength was also intensified in nano-silica containing hydrogels. Proliferation and viability of encapsulated MG-63 cells were evaluated by trypan blue staining and MTT assay. Alg/ gel/ $nSiO_2$ microcapsules were biocompatible when tested to MG-63 cell line. At the end of 28 days no dramatic discrepancy in the amount of cell numbers of alg/ gel/ $nSiO_2$ and alg/ gel microcapsules was observed. Alkaline phosphatase activity and alizarin red staining analysis demonstrated that introduction of nano-silica into conventional alg/ gel hydrogel microcapsules can encourage better matrix mineralization and calcium deposition. Thus, these results prove that prepared alg/ gel/ $nSiO_2$ hydrogel microcapsules can induce better bone generation and would be a forthcoming candidate for bone tissue engineering applications.

a92672

## 03-P223 The chondroprotective effects of diosmin on hydrogen peroxide-stimulated chondrocytes

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Oxidative stress and reactive oxygen species (ROS) are involved in the pathology of osteoarthritis (OA). Investigating the deregulation of anti-oxidative capacity in human chondrocytes may provide a better understanding to the role of oxidative stresses in the OA progression. **Diosmin** is a member of flavonoids with the property of anti-oxidation. We therefore demonstrated the therapeutic potentials of **diosmin** on human OA chondrocytes**.** The dose-dependent cytotoxicity and viability of **diosmin-treated cells** were evaluated. **The** chondrocytes were stimulated by $H_2O_2$ for 30 mins and subsequently treated with **diosmin**. The phenotype, chondrocyte-specific transcriptional factors as well as proteins were analyzed. The antioxidant enzymes were also tested to the treated cells. We found $H_2O_2$ stimulation caused a hypertrophic transformation and impaired the antioxidant capacity to chondrocytes. However, **diosmin supplement can effectively reduce oxidative stress and partially restore antioxidant capacity in hydrogen peroxide-stimulated chondrocytes. In conclusion, diosmin has the potential to ameliorate the dysfunctional anti-oxidative system in OA patients.**

a92073

## 03-P224 Silver, Silicon Co-Substituted Hydroxyapatite in Mediating Bacteria-Cell Competition for Enhanced Tissue Regeneration

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Implant-related infection is a devastating complication that significantly impedes tissue regeneration, which reduces the chances of a successful patient outcome. This is due to bacteria winning the race for implant surface. To better mimic the race for surface between microbial colonisation and tissue integration, this study integrated three components: *Pseudomonas aeruginosa* (*P. aeruginosa*), human derived-bone marrow mesenchymal stem cell (hBMSCs), and silver, silicon co-substituted hydroxyapatite (Ag,Si-HA). Ag,Si-HA, a newly developed bone tissue engineering material that aimed to overcome potentially two challenges: lack of native tissue integration and implant-related infection, was exposed simultaneously to *P. aeruginosa* and hMSCs to simulate surgical site infection, in which the responses of Ag,Si-HA in mediating bacteria-cell competition was evaluated. Ag,Si-HA inhibited the growth of *P. aeruginosa*, and at the same time showed well proliferation of hMSCs over a period of 7 days. Well-spread cells with no bacteria were also found attaching on Ag,Si-HA. On the other hand, hMSCs could not proliferate on HA when co-cultured with *P. aeruginosa*. Furthermore, bacteria colonies were also increasing on the surface of HA with culture period. Despite being in a bacteria contaminated environment, Ag,Si-HA supported the proliferation of hMSCs, while simultaneously prevent microbial adhesion and biofilm formation, and with these functional properties, Ag,Si-HA could potentially facilitate bone tissue regeneration. [NMRC Open Fund Individual Research Grant Number OFIRG15nov026]

a93269

## 03-P225 Fucoidan inhibits new blood vessels formation in a tumor microenvironment

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The generation of new blood vessels (angiogenesis) is an essential requirement for cancer cells survival and cancer progression. Therefore, one of the therapeutic routes quests for anticancer agents able to block this phenomenon. Fucoidan seems to be a promising natural-origin alternative to current cancer chemotherapeutics. However, a previous work demonstrated that different fucoidan extracts may present disparate biological responses based on their chemical features. Considering that the angiogenic potential of fucoidan is still controversial, since both pro- and anti-angiogenic responses have been reported in literature, an effective anticancer extract was herein tested.

Cytotoxicity results set 0.5 mg/mL as the selective fucoidan concentration (toxic over human breast cancer but not over endothelial cells) to be further explored while evaluating angiogenesis *in vitro* (tube formation assay) and *in vivo* (chicken embryo chorioallantoic membrane – CAM – assay). Tube formation assay demonstrated that fucoidan, either administered while endothelial cells seeding or after their adhesion, inhibited or disrupted the formation of tubular-like structures. Furthermore, fucoidan did not significantly reduced VEGF secretion, but significantly reduced the expression of PDGF when compared to the control condition (without fucoidan). Therefore, in the presence of the effective anticancer fucoidan extract, endothelial cells are metabolic active and expressing VEGF, despite not capable of forming mature tubular-like structures. To evaluate the fucoidan activity in an *in vivo* angiogenesis model, a multicellular tumor spheroid was onplanted into the CAM. Although the number of blood vessels seems to not be affected *in vivo*, the size of the tumors significantly decreased in the presence of fucoidan.

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a91486

## 03-P226 From Tissue Engineering to Tumor Engineering – Application of the Arteriovenous Loop Model for the Generation of a Fully Vascularized Tumor in an Animal Model

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Traditional animal tumor models are normally based on the direct injection of tumor cells into the vascular system or the target tissue. These approaches lead to the generation of a tumor that is highly influenced by the surrounding animal tissue. This makes it challenging to analyze the contribution of single cell types and the vascularization of the tumor itself. Until now there is no *in vivo* tumor model that studies tumorigenesis and angiogenesis under standardized conditions in an environment that is 'isolated' from the surrounding animal tissue.

As a structural support for tumor cells, electrospun PCL-scaffolds combined with fibrin and alginate matrices, were produced. Different cell types, such as MDA-MB231, and further normal breast cells or adipose-derived stem cells, were seeded onto the scaffolds or encapsulated in the matrices for 3D *in vitro* and *in vivo* subcutaneous experiments. For the establishment of an *in vivo* breast cancer angiogenesis model, the breast cancer cell line MDA-MB231 encapsulated in a fibrin matrix was implanted into the arteriovenous (AV) loop model within an isolated chamber for up to 8 weeks. 3D-imaging (MRI/PET-CT) was performed over time and immunohistochemistry at time point of explantation.

Tumor cells could properly adhere and proliferate both on scaffolds and within the matrices. In subcutaneously implanted alginate-fibrin, tumor cells highly proliferated and formed small colonies after 4 weeks shown by PET-CT and immunohistochemistry. In the AV loop model, initial tumor formation and vascular sprouting were visible after 4 weeks. In some animals at 8 weeks using immunohistochemistry we detected cytokeratin-18-positive cells throughout the entire tumor. CD31 and alpha-smooth muscle actin staining revealed a dense vascularization within the tumor and surrounding tissue with connection to AV loop vessels. In ongoing experiments, tumor cells grown on scaffolds are implanted within the hybrid hydrogel in the AV loop model.

In the current study the AV loop was adapted as novel *in vivo* tumor model in an encased isolated chamber. It could be demonstrated that it is possible to generate a fully vascularized tumor only directly influenced by the animal's vascular system and not by the surrounding tissue. We believe that this model can serve as a novel xenograft model for providing significant benefits for validation of cancer drugs and development of new cancer therapies individually adapted to the patient's requirements.

a92017

## 03-P227 *In Vivo* Modelling of Prostate Cancer Metastasis using Tissue-Engineered Humanized Bone

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Prostate cancer (PCa) preferentially metastasises to bone, at which stage the disease is generally considered incurable. Yet, the development of effective therapies is hampered by a lack of adequate *in vivo* models recapitulating the hallmarks of PCa metastasis, including cancer cell dissemination from an orthotopic primary tumour, vascular invasion, and homing to bone. Here, we employed tissue engineering approaches to create an ectopic humanized organ bone (hTEBC) with a humanized vascular bone marrow niche which allows for the species-specific investigation of the interaction between human cancer cells and human bone within a murine host.

To achieve this, we used melt electrowriting to fabricate tubular polycaprolactone (PCL) scaffolds which were coated with calcium phosphate and pre-cultured with osteoprogenitor cells from human bone. To engineer a vascular compartment within the developing bone, human mesenchymal stem/stromal cells and umbilical vein endothelial cells embedded in gelatin methacryloyl (GelMA) hydrogels were placed inside the tubular bone scaffolds prior to implantation *in vivo*. CT imaging and histological analysis confirmed the formation of bone with human derived extracellular matrix (ECM) components and bone marrow in the hTEBC.

Luciferase-expressing human PCa cell lines (LNCaP or PC3), together with human prostate fibroblasts, were orthotopically implanted into the mouse prostate, and primary tumour development was tracked using bioluminescence imaging. We found that after up to 10 weeks *in vivo*, human PCa cells consistently metastasize to the hTEBC and mouse organs from the primary prostate tumour in immunocompromised (NSG) mice.

a91912

## 03-P228 Tissue engineering of an orthotopic humanised bone-organ as a platform for preclinical multiple myeloma research

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Multiple Myeloma (MM) is a B cell neoplasm that still remains largely incurable. Despite numerous efforts to develop new therapeutic strategies for MM, most drugs fail clinical trials despite successful preclinical studies. This is mainly due to the lack of clinically predictable animal models. Hence, there is an unmet need to develop a model that mimics key aspects of MM, such as tumour-microenvironment interactions.

Here we developed a fully personalised MM animal model that is able to engraft patient cancerous cells into an orthotopic and humanized tissue-engineered bone construct (ohTEBC) to create a fully functional humanized bone marrow (hBM) niche and a human haematopoietic system.

The ohTEBC is generated from melt electrospun medical-grade polycaprolactone (mPCL) tubular scaffolds coated with calcium phosphate and seeded with human bone osteoprogenitor cells, while the hBM niche is engineered with the patient's own BM cells combined with a fibrin glue hydrogel. This ohTEBC also contains human haematopoietic stem cells (HSCs) that will contribute to form a humanized immune system as well as mesenchymal stem cells (MSCs), which play a key role in the cross-talk with myeloma cells. After orthotopic implantation around the right femur in NSG mice, the ohTEBC formed an organ bone containing a cortical shell infiltrated with BM and was composed of human cell and extracellular matrix components. Patient-derived MM cells induced large local osteolytic lesions within the ohTEBC, which is reflective of the clinical disease. Therefore we demonstrate that this tissue-engineered MM model holds the potential as a unique and patient-specific drug testing platform, not only for common drugs, but also for immunotherapy and specific drugs targeting the dormant cell population.

a92859

## 03-P229 Efficient Isolation of Exosomes Derived from Stem Cells Using Porous Glass Filter

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Exosome is a type of extracellular vesicles which contain some kinds of proteins and nucleic acids such as mRNA and miRNA. Exosome exists in bodily fluids and has recently been considered as a biomarker for cancer. Moreover, recent studies have shown that stem cells produce many exosomes, and the exosome is also attracting attention as a new biomarker for regenerative medicine. Some methods for exosome isolation already have been known, however, efficient and simple methods for exosome isolation still have not been developed. In this study, we report an efficient and simple method for exosome isolation with porous glass device. Porous glass has three-dimensional nanostructure. According to heating time and temperature, we can control the pore size of porous glass easily.

It is possible to isolate exosomes from culture supernatant of cells or bodily fluids by using the porous glass as filter. Exosomes derived from HepG2 cells were collected using ultracentrifugation. Collected exosomes were labeled by fluorescence probe (PKH67) and the isolation efficiency was calculated from the fluorescence intensity of labeled-exosome sample (500 $\mu$L) before and after filtering. Isolated exosomes on the porous glass surface were observed using fluorescence microscope. Furthermore, shape and distribution of exosomes in the porous glass filter were confirmed with scanning electron microscope (SEM).

Porous glass filter enabled us to isolate the exosomes from cell-culture supernatant with high efficiency (>80%). In addition, rapid exosome isolation was achieved within 10 min by centrifugation at a lower spin speed (<6000g). These data suggest that the porous glass device is useful for the efficient and simple exosome isolation.

a91347

## 03-P230 Colorectal tumour organoids: advantages of biofabrication methods for high throughput in vitro models

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In cancer research, costs, time and effectiveness of chemotherapies are always questioned due to the low success in drug development. One of the main factors of such poor success is the lack of the translatability of findings form pre-clinical cancer models to patients. Routine drug tests use 2D in vitro models, which are well known for their limited ability in reproducing the tumour microenvironment, e.g. cell-cell interaction, cell-stroma interaction, extracellular matrix. We here address such challenge, conceiving a more relevant 3D *in vitro* model to study cell-cell and cell-stroma interactions in the colorectal tumour site.

In particular, we focused on the expression of CD44: 1) receptor abundantly expressed in solid tumours and with increased expression in tumour development and 2) 'hot' target in drug delivery. We believe that tumour spheroids are not appropriately modeling colorectal tumouringenesis in chemotherapeutics screening, being challenging in 1) control size and cell growth, as well as 2) mimic the phenotypic expression of cancer cells. In this study we used a simple manufacturing technique to fabricate 3D cancer *in vitro* models by means of alginate-based hydrogel microspheres encapsulating HCT-116 cells. Biofabrication steps were characterized to obtain tunable engineered tumour organoids, i.e. hydrogel microspheres encapsulating tumour cells. Microbeads were characterized in size, shape, initial cell number, cell viability and growth. HCT-116 engineered tumour organoids behavior was compared to the gold standards TCP and spheroids. Expression of CD44 was found similar in both 3D models (engineered tumour organoids and spheroids), and notably low HIF-a expression was reported in encapsulated HCT-116 cells. We showed that alginate-based hydrogels - with properties similar to the tumour microenvironment - better represent the physical barriers for drug delivery, and help in investigating new strategies for the effective delivery of drugs to the tumour. We believe that the use of engineered tumour organoids will enable to speed-up the pre-clinical phase.

a90293

## 03-P231 Human Colon Cancer Screening by using Fluorescence-based Wax Physisorption Kinetics

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tructure of cancer-associated alterations of cell surface glycome (oligosaccharide residues of glycoprotein) is demonstrated to be elongated or branched during the progression of cell carcinogenesis[1]. Wax-physisorption-kinetics-based FTIR imaging has been successfully demonstrated that n-pentosane ($C_{25}H_{52}$) and beeswax ($C_{46}H_{92}O_2$) were utilized as antibody-like probe to differentiate malignancy from normal tissue [2-4]. Based on the principle of Wax Physisorption Kinetics (WPK), a series of n-alkanes (C22-C34 frame) was further employed as wax adsorbents for normal colon fibroblast cell and colon cancer cells HT-29 (Unknown grade), SW-1116 (Dukes 'type A, grade III), SW-480 (Dukes' type B), SW-403 (Dukes' type C, grade III) and LoVo (Dukes' type C, grade IV) for selecting an optimal wax adsorbent with appropriate carbon chain length. The WPK results showed that normal colon fibroblast cell (CCD-18Co) has a greater physisorption with docosane ($C_{22}H_{46}$) according to greater docosane residue was found onto CCD-18Co; inversely, triacontane ($C_{30}H_{62}$) has a greater residue adhering onto colorectal adenocarcinoma cell lines, as shown in Fig. 1. Therefore, docosane and triacontane could be potential cancer diagnostic probes for screening colon cancer. In this study, a fluorescence-wax probe was utilized for screening CCD-18Co, SW-480 and SW-403. The preliminary results of fluorescence-based WPK showed that the fluorescence intensity of advanced grade cancer cell SW-403 was than lower grade cancer cell SW-480 and normal fibroblast cell CCD-18Co. An innovative fluorescence imaging combine infrared wax-physisorption kinetic method for screening human colon cancer have been successfully developed based on physisorption between diagnostic wax agents and oligosaccharide of membrane glycoprotein on normal, early and advance cells.

a91466

## 03-P232 *In silico* breast cancer model for understanding tumor development

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Cancer leads among the deadliest diseases that we face today. After decades of experimental research on cancer, we are still far away from a cure. We lag behind in the fight against cancer in multiple ways. The need for a better treatment is dire with an ever increasing number of cancer afflicted population. Drugs have been developed for cancer however they have serious health implications. *De novo* drug development is prohibitively costly therefore, we need to find better strategies of using the available drugs. Computational models can alleviate many drawbacks in the traditional research methodologies. A large amount of high throughput data on cancer is available that needs to be incorporated in the *in silico* models for better predictions. Mathematical models are already used to study various aspects of cancer and its treatment, like chemotherapy, tumorigenesis and progression. To build an *in silico* cancer model, we are using agent-based modeling (ABM) approach. Several computational studies have shed light on cancer morphogenesis, metastasis and effects of chemotherapy. Here, we develop an agent based *in silico* model for cancer, wherein cancer cells can display duplication, motion and death under certain specific conditions. The effect of gradients (drug concentration or gel density) can easily be mimicked using this model. Mathematical modeling of population dynamics and treatment responses provides mechanistic insights regarding cancer and can be used to predict better treatment regimens. Efforts are underway to incorporate experimental data on the efficacy of various drugs into this model and design novel cancer treatment strategies. This *in silico* model is expected to provide us the capability to perform *in silico* clinical trials in future.

a90011

## 03-P233 Network pathways of PTCH1, ERBB3, CTNNB1 and EFNA1 in gastric cancer and mesenchymal stem cells

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Network signaling pathways are activated in several disease status and differentiation of stem cells. The epithelial-mesenchymal transition (EMT) is involved in cancer metastasis, malignancy and anti-cancer drug resistance. To elucidate the molecular network pathways in cancer and stem cells, gene expression and networks were profiled in diffuse-type gastric cancer (GC) and mesenchymal stem cells (MSCs). Network pathways including beta-catenin (CTNNB1), ephrin A1 (EFNA1), erb-b2 receptor tyrosine kinase 3 (ERBB3), patched 1 (PTCH1) were generated, and the gene expression analysis of molecules in the networks revealed that slit guidance ligand 2 (*SLIT2*) in EFNA1 network was up-regulated and growth factor receptor bound protein 7 (*GRB7*) in ERBB3 network, cadherin 1 (*CDH1*) and Wnt family member 5A (*WNT5A*) in PTCH1 network, and *ERBB2* in CTNNB1 network were down-regulated in diffuse-type GC compared to intestinal-type GC. These results demonstrate that the molecules in the network pathways are differently regulated in the disease and cellular conditions.

a92134

## 03-P234 Combining high-resolution 3D optical imaging, computational modelling and tissue engineering to investigate patterns of cancer invasion

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The tumour microenvironment plays an integral role in the inception, growth and invasion of cancerous cells (1). Engineered, 3D *in vitro* cancer models provide controllable and reproducible environments for investigating patterns of cancer invasion in varying tumour microenvironments. We have combined novel, 3D imaging of an *in vitro* tumour model, with computational modelling to develop a platform for studying the biophysical processes that govern cancer invasion.

We used High-Resolution Episcopic Microscopy (HREM) (2), to image an established, 3D *in vitro* colorectal cancer model (3). HREM is a blockface imaging technique, in which samples embedded in resin are serially sectioned within a customised fluorescence microscope.

Five *in vitro* tumours were imaged with HREM, at days 1, 7 ,14, 21, and 28 to quantify tumour cell invasion into the surrounding a-cellular regions. Images were segmented via machine learning and quantified on the basis of various geometric measures including volume and surface area.

Three dimensional *in silico* simulations of *in vitro* growth were performed using FEB3, an open-source finite element platform developed by the authors (4). Here we use FEB3 to solve coupled reaction-diffusion equations describing multi-species growth, and directly specify the initial and boundary conditions (cell density and sample geometry) to best simulate the *in vitro* data.

Geometric measures were compared between *in vitro* and *in silico* data and found to

be concordant. This work has demonstrated the novel utility of HREM in imaging 3D in vitro cancer models, and its potential use with 3D in silico cancer models.

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a90621

## 03-P235 Local delivery of type 1 interferon via myeloid cells from induced pluripotent stem cells elicits systemic antitumor immunity

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We previously established a method to generate myeloid cells with proliferation capacity from induced pluripotent stem cells (iPSCs) (Haruta M. Gene Ther 20: 504, 2013, Zhang R. Cancer Immunol Res. 3: 668, 2015). This system can be used for gene manipulation to enhance their efficacy and safety, and can stably provide the desired amount of cell preparation of validated quality, curtailing expenditure, labor, and time. Genetically engineered type 1 IFN-expressing human iPSC-derived proliferating myeloid cells (iPSC-pMCs) exhibited therapeutic effects against peritoneal dissemination of gastric and pancreatic cancers and melanoma (Koba C., PLoS ONE 8: e67567, 2013, Miyashita A., Cancer Immunol. Res. 4: 248, 2016). However, these studies employed xenografted tumors in T cell-deficient mice, focusing mainly on potentially direct effects of type 1 IFNs on tumor growth, and the role of adaptive immunity in the effectiveness, critical target, and in vivo safety of these cells remained to be elucidated. Here, we show that local administration of type 1 IFN-producing iPSC-pMCs alters the tumor microenvironment and propagates molecular signature associated with type 1 IFN, leading to the distant tumor control. Although it is restricted at the site of treatment, they directly inhibited the tumor growth via the type 1 IFN receptor signaling. Type 1 IFN-producing iPSC-pMCs actively influence on host DC function to enhance CD8+ T cell priming and provide effector functions to CD8+ T cells. The CD8+ T cells given effector function infiltrate into distant tumor, which result in the systemic tumor control. The adoptive immune response for the distant tumor control is dependent on transcription factor IRF7 but not on STING-IRF3 pathway. The local administration of IFN-α-iPSC-pMCs in combination with immune checkpoint blockade overcomes resistance to single-treatment modalities, and generates a long-lasting antitumor immune response in certain tumor models, thereby further extending their therapeutic potential. These preclinical data provide the mechanistic rationale for clinical trial of type 1 IFN-producing iPSC-pMC therapy.

a92570

## 03-P237 Intratracheal delivery of immunostimulatory oligonucleotides using biodegradable polyketal nanoparticles: effect of long-term immune responses on murine lung cancer

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Recent advances in immunotherapy, such as immune checkpoint inhibitor (ICI) therapy for lung cancer, have shown impressive clinical results, although there is risk of serious ICI-related systemic adverse events, such as thyroid diseases, pneumonitis, and type 1 diabetes. Thus, immunotherapy should be directly applied to the primary tumor site to enable higher retention while limiting systemic toxicities by administering relatively small amounts of immune modulators. To this end, we have developed inhalable microparticles (MP) incorporating immunostimulatory oligodeoxynucleotides (IS-ODN) for treating lung cancer.

Biodegradable polyketal nanoparticles (poly-[1,4-phenyleneacetone dimethylene ketal] matrix) nanoparticles (particle size, 200-600 nm), which allow for safe and efficient intratracheal administration of therapeutic agents, were used as IS-ODN carriers (conjugation ratio, 1:27-30; loading efficacy, 80%). These particles were further assembled to microparticles by freeze-drying for intratracheal administration. To evaluate the efficacy against lung cancer, we developed a fatal model mimicking primary lung cancer by intratracheal instillation of $1x10^6$ Lewis lung carcinoma (LLC) cells in C57BL/6 mice, resulting in peribronchial tumor formation with an approximately 22-day median survival.

In this study, at 60 days, intratracheal MP-IS-ODN-treated primary lung cancer model showed significant improvement in survival compared with those treated with free IS-ODN (82% vs. 38%). Histological analysis revealed that intratracheal MP-IS-ODN changed the tumor microenvironment by decreasing the number of PD-1 positive cells, tumor-suppressive Treg, and M2 macrophages, while increasing the number of tumoricidal T cells and M1 macrophages. The mice who survived were further re-challenged with $2x10^6$ LLC cells for evaluating long-term tumor immunity. The mice previously treated with MP-IS-ODN showed 100% survival at 1 year, whereas mice previously treated with non-MP-IS-ODN and/or low-dose MP-IS-ODN showed 50% survival after the re-challenge with LLC cells. Although mild-to-moderate alopecia was seen in about 30% of mice that survived for 1 year after previous MP-IS-ODN treatment, other markers or symptoms of organ damage were not apparent. Our results suggest that MP-IS-ODN was able to confer a long-term survival advantage of 80% by maintaining long-term (memory) tumor immunity, thus preventing cancer recurrence.

a93047

## 03-P238 A pH-Responsive Hollow Microsphere System that Can In Situ Self-assemble Micellar Depots to deliver NO, Reversing Hypoxia-induced Radioresistance

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The microenvironment of solid tumor tissues is generally characterized by hypoxia, which has been regarded as the major cause of resistance to radiotherapy. Nitric oxide (NO) is considered as a radiosensitizer, owing to its capability on fixation of radiation-induced DNA damage. As directly administering gaseous NO molecules is difficult, attempts have been made to deliver exogenous NO donors. Nevertheless, the in vivo half-life of NO that is generated from NO donors is extremely short when exposed to heme-containing proteins such as hemoglobin (Hb), restricting its application for the radiosensitization. To extend its half-life, the generated NO must be immediately protected from the access of Hb. In an attempt to address this concern, a hollow microsphere (HM) system that comprises a PLGA shell and an aqueous core containing a pH-responsive NO donor (DETA-NONOate; NONOate) and a surfactant molecule (sodium decanoate; SD) is proposed. In acidic tumor tissues, environmental protons infiltrate the PLGA shell of the HMs and react with their encapsulated NONOate to generate NO bubbles spontaneously; meanwhile, the surfactant molecules SD actively trap the generated NO bubbles to form micellar depots within the HMs (Micelles@HM) in a condition that is absence of Hb. The NO-containing micellar depots that are self-assembled by NONOate and SD in bulk solutions (Micelles@BS) and the NO generated from free NONOate in bulk solutions, in which Hb is present, are used as controls. In the former case, the generated NO is immediately protected within the Micelles@HM, while in the latter two cases, part of the generated NO may undergo degradation owing to the presence of environmental Hb. Our results demonstrate that the NO level generated by Micelles@HM was significantly higher (82.5 ± 4.3 %) than those generated by Micelles@BS (40.2 ± 3.8 %) and free NONOate (21.7 ± 3.2 %), suggesting that the Micelles@HM formed in the absence of Hb largely improves the stability of NO. The NO gas could then be gradually released from Micelles@HM, acting as a radiosensitizer and enhancing radiation-induced cell death. The concept of this study provides a useful strategy for reversing hypoxia-caused radioresistance.

a93056

## 03-P239 Bubble-Generating Carrier Systems for Therapeutic Applications

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We have recently been devoted to developing a few bubble-generating carrier systems for different therapeutic applications. We will first report a bubble-generating agent, sodium bicarbonate, which is encapsulated in a carrier system that can generate $CO_2$ bubbles upon responsive to changes in intracellular/extracellular pH for actively triggering localized drug release. Additionally, a carrier system that contains NONOate and an anticancer agent CTP-11 is formulated. In an acidic, aqueous environment, NONOate decomposes and generates NO bubbles, triggering localized drug release; meanwhile, NO can be used to inhibit the expression of P-glycoproteins in tumor cells to overcome the multi-drug resistance, enhancing the efficacy of cancer therapy. An $O_2$-generating system for the enhancement of the therapeutic efficacy of doxorubicin toward hypoxic tumor cells is also reported. Furthermore, a sustained $H_2S$-release system is established as a powerful therapeutic system for chronic wound healing in diabetic rats. Given the exciting advances that are mentioned above, we believe that these bubble-generating carriers may lead to significant technological breakthroughs in the treatment of various human pathologies.

a91051

## 03-P240 Local Co-Delivery of Anticancer Drug and Tumor-Suppressing Gene Based on Bioinspired Sticky Protein Nanoparticles

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Tremendous strategies for cancer therapy have been widely developed in the past decades to improve therapeutic efficacy and to reduce undesired side effects. Even though single therapeutic agent delivery systems showed considerable anticancer activities in preclinical trials, insignificant progress has been reported in clinical studies due to molecular complexity and abnormalities of cancer. In this work, we designed local co-delivery system of anticancer drug and cancer-suppressing gene based on bioinspired sticky protein nanoparticles (NPs) for effective cancer therapy. We fabricated sticky NPs with co-loading of anticancer drug doxorubicin (DOX) and tumor-suppressing p53 gene through a electrospraying process of 3,4-dihydroxyphenylalanine (DOPA)-containing bioengineered mussel adhesive protein (MAP). The DOX- and p53-co-loaded MAP NPs (MAP@DOX/p53 NPs) showed higher anticancer activity through synergistic effects than sole delivery of either DOX or p53 gene. In addition, the MAP@DOX/p53 NPs could provide local delivery of co-loaded therapeutics onto tumor tissues via simple and facile spray process due to their adhesive properties, and thus increasing therapeutic efficiency. This MAP NPs-based local combination therapy can promote tumor ablation and can be used for further clinical applications.

a91052

## 03-P241 Bioinspired Sticky Protein Nanoparticle-Based Spray Delivery System for Focal Cancer Treatment

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Systemic chemotherapy has been widely used to remove invisible residual cancer cells and prevent cancer recurrence after removal of primary tumor. However, there still remains clinical challenges such as insufficient accumulation, metabolic clearance, and systemic toxicity. Therefore, localized drug delivery system has received considerable attention to achieve improved accumulation to the site of action and avoid undesired side effects. Here, we propose novel spraying system using mussel adhesive protein (MAP)-based sticky nanoparticles (NPs) to provide local delivery of anticancer drugs. We confirmed that the MAP NPs were successfully sprayed onto target sites and exhibited great retention through adhesive property. The doxorubicin (DOX)-loaded MAP NPs (MAP@DOX NPs) showed effective cytotoxicity on cancer cells through selective drug release by pH-responsive $Fe^{III}$-DOPA complexes. In addition, we exploited the sprayed MAP@DOX NPs to inhibit further growth of tumor *in vivo*, indicating the potential applicability as a localized drug delivery system. We anticipate that the MAP-based sprayable sticky NPs provides a promising approach for focal drug delivery in cancer treatment that require improved locoregional control for increasing therapeutic effects.

a93193

## 03-P242 Novel Biodegradable PEG-GATGE Dendritic Block Copolymers as siRNA Vectors

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The unique structural characteristics of the dendritic nanostructures, namely globular, well-defined and very branched structure, multivalency, low polydispersity and controllable nanosize, make them into promising carriers for different bioactive molecules. Particularly relevant is their capacity to complex and protect nucleic acids (NAs) in compact nanostructures ("dendriplexes") with application as non-viral vectors in gene therapy.

Despite the progress in the design of dendritic structures with enhanced features for biomedical applications, one of the main drawbacks of the most currently used dendritic formulations is their non-degradability under physiological conditions that can result in cytotoxicity/ complications induced by the accumulation of nondegradable synthetic materials inside cells or in tissues. Moreover, in the gene therapy field, vector stability can further hinder the intracellular release of the NAs from the dendriplex, consequently leading to low transfection efficiencies. Therefore, biodegradable cationic dendritic structures have been eagerly awaited. However, the development of these dendritic nanocarriers is challenging because of the undesired and/or premature degradation observed during their synthesis and/or application.

In the present work, we describe the synthesis and characterization of a novel family of biodegradable, biocompatible, water-soluble and non-toxic PEG-GATGE dendritic block copolymers, based on a gallic acid (GA) core and triethylene glycol (TG) butanoate arms, incorporating ester bonds (E) at the dendritic arms/shell.

Their successful functionalization by "click" chemistry with unprotected alkynated amines allowed to complex and deliver siRNA. The hydrophobic character of the GATGE building unit confers to these hydrolyzable dendritic bionanomaterials a great ability to complex, protect and mediate the cellular (U2OS) internalization of siRNA. Moreover, the localization of the degradation points at the dendritic periphery, close to the complexed siRNA, was found important for the NA release from the nanoparticles, rendering a significant improvement of the transfection efficiency compared to their hydrolytically stable PEG-GATG copolymer counterparts. The present study puts forward these systems not only as suitable vectors for NA, but also opens new avenues for further developments exploring their use in theranostics.

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a93268

## 03-P243 Multivalent DNA Biochips for Single-Molecule Investigations on Cancer Cell Adhesion

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DNA assembles according to precise base-pairing rules. This allows for the construction of DNA nanostructures (DNA origami) in which DNA is folded into various shapes with a high degree of positional order allowing for complex arrangements to be obtained. In addition, DNA origami can be employed as scaffolds to pattern multiple bioactive structures and nanostructures (e.g. peptides, antibodies, etc.) at the scale of individual molecules. In order to take fully advantage of this approach we need to control the organization of DNA origami on surfaces into ordered array configurations. This in turn can allow for the fabrication of biochips for single-molecule investigations. Combining our skills of nano-fabrication, DNA nanostructure engineering, and molecular-specific cancer targeting, we present a platform for in vitro cell-surface investigations with single-molecule resolution. Multivalent DNA origami were organized into order arrays on surfaces, exhibiting individual integrin binding sites and epidermal growth factor at specific nanoscale spacing. Spectral separation via Super-Resolution microscopy was employed to confirm the arrangement and geometric of single molecular modified DNA origami. We explored how the geometric organization and cooperation of cancer-promoting $\alpha v \beta 6$ integrin binding peptides and EGF affect cell adhesion and spreading. We showed that $\alpha v \beta 6$ integrin binding peptides and EGF nanospacing affects spreading and direct cancer cell fate. The cooperation of multi-target was investigated at the same time. This approach is the first platform that allow for multivalent diagnostic target and intracellular signalling studies at the nanoscale, and is ideal for the development of novel cancer therapeutics platforms.

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Huang, D., Freeley, M. & Palma, M. "DNA-Mediated Patterning of Single Quantum Dot Nanoarrays: A Reusable Platform for Single-Molecule Control," Sci. Rep. 7, 45591 (2017).

a90998

## 03-P244 Improved photodynamic therapy of melanoma with PPIX@CD: a carbon dot – protoporphyrin IX host-guest conjugate

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Photodynamic therapy (PDT) is an alternative treatment method for cancer which uses light in combination with excitable photosensitisers (PS). However, compounds such as protoporphyrin IX (PPIX) are limited in their effectiveness by factors such as low compound solubility, inefficient accumulation in target tissue, high dark toxicity, and photobleaching. Carbon dots (CDs) are nanoparticles with high biocompatibility, no photobleaching, and rapid cellular uptake, which makes them suitable platforms for conjugates in biomedical applications.

In this work, we compare the PDT effect and efficiency of three carbon dot-based conjugates: PPIX@CD, a newly synthesized conjugate with an encapsulated PPIX, PPIX-CD, a previously reported crosslinked conjugate, and its less soluble fraction (PPIX-CD)p. PPIX@CD was synthesised through one-step microwave pyrolysis of citric acid, ethane-1,2-diamine and PPIX. FT-IR and UV-Vis spectra confirm PPIX embedding into the CD core. Total porphyrin content was estimated to be 30.59% in PPIX@CD, 43.03% in PPIX-CD and 52.98% in (PPIX-CD)p through the use of a calibration curve. TEM images show a significant difference in PPIX@CD aggregate morphology when compared to unmodified and crosslinked particles.

Confocal microscopy was used to observe conjugate uptake and localisation. Conjugates demonstrated significantly reduced dark toxicity. Additionally, PPIX-CD and PPIX@CD showed increases of 48.34% and 157.15% of PDT efficiency in comparison to PPIX, despite reduced PS content. Cancer spheroids were also utilised to observe conjugate penetration and distribution within a 3D environment. These results indicate host-guest encapsulation is a viable alternative to crosslinking for the synthesis of new carbon dot-based conjugates in photodynamic therapy and imaging.

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a91890

## 03-P245 Nanotherapeutic and Biomaterial Scaffold Design for Cancer Immunotherapy

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NanoBioengineering holds enormous promise in the diagnosis and treatment of many human diseases with unmet medical needs, such as cancer. To this end, the overarching goal of my research is to develop novel nanotherapeutic platforms for use in targeted drug / gene delivery and is also to design functional biomaterials for various applications in cancer immunotherapy. The strategy involves developing versatile nanovectors that can effectively overcome multidrug resistance and elicit a potent antitumor immune response. It also includes engineering functional bio-inspired material to modulate immune regulation in the tumor microenvironment for cancer immunotherapy.

In addition, inefficient delivery of drug/gene to target cells in vivo environment always remains obstacles for the clinical translation of therapeutic vectors. To overcome such challenges, we proposed a novel strategy for in vivo modulation of target cells by using a biomaterial scaffold to create a physical microenvironment where target cells can be recruited and proliferated, demonstrating that this approach could remarkably enhance the delivery efficacy of drug/gene to target cells in vivo. In this presentation, many pivotal components of these therapeutic strategies will be discussed in more detail.

a92705

## 03-P246 Hybrid nanoparticles inhibited the growth of liver cancer stem cells

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Cancer stem cells (CSCs), also called tumor-initiating cells, are involved in tumor progression, metastasis, and drug resistance. Hybrid liposomes (HLs) are nano-sized liposomal particles and can be prepared by simply ultrasonicating a mixture of vesicular and micellar molecules in buffer solutions. HLs composed of L-α-dimyristoylphosphatidylcholine (DMPC) and polyoxyethylene(23) dodecyl ethers ($C_{12}(EO)_{23}$), without any anti-cancer drugs, have remarkable inhibitory effects on the growth of various tumor cells, and stimulate apoptosis of tumor cells *in vitro*, *in vivo*, and in clinical settings[1,2]. HLs distinguished between the liver cancer cells and normal cells which had higher and lower membrane fluidities respectively, then fused and accumulated preferentially into the membranes of liver cancer cells [3]. In this study, we investigated the inhibitory effects of HLs on the growth and CSC subpopulations of liver cancer cells (HepG2) in vitro. HLs markedly inhibited liver cancer cell growth. Notably, reduction of the CSC subpopulations of CD133+ /EpCAM+ liver cancer cells treated with HLs was obtained using flow cytometric analysis. Furthermore, HLs markedly decreased the number of colony-forming cells, as seen by the soft agar colony formation assay. Finally, we observed the fusion and accumulation of HLs including fluorescent labeled lipid (NBDPC) into the cell membranes of CSC using a flow cytometer. Significant increases in accumulation of HLs/NBDPC into the CSC (especially EpCAM+ cells) was observed in a dose dependent manner. These results suggest that HLs could be a novel nanomedical therapeutic agent for targeting CSCs in liver cancer therapy.
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a93061

## 03-P247 An NIR-absorbing Nanoparticle System Loaded with TLR-7/8 Ligand for Combinational Photothermal Immunotherapy

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Recent studies have highlighted the combination of conventional treatment modalities with immunotherapy, which have the potential to reduce cancer metastasis and improve survival. In this work, a nanoparticle (NP) system that is composed of polyaniline-conjugated glycol chitosan (PANI-GCS) was prepared to encapsulate an insoluble toll-like receptor (TLR) 7/8 ligand for combinational photothermal immunotherapy. The hydrophobic PANI was covalently grafted on the hydrophilic GCS via its highly reactive amine groups to form an amphiphilic polymer (PANI-GCS), which could self-assemble into NPs in an aqueous milieu. The conjugated PANI can be utilized as nano-localized heat sources, remotely controlled by using near-infrared (NIR) light, for cancer cell ablation, while TLR7/8 ligand is applied to induce the maturation of dendritic cells (DC), which may subsequently enhance the activation of antigen-specific T cell responses. Our TEM images reveal that the size of the TLR7/8 ligand-loaded PANI-GCS NPs was approximately 160 nm, which could be effectively internalized by bone-marrow derived dendritic cells (BMDC), resulting in the upregulation of cell surface costimulatory molecules and the secretion of proinflammatory cytokines. In the animal study, we found that intratumoral injection of the TLR7/8 ligand-loaded PANI-GCS NPs was able to generate localized hyperthermia (~ 45° C) upon exposure to NIR, and was able to potentiate the effect of TLR7/8 ligand, resulting in the suppression of the growth of CT26 tumor cells and a significant improvement in the survival of mice. Additionally, the mice that had previously rejected the first tumor induced tumor-specific $CD8^+$ T cell responses that are capable of protecting mice from the same tumor rechallenge. These analytical results suggest that the TLR7/8 ligand-loaded PANI-GCS NPs may serve as a platform for combination of hyperthermia with immune stimulation for more effective cancer treatment.

a90110

## 03-P248 Tumor organoids; basic cancer biology and personalized medicine applications

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Tumors consist of a heterogeneous cell population, resulting in large differences of responses to particular therapies. Precision, or personalized, medicine, in which mutations identified in a patient's tumor, are employed to drive targeted therapies to address this problem. However, even after identification of key mutations, oncologists are often left with several drug options, with no guarantee that the therapy prescribed will be the best, or work at all in a particular patient. This problem is further exacerbating by the complex tumor microenvironment, consisting of a number of stimuli such as: stromal cells, signaling and paracrine factors, as well as extracellular matrix (ECM) components. Each of these factors push and pull cancer cells in different directions and the sum of the interactions defines a cancer cell's final disposition. In terms of the mechanical interaction of the ECM and the cancer cell, a number of observations have been made, such as: stiffer environments produce more aggressive cancer cells and facilitate migration and invasion, and aligned matrices are associated with healthy tissues while unaligned matrices are associated with poor prognosis. Although there is a wealth of literature describing the interaction of both stiffness and alignment, there are few studies that determine the relative input of either stimuli. In this study, we produce tissue constructs with varying ECM alignments and stiffnesses to generate a toolbox of ECM compositions. Stiffness is assayed using rheometry to produce stress-strain relationships while alignment is derived from picrosirius red imaging and image segmentation. Using tumor cell spheroids, we then integrate a tumor compartment into the submucosal construct. We then use WNT activity and epithelial-to-mesenchymal transition (EMT) markers to assess the relative effects of stiffness and alignment on cancer cell progression. In all, we observe matrix alignment produces a dominating effect on cancer cell phenotype across the stiffnesses assayed. Higher stiffness is associated with increased WNT activity, but this activity is significantly decreased when spheroids are cultured in highly aligned matrices. Finally, we integrated patients' derived tumor cell spheroids within the tissue constructs and measured their response to chemotherapy under conditions of different ECM mechanics.

a91561

## 03-P249 Patient Specific Microfluidic Model to Unveil Novel Molecular Interactions Between Stromal and Cancer Cells During Invasion

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Interactions between the tumor and stroma significantly influence cancer progression and metastasis. Tumor cells and fibroblasts make up a large part of these interactions, but their exact consequences on cancer progression are still being elucidated. To date, numerous *in vitro* and *in vivo* models have been utilized to recapitulate tumor-stroma interactions. However, conventional *in vitro* models still do not provide enough context to reflect the 3D human tumor microenvironment, while animal models are too complex to develop causal relationships between the microenvironmental cues (e.g., fibroblasts) and cancer invasion. Notably, microfluidic models have been developed to bridge this gap to better recapitulate the tumor-stroma niche while providing better control over the microenvironmental cues.

In this work, we developed a 3D *in vitro* microfluidic model that mimics the tumor microenvironment by conserving native cellular arrangement while integrating patient-derived stromal cells. Using our model, we performed single-cell imaging-based assays consisting of proliferation and invasion analyses which were coupled with RNA-seq to unveil novel targets during cancer cell invasion. Cancer-associated fibroblasts (CAFs) derived from patient tumor biopsies, when incorporated into the stromal region of the microfluidic device, promoted migration and proliferation of cancer cells. Importantly, analysis of gene expression using gene ontology demonstrated enrichment for adhesion and extracellular matrix organization processes. On the other hand, primary normal fibroblasts (NFs) were found to reduce cancer migration and proliferation while gene ontology showed enrichment for inflammatory response processes. Notably, based on the gene expression analyses of both CAFs and NFs within the proposed microfluidic platform, we were able to generate a list of differentially expressed genes (DEGs) exposing possible targets to inhibit cancer progression.

Overall, these results demonstrated the ability of our model to recapitulate patient specific tumor microenvironment to investigate the consequences of tumor-stroma interactions at a cellular and molecular level on invasion. Our future work will involve validating the DEGs as well as performing functional studies through knockdown and overexpression of the novel DEGs to better understand their role on cancer invasion.

a93435

## 03-P250 Bioprinting a tumour microenvironment on a chip

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Cancer is one of the major causes of morbidity and mortality. According to the American Cancer Society, over 1.7 million people were diagnosed with cancer in 2016, and approximately 0.6 million people lost their lives to it in the same year (1). It is further expected that the annual cancer incidences will rise from 14 million in 2012 to 22 million within the next 20 years (2), leading to significantly increased healthcare costs and the great need to better understand cancer to improve therapy. The cancer microenvironment is highly complex, and is highly dynamic with distinctive key features (*e.g.* cellular and ECM compositions, matrix stiffness, and degree of vascularization) present at each of the different stages of the disease (3, 4). In particular, the largescale growth of a tumour ultimately requires a blood supply (5). To fully grasp the complexity of the tumour microenvironment, as well as screening of various anticancer drugs, it has been increasingly realized that *in vitro* engineered human cancer models are strongly desired. In particular, three-dimensional (3D) cancer models are anticipated to precisely mimic the *in vivo* tumour microenvironment in human patients by recapitulating the proper tumour cell/matrix composition and other key parameters that match both the type and stage of the disease, and therefore provide accurate mechanistic studies as well as a tool for personalized anti-cancer therapeutics studies. In this work present a tumour-on-a-chip device, which integrates a microfluidics biochip, tissue engineering and biomaterials. We demonstrated the bio printability of endothelial and tumour cells inside the microfluidics biochip.

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a91499

## 03-P251 Novel Insights on Wilm's Tumor: Using Human Nephron Progenitors

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"INTRODUCTION AND OBJECTIVES: Wilms tumor (WT), a pediatric cancer also known as nephroblastoma, accounts for 95% of renal malignancies in children. Currently little is known of WT formation, and less than half of these tumors are diagnosed at Stage 1 due to their asymptomatic nature. The presence of nephron progenitors (NP) characterized by the expression of CITED1 and/or SIX2 determine the aggressive state of the tumor. However, due to past inability to isolate live human WT CITED1 and/or SIX2 positive cells, little is known about their cell biology and involvement in tumor progression.

METHODS: To answer these questions, we used immunofluorescence to characterize WT samples and identified CITED1+, SIX2+ and CITED1SIX2+ cells within WT samples and compared our findings with NP isolated from human fetal renal samples. In addition, using our established Smartflare isolation methods, we obtained live CITED1SIX2 positive cells from WT samples, isolated RNA and performed RNA-Seq.

RESULTS: We confirm the expression of CITED1 and SIX2 positive cells within the WT samples, and for the first time we also established the existence of a side population expressing both CITED1 and SIX2, as observed in the true NP within the human fetal kidney at week gestation 16. Further characterization of the WT also identified the absence of mature renal cell markers such as Nephrin and Aquaporin along with the absence of renal developmental markers such as Calbindin and LEF1. However, unlike NP from fetal kidney, some WT cells express NPHS2 and WT1 similarly to developing structure such as the renal vesicle, C- shaped bodies, S-shaped bodies, and glomeruli. Using Smartflare technology we have identified on average 6.7% SIX2+, 0.1% CITED1+, and 12.43% CITED1SIX2+ cells, while human fetal samples express .16% of CITED1SIX2+, 19.8% of SIX2+ and hardly any CITED1+ cells. Our RNA-Seq analysis identified different pathways expressed between NP isolated from fetal kidney and WT, specifically in relation to their cell cycle and cell cycle regulators.

CONCLUSIONS: This work suggests that we can identify and select for the first time CITED1SIX2 cell present within the WT. RNA-Seq data showed the presence of mechanisms essential for tumor formation. The discovery of this mechanism can help the development of new strategies aimed at halting tumor progression.

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a93478

## 03-P252 The regulatory mechanism of PAX3/p53 pathway in glioma stem cell renewal and differentiation

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In GBM, the brain glioma stem cells (BGSCs) were identified and played a crucial role in resistance of GBM to conventional therapies described above. PAX3 was identified as a diagnostic/prognostic marker and a therapeutic regulator in the therapy of GBM. Here, we hypothesized HIF-1 α mediated PAX3/p53 axis promoted the process of dedifferentiation under hypoxia, contributing to the cancer stem cell properties, such as proliferation and migration. Immunofluorescence of p53 was shown activated following BGSCs differentiation. We further identified that PAX3 might specifically bind to the promoter of p53 gene, and transcriptionally repressed p53 expression. ChIP assay further confirmed that PAX3/p53 axis regulated the differentiation process of BGSCs. Then, the function of PAX3 in BGSCs were sequentially investigated in vitro and in vivo. Ectopic PAX3 expression promoted BGSCs growth and migration while PAX3 knockdown suppresses BGSCs growth, migration in vitro and in vivo. Similar to PAX3 overexpression, p53 inhibition also showed increase in growth and migration of differentiated BGSCs. Regarding the functional interaction between PAX3 and p53, PAX3 knockdown mediated decrease in proliferation was partially rescued by p53 inhibition. Our work may provide a supplementary mechanism in regulation of the BGSCs differentiation and their functions, which should provide novel therapeutic targets for GBM in future.

a93079

## 03-P253 4D Tumoroid Cultivation for Sequential Assessment in Microenvironmental Context

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Tumors are regarded as dynamic abnormal organs and show stepwise changes during progression. As tumorigenic processes are closely interrelated, it is important to comprehensively assess the overall process of tumorigenesis to properly characterize tumor features. In particular, since tumors in vivo are exposed to heterogeneous gradient of nutrient and oxygen that spontaneously form hypoxic core within the tumor mass, it is important to properly understand the hypoxic features of tumors within a biologically relevant context. Here, we present a four-dimensional (4D) tumoroid cultivation methods to sequentially observe and assess the tumor features using biophysical parameters. By observing the time-varying processes of the tumoroid array, we found a positive correlation between parametric features of growth and invasion only in cases of tumoroids containing a hypoxic core, even for heterogeneous populations of tumoroids, showing higher expression of hypoxia inducible factor 1α (HIF1α). Within a bioengineered 3D microenvironment which consists of hyaluronic acid-based hydrogels and the stromal cells such as endothelial cells or immune cells, we visualize stromal interactions of hypoxia-induced tumoroids. In addition, we could confirm that the microenvironmental effects impact on enhancing invasive capacity and chemo-resistance in hypoxia-controlled tumoroids. Taken together, our platform enables prediction of tumoroid invasion by simply assessing the morphological features of the tumoroid. Our findings may be useful both for standardization of in vitro platforms for efficient cancer drug screening and for the discovery of novel drugs targeting tumor microenvironments.

a92260

## 03-P254 Focus on Bone Cancer Model Via Biomimetic Materials

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Worldwide cancer remains the second-most common cause of death, despite the advances in prevention, early detection, treatment and Osteosarcoma (OS) is the most common type of bone cancer, especially in children and young adults[1].
In the last decades, has been demonstrated the central roles of cancer stem cells (CSCs) in tumour recurrence, metastases and chemo resistance via self-renewal and differentiation; moreover, the interaction with extracellular matrix (ECM) appeared to be fundamental for CSCs signalling pathways[2]. For that reasons, 3D cell culture models are gaining more and more significance for the study of cancer biology because these models are recapitulating the in vivo situation of cells much better than the standard monolayer cultures. In the present study, we proposed a more complex in vitro 3D model mimicking the in vivo tumour microenvironment, with particular focus on osteosarcoma stem cells niche; CSCs and cancer cells were cultured in tradition 2D system and on a natural-inspired hydroxyapatite based scaffolds in order to obtain a 3D cancers stem cells model. CSCs were obtained staring from MG63 and SAOS2 cells line by sarcosphere formation assay[3], and long term culture were assess in static and dynamic condition.
The 3D CSCs model were characterised by assessing the proliferative and apoptotic state, the expression of osteosarcoma cancer cells related markers (Oct4, NANOG, SOX2, etc) and by evaluating the interaction of CSCs with biomaterial that guiding the plethora of signalling pathways involved in cancer developing.
In contrast to traditional 2D culturing systems, 3D cultures create an environment that enables cells to contact and interact in all dimensions with the scaffold and other cells in culture.
The exiting results leads to the conclusion that 3D CSCs models resulting in a great advancement for preclinical in vitro study, including new drug screening in OS, with the ultimate goal to be applied in personalized medicine.

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a90680

## 03-P255 Developing Tissue Engineered Model for Ameloblastoma

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Ameloblastoma is usually a benign tumour and associated with excess growth in the jaw area. It is characterized by locally aggressive behaviour and associated with high rate of recurrences. Removal of tumour minimizes the risk of recurrence and failure to remove the tumour properly can cause deaths due to metastasis to different organs. Thereby, alternative therapies should be developed to overcome recurrence cases. Information on the molecular mechanisms of ameloblastoma development and ameloblastoma-associated bone resorption is limited and therapeutic agents are not commonly used in ameloblastoma.
This project aims to develop a three-dimensional (3D) tumour model to understand the underlying mechanisms of ameloblastoma. AM-1 cells, which are mandibular-derived ameloblastoma cells, are used to develop a tissue engineered model. This model was designed as an environment, which accurately represents the human environment by incorporating bone mineral components to 3D model. An artificial cancer mass (ACM) composed on AM-1 cells was created and integrated into a collagen-only stroma to observe how AM-1 cells invades stroma.
Bone mineral components were also incorporated to this 3D model. This was achieved via using NuOss Cancellous Granules, which are made up of decellularised bone. By integrating ACMs to different stromal environment, changes in the invasion pattern of AM-1 cells and invasion-induced mineral breakdown are being observed.
By establishing an ameloblastoma 3D model, it can become possible to observe the cell-to-cell interactions that lead to ameloblastoma development as well as how the cells invade the bone tissue. Understanding these mechanisms is useful in developing and testing novel treatments.

a93374

## 03-P256 Biomimetic scaffolds to recreate hypoxic-induced tumor progression and drug resistance

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The complex interactions between a developing tumor and the surrounding microenvironment substantially contribute to the phenotype and behavior of cancer cells and govern processes as progression and resistance to therapies. For decades our understanding of cancer biology has relied on monolayer cultures grown on inert supports where the environmental cues of in vivo tissues are lost. On these substrates cancer cells alter some of their essential features, partially justifying the discrepancies that persist between preclinical and clinical results. Engineered in vitro models have led to new insights into the pathogenesis, prognosis and treatment of human diseases, allowing to investigate cells in a physiological-like context. This is crucial in oncology, as the fate of tumors depends either on the intrinsic characteristics of cancer cells and on the environmental conditions where they reside and grow. Here, we develop a biomimetic scaffold-based model of breast cancer that recreate mechanisms and processes involved in the tumor evolution and chemotherapy resistance acquisition. We selected two subtypes of breast cancer cells characterized by opposite clinical outcomes and a different molecular pattern, and probed specific responses during culture, performing comparisons with standard monolayers, xenotransplanted cells and validation in patient samples. We show that cancer cells in biomimetic conditions display in vivo-like growth dynamics and create an hypoxic niche that induce the modulation of gene expression, the selection of aggressive cells and the acquisition of chemotherapy resistance. This model may provide a high- throughput technology for disease progression modeling, biomarker detection and drug screenings with enhanced clinical relevance.

a93087

## 03-P257 Morphology-based Categorization for Evaluation of 3D cultured Tumor-derived Cells

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Recently, three dimensionally (3D) cultured cells, such as cell-aggregates, spheroid, and organoids, are expanding the capability of in vitro cell-based assays for drug developments. Compared to the two dimensionally culture cells, 3D cultured cells are known to show higher functions that can represent partial performance of organs. Therefore, there are growing attentions to establish new assay platforms using spheroids for more functional drug screening. Especially to understand the heterogenic and complex cellular response of cancer/tumor tissues, 3D cultured cells derived from cancer/tumor cell lines had been widely studied. By the development of multiple 3D culture protocols, there are growing reports that indicates effective assay performance.

However, in spite of the growing expectations of 3D cultured cell applications, the homogeneity/heterogeneity of produced multiple spheroids for assay have been still unclear. Especially, in the situation of primary cultured tissue-derived cells for their cancerous risk assessment, image-based morphological technology can offer a great potential for the high throughput and quantitative profiling technology to assess their heterogenic responses. Here, we introduce label-free live morphology-based analysis to cluster and discriminate the sub-category populations in 3D cultured cells, and its effective application for analyzing such morphologically irregular clone with “Image-cell picker”, the automatic platform we have developed.

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a91086

## 03-P258 Bioengineering the human bone niche for advanced cancer – *In vitro* assessment of anti-androgen therapies in metastatic prostate cancer

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In the last 20 years, cancer models have been helpful in unravelling important molecular mechanisms involved in different subtypes and stages of cancers[1]. With the rapid expansion of tissue engineering technologies, tumour engineering itself has grown briskly, to provide more sophisticated microenvironments, enabling faster and more efficient drug regimen selection prior to clinical trials[2]. Yet, current models are still lacking reproducibility, in-depth characterization and patient specificity necessary to successful implementation as effective drug screening platforms. In our lab, we have been recreating metastatic prostate cancer in the bone compartment, with a tissue engineered 3D *in vitro* model using patient derived progenitors, grown for several months to achieve bone mineralization mimicry. At the metastatic stage of prostate cancer, anti-androgens therapies are widely used for treatment, despite 5-year survival rates being below 3%, and no reduction in the metastatic burden. By utilising our metastasis bone model, we have been able to quantify in depth the effects of standard therapies (Enzalutamide (Xtandi®), Bicalutamide (Casodex®)) in the bone metastatic context. We are showing that anti-androgen therapies have a detrimental effect on metastasized prostate cancer cells, by gene and proteins analysis and novel methodologies using real live imaging, morphometry, and migration in 4D. By deregulation of the bone markers under these treatments (COL1, ALP, SOST, PTH1R) we are able to show that these therapies favour bone overproduction and cancer cell proliferation and migration, the results of an adaptive response induced by anti-androgen therapies. The model and innovative quantitative methodologies presented here are a powerful platform to study cancer cell behaviour in a relevant therapeutic context, ultimately maximising translation to patients.

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a93481

## 03-P259 Raman spectroscopic analysis of breast cancer tumour microenvironment

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Breast cancer incidence has risen significantly in the past years becoming the most frequent cancer among women. Raman spectroscopy (RS) has been successfully used to identify chemical changes in the breast tissue that occur due to development and progression of BC [1]. Invasion of breast cancer cells from the in situ tumour involves changes of the extracellular matrix and surrounding tissues [2]. During this study, 10 breast cancer samples and 10 normal breast samples arranged in Tissue microarrays (TMAs) have been analysed with Raman imaging. Cancerous and normal epithelia and its microenvironment have been analysed. For each area, imaging maps were collected and analysed with supervised and unsupervised algorithms. Biochemical characterisation and differentiation of cancerous and normal surrounding tissue was successfully achieved based on principal component analysis (PCA). The classification was achieved based on the differences in protein conformations, tryptophan configurations and increased glycogen content. RS allows the identification of changes in the breast tumour perimeter and the classification and progression of the disease.

a93460

## 03-P260 High-throughput Screening of Brain Cancer-ECM Interactions in 3D using Gradient Hydrogels

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Brain cancer is a devastating disease given its extreme invasiveness and delicate location, yet effective therapies remain elusive. Glioblastoma multiforme (GBM) is one of the most fatal forms of brain cancer. One key hallmark of brain tumors is the significantly increased tissue stiffness as the cancer progresses, however, how varying matrix cues modulate GBM progression in 3D remains largely unknown. The objectives of this study is to develop a facile method that allows fabrication of 3D gradient hydrogels with brain-mimicking biochemical cues and tunable stiffness as in vitro brain tumor models, and apply such biomaterials platform for evaluating GBM-niche interactions in a high-throughput manner. Gradient hydrogels were fabricated using our recently published method. The resulting gradient hydrogels exhibit brain-mimikcing stiffness ranging from ~40 Pa to 1000 Pa. While all zones support high GBM viability and proliferation, increasing hydrogel stiffness led significant decreases in GBM spreading in 3D, as shown by actin staining. This is coupled by stiffness-dependent upregulation of genes responsible for the ECM degradation, which is essential for cancer metastasis. When encapsulated in gradient hydrogels, GBM 270 exhibited differential resistance to Temozolomide (TMZ), a drug clinically used for treating GBM, highlighting the important role of matrix cues on drug responses of GBM. Together, our results validate such 3D gradient hydrogels as a biomimetic cancer cell niche, which enables high-throughput screening studies to accelerate the discovery of the role of niche cues in cancer progression using reduced materials, cells and time.

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a91000

## 03-P261 Brain-Mimetic Scaffolds Enable Investigation of Therapeutic Resistance and Invasion in Glioblastoma

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Glioblastoma (GBM) is the most lethal, yet common, cancer originating in the brain. The tumor microenvironment sustains the highly migratory and treatment resistant properties of a sub-population of stem-like cells in GBM (GSCs), which are thought to be largely responsible for tumor recurrence. Interactions between hyaluronic acid (HA) – a major component of the brain extracellular matrix (ECM) further overexpressed in GBM – and its receptors – including CD44, which is upregulated by GSCs – are reported to facilitate characteristic treatment resistance and aggressive infiltration adjacent to the brain microvasculature. However, the majority of pre-clinical studies do not account for the tumor microenvironment, compromising their clinical relevance. Thus, we have developed HA-based culture platforms that provide a controlled experimental context in which to characterize how the GSC microenvironment facilitates GBM tumor aggression.

Multiple patient-derived GSC lines were cultured in HA-based hydrogels with varied compressive moduli, HA content, and ECM peptides. While GSCs were sensitive to erlotinib – an epithelial growth factor receptor (EGFR) inhibitor – when cultured as gliomaspheres, those in hydrogels mimicking normal brain acquired cytotoxic and cytostatic resistance, which was dependent on HA-CD44 interactions. GSCs cultured in soft, HA-rich hydrogels increased CD44 expression and EGFR phosphorylation, indicating that HA-bound CD44 and EGFR interacted to facilitate resistance. In addition, luciferase-encoding reporters for transcription factor activity showed that erlotinib treatment reduced activity of apoptotic factors and increased activity of oncogenic factors. Integrin binding peptides, such as RGD, provided further protection against cytostatic and cytotoxic effects of erlotinib. Migratory activity of GSCs was further enhanced when cultured in hydrogels with peptides derived from integrin-binding sialoprotein, which we have identified to be uniquely upregulated by tumor vasculature.

In sum, we report biomaterial cultures of patient-derived GSCs in which clinical phenotypes – including 1) kinetics of acquired resistance to inhibition of EGFR and 2) vasculature-associated infiltration – are preserved. Ultimately, we aim to identify specific GSC-ECM interactions whose targeted disruption represents a viable clinical strategy to prevent and/or delay both therapeutic resistance and vessel-mediated tumor infiltration.

a92347

## 03-P262 Specific ECM composition regulates Smad dependent - Transforming Growth Factor- β1 (TGF β1)–induced EMT response in HepG2 cells engineered in cirrhotic and healthy liver 3D scaffolds

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**Background:** Hepatocellular carcinoma (HCC) is the second cause of cancer deaths motivating the investigation of new therapeutic targets to provide alternative treatments. A new 3D model was developed by using decellularized human liver 3D scaffolds which maintains the original tissue-specific 3D architecture, biochemical-and mechanical properties. Cirrhotic ECM specifically induced an EMT-related gene signature in HepG2.

**Aims:** This study investigates further the effect of cirrhotic and healthy ECM on TGF β1-induced SMAD2/3 signalling in EMT-related HCC cell behaviour and on the assessment of drug sensitivity.

**Method:** Human liver 3D scaffolds were obtained by decellularization of healthy and cirrhotic human livers and were repopulated with HepG2 cells for 7 days followed by TGF β1 (5-10ng/ml) treatment. Changes in TGF β1 response were investigated by pre-treatment/treatment with TGF β-R1 kinase inhibitor Galunisertib (10μM) for 48hrs. Protein expression was assessed for the canonical TGF β1 signalling pathway proteins P-SMAD2/3, SMAD2/3 and non-canonical signals of Erk, Akt and JNK in HepG2 cells cultured in cirrhotic and healthy scaffolds. Exogenous TGF β1 uptake and release was analysed by ELISA.

**Results:** P-SMAD3 had a low expression in HepG2 cells cultured on healthy scaffolds while being highly expressed on cirrhotic scaffolds. In contrast, P-SMAD2 had a low expression in HepG2 cells cultured on cirrhotic scaffolds, while increased in HepG2 cells cultured on healthy scaffolds. SMAD2/3 proteins were expressed in HepG2 cells cultured on both cirrhotic and healthy scaffolds. Each protein was differently affected by exogenous TGF β1 treatment. Pre/simultaneous treatment of TGF β1 with Galunisertib affected protein expression depending on the ECM-scaffold used. TGF β1-Galunisertib treatment reduced albumin protein expression in HepG2 cultured on cirrhotic scaffolds. Erk protein expression was similar on both types of scaffolds, while Akt showed a higher expression on healthy scaffolds. Uptake/release of TGF β1 showed marked differences between healthy and cirrhotic scaffolds with an increased uptake and release in time by cirrhotic 3D scaffolds.

**Conclusion:** This study suggests that the ECM composition of the 3D liver scaffold itself can affect gene/protein expression which can be further modified by TGF β1 treatment. This study suggests that TGF β1 and its downstream signaling pathway(s) represent potential targets for the treatment of HCC.

a90515

## 03-P263 Establishment of patient-specific cancer cell lines from colon cancer tissues by membrane Filtration Method via Nylon mesh filter and PLGA-silk screen membranes

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Cancer stem cells (CSCs) typically comprise 1%–5% of the total tumor cell population. This cell population is considered to be primarily responsible for tumor initiation, growth, and metastasis. However, it is hard to distinguish cancer cells from other cells (i.e. fibroblasts, red blood cells…etc.) in primary tissue after enzyme digestion process. Therefore, we develop membrane filtration and migration method to target and purify rare primary CSCs based on their high migration mobility characteristics compared with other tissue cells. We expect to establish the primary colon cancer cell line from primary tumor tissue by the membrane filtration and migration method for the development of patient specific therapy in clinical application.

We designed membrane filtration method to purify and further establish primary colon cancer cell line from human colon cancer tissue. The colon cancer tissue was digested by collagenase type IV at first. After digestion of the tissue, the primary colon cancer cell solution was permeated through the membranes with different pore size and different degree of biocompatibility. We used Nylon mesh filter[1] with pore size of 11 μm and 20 μm and PLGA-silk screen membrane with different PLGA concentration 3%, 5%, 10% as the membranes in this study. With increasing PLGA concentration, the pore size of the membranes decreased. These membranes were used to capture primary colon cancer cells, and the cells on the membranes were expected to migrate into the culture dishes during the cultivation of the membranes in the culture medium (i.e., membrane migration method) after the filtration of colon cancer tissue solution through the membranes. Afterwards, we analyzed the expression of cancer stem cell markers, CD133 and CD44 by flow cytometry. Furthermore, the tumorigenesis potential of primary cells and the cells purified by the membrane filtration and migration method was evaluated by soft agarose colony forming assay. We expect to establish patient-specific colon cancer cell line by the membrane filtration and/or migration method for benefit of the cancer therapy in the future.

a92461

## 03-P264 Liposomal drug delivery in an in vitro 3D bone marrow model for multiple myeloma

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Multiple myeloma is an incurable malignancy of the plasma cells that develops in the bone marrow (BM). To assess the therapeutic potential of experimental anticancer drugs against myeloma *in vitro,* 2D cell line cultures are commonly used. These 2D cultures do not mimic the dependency of primary myeloma cells on the surrounding BM, nor the BM itself. The BM supports myeloma progression and resistance to therapy, essential characteristics that need to be taken into account when assessing the therapeutic potential of experimental anticancer drugs. Also the effects of experimental drugs on the surrounding BM should be assessed. This can be done using a 3D BM model for the culture of myeloma cells.

A previous developed 3D BM-myeloma model was used to assess the delivery, migration and overall effect of both an established chemotherapy and a novel therapy for multiple myeloma: liposomal drug delivery. Targeted drug delivery using liposomes has the advantage of intensified drug activity, while decreasing off-target effects. The feasibility to test such a nanomedicine delivery system in a 3D tissue model, and thereby establish its role as a predictive tool, was subject of this study.

Liposomes of different sizes (on average 70 nm, 100 nm and 200 nm) were tested in a 3D BM-myeloma model. This model is based on mesenchymal stromal cells, endothelial progenitor cells and myeloma cells co-cultured in hydrogel. Only the 70 nm and 100 nm liposomes were capable of passively diffusing through the 3D model after injection, but not after addition to the medium. Cellular uptake of the liposomes was observed throughout the model, displaying a concentration gradient after injection. When comparing free drug therapy to liposomal drug therapy (doxorubicin), similar killing effects towards myeloma cells were observed. However, the free drug therapy induced significantly more BM cell death compared to the liposomal drug therapy.

In conclusion, the previously developed 3D BM model allows the study of nanomedicine delivery system on (primary) myeloma cells. The model could be used to identify the optimal delivery method *in vitro*, showing cellular targeting throughout the model and general effectiveness of the added therapy by analysis of both on- and off-target effects.

a92218

## 03-P265 Development of an *in vitro* three-dimensional tumor model for drug screening

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The use of cell-derived matrices (CDMs) is a promising alternative to decellularized tissues/organs because these are bioactive and biocompatible materials consisting of a complex assembly of proteins, matrix macromolecules and growth factors. The ability to create different CDM based on the cell source and culture methods, offers the possibility to tailor-made bioactive materials for desired applications as the creation of a 3D printable bioink, for tissue engineering applications. Cell cultured microparticles that favor its adhesion, proliferation and CDM production enhanced by the Macromolecular Crowding (MMCs) effect will be used for the creation of colon tumor CDM[1].

Poly-lactic acid (PLA) microcarriers are made by jetting this polymer through a coaxial needle[2] and coating them with Fibronectin to enhance cell adhesion.

hAMSCs, colon tumor cells and CAFs[3] cells are seeded in co-culture at microparticles' surface in spinner flasks with intermittent agitation to enhance nutrient transfer and avoid dead areas. Then, cell-seeded particles are cultured for 21 days with MMCs and with permanent agitation. Results are analyzed in terms of Total DNA, Total protein production, surface properties by SEM, mechanical properties by AFM and protein expression by immunostaining and confocal microscope imaging and qRT-PCR.

MMCs cultured tissues present significantly higher amounts of total protein in comparison to the same tissues cultured in absence of this substance. Fibrillary proteins (such as Collagen types I and III) present in colon tumor extracellular matrix (ECM) are highly expressed after 21 days of culture. Therefore, tissues density and size is greater, more protein is observed when topography is studied at the SEM and tissue stiffness is increased.

Furthermore, CDM can be used to understand various mechanisms promoting cancer progression by decellularizing bioengineered tissues and by the recellularization of them, as a tumoral model to develop drug screening assays or identifying therapeutic targets.

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a93201

## 03-P266 Design and Implementation of a 3D Hydrogel System for Chemo-Sensitivity Testing and Diagnostics Using Growth Model Tumors and Human Tumor Biopsies

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We developed a customized growth model system for *in-vitro* chemosensitivity and cancer drug screening. Extracellular matrix biomaterial analogs made from PEG-fibrinogen hydrogels were used to encapsulate tumor biopsies, in order to enhance the outgrowthof cancer cells over mesenchymal cells into the 3D hydrogel. After establishing the optimal hydrogel composition for the best cancer cell outgrowth using artificial tumors, preliminary experiments were performed with human biopsie. In the first experiments, biopsy samples were encapsulated using the same conditions proven successful for the artificial tumors, but in most samples, no cell outgrowth was visualized.To induce cell outgrowth from the human biopsies, we alterated the matrix stiffness and fibrinogen concentration, as well as added fibronectin, Laminin-511, or fibroblast cells into the hydrogel matrix.

Whereas most of the condition alone failed to promote cancer cell invasion, highr matrix stiffness together with a lower fibrinogen concentration did lead to improved cancel cell invasion.

a91256

## 03-P267 In vitro preclinical primary pancreatic cancer models: microenvironment re-modelling during tumour progression

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Pancreatic adenocarcinoma (PA) is a common cancer with annual mortality rate similar to the incidence rate. PA prognosis is very poor, and there is an urgent need to design and test interventions in a more effective manner. PAs are stroma-rich cancers in which the dense extra-cellular matrix (ECM) is ascribed to reduce drug perfusion, hence drugs are poorly delivered to cancer cells and lack of efficacy.

Currently chemotherapies are tested with simple 2D in vitro models, which fails in recreating the pancreatic tumour microenvironment (TME) hence predicted drug efficacy is far from the real values. The dense TME in facts forms a physical barrier that blocks the penetration of drugs and reduces their efficacy. Novel strategies are now trialled, combining standard chemotherapies with actives targeting the stroma with the ultimate intention to facilitate drug delivery to cancer cells and block tumour growth.

In this study we modelled hydrogel composition to mimic different stages of PA, from the early stages (T1, less than 5% of tumour tissue) to later and metastatic stages (T3, tumour tissue start to growth and invade neighbours tissues). Hydrogels with defined composition (alginate, gelatin, hyaluronic acid) and stiffness values matching different stages of PA development (T1-T3, from 2 kPa to 20 kPa respectively) are used as substrate for cell culture. Pancreatic cancer cell lines are characterised in biomarkers expression e.g. CD44, metastatic potential and drug resistance.

a92952

## 03-P268 Extracellular Matrix Hydrogel Downregulates Neoplastic Esophageal Cell Phenotype

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Tumor resection requires a suitable biomaterial that will not promote infection or re-occurrence of disease, and ideally reconstruct the tissue; but limited options exist. In 2011, our group treated 5 esophageal adenocarcinoma (EAC) patients (T1A) with a heterologous, xenogeneic extracellular matrix (ECM) bioscaffold after aggressive mucosal resection. The patients functionally preserved their esophagus, did not stricture, and most importantly, did not show reoccurrence of cancer to date. Since then, 9 more patients have been treated with similar results, and are 1-8 years disease-free. The described study is in a Phase I Clinical Trial, but before widespread clinical translation, an understanding of the molecular mechanisms is needed. The objective of the present study was to evaluate the effect of ECM bioscaffold degradation products (formed by pepsin-solubilization of the bioscaffolds) from non-malignant, decellularized tissue on normal (Het-1A), cancer pre-cursor (CP-A), and neoplastic (SK-GT-4, OE33) esophageal epithelial cells. ECM from heterologous urinary bladder matrix (UBM) and esophageal mucosa (eECM) were evaluated as candidate therapies. The two ECM tissue sources showed similar but distinctive effects on cell morphology, cell function (metabolism, proliferation, apoptosis), and EAC signaling pathways (gene, protein expression) compared to pepsin control. Both ECM tissue sources decreased OE33 cell proliferation and EAC signaling pathway PI3K-AKT gene expression at 24h, and decreased OE33 phosphorylated AKT as early as 6h for eECM and at 24h for UBM. The two tissue types also showed distinctive effects to downregulate neoplastic phenotype: UBM decreased OE33 and SK-GT-4 cell metabolism and increased CP-A cell apoptosis; while eECM decreased SK-GT-4, CP-A, and Het-1A cell proliferation; and showed robust downregulation of OE33 cell cycle/DNA replication signaling. The present study supports the continued investigation of ECM bioscaffolds to treat EAC patients in the Phase I clinical trial.

a90750

## 03-P269 Identification of Colon Cancer Cells and Cancer Stem Cells from Cancer Cell Line for Establishing Patient-Specific Targeted Therapy

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Cancer stem cells (CSCs) demonstrate the characteristics of normal stem cells, which own the ability to self-renewal and differentiate. Cancer stem cells are considered to be responsible for tumor generation, relapse, and metastasis. The purification and identification of cancer stem cells help to construct a specific targeted therapy, and improve the quality of life of patients in clinical application.

In this study, we investigate the optimal cell culture materials for capturing CSCs. To investigate the interaction between low malignant colon cancer cell (LoVo cells) and extracellular matrix, we culture LoVo cells under various types of 2D and 3D microenvironments. For 2D culture materials, different types of extracellular matrix (Matrigel, recombinant vitronectin, synthemax type II, Cellstart, collagen type I, fibronectin, and mouse embryonic fibroblasts)-immobilized dishes, poly(NIPAAM-co-BA)-coated dishes, and polystyrene dishes have been used. On the other hand, for 3D culture materials, ultra low attachment plates have been applied for spheroid culture. The suppression and enrichment of LoVo cells on these materials can be observed and evaluated under microscopy. LoVo cell showed different phenotypes while culturing on different extracellular matrix environments. After three passages, we examined the expression of cancer stem cell marker, CD133 and CD44, by flow cytometry. Furthermore, tumorigenesis potential of LoVo cells on different culture materials were evaluated by using soft agarose colony forming assay.

We identify the optimal extracellular matrix for isolating CSCs from low malignant cancer cells and develop a specific targeted therapy for patients in the future.

a90474

## 03-P270 Examining the Interaction Between Adipose Derived Stem Cells (ADSC) and MCF-7 Breast Cancer Cells Using Co-Culture Technique

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Introduction: Breast cancer is the most common cancer worldwide with nearly 1.7 million new cases diagnosed each year. The gold standard autologous breast reconstruction following mastectomy often involves 'lipofilling' or fat transfer which, although cosmetically successful, contains significant numbers progenitor cells with the potential for malignant transformation. Both *in vitro* and *in vivo* studies have suggested that Adipose Derived Stem Cells (ADSCs) confer a malignant advantage to cancer cells via the release of cytokines into the surrounding area and through direct cell-to-cell interaction, affecting phenotype, morphology and growth rates. This has caused surgeons to be cautious when using this technique for reconstruction, however no such advantage has been observed in the clinic.

Hypothesis: ADSCs isolated from breast cancer patients exhibit different cytokine excretion profiles from those harvested from healthy patients, and therefore are less likely to support cancer growth and progression.

Methods: ADSCs harvested from healthy and cancer patients were fully characterised. Media conditioned by the ADSCs was incubated with the breast cancer cell line, MCF7, and alterations in proliferation (iCELLigence), migration (scratch assay with cell culture inserts), invasion (collagen membranes) and bioenergetics (seahorse) were examined.

Results: We have shown that conditioned media from healthy ADSCs had a greater impact on the proliferation, migration and invasion of MCF7 cancer cells when compared to those extracted from patients who have been diagnosed with cancer.

Conclusion: Previous scientific studies have examined only healthy ADSC populations, therefore the unique focus of this work aimed to better replicate the current patient model and has determined that the presence of cancer has a significant impact on the pro-cancer cytokine production of this progenitor population. This indicates that ADSCs utilised for reconstruction in breast cancer patients do not confer the malignant advantage to the cancer cells observed in previous studies.

a92138

## 03-P271 Indole-6-carboxaldehyde inhibits the PMA-induced metalloproteinase matrix (MMP)-9 and invasion of HT1080 human fibrosarcoma cell

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*Sargassum thunbergii*, brown algae, has several compounds with biological activities. It is known that *S. thunbergii* methanol extract and phlorotannin inhibited matrix metalloproteinase (MMP)s. However, there is no study whether indole derivatives isolated from *S. thunbergii* was inhibited the cell migration related to the antitumor activity. Therefore, the aim of the present study was to investigate the cell migration inhibitory effect of indole derivatives from *S. thunbergii* through down-regulation of the phorbol 12-myristate 13-acetate (PMA)-induced MMP-9 in HT1080 fibrosarcoma cell and the associated mechanism of action. Among indole derivatives, indole-6-carboaldehyde (ICA) significantly inhibited cell migration and suppressed MMP-9 expression on PMA-stimulated HT1080 fibrosarcoma cell. The ICA suppressed the phosphorylation of c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK). In addition, ICA suppressed the phosphorylation of inhibitor of $\kappa$ B$\alpha$ (I $\kappa$ B$\alpha$) and nuclear translocation of p65 in PMA-stimulated HT1080 fibrosarcoma cell. Based on the results, ICA inhibits cell migration via down-regulation of MMP in PMA-sitmulated HT1080 fibrosarcoma cells through suppressing the phosphorylation of JNK and ERK and the translocation of nuclear factor-$\kappa$ B (NF-$\kappa$ B).

a92143

## 03-P272 The heptameric peptide purified from marine microalgae inhibited PMA-induced MMP-9 in human fibrosarcoma HT1080 cells

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In this study, a heptameric peptide obtained from marine microalga, *Pavlova lutheri* and its inhibitory effect and the associated mechanism of action on matrix metalloproteinase-9 (MMP-9) was evaluated in human fibrosarcoma cell line (HT 1080). Consecutive purification, a potent MMP-9 inhibitory peptide is composed of seven amino acids, Leu-Leu-Ala-Pro-Pro-Glu-Arg (796.4 Da). The mRNA expression levels of both MMP-9 was inhibited by the purified peptide. In addition, we also noted that the peptide inhibited PMA-induced translocation of nuclear factor-κ B (NF-κ B) by suppressing I κ Bα phosphorylation and it suppressed the phosphorylation of c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK). Therefore, these results suggested that the purified peptide obtained from *Pavlova lutheri* has a potential as valuable natural preventive agents for MMP-related diseases.

a91806

## 03-P273 A hematopoietic origin of pericytes during angiogenesis – Implications for cell-based therapies

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Pericytes wrap around endothelial cells in small blood vessels and are indispensable for proper vessel function. A couple of years ago pericytes have been identified to be mesenchymal stem cells. Mesenchymal stem cells, and especially their specialized subpopulation of pericytes, represent promising candidates for therapeutic angiogenesis applications, and have already been widely applied in pre-clinical and clinical trials. However, cell-based therapies of ischemic diseases have not resulted in significant long-term improvement. Interestingly, just recently pericytes from a hematopoietic origin were observed in embryonic skin. Additionally, a pericyte sub-population expressing leukocyte and monocyte markers was described during adult angiogenesis *in vivo*. Since mesenchymal stem cells do not express hematopoietic markers by definition, the latter cell type might represent an alternative hematopoietic pericyte population relevant to angiogenesis.

We therefore sourced blood-derived angiogenic cells (BDACs) from monocytes that closely resembled hematopoietic pericytes *in vitro*, which had only been observed *in vivo* thus far. BDACs displayed many pericytic features such as pericyte markers PDGFRb and NG2, while expressing leukocyte markers CD45 and monocyte markers CD11b. At the same time, they lacked properties inherent to monocytes and macrophages such as the ability to phagocytose or the ability to be polarized. They also enhanced angiogenesis *in vitro* and *in vivo* and enhanced revascularization and functional tissue regeneration in a pre-clinical model of critical limb ischemia. Comparison between BDACs and mesenchymal pericytes in functional *in vitro* assays revealed that in direct co-culture BDACs enhanced, while mesenchymal pericytes impaired endothelial sprouting. In contrast only mesenchymal pericytes consistently stabilized endothelial tubular networks.

We therefore concluded that BDACs (while resembling hematopoietic pericytes) enhanced early stages of angiogenesis, while mesenchymal pericytes were responsible for blood vessel maturation and homeostasis. Since the formation of new blood vessels is crucial during therapeutic angiogenesis or during integration of implants into the host tissue, hematopoietic pericytes (and therefore BDACs) might offer an advantageous addition or even an alternative for cell-based therapies.

a93385

## 03-P274 Targeted delivery of nitric oxide via "Bump-and-hole" based enzyme/progdrug pair

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Nitric oxide (NO) is a versatile endogenous messenger whose spatio-temporal generation is under precise control. Despite its therapeutic potential for a wide range of diseases, NO-based therapies are rarely limited clinically due to lack of effective strategies that enable the precise delivery of NO to specific site. In the present study, we developed a novel NO delivery system via modification of enzyme/prodrug pair of galactosidase/galactosyl-NONOate using a "bump-and-hole" strategy. Precise delivery to targeted tissues has been clearly demonstrated by *in vivo* near infrared imaging assay. The therapeutic potential was evaluated in rat hindlimb ischemia and mouse acute kidney injury models, respectively. Targeted delivery of NO evidently enhanced therapeutic efficacy that helps to tissue repair and function recovery, and abolished side effects due to the systemic release of NO. The protocol developed in this study holds broad applicability in the targeted delivery of important gaseous signaling molecules ($H_2S$, $SO_2$, CO), and offers a potent tool for the investigation of relevant molecular mechanism.

a91257

## 03-P275 Flow-Induced Vascular Network Formation and Maturation in Three-Dimensional Engineered Tissue

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Engineered three-dimensional (3D) constructs have received much attention as *in vitro* tools for the study of cell-cell and cell-matrix interactions, and have been explored for potential use as experimental models or therapeutic human tissue substitutes. Yet, due to diffusion limitations, lack of a stable and perfusable blood vessel networks, jeopardizes cell viability once the tissue dimensions extend beyond several hundred microns. Direct perfusion of 3D scaffold cultures has been shown to enhance oxygen and nutrient availability. Additionally, flow-induced shear stress at physiologically relevant levels, positively impacted endothelial cell migration and alignment in various two-dimensional (2D) culture models and promoted angiogenic sprouting in microfluidic systems. However, little is known about the effect of flow on vascularization in implantable 3D engineered tissue models. The present study investigated the effect of direct flow-induced shear stress on vascularization in implantable 3D tissue. The differential effect of various levels of shear stress, applied while maintaining constant culture conditions, on vascular parameters was measured. Samples grown under direct flow conditions showed significant increases (>100%) in vessel network morphogenesis parameters and increases in vessel and extracellular matrix (ECM) protein depth distribution, as compared to those grown under static conditions. Enhanced vascular network morphogenesis parameters and higher colocalization of alpha-smooth muscle actin ($\alpha$-SMA) with endothelial vessel networks characterized the specific contribution of direct flow to vessel network complexity and maturation. These observations suggest that flow conditions promote 3D neovascularization, and may be advantageous in attempts to create large-volume, clinically relevant tissue substitutes.

a93367

## 03-P276 Electrospun patch functionalized with nanoparticles allows for spatiotemporal release of VEGF and PDGF-BB promoting *in vivo* neovascularization

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The use of nanomaterials as carriers for the delivery of growth factors has been applied to a multitude of applications in the field of tissue engineering. Growth factors delivered with tailored spatiotemporal characteristics are leveraged to induce cellular signaling which can have downstream remodeling effects. However issues of toxicity, stability and systemic effects of these platforms have yet to be fully understood, especially for cardiovascular applications. In this study we proposed a delivery system composed by poly(DL-lactide-co-glycolide) acid (PLGA) and porous silica nanoparticles (pSi) to deliver vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF).

The tight spatiotemporal release of these two proteins has been prove to promote neovascularization and could be easily applied to ischemic tissue. In order to avoid tissue toxicity, augmenting the localized release, and stability of the platform we conjugated them to and electrospun (ES) gelatin patch patches. When compared to freely disperse particles, ES gelatin patches were shown in vitro cultured with neonatal cardiac cells to have significantly less internalization of PLGA-pSi particles compared to free PLGA-pSi or pSi groups. Internalization was positively correlated to late stage apoptosis resulting in PLGA-pSi and pSi groups with over 15% of cells apoptotic compared to approximately 4%in the ES gelatin group after 3 days in culture.

Release of growth factors from the ES gelatin patch was shown to be sustained and with the sequential release of VEGF and PDGF over time based on different PLGA encapsulation formulations. When implanted subcutaneously, ES gelatin patches were shown to have greater neovascularization than controls evidenced by increased impression of α-SMA and CD31 after 21 days. qRT-PCR results support increased angiogenesis by upregulation of VEGFA, VEGFR2, Vwf and COL3A1 exhibited a synergistic effect with the release of VEGF and PDGF after 21 days in vivo. The results of this study proved that PLGA-pSi particles crosslinked to ES gelatin reduced cellular toxicity and could be tailored to have a dual release of growth factors promoting localized neovascularization.

a93404

## 03-P277 Re-vascularising an ischemia limb- Can hAEC exosomes augment angiogenesis

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**Background:** Cardiovascular disease (CVD) remains the leading global health problem affecting individuals from every socioeconomic scale. Progressive tissue hypo-perfusion results in ischemic ulceration leading to chronic critical limb ischemia (CLI). Ischemic hypoxia causes oxidative stress initiating inflammation and necrosis of the extremities, leaving amputation as the only option. Human amnion epithelial cells (hAECs) have been shown to secrete pro-resolution and angiogenic factors to subdue inflammation and accelerate vascularisation suitable for preservation of ischemic limbs.

**Methods:** SCID mice underwent surgical ligation of femoral artery to induce hind limb ischemia and were treated with 10ug of hAEC Exo intramuscularly. Neovascularisation was assessed using 99mTc-RGD SPECT imaging, and macrovascular volume was assessed by quantitative time of flight MRI. Histological assessment of capillary formation and muscle loss was measure with CD31 and H&E staining.

**Results:** hAEC Exo at 10ug increased 99mTc-RGD retention in the ischemic hind limb by day 7 post-surgery, with signifcant retention at day 14. Histological analysis showed significant muscle recovery at day 14 with increased in capillary density ($CD31^{+}$) at day 14.

**Conclusion:** Human amnion epithelial cell exosomes possess angiogenic properties suitable for revascularisation of hind limb ischemia.

a91449

## 03-P278 An electrospun vascular scaffold for cellularized small diameter blood vessels: a preclinical large animal study

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The strategy of vascular tissue engineering is to create a vascular substitute by combining autologous vascular cells with a tubular-shaped biodegradable scaffold. We have previously developed a novel electrospun bilayered vascular scaffold that provides proper biological and biomechanical properties as well as structural configuration. In this study, we investigated the clinical feasibility of a cellularized vascular scaffold in a preclinical large animal model. We fabricated the cellularized vascular construct with autologous endothelial progenitor cell (EPC)-derived endothelial cells (ECs) and smooth muscle cells (SMCs) followed by a pulsatile bioreactor preconditioning. This fully cellularized vascular construct was tested in a sheep carotid arterial interposition model. After preconditioning, confluent and mature EC and SMC layers in the scaffold were achieved. The cellularized constructs sustained the structural integrity with a high degree of graft patency without eliciting an inflammatory response over the course of the 6-month period in sheep. Moreover, the matured EC coverage on the lumen and a thick smooth muscle layer were formed at 6 months after transplantation. We demonstrated that electrospun bilayered vascular scaffolds in conjunction with autologous vascular cells may be a clinically applicable alternative to traditional prosthetic vascular graft substitutes.

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a92950

## 03-P279 Construction of intelligent antithrombotic small diameter tissue engineered blood vessel in vivo by utilization host response

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Thrombosis is one of the biggest obstacles in the clinical application of small-diameter TEBVs. The implantation of an unmodified TEBV will lead to platelet aggregation and thrombosis, in which the high concentration of adenosine diphosphate (ADP) that is released by platelets plays an important role. Before endothelializaton of TEBVs in vivo, how to prevent thrombosis is a key problem. Recent studies have proved that low levels of inflammatory response could promote mobilization and homing of early EPCs. However, excessive inflammation would reduce the EPCs and cause thrombosis. So it's critical to promote inflammation resolution of TEBVs in time and facilitate the growth and differentiation of EPCs towards ECs.

We designed a reduced graphene oxide based dual-enzyme biomimetic cascade to successively convert ADP into AMP and AMP into adenosine.1 We used RGO as a support and bound apyrase and 5-nucleotidase on its surface through covalent bonds, and then, we modified the surface of the collagen-coated decellularized vascular matrix with the RGO-enzyme complexes, in which RGO functions as a platform with a large open surface area and minimal diffusion barriers for products to integrate two catalytic systems for cascading reactions. And we found a novel mechanism underlies netrin-1 regulating inflammation may acting on MΦ.2 Our results show that netrin-1 treatment of MΦ induced CD163 expression and suppressed LPS-induced CD86 expression, further studies showed netrin-1 activated STAT6, PPAR$\gamma$ and glucocorticoid pathways.

We successfully constructed RGO-enzyme-coated TEBVs for antiplatelet function and endothelialization. These TEBVs reached a patency rate of 90% 7 days after grafting. Furthermore, we found that netrin-1 promotes MΦ reprogramming to induce inflammation resolution and the release of exosomes from MΦ to target and regulate EPCs and improve the long term patency rate of small diameter TEBV. So we constructed intelligent anti-thrombosis small diameter tissue engineered blood vessel in vivo by utilization host response.

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a90968

## 03-P280 Small Diameter Nanofibrous Scaffolds for *In Situ* Vascular Tissue Engineering

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Over 17 million deaths were attributed to Cardiovascular disease (CVD) in 2008, of these deaths, 50 percent were the direct result of vascular damage induced by plaque buildup and accompanying lesions. Currently, the standard treatment utilizes synthetic and autografts from Dacron and Teflon or the saphenous vein. However, patients suffering from CVD may not have healthy vessels, and synthetic grafts do not allow cellular remodeling, falter over time, and when less than 6 mm in ID experience deleterious interactions with the circulatory proteins, leading to thrombosis. To overcome the shortcomings described, a biomimetic bi-layer nanofibrous tubular scaffold with tunable diameter and pore size has been developed using chemically modified poly(l-lactic acid) (PLLA) and electrospinning Poly( $\varepsilon$ -caprolactone). The inner layer was fabricated through a thermally induced phase separation technique and using a sugar template to produce an architecture that is porous and interconnected and able to mimic the supportive structure of the extracellular matrix (ECM). The scaffolds were fabricated with dimensions of 0.9 mm ID, 1.6 mm OD, and 5.0 mm in length and conjugated with anticoagulant molecules (heparin, PEG) before *in situ* implantation as a replacement for the descending aorta in female rat models (250 – 350 g). Doppler ultra sound was used to evaluate the scaffolds and showed no issues of thrombosis or aneurysm formation in 30 early rat models. At 1 and 3 months, the rats were sacrificed, and the scaffolds were harvested. Through H&E, Masson's trichrome, and Verhoeff's stainings, excellent cellular infiltration, proliferation, and migration from the sites of anastomosis towards the midpoint of the scaffold were observed. More importantly, there was cellular remodeling with laminar cellular arrangement, excellent ECM deposition, and the beginning of the same phenomena in the midpoint of the scaffold as early as 1 month. The gene expression of SM22-$\alpha$, CNN-1, and MYH-11 of the inner layer was quantified and showed elevated levels of these markers compared to native vasculature. We believe, therefore, that we have developed an effective vascular graft producing platform to generate mature small diameter blood vessels that can function in animal models.

a90570

## 03-P281 A silk fibroin-polyurethane hybrid and semi-degradable arteriovenous graft for hemodialysis

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In the field of hemodialysis there is the urgent, unmet, clinical need of a vascular access that is easily accessible (available off-the-shelf and suitable for early cannulation like synthetic arteriovenous grafts) and durable (patent on the long term like native fistulae). To meet this need, herein it is proposed a novel hybrid and semi-degradable vascular access, combining a medical-grade non-degradable polyurethane (PU), conferring elasticity and puncturability, with silk fibroin (SF), providing biocompatibility, bioactivity, and remodeling capacity.

Grafts (L≈22cm, ID≈7mm, thickness≈400mm) were fabricated by electrospinning concentric layers of SF around a core of blended SF and PU (Silkothane®). Following crystallization in ethanol solutions, vacuum drying and ethylene oxide sterilization, prototypes were characterized in terms of morphology, mechanics, physico-chemistry and cytotoxicity, according to international standards and in comparison with commercial grafts when applicable.

Silkothane® grafts revealed a fibrous morphology with diameters between 0.3±0.1$\mu$m and 1.0±0.2$\mu$m. Dynamic compliance was 5±1%, between the saphenous vein (4%) and carotid artery (8%), and burst pressure was 840±117mmHg. Suture retention strength was 3.0±0.5N. Water permeability before and after puncture were $1.8x10^{-17}$ ± $1.4x10^{-17}$Darcy and $9.9x10^{-14}$ ± $2.8x10^{-14}$Darcy, respectively, in line with a commercial PU graft ($2.0x10^{-17}$ and $8.2x10^{-14}$Darcy) and superior to two commercial polytetrafluoroethylene grafts (both before puncture – 0 Darcy; after – $1.9x10^{-13}$ and $1.1x10^{-12}$Darcy). Cytotoxicity level was equal to the negative control.

Overall, *in vitro* characterizations showed that Silkothane® grafts possess excellent properties to be used as off-the-shelf and early-puncturable hemodialysis vascular accesses, possibly favoring tissue colonization and remodeling on the long term, and realizing the so-called 'endogenous tissue regeneration' approach to tissue engineering. Further tests are underway to assess the grafts' behavior i) upon *ex vivo* contact with blood, ii) upon anastomosis to native arteries, and iii) when cultured with smooth muscle cells under arterial hydrodynamic conditions.

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a91608

## 03-P282 Developing Imaging-Compatible 3D-Printed Bioresorbable Scaffolds

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It is estimated that approximately 8-12 million people in the United States have peripheral artery disease (PAD).[1] Problems (e.g. restenosis, thrombosis, and impaired vasomotion) associated with bare and drug-eluting metal stents have prompted the development of bioresorbable vascular scaffolds (BVS). Currently, only the beginning and end of polymeric commercially available BVSs can be visualized using conventional X-ray imaging equipment. The stent placement within the vessel wall and degradation can only be assessed using complex intravascular imaging methods. The objective of this study is to develop a biocompatible BVS that can be fully visualized using non-invasive clinical imaging modalities such as X-rays.

We investigated the feasibility of printing BVS that contained iodixanol, a commercially available iso-osmolar contrast agent, which is commonly used during fluoroscopy and angiography. 5.5, 4 and 2% (w/v) of iodixanol were successfully incorporated into the biomaterial ink and used to fabricate BVSs. BVSs with and without contrast agent were imaged using microCT. We further evaluated the radiopacity of BVSs in a chicken leg to test how tissue affects the optical properties using a clinical digital radiograph. The BVS containing 5.5% contrast agent showed the most promising results and will be evaluated for biocompatibility and visibility *in vivo*.

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a90329

## 03-P283 Biofabrication and Bioprinting of Vascular Spheroids

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### Introduction

The most critical challenge in bioprinting is the integration of a vascular network needed for viability and maturation of the fabricated tissue. We hypothesized that vascular spheroids can be used as building blocks for bioprinting a vascularized macrotissue. In this study, vascular spheroids were biofabricated, encapsulated in photo-crosslinkable gelatin, and subsequently bioprinted into a 3D construct.

### Materials & Methods

Vascular spheroids combining endothelial cells with supporting cells (fibroblasts and/or adipose tissue derived stem cells (ADSC)) were created by seeding $0{,}5*10^6$ cells in high throughput agarose microwells (2865 pores, diameter 200 $\mu$m) and cultured in endothelial culture medium for 10 days. These vascular spheroids were encapsulated in Matrigel or 10w/v% photocrosslinkable gelatin methacrylamide (Irgacure 2959 as photoinitiator) to study viability and fusion. 3D bioprinting of vascular spheroids in gelatin methacrylamide was performed with the 3D Discovery (RegenHu).

Morphology, extracellular matrix production, lumen formation and spheroid fusion,... were evaluated by various techniques: phase contrast/fluorescence microscopy, immunohistochemistry, transmission electron microscopy (TEM).

### Results

When applying the correct coculture ratio, viable spheroids were obtained in which endothelial cells spontaneously formed lumina and a capillary like network, as shown by immunohistochemistry and TEM. Especially the presence of ADSC in the spheroid led to a higher vascularization of the spheroid and more extracellular matrix production.Moreover, the spheroids were able to fuse in suspension and in hydrogels, creating a macrotissue. During fusion, cells reorganized, creating a branched capillary network throughout the entire fused construct by inoculating with capillaries of adjacent spheroids. Bioprinting of vascular spheroids encapsulated in photocrosslinkable gelatin resulted in high viability of the macrotissue and fusion of the vascular spheroids into a vascular network.

### Conclusions

In conclusion our findings showed that (1) agarose microwells are well suited for large scale production of uniform, high quality vascular spheroids, (2) encapsulated vascular spheroids have the capacity to fuse into a macrotissue, (3) bioprinting of vascular spheroids with gelatin methacrylamide as bioink is successful and leading to a vascular network.

### Acknowledgements

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a91546

## 03-P284 3D Bioprinting a Multilayered Heart Valve Scaffold Using Polycaprolactone and Gelatin Methacrylate-PEG Bioinks

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Heart valve disease is an increasing clinical burden associated with high morbidity and mortality. The mechanical strength of valves is attributed to the unique microstructure of each valve leaflet layer. To recapitulate the structure and function, a multilayer leaflet can be developed using 3D bioprinting. Thus, the aim of this project is to first develop suitable bioinks for 3D bioprinting and then develop the multilayered leaflets. A hydrogel-based bioink composed of varying concentrations of poly(ethylene glycol) diacrylate (PEGDA) and gelatin methacrylate (GelMA) was developed to be suitable for 3D bioprinting and cell encapsulation. Meanwhile, polycaprolactone (PCL) was optimized for 3D bioprinting and utilized to match the mechanics of the native valve leaflets. Bioink properties were characterized by degradation, swelling ratio, and compression test. While, cell viability was assessed through the Live/Dead Assay. The printability of each bioink was determined by performing a shape fidelity test. A formulation of 10% w/v of GelMA or a combination of GelMA/PEGDA led to a white-light crosslinkable bioink. While the 10% w/v GelMA scaffold degraded within 5 days, the combination of GelMA/PEGDA addressed the issue of degradation, with no degradation after 7 days. Furthermore, both 10% GelMA and GelMA/PEGDA bioinks had over 80% cell viability. The GelMA and GelMA/PEGDA bioinks exhibited 75% print accuracy when compared to CAD model, suggesting these bioinks are suitable for 3D bioprinting. The modulus of 3D bioprinted GelMA scaffolds were 5 kPa, which is comparable to the modulus cells experience in native leaflets. As for the load-bearing component of the leaflet, PCL can be 3D bioprinted into scaffolds that are flexible and have a physiological modulus of 4-6 MPa. Furthermore, PCL and the GelMA/PEGDA bioinks can be crosslinked together to form a multilayered scaffold. Combined, we have developed two bioinks suitable for 3D bioprinting and with the mechanical properties necessary to generate a multilayered leaflet for heart valve tissue engineering.

a90516

## 03-P285 Mechanical behavior of small diameter polyurethane - based vascular grafts produced by electrospinning

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Incoherence of the mechanical properties of the native artery and synthetic vascular grafts (VG) are known to cause intimal hyperplasia, followed by graft failure. Design and production of a synthetic tissue-engineered VG with viscoelastic properties similar to those of a human artery is a critical objective of vascular engineering[1-3]. In the present study we have investigated the mechanical properties of VGs produced by electrospinning from blends of 3-5% Tecoflex EG-80A polyurethane with 10÷15% gelatin in hexafluoro-2-propanol. Mechanical properties and compliance of glutaraldehyde-treated (GA) and untreated VGs produced from Tecoflex and gelatin under variable hydrodynamic loading were studied immediately upon VG production and after 2 and 4 weeks of incubation in PBS at 37° C. Rat abdominal aorta (diameter 1.8-2 mm) and ePTFE grafts (diameter 2.4 mm, Zeus Inc., USA) were used as controls.

The ePTFE VGs, commonly used in clinical practice, exhibit high tensile strength, but pressure up to 200 mmHg virtually does not inflate the VGs. Tensile strength of Tecoflex-gelatin VGs was only marginally influenced by aging, whereas its compliance decreased during the incubation. In addition to being more compliant than other tested VGs, GA treated VGs from 3% Tecoflex with 15% gelatin were more responsive to low pressure and retained their compliance after the long term incubation. Evidently, guided protein crosslinking prevents additional undesirable crosslinks between proteins in a fiber and between adjacent fibers. In-depth physical and chemical analysis of fiber structure and inter-fiber contacts is necessary to explain the good compliance of GA treated VGs and allow us to improve the compliance and durability of electrospun polyurethane-based VGs.

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a90036

## 03-P286 Cryopreservation and its Influence on the Differentiation of Adipose Derived Stromal Cells Towards Vascular Smooth Muscle Cell-like Cells: Implication of ERK1/2, IFN γ and Smad2/3

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Vascular smooth muscle cells (VSMCs) play essential roles in regulating blood vessel form and function, and are required for tissue engineering applications. Regeneration of functional vascular smooth muscle tissue to repair diseased vessels requires access to a VSMC source that is effective, efficient and safe. Multipotent adipose derived stromal cells (ASCs) can be readily harvested from abundant fat tissue and differentiated into VSMC-like cells. While the derivation of VSMCs from ASCs is promising, it is not known whether cryopreservation affects ASC differentiation towards VSMC. The objective was to investigate the effect of cryopreservation on ASC differentiation into VSMCs. It was hypothesized that short-term cryopreservation will not have a negative effect on the ASCs' ability to differentiate towards a VSMC cell fate. Cryopreserved passage 5 ASCs were generated by suspending fresh passage 4 ASCs in cryopreservation medium (10% FBS, 10% DMSO and 80% low-glucose DMEM) and frozen at -80° C for one month, then thawed and cultured for one passage before differentiation. Both fresh ASCs (FA) and cryopreserved ASCs (CA) were treated with VSMC differentiation factors (5 ng/ml transforming growth factor $\beta$1 and 1 $\mu$M retinoic acid) for 1 week, and expression of VSMC genes/proteins, and cell contractility were investigated (N=3, n=3). mRNA and protein levels of the late stage contractile marker smoothelin were significantly higher in VSMC-like cells derived from FA than from CA ($p<0.05$). FA generated VSMC-like cells contracted collagen gels more ($p<0.05$) in response to norepinephrine and vasopressin, when compared with the those derived from CA. Cytokines (TNF$\alpha$, IFN $\gamma$ ), total and phosphorylated signalling proteins (MEK/ERK, p38, JNK, RhoA, Smad2/3) involved in VSMC differentiation were quantified in FA vs. CA. CA demonstrated significantly less phosphorylation of ERK1/2 and Smad2/3, and released more IFN $\gamma$ than FA. This could account in part for the decreased expression of smoothelin gene/protein and reduced contractility of CA-derived VSMC-like cells. This study provides significant insights into how CA could be potentially pre-treated before being differentiated for subsequent vascular tissue regeneration. **Acknowledgement** CIHR operating grant #230762, OGS scholarship and NSERC PGS-D scholarship.

a92651

## 03-P287 AAV-mediated Therapeutic Strategy for Ischemic Heart Disease

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Recent work in cellular reprogramming of fibroblasts into cardiomyocytes has demonstrated therapeutic potential for cardiovascular disease, in which viruses were utilized as biomaterials for successful transgene delivery. Retrovirus- or lentivirus-mediated transgene delivery into the cardiovascular system was majorly used and is not yet feasible for application to humans. Here, We propose to utilize AAV-mediated gene delivery as a reprogramming and thymosin beta4 (Tb4) - mediated heart regeneration in a myocardial ischemia model. AAV-GMT promoted a gradual increase in expression of cardiac-specific genes, including Actc1, Gja1, Myh5, Ryr2, and cTnT, with a gradual decrease in expression of a fibrosis-specific gene, procollagen type I. Vessel growth was enhanced in fibrotic areas in the AAV-Tb4 co-treatment group compared to the AAV-GMT or AAV-Tb4 only group; enhanced cell survival was also accompanied by AAV-Tb4 co-injection. Histological assessment of heart also showed decreased myocardial fibrosis and increased wall thickness in the AAV-Tb4 co-injection group, with consequent improved cardiac function, as shown by echocardiography. Taken together, the combined AAV-mediated GMT and Tb4 therapy enhanced the recovery of heart structure and function via enhanced heart regeneration and blood vessel growth in an acute myocardial infarction model. [This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (H16C1067)]

a90931

## 03-P288 MicroRNA-145-loaded poly lactic-co-glycolic acid nanoparticles attenuate venous intimal hyperplasia in a rabbit vein graft disease model

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BACKGROUND: Vein grafts implanted under arterial pressure are prone to atherosclerosis, leading to vein graft disease. A key mechanism is intimal hyperplasia, which is caused by a phenotypic modulation of vascular smooth muscle cells (VSMCs). MicroRNA-145 (miR-145) is reported to regulate the phenotype of VSMCs from proliferative to differentiated state. Viruses or plasmids are generally adopted to transduce the microRNA. We hypothesized that an *ex vivo* microRNA-delivery system using miR-145-loaded poly lactic-co-glycolic acid (PLGA) nanoparticles (NPs) could attenuate venous intimal hyperplasia.

METHODS: Vein grafts of male Japanese white rabbits were harvested and soaked in PBS, scrambled control-microRNA (cont-miR)-loaded PLGA NPs solution, or miR-145-loaded PLGA NPs solution for 30 min before implantation. The rabbits were divided into 3 groups: PBS-treated ($n$ = 8), cont-miR-treated ($n$ = 7) and miR-145-treated group ($n$ = 7) and underwent the replacement of the carotid artery with the vein graft. Two weeks after the surgery, the vein grafts were explanted and examined.

RESULTS: The miR-145 expression level evaluated by qPCR was significantly higher in the miR-145-treated group (PBS-treated vs. cont-miR-treated vs. miR-145-treated group; 0.69 ± 0.17 vs. 0.70 ± 0.19 vs. 1.03 ± 0.28; $p$ = 0.02 in miR-145-treated vs. cont-miR-treated group). The neointimal area was significantly smaller in the miR-145-treated group (1.63 ± 0.52 vs. 1.75 ± 0.48 vs. 0.93 ± 0.32 $mm^2$; $p < 0.01$ in miR-145-treated vs. cont-miR-treated group). The immunohistochemical analysis of Ki-67 staining revealed significantly lower VSMC proliferative activity in the miR-145-treated group (proportion of Ki-67-positive cell counts; 41.3% ± 7.4% vs. 40.4% ± 5.5% vs. 27.7% ± 3.3%; $p < 0.01$ in miR-145-treated vs. cont-miR-treated group; $n$ = 5 for each), indicating the phenotypic alteration of VSMCs into differentiated state by the miR-145-loaded PLGA NPs treatment. The analysis of qPCR showed that the mRNA expression of myocardin was significantly upregulated in the miR-145-treated group (0.58 ± 0.67 vs. 0.61 ± 0.46 vs. 1.70 ± 0.72; $p$ = 0.02 in miR-145-treated vs. cont-miR-treated group; $n$ = 6 for each), suggesting that miR-145 retained the VSMC phenotype in contractile state.

CONCLUSIONS: miR-145-loaded PLGA NPs attenuated the intimal hyperplasia via regulation of the phenotype of VSMCs. This simple technique without viral or plasmid vectors appears promising for clinical application.

a91501

## 03-P289 An engineered perfusable microfluidic platform for recapitulating *in vivo* nanoparticle translocation

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Accumulating evidence indicates that air pollutants, more specifically ultrafine nanoparticles, contribute to serious and fatal damage to the cardiovascular system. However, the mechanisms that drive air pollutants associated cardiovascular disease and dysfunction remain unclear. Two-dimensional cell culture systems fail to predict nanoparticle toxicity *in vivo*, yet they remain the platform of choice for nanoparticle assessment. In an effort to create a more predictive *in vitro* model, we have developed the 3D platform with physiological shear stress, and supports the combination of dense parenchymal tissue and vascular interface on a single platform to unravel the impact of air pollution-derived nanoparticles on endothelial cells and cardiac tissue. When endothelial cells were exposed for 24 hours to 12-nm silica nanoparticles that are commonly used to model the toxic effect of ultrafine airborne particles, cytotoxicity and vascular permeability was significantly lower under flow conditions than that measured in static Transwell condition. This illustrates that vascularization with physiological shear stress is a crucial factor in terms of nanotoxicity evaluation towards real-life human exposure. To further understand the effect of nanoparticles on the cardiovascular system, human iPSC-derived cardiomyocytes were seeded around the vascular scaffold. We measured the amount of nanoparticle translocation from vascular space to cardiac tissue using inductively coupled plasma mass spectrometry (ICP-MS), and their possible relationship to functional properties of cardiac microtissue (contractile force and frequency) were analyzed. This microengineering approach may offer new opportunities to more accurately model cardiovascular response to nanoparticles and may help us to understand whether any safe nanoparticle dose exists that eliminate the cardiovascular effect.

a91931

## 03-P290 Investigation on microvascular toxicity and extravascular transport of nanoparticles using perfusable 3D microvessel model

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Vascular networks are the first site exposed to cationic polymer nanoparticles (NPs) when administered intravenously, and function as a barrier for NP to reach the target organ. While cationic polymer NPs have been intensively studied as non-viral delivery systems, its biological effects in human microvessel have been poorly investigated due to lack of appropriate *in vitro* systems. Here, we employed the three-dimensional mirovessel on chip which accurately models the *in vivo* state. An open and perfused microvessel surrounded by pericyte was proven to reproduce important features of living vasculatures like barrier function and biomarkers. Using microvessel chip, we observed the constriction of microvascular lumen induced by perfused polyethylenimine (PEI)/DNA NPs. We demonstrated that oxidative stress that occurs when exposed to PEI NPs led a rearrangement of microtubules resulting in microvessel constriction. Furthermore, the transcytotic behavior of PEI NPs was analyzed in microvessel by monitoring the escape of PEI NPs from microvascular lumen to perivascular region, which was not available in 2D culture system. With the understanding of different behaviors of cationic polymer NPs depending on their transcytotic route, we suggested a powerful route for efficient extravascular transport.

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a92046

## 03-P291 The Next Generation of Synthetic Vascular graft: 3D Plasma Ion Immersion Implantation

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Current synthetic graft materials have significant limitations that preclude their use in low flow settings and at diameters less than 6mm. Expanded polytetrafluoroethylene (ePTFE) is the most commonly used graft material, despite its poor vascular compatibility. The chemically inert surface is high hydrophobic and presents an unfavourable substrate for re-endothelialisation while promoting thrombus formation. We aimed to use plasma ion immersion implantation (PIII) to modify the surface of ePTFE. PIII is well established for activating inert polymers, but only characterised for two-dimensional substrates. Here we developed a novel PIII system to treat the inside of three-dimensional materials, including small diameter ePTFE vascular grafts.

6mm ePTFE conduits were treated by the PIII system using 4, 6 or 8kV voltage. Under nitrogen ion bombardment, reactive radical groups were created, which facilitate covalent immobilisation of molecules and reduced hydrophobicity. To confirm homogeneous PIII treatment, grafts were separated into three 1cm sections and surface chemistry assessed by FTIR and XPS. Contact angle measurements, used to test surface wettability, revealed a 28° (±2) reduction after treatment, down to 94°. Replicate samples were subjected to ELISA for protein attachment, and an endothelial cell attachment assay. Following PIII treatment, ELISA demonstrate a 120±X22% increase in protein attachment at 4kV, 172±21 % for 6kV and approximately 231±13 % for 8kV when compared to untreated samples. Endothelial cell attachment also increased 136±17% throughout all voltage regimens. All three sections were shown to be equivalently treated, with no significant differences observed in ELISA or attachment assay. Blood compatibility was examined by incubating samples with heparinised whole blood (0.3 U/ml) for 60 min at 37 ° C whilst rocking. Thrombogenicity under flow conditions was investigated using a modified Chandler loop rotated at 34 rpm at 37 ° C for 60 min. Thrombus weight was dramatically reduced in treated samples for both static (ePTFE 70.3 mg ± 13.7 vs PIII 47.3 mg ± 9.2), and loop (ePTFE 82.2 mg ± 12.6 vs PIII 51.3 mg ± 14.7) assays.

Using the new PIII system, homogeneous surface treatments were achieved on the luminal side of ePTFE grafts, with protein binding and cell attachment increased post-treatment. This significantly enhances the applicability of PIII treatment to synthetic conduits and other three-dimensional substrates.

a91003

## 03-P292 The role of 3D collagen and 5-azacytadine on the modulation of human bone marrow-derived mesenchymal stem cell secretome for enhanced cardiac regeneration

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Myocardial infarction (MI) is one of the leading causes of death and could result in loss of up to 50 g of cardiac tissue and death of billions of cardiomyocytes. Mesenchymal stem cells (MSC) are a promising cell source for cardiac repair as they secrete a wide range of signaling molecule. Here we investigate the role of 3D collagen matrix and 5-azacytidine (aza) on the modulation of human bone marrow-derived MSC (HBM-MSC) secretome pertinent to cardiac regeneration. HBM-MSCs spheroids were formed from loose cells using hanging-drop protocol and after 24 h, cultured within type-1 human collagen hydrogels (2 mg/ml) in the presence of 5-aza for up to 4 weeks. Essential cardiac proteins (e.g. collagen III, laminin, eNOS, elastin, hyaluronic acid, MMPs, TIMPs, cytokines, chemokines) were qualitatively and quantitatively analyzed at various intermediate time-points from these cultures. Results showed that spheroid formation, optimal collagen gel concentration (2 mg/mL) and aza dosage (10 mM) caused significant upregulation in the release of ECM components with concomitant downregulation in the release of inflammatory cytokines, MMPs and TIMPs, when compared to loose MSC cultures in TCP. Our results attest to the role of 3D stem cell microenvironment in positively tuning the secretome profile of human MSCs needed for cardiac tissue regeneration following cardiac inflammation and diseases such as MI.

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a93388

## 03-P293 Elastic fiber assembly in 3D tissue models by vascular smooth muscle cells derived from human mesenchymal stem cells

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Fabrication of a synthetic scaffold incorporated with functional elastic fibers is a critical design goal for successful vascular tissue engineering (VTE). Human bone marrow-derived mesenchymal stem cells (MSCs) are among the most promising stem cell types in VTE being able to differentiate into smooth muscle cells (SMCs), which are not only the predominant component of blood vessel medial layer but also the major sources for tropoelastin biosynthesis. However, traditional culture approaches through a continuous supply of biochemical factors has limitations including the high costs associated with prolonged culture as well as potential development of pathological state. In contrast, MSCs-derived SMCs through emerging culture approaches such as 3D topography or biomechanical forces represent an immature phenotype characterized by solely up-regulation of early-phase SMC genes and proteins. The immature phenotype may hinder the biosynthesis of tropoelastin.

Thus, the capability of tropoelastin biosynthesis and elastic fiber assembly by MSCs-derived SMCs is still uncertain. A combination of biochemical factors and biomechanical stimulation is a promising platform in directing stem cell differentiation toward a matured cell type. In this presentation, we will demonstrate the synergistic effect of pre-treatment with either transform growth factor-beta 1 (TGF-$\beta$1) or L-ascorbic acid and dynamic pulsatile perfusion in bioreactor on smooth muscle differentiation and maturation in 3D scaffolds. We also investigated the effect of MSCs-derived conditioned medium (MSC-CM) and SMC-derived conditioned medium (SMC-CM), as well as their combination on SMC maturation particularly, elastic fiber assembly in scaffolds.

a93480

## 03-P294 Cardiac Stem Cells as Regulator of Adult Cardiomyocyte Homeostasis and Their Myocardial Niches

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The adult heart was known as a terminally differentiated organ which was unable to reproduce and regenerate myocardial cells in the past. However, about 1% of myocardial cells are lost annually and regenerated into new cells, which is very low in cardiac activity, It is suggested that the mechanism exists. Two mechanisms have been proposed that adult cardiomyocyte homeostasis is regulated by cell renewal and cardiomyogenic differentiation of cardiac stem cells (CSCs), or cell division of pre-existing cardiomyocyte. However, the origin of renewed cardiomyocytes and its regulating mechanism are still uncertain. To investigation these uncertainties, We have reproduced myocardial homeostasis using a fibrin-supported three-dimensional culture method, and developed a new method for isolating, amplifying and culturing cardiac stem cells derived from myocardium. In this study investigates the origin of renewed cardiomyocytes and its regulating mechanism in the adult heart. The role of CD200+ cardiac stem cells that have been discovered through exploratory research in cardiomyocyte renewal. Therefore, the in situ localization, immunophenotypic identity, and interaction with surrounding cells of CD200+ CSCs in the adult myocardium. Finally, the biological characteristics of isolated CD200+ cardiac stem cells will be analyzed to confirm the ability and role of CD200+ cardiac stem cells in myocardial infarction animal models through in vivo cell tracking.

a92657

## 03-P295 Design and fabrication of perfused micro-bioreactor for three-dimensional culture of cardiomyocytes with electrical stimulation

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There is an urgent need for in-vitro cardiac tissue models for physiological studies and for toxicity and efficacy testing in drug development. Perfused micro-bioreactors enable cardiomyocytes to be cultured three-dimensionally with functional stimulation response and measurements with technical advantages for high throughput and parallel testing[1].

In this study, parallel perfused micro-bioreactors were fabricated with Polydimethylsiloxane (PDMS). Neonatal rat cardiomyocytes and human iPSC derived cardiomyocytes were cultured in 200mg/ml fibrinogen gel with initial cell densities of 0.5 million/ml. Cardiomyocytes embedded fibrinogen gels were formed in 24-well culture dish before transferred to the perfused micro-bioreactors with carbon electrodes to provide electric stimulation of 5V/cm. To determine contractile force, the most important function of cardiomyocytes in vivo, casted rectangular fibrinogen gels with cells were attached onto two flexible PDMS pillars with a diameter of 1mm and length of 12mm. Their deformation was measured and used to estimate cellular contractile force.

Culturing of cardiomyocytes within the perfused bioreactor increased cell viability and survival for longer period of time (6 weeks to 8 weeks). Also the cells showed aligned morphology and it took less time for them to mature. Comparing with conventional culture based on 24-well culture dish, the perfused micro-bioreactors provide more stable and in-vivo like environment for cardiomyocytes. The existence of mechanical and electrical stimulation was shown to have significant effects on cell functions. The fabrication and preliminary experimental results of this perfused micro-bioreactor could be exploited in the application of drug testing and screening on cardiac tissues.

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a92109

## 03-P296 Contractile Force Measurement of Human iPS Cell-Derived Cardiac Cell Sheet-Tissues and Its Application to *In Vitro* Drug Testing

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We have developed our original tissue engineering technology "cell sheet engineering" utilizing temperature-responsive culture dishes. The cells are confluently grown on a temperature-responsive culture dish and can be harvested as a cell sheet by lowering temperature without enzymatic digestion. Cell sheets are high-cell-density tissues similar to actual living tissues, maintaining their structure and function. Based on this "cell sheet engineering", we are trying to create functional cardiac tissues from human induced pluripotent stem cells, for regenerative therapy and *in vitro* drug testing. Toward this purpose, it is necessary to evaluate the contractility of engineered cardiac cell sheets. Therefore, in the present study, we developed a contractile force measurement system and evaluated the contractility of human iPS cell-derived cardiac cell sheet-tissues. We used human iPS cells in which puromycin-resistance gene under the control of α-myosin heavy chain promoter was transferred. By cardiac differentiation inducing culture using bioreactor system and puromycin treatment, human iPS cell-derived cardiomyocytes were prepared. The cardiomyocytes were cultured confluently on temperature-responsive culture dishes (UpCell; CellSeed, Tokyo, Japan). The cardiomyocyte sheets were transfered onto fibrin gel sheets by our stamp technique previously developed. The cardiomyocyte sheet-tissues were vertically mounted to the force measuring device, and contractile force due to the spontaneous beating was measured successfully. Drug testing was performed by administering several drugs. We confirmed the increase of contractile force due to the administration of adrenaline, isoproterenol, and Bay K8664, and decrease of contractile force due to the administration of verapamil and nifedipine. Additionally, we also confirmed the continuous decrease of contractile force due to the administration of a cardiotoxic anticancer drug adriamycin. In conclusion, the force measurement system developed in the present study is valuable for the evaluation of engineered cardiac cell sheet-tissues, and for *in vitro* cardiac drug testing.

a91503

## 03-P297 Functional Contractile and Permeability Deficits Identified in the Principal Cardiovascular Disease Risk Locus in Human iPSCs

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Genome-wide association studies have found that single nucleotide polymorphisms (SNPs) in the 9p21 gene locus–a locus that only emerged in non-human primates–are highly correlated with coronary artery disease (CAD). Homozygous carriers of 9p21 SNPs account for 23% of the US population and have a 60% increased risk of CAD. However, the mechanisms responsible are poorly understood. To better understand the function of 9p21 SNPs in CAD, we differentiated induced pluripotent stem cells (iPSCs) into endothelial cells (ECs) and smooth muscle cells (SMCs) from patients who were homozygous for the risk haplotype (R/R) or homozygous for the non-risk haplotype (N/N). In addition, the 9p21 gene locus was deleted from these cell lines to produce knockout (KO) lines compared to wild type (WT) lines. RNA sequencing identified a number of adhesion and contractility deficits in only the R/R WT SMCs compared to the R/R KO, N/N WT, and N/N KO lines. Functional assessments for adhesion strength, cell contractility, and collective contraction and matrix remodeling all showed that impairment correlated with presence of the risk locus. Interestingly, contraction could be stimulated with the agonist bradykinin, indicating that cells containing the risk locus possess but cannot use their contractile capabilities due to the presence of the risk interval. Concurrently, we found that differentiated ECs displayed similar phenotypic shape and size while maintaining expression and localization of ZO1 and VE-Cadherin. However, R/R WT EC monolayers were significantly more permeable compared to ECs lacking the risk locus. Under stress, EC monolayers responded similarly to TNFalpha signaling, significantly increasing permeability for all VECs and maintaining significantly more permeable monolayers of R/R WT ECs in comparison to R/R KO and N/N WT ECs. Because of these deficits found in adhesion, cell and collective contraction of SMCs as well as permeability and stress responses of ECs containing the 9p21 SNPs, we believe the principal cardiovascular disease risk locus plays a key role in impairment of vascular functionality and thus is influential in the procurement of CAD.

a91489

## 03-P298 Investigation of Pro-inflammation and Wound-healing Processes in the Co-culture of Human Monocytes with VSMCs Differentiated from ASCs

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Multipotent adipose derived stromal cells (ASCs) can be readily harvested from abundant fat tissue and differentiated into vascular smooth muscle cell (VSMC)-like cells for vascular tissue regeneration. Monocytes are among the first groups of cells present after biomaterial-based vascular tissue construct implantation. Depending on the activation states of monocytes (inflammatory or wound-healing), they could have different effects on tissue-specific cells and contribute to prolonged inflammation or tissue repair and wound-healing. Although activation of monocytes towards a pro-inflammatory phenotypic state can lead to the over-proliferation of VSMCs and ultimately potential stenosis and failure of vascular grafts, it was shown that when co-culturing primary VSMCs with monocytes at a 4:1 ratio on a pro-wound healing polyurethane (D-PHI), tissue regeneration could occur. However, the latter has not yet been demonstrated to be the case for ASC derived VSMCs (ASC-VSMCs) and monocytes. The objective was to develop a 3D ASC-VSMC/monocyte co-culture system *in vitro* using porous D-PHI scaffolds, in order to investigate the pro-inflammatory/wound healing processes over a 4-week period. It was hypothesized that cytokine release profiles would change with a temporal inflammatory/tissue regeneration profile similar to that of relatively normal wound repair. Fresh monocytes were isolated from human blood. ASC-VSMCs were generated by treating human ASCs with VSMC differentiation factors (5 ng/ml transforming growth factor $\beta 1$ and 1 $\mu$M retinoic acid) for 1 week. ASC-VSMCs and monocytes (4:1 ratio, 300,000 ASC-VSMCs + 75,000 monocytes) were co-cultured on a 3D D-PHI scaffold for 0 (i.e. 24 h post seeding), 1, 2 and 4 weeks. Proinflammatory cytokines (IL-6, IL-1$\beta$, TNF-$\alpha$) and pro-wound healing cytokines (IL-10, MCP-1, MCP-2, GCSF, IL-4) were analyzed using Ray Biotech growth factor array. It was found that the release of IL-6, IL-1$\beta$, TNF-$\alpha$ all increased at 1-week time point (vs. 0 week), and then decreased at 2, 4 week time point (vs. 0 week), while release of IL-10, MCP-1, MCP-2, GCSF and IL-4 increased after 0 week and were still elevated at week 4. This study suggests a switch from an initial inflammation phase to a regeneration phase could occur during the culture of ASC-VSMCs in the presence of monocytes on D-PHI. **Acknowledgement** CIHR operating grant #230762, OGS scholarship and NSERC PGS-D scholarship.

a92444

## 03-P299 Elastogenicity of cBM-SMCs in a 3D collagenous milieu: Identifying a potential cell source for cell therapy in AAAs

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Restoring elastin homeostasis in progressing abdominal aortic aneurysms (AAAs) is a major yet un-addressed challenge due to poor tropoelastin (precursor molecule) mRNA synthesis by adult vascular smooth muscle cells (SMCs), their impaired ability to assemble new elastic fibers and their inability to overcome chronic proteolysis in the AAA wall. We recently discovered that SMC-like cells (cBM-SMCs) differentiated from rat bone marrow mesenchymal stem cells (BM-MSCs) on a fibronectin substrate in presence of the growth factors TGF-$\beta$ and PDGF, exhibit superior elastogenic and anti-proteolytic properties in a long-term 2D culture relative to undffierentiated BM-MSCs, healthy rat aortic SMCs (RAMSCs) and other derived SMC phenotypes. We also found these cells to provide paracrine pro-elastogenic and anti-proteolytic impetus to cocultured aneurysmal SMCs. While these outcomes in 2D cultures are promising towards use of cBM-SMCs for cell therapy for AAAs, to provide greater relevance to our intended application, in the present study, we investigated matrix synthesis ability of the cBM-SMCs within a 3D collagen-rich, de-elasticized microenvironment evocative of the aneurysmal aorta. Acid solubilized type-I collagen was mixed with a suspension of RASMCs, rat cBM-SMCs and rat BM-MSCs either standalone and or with a mixture of each of these cell types with rat aneurysmal SMCs (EaRASMCs) to generate a final mixture containing 2mg/ml of collagen, 250k cells/ml (for biochemical assays) and $1 \times 10^6$ cells (for histology), and 20% v/v fetal bovine serum (FBS). The cell-seeded constructs were cultured for 21 days in DMEM containing 10% v/v FBS post gelation. Gel contraction, elastic matrix contents, protein synthesis and enzyme activity of matrix-metalloproteases (MMPs) 2 and 9, desmosine crosslinks and lysyl oxidase protein and enzyme activity were measured after 21 d. Elastic matrix amounts, fiber formation, and desmosine-mediated crosslinking of elastin, besides contractility of constructs was significantly higher in cBM-SMC cultures and also in EaRASMCs /cBM-SMCs co-cultures versus controls. Morphometric analysis of Verhoeff-Van Gieson-stained sections also showed higher density of elastin in constructs seeded with cBM-SMCs and separately in EaRASMCs /cBM-SMCs co-cultures compared to controls. Our study strongly supports the utility of cBM-SMCs for cell therapy aimed at restoring elastic matrix homeostasis in the AAA wall towards achieving growth arrest or regression.

a90287

## 03-P300 Differentiation of Human Pluripotent Stem Cells into Cardiomyocytes Cultured on Hydrogels having Different Elasticity

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Stem cells are an attractive prospect for tissue engineering and regenerative medicine for their unique biological properties.[1] The coating materials were based on extracellular matrix (ECM), such as Matrigel, recombinant Vitronectin (rVN), Synthemax II, Cellstart and Laminin-521 are commonly used as the cell culture substrates for human embryonic stem cells (hESCs). Our previous study showed that polystyrene tissue culture (TCPS) dishes coated with Laminin-521 has higher survival rate for hESCs differentiated into cardiomyocytes compared with other ECM-coated dishes. In addition, Laminin-521 is derived from natural laminin and therefore facilitates self-renewal of hESCs and induced pluripotent stem cells (iPS cells) in a chemically defined, xeno-free stem cells culture system. We designed different stiffness of polyvinylalcohol-co-itaconic acid (PVA-IA) hydrogel conjugated with Laminin-521 and oligopeptides derived from laminin as hESCs culture substrate and we evaluated hESCs attachment and pluripotency cultured on these hydrogels. Furthermore, we differentiated hESCs into cardiomyocytes to investigate the survival rate and expression of cardiomyocyte surface marker (cTnT).

The PVA-IA hydrogels in this research were prepared by coating aqueous PVA-IA solution on the TCPS and were crosslinked with glutaraldehyde to control the elasticity of the PVA-IA hydrogels. Laminin-521 and oligopeptides derived from laminin was covalently bound on PVA-IA hydrogels. We investigated the effect of different crosslinking degree of PVA-IA hydrogels grafted with Laminin-521 and the oligopeptides to investigate the differentiation ability of hESCs into cardiomyocytes. We further evaluated the pluripotency and differentiation ability of hESCs after long-term culture of hESCs on PVA-IA hydrogels (more than 10 passages).

a92666

## 03-P301 Development of tissue engineered decellularized connective tissue membrane for allogeneic arterial patch implantation

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**Objectives:**

In the development of new biomaterials for cardiovascular grafts, decellularized connective tissue membrane presents potential for new applications in regenerative medicine.

Previously, we have developed *in vivo* tissue-engineered autologous small-caliber vascular grafts, named Biotubes, which is constructed by a novel concept of regenerative medicine. We have reported Biotubes withstood systemic blood pressure and exhibited excellent performances as small caliber vascular prostheses in animal models. However, as it takes 4 weeks even in the healthy animals to fabricate Biotubes, it is expected to be problematic to apply them to the severely diseased patients whose regenerative activity is suppressed because of immaturity in the fabricated tissues. Therefore, possibility of the allogeneic implantation of the Biotubes from the healthy donor to the diseased patient should be evaluated. The objective of this study is to fabricate off-the-shelf cardiovascular grafts using allogeneic animals.

**Methods and Results:**

Silicone rod molds (diameter: 5mm, length: 10 cm) were placed into subcutaneous pouches of beagle dogs, and after 4 weeks the implants with their surrounded connective tissues were harvested. Tubular connective tissues with internal diameter of 5 mm were obtained after pulling out the impregnated molds. Tubular connective tissues were perfused with 1% sodium lauryl ether sulfate (SLES), 1% Triton-X, and 500 U/ml of DNase in phosphate-buffered saline (PBS). Decellularized tubular connective tissues were stored in PBS at -20 degrees for 1 week.

Decellularized tubular connective tissues were cut open and trimmed to elliptical sheets of 15 x 8 mm. Those were transplanted to the carotid arteries of another beagle dogs as vascular patches (n = 6).

Echocardiography revealed no aneurysmal changes of the grafts. Vascular patch grafts were resected 1, 2 and 4 weeks after implantation (each group n = 2) and histologically evaluated. Histological evaluation revealed that patch grafts formed neointima on the luminal surface and graft walls had cell infiltration over time.

**Conclusions:**

Decellularized connective tissue membranes can be prepared and stored beforehand and can be used as allogeneic grafts. It was suggested that the decellularized connective tissue membranes have sufficient strength to withstand arterial pressure and can self-assemble rapidly after implantation. They could be one of the ideal allogeneic cardiovascular grafts.

a91035

## 03-P302 Exogenous nitric oxide delivery promotes matrix deposition and suppresses inflammation in adult human aneurysmal smooth muscle cell cultures

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Vascular diseases such as aneurysms are characterized by endothelial dysfunction, variation in nitric oxide (NO) release, over-proliferation of aortic smooth muscle cells (SMCs), upregulation of matrix degrading enzymes, degradation of elastin matrix fibers, and compromise in SMC-elastin signaling homeostasis. Here, we investigate the benefits of exogenously delivering NO cues to regulate cell proliferation, matrix synthesis and deposition (glycosaminoglycans, elastin, desmosine), lysyl oxidase enzyme, and cytokines/ chemokines release by adult human aortic aneurysmal SMCs (AA-SMCs) within 3D biomimetic cultures, using biochemical assays, western blot analysis, RT-PCR, and cytokine/ chemokine assays. Adding GSNO marginally suppressed AA-SMC proliferation and promoted multi-fold increase in total protein deposited in cell matrix compared to controls ($p < 0.01$). Significantly higher tropoelastin and sGAGs were released and deposited as matrix within 3D cultures, compared to controls. GSNO induced 2.5-fold increase in LOX activity within 3D cultures, which is encouraging from a tissue engineering and in situ elastin regeneration standpoint within aneurysms. Elastin gene expression in adult AA-SMCs in the presence or absence of NO was significantly lower compared to healthy aortic SMCs. iNOS and TIMP-1 amounts were enhanced within 3D cultures receiving GSNO, and comparable to that noted in healthy SMC counterparts. Base-level expression (i.e., 0 nM GSNO) of fibrillin-1 gene in AA-SMCs was modestly higher compared to their healthy counterparts. Results attest to the benefits of delivering 100 nM NO cues to suppress AA-SMC proliferation and promote robust ECM synthesis and deposition by adult human AA-SMCs, with significant applications in tissue engineering, biomaterial scaffold development, and drug delivery.

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a91923

## 03-P303 Stents with Inducible VEGF/HGF-secreting MSCs enhanced Re-endothelialization and decreased Restenosis in Swine Model

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Plaques caused by atherosclerosis within the vasculature can eventually leads to heart failure. Currently cardiac stenting is the most effective and a less invasive approach to treat the disease. However, in-stent restenosis has been the most complex and chronic side-effect of the stenting treatment. In this study, to reduce the restenosis of stent and induce re-endothelialization within the artery, we applied coronary stents coated with stem cells secreting angiogenic growth factor in an inducible genome-editing system. After confirming the characteristics of the cells and adhesion property on stents, we transplanted the stents into swine model to evaluate the re-stenosis and potential therapeutic use of the stents with stem cells. Restenosis was evaluated via optical coherence tomography (OCT), micro-computed tomography (mCT) and angiography, and re-endothelialization by immunostaining after cardiac stent treatment. Compared to bare metal stent or parental UCB-MSC coated stent, the stents with the stem cells of controlled and combined release of hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF) successfully reduced the re-stenosis within the stent and induced natural re-endothelialization. Furthermore, UCB-MSC had ability to differentiate into endothelial cells in matrigel condition and HGF and VEGF improved the differentiation. Our study indicates that the stents coated with the UCB-MSCs secreting VEGF/HGF would reduce restenosis side effects of cardiac stenting with improved re-endothelialization.

a92316

## 03-P304 Antihypertensive effect of an enzymatic hydrolysate from *Styela clava* flesh tissue in type 2 diabetic patients with hypertension

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In this randomized, placebo-controlled, double-blind study, we evaluated the antihypertensive effects of enzymatic hydrolysate from *Styela clava* flesh tissue in patients with type 2 diabetes mellitus (T2DM) and hypertension. *S. clava* flesh tissue hydrolysate (SFTH) (n = 34) and placebo (n = 22) were randomly allocated to the study subjects. Each subject ingested two test capsules (500 mg) containing powdered SFTH (SFTH group) or placebo capsules (placebo group) during four weeks. In the SFTH group, systolic and diastolic blood pressure decreased significantly 4 weeks after ingestion by 9.9 mmHg ($P < 0.01$) and 7.8 mmHg ($P < 0.01$), respectively. In addition, the SFTH group exhibited a significant decrease in hemoglobin A1c with a tendency toward improvement in homeostasis model assessment of insulin resistance, triglyceride, apolipoprotein B and plasma insulin levels after 4 weeks. No adverse effects were observed in other indexes, including biochemical and hematological parameters in both groups. The results of our study suggested that SFTH exerts a regulatory, antihypertensive effect in patients with T2DM
and hypertension.

a91626

## 03-P305 A Platform to Study 4D Hemodynamics and Morphology in Artery Models Using Ultra-High Field MRI (17.6 T)

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The knowledge of the correlation between arterial pulse-wave-velocity (PWV) and endothelial wall shear stress (WSS) is of great relevance to understand the interaction of arterial stiffness and pathologic flow patterns (e.g. low/oscillatory WSS) in vascular diseases. Non-invasive measurements of these parameters using flow Magnetic Resonance Imaging (MRI) *in vivo* are challenging due to long measurement times and limited spatiotemporal resolution. At this point, tissue engineering poses a potential alternative to conventional animal studies since tissue-engineered arteries have physical properties similar to native arteries and enable the study of pathologies in controlled environments.

In this work, we combined non-invasive ultra-high field MRI with Tissue Engineering using carotid artery models, cultured in a modular bioreactor-platform in order to create a potential in vitro test system for atherosclerosis research and a suitable tool for *ex vivo* and *in vitro* studies of arterial elasticity and hemodynamics.

A 3D-printed tissue chamber, suitable for MRI measurements and long-term culture of arteries, was developed and connected to the bioreactor platform with adjustable flow/pressure rates and definable pump frequencies. Flow and morphology measurements of carotid arteries were performed using radial 4D Phase-Contrast-MRI. Local PWV was determined using the Q-A method. WSS was derived from the 3D-velocity gradients at the vessel wall. 4D flow dynamics (volume + peak flow, PWV and WSS) as well as 3D vessel wall morphology were obtained from one single measurement. Furthermore, the obtained WSS values were separated into all three components (longitudinal, circumferential and radial vectors), allowing the accurate analysis of WSS patterns and their potential correlation with plaque development and stiffening in the whole artery.

To create tissue engineered arteries with physical properties similar to native ones, we furthermore developed a gentle decellularization method for porcine carotid arteries, enabling the recellularization with human vascular cells and the creation of atherosclerotic artery models in future studies.

These tissue engineered arteries, combined with the fast and non-invasive flow quantification setup, offer the possibility to examine the effects of specific biological modifications on the physical, flow-dynamic properties as well as changes in vessel wall morphology and vice versa in a fully controllable environment.

a92032

## 03-P306 Fast Self-Navigated Wall Shear Stress Measurements in the Murine Aortic Arch Using Radial 4D-PC-MRI at 17.6 T for Tissue Engineering Approaches

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4D phase contrast (PC)-MRI is a non-invasive tool for the assessment of cardiovascular hemodynamics and Wall Shear Stress (WSS) to study vascular functions *in vivo* and is highly valuable for the investigation of tissue-engineered vascular implants in animal models . Major limitations of conventional triggered methods are the long measurement times needed for high-resolution data sets and the requirement of stable ECG triggering.

In this abstract we present an advanced method based on a self-navigated 4D radial PC-cine FLASH acquisition that enables high-resolution (100 $\mu$m)$^3$ flow measurements of the complete aortic arch in 32 minutes. 4D-flow dynamics, aortic cross sectional areas, longitudinal, circumferential and radial wall shear stress components as well as the oscillatory shear index (OSI) can be determined from the acquired data. The use of retrospective reconstruction and self-gating enables a very flexible data analysis and increases the robustness since prospective ECG triggering is no longer needed.

All data were acquired on a vertical 17.6T small animal MRI scanner. 4D flow and WSS was measured in the aortic arch of 12-weeks-old wild-type C57/BL6J mice (n=7) with a radial 4D PC-MRI sequence. Flow measurements were conducted without triggering during free breathing and required a total scan time of 32 minutes. After segmenting the aortic lumen semi-automatically, time-resolved WSS-components and OSI were calculated from the spatial velocity gradients at the lumen surface at 14 locations along the aorta. Mean and median of all 3 WSS components as well as the time-resolved WSS and OSI over the whole heart cycle were assessed at 14 analysis planes along the aortic arch.

We demonstrate the feasibility of fast self-navigated 4D flow and WSS measurements, shown here for the murine aortic arch, with very high temporal (30 frames / cardiac cycle) and spatial resolutions (100 $\mu$m$^3$). The proposed method does not require ECG signals for motion synchronization and hence leads to higher robustness and an improved animal handling. In future applications, this new method is planned to be used to study the hemodynamics and WSS in atherosclerotic mouse models as well as animal models of implantation in the field of cardiovascular tissue engineering.

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and the Comprehensive Heart Failure Center (CHFC).

a90744

## 03-P307 Cardiomyogenic Differentiation of Mesenchymal Stem Cells Using Inducing Agents and an Anisotropic Scaffold

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Mesenchymal stem cells (MSCs) have been identified as a potential cell source for cardiac tissue engineering because of their plasticity, ease of harvest & isolation relative to other cell sources and ability to be utilized for both autogenic and allogenic transplants. They are highly proliferative and can be maintained in a simple culture medium in vitro without any karyotype alterations for several passages. The ability to differentiate MSCs into cardiomyocytes is recently being investigated because of their promising advantages as a cell source. In this study, 4 inducting agents (5-azacytidine, zebularine, endothelin-1 and ascorbic acid) were tested for their potential to chemically induce porcine bone marrow-derived MSCs into the cardiomyogenic lineage either alone or in combination with another. Using immunocytochemistry and relative gene expression studies done using RT-PCR, zebularine was identified to be the best inducing agent among the cocktails tested due to the upregulation of early cardiac markers (cx3, tbx5, desmin), outperforming the most commonly used agent, 5-azacytidine. Furthermore, culture on anisotropic silk scaffolds fabricated using unidirectional freezing further upregulated the expression of these markers relative to cells cultured on tissue culture plates. It was however noted that the differentiated cells were of an immature phenotype as contractile proteins (MHC, mlc2v, cardiac actin, troponin T, cardiac troponin I, actn2) characteristic of mature cardiomyocytes were not expressed. Nevertheless, the appropriate degree of differentiation necessary for transplanted cells is still under debate. The choice of using of porcine cells in this study promotes utilizing larger animal models in cardiac studies as pigs have been identified as a better model than the usual small animal models like mice and rats.

a91820

## 03-P308 Noninvasive OCT Imaging of Three-Dimensional Cardiac Tissues Derived from Human Induced Pluripotent Stem Cells

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Engineered three-dimensional (3D) tissues have the potential to be used in regenerative medicine or in vitro screening. Advancements in the development of engineered cardiac tissue show promise considering the global impact of cardiovascular diseases and the recent progress in methods of deriving cardiomyocytes from iPS cells. In view of increasing needs for the functional evaluation of 3D tissues, noninvasive methods for 3D tissue characterization are expected to play a key role in quality assurance.

In this study, 3D cardiac tissues were constructed using Layer-by-Layer (LbL) methods. After confirming the initiation of spontaneous beating, the structure and function of the cardiac tissues were evaluated using high-speed video microscopy and optical coherence tomography (OCT). High-speed video microscopy detects the beating motion at the surface of the cardiac tissue and OCT noninvasively measures its thickness. The results suggest that there is a relationship between the speed of the surface beating and the tissue thickness.

Furthermore, the tissue internal beating state was analyzed by measuring the maximum contraction speed and the contraction-relaxation distance through image analysis of OCT data. In general, the data generated from the motion speed analysis of OCT images are not sufficient for quantification because of difficulties in correlating low frame rate images to real-time beating motion. . To overcome this, OCT images were obtained with a high-speed frame rate and the internal beating movements were corrected using the tissue surface movements obtained by high-speed video microscopy. Combining these methods, preliminary testing suggests that different speeds may be observed at different depths throughout the tissue. These results indicate that tissue thickness and depth information are needed to evaluate 3D tissues. To further confirm these evaluation methods, variations in tissue beating were analyzed after drug treatment.

a91688

## 03-P309 Drug response of scaffold-free cardiac constructs fabricated using bio-3D printing

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**Background:** One of the key aspects of drug development is toxicity testing. Our aim was to develop a 3D bioprinting system able to produce cardiac constructs for drug testing, as well as a possible alternative to tissue transplantation in the future. Cell spheroids were printed onto a needle array according to the desired 3D design. Here, we present the method for fabrication of cardiac constructs using iPS-derived cardiomyocytes and drug response. **Materials and Methods:** Cardiomyocytes derived from human iPS cells (iCell), Human Umbilical Vein Endothelial Cells (HUVECs) and Normal Human Dermal Fibroblasts (NHDFs) were mixed and seed into ultra-low attachment 96 U-well plates to make cardiac spheroids. These were used to make cardiac tubular constructs, by means of a 3D-Bio printer (Regenova; Cyfuse Biomedical K.K. Tokyo, Japan). The constructs were cultured on needle arrays in bioreactor for 7 days, after which they were treated with isoproterenol and the drug response was evaluated. For that purpose, the movement of the top of the needle array was recorded and analyzed. **Results:** Spheroid fusion and contractibility of cardiac tubular constructs were observed after fabrication, on the needle array. Immediately after the addition of isoproterenol to the printed tubular cardiac constructs, the movement of the needle array has increased. In addition, the beating rate of cardiac constructs has also increased, compared to the initial state, before the addition of isoproterenol. 30 min after the removal of isoproterenol, the movement of the needle array returned to the initial base levels. These results indicate that the tubular cardiac constructs are able to display reversible drug response **Conclusion:** We have successfully fabricated tubular cardiac constructs and evaluated their response to isoproterenol. In future work, these constructs will be used to test new drugs being developed to treat cardiac diseases.

a93420

## 03-P310 Bioprinted Patch with Cardiac Extracellular Matrix and Human Cardiac Progenitor Cells for Heart Regeneration

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The heart has a limited capacity for regeneration after ischemic injury or surgical intervention in congenital patients. Human cardiac progenitor cells (hCPCs) have the potential to regenerate the myocardium by releasing pro-regenerative and anti-inflammatory paracrine signals such as growth factors and exosomes. Cardiac extracellular matrix (cECM) has also been found to be beneficial to regenerating adult hearts post-infarct when injected directly into the myocardium. Other biomaterials commonly used for cardiac applications lack the complexity and specificity of cECM. Additionally, cECM has been shown to induce hCPC differentiation towards a regenerative phenotype, particularly when cultured in 3D systems. To this end, we have developed a heart patch with both pediatric hCPCs and cECM which, when attached epicardially on the damaged myocardium of pediatric patients, will allow for effective regeneration through release of paracrine factors. The patch was generated through a 3D bioprinting methodology, which allows for high degrees of shape and fiber control, as well as target tissue specificity. The inclusion of bioactive GelMA (gelatin methacrylate) allowed for high fidelity printing through viscous bioink formation at low temperature, followed post-printing by white light radial polymerization to hold the patch shape when reintroduced to physiological temperatures. The printed construct had improved printability with the inclusion of cECM compared to printing pure GelMA and showed homogeneous distribution of cECM and hCPCs. The patch supported viable and proliferative hCPCs, which in turn upregulate paracrine signal production for regeneration, while also having a high degree of swelling and porosity, indicating an effective environment for cell growth and signaling release. The patch did not degrade via hydrolysis after several weeks *in vitro*, allowing for maintenance after implantation, and matched the fibrous structure and mechanical moduli of native myocardial tissue, indicating potential effective integration into host tissue during the therapeutic period. Through all these factors, the patch showed a high degree of continuous paracrine signaling release, particularly factors for improvement of angiogenesis, tissue remodeling, reduction of inflammation, and cardiomyocyte proliferation. Future studies will focus on the *in vivo* testing of the construct for restoration of myocardium and contractile function.

a91402

## 03-P311 $^{19}$F Magnetic Resonance Imaging of Tissue Inflammation and T1 Imaging of Fibrosis after Myocardial Infarction in Rats

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Although modern interventional medicine decreased deaths by acute myocardial infarction (MI), it has led to an increase in the rate of heart failure with consequences ranging from high mortality rate, high medical fees and massive impairments of life quality for MI survivors. While the causal events are still hardly understood, it is suggested to be correlated with myocardial inflammation and wound healing processes which occur after the MI event. Unfortunately, in some cases this remodeling process leads to a widespread stiffening of the surrounding muscle tissue, causing diastolic and systolic dysfunction and ultimately resulting in heart failure.

Magnetic resonance imaging (MRI) methods provide a non-invasive way to elucidate underlying processes in the inflammation and subsequent remodeling of myocardial tissue. The high spatial and temporal resolution in combination with contrast agents such as $^{19}$F enables the investigation of macrophage movement and fibrosis development over time. By measuring T1 pre and post administration of a contrast agent (CA) to determine extracellular volume (ECV) or regional blood volume (RBV) within the region of interest, we are able to monitor myocardial fibrosis in reperfused MI Wistar rats. The change of ratio between ECV and RBV is directly correlated to the quantitative change in the connective tissue which is predicted to increase due to fibrosis and ventricle remodeling. Furthermore, by measuring inflammation indirectly using $^{19}$F to label macrophages, a correlation is established which will also be compared and verified by immunological methods such as flow cytometry and histology.

While the importance of a non-invasive powerful diagnostic tool is primarily critical for MI patients, for which conventional surgical procedures carry risk or may not be feasible at all, it must also be mentioned that non-invasive methods such as MRI may also prove to be a valuable tool to analyze samples and situations in the future of the rapidly advancing field of tissue engineering.

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a91732

## 03-P312 Fibrous matrix transmitted force contributes to local fibroblasts activation during cardiac fibrosis expansion

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Fibrosis expansion is responsible for poor long-term prognosis of myocardial infarction. However, understanding on roles of different factors in directing local fibrosis expansion has been extremely hindered by the complicated biochemical and biomechanical environment *in vivo*, which is difficult to be decoupled and monitored in animal models. Here, we constructed a three-dimensional (3D) biomimetic model for cardiac fibrosis and recreated the dynamic ventricular remodeling process *in vitro*. Based on the model, we elucidated a possible mechanism for local fibrosis expansion, in which the force generated by pre-activated myofibroblasts and transmitted through extracellular matrix (ECM) fibrils contribute to neighboring quiescent fibroblasts activation, resulting in far-end infiltration of fibrotic edge. A mathematical model in which only fibrous matrix properties, cell generated force and mechanical activation of fibroblasts are involved could simulate the fibrosis expansion process of our 3D biomimetic model to a great extent also confirmed the sufficiency of the fibrous matrix transmitted force theoretically. Moreover, disruption of collagen crosslinking by BAPN to attenuate force transmission *in vivo* significantly decrease the rate of fibrosis expansion, indicating the importance of fibrous matrix transmitted force in promoting fibrosis expansion, and potentially offering a novel therapeutic strategy for cardiac fibrosis.

a92429

## 03-P313 Influence of different bioreactor parameters on whole heart decellularization

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Bioreactors enable control over physicochemical and physiological parameters useful to organ and tissue engineering. The objective of this study was to use a commercial bioreactor platform to investigate the influence of flow rate, pulsatility, stir speed and time parameters for whole heart decellularization. The need for advanced cardiac regenerative medicine strategies is founded in the fact that cardiovascular disease is now the leading cause of death (31%). The heart is a complex organ with limited regenerative capacity, and transplantation carries drawbacks and may not be a viable therapeutic strategy to satisfy population demand. A widespread technique of obtaining a cardiac scaffold is whole heart decellularization, which generates an acellular cardiac matrix that can be recellularized using bioreactors.

This research focused on obtaining a decellularized pig heart, testing different operational conditions and evaluating the influence of different parameters on the process. Decellularization fluid composed of 1% (v/v) SDS, was perfused through the bulk tissue of a pig heart by antegrade coronary perfusion using the Eva Luxor™ bioreactor (Eva Scientific Ltda.), operating in "decell-mode" with different operational conditions, such as flow rate, agitation, regime (constant/pulsatile) and use of recycled fluid.

In each operational condition, the results of the decellularization process were gradually obtained and evaluated, allowing the analysis of the influence of different parameters. The change of parameters, from one set of conditions to the next, was backed up by the results obtained from the previous operational conditions. The best results were observed at operational conditions that included agitation (350 rpm) and a high perfusion flow rate (500 mL/min). At the end of the experiment, the heart showed typical characteristics of a successful decellularization such as whitish coloration, flaccidity, change in size, absence of cells and intact anatomy (such as coronary vessels, observed by the antegrade dye injection technique).

In summary, decellularization parameters play an important role in robustly obtaining scaffolds conducive to cardiac regeneration and evaluating the process parameters using sophisticated control, monitoring and data-logging systems is an important step in the quest for optimization of this technique and its results.

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a91105

## 03-P314 Specific detachment and efficient collection of beating iPS-derived cardiac cells on fluidic device system with visible light

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Recently, specific cell sorting techniques are required with the development of regenerative medicine. Here, we report the specific and speedy cell detachment system with photo-responsible cell scaffold, fluidic device, and irradiating visible light.

Photo-responsible cell scaffold was prepared using gold nanoparticles (AuNP) embedded collagen gel. The acryl fluidic device was coated with this gel, and cardiac cells differentiated from human-induced pluripotent stem cell (hiPSC) in the bioreactor[1] were transferred and cultured on this fluidic device. After 4 days culture, the beating cardiomyocytes on this device were detected by an inverted microscope and detached by a 561nm laser irradiation with following medium/air replacement. The detached cells were collected by medium injection by a syringe pump.

The probability of specific detachment of the beating cardiomyocytes is around 90% with medium/air replacement but decrease to 10% without medium/air replacement. It is considered that gel surface temperature is rising faster because the heat capacity of air is smaller than that of medium.

This technique shows 350 beating cells/hour of collection efficiency, and can be expected the applications for regenerative medicine including cardiac cell sheet tissues.

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a91018

## 03-P315 Stem Cell Therapy for Myocardial Infarction Using Blood-Immiscible, Injectable, Sticky, and Bioactive 3-Dimensional Cell Carrier Based on Mussel Protein Glue

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Myocardial infarction (MI), one of the major cause of death worldwide, has resulted in the loss of cardiac muscle and left ventricular dysfunction. For restoring scarred cardiac tissue with limited self-regeneration capacity, exogenous stem cells should be efficiently delivered by various therapeutic strategies such as cell injection, cell patch, and cell sheet engineering. However, needle injecting stem cells would be invasive to thinned myocardium with low delivery efficacy due to blood circulation with high pressure of heart. In addition, therapeutic efficacy of attaching cell patch or sheet on the surface of myocardium would not be curative for inner endocardium because of epicardium thickness and scar. Thus, future therapeutic methods for MI aim the increasing cell retention after injection, which is directly related to efficacy improvement. In this work, we proposed a novel stem cell therapy for MI using injectable sticky mussel adhesive protein (MAP)-based 3-dimensional (3D) stem cell carrier with water (blood or body fluid *in vivo*)-immiscibility. With high encapsulation efficiency and survival rate of encapsulated mesenchymal stem cells, the MAP-based cell carrier could be well-injected and evenly distributed throughout scarred myocardium *in vivo*. Its underwater adhesiveness and biocompatibility fostered the integration between grafted stem cell carrier and damaged tissue, resulting in high stem cell retention and maximized paracrine effects. Growth factors and cytokines released from stem cells encapsulated in MAP-based cell carrier induced angiogenesis in damaged myocardial tissue, prevented aggrevation of myocardial wall, induced contractive force recovery, and prevented remodeling and scar formation, determined by histological, immune-histochemical, and echocardiographic analysis. Collectively, novel stem cell therapeutic strategy using MAP-based cell carrier can be potentially applied for recovering infarcted cardiac tissue with injured structural and contractive functions.

a90566

## 03-P316 Hydrogels based on hyaluronan derivatives combined with implantable device for heart regeneration after myocardial infarction

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Myocardial infarction is one of the leading disease causes significant morbidity and mortality worldwide. Cardiac regenerative therapy has progressed from delivering simple suspensions of cells or molecules to more advanced biomaterial scaffolds. Hydrogels with incorporated cells is promising new therapeutics for patients with damaged and ischemic myocardium.

In our previous work, we presented synthesis of new hyaluronan derivative (HA-PH-RGD) bearing both hydroxyphenyl moiety and RGD cell adhesive sequence. The system is capable to form covalently crosslinked hydrogels by horseradish peroxidase (HRP 0,2–0,5 U/ml) mediated reaction. Crosslinking reaction is initiated by addition of highly diluted $H_2O_2$ (0,5–1,7 mM)[1,2]

To achieve therapeutic effect, high number of stem cells ($20{\cdot}10^6$ of human Adipose Derived Stem Cell ADSC) was incorporated into the hydrogel. Developed material was combined with unique implantable device[3] (patch-like substance carrier) adapted for the locationally accurate delivery and administration of substances onto the surface of the heart.

Viscoelastic and mechanical properties of hydrogels were evaluated for homogenization machine (Benchtop type) and application part of implantable device. Cell viability and distribution through the implantable device was evaluated *in vitro*.

There were no statistical differences between the elastic modulus G´, Young's modulus Y and swelling coefficient Q for the hydrogels prepared by the application part of implanted device compared to homogenization machine (Benchtop type).

20 million ADS cells per ml of hydrogel were successfully incorporated into the matrix. Average cell viability in hydrogel was 76 %. No significant difference in cell distribution through the implantable device was observed.

Described scaffold was suitable for incorporation of high number of cells which is required to regenerate heart tissue after myocardial infarction. HA-PH-RGD based hydrogel can be applied into the implantable device and used for *in vivo* study.

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a90569

## 03-P317 Heart-on-chip platform to assess the influence of culture media perfusion on *in vitro* cardiac tissue maturation

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Politecnico di Milano

Heart-on-chip technology has gained attention overtime due to its potential to leverage the effectiveness of preclinical trials on drug-induced cardiotoxicity screening. However, the major hurdle in modelling cardiac disease remains: cell immaturity [1]. Mechanotransduction has proven the crucial role of mechanical forces in regulating cell fate, tissue formation and functionality. Thus, complementing chemical cell manipulation with mechanical stimuli appears an attractive approach to recreate *in vitro* a phenotypically stable cardiac cell population resembling the native myocardium [2]. Here a microfluidic platform for the assessment of perfusion on 3D cardiac ventricular microtissues was developed.

The device design integrates an on-chip peristaltic pumping system, allowing an automated, fine control over flow profiles on side channels that flank a central 3D cardiac culture chamber. Cardiomyocytes were embedded in fibrin gel, seeded in 3D in the microfluidic platform and cultured for 7 days under different profiles of medium perfusion. Maintenance of cell viability was monitored by Live/Dead assay along time at different flow conditions. The effect of perfusion on contractility was assessed by analysis of autonomous beating profile, specifically beating rate and beating velocity. Effects on electrical functionality regarding excitation threshold and maximum capture rate was assessed as well. Cell-cell communication was evaluated through quantification of gap junctions. Static conditions were used as control.

Cells cultured on the microfluidic platform showed high viability after 7 days in culture. After one day in culture, autonomous synchronized beating was observed on perfused cultures, while static condition was quiescent. Perfused cultures showed an increased presence of gap junctions, and enhanced functional and electrical maturity.

The integration of a peristaltic pump on chip represents an attractive approach as compared to traditional bulky electro-mechanical off-chip fluid handling systems. Dynamic flow facilitates the nutrient and metabolites exchange, resembling the native environment of the heart.

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a91289

## 03-P318 Integrating Traction Force Microscopy into Organ-on-a-Chip platforms: *In situ* characterization of contractile forces in 3D cardiac µ-tissues

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Over the last years, microfluidic Organ-on-a-Chip (OoC) systems evolved from a conceptual idea to a feasible alternative for animal models. OoCs combine human induced pluripotent stem (iPS) cells with microfabrication technologies to create microphysiological *in vitro* models featuring human genetic background, physiologically relevant tissue structure and "vasculature-like" perfusion. As cardiovascular diseases constitute a dominant cause of death, engineered cardiac tissues are intensively investigated, resulting in the emergence of a variety of Heart-on-a-Chip systems. Those systems are able to create viable, functionally beating 3D microtissues and study their reaction onto different administered drugs.

However, most of these systems lack the ability to analyze the integrated tissues in a quantitative way. Current models optically determine the beating rate, thereby gaining a temporal contraction profile, but fail to exactly determine twitch forces, being the most important characteristic of cardiac tissues. Other devices make use of micropillar arrays to determine an exact force distribution with the disadvantage of influencing the tissues' environment by introducing additional artificial elements.

We present a novel integrated device enabling the parallelized cultivation of human cardiac microtissues and simultaneous characterization of their traction forces in a physiological, precisely controlled structure and environment. By combining Traction Force Microscopy (TFM), which is already widely used as a standard for studying traction forces on a cellular level, with microfluidic OoC-systems, we are able to extract information about spatial and temporal contractile forces in cardiac microtissues *in situ*. A deformable bottom layer of known elastic properties is integrated into the microfluidic chip system and its tissue-induced deformation is mapped by help of minimally invasive fluorescent beads.

We highlight that the presented system utilizes a next-generation cell loading technique enabling tissue generation devoid of air bubbles and allowing exact aliquoting of iPS cell-dervied cardiac cell types for the creation of multiple cardiac tissues in parallel. The developed system enables the multiplexed automated analysis of contractile forces in many individualized cardiac tissues for drug testing, hence bridging the gap from bare tissue creation to viable big data collection and can be regarded as universal system, also applicable to other tissue types.

a91796

## 03-P320 Toward in vitro construction of iPSC-derived human heart-like tissue

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To date, research on heart development has focused on generating cardiomyocytes (CMs) with the goal of cell transplantation to cure myocardial infarction. In recent years, engineering of myocardial tissue rather than a pure CM population to study cardiac function and transplantation potential has become more prominent. However, using mainly primary tissue or human iPSC-derived CMs in combination with HUVEC and/or fibroblasts, such models do not represent the human physiological system very well.

Here, we aim to construct human heart-like tissue by combining a series of human iPSC-derived cells including CMs, endothelial cells and epicardial-like cells or cardiac fibroblasts. Directed differentiation of human iPSC enables cardiac cell generation from a common progenitor pool (pre-cardiac mesoderm), which represents human heart development further increasing the correlation with human tissue. Moreover, the possibility of genetically engineering iPSCs easily can be used to generate novel disease models. Because 3D cell culture systems permit us to mimic the physiological state more closely than common 2D-monolayer culture systems, we cultured cardiac cells in 3D via two unique approaches: (i) organ bud formation using mesenchymal stem cells; and (ii) spheroid formation via cell aggregation. Furthermore, we generated various reporter cell lines to characterize each cell type. CMs could be easily identified by MYH6 promotor-driven eGFP expression, while sorted cardiac endothelial cells were derived from iPSCs constitutively expressing fluorescent proteins (i.e. mCherry, BFP). Taking advantage, self-patterning of cells can be tracked and cellular behaviour at the transcriptional, translational and epigenetic levels will be analysed.

Further research on spheroids representing the other layers of the heart will enable us to gain more insight into unexplored heart tissues. 3D bioprinting is a technique that allows scaffold-free controlled arrangement of spheroids. During human development, the heart forms a tube structure. Mimicking this state using 3D bioprinting is expected to aid in comprehending human heart developmental stages better.

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a91672

## 03-P321 Engineering multiscale models of the myocardium for investigating structure-function relationships in the human left ventricle

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Using a series of novel bioengineered cardiac culture platforms of increasing complexity, we have been investigating whether recapitulation of multiscale structural features of the human heart wall can produce functional profiles that more closely model heart function *in vivo*. We aim to create a multifaceted screening system using several core technologies developed by our group to evaluate different aspects of myocardial electromechanical function. Specifically, we posited that the use of a targeted set of functional assays in combination with appropriate maturation stimuli would provide a more comprehensive understanding of how structural organization regulates myocardial function. Nanotopographic microelectrode arrays were used to evaluate electrophysiological function, while nanopatterned cell sheet stacking technology was employed to create 3D cardiac patches for analyzing contractile performance. Finally, we developed a tissue-engineered tubular ventricle model for assessing pressure generation in a 3D model of the left ventricle. The analysis of multiple functional endpoints using separate optimized assays enables more detailed analysis of how cellular and tissue level organization contributes to the development of electromechanical profiles in heart tissue. The development of such models not only provide new information on the role of structural organization in the development of heart function, but also offers a series of new preclinical platforms for evaluating the effect of specific disease states and/or drug treatments on human cardiac performance. The movement from platforms with higher levels of throughput to those with higher degrees of biomimicry, transitioning from 2D sheets to 3D sheets to 3D ventricular structures, constitutes a natural "funneling" of the preclinical screening process. Drug candidates and disease-relevant phenotypes characterized using simpler multiplexed models can then be re-evaluated using systems that offer closer representations of the native tissue, and provide physiological endpoints analogous to those monitored in patients. As such, the developed methods for studying maturation and electromechanical function of human cardiac tissue could provide the framework for a next generation screening process geared towards replacing animal testing with increasingly physiologically representative models of the myocardium.

a92704

## 03-P322 Torsade de Pointes arrhythmia model *in vitro* with 3D human iPS cell-engineered heart tissue

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BACKGROUNDS: Torsade de Pointes (TdP) is a life-threatening arrhythmia that compromises clinical health care and drug discovery. Simple 2D cell culture system was insufficient to reproduce actual occurrence of TdP. Here we show an *in vitro* TdP model using hiPSC-derived cardiac tissue sheets (CTSs).

METHODS & RESULTS: With the use of temperature-responsive culture dishes, we prepared CTSs as a 3D structure with 5-6 of cell layers that contained a mixture of hiPSC-derived cardiomyocyte (CM) and non-cardiomyocyte populations. We simultaneously monitored the extracellular field potential (EFP) with a multi-electrode array and the cellular contractile movement with a live cell imaging system. Upon treatment of an IKr channel blocker, E-4031, CTSs exhibited TdP-like tachyarrhythmia not only as a typical polymorphic EFP pattern but also as the reentrant spiral wave propagation accompanied by meandering of the spiral wave center which is a characteristic of TdP. The induction efficiency of TdP-like waveform in CTSs with the mixed cell populations was 80% (12/15), whereas that in CTSs with CM alone was 0% (0/18) ($p<0.001$). The TdP-like waveform induction efficiency in 2D culture condition with the mixed cell populations was only 30% (3/10) ($p<0.05$). These results indicate that the heterogeneity of the cellular components and the 3D structure are both essential factors for reproducing TdP-like arrhythmia in vitro. We also found that Cx43 expression in CTSs was mainly localized in CMs that results in heterogeneous structure formation. The 3D structure seemed to contribute to reduce the excessive deviation in the cellular heterogeneity and provide "appropriate heterogeneity" to be a functional reentry center that can move around in the CTSs. Then TdP can be maintained for longer periods.

CONCLUSION: We succeeded in inducing and visualizing TdP-like arrhythmia in 3D *in vitro* model using entirely stem cell-derived human heart tissue. This model will broadly contribute to the elucidation of TdP pathophysiology, drug discovery and cardiac safety tests.

a92745

## 03-P323 In vitro modeling of cardiac fibrosis for drug screening using simulated extracellular matrix in human induced pluripotent stem cells derived cardiac construct

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**Background:** Cardiac fibrosis is one of major etiologies in heart failure and development of new medications is crucial. However, assay system for discovering new medications mainly depend on animal model in which some drawbacks such as high cost exist and new in vitro assay using human cells should be developed. In vitro culture system composed of only fibroblasts cannot evaluate the influence for cardiomyocytes nor interaction between cardiomyocytes and fibroblasts. We hypothesize cardiac tissue like structure (CTS) using human induced pluripotent stem cell derived cardiomyotes (hiPSCs-CMs) may provide the useful tools for in vitro drug screening.

**Methods and Results:** Cardiomyogenic differentiation was induced in hiPSCs to produce cardiac CTS including iPSC-CMs and fibroblasts. After stimulation to CTS by pro-fibrotic protein such as TGF-b, expression of Extracellular matrix (ECM; Collagen type I, Collagen type III, Fibronectin) and proteases (MMP-2) was significantly increased, as assessed by real-time PCR and immunohistochemistry. In addition, treatment of TGF-b decrease the cardiac contractile/relaxation velocity (without vs with TGF-b; 27.4±6.1 vs. 20.9±4.3, 8.4±3.0 vs. 3.9±1.5μm/sec, respectively; $P<0.05$) as assessed by image-based motion analysis. Furthermore, we analyzed whether CTS could response to anti-fibrotic factor, HGF. As a result, treatment of HGF decreased the expression of ECM and increased the contractile/relaxation property. Finally, we generated the different ratio (50, 60, 70 and 80%) of cardiomycoytes in CTS to compare sensitivity to pro-fibrotic stimulation. As a result, the increase of the gene expression of fibrotic marker was more abundant in CTS containing 50 to 70% cardiomyocytes.

**Conclusion:** CTS generated by iPSC-CM and non-CMs response to both pro- and anti- fibrotic stimulation and have a possibility to propose simulated cardiac ECM in vitro, suggesting that this system may provide the useful tool for anti-fibrotic drug screening.

a92804

## 03-P324 An Injectable Self-doping Conductive Hydrogel that Improves Electrical Coupling of Isolated Cardiomyocytes and Restores Heart Function after Myocardial Infarction

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Myocardial infarction (MI) induces permanent loss of cardiomyocytes and forms fibrous scar tissues. The nonconductive nature of fibrous scar tissues can cause desynchronized cardiac contraction, owing to the electrically uncoupling viable cardiomyocytes in the infarct region. In this work, a self-doping conductive polymeric hydrogel (Gel-PAMB) was synthesized by grafting the conductive poly-3-amino-4-methoxybenzoic acid (PAMB) on biocompatible gelatin (Gel) and crosslinked by 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) in combination with N-hydroxysuccinimide (NHS), which can be injected to the MI tissues to support electric conduction, thus improving cardiac function. The results of the *in vitro* study demonstrated that Gel-PAMB facilitated electrical signaling propagation, as confirmed through the calcium signaling and multielectrode array analysis. The velocity of electrical signaling propagation was faster and better synchronized for cardiomyocytes on Gel-PAMB relative to cells on Gel. Furthermore, The Gel-PAMB was injected into the MI area of rats, and the preliminary *in vivo* experiments showed that a significant improvement of heart functions, such as narrower QRS interval, reduced induced arrhythmia and spontaneous arrhythmia, improved conduction velocity, and increased fractional shortening, was achieved compared to Gel treatment. Overall, both *in vitro* and *in vivo* results clearly suggests that the Gel-PAMB can synchronize cardiac contraction by electrically bridging isolated viable cardiomyocytes within the scar tissues, restoring the global heart function.

a91381

## 03-P325 The cardiac ECM as a template for tissue engineering scaffolds

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**Introduction** The success of tissue engineering scaffolds is heavily dependent on their similarity to the extracellular matrix (ECM) of the respective organ. In order to construct a sufficiently biocompatible tissue engineering scaffold, it is critical to have a detailed understanding of microscopic, three-dimensional structure of the ECM of interest. However to date the structure of the ECM of the heart is relatively poorly understood;
Here, we provide comprehensive characterisation of the cardiac ECM using decellularized pig hearts.

**Materials & Methods** Porcine hearts were obtained from adult slaughter-house pigs. Cylinders were cut from the left ventricle, using a standard biopsy-punch. These cylinders were decellularized using a SDS/Triton X-100-based protocol.[1] Decellularized tissue was characterised with regard to its mechanical, swelling, and three-dimensional design properties. Furthermore, tissue was homogenised, made into films, and seeded with cardiomyocytes to test for cell attachment. Collagen I films served as a control.

**Results** Imaging showed an average pore size of 22.9 ± 3.6 µm and revealed the inherent micro-vessel structure. Vessels were imaged over 8 mm and showed several bifurcations. The scaffolds showed a significant swelling capability of around 1000% and a compressive modulus of 8 ± 0.4 kPa after 2 hours in water. Films made from homogenised tissue showed increased cell attachment after 2 hours, compared with pure collagen films.

**Discussion** The decellularization protocol used for this study was shown to preserve three-dimensional architecture, with its interconnected pore-system and innate vasculature, as well as cell binding sites, showing a higher cell attachment after 2 hours compared with the commonly used collagen I. The characterization of the decellularized tissue provided a template for the creation of artificial tissue engineering scaffolds.
In future work, the properties of engineered tissue should be matched to the range of values characterized here in order to maximize the probability of success.

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a92591

## 03-P326 Culture-free on-site Implantation of Allogeneic Adipose-Derived Human Mesenchymal Stem Cell Spray Boosts Convenience of Regenerative Therapy in Cardiovascular Disease

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Background:
Although remarkable progress has been made in myocardial regeneration therapy, how to deliver cells to damaged heart less invasively and more effectively without cardiac tissue destruction may be crucial in the cellular therapy for heart failure. We hypothesized that culture-free on-site implantation of allogeneic Adipose-Derived Human Mesenchymal Stem Cell (hADSC) using surgical fibrin glue spray might have impact on the recovery of cardiac function in porcine ischemic cardiomyopathy.

Methods and Results:
Most of the hADSC isolated from human fat tissue expressed CD73 and CD90, and were negative for CD31 and CD45, as measured by flow cytometry.
In graft preparation, fibrin and thrombin solution were prepared using a Manufactured Combi-Set. ADSC ($1x10^8$/graft) were mix with fibrin solution before implantation, and then they were sprayed over the infarct area of the myocardium to deliver cells.
ELISA analysis revealed that ADSC graft secreted various cytokines such as VEGF and HGF in vitro. We induced myocardial infarction in porcine model and divided into the ADSC-transplanted groups (A group, n=6) and the control (C group, n=6). At 4 weeks after the transplantation, US revealed that A group had significantly greater ejection fraction compared with the C group (54.9±1.5, and 41.0±2.6%, $P<0.05$ respectively) with improvement longitudinal strain and LV reverse remodeling assessed by MRI. $NH^3$ positron-emission tomography depicted that coronary flow reserves were 1.5 to 2 times greater in the A group.
Histological analysis revealed that the graft had survived for at least four weeks and that the significant reduction in hypertrophy, interstitial fibrosis, and higher vascular density in the A group. The mRNA levels of VEGF, b-FGF and SDF-1 in infarct border and remote area were significantly higher in group A.

Conclusions:
Culture-free on-site implantation of allogeneic ADSC by using fibrin glue spray method could easily deliver allogenic cells to epicardium with adequate functional and histological recovery, suggesting that this new cell delivery method have some impacts on cell-based therapy in clinical scenario.

a91965

## 03-P328 Design and Development of a Miniaturized Imaging Window for Intravital Nonlinear Microscopy

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**Introduction**: According to the ISO 10993 normative set, new biomaterials must be tested in vivo by subcutaneous implantation. This implies remarkable costs, animal use and unethical procedures.

Intravital microscopy [1] has been used to quantify the foreign body reaction by observing a single animal in multiple timepoints [2], thus reducing costs and animals. To this end, specific transparent window chambers and micrometric scaffolds were proposed. However, no device providing a specific tracking geometry for repeated analyses exists.

In this work we have developed and validated in vivo a miniaturized imaging window (Microatlas) that can be imaged without a percutaneous access. It acts as a scaffold for tissue regeneration and allows 3D repositioning.

**Materials & Methods**: Microatlas devices were fabricated on glass coverslips via two-photon laser polymerization in a hybrid biocompatible photoresist. They consist in micro grids with micrometric cubic pores, acting as scaffolds for cell migration. The spatial repositioning of the microscope is assured by polymerized landmarks. Microatlas devices can be paired to a millimetric portion of a biomaterial, sealed and implanted. We paired the Microatlas with glass coverslips and implanted in chick embryos.

**Results and Discussion**: Confocal microscopy confirmed the tendency of tissue to grow inside the microgrids. Two-photon excitation fluorescence acquisitions of non-immunolabelled specimens showed the presence of a thin layer of collagen I. Immunofluorescence analyses revealed a commitment of embryonic cells toward the endothelium lineage and the presence of inflammation-related cells. Standard coverslips did not induce an inflammatory response.

**Conclusions**: Microatlas devices guided the formation of a new tissue and allowed repeated intravital observations of host immune response to a biomaterial. These results represent a starting point to optimize the Microatlas to approach the ISO 10993 requirements.

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**Acknowledgments:**

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a91362

## 03-P329 Tunable angiogenesis and host immune response on Elastin-like recombinamer based scaffolds on soft tissues regeneration

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One of the most important facts for a tissue regeneration based on engineered scaffolds is a proper angiogenesis. Many efforts have been done to control angiogenesis, improving it but without obtain aberrant formations as angiomas. We present a bioactivated material capable to promote or inhibit angiogenesis despite the presence of highly angiogenic cell source without the exogenous supplementation of angiogenic factors. We show how ELR-based hydrogels with functional RGD and REDV sequences will promote cell adhesion and enhance the SVF response in terms of in vitro angiogenic factor release and endothelial cell organization, improving also the *in vivo* angiogenic potential and integration with the host tissue.

2 million human SVF cells were encapsulated in not functionalized (NF) (control group) or RGD-REDV (RR)- based ELR hydrogels and then cultured either in static or under perfusion conditions. Constructs were inserted in subcutaneous pockets created in the back of male nude rats. DNA analyses were performed. Pro- and anti- angiogenic factors were evaluated by ELISA analyses, production of VEGF was measured, histology (H&E) and staining against vimentin, CD31 and CD45 were performed. Quantification of vessel length density (VLD) was carried out. Perfusion-culture showed to promote a superior cord-like organization by endothelial cells, so we cultured the SVF cells in perfusion condition to investigate the role of hydrogel functionalization and architecture in terms of angiogenic potential and host response in a nude rat subcutaneous pocket model.

The change of the bioactive properties of the ELR hydrogels can on its own significantly modulate the implant interaction with the host and the vascularization potential of highly angiogenic cells, as SVF. In our study, we found that the open structure and the functionalization allowed an increasing infiltration of host cells with time *in vivo* and a resorption of the hydrogel without generating a severe foreign body reaction, creating new vascular vessels. Although NF hydrogels did not degrade and were not colonized by host cells, no inflammatory reaction was observed around the grafts.

Our study showed how angiogenesis and host integration of the engineered tissues can be controlled by modulating the functionalization of the hydrogel alone. The proposed strategy could be easily implemented and adapted to the specific type of tissue/organ regeneration further progressing towards a successful regenerative medicine.

a92749

## 03-P330 Tissue-resident CD169+ macrophages are crucial drivers of innate and adaptive immunity during blood-stage Plasmodium infection

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Siglec-1/CD169 identifies a subpopulation of self-renewing macrophages amongst a highly plastic and heterogeneous macrophage family. Tissue-resident CD169+ macrophages are mostly strategically positioned in close proximity to vasculature and specialized to capture blood borne sialylated pathogens but the immunomodulatory role of these macrophages in blood-stage malaria remains unknown. Herein, we exploited CD169-DTR transgenic mouse, which enables specific and efficient ablation of CD169 expressing macrophages across lymphoid and non-lymphoid organs in steady state and during Plasmodium infection to unravel their role during malaria. Strikingly, CD169+ macrophage deficient mice show accelerated onset of malarial syndromes such as acute lung, liver, kidney injury, amongst others and succumb to infection during P. berghei ANKA (PbA) and self-resolving non-lethal P. yoelii (Py) malaria. The observed widespread tissue inflammation in Plasmodium infected CD169-DTR mouse was due to failure to restrain parasite dissemination, exacerbated tissue hemozoin deposition, establish appropriate pro/anti-inflammatory cytokine milieu suggesting CD169+ macrophages as critical innate regulatory players that curb malaria induced inflammation. Henceforth, we show, CD169+ macrophages possess anti-inflammatory attribute and are constant cellular sources of IL-10 during malarial infection. Further, we not only demonstrate the involvement of CD169+ macrophages in mediating cytotoxic T cell responses during Plasmodium infection but also parasite specific antibody responses during Py malaria. Collectively, our results highlight previously unappreciated pleiotropic functional virtues of CD169+ macrophages in orchestrating innate as well as adaptive immune responses for resolution of infection during acute and severe malaria.

a90946

## 03-P331 Pro-healing Macrophage Phenotype Inducing cytokine cocktail loaded thin gelatin hydrogels for controlling innate immune response in vivo in tracheal patch model

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Once a material is implanted into the body, an immune reaction is triggered leading to inflammation. Depending on the response of innate immune system, this process can lead to healing around the biomaterial or chronic inflammation/ failure. In order to prevent excessive inflammation and promote tissue integration after implantation, interactions of the materials with the host immune system must be controlled. Macrophages are the main cells involved in the innate immune response. Due to their plasticity they can either be polarized in pro-inflammatory or pro-healing, anti-inflammatory phenotypes.

In this study, a cytokines cocktail based on pro- healing Interleukins[2], was developed to polarize incoming macrophages that are mobilized after implantation into pro-healing phenotype to modulate inflammation and promote tissue regeneration. This cocktail was loaded in gelatin hydrogels[1] to develop an immunomodulatory hydrogel. As a proof of concept, this immunomodulatory hydrogel was used as a surface coating for 3D printed silicone tracheal patch, used to repair tracheal defects, in order to modulate immune response after implantation.

An *in vivo* study on rats was carried out to evaluate the effect of the coating on the integration of the tracheal patch. Rats were divided in three groups of animals: i) implant, ii) implant coated with hydrogel and iii) implant coated with immunomodulatory hydrogel.

The stability of the coating on the implant and its ability to release anti-inflammatory cytokines was demonstrated for at least 14 days. It was also shown that the immunomodulatory hydrogel significantly decreased the overall inflammation response both on local and systemic levels after implantation. The systemic inflammation was assessed with the quantification of pro-inflammatory cytokines in the blood (IL-1 $\alpha$ and $\beta$, CXCL-1/KV, CCL2/MCP-1) and the local inflammation was estimated with histology.

With this work, we have demonstrated the ability of our immunomodulatory hydrogel to control the level of inflammation once applied on tracheal patch. Such thin coatings can be used to diminish the initial immune response to tissue engineering scaffolds and improve their long-term integration and functionality.

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a92916

## 03-P332 Inhibiting IL-1 Signaling Promotes Growth Factor-Based Regeneration

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While growth factors have a great potential in regenerative medicine, numerous clinical trials have failed to show sufficient safety or cost-effectiveness, mainly due to the high doses of growth factors required to promote regeneration. One of the challenges when delivering growth factors into an injured tissue is to provide a controlled release that will trigger a precise signalling to cells[1].

In addition, growth factor signalling may be modulated by the inflammatory microenvironment of the tissue in which they are delivered. Recently, we have shown that the regenerative capacity of mesenchymal stem cells (MSCs) is inhibited by the inflammatory cytokine interleukin-1 (IL-1)[2].

In the present study, we found that the innate immune response to bone injury via the IL-1 receptor 1 (IL-1R1) signaling axis impairs the regenerative capacity of both bone morphogenetic protein-2 (BMP-2) and platelet-derived growth factor-BB (PDGF-BB) in the mouse. Mechanistically, we revealed that IL-1 impairs BMP-2 effect via stimulating Smad proteins degradation, which are critical for BMP signalling, while PDGF-BB effect is inhibited via dephosphorylation of the key kinase Akt. Then, we thought of co-delivering growth factors with IL-1 receptor antagonist (IL-1Ra) to stimulate bone regeneration. In order to deliver IL-1R1, BMP-2 and PDGF-BB, we engineered variants to contain a sequence derived from placenta growth factor-2 (PlGF-$2_{133-141}$) that provide super-affinity to extracellular matrix proteins[3]. Co-delivering low doses of the engineered growth factors with IL-1Ra/PlGF-$2_{133-141}$ stimulated robust bone regeneration in a cranial bone defect and in a femur critical-size defect models in the mouse, while growth factors delivered alone had a much lover effect.

This study highlights the importance of controlling the immune microenvironment in which growth factors are delivered to promote efficient regeneration.

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a92009

## 03-P333 Engineered growth-factors improve wound healing in the non-obese diabetic mouse model of type-1 diabetes

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Diabetes is one of the modern major health scourges and affects more than 350 million people worldwide. 12% of all global health expenditures are spent on diabetes and diabetes-related health problems, including chronic non-healing wounds. The treatment of chronic non-healing wounds has been estimated to take up 33% of the total amount of money spent treating diabetes [1].

Chronic non-healing wounds are caused by unresolved inflammation in the wound-healing cycle. Clinical application of growth-factors (GFs) has failed to improve wound healing, largely due to the amount of GF necessary to apply to the wound, as well as off-target effects caused by GF's potential escape into the bloodstream [2, 3]. The Hubbell lab has created engineered growth-factors (eGFs) that contain a heparin-binding domain from placental-derived growth factor (PlGF) that binds strongly to multiple extracellular matrix (ECM) proteins [4]. As a result of this binding to matrix proteins, eGFs improve wound healing at a lower dose and are retained longer within a wound, compared to wild-type (WT) GFs. These eGFs speed wound healing, can be used at lower concentrations, and are safer than WT GFs.

Here we show that several eGFs improve wound healing in the type 1 diabetes (T1D) non-obese diabetic (NOD) mouse model, including vascular endothelial growth factor-A (VEGF-A), platelet-derived growth factor BB (PDGF-BB), stromal-derived factor-α (SDFα, CXCL12), and heparin-binding EGF-like growth factor (HB-EGF). Several individual eGFs, and combinations of eGFs, improve wound closure and tissue regeneration in the NOD skin.

eGFs also change the cellular milieu of the wound, increasing the number of arginase+ macrophages, amphiregulin+ T cells, decreasing the number of neutrophils, and changing the ratio of classically-activated macrophages to wound-healing macrophages. Interestingly, eGF-induced changes to the wound's cellular milieu differ based on the which eGFs are added to the wound, and the timepoint at which the wound is analyzed. These differences suggest that each eGF may be improving wound healing by different mechanisms over different stages of wound-healing.

These results suggest that eGFs may be a potential therapeutic for chronic non-healing wounds, and also suggest a mechanism-of-action for how different eGFs may improve wound healing.

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a92827

## 03-P334 Clarification of Interaction Mechanism between Transplanted Stem Cells and Immune Cells using Quantum Dots Imaging Technique

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It is important to understand the influence of immune cells and cancer cells on stem cells in order to improve the safety and therapeutic effect of regenerative medicine for cancer patients. We have already developed *in vivo* imaging technology for transplanted stem cells using quantum dots (QDs), which are advanced fluorescent nanomaterials applied to 4K / 8K displays. In this study, the optimal ratio of QDs and cell penetrating peptide for labeling cancer cells (A549 cells) and macrophages were determined, and then *in vivo* fluorescence imaging of QDs-labeled A549 cells and macrophages. QDs fluorescence derived from A549 cells was confirmed to maintain at least 3 weeks in the tumor site of A549 tumor-bearing mice. QDs labeled macrophages were transplanted into A549 tumor-bearing mice through the tail vein, and then were succeeded in imaging the accumulation of the macrophages to several organs. QDs labeling and *in vivo* imaging technology to stem cells, cancer cell, and macrophages enabled us to discriminate the accumulation site of each cell due to the difference in fluorescence wavelength of QDs. These result suggest that the interaction between immune cells and stem cells and cancer cells can be confirmed and analyzed by the fluorescence imaging technology. In the future, we will investigate the influence of cancer and immune cells on stem cells.

a91834

## 03-P335 Early diagnosis of progressive glomerulonephritis by using Wax-Physisorption-based FTIR Micro-spectroscopy

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Altered glycosylation of glycoprotein is a common phenotypic change in cancer and inflammation cells [1-2]. Wax-physisorption-kinetics-based FTIR (WPK-FTIR) imaging has been developed for investigating the variation level of glycosylation based on physisorption capability between glycan attached to glycoprotein on surface of cancerous sample and n-alkanes in the range C20-C34 as glycan adsorbents. WPK-FTIR imaging was successfully demonstrated that aberrant glycan of glycoprotein on malignant sample surface exhibited a strong physisorption with longer chain n-alkane than those of shorter n-alkanes therein [3-5]. Herein WPK-FTIR imaging was utilized for inspecting the inflammation level caused by altered glycosylation of glycoprotein of the glomerulus of rat kidney tissue for assessment of chronic kidney disease status, and n-$C_{22}H_{46}$, n-$C_{25}H_{52}$, n-$C_{28}H_{58}$, and n-$C_{30}H_{62}$ were utilized as glycan adsorbents. The result of WPK-FTIR imaging of the glomeruli revealed a greater physisorption with n-$C_{22}H_{46}$ for rats before treatment of rabbit anti-glomerular basement membrane (anti-GBM) serum than other n-alkane adsorbents herein. On the other hand, the glomeruli of rats after treatment of rabbit anti-GBM serum exhibited a greater physisorption with n-$C_{28}H_{58}$ adsorbent than other n-alkane adsorbents herein, as shown in Fig.1. Furthermore, increasing IR absorbance ratio, $A_{22}$ (n-$C_{22}H_{46}$ remain)/$A_{28}$ (n-$C_{28}H_{58}$ remain), of glomeruli was observed after treatment of rabbit anti-GBM serum and which was 0.51 ± 0.27, 1.08 ± 0.58,1.34 ± 0.92 and 2.04 ± 0.85 at the day prior to treatment, day 8 (early inflammation), day 21 (early renal fibrosis), and day 28 (late fibrosis and renal fibrosis), respectively. Consequently, we suggested the $A_{22}$ /$A_{28}$ of glomeruli should be a signpost of the level of inflammation and glomerular fibrosis.

a92062

## 03-P337 Surface modification on polyester urethane acrylate films to direct foreign body response

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A foreign body response (FBR) developing a fibrocellular capsule generally composed of fibroblasts and macrophages is a result of an inflammatory response upon implantation of a biomaterial device. In the field of tissue engineering, researchers aim to reduce the FBR.
We, on the other hand, aim to make use of such a response to direct cell migration and tissue formation/plasticity, for regeneration of damage tissues using the *in vivo* bioreactor strategies. Since surface properties of biomaterials are a critical factor in modulating the FBR, we modulate initial cell and material interaction by tailoring hydrophilicity and surface topography on polyester urethane acrylate (PEUAc) films. Here, we analyzed alteration of polymeric film's surface chemistry and topography by solvent etching and plasma treatments. High-resolution scanning electron microscopy (SEM) and contact angle measurement showed different surface topography and wettability between the different surface treated films. Total amount of proteins absorbed by the films were quantified and resulted in an increase in absorption due to the surface modifications applied. *In vitro* studies were performed to evaluate the effect of surface modification using fibroblast and macrophages.
DNA and presto blue assay demonstrated difference in cell proliferation and metabolic activity between the different surface modifications. Live/dead assays and methylene blue staining, as well as SEM images confirmed quantitative data.
Focal adhesion analysis through vinculin was performed to further confirm the entity of cell-material interactions and cytocompatibility. Cytokine analysis using IL-1$\beta$, IL-6, IL-10, MCP-1 and TGF-$\beta$1 was done to connect surface properties to pro- and anti-inflammatory (M1 and M2) macrophages, showing a correlation between hydrophilicity and topography on M1 and M2 conversion. Finally an *in vitro* co-culture study seeding both macrophages and fibroblasts was done in the attempt to mimic part of initial inflammatory events that lead to a fibrocellular capsule formation *in vivo*.

a91389

## 03-P338 The effect of different types of stromal cell sheets on the inflammatory state of macrophages *in vitro*

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Recently, cell sheets were introduced as new method of delivering stromal cells to damaged tissue. Excessive inflammation however can impair the healing response. Previous *in vitro* studies have shown that mesenchymal stromal cells can modulate macrophage-induced inflammation and therefore are promising candidates for cell-based therapies aimed at promoting tissue healing. The goal of the current study was to compare the effect of different types of stromal cell sheets on the inflammatory state of macrophages *in vitro*.
We compared the effects of human adipose tissue-derived stromal cell (ASC) sheets, bone marrow derived stromal cell (BMSC) sheets and fibroblast sheets on macrophage functional phenotype. These stromal cell sheets were cultured with and without interferon g (IFNg) and tumor necrosis factor a (TNFa) to simulate an inflamed environment. Conditioned medium (CM) from these sheets was used to culture human primary macrophages for 6 days. The macrophages were analysed using flow cytometric analysis and gene expression. Sheets were examined for their production profile, viability and senescence.
Macrophages cultured in CM from ASC sheets that we cultured with IFNg/TNFa displayed a higher median fluorescence intensity (MFI) of the anti-inflammatory cluster of differentiation 206 (CD206) surface marker than when cultured in CM from BMSC sheets with IFNg/TNFa, and expressed more chemokine (C-C motif) ligand 18 (*CCL18*) and interleukin 1 receptor antagonist (*IL1RA*) than when cultured in CM from fibroblast sheets with IFNg/TNFa. When comparing the effects of sheets without inflammatory stimulus, BMSC sheet CM induced the highest transforming growth factor b (*TGFB*), *CCL18*, and *IL1RA* expression. Interestingly, ASC sheets had higher cell viability and less senescent cells than BMSC sheets and fibroblast sheets, with and without inflammatory stimulus. Prostaglandin E2 ($PGE_2$), IL1RA, and L-kynurenine were low or undetectable without the presence of IFNg/TNFa and increased in all sheets when these cytokines were present but without statistical significant differences between sheet types.
Taken together, ASC and BMSC sheets can stimulate the anti-inflammatory macrophage (M2) phenotype to a better extent than fibroblast sheets. It is suggested that ASC sheets might outperform BMSC sheets in an inflammatory situation since ASC sheet CM induced-macrophages have more M2 characteristics and ASC in the sheet were more viable.

a90941

## 03-P339 Hydrogels for Delivering the Immunomodulatory Properties of Mesenchymal Stromal Cells

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Understanding the mechanism by which mesenchymal stromal cells (MSCs) regulate inflammation can help design efficient therapies for inflammatory and auto-immune conditions. We developed an *in vitro* bioassay to assess the immunosuppressive behaviour of MSCs. We assessed the effect of allogeneic Balb/c mouse MSCs on the proliferation of Concanavalin A-activated splenocytes (SPL) *in vitro*. We compared the immunosuppressive potential of the MSC monolayers in the direct (cell-cell contact) vs. indirect (paracrine) mechanism with the SPL. Following this we assessed the effect of encapsulating MSCs within Alginate and Fibrin on immunosuppression. In addition, we looked at the MSC genes implicated in immunomodulation and how their expression is affected when the cells are encapsulated.

SPL were stained with cell tracker violet dye and added directly to MSCs monolayers or within a trans-well (indirect). Following 72h incubation the proliferation of the SPL was assessed in a flow cytometer. For the encapsulation experiments, the MSCs were loaded into alginate and prepared as macro-beads or loaded into Fibrin clots. These were co-cultured with the SPL directly for 72h before analysis. The gene expression studies were performed on MSCs activated using Tumour necrosis factor-$\alpha$, Interferon-$\gamma$ or both and PCR performed on samples isolated 24h later.

An almost 99% reduction in proliferation for the direct mechanism vs. ~50% for the indirect was observed. Repeated immunosuppressive potential of MSC monolayers was also consistent at ~50% for up to 3 exposures. Alginate was found to be inert while Fibrin showed immunosuppressive effects. Both MSC-loaded alginate and Fibrin showed immunosuppressive effect with the latter being much stronger than the alginate material albeit not as potent as MSC monolayers. By comparing the effect the biomaterials have on the immunomodulatory behaviour of the MSCs the best material and design can be identified for further *in vivo* delivery of the cells.

a90648

## 03-P340 Effects of Exosomes Released by Mesenchymal Stem Cells for Immune Regulation

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Intercellular communication is the first prerequisite for a functional immune surveillance in order to obtain a healthy organism. Mesenchymal stem cells (MSCs) are multipotent stem cells that can be differentiated into a variety of cell types, incuding adipocytes, chondrocytes, osteocytes, and neuronal cells. These MSCs have been reported to exhibit immunomodulatory effects in many diseases. Many studies have reported that MSCs have distinct roles in modulating inflammatory and immune responses by releasing bioactive molecules. Exosomes are homogeneous group of nanovesicles with a size of approximately 50-100 nm. Exosomes are cell-derived vesicles present in biological fluids, including the blood, urine, culture medium of cell cultures. In this study, we investigated the immunomodulatory effects of mouse adipose tissue-derived MSCs (mAD-MSCs), cultured medium of mAD-MSCs (MSC-CM), and mAD-MSC-derived exosomes (MSC-Exo) on LPS-stimulated RAW 264.7 cells. We observed that the expression levels of IL-1beta and TNF-alpha were significantly increased in LPS-stimulated RAW 264.7 cells compared to those in LPS-unstimulated RAW 264.7 cells. Also, these values were significantly decreased in mAD-MSCs/RAW 264.7 cell co-culture groups, MSC-CM-treated groups, and MSC-Exo-treated groups. MSCs can modulate the immune system in part by secreting cytokines. We observed that immunomodulatory factors such as IL-1beta and TNF-alpha were secreted by mAD-MSCs under co-culturing conditions of mAD-MSCs with activated RAW 264.7 cells. In addition, mAD-MSC-derived exosomes exhibited similar immunomodulatory effects in activated RAW 264.7 cells. Therefore, our results suggest that mAD-MSCs have an immunomodulatory function through indirect contact.

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a91265

## 03-P341 Cell surface engineering for immunocamouflage of single cells via layer-by-layer coating technique

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Cell surface engineering has a great potential in cell-based therapies including transplantation, transfusion, and targeted cell delivery. Immunological rejection, one of the most challengeable issue for cell-based therapy, is known to be resolved by immunocamouflage. In this study, we report a cell surface modification strategy to form an immunoevasive layer via diverse chemical methods, for example, thiol-maleimide conjugation, tyrosinase-mediated enzymatic reaction, and electrostatic force on cell surface. We have coated chemical moieties of glycosaminoglycans(GAGs), such as chondroitin sulfate(CS) and hyaluronic acid(HA), on human mesenchymal stem cells (hMSC). No morphological changes have been observed by the cell surface modification of the hMSCs. To evaluate immunoprotection, we performed anti-CD44 treatment to see whether the antibody can penetrate glycan films and recognize cell membrane antigen inside. Furthermore, when the engineered hMSCs were co-cultured with lymphocytes derived from immunocompetent mice, the viability of engineered cells were highly preserved compared to the cells without coating layer, suggesting that the cell surface modification effectively protected the cells from immune response. With our methodology, varieties of cells are expected to be coated to avoid immune response. Overall, we hypothesize our biocompatible and facile cell camouflaging strategy can provide a new platform for clinical applications of engineered-cell therapy.

a90731

## 03-P342 Metallosis: The Battle of Five Armies

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Metals and their alloys have been widely used as implantable materials such as artificial joint prosthesis; internal fixation nails, plates and rods; dental implants and stents. Metallosis including adverse reactions to metal debris (ARMD) in hip arthroplasty is a combination of aseptic fibrosis, local necrosis, inflammation, or loosening of an implanted device secondary to metallic corrosion and release of wear debris. The mechanism of inflammation in metallosis is controversial due to the complexity of the morphology, composition of the wear particles and cell/tissue responses involved. Metal allergy can partially explain some clinical cases but the causes of many are still unknown. We have recently analyzed 285 cases of metallosis from hip replacement. It is identified that five key factors have played an important role in metallosis: (1) wear metal nanoparticles; (2) metal corrosion products; (3) macrophages; (4) master cells; and (5) lymphocytes. In the cases of hip arthroplasty, there are distinct differences between wear metal particles and metal corrosion products, both are recognized as foreign body invasion and induce a battle led by host macrophages, master cells or lymphocytes. Macrophages respond to both types of metal materials by phagocytosis and engulfment in all cases with metallosis. Master cells are seen in some cases and located between macrophages/metal particles and blood vessels. Lymphocytes are dissociated with metal particles but with more severe clinical manifestation following their infiltration. Consequently, it results in large area of cell apoptosis and necrosis, inflammation, fibrosis or pseudotumour formation and failure of the implants. It is still not clear of the key mechanism that how inorganic metal debris can induce antigen related adaptive immune responses involving in lymphocytes including T and B cells in metallosis. The understanding and solution of the mechanism may ultimately extend the application of metallic materials for regenerative medicine due to the unique mechanical property and durability of metals.

a91373

## 03-P343 Microparticles Maintain Anti-inflammatory Macrophage Phenotype in Inflammatory Conditions

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Monocyte-derived macrophages (MDM), the primary innate immune cells, are essential for tissue regeneration following trauma, which they orchestrate by switching from inflammatory to anti-inflammatory phenotypes. However, in many forms of failed tissue regeneration, such as traumatic brain injury, myocardial infarction, and chronic skin wounds, MDM become stalled in a prolonged inflammatory phenotype that prevents tissue regeneration and exacerbates pathology. Attempts to administer anti-inflammatory MDM to injury sites have failed because the wound microenvironment causes MDM to revert to an inflammatory phenotype. Development of a strategy that promotes and preserves an anti-inflammatory MDM phenotype may provide a vital treatment for these injuries. Here, we made poly(lactic-co-glycolic acid) particles (0.7-2.0μm) that are phagocytosed by MDM, stored intracellularly, and subsequently release polarizing molecules to direct MDM behavior. To determine the effects of particle properties on intracellular longevity, particles loaded with the model drug Nile Red were coated with chitosan to increase surface charge from -20 to 13mV. Negatively charged, uncoated particles were fluorescently detected intracellularly for more than 15 days *in vitro* while chitosan-coated particles reduced MDM viability within one week. To explore this platform's utility to control macrophage phenotype, we loaded uncoated particles with Dexamethasone (DEX) because DEX is anti-inflammatory, promotes phagocytosis, crosses membranes, and has cytoplasmic receptors. MDM loaded with DEX-particles upregulated the phagocytic receptor CD163 for one week *in vitro* relative to untreated MDM, suggesting that released DEX altered MDM behavior. To examine if DEX-particles could prevent MDM inflammatory polarization even in the presence of pro-inflammatory stimuli (lipopolysaccharide and interferon gamma), DEX-particles were fabricated with increasing amounts of DEX (0-56% w/w) and administered to MDM. Subsequent treatment with inflammatory stimuli precluded inflammatory polarization for up to one week *in vitro* in the cells treated with 56% w/w DEX particles. This work suggests that exogenously loading MDM with particles can generate and maintain anti-inflammatory MDM behavior even in the presence of inflammatory stimuli. Because of the ubiquitous influence of MDM on tissue regeneration, this platform for sustained modulation of MDM behavior may have applications across an array of pathologies.

a93036

## 03-P344 Self-assembled chitosan-glycyrrhizin nanoparticle as an anti-inflammatory drug to treat inflammatory bowel disease

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Inflammatory bowel disease (IBD) is a chronic, recurrent inflammatory disease of the digestive tract and its incidence is increasing. It is difficult to treat the IBD because the reason of its incidence has yet to be revealed. Most of the currently used IBD treatments have many limitations because they cause serious side effects. In this study, we used glycyrrhizin that is known to have excellent anti-inflammatory effects. Glycyrrhizin, a natural product extracted from licorice roots, can inhibit TLR4 (Toll-like receptor 4) translocation by inhibiting the formation of lipid rafts, thereby significantly reducing the LPS (lipopolysaccharide)-induced inflammatory response. However, since the half-life of glycyrrhizin is very short (6 h), here it was chemically conjugated with chitosan for enhancing glycyrrhizin effect onto the intestine and in the bloodstream. This chitosan-glycyrrhizin conjugate made self-assembed nanoparticle (~100 nm size) due to hydrophilic region of Glycyrrhizin and hydrophilic region of chitosan. Glycol chitosan-glycyrrhizin (chitosan-GL) and chitosan oligosaccharide-glycyrrhizin (COS-GL) were prepared to investigate cell viability and inflammation inhibition effect according to the molecular weight of chitosan. Both chitosan-GL and COS-GL did not affect the viability of non-activated RAW264.7 immune cells and intestinal caco-2 cells. Also, it reduced secretion of TNF-a cytokine from RAW264.7 cells (>65%). In addition, LPs-induced damage of tight junction through caco-2 cell monolayer was improved by treatment with chitosan-glycyrrhizin, which was caused by modulation of calcium content in the cells. In ongoing study, we will carry out the anti-inflammatory effect of chitosan-glycyrrhizin conjugates in DSS (dextran sulfate sodium)-induced IBD animal models.

a92700

## 03-P345 Preparation of gelatin hydrogels for dual drug release to modify macrophages polarization

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Macrophages have two polarization states pro-inflammatory and anti-inflammatory phenotypes. Anti-inflammatory macrophages have an inherent nature to promote tissue repairing and remodeling. Macrophages are differentiated from monocytes circulating in blood. If the anti-inflammatory macrophages can be accumulated at a target site, it is expected that the regeneration and repairing of the site are promoted. This study is undertaken to experimentally confirm this idea of macrophage-assisted tissue regeneration. In this study, SEW2871 of a monocyte recruitment agent and pioglitazone of a macrophage polarization modifier were used. A hydrogel system for the two drugs release was designed, and the drugs release profiles and the effects of cell recruitment and polarization were investigated.

Cholesterol was chemically grafted to gelatin. Micelles formation of the cholesterol-grafted gelatin allowed the hydrophobic SEW to solubilize in water (SEW-micelles). Poly(lactic-co-glycolic acid) spheres incorporating pioglitazone (pio-spheres) were prepared by the conventional solvent evaporation method. To evaluate the macrophages polarization, pio-spheres were incubated with mouse bone marrow-derived macrophages. The gelatin solution containing SEW-micelles was closslinked to the gelatin hydrogels incorporating SEW-micelles, followed by the addition of the pio-spheres to obtain the gelatin hydrogels incorporating SEW-micelles and pio-spheres (SEW-pio-hydrogels). The in vivo and in vitro release profiles of SEW and pioglitazone from the SEW-pio-hydrogels were estimated. After the implantation of SEW-pio-hydrogels into a bone defect of rat ulna, the bone regeneration was evaluated.

The amount of water-solublized SEW changed with the ratio of SEW2871 added initially to the cholesterol-grafted gelatin. The amount of pioglitazone incorporated in the pio-spheres was constant, irrespective of the spheres size. Upon incubating macrophages with the pio-spheres, the IL-10 production and the activity of arginase-1, a measure of anti-inflammatory macrophages, increased. Both the SEW and pioglitazone were released in vitro with time from the SEW-pio-hydrogels. The results of animal experiments will be reported.

a92843

## 03-P346 Stem cell-derived Nano-Ghosts with innate targeting and immunomodulatory capabilities: A new approach for cartilage regeneration

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**Introduction:** Osteoarthritis (OA) is the most common degenerative disease of the joints, leading to cartilage degradation and sclerosis of the underlying bone. One of the main hallmarks of OA is the extracellular matrix degradation which promotes inflammation and leads to further cartilage loss via an increase in matrix degrading enzymes. Mesenchymal stem cells (MSC) play an important role in inflammation reduction and their use for cartilage repair is increasing, due to their proposed aptitude to home, colonize and promote regeneration of injured tissues. We designed a new kind of nanoparticles, termed Nano-Ghosts (NG), derived from the cytoplasmic membrane of MSCs. Retaining MSCs' surface properties, NGs are expected to target damaged tissue and lacking internal machinery they are also safer, as drug delivery system, compared to synthetic or viral vectors. In this study we demonstrate that NGs can target human articular chondrocytes (AC), cartilage explants and prevent inflammation.

**Methods:** NGs' anti-inflammatory effects were studied *in vitro*, on both TNFα-stimulated and non-stimulated ACs and on human cartilage explants *ex vivo*. Smooth muscle cells (SMC)-NGs were used as a non-mesenchymal control. Targeting was evaluated by flow cytometry and confocal microscopy. Anti-inflammatory effect was assessed by qPCR of both cartilage and inflammation markers and ELISA.

**Results:** Flow cytometry showed that MSC-NGs target osteoarthritic ACs 2 times more efficiently than SMC-NGs. Moreover, MSC-NGs showed 4 times higher targeting when ACs were TNFα stimulated. Targeting was confirmed using confocal microscopy and imaging flow cytometry, which showed that NPs bound the membrane and penetrated the cells. Similarly, in human explant cultures NGs binding was 5 times higher when the explants were TNFα stimulated, confirming the monolayer results. The anti-inflammatory effects were tested on different markers such as NO production, IL6 and PGE2 production. Our data showed that NGs reduced inflammation by more than 50% at both RNA and protein levels.

**Conclusion:** Here, we show proof-of-concept of the use of NGs with intrinsic targeting abilities for targeted cartilage regeneration. Further studies will focus on loading the NPs with drugs that can further decrease inflammation and promote cartilage regeneration. NGs could open new avenues towards the treatment of OA as a carrier for targeted delivery of therapeutics, such as anti-inflammatory agents and growth factors.

a91980

## 03-P347 A three-dimensional *in vitro* model of lymphangiogenesis in tumor microenvironment

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Lymphangiogenesis is process of generating new lymphatic vessel in disease such as tumor metastasis and inflammation.[1] The new lymphatic vessel promotes cancer metastasis and have a key role of dendritic cell (DC) migration for adaptive immunity.[2] Since this process is not shown in normal physiology, understanding lymphangiogenesis can provide new therapeutic potential.

However, the mechanism of cancer metastasis and DC migration remain poorly understood and biophysically relevant three-dimensional lymphangiogenesis model is demand for understanding the mechanism.

Previously, micro-sized platform, called microfluidic platform, was used for three-dimensional *in vitro* model and that platform was very useful for real time monitoring and constructing complex models. However, the platform was hard to incorporate tumor spheroid having hypoxic core and had a limitation for biological analysis such as qRT-PCR and western blot.[3]

Here, we presented extracellular matrix (ECM) incorporated new macro-fluidic platform for physiological relevant lymphangiogenesis model which has lumen structure and button-like junctions. Using this model, we successfully developed tumor microenvironment for tumor metastasis applying cancer spheroid which has hypoxic core. Interestingly, vascular endothelial growth factors (VEGFs) combination and induced interstitial flow in tumor site facilitate lymphangiogensis and promote cancer metastasis. Furthermore, this platform has a possibility for adaptive immune model since DC shows migration to new lymphatic vessel and transmigration through the lymphatic endothelium. Moreover, the macro-fluidic platform can be performed biological analysis on chip, such as qRT-PCR, western blot, ELISA, immunostaining and monitoring in real time for cell development and migration.

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a90997

## 03-P348 An *In Vitro* Testing Platform of Macrophage Polarization in Hydrogel Scaffolds

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Biomaterials for tissue engineering and regenerative medicine applications need careful scrutiny to ensure that the materials do not induce any adverse response from the body's immune system once implanted. Macrophages are known for their foreign body response and polarized phenotypes when interacting with biomaterials. Through paracrine signaling, macrophages further regulate the phenotype of fibroblasts to fibrotic or non-fibrotic, dictating the eventual host-biomaterial integration. An *in vitro* testing platform analyzing macrophage-fibroblast-biomaterial interactions is needed to evaluate how material surfaces modulate the polarization and cell signaling of macrophages in a systematic and controlled fashion before *in vivo* investigation.

The primary goal of this study was to complete the first step towards a functional testing platform through characterization of the immunological response within THP-1 macrophages induced by a chosen biomaterial, namely, a chitosan-glycol based hydrogel. The glyoxal crosslinker concentration was varied to alter stiffness and porosity of the hydrogel so that cellular response – notably macrophage polarization – could be evaluated as a function of crosslinker concentration.

Monocultures of PMA induced THP-1 derived macrophages (M0) were seeded on the surface of chitosan-glycol hydrogels. Glyoxal crosslinker concentrations were varied between 0.005% and 0.02% within the *in vitro* platform over a three day period. M0 cells cultured on tissue culture plastic were the controls. Enzyme-linked immunosorbent assays for tumor necrosis factor (TNF)-$\alpha$ and interleukin (IL)-10 secreted proteins, confocal microscopy for cell viability, and flow cytometry examining pro-inflammatory M1 (CD16, CD64) and anti-inflammatory M2 (CD 163, CD206) cell phenotype markers were performed.

This study combined multiple analytical tools to profile the immune reaction of macrophages to a chitosan-based hydrogel whilst varying crosslinker concentration. Results showed that as crosslinker concentration was reduced, an increased pro-inflammatory response was observed from the macrophages. Such a response is likely associated with the alteration of gel stiffness and porosity that are potently controlled by crosslinker concentration. The proposed platform will be extended for macrophage-fibroblast coculture and other biomaterial testing to help accelerate the design of immune-instructive biomaterials and reduce the number of animals used in experiments.

a90961

## 03-P349 IMMUNE ASSISTED ARTIFICIAL TISSUE DEVELOPMENT VIA CO-ENCAPSULATION OF FIBROBLAST/ENDOTHELIAL CELLS AND MACROPHAGES IN GELATIN HYDROGELS UNDER CYTOKINE STIMULATION

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Tissue functions are dependent of their interactions with the other systems of the body such as blood circulation, nervous network and immune system. Resident macrophages in tissues play a role in tissue homeostasis and also provide a mean to re-establish homeostasis following tissue damage. As a consequence incorporation of phenotype controlled autologous macrophages in artificial tissues[1] can facilitate the resolution of inflammation application. In this work, we have elaborated two potential strategies to assist and improve ,via the incorporation of phenotype controlled macrophages, the 3D remodelling of an artificial soft tissues with gelatin hydrogels[2] containing fibroblasts and/or endothelial cells i) with supplementation of IL-4 in the presence of macrophages and ii) in tri-culture via incorporation of naive monocytes or differentiated macrophages. IL-4 supplementation had a boosting effect on fibroblast proliferation with significant upregulation of secretion of activin, IL-1RA, TNF alpha and IL-1beta in the presence of differentiated macrophages. IL-4 supplementation in endothelial/macrophage co-culture condition accelerated the organization of the capillary sprout-like structures and increase the rate of proliferation at day 1. At the earliest stage of culture, an up-regulation of certain cytokines such as IL-6, IL-1RA in the presence of differentiated macrophages was observed thus creating a favorable microenvironment for angiogenesis. The incorporation of either naïve monocytes or macrophages with fibroblasts and endothelial cells in a tri-culture configuration have led to denser, tissue like structures with highly degree of hydrogel remodelling. In the condition of tri-culture with differentiated macrophage, we have observed an upregulation of angiogenic cytokines such as IL-6, IL-8 without additional IL-4 supplementation. This work have proved that incorporation of phenotype controlled macrophages in a resident macrophage-like function had significant effects on the maturation and cytokine microenvironment of 3D cell type-laden hydrogels. This approach can be used to improve the integration of tissue engineering scaffolds and also for the development of more relevant in vitro tissue models.

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a90869

## 03-P350 Modulation of Inflamed Synovium and its Residing Macrophages with Triamcinolone Acetonide Improves Chondrogenesis of Mesenchymal Stem Cells

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**INTRODUCTION:** Joint injuries may result in cartilage defects and inflammation of the joint. When inflamed, the synovium in the knee joint secretes factors that stimulate cartilage degradation and inhibit cartilage repair by suppressing stem cell chondrogenesis. Pro-inflammatory macrophages residing in the synovium are suspected to contribute to this effect. To improve cartilage repair, joint homeostasis should be restored. We aimed to counteract the anti-chondrogenic effect of inflamed synovium by modulating synovial macrophages with the anti-inflammatory drug Triamcinolone Acetonide (TAA).

**MATERIALS & METHODS:** Synovium tissue obtained from osteoarthritic patients undergoing a total knee replacement was cut into small pieces and either used to culture as explants or to allow outgrowth of fibroblasts. Explants and cells were cultured with or without 1 uM TAA for 72 hours. The anti-inflammatory effect of TAA on gene expression of synovium explants and synovial fibroblasts was evaluated by qPCR. Also, the inflammatory surface markers on synovial macrophages were analyzed with flow cytometry. Conditioned medium (CM) from the explants was assessed for its effect on chondrogenesis of human bone marrow mesenchymal stem cells (MSC) by gene expression of cartilage matrix components.

**RESULTS:** TAA significantly decreased gene expression of tumor necrosis factor-alpha (*TNFA*), interleukin(IL)-1$\beta$ (*IL1B*) and *IL6* in synovium explants and synovial fibroblasts, and increased expression of chemokine (C-C motif) ligand-18 (*CCL18*), interleukin-1 receptor antagonist (*IL1RA*) in synovial explants. The expression fibrotic gene alpha-SM-actin (*aSMA*) upregulated following the TAA treatment. On the other hand, TAA decreased pro-inflammatory CD14+/CD80+ and CD14+/CD86+ cells in the synovium. The decrease of collagen type-2 (*COL2A1*) and aggrecan (*ACAN*) expression in chondrogenic MSCs cultured with synovium CM was reduced by treating synovium with TAA during the production of the CM.

**DISCUSSION & CONCLUSION:** Reducing synovial inflammation by modulating its residing macrophages with TAA decreased the anti-chondrogenic effect of synovium on MSC chondrogenesis. This might eventually improve the joint environment for better cartilage repair.

a91155

## 03-P351 Immunomodulatory and Antimicrobial coatings for medical devices

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All implantable biomedical systems face several risks once in contact with the host tissue: excessive immune response to the implant and development of bacterial biofilms. A multifunctional surface coating that can address all these two issues concomitantly would significantly improve clinical outcomes. We hypothesized that polyarginine (PAR), a synthetic highly cationic polypeptide, can act on macrophages to control innate immune response because arginine is an important component of macrophage metabolism. Moreover, PAR is susceptible to act as an antimicrobial agent due to its positive charges. We developed a new polyelectrolyte multilayer films based on PAR and hyaluronic acid (HA). The layer-by-layer PAR/HA films have a strong inhibitory effect on the production of inflammatory cytokines released by human primary macrophages subpopulations (1). This could reduce potential chronic inflammatory reaction following implantation. Next, we show that PAR/HA films were very effective to inhibit Gram- positive and Gram-negative pathogenic bacteria associated with infections of medical devices. We demonstrate that exclusively films constructed with poly(arginine) composed of 30 residues (PAR30) acquire a strong antimicrobial activity (2). Moreover, changing HA by another synthetic or natural polyanion did not provide any more antimicrobial activity (3). The cytocompatibility of the PAR/HA films was assessed with several cell types playing a major role in tissue engineering. This all-in-one system that limits strong inflammation and prevent pathogen's infections on implants constitutes an original strategy easy to scale up.

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a92571

## 03-P352 Monocyte/Macrophage response to calcium phosphate, chitosan and hyaluronic acid – biomimetic substrate

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A promising approach in tissue regeneration is the use of biomaterials possessing bioactive components that control the new tissue formation. While an optimal environment with moderate inflammatory signals presents a key feature in initiating healing process, an exacerbated and/or chronic inflammation may hamper tissue repair. Amalgamation of organic and inorganic materials for use in bone regenerative medicine has drawn lots of attention due to their great potential. Using a new and straightforward method based on simultaneous spray coating of interacting species process, we designed a bioactive organic/inorganic substrate made of chitosan (CHI) and hyaluronic acid (HA)/ calcium phosphate (CaP). The resulting CaP-CHI-HA substrate boosts the early stem cell differentiation into osteoblast-*like* lineage, maintains stem cell paracrine production of osteoprotegerin, and induces paracrine secretion of angiogenic growth factors (VEGF and bFGF). However, understanding the interactions between CaP-CHI-HA and monocytes/macrophages was not investigated so far. Therefore, we studied the influence of CaP-CHI-HA on monocyte behaviour and elucidate the possible underlying mechanisms. Using THP-1 cells, cytocompatibility of CaP-CHI-HA was firstly monitored by WST-1 assay, DNA quantification and intracellular accumulation of reactive oxygen species (ROS). The low metabolic activities and DNA content of THP-1 on CaP-CHI-HA in addition to the absence of an increase of intracellular accumulation of ROS compared to LPS positive control confirmed the biocompatibility of the build-up substrate. Adhered THP-1 on CaP-CHI-HA substrate exhibited a sub-membranous F-actin localization with a prominent and abundant distribution of vinculin throughout the cytoplasm and the membrane. In contrast, on LPS control, F-actin was mostly arranged as spike-*like* protrusions and protruded the cell membrane with vinculin evenly localized at peri-nuclear region. In contact with CaP-CHI-HA substrate, THP-1 increased the secretion of pro-inflammatory cytokines as IL-6 and IL-8 ($p$=0.042 and $p$=0.026, respectively, Mann Whitney test); concomitant with an increase in the secretion of IL-10 anti-inflammatory cytokine ($p$=0.02, Mann Whitney test). In conclusion, the present results showed that the CaP-CHI-HA seems to activate monocytes commitment into an inflammatory profile with an important secretion of IL-10 cytokine; which plays beneficial roles at certain stages of tissues healing and repair.

a91977

## 03-P353 In Vitro Effect of Maitake (*Grifola frondosa*) Particle on Cellular Immune-Enhancing Function

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β-glucan is a macromolecular substance in which the glucose unit, which is a type of biopolymer, has a β-glycosidic bond at position 1.3. β glucan, which is present in mushrooms, was known as a natural immunomodulator without tolerance by enhancing immunity. It has also been reported to have anticancer and antioxidant efficacy. β-glucan slightly differs in the structure and physical properties of glucose. Especially *Grifola frondosa* has a structure of β- (1, 3) and β- (1,6) bonds and is different from structure of other medicinal mushrooms. The content of β -glucan also varies depending on the particle size. However, the content of β-glucan and immunological activity by particle size of *Grifola frondosa* has yet to be fully elucidated. In this study, β-glucan contents were analyzed according to particle size of leaf mushroom. Thereafter, cell activation and immunoactivity were measured. The highest β-glucan content was observed at particle sizes of 20-30 μm (27.65 ± 0.30 w / w). All samples showed at least 104% cell activation compared to control and showed the best cell activity at 20-30 μm particle size (120.6%). Finally, particle sizes of 20-30 μm further increased the expression of the cytokine over control. This study suggested that the optimal particle size of *Grifola frondosa* could be used as a health supplement foods and food additive such as immune boosters, hypotensive agent and hypoglycemic agent.

a91813

## 03-P354 Cardiac Extracellular Matrix Aging Impacts Macrophage Phenotype and Function

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Clinical interventions following myocardial infarction (MI) are limited in efficacy due to the limited proliferative capacity of post-mitotic, adult mammalian cardiomyocytes, which are depleted in large quantities during MI events. However, potent cardiac regeneration in neonates and limited cardiomyocyte proliferation in aged individuals following MI has been observed. This neonatal cardiac regeneration is not holistically dependent on non-terminally differentiated cardiomyocytes but is also dependent on the macrophage populations present in the tissue. Cardiac macrophages are derived from three unique sources and colonize cardiac tissue during distinct periods of development. However, the factors governing the maintenance and recruitment of these macrophages in aged individuals remains unclear. The local microenvironment composed of extracellular matrix (ECM), growth factors, chemokines, and numerous additional soluble factors plays a substantial role in determining macrophage development and phenotype. Thus, this study sought to examine the potential role cardiac ECM aging plays in altering macrophage phenotype and functionality. A decellularization protocol was optimized to isolate murine cardiac ECM. Biochemical and DNA quantification assays were performed to confirm decellularization. Bone marrow derived macrophages isolated in culture were exposed to either young or aged cardiac ECM degradation products. Following 24 hour ECM exposure, macrophage nitric oxide production and gene expression levels were assessed at baseline and following exposure to canonical M1 or M2 polarizing cytokines. Macrophages exposed to aged cardiac ECM degradation products exhibited increased nitric oxide production compared to young ECM-exposed groups, a functional response exhibited in pro-inflammatory macrophages. Additionally, qRT-PCR analysis revealed several alterations in gene expression following ECM exposure with subsequent cytokine polarization. These results indicate that macrophages exhibit differential responses to young and aged cardiac ECM degradation products *in vitro.* Acknowledgements**:** NIH T32 EB001026

a92851

## 03-P355 Immune modulating scaffolds for bone tissue engineering

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**Introduction:** The immune response to scaffold implantation plays a central role in tissue regeneration. Micro and nano sized hydroxyapatite particles are widely used for bone tissue engineering applications due their biocompatibility and inherent osteo-inductivity. Furthermore, it is well known that the immune response to these particles strongly depends on size and morphology. The first objective of this study was to elucidate the response of primary human macrophages to micro and nano sized HA particles. Furthermore, we sought to determine whether macrophages exposed to HA particles can influence MSC differentiation along the osteogenic lineage *in vitro*. Finally, we sought to characterise the *in vivo* immune response to scaffolds functionalised with different HA particles following implantation into critically sized rat femoral defects.

**Results:** We demonstrate that micron sized HA particles preferentially drive M1 macrophage responses, while nanoHA particles polarize macrophages towards an anti-inflammatory M2 phenotype. Conditioned media (CM) from nanoHA treated macrophages significantly enhanced expression of the osteogenic genes, BMP2 and ALP, in MSCs, while enhanced mineralisation was observed in the nanoHA CM group compared to control. Moreover, the enhanced osteogenic gene expression/mineralisation in MSCs was found to be dependent on IL-10 production by macrophages.

**Discussion:** This work highlights how the innate immune response can modulate stem cell regenerative capacity and emphasises the importance of integrating control of the host immune microenvironment into regenerative strategies. Such studies may allow for optimisation of biomaterials on the bench prior to clinical trials and furthermore, may act as a predictor of success for implanted devices. This in turn may reduce the incidences of devices failing clinical trials and therefore, reduce the economic burden of bringing new devices to market.

a90577

## 03-P356 Immunological Compatibility of Human Recombinant Decorin- a Prerequisite for Novel Scaffold Design

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The small, leucine-rich proteoglycan decorin is a promising structural component for the bio-functionalization of tissue-engineered scaffolds. Recently the enhanced attraction of endothelial progenitor cells was demonstrated [1]. To assess immunological compatibility of human decorin in its full recombinant form, we developed *in vitro* test systems to characterize the interaction of human recombinant decorin with different human immune cell subsets to predict compatibility after implantation.

First, the chemotactic effect of decorin on monocytes and polymorph nuclear cells was analyzed. In addition, the influence of decorin (50 µg/mL) on the surface marker expression level of activation/differentiation markers (CD16) as well as M1-type (CD80, HLA-DR) and M2-type (CD163, CD206) polarization markers on co-cultured monocytes and macrophages was measured by flow cytometry. The potential of decorin to modulate low-dose anti-CD3-induced T cell proliferation was analyzed by a carboxyfluorescein succinimidyl ester (CFSE)-based assay. In both setups, cytokine secretion was quantified by an enzyme immune assay for IL-10, TNF$\alpha$, IFN$\gamma$ and IL-6.

Human recombinant decorin dose-dependently triggered migration of monocytes and polymorph nuclear cells in chemotaxis assays, with a maximum at 10 µg/mL. Monocytes did not up-regulate CD16 in co-cultures with decorin and rather increased expression of CD206, a known M2-type macrophage marker. When co-cultured with macrophages, the expression level of CD80, but not of HLA-DR was increased. Moreover, secretion of TNF$\alpha$, IL-6 and IL-10 was induced that was not inhibited by Polymyxin B. In five-days, co-cultures with human peripheral blood mononuclear cells (PBMC) revealed, that decorin significantly inhibited the proliferation of CD8+ T cells when compared with the anti-CD3-triggered control, and reduced the level of IL-10.

In conclusion, we developed reliable test systems to analyze the crosstalk of decorin with human immune cells. Decorin can attract monocytes as well as polymorph nuclear cells, and influences the macrophage polarization status, which is important for remodeling processes of the extracellular matrix. Therefore, decorin could a promising protein for the generation of off-the-shelf biomaterials.

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**Acknowledgments:**

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a92850

## 03-P357 Immunomodulation and osteogenic response on mineralizedcollagen during bone tissue regeneration

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**Abstract:** The immune response to implanted materials remains a critical challenge for the development of biomaterials used in medical devices and regenerative medicine[1,2]. Understanding this response and designing better biomaterials requires a multidisciplinary approach involving materials engineering and immunology[3]. Through transformation of the microstructure of bone repair materials, observation effects on macrophage polarization direction and function, exploration bone restoration, response and significance of microstructure on the body's immune isone of a new direction for bone repair material technology. The goal of our work is based on the forefront of bone repair material research and aims at the clinical problem. We designed and fabricated mineralized collagen with different nanostructures and investigated the influence of mineralized collagen withdifferent microstructure on osteoblast in microenvironment of bone defect site.Further, the relationship between the cells treated by mineralized collagen with different nanostructures and M2 polarization direction will be disclosed. Under the conditions of mineralized collagen with different nanostructures regulating the balance of M1 to M2 will be sought, through exploring the effects of M1/M2 balance and bone repair. Ultimately, a way to impair pathological inflammation though regulating macrophage function by composition composited will be explored,providing new thought for research, development, evaluation and application of newbone repair materials.

**Keywords:** Immune response, mineralized collagen, M1/M2 polarization

**Reference:**

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a91783

## 03-P358 Engineered Antigen Specific *In Vitro* System for Characterizing Immunological Responses to Encapsulated Cells

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Alginate encapsulation has been extensively studied to protect transplanted cells from host immune recognition and destruction. Despite widespread use, the mechanisms by which alginate encapsulation affects host immune responses to transplanted cells remain unknown. The biomaterial field has long suspected that alginate encapsulation blocks contact-dependent direct antigen recognition; however, it is unknown if indirect recognition, where donor antigen shed from the capsule is presented and recognized by the host, occurs in these systems.

To explore these mechanisms, we generated an in vitro platform capable of delineating both direct and indirect recognition using ovalbumin (OVA) as a model antigen. Cells isolated from membrane-bound OVA (mOVA) mice were used as stimulators and co-cultured with purified immune populations of antigen-specific responders (OTI). Proliferation and activation of the clonally-specific OTI CD8+ T cells were quantified via flow cytometry, while encapsulated cells viability was assessed by Live/dead and Caspase 3/7 imaging. To mimic direct recognition, unencapsulated or encapsulated mOVA cells were co-cultured with purified OTI CD8+ T cells for 3 days. As expected, this purified CD8+ T cell population was highly activated by free mOVA cells (> 90% viable, granzyme B+, CD8+ T cells). Alternatively, CD8+ T cells were nonresponsive to encapsulated mOVA cells; statistically equivalent to negative controls. To examine if immune cells can respond to encapsulated cells via the indirect pathway, this purified CD8+ T cell population was supplemented with cross-presenting dendritic cells (CD8+DCs; professional antigen presenter for CD8 T cells), which provides a means for indirect activation of CD8+ T cells. This co-culture system resulted in robust activation for both unencapsulated and encapsulated mOVA cells; illustrating that indirect antigen presentation is not only feasible but can result in aggressive conversion of naïve T cells to an effector T cell profile.

This work provides a means to examine, in detail, the impact of alginate encapsulation on host immune responses, revealing the possible failure mechanisms of alginate encapsulated cell transplant. Also, it establishes an *in vitro* platform to efficiently screen the immune impact of various encapsulation approaches and/or immunomodulatory agents.

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a93334

## 03-P359 Promotion of Regulatory Tcell Induction via Engineered Bio-active Synthetic Polymers for Islet Allograft Tolerance

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Cellular-based therapies for the treatment of Type 1 Diabetes (T1D), have the potential to restore endogenous glycemic control for patients. Yet, the activation of pathogenic T effector (Teff) cells and the subsequent stimulation of allo and auto responses has hindered its applicability. Immunotherapies are an attractive approach in regulating local immune responses in clinical transplantation. At the forefront of immunotherapies are T regulatory cells (TRegs), a specialized subset of T cells in charge of peripheral self-tolerance and the regulation of pathogenic immune responses. We have engineered surface functionalized synthetic materials, for the dual delivery of two potent bio-active signals for the local regulation of the immune response: PD-L1, an immune checkpoint pathway involved in TReg balance, and VEGF a potent angiogenic factor with possible implications in TReg regulation. Herein, we sought to evaluate the effect of these engineered materials on TReg induction *in vitro* as well as graft acceptance in an animal model of diabetes. *In vitro*, co-delivery of SA-PD-L1 with VEGF lead to enhanced conversion of naïve Teff into Treg cells, in the absence of stimulatory TGF-beta. Furthermore, *in vivo* assessment of syngeneic pancreatic islets co-transplanted with functionalized SA-PDL-1 microgels, led to diabetes reversal in all transplanted animals (n=5); glucose tolerance tests, performed 4 weeks post-transplantation, demonstrated glucose clearance rates comparable to naïve animals of the same age, indicating no adverse effects of microgels to engraftment. Overall, these results demonstrate the potential of this technology to heighten the performance of islet allografts in the absence of chronic systemic immunosuppression.

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**Disclosures**: D.M.H. and A.J.G have a patent on microparticle fabrication technology.

a91772

## 03-P360 Anti-inflammatory property of Curcumin-Albumin conjugate established using primary cell cultures

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Chronic inflammatory disease conditions identified in humans are atherosclerosis and osteoarthritis. In the former, inflammatory changes and dysfunction of endothelial cells (EC) and in the latter inflammation of chondrocytes trigger the disease. Currently available anti-inflammatory drug is hardly effective for reversing the diseases. Therefore, attention is required to develop novel therapies for these diseases. The anti-inflammatory property of purified curcumin has been described in literature. However, it has not become therapeutic molecule because of its poor aqueous solubility. Efforts to conjugate curcumin with albumin (Curc-Alb) resulted in enhanced aqueous solubility and bioavailability (C Thomas et al, 2014). However, anti-inflammatory property of the conjugate has not been explored. Current study aims to determine the optimum & safe concentration that maintain viable cells showing anti-inflammatory effect of Curc-Alb. Human umbilical cord vein endothelial cells (HUVEC) and human adipose derived mesenchymal stem cells (h ADMSCs) differentiated to chondrocytes were used to study reversal of induced inflammation. Both MTT and proliferating cell nuclear antigen assays estimated safe dose without causing cell death. Fluorochrome-tagged Curc-Alb internalization was established by qualitative/quantitative assays indicating possible intra-cellular pathways to control inflammation. Inflammation induced in both cell types using Interleukin-6 (IL-6), Tumor necrosis factor (TNF-$\alpha$), Interleukin-1$\beta$ (IL-1$\beta$). The inflammatory marker expression was estimated with and without Curc-Alb by qRT-PCR and dose response was established. The results indicate that anti-inflammatory property of Curc-Alb could be considered for therapy at safe dose determined. Therefore, regeneration of experimentally damaged cartilage using Curc-Alb has been designed with IAEC approval and is in progress.

a92927

## 03-P361 Nitrate supplementation ameliorates DSS-induced colitis

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Inflammatory bowel diseases (IBD) are typified by chronic inflammation, loss of epithelial integrity and gastrointestinal microbiota dysbiosis. In order to treat these disorders, we used inorganic nitrate, a potent NO donor and microbiota regulator, in a murine model of Dextran sodium sulfate (DSS) induced colitis. $NaNO_3$ was pretreated in drinking water for five days and NaCl was used as control. Results showed that oral administration with dietary nitrate evidently maintained the consistency of colon, improved the reduced colon length, kept the body weight, decreased the apoptosis rate of colon epithelial cells as well as ameliorated the inflammatory infiltration. Faeces were collected for microbiota analyses. Microbiota profiling revealed that microbiome compositions in nitrate treatment group are more stable. The present study shed a light on the preventive effects of nitrate on the DSS-induced colitis, which may through the mechanism of homeostasis of intestinal flora.

a91117

## 03-P362 An Experimental Pathway for Assessing the Immunomodulatory Property of Biomaterials Using Lipopolysaccharide

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The design paradigm of biomaterials has been changed to ones with favourable immunomodulatory effects, indicating the importance of accurately evaluating the immunomodulatory properties of biomaterials. Among all the immune cells, macrophages receive most attention, due to their plasticity and multiple roles in the materials and host interactions; thereby become model immune cells for the evaluation of immunomodulatory properties of biomaterials in many studies. Lipopolysaccharides (LPS), a polysaccharide in the outer membrane of Gram-negative bacteria, and elicit strong immune responses, which was often applied to activate macrophages, resulting in a pro-inflammatory M1 phenotype, and the release of pro-inflammatory cytokines, including tumour necrosis factor alpha (TNF$\alpha$), interleukin (IL)-1, and IL-6. However, there is no consensus on how to apply macrophages and LPS to detect the immunomodulatory properties of biomaterials. The lack of scientific consideration of this issue has led to some inaccurate and insufficient conclusions on the immunomodulatory properties of biomaterials, and inconsistences between different research groups. In this study, we carried out a systemic study to investigate the stimulatory effects of LPS with different times, doses, and conditions on the activation of macrophages. An experimental pathway was proposed accordingly for the activation of macrophages using LPS for assessing the immunomodulatory property of biomaterials.

a94026

## 03-P363 Magnetic modulation of immunological microenvironment inside scaffolds to enhance bone tissue regeneration

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Cells living in physiological conditions experience certain mechanical stimulations depending on their development stages. In the research of tissue engineering and regenerative medicine, mechanical forces are applied by exposing cells seeding in the scaffold to shearing fluids or applying strains on the scaffold in bioreactors. In these cases the scaffolds themselves are not able to provide direct mechanical stimulations to the cells. Here we reported a strategy of building "dynamic" scaffolds by integrating super-paramagnetic scaffolds (mag-S) and applied static magnetic fields (MF) to generate mechanical forces directly on various cells, which effectively modulated immune cells including macrophages and fibroblasts as well as mensenchymal stem cells. The mag-S was fabricated by electrospinning with the solution of composite composed of polylactic acid, hydroxyapatite nanoparticles and iron oxide nanoparticles.

We showed that macrophages, fibroblasts or mensenchymal stem cells could sense the mechanical stimulation generated by the integration of mag-S and MF (mag-S+MF). Under these conditions, the macrophages or fibroblasts were likely to exhibit the wound healing phenotype while the inflammatory phenotype was suppressed, and mag-S+MF could induce the differentiation of the stem cells even in the absence of inducer. It was demonstrated that the platform of mag-S+MF modulated the phenotype polarization of the macrophages or fibroblasts through activating signaling proteins of VEGFR2/HIF-1$\alpha$ or integrins/FAK/ERK respectively, meanwhile inhibiting the activation of TLR4/NF-$\kappa$B. In conclusion, the platform promoted the wound healing phenotype of the immune cells, which benefited the strategy of scaffold-guided tissue regeneration.

a90176

## 03-P364 *In Vitro and In Vivo* Evaluation of Dual Growth Factor-Loaded Alginate/Hyaluronic Acid Hydrogel for the Treatment of Liver Cirrhosis

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Liver cirrhosis is defined as irreversible end result of fibrous scarring and involves loss of liver cells. As cirrhosis progresses, more scar tissue forms, making it difficult for the liver to function. When cirrhosis is going on, it causes various complications and when continues, cause cancer. There are many causes of cirrhosis including viruses, alcohol, and immunology factors. Recent treatment of cirrhosis includes the injection of intravenous antibiotics, and liver transplantation is an option of last resort. In this study, we prepared growth factor [hepatocyte growth factor (HGF) and/or basic fibroblast growth factor (bFGF)]-loaded *in situ* gelling alginate (ALG)/hyaluronic acid (HA) hydrogels with a controllable gelation rate using $CaSO_4$ as a crosslinking agent and $Na_2HPO_4$ as a crosslinking retardation agent. HGF is recognized enhancing hepatocytic differentiation and relaxing fibrotic tissue, and bFGF is promoting proliferation and growth of cells. The ALG/HA hydrogels provided sustained release of HFG and bFGF immobilized in the hydrogels over 4 weeks. The dual growth factors-loaded ALG/HA hydrogels with different ALG/HA ratios were investigated for their *in vitro* hepatocytic differentiation of adipose stem cells (ASCs) and *in vivo* animal study using an acute cirrhosis BALB/c mice model (direct injection of the hydrogel in the liver cirrhosis site). The growth factors were continuously released from the hydrogels for more than 35 days. The dual growth factor-loaded ALG/HA hydrogel showed the enhanced hepatocytic differentiation of ASCs compared to the hydrogels with single growth factor loading or those without growth factor (by the analyses of DNA contents, RT-PCT, western blot, and immunostaining). For single growth factors, HGF-loaded hydrogel showed better hepatocytic differentiation than the bFGF-loaded hydrogel. The dual HGF/bFGF-immobilized ALG/HA hydrogel allowed greatly enhanced treatment effect for liver cirrhosis (by the evaluations of gross view, H&E staining, Sirius red staining, and immunostaining). From the results, dual HGF/bFGF-loaded ALG/HA hydrogel may be a good system for the treatment of liver cirrhosis.

a92300

## 03-P365 Construction of a fiber-type hepatic tissue by bottom-up method using multilayer spheroids of hepatocytes, endothelial cells and mesenchymal cells

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### Introduction

Liver regenerative medicine using cultured hepatocytes or hepatic tissues has the potential to replace liver transplantation. However, this therapeutic strategy has challenges to overcome, including in construction of the hepatic tissues. As an approach to fabricating functional 3D hepatic tissues, we focused on hepatocyte spheroids, which have a high cell density and maintain high liver-specific functions. We employed a bottom-up method using spheroids, arranging hepatocytes, endothelial cells and mesenchymal cells regularly at the time of tissue construction. This approach enabled a vascular network to be formed within the three-dimensional hepatic tissue.

### Materials and Methods

We fabricated hepatocyte spheroids covered with human umbilical vein endothelial cells (HUVECs) and NIH/3T3 cells. The spheroids were seeded into hollow fibers, and then the spheroids were packed at high density by centrifugation to induce the formation of a cylindrical hepatic tissue in the lumen of hollow fibers. We then performed histological and functional analyses of the hepatic tissues. We also performed animal experiments using liver failure mouse. SCID mice were subjected to partial hepatectomy (85%). Subsequently, a cylindrical hepatic tissue collected from hollow fibers were transplanted onto the surface of the remaining liver. We evaluated the survival time of the mouse after transplantation. We also performed histological analysis of the transplanted tissue.

### Results and Discussion

The stacking spheroids formed cylindrical hepatic tissue inside hollow fibers. The tissue was able to collect from the hollow fibers and was sufficiently strong for transplantation. The hepatic tissues showed high liver-specific functions of ammonia removal and albumin secretion. In the histological analysis, we found that the HUVECs were regularly distributed within the tissue. The NIH/3T3 cells proliferated and were distributed uniformly throughout the tissue. Hypoxia-inducible factor-1α (HIF-1α) expression was suppressed in the hepatic tissue throughout the culture period. In the animal experiments, we found that the survival time of mice with liver failure could be prolonged by transplantation. We also confirmed the existence of erythrocytes in the transplanted tissue, indicating functional vessel formation. In summary, we fabricated a functional 3D hepatic tissue by the bottom-up method using hepatocyte spheroids.

a91987

## 03-P366 Time-dependent liver-specific gene expressions of vascularized subcutaneous human liver tissue

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Subcutaneous transplantation of engineered hepatocyte/fibroblast sheets (EHFSs) is a low invasive and safe approach to construct vascularized subcutaneous human liver tissue (VSLT) (Sakai Y, *et al*., *Biomaterials*, 2015). However, the liver-specific structures and functionalities in the development process of VSLTs in mice remains poorly understood. In the current study, describe time-dependent characteristics of the formation of the vascular network, cell–cell adhesions molecules, liver transporters at the apical and basal membranes, liver-specific protein synthesis, and metabolizing activities.

The EHFSs formed multi-layered thick tissues by rapid neovascularization allows overcoming extremely difficult problems, such as the lack of oxygen supply on the formation of three-dimensional primary hepatocyte tissue under the skin. The blood vessels consisted of mouse-origin endothelial cells (ECs) (mVEGFR2) from the subcutaneous space at 1–7 days and the following formation of the vascular network was performed by human-origin ECs (hVEGFR2). Many varieties of liver-specific gene expressions increased with the construction of the VSLTs: cell–cell adhesion molecules (CDH1, CLDN3, and CX32), transporters at basal (OATP1A1, OCT1, and NTCP) and apical membranes (MRP2, MDR1, and BSEP), blood coagulation factors (F8 and F9), and urea synthesis (OPS1, OTC, and ARG1) and metabolism enzymes (CYP7A1, CYP1A2, CYP2B6, CYP3A4, and UGT1A1).

We demonstrate that the EHFSs using engineered hepatocyte/fibroblast system facilitated the construction of functional VSLTs by overcoming various extremely challenging problems, such as the lack of oxygen supply on formation of 3D primary hepatocyte tissue under the skin. The VSLTs could be a second liver organ because of the construction of abundant vascular networks, cell polarity, and maintenance of high expressions of liver-specific functions. Therefore, the EHFSs could be established as potential treatments of liver diseases.

a91100

## 03-P367 Development of miniature human liver with mouse decellularized liver

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The extracorporeal reconstruction of human liver is needed to replace an animal experiment in drug screening field and to elucidate the mechanism of some disease such as cancer metastasis. However, human liver weighs more than 1 kg and contains a large amount of hepatocytes. The large amount of cells in need, the steps of organ creation and the culture of whole liver make it difficult to reconstruct human liver on the original scale. Therefore, our aim of this study is to reconstruct human liver *in vitro* in small scale based on the concept of whole organ engineering.

We tried to construct miniature human liver by using decellularized mouse liver. It is estimated that the required cell number per a liver construction can be reduced to about from$10^6$ to $10^7$ cells by this scale down. As a cell source, we tried to isolate primary human hepatocytes from surgically resected human liver tissue. Primary human hepatocytes were isolated at more than 70% viability by using collagenase solution. Additionally, we developed the decellularization method of mouse right lobe based on that method of rat as a scaffold of miniature human liver. The mouse liver was removed blood by perfusion of the buffer *in vivo.* Then, the only right lobe was obtained and decellularized by the perfusion of the 4% Triton X-100 solution via portal vein and the DNase/RNase treatment. Then, the decellularized mouse right lobe was obtained. The tubular network structure was observed by injection of resin via portal vein, and it revealed that the decelllularized liver maintained the structure of the vascular network. Furthermore, the histological analysis of the tissue was performed and it was confirmed that cell components were removed from the tissue. Therefore, we tried to seed the liver cells into the obtained tissue and culture.

In conclusion, we achieved the development of the preparation method of decellularized mouse right lobe. Then, it was suggested that combining the obtained human primary hepatocytes with mouse decellularized liver is expected to create a miniature human liver.

a90789

## 03-P368 Biofabrication of Micro Blood Vessel Network from Hydrogel Beads

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Micro blood vessel network is one of essential issues in biofabrication of bulk tissues, artificial organs, organ-on-chips, etc. It is extremely difficult to build micro blood vessel network by conventional tissue engineering methods like induced differentiation from stem cells. Even with biofabrication technique, which also known as 3D bio-printing, due to the limitation of printing precision, blood vessel network below 50 $\mu$m are still hard to achieve. Herein, we will report a facial method to build micro blood vessel network from bio 3D printed hydrogel beads. The hyaluronic acid based biopolymer hydrogel (HA) was first printed into tiny drops with controlled diameter between 10 – 50 $\mu$m through a 3D bio-printer and cross-linked by enzyme reaction. Formed beads were later mixed with hepatic cells and endothelial cells and inoculated into a PDMS-based micro-well for co-culture. These HA beads were functioned as spacers among the randomly packed cells during aggregation. After 14 days of co-culture, HA beads were digested by hyaluronidase and left micro-channel with tens of micrometers in diameter, which could be utilized as micro-blood vessel network. Finally, the mass transfer efficiency for hepatic cells was enhanced as-expected.

a91665

## 03-P369 Effect of temperature and oxygen supply to liver function expression in hepatocyte culture toward to organ culture

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Liver expresses the functions in vivo and it is kept at 37° C. Therefore, it was thought that liver should express the functions under the same condition as in vivo and the liver culture system has been tried to be developed based on the concept. It is called normothermic liver preservation and has been studied since 1930s. However, the liver culture systems which can keep the liver functions completely for a few days in vitro have never been developed. Presently, the liver for transplantation is preserved by replacing from blood to ice-cold organ transplantation solution and it is transported to recipient in ice storage based on the method which was proposed by Collins in 1969. This method is widely used in organ transportation for organ transplantation and the ischemia time from resect to reperfusion is limited depending on each organs. The ischemia time of liver is limited within 12 hours. If it was prolonged, the patient survival rate is significantly decreased. Therefore, the transplantable place is limited. On the other hand, there are some reports that the liver preservation will be achieved by perfusion at around 20° C with culture medium containing red blood cells. In this study, our aim to reveal the effect of temperature and oxygen supply to the expression of liver functions. The gel culture of hepatocyte was used in our study because hepatocyte can express the specific functions there as high as spheroid culture which is called miniature liver. The primary rat hepatocytes were cultured in collagen gel at some temperatures and the ammonia metabolic as a liver specific function was analyzed. In this time, the construction of a culture condition with enough oxygen supply was also tried by using diluted blood with culture medium. As the results, the expression of liver function was different depending on the temperature. In addition, it was suggested that the function expression will increase by increasing the oxygen supply. The obtained results are expected to be useful for the improvement of liver function expression in the treatment of liver support system with hepatocytes and the development of liver construction system based on whole organ tissue.

a91080

## 03-P370 Generation of Functional Human Liver Tissues in Vitro via Contraction of Hepatic Cell Sheets

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Primary human hepatocytes (PHHs) cultured as suspension and monolayer are considered as the gold standard to study drug transport and metabolism. However, PHHs only maintain the liver-specific functions for a few hours in suspension and a few days in monolayer. There are many methods that have been developed for culture PHHs in vitro ranging from 2D collagen coated dish, collagen gel sandwich culture to 3D spheroids. However, how to maintain active hepatocyte functions in vitro in long term is still a big challenge.

Here we demonstrate a simple method to generate functional stratified human liver tissue in vitro. PHHs were co-cultured with normal human dermal fibroblasts (NHDFs) on the temperature responsive cell culture plates. After 4 days culture, PHHs/NHDFs mono-layer co-cultures were harvested as floating cell sheets by reducing the temperature to 20℃ for 30 min. The floating cell sheets were condensed to form multilayered assemblies due to contraction of cells, in particular, fibroblasts. The contracted cell sheets were re-attached and cultured on other dishes for 10 days. It was found that the albumin secretion of these contracted cell sheets were two times higher than that of normal co-cultures. Densely distribution of hepatocytes and fibroblasts in the contracted cell sheets allows the enhanced homotypic and heterotypic cell-cell interactions, which may in turn result in increase of hepatocyte functions.

We also found the seeding density and co-culture ratio could affect morphology and functions of hepatocytes in the contracted cell sheets. We are now trying to optimize the conditions to fabricate contracted hepatic cell sheet-based models for drug screening.

a90625

## 03-P371 HGF/Heparin-immobilized Decellularized Liver Matrix as Substrates for Hepatocytes Regeneration in Acute Liver Injury model

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In order to overcome the shortage of organ donation and injury of patients after partial hepatectomy (PH) treatment, we aimed at developing a liver film for hepatocytes regeneration through liver tissue engineering. In this study, a HGF/heparin-immobilized decellularized liver matrix (DLM) film (HGF/heparin complex coated on DLM film) was developed for hepatocytes regeneration in liver injury. In the result, (1) the amounts of immobilized heparin on DLM film was 30$\mu$g/cm$^2$ when the initial heparin concentrations were 1 mg/ml. (2) The relative cell viability and albumin synthesis of the hepatocytes on HGF/heparin-immobilized DLM film was 20-30% and 20% superior than on normal dish at 3 days of culture, respectively. (3) The lactate dehydrogenase activity of the D-galactosamine-induced injury of hepatocytes on HGF/heparin-immobilized DLM film cultures was 10 milliunits/mL, which was 50% lower than that in the D-galactosamine-induced injury of hepatocytes on normal dish cultures, and albumin synthesis can recover to the same level as non-toxic hepatocytes cultured on normal dish. The HGF/heparin-immobilized DLM film showed highly potential in maintaining hepatocyte culture and also in repairing injured hepatocytes from D-galactosamine. It is believed that this HGF/heparin-immobilized DLM film has promising potential for hepatocyte transplantation, and could be applied for future use in liver tissue engineering use.

a91165

## 03-P372 Mesenchymal stem cells and induced bone marrow macrophage combination therapy for liver cirrhosis mouse model

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Division of Gastroenterology and Hepatology, Niigata University

**Background & Aims:** Decompensated liver cirrhosis often progresses even after treatment of causes such as hepatitis C viruses. Thus, novel therapeutic approaches are urgently needed. We describe a novel therapeutic approach for decompensated cirrhosis using mesenchymal stem cells (MSCs) and induced bone marrow-derived macrophages (id-BMMs), and analyze the mechanisms underlying liver fibrosis improvement and regeneration. **Methods:** Mouse MSCs and id-BMMs were cultured from mouse bone marrow. The mRNA expression after addition of serum from liver-damaged mice was analyzed by microarray and real-time PCR. Co-culture and phagocytosis assays were performed. MSCs, id-BMMs, and a combination therapy using MSCs and id-BMMs (50/50) were administered to mice with CCl4-induced liver cirrhosis, and fibrosis regression and liver regeneration were evaluated. The behavior of administered cells was evaluated by intravital imaging. Host migrated cells in the liver were quantified by immunohistochemistry and flow cytometry. **Results:** After addition of serum from liver-damaged mice, id-BMMs expressed higher amounts of anti-fibrotic MMPs, pro-regenerative factors, and chemoattractant, while MSCs upregulated the anti-inflammatory factors and macrophage modulating factors. In co-culture, MSCs induced switching of id-BMMs toward the M2 phenotype. M2-polarized id-BMMs gained high phagocytic ability. The combination therapy effectively increased liver fibrosis regression (27.3% regression compared to control, $p < 0.01$) and PCNA-positive cells, and decreased serum levels of hepatobiliary enzymes. Intravital imaging showed that a large number of id-BMMs, which phagocytosed hepatocyte debris, and few MSCs migrated to the fibrotic area in the liver. Host macrophages and neutrophils gathered after combination therapy and produced anti-fibrotic MMPs, followed by production of pro-regenerative factors from phagocytosing id-BMMs. **Conclusions:** Indirect effector MSCs and direct effector id-BMMs synergistically improved liver cirrhosis with recruiting the host cells to the liver in mice. This concept paves the way for new treatments for decompensated liver cirrhosis.

a90558

## 03-P373 Development of a novel targeted drug delivery system for liver tissue regeneration

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Mesoporous silica nanoparticles (MSNs) are solid materials possessing a honeycomb-like porous structure and hundreds of empty channels, it has been developed recently and regarded as an efficient drug carrier in the targeted drug delivery system (TDDS). However, most of the TDDS are designed to kill the tumor cells, and there are fewer related-reports about the cell-targeted strategy for enhancing the functionality of targeted cells. In this study, we applied MSNs to carry lysine for hepatocyte culture since lysine has been proved to enhance the mitosis of cell. Moreover, MSNs were encapsulated with chitosan to enhance the biocompatibility, and glycyrrhizin (GL) were conjugated to MSNs for hepatocyte targeting. Results have shown that: (1) MSNs showed no cytotoxicity to the hepatocytes in low concentration (<0.1mg/mL). (2) The albumin concentration in hepatocyte cultures with 0.1 mg/mL of lysine was increased 30% higher than that in control condition. (3) The maximum loading capacity of lysine on MSNs, chitosan-MSNs, and GL-chitosan-MSNs are 80%, 70% and 70%, separately. (4) MSNs particles showed a negative zeta potential of -31.8mV compared to the chitosan-MSNs and GL-chitosan-MSNs, which showed highly positive zeta potentials of 48.9mV and 34.4mV, respectively. The average particle size of GL-chitosan-MSNs, chitosan-MSNs were found to be relatively larger than MSNs (246nm, 222nm and 148nm, separately). (5) GL-chitosan-MSNs-lysine was found largely in hepatocyte than that in other cells. (6) GL-chitosan-MSNs-lysine enhanced hepatocyte functionality in 7 days of cultures. In conclusion, GL-chitosan-MSNs-lysine has a high potential in hepatocyte-target study.

a91703

## 03-P374 Construction of engineered human liver organoid by stem cell incorporating with the cadherin modified biomaterials

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Mesenchymal stem cells (MSCs) have emerged as a promising source of multipotent cells for various cell-based therapies due to their unique properties and 3D MSC aggregates has been explored as a potential strategy to enhance therapeutic efficacy. In this study, 3D MSC aggregates incorporated with human E-cadherin-Fc modified poly PLGA(hE-cad-PLGA) microparticles have been fabricated. The results show that, compared with the plain PLGA, the hE-cad-PLGA microparticles distribute within the aggregates more evenly and further result in a more significant improvement of cellular proliferation and secretion of a series of bioactive factors due to the synergistic effects from the bioactive E-cadherin fragments and the PLGA microparticles. Meanwhile, hE-cadherin-Fc can upregulate the phosphorylation of EGF receptors and activate the AKT and ERK1/2 signaling pathways inner the aggregates. Additionally, hepatic differentiation of aggregates were enhanced by the synergistic effects of E-cadherin with cytokines. Furthermore, liver organoid were constructed by the MSCs aggregates incorporating with E/VE-cadherin modified PLGA microparticles. The cooperation of E/VE-cadheirn as matrixes in the cell aggregates markedly enhanced vascularization and hepatic functions *in vitro*. Therefore, engineering 3D cell aggregates with hE/VE-cad-PLGA microparticles can be a promising method for ex-vivo multipotent stem-cell expansion, differentiation and the construction of organoid for clinical applications.

a90620

## 03-P375 Liver-on-chip: primary rat small hepatocytes in a microfluidic platform

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Department of Bio-Industrial Mechatronics Engineering

The liver is an organ with vital functions, including energy storage, secretion protein synthesis, and especially metabolism of pharmaceutical drugs. However, *in vitro* studies of drug test are usually limited to precisely evaluate the real influences on hepatic tissue because it is an obstacle to develop a platform which can sophisticatedly mimic *in vivo* hepatic environment.

Thus, in this study we established a microenvironment-mimicking liver-on-a-chip (LOC) platform for *in vitro* hepatotoxicity test. Small hepatocytes, which have been identified in primary hepatocyte cultures with high potential for proliferation and differentiation into mature hepatocytes, was used as cell source for LOC platform. The result showed that small hepatocyte on collagen coating dish can expand in 2D primary cultures, and formed colonies which is about 300-400$\mu$m wide. Compared to primary hepatocytes, which normally maintain their function about 7 days, small hepatocytes can live at least 4 weeks. We analyzed the gene expression of small hepatocytes by q-PCR. Expression of albumin and Tryptophan 2,3-dioxygenase (marker of primary hepatocytes) is higher, and Follistatin (marker of small hepatocytes) expression is lower after 3 weeks' culture.

The small hepatocyte-derived liver-on-a-chip platform therefore can simulate the real environment in model animals, and also build toxicology database and make safety assessment of drugs, chemicals and pesticides.

**Keyword : Live-on-a-chip, Microfluidic, Small hepatocyte**

a91620

## 03-P376 Cryogel-integrated hepatic cell culture microchips for liver tissue engineering

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Recently, a number of studies have highlighted that the cell microenvironment plays a critical role in various biological processes. In this context, 3D cell culture systems have been the focus of much attention to mimic more closely the natural environment found in vivo. To this end, we have developed an innovative technology **combining biomaterials and microfluidics**. A polydimethylsiloxane (PDMS) **microchip containing a macroporous alginate hydrogel** was designed for hepatocyte culture such as HepG2C3a and HepaRG cells.

A microfluidic device was successfully engineered to ensure a homogeneous perfusion while providing a micro-sized cell culture chamber in 3D. Alginate was **covalently crosslinked** at subzero temperatures inside the microchip device to create a macroporous scaffold for cells. Integrated cryogels can be **functionalized with peptides or proteins** to promote cell-matrix interactions.

The physical properties of the integrated cryogels were characterized. The cryogels showed an **open and interconnected macroporous structure** with remarkable elastic properties sustaining flow induced shear stress and pressure drop. Additionally, cryogels have **soft mechanical properties**, similar to healthy liver tissues.

The dynamic 3D cell culture showed that **cells spread** when the alginate is functionalized, they form **aggregates** in the other case. Thus, two ways of culture can be done depending on the application.

Looking into the future, this innovative platform may facilitate cell-based validation in the **drug discovery process**, **decreasing the cost** and **increasing the speed in screening** large numbers of compounds.

a92377

## 03-P377 Electric-field assisted microfluidic synthesis of tailorable porous microbeads as cell carriers for tissue engineering applications

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A promising approach in tissue regeneration is the injection of miniaturised scaffolds directly to the site of treatment which allows to avoid more invasive procedures associated with introduction of macroscopic implants. In this article, we present a novel and highly efficient method for the manufacture of porous microbeads of tailorable dimensions (in the range ~ 300-1500 um) and with a uniform and fully interconnected internal porous texture. The fabrication method proceeds through a preliminary generation of a monodisperse oil-in-water emulsion by using a flow-focusing microfluidic device. The emulsion, after being discretised into droplets through the application of a pulsed electric field, serves as a template for the porous microbeads. By tuning the rates of flow as well as the strength and frequency of the electric field, microbeads of controlled dimensions are produced with high reproducibility. We model the process of the drop formation and predict drop sizes as a function of the applied voltage with good agreement with the experimental data. In addition, we develop a 'phase diagram' indicating various operational regimes of the device depending on both the voltage and the frequency of electric pulses. Finally, we demonstrate the potential of the porous microbeads as cell carries: we seed them with human mesenchymal stem cells (hMSCs) and hepatic cell line (hepaRG) monitoring their viability, degree of microbeads colonization, and retention of stemness character up to three weeks. Finally, we benchmark the presented porous microbeads against conventional microbeads with polydisperse pores.

a91163

## 03-P378 Microfluidics-based droplet platform for rapid spheroid production and liver tissue engineering

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An attractive option for tissue engineering is to use multicellular spheroids as 3D microtissues, either as building block or for direct cellular injection. Conventional approaches of fabricating spheroids suffer from low throughput and polydispersity in size, and fail to supplement cues from extracellular matrix (ECM) via biochemical and biophysical signaling. Moreover, translation of any inventions into products requires the establishment of standardized processes without incurring prohibitive cost. Microfluidic technologies present many advantages to improve the quality of biomanufacturing. We have developed a microfluidics-based water-in-oil-in-water double-emulsion (DE) droplet platform as pico-liter sized bioreactor for rapid cell assembly and well-controlled microenvironment for spheroid culture. Cells aggregated to form size-controllable (30–80 mm) spheroids in DE droplets within 150 min. Moreover, precursor hydrogel solution can be adopted as the inner phase to produce spheroid-encapsulated microgels after spheroid formation. As the first example, the encapsulation of human mesenchymal stem cells (hMSC) spheroids in alginate and alginate-arginine-glycine-aspartic acid (-RGD) microgel was demonstrated, with enhanced osteogenic differentiation exhibited in the latter case. To enhance functions and viability of hepatocyte for liver tissue engineering, we also generated microencapsulated homotypic or heterotypic (co-cultured) hepatocyte spheroids. The composition of the microgel is tunable as demonstrated by improved hepatocyte viability and functions during 24 day culture (albumin secretion, urea secretion, and cytochrome P450 activity) when alginate-collagen composite microgel was used instead of alginate. Hepatocyte spheroids in alginate-collagen also performed better than hepatocytes cultured in 2D collagen-sandwich configuration. Moreover, hepatocyte functions were significantly enhanced when hepatocytes and endothelial progenitor cells (used as a novel supporting cell source) were co-cultured to form composite spheroids, which could be further boosted when encapsulated in alginate-collagen microgel. Our DE platform for spheroid production and microencapsulation with high yield, versatility, and uniformity is envisioned to be an enabling technology for liver tissue engineering as well as biomanufacturing.

a91350

## 03-P379 Functional Evaluation of a Hepatic Tissue Constructed from Multicellular Spheroids

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Liver transplantation is the only operation for patients with severe liver failure currently, however, it is hindered by the shortage of donors. The Bioartificial Liver (BAL) support system may serve as a supportive device, inducing the liver regeneration and bridging the individual's liver functions until the transplantation is possible.

One great challenge of the clinical application of BAL system is to secure a cell source, which could be easily obtained at a large amount with high functionality. In this research, we focused on the genetically engineered hepatoma cell line, Hepa/8F5 cells [1], which are capable of switching from infinite proliferation to expression of significant liver-specific functions by over-expression of introduced genes.

Spheroids, a typical 3D culture model, was adopted in this research for further construction of a hepatic tissue inside hollow fibers (HFs), which were usually applied as the scaffold for BAL system. What's more, in order to improve the mass transfer inside the dense tissue, we co-cultured the spheroids with Human Umbilical Vein Endothelial Cells (HUVECs). In details, we first constructed spheroids by gyratory culture; then coated collagen on the surface of spheroids for further attachment of endothelial cells; after co-culturing the spheroids with HUVECs, we stacked the multicellular spheroids inside the HFs, and incubated until the construction and maturation of the tissue, mainly by self-assembly of these multicellular spheroids inside HFs.

We compared the viability and functionality of 3 groups inside HFs with the same diameter: 1) mono-dispersed Hepa/8F5 cells; 2) Hepa/8F5 spheroids and 3) HUVECs-coated Hepa/8F5 spheroids. Group 3 showed the highest viability, which could be maintained around 65% for at least 6 days, whereas Group 1 and 2 showed a lower viability with further deterioration. Group 3 also showed a higher level of ammonia removal and albumin secretion ability (around 1.5-fold) than Group 2. Interestingly, although Group 1 and 2 showed similar viability, no ammonia removal was confirmed in Group 1, which could be attributed to the effect of 3D spheroids formation.

In a word, Hepa/8F5 cells and this bottom-up approach using multicellular spheroids are suitable for construction of hepatic tissue, and the co-cultured HUVECs played an important role in improving the performance of constructed hepatic tissue.

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a92373

## 03-P380 The inhibitory effect of Adrenomedullin (ADM) on hepatic NF-kB activation in 2D and 3D hepatic cell cultures

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**Background:** Adrenomedullin (ADM) is a neuropeptide exerting multiple effects through autocrine/paracrine mechanisms. ADM plays an immunomodulatory role and has anti-inflammatory activity in various diseases but its role has not been investigated in liver diseases.
**Aims:** We assessed the hepatic ADM expression in different inflammatory liver diseases (HCV, AIH, NASH) and the mechanism(s) by which ADM affects NF-kB activation in classical 2D and a new 3D model.
**Method:** Immunofluorescence analysis was performed on liver tissue samples to assess ADM expression and $\alpha$-SMA colocalization. HepG2 and LX2 were exposed to LPS (1ng/mL), ADM (10-7M) for 24hrs, ADM (10-7M) for 4hrs followed by LPS for 24hrs. ICC for p65 nuclear translocation and QRT-PCR was performed. Human liver 3D scaffolds were obtained by decellularization of healthy and cirrhotic livers. LX2 cells or primary hHSCs were cultured on scaffolds for 10 days. Primary hHSC in healthy scaffolds were treated with LPS for 1-3hrs, PDGF-BB (1-10ng/mL), or TGF$\beta$1 (2-5ng/mL) for 24hrs. hHSCs in cirrhotic scaffolds were exposed to ADM for 4hrs. QRT-PCR was performed.
**Results:** Human HSCs ADM-related fluorescence intensity in NASH patients (n=5) was less than in HCV (n=5) and AIH (n=5) patients, but the degree of colocalization was similar in all patients. ADM pretreatment of LX2 and HepG2 in 2D, followed by LPS exposure significantly reduced p65 nuclear translocation and increased NF-kB inhibitor IkB$\alpha$ gene expression. ADM expression decreased in LPS-treated HepG2 whereas exogenous ADM pretreatment counteracts this effect. ADM gene expression was decreased in TGF$\beta$1 and LPS-treated hHSCs cultured in 2D and was upregulated in 3D vs 2D. In contrast, TGF$\beta$1 and PDGF-BB-treated hHSCs in 3D showed a reduced ADM gene expression. ADM pretreatment in hHSCs in healthy scaffolds increased ADM expression, reduced NFKB1, but not NFKB2, and completely abrogated the effect of subsequent exposure to all stimuli. ADM expression was upregulated in hHSCs in cirrhotic scaffolds vs healthy scaffolds and exogenous ADM treatment favoured hHSCs deactivation in cirrhotic liver scaffolds.
**Conclusion:** This study shows that ADM expression changes with respect to the aetiology of liver inflammation. ADM leads to a reduction in activation of the canonical NF-kB pathway in hepatic cells in both 2D and 3D cultures. These findings suggest that the ADM system as a possible pharmacological target for the management of inflammatory liver diseases.

a92028

## 03-P381 *C. sinensis* infestation increases collective migration of cholangiocarcinoma in three-dimensional tumor microenvironment

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Cholangiocarcinoma (CCA) is an aggressive malignancy of the bile duct epithelia associated with local invasiveness and a high rate of metastasis and the second most common primary hepatic tumors. Infection with the liver flukes *Opisthorchis viverrini* and *Clonorchis sinensis* is considered an established risk factors for CCA and which leads to the highest incidence rates of CCA in Southeast Asian countries [1]. During this host-parasite infection, liver flukes continuously release their excretory-secretory products (ESPs) into bile duct and surrounding liver tissues they were present, and chronically influence the neoplastic and normal epithelium. Indeed, CCA cells and normal biliary epithelial cells exposed to liver fluke ESPs display diverse pathophysiological responses. In CCA microenvironment, diverse response of tumor and normal biliary epithelial cells by C. sinensis may cooperatively affect tumor invasion and/or metastasis, especially regulation of cytokines and chemokines such as interleukin-6 (IL-6) and transforming growth factor-beta (TGF-$\beta$). IL-6 is a crucial mediator of hepato-biliary response to systemic inflammation and the production of IL-6 can be promoted by ESPs of carcinogenic liver fluke in human cholangiocyte cells *in vitro* [2]. As generally known, in tumor microenvironment, many cell types are responsive to TGF-$\beta$ signaling leading to complex effects on cancer initiation and progression with the induction of a mesenchymal phenotype in epithelial tumor cells, also known as epithelial-to-mesenchymal transition (EMT) after prolonged exposure to TGF-$\beta$ [3]. So we hypothesized that IL-6 and TGF-$\beta$ induced by liver flukes in tumor microenvironment with *C. sinensis* infestation, especially from normal cholangiocyte, may play an important role in highly lethal malignancy of CCA. So we established three-dimensional co-culture model of normal cholangiocyte and CCA cells using microfluidic platform as a tumor microenvironment and assessed migration and invasion of CCA cells with ESPs treatment, in direct and gradient condition. Single cell invasion and migration of CCA cells were significantly increased. Also IL-6 and TGF-$\beta$ expression in normal cholangiocyte cells were elevated by ESPs treatment.

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a91446

## 03-P382 Revascularization of liver scaffolds using vascular induction and maturation cycles

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Liver transplantation is the only effective treatment to extend the life of patients with terminal hepatic diseases. Organ bioengineering and regenerative medicine are promising new technologies that can help increase the number of available organs for transplantation. However, up to date, all the bioengineered livers lack a functional and patent vascular network which could enable their long-term transplantation into a living animal. Hence, in this study, we sought to recreate a functional re-endothelialized vascular tree *in vitro* in a decellularized liver scaffold.

To achieve this, we recellularized an acellular rat liver scaffold with human umbilical vein endothelial cells (hUVECS) and human mesenchymal stem cells (hMSCs) in a bioreactor perfusion system. Cells were perfused at several discrete pressure conditions, showing excellent scaffold distribution. Furthermore, we applied two sequential experimental conditions that enabled the induction of cell growth and vascular maturation.

Two weeks after seeding, we were able to observe a branched and large vascular network with the presence of vascular structures with different diameters, showing the formation of large, intermediate and small vessels. These structures were completely lined by endothelial cells, which were surrounded by hMSCs, as observable in native vessels. This reconstituted vascular tree was able to secrete prostacyclin, and capture Dil-Ac-LDL and FITC-conjugated Ulex europaeus lectin. It was also able to sustain heparinized blood flow for 30 minutes with minimal platelet adhesion and aggregation.

These results unveil a novel strategy to target organ scaffold revascularization yielding the generation of functional vessels with the potential to withstand blood perfusion *in vivo*.

a91666

## 03-P383 Development of liver-specific ECM electrospun nanofiber as a potential substrate for primary hepatocytes

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Electrospinning has gained a lot of attention because of its ease of producing fibrous scaffolds, cost-effectiveness, and tunability. The fabricated scaffold obtained highly interconnected nonwoven fabrics which are very similar to the fibrillar structure of the extracellular matrix. Bicomponent gelatin and polycaprolactone (PCL) nanofibers have long been explored as scaffold material because of its excellent biocompatibility and biodegradability. However, the cell culture system is associated with dedifferentiation and decreased tissue-specific functions. Studies have suggested that tissue-specific extracellular matrix can promote site-appropriate differentiation, and maintain site-appropriate phenotype *in vitro* culture systems. In this study, liver-specific extracellular matrix (L-ECM) was added to the gelatin-PCL blend to improve its characteristics and to determine its advantage as hepatocyte substrate for liver regeneration. First, L-ECM was obtained from decellularization and solubilization procedures of the porcine liver. Through the conventional electrospinning method, nanofibrous scaffolds were fabricated using different volume/weight ratios of solubilized L-ECM, gelatin, and PCL. The fabricated fibers were characterized via scanning electron microscopy (SEM), FTIR spectroscopy, tensile strength and water contact angle. Hepatocytes were then cultured for 7 days to determine its efficiency and biocompatibility. Results showed that the optimized decellularization procedure removed up to 93% of cells and preserved most of the key components (e.g. collagen, laminin). SEM analysis clearly indicated a strong influence of L-ECM on fiber diameter, pore size, and porosity. FTIR spectroscopy showed characteristic peaks of both gelatin and PCL. While tensile strength and water contact angle showed favorable mechanical and hydrophilic features of the scaffolds. SEM images and phase-contrast microscopy of the cell-seeded scaffolds demonstrated cell attachment on its surface. And, the liver-specific function such as CYP1A1 enzyme induction was maintained from day 5 to day 7 of cell culture in L-ECM containing nanofibers compared to PCL-gelatin nanofibers. Collectively, the addition L-ECM induced an environment suitable for survival and maintenance of liver-specific functions of hepatocytes. This study also demonstrated the feasibility of the L-ECM containing nanofibers can provide functional biomaterial platforms for liver tissue engineering.

a92613

## 03-P384 Granulocyte-macrophage colony-stimulating factor reduces liver fibrosis in dimethylnitrosamine-induced liver injury in rats

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Granulocyte-macrophage colony-stimulating factor (GM-CSF) exerts several therapeutic pharmacological effects, but its role under liver fibrosis has not yet been studied. Here, we investigated the inhibitory effects of GM-CSF on Dimethylnitrosamine (DMN)-induced liver fibrosis in rats. In this study, animal model for liver fibrosis was induced in Sprague-Dawley rats by the intraperitoneal injections of DMN(10 mg/kg of body weight) for 3 consecutive days per week for 4 weeks. To see the inhibitory effects, GM-CSF(50 μg/kg of body weight) were injected for 2 consecutive days per week for 4 weeks along with DMN. We found that the DMN administration induced severe liver pathological alterations and loss in body and liver weights. However, GM-CSF improved the liver conditions and significantly reduced the body and liver weight loss. GM-CSF has also inhibited the elevation of aspartate aminotransferase (AST), total bilirubin level (TBIL), and increased albumin level (ALB). Assessment of the Hematoxylin and Eosin (H&E) staining showed reappearance of normal arrangement of the hepatic plates and disappearance of fibrosis. In addition, Masson's trichrome staining revealed that GM-CSF successfully attenuated the collagen levels compared to DMN-treated group. These results suggest that GM-CSF exhibited hepatoprotective effects against the DMN-induced liver fibrosis. Thus, we conclude that GM-CSF can be studied further as an anti-fibrotic compound for the treatment of liver fibrosis.

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a90144

## 03-P385 Ascorbic Acid-2-phosphate protective effect against ROS on pancreatic β -cell spheroids

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One of the permanent concerns when fabricating 3D tissues *in vitro* is oxygen supply. A lack of vasculature restricts oxygen supply for layers of cells through culture medium, leading to potential cell hypoxia in the core of 3D tissues thus limiting their achievable size. One potential approach to improve oxygen supply is the use of oxygen permeable microarray device for culturing 3D tissues. Our culture device made of polydimethylsiloxane provides oxygen to spheroids not only through the culture medium but also through the bottom of the culture device. We demonstrated that this approach allows decreasing hypoxia level in pancreatic β-cell spheroids in 2 times (compared with the same design device made of oxygen impermeable polymethylmethacrylate) and improving expression of the INS1 gene (2.2 times). However, insulin secretion was not greatly improved. The latter is probably attributed to reactive oxygen species (ROS) because the amount of ROS generated inside cells significantly increased in culture using the culture device. To reduce ROS concentration for the protection of pancreatic spheroids, we added a physiological amount of ascorbic acid-2-phosphate (AA2P, long-lasting vitamin C derivative) to the culture medium. AA2P similarly to NAC and DTT (widely used antioxidants) led to 36% decrease in ROS concentration inside cells. Moreover, the addition of AA2P led to additional 2 times upregulation of INS1 gene expression. Interestingly, almost the same improvement was observed with the addition of 60 times more concentrated NAC solution showing the difference in the effectiveness of these two antioxidants in case of pancreatic β-cells. These results show the importance of controlling oxygen and antioxidant culture conditions for fabrication of pancreatic tissues.

a91944

## 03-P386 Study on islet purification and viability and function maintenance

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Diabetes mellitus currently affects about 350 million people worldwide, and the World Health Organization (WHO) predicts that death due to the complications of diabetes will likely increase by more than 50% over the next 10 years. Type I diabetes mellitus, which represents approximately 10% of all diabetes cases, requires daily insulin injections. This treatment, however, is demanding for the patient, can be painful, and does not eliminate the risk of diabetic complications. Islet transplantation is a promising treatment for type I diabetes mellitus that achieves an insulin-independent, constant normoglycemic state and avoids diabetic complications. While islets are biologically active substances, so a successful transplantation depends on optimizing islet viability in vitro, in culture after isolation from the pancreas, and during transportation. So the aim of this paper was to establish a new islet purification process to improve the purity of islets for implantation, and Alginate gels (ALGs) encapsulation technology was used to improve the islet purity. In this study, the hypertonic-hypotonic treatmentwas effectivelyimproved the purity of islets; ALG bead was an efficient microenvironment culture system, which might provide a useful platform for protecting islets from mechanical damage during shipment and establishing a new engineering islet to maintain high viability of islets, and promote the development of islets transplantation. ×

a92531

## 03-P387 Engineering islet for improved performance by optimized reaggregation in alginate gel beads

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Islet transplantation is a promising treatment for type I diabetes mellitus that achieves an insulin-independent, constant normoglycemic state and avoids diabetic complications. Islets are biologically active substances, so a successful transplantation depends on optimizing islet viability in vitro, in culture after isolation from the pancreas, and during transportation. After islet isolation, diffusion has become the main mechanism to transport oxygen and nutrients into the core of islets. However, diffusion has limitations, by which nutrients cannot effectively reach the core of large islets and can eventually cause core cell death and islets loss. This problem can be resolved by dispersing islets into single islet cells, but single islet cells do not exhibit insulin release function in vitro culture. In this study, we intended to establish a new islet engineering approach by forming islet cell clusters to improve islet survival and function. Therefore, alginate gels were used to encapsulate islet cells to form artificial islets after dispersion islets into single cells. The shape of the islet cell clusters was similar to native islets, and the size of the islet cell clusters was limited to a maximum diameter of 100 $\mu$m. By limiting the diameter of this engineered islet cell clusters, cell viability was nearly 100%, a significant improvement over natural islets. Importantly, islet cell clusters express the genes of islets, including Isl-1, Gcg, and insulin-1, and insulin secretion ability was maintained in vitro.

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a92188

## 03-P388 3D Bioprinting of biomimetic pancreas

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The prevalence of diabetes is expected to increase 55% in the next 20 years, rising from 382 million people living with diabetes in 2013 to 642 million by 2040. Type 1 diabetes (T1D) is an autoimmune disease caused by progressive destruction of the beta cells in the pancreas, leading to insufficiencies control of blood sugar ending in life-threatening secondary complications such as arteriosclerosis, kidney failure and impaired vision. Islet transplantation is a minimally invasive beta cell replacement therapy for patients with T1D considered as a potential to cure the disease but is hampered by the loss of islet grafts due to transplantation into the portal vein. Developing a scaffold that could favor islet survival and allow for transplanted into an alternative site such as subcutaneously is one solution to complete a cure for everybody suffering from T1D. The first study of 3D printing of islets using modify alginate shown good viability and morphology (1). Strategies to modify bioinks with supporting stem cells such as adipose-derived stem cells (ASCs) could favor the cell survival, reduce cellular stress, and increase angiogenesis into the scaffold grafts. In this study we designed, biofabricated and evaluated an implantable device of 3D bioprinted human ASCs together with human islets. Cells were embedded in alginate based bioink followed by 3D bioprinting of constructs using INKREDIBLE 3D Bioprinter from CELLINK AB. In vitro evaluation showed improved cell viability in 3D bioprinted constructs with a combination of ASCs and islets compare to islet alone. 3D bioprinted constructs with islets alone or islets with ASCs showed ability to secret insulin upon a glucose challenges (GSIS) indicate preserved functionality of islets after 3D bioprinting. This study showed that islet´s survival was improved by creating a favorable microenvironment of human adult stem cells in the scaffold.

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a93024

## 03-P389 Formation of 3-D multilayered cell sheet enhances therapeutic capacities of differentiated insulin producing cells for diabetic control

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Transplantation of pancreatic islet is the ideal therapy for diabetes patients. Although the experiences with islet transplantation for diabetic control have markedly improved, there are still several obstacles for successful clinical applications. The main limitations of this approach are the shortage of donor and the low engraftment efficacy. In this study, we prepared insulin-producing cells (IPCs) from adult human liver cells as a new islet source. Liver cells are expanded and trans-differentiated to IPCs using adenovirus vectors carrying the human PDX-1, NeuoD and MafA genes, which are ß cell-specific transcription factors. Multiplicity of genes and treatment condition were optimized based on promoter assay and insulin gene expression. To confirm the ß cell specific function of IPCs, immunohistochemistry, endocrine-specific gene expression, and insulin contents were assessed at 5 days after the initial exposure to the viral treatment. In this study, 3-layered IPCs sheet were prepared to enhance the maturation IPCs and increase graft survival. To make a IPCs sheets, IPCs seeded onto temperature response culture dish. The IPCs sheet was pick up using a cell shifter and stratified onto another IPC sheet twice to make a 3-layered sheet. mRNA expression of endocrine specific hormones and transcription factors showed to ß cell of IPCs were significant enhanced in IPCs sheet group than monolayer culture. Single IPCs were injected by portal vein (PV) and IPCs sheet were transplanted onto the liver surface of the diabetic immune deficient mouse. Biodistribution of cells were assessed by bioluminescence image tagging with Qtacker 800 and mRNA level of human Alu gene. Mouse transplanted with IPCs sheets showed significantly high engraftment efficiency and reduced unexpected distribution of transplanted cells in organs other than the liver compares to that PV injection. Blood glucose level in IPCs sheet transplanted diabetic mouse was decreased and body weight was increased although they did not restore normoglycemia level. However, single cell injection group and diabetic mouse showed decrease of body weight. After 1 and 2weeks of transplantation, immunohistochemical analyses showed that IPCs transplanted liver stained positively for insulin. Also, cell sheet transplantation technique onto liver reduced the liver toxicity of PV injection. As a these results, multilayered cell sheet formation enhanced the graft survival and differentiation function of IPCs.

a92643

## 03-P390 Subcutaneous Transplantation Of MIN6 Beta Cells Embedded In mPEG-Ala Hydrogel

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The liver is the current site of choice for clinical islet transplantation, but many intraportal grafts were lost in the early stage of the transplantation. Other disadvantages of liver site include difficulty of monitoring the islets, and procedure- associated complications. In contrast, the subcutaneous space offers the advantages of a large space and a minimally invasive procedure performed and easy graft monitoring and removal. However, islet transplantation into an unmodified subcutaneous site has poor efficacy, possibly due to poor oxygenation and inadequate vascularization of the transplanted tissue. In this study, we test the feasibility of subcutaneous transplantation of MIN6 beta cells embedded in temperature-sensitive Poly(ethylene glycol) methyl ether (mPEG)-Ala hydrogels as scaffolds. After 14-day culture, the viability of MIN6 beta cells in mPEG-Ala hydrogel was comparable with those in medium, either assayed by MTT or LIVE/DEAD. Static incubation showed both had comparable stimulation index. For in vivo experiments, we infected MIN6 beta cells with luciferase by AAV and then transplanted $5*10^6$ cells embedded in mPEG-Ala hydrogel into subcutaneous tissue of the upper back of each nude mouse. Positive images of In Vivo Imaging System (IVIS) and positive insulin staining of tissue histology were observed up to 41 days after transplantation. Besides, we overnight incubated MIN6 beta cells with chitosan-coated superparamagnetic iron oxide (CSPIO) and then transplanted $5*10^6$ MIN6 beta cells into subcutaneous tissue of the left flank of a nude mouse. The graft of CSPIO-labeled MIN6 beta cells were visualized on Magnetic Resonance (MR) scans as distinct hypointense spots on T2-weighted images located at the subcutaneous site at day 20. These results indicate subcutaneous transplantation of MIN6 beta cells embedded in temperature-sensitive mPEG-Ala hydrogels is feasible. Moreover, the IVIS and MR imaging are useful tools for detecting and monitoring beta cells at subcutaneous site.

a90738

## 03-P391 Pancreatic Beta Cell Differentiation of Human Tonsil-derived Mesenchymal Stem Cells by Regulating Cell-Matrix Interactions

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Human tonsil-derived mesenchymal stem cells (T-MSCs) possess a great potential to differentiate into multiple lineages and self-renewal capacity, allowing them to be utilized as patient-specific cell-based therapeutics. Although various stem cells-derived pancreatic beta cells or insulin-producing progenitor cells has been proposed as a novel approach for treating diabetes mellitus, an efficient method for establishing functional insulin-producing cells is still a daunting task. Here, we aim to develop a novel cell culture platform that could regulate important cell-cell/cell-matrix interactions by introducing a growth factor immobilized matrix that can support the adhesion, proliferation, and differentiation of T-MSCs into insulin-producing cells. Our findings demonstrated that cells cultured on a growth factor-immobilized matrix were able to become insulin-producing beta cell-like progenitors, as shown by the upregulation of pancreatic beta cell-specific markers, such as PDX-1, Insulin, and Glut-2. Such a cell culture platform can offer novel strategies to achieve functional pancreatic beta cells from a patient-specific cell source to treat diabetes mellitus.

a91597

## 03-P392 Bioprinted Pancreas-On-A-Chip Model For Drug Screening In Diabetes Therapy

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Due to the limited supply of organs for transplantation, engineering a fully functional human organoid will be a milestone in the field of tissue engineering and regenerative medicine. Some approaches have been investigated for vascularization of three-dimensional (3D) soft tissues, highly essential for successful fabrication of physiologically-relevant tissue models. This research focuses on creation of vascularized pancreatic islets, which brings new perspectives in treatment for type I diabetes or drug testing. This research is significant since diabetes type I will double over the next decade if current rates of increase continue. In this research, pancreatic spheroids were formed by mouse insulinoma B-cells (BTC-3) and rat heart microvascular endothelial cells (RHMVECs). Co-cultured spheroids provide a vascularized network within the fibrin scaffold that supplies nutrients to cells and drains metabolites, including insulin. The presented tissue model is arranged for a long-term culture that provides data of changes accompanying the tissue formation and maturation. The tissue spheroids were precisely deposited in a perfusable, hydrogel-based device by custom-designed 3D-bioprinter. The active perfusion of the engineered vascularized pancreas tissue within the device provides dynamic culture conditions for a month. The expected outcome is cell response to glucose and detection of insulin in real-time at the outlet of the device. It is possible by creating a hollow vasculature from the tissue spheroids to the channel that will allow quick exchange of fluids rather than diffusion through the hydrogel. This approach combines fabrication of vascularized engineered islets and 3D bioprinting with preserved functionality for treatment of type I diabetes. The constructed device will be also a useful tool for drug screening.

a92457

## 03-P394 ENGINEERED 3D HUMAN PANCREATIC CANCER MODELS: THE ROLE OF TISSUE-SPECIFIC EXTRACELLULAR MATRIX IN TUMOR PROGRESSION AND THERAPY-RESISTANCE

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**Background and aims:** Over 80% of patients with pancreatic ductal adenocarcinoma (PDAC) are diagnosed with concurrent metastases. Conventional treatment approaches have had little impact on the course of this disease over the last 50 years. Therefore, the development of new treatment strategies to control PDAC is needed. We propose the use of 3D extracellular matrix (ECM) scaffolds that could redefine *in vitro* models of PDAC and preclinical testing of novel therapies.

**Methods:** Decellularised human pancreata and livers were extensively characterised for the elimination of cellular material and preservation of ECM proteins and micro-architecture (n>7) using histology, SEM, Raman Spectroscopy and quantification kits. Both primary (PANC-1, MIA PACA-2, and patient-derived cells) and metastatic pancreatic tumour cells (PK-1) were seeded onto 5 $mm^3$ scaffolds, as well as 2D cultures. Histological analyses were used to confirm cell attachment and migration. Further, gene and protein expression were evaluated at 14 days. Additionally, viability and FACS analyses were performed to test therapy-resistance in both 2D and 3D systems upon treatment with doxorubicin and gemcitabine, as well as novel photodynamic therapy (PDT) and immunotherapy using stimulated NK cells.

**Results:** All primary cells were able to migrate and invade the pancreas scaffolds whereas several of these cells were only able to attach superficially onto the liver scaffolds. PK1 cells were able to exclusively migrate and invade the liver scaffolds and only attached superficially onto the pancreatic scaffolds. These differences were supported by significant deregulations in gene and protein expression (i.e. MMP9, WNT1, $\beta$CATENIN) between pancreas scaffolds, liver scaffolds, and 2D culture. Interestingly, both primary and metastatic cells were found significantly more resistant to all treatments (chemotherapy, PDT, and immunotherapy) in the 3D models when compared to 2D cultures (n=4, $p<0.001$), even though confocal microscopy confirmed the uptake of drugs into the cells.

**Conclusion:** Our results suggest that primary and metastatic pancreatic cancer cells manifest a conserved invasive behaviour depending on the 3D ECM structure of origin. Moreover, there is an evident alteration in cell response to different cancer-therapies in the presence of a natural ECM niche. These observations provide a proof of concept for the development of an effective bioengineered model for drug screening and biomarker discovery.

a93118

## 03-P395 Non-degradable cell encapsulation of pancreatic islets with alginate-EGCG conjugation

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Immunoprotective microencapsulation techniques with biocompatible alginate have been developed for pancreatic islet transplantation. However, pure alginate microencapsulation techniques have a limitation in that $Ca^{2+}$-alginate hydrogel slowly dissolve in the body and induce hypoxia to encapsulated islets. To overcome these limitations, we conjugated (-)-epigallocatechin gallate (EGCG) to alginate, which is capable of forming aggregates by auto-oxidation, independent of $Ca^{2+}$, as well as radical scavenging and HMGB1 (cell damage molecule) capturing. The bonding reaction was carried out by preparing an ethylamine-bridged EGCG dimer through an aldehyde-mediated condensation reaction and then forming an amide bond through an EDC/NHS reaction. First, we introduced the EGCG to the surface of alginate microcapsule through alginate-EGCG conjugation, which was confirmed that the thin layer was formed by auto-oxidative coupling of EGCG on the surface. And the improvement of stability by surface modification was also confirmed. Then we confirmed that the surface modified microcapsules could have a hollow structure by $Ca^{2+}$ chelating action of EGCG. Hollow microcapsule could solve hypoxia problem, but its structure was vulnerable to physical damage due to their weak physical properties. However, we confirmed that the surface-modified microcapsules could have stronger physical properties than the conventional alginate microcapsules. In addition, the surface modification of the microcapsules was performed once again using alginate for the suppressed immune response. Currently, cell experiments are underway to confirm the effect of radical scavenging and cell release-HMGB1 capture function of EGCG on cell survival. Subsequently, the immune response of the body through the transplantation of surface-modified microcapsule with islets and their therapeutic effect on diabetic animals will be confirmed.

a93034

## 03-P396 Evaluation of polylactic acid/ polyglycolic acid covering material after pancreatic surgery using rat pancreatic fistula model

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Pancreatic fistula is frequent and devastating morbidity after pancreatic surgery. When patients developed pancreatic fistula, leaked pancreatic juice damage surrounded tissue and cause sepsis or critical hemorrhage. Prevention of pancreatic juice leakage from pancreatic stump/ anastomosis is very important for the sake of safe pancreatic surgery. Covering the cut surface by biomaterials is one of the promising way to prevent pancreatic juice leakage, however, past effort for this has not fully succeeded. One of the reason for failure may be inappropriate degradation profile induced by pancreatic enzymes. Although degradation profile is one of the most important aspect of biomaterials and pancreatic juice possibly affect this, there were little previous reports about biomaterials considering changes by exposure to pancreatic juice. Polylactic acid/ polyglycolic acid is promising biomaterial because we can adjust its character in various way by changing their composition, type of filaments. We evaluated various poly lactic acid/ poly glycolic acid materials using rat pancreatic fistula model. Here we want to discuss the optimal character of biomaterials aiming prevention of pancreatic fistula.

a90416

## 03-P397 Effects of Soybean Isoflavones on the Survival of Random Skin Flaps in Rats

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**Background:** Random flap transplantation has been widely used in the repair and rebuild of skin soft tissue flap. However, such flaps exhibit poor survival. It's quite important to improve the flap survival in plastic surgery.

**Objective(s):** This study aims to investigate the effects and the potential mechanism of soybean isoflavones on the survival of random skin flaps.

**Materials and Methods:** Overlength random skin flap models(measured 9cm*3cm) were set up on 50 healthy male SD rats. They were divided into two groups. One group was assigned to be treated with soybean inflavones by gavage(test group) and the other(control group) with saline. Normal saline or soybean inflavones [240mg/kg] was instrastrically administrated daily. On postoperative day 4, malondialdehyde(MDA) and superoxide dismutase(SOD) were inspected by test kits .On postoperative day 15, the flap survival area was measured with transparent graph paper under direct visualization. The levels of microvessels were evaluated by histologic examination(HE). The expression of VEGF was immunohistochemically assessed .Angiogenesis was assessed via lead oxide-gelatin angiography, and the blood flow on models was examed by laser Doppler flowery.

**Results:** In the test group compared with the control group, the flap survival rates are much higher. SOD activity is increased markedly, when MDA level decreased. HE reveales the inflammation reaction are significantly attenuated. It as well promotes the expression of VEGF and increases skin flap angiogenesis in the tested group.

**Conclusion:** Soybean isoflavones can inhibit the ischemical reperfusion injury and increase angiogenesisto improve the flap survival rate in rats.

a90836

## 03-P398 N-acetyl cysteine loaded nano graphene oxide reinforced collagen – a 3D biosponge for skin regeneration application

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Skin regeneration after deep dermal injuries is a major clinical problem due to the current therapies limited to poor healing results with persistent oxidative stress , slow fibroblast immigration, poor epithelization and insufficient vascularization.Functionalized graphene oxide(GO) offers many advantages as a biomaterial in various biomedical applications and antioxidant N-acetyl cysteine (NAC) is known to promote endothelial cell function and angiogenesis which may have therapeutic benefits in the setting of skin regeneration. In this study, GO was incorporated with collagen I and NAC to make a drug-release 3D scaffold as a potential skin regeneration biosponge in both in vitro and in vivo studies. The porosity, water retention and water absorption of the prepared Collagen-Graphene Oxide loaded with NAC(CGN) drug release biosponge is measured by dewatering method, and the CGN biosponge is characterized by X-ray diffraction, attenuated total reflectance (ATR)-FTIR and Raman spectroscopy. The surface property of the CGN biosponge is characterized by employing transmission electron microscopy (TEM) and scanning electron microscopy (SEM). The mechanical stability of the CGN biosponge is great as biomaterial. An in vitro cell study reveals CGN biosponge can promote cell immigration against human fibroblast. In vivo skin regeneration studies showed faster skin regeneration efficiency of CGN biosponge than that of Collagen(C), Collagen-Graphene Oxide(CG) and Collagen loaded with NAC(CN),including faster epithelization ,better vascularization and lower IL-7 expression. These findings suggest that CGN drug release biosponge could serve as a better platform for skin regeneration applications.

a91500

## 03-P399 Functionalized Collagen Scaffolds Result in Sustained Postnatal Regeneration after *in utero* Closure of Skin Defects in Sheep

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### Introduction

For a number of congenital anomalies, like spina bifida, it may be advantageous to close the defect in the prenatal period. In case of spina bifida, this would prevent *in utero* degeneration of the exposed spinal cord tissue and improve neurological outcome.

### Materials & Methods

In this study, three full-thickness skin defects were created in sheep at 79 days post gestation of which one was treated with a collagen scaffold (COL), one with a collagen scaffold loaded with heparin, vascular endothelial growth factor (VEGF) and fibroblast growth factor 2 (FGF2) (COL-HEP/GF) and one was left untreated. Skin regeneration was evaluated 1 and 6 months postnatally using semi-quantitative histological analysis on excised skin specimens and comprehensive gene expression microarray analysis on laser-dissected dermis and epidermis.

### Results

Histology showed that the addition of heparin and growth factors to the collagen scaffold significantly increased the total surface area of regenerated skin with hair follicle generation. One month after birth epidermal thickness was increased, but at six months this was reduced to near normal thickness. In COL-HEP/GF, scaffold degradation was significantly decreased.

Gene expression microarrays showed a shift in the total number of differentially expressed genes from the epidermis at one month to the dermis at six months. At one month in the epidermis, GO-terms "mitochondrial translational termination" and "mitochondrial translational elongation" were enriched in COL-HEP/GF compared to the untreated defect. At six months in the dermis, both collagen scaffold treatments led to enrichment of GO-terms related to extracellular matrix organization compared to native skin. Furthermore, fold change analysis revealed that the gene expression pattern of COL-HEP/GF more closely resembled normal skin than COL.

### Conclusion

Our data indicate that *in utero* intervention during skin wound healing with functionalized collagen scaffolds loaded with heparin, VEGF and FGF2 has long-term beneficial effects on skin regeneration. This approach offers new possibilities for the treatment of closure defects such as spina bifida.

### Acknowledgement

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a91814

## 03-P400 The construction of full-thickness skin flap for the treatment of deep wound

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**Background**: Deep wounds have always been a thorny issue in plastic and reconstructive surgery for poor blood supply. Skin flaps, with self-blood supply and integral structure of epidermis, dermis and subcutaneous soft tissue, have usually become the fisrt choice for the treatment of deep wounds. However, the inevitable secondary injury in donor site impede the application of autologous skin flap transplantation. Skin flap constructed by tissue engineering technology has brought hope to solve this problem.In response to the problems above, our group took the lead in using tissue engineering technique to construct composite skin fla

**Object**: To establish a rat model Cconstructing full-thickness skin flap with allogeneic epidermal sheet, porcine acellular dermal matrix and autologous adipose tissue and exploring an effective vascularization strategy.

**Methods:** First, we used porcine acellular dermal matrix(PADM) as a dermal substitute and applied laser punching technique to improve the permeability of PADM. After that, stromal vascular fraction(SVF) extracted from inguinal adipose was co-cultured with PADM for 4 weeks *in vitro*, developing an initial vascular network to ensure the early blood supply of PADM after transplantation. Then silica gel,allogeneic epidermis and microporous PADM was embedded into the back of rats in one step. After the tissues above wereas fully vascularized, adipose tissue with ADSCs was injected thereon. Finally, the prefabricated full-thickness skin flap was used to repair the adjacent deep wound.

**Result**: The laser-perforated PADM had significant vessel ingrowth in the hole after transplantation, and there was no obvious contracture of PADM postoperation which meant laser punching did not affect the intensity of PADM. The surface of PADM could form microvascular network after 4 weeks'co-culture with SVF, ensuring the early blood supply of PADM after transplantation. After two weeks'incubation, adipose tissue could survive and connect with PADM through the fascia. The skin flap almost entirely survived 6 weeks postoperatively, and epidermalization could be seen. HE staining confirmed that the tissue engineering skin flap, with complete structure of epidermis, dermis and subcutaneous soft tissue, was well compatible with the surrounding normal skin.

**Conclusion**: Our pioneering research demonstrates the feasibility of fabricating a tissue engineering comnposite skin flap and its application in deep wound repairing.,

a92121

## 03-P401 In vivo sheep study on the effects of ITAP's porous size on soft tissue attachment

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Intraosseous Transcutaneous Amputation Prosthesis (ITAP) is a new generation of percutaneous implants, attached by osteointegration to the skeleton and protruding through the skin, allowing the mechanical forces to be directly transferred to the body and the external skin being freed from frictions and infections. The skin-implant interface is maintained with a flange, which resides below the epithelium, and is designed with pores to enhance sub-epithelial attachment.

This study is to investigate the effect of pore size of the flange on soft tissue attachment. Twenty ITAPs of two different flange designs (3D printed porous and non-printed plain with pores) were implanted into the tibia of sheep (n=10). Implants were retrieved 3 months post-operation and analysed for sub-epithelial tissue attachment within the flange and pylon. Signs of infection were also analysed.

It was observed that the skin around the implants was dry and clean, and no infection was observed in all ten sheep. The histology examination revealed significant improvement in terms of epithelial downgrowth and epithelial and sub-epithelial attachment in the 3D flange group compared with plain flange group. However, the soft tissue attachment and epithelial attachment are varied between implants and no statistically significant differences were observed. It is postulated that flange design is not the only factor influencing the soft tissue attachment and epithelial attachment, but the surface morphology and surface chemistry are also fundamental factors. As future work, the flange surface will be modified with TiO2 nanotubes in order to improve the cell attachment. Preliminary work on TiO2 nanotubes has demonstrated their potential in enhancing cells attachment and will be further investigated.

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a92392

## 03-P402 Sandwich-type Collagen-Coated Antioxidative Nanofibrous Membrane for Wound Dressing Application

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Wound dressings are used for providing a suitable environment to facilitate skin regeneration[1]. Many investigations of the electrospun nanofibrous membrane used as wound dressing have been conducted because of its innate properties, such as high porosity and large specific area[2].Nevertheless, the nanofibrous membrane is too thin to absorb fluids such as blood and exudation and it is necessary to keep wound clean and dry as wound dressing[1]. Moreover, the nanofibrous membrane cannot directly accelerate the process of wound healing particularly in disease condition, such as diabetes and usually requires further cross-linking steps to keep a functional biomaterial as wound dressing[2].

Collagen is widely used not only as a material as wound dressing owing to its favorable characteristics, which include biocompatible, non-toxic, biodegradable, hemostatic, and great water-absorbing quality, but also as a appropriate material for drug delivery system[3]. It has been reported the N-Acetylcysteine (Nac) accelerates wound healing owing to its ability to scavenge overproduced reactive oxygen species[4]. In this study, collagen had been crosslinked with Nac to make an antioxidative drug-loaded collagen, which made it possible to keep effective drug concentration on wound area. However, the antioxidative drug-loaded collagen has its limitation that it's too weak to bear stress as wound dressing[5].

To overcome these limitations, we presented a class of wound dressing which was constructed in the form of a 'sandwich': consisting of collagen loaded with N- acetylcysteine on the top and bottom and the electrospun nanofibrous membrane at the core.The sandwich-type antioxidative nanofibrous membranes have a great potent on wound dressings and may provide a good solution for the treatment of chronic wound including diabetic ulcer and pressure ulcer.

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a92157

## 03-P403 A Histological and Functional Comparison of Dermal Scaffolds for Cutaneous Wound Healing in the Porcine Animal Model

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Cutaneous wound healing requires a complex interplay of cell action to orchestrate haemostasis, inflammation, deposition and remodelling processes to repair a wounded site[1]. Dermal scaffolds aim to improve the final appearance and function of skin after completion of the healing process by providing biocompatible exogenous dermal material to aid endogenous processes[2]. In this study, the functional and aesthetic outcomes of treatment with the collagen-based scaffolds, and fibrin-based SmartMatrix® dermal scaffolds was studied on 4cm diameter circular full thickness wounds, in comparison to healing by secondary intention or with a split thickness skin graft.

Biomaterial performance was assessed histologically using wound biopsies taken regularly over a period of 6 months, allowing assessment of healing rate, graft take, epidermal coverage, angiogenesis and inflammation. Each biomaterial furthermore showed characteristic individual behaviour in vivo, showing unique aspects of persistence, cell infiltration and associated cell behaviour within or adjacent to the biomaterial.

Finally, the macroscopic phenotype of treated wounds was observed, with changes in superficial blood perfusion in early wounds measured using laser doppler flow assessment, aesthetic outcome appraised and compared, and changes in wound area, volume and depth measured using Eykona 3D imaging. This study documents significant differences between commonly used dermal scaffolds and a novel fibrin-based dermal scaffold likely to show clinically significant differences in wound healing outcome.

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a91139

## 03-P404 Bioactive Peptide Amphiphile Nanofiber Gels Enhance Burn Wound Healing: In Vitro and In Vivo Studies

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**Objectives:** Burns are physically debilitating and potentially fatal injuries. The standard-of-care for burn wounds is the coverage with gauze dressings designed to minimize trauma to the regenerating epidermis and dermis during dressing changes. However, deep-partial and full thickness burns always heal slowly when standard wound care alone is performed. We have previously reported that peptide amphiphilic (PA) gels, pH-induced self-assembling nanostructured fibrous scaffolds, promote cell proliferation and have great potential in regenerative medicine for rapid repair of tissues. In this study, we hypothesized that the PA gels are capable of accelerating wound healing in burn injury.

**Methodology:** Artificially generated thermally-damaged fibroblasts and human umbilical vein endothelial cells were seeded onto the various PA nanofiber gels including backbone-PA (C16-VVVAAAEEE), E2-RGDS PA (C16-VVAAEERGDS) and E3-RGDS PA (C16- VVVAAAEEEGGGGGRGDS). Cell proliferation was assessed at different time points and thermally-damaged fibroblasts and HUVECs manifested increased proliferation with time when cultured with various PA gels. To determine in vivo effects, burn wounds of rats were treated with the bioactive RGDS modified PA (E3-RGDS-PA) gel that showed greater cell proliferation in vitro. The wound closure was observed and skin samples were harvested for histologic evaluation.

**Results:** Cell proliferation using the RGDS-PA gel was significantly higher than that observed in other gels. The RGDS-PA gel significantly enhanced re-epithelialization during the burn wound healing process between days 7 and 21. Application of PA gels accelerates the recovery of deep partial thickness burn wounds by stimulation of fibroblasts and the creation of an environment conducive to epithelial cell proliferation and wound closure.

**Significance:** This biomaterial represents a new therapeutic strategy to overcome current clinical challenges in the treatment of injuries resulting from burns.

a91312

## 03-P405 A Highly Programmable and Non-invasive Biomaterial to Aid Wound Healing

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We are working towards an effective, safe therapeutic solution in the management of complex (partial and full thickness) wounds. Non-healing ulcers, among other problems, can lead to infection and disability and current wound management protocols are not satisfactory. Wound healing is promoted by a number of growth factors (GFs) including platelet-derived GF-BB (PDGF-BB) and bone morphogenetic protein 2 (BMP2). Current GF products have a large associated cost and potential adverse effects (e.g. PDGF containing Regranex® warns not to use more than 3 times due to health warnings such as death secondary to malignancy and BMP2 containing InFuse has a safety warning issued).

Appropriate interactions of GFs with the extracellular matrix are critical to regulation of signalling and effectiveness. Our approach utilizes poly(ethyl acrylate) (PEA) that promotes self-organization of fibronectin (FN) into biological nanonetworks, unravelling the FN molecule to reveal cell adhesive and GF binding domains. We have previously shown successfully effectiveness of this system to deliver BMP2 for bone regeneration (1). Here, we use PEA to facilitate exposure of $FNIII_{12\text{-}14}$ to bind PDGF-BB in close apposition to the integrin binding $FNIII_{9\text{-}10}$ domain. The FN nanonetworks sequester PDGF-BB at very low concentration (100 ng/ml) compared to GF containing products (approx. 300 fold lower). A newly approach is the introduction of cytokines that are critical to wound healing, making use of the $FNI_{1\text{-}5}$ domain that has been demonstrated to bind and efficiently present cytokines/chemokines (2). The biological activity of the system with the GF PDGF-BB and chemokines CXCL11 and CXCL12 has been evaluated *in vitro* using fibroblasts (L929 and hTERT) to correlate enhanced cellular migration to the wound site with the synergistic presentation of GFs and chemokines using wound healing assays. The future implantation of the system in clinics in a bandage form has been studied looking at the release of the PDGF-BB, CXCL11, and CXCL12. Further experiments using keratinocytes have been performed to analyze their migration and maturation. The accelerated cell migration coupled to low dose PDGF-BB delivery will provide cost-effective, safe, enhanced wound closure.

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a93322

## 03-P406 Bioactive nanovesicles from umbilical cord blood controlled delivery system for chronic wounds

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Chronic wounds are a worldwide problem with high impact on public health and economy. About 15% of older adults suffer from this condition in the US, with increasing incidence of 2 to 3 million a year [1, 2]. And it is estimated that 12% of these patients will need amputation [1]. The rising tendency of chronic wounds demands more effective therapies. Exosomes are vesicles with a diameter of 30-200nm originated in multivesicular bodies and contain bioactive proteins, lipids and RNA [3].

In the present work, we hypothesized that umbilical cord blood mononuclear cells (hUCBMNCs) can be a source of bioactive nanovesicles (NVs, including exosomes) with regenerative potential for chronic wounds that could be potentiated by a controlled delivery platform.

NVs obtained upon sequential ultracentrifugation have typical exosome morphology, size, zeta potential, and markers. NVs were internalized by skin cells (keratinocytes, fibroblasts, and endothelial cells), and demonstrated bioactivity by increasing cell proliferation, survival, migration in a scratch assay and endothelial cells tube formation. *In vivo*, a twice a day topical administration of low amounts of NVs enhanced wound healing kinetics in diabetic and non-diabetic mice, as compared to control (PBS) and PDGF-BB (FDA-approved advanced therapy). Moreover, we developed a hyaluronic acid (HA) photo-sensible hydrogel that allowed the controlled release of NVs in the wound site and further improved healing kinetics, as evaluated by macroscopic observation and histological analysis, by modulation miRNA expression in the skin. RNA-Seq analysis of the NVs revealed a very complex miRNA composition and *in vitro* and *in vivo* tests demonstrated that the most prevalent miRNA was partially responsible for their bioactivity.

In summary, a new platform for delivery of therapeutic nanovesicles was successfully developed and represents a promising tool for the treatment of chronic wounds.

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a91485

## 03-P407 Platelet derivatives, alginate and sericin: an activated biomembrane as medical patch in skin regeneration

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Chronic skin wounds with different etiophatology as pressure or foot/leg diabetic ulcers, heavily compromise the patients' life quality and represent a high and constantly growing cost for National Health Services. Platelet derivatives (PL) has been used in clinical practice for reparative treatments of these pathologies, given their capacity to activate cell recruitment, proliferation, differentiation, as well as to regulate angiogenesis. Previous work of ours (Spanò et al, 2016) demonstrated that Platelet Rich Plasma based biomembranes, with a standardized platelets concentration, can play a critical role in skin regeneration. In this context, we here report the development of a sericin/alginate sponge-like membrane as a new delivery system of PL.

Sericin, alginate and PL were solubilized in distilled water and cast into freeze-dried molds. An *in vitro* test was performed to quantify the growth factor release from sponge/PL membrane by ELISA kit and the biological activity of the released factors was tested on BMSCs by cell proliferation and wound scratch assay. *In vivo* efficacy of sponge/PL was evaluated by skin mouse model. The lesions were treated with sponge membranes, with or without PL, and covered by Tegaderm™. The animals were sacrificed at different times (3, 7 and 21 days and a histological investigation was performed.

*In vitro* results indicated that the release of growth factors and the biodegradation of sponge membrane occurred within 48 hours, a time optimized to burst the healing process. The biological activity of the factors released by the sponge membranes containing PL, characterized by cell proliferation and migration, was maintained. The *in vivo* experiments demonstrated that membranes containing PL promoted a more rapid wound closure compared to the lesion treated with sponge without PL. In particular the histological analysis showed a strong inflammatory reaction in the sponge/PL implants at day 3, in agreement with the effect of PL observed in vitro. However a more evident reconstitution of the subcutaneous layer was observed in the following days.

In conclusion the sponge/PL membrane including PL, represents a powerful tool for the treatment of skin ulcers being able to reduce the length of the inflammatory phase, to accelerate the resolution phase and to promote a more efficient closure of the wound.

a93459

## 03-P408 Function Of Latent TGF-Beta-1 Binding Protein-2 (LTBP-2) In Modulation Of Growth Factor Storage, Expression And Activity In Normal And Fibrotic Tissues

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LTBP-2 belongs to the fibrillin-LTBP superfamily of extracellular matrix proteins. Unlike other LTBPs, LTBP-2 does not covalently bind TGF-beta and its molecular function remains unclear. The aims of this study were to determine the function of LTBP-2 on a) elastic fibre assembly b) FGF-2 storage and activity and c) TGF-beta expression in fibroblasts. Additionally the expression and distribution of LTBP-2 in fibrotic skin disorders was determined. We have previously presented evidence that LTBP-2 is a negative modulator of elastic fibre assembly. New data using ear cartilage chondrocyte cultures shows that this inhibition is largely confined to the stage of elastin deposition onto the fibrillin microfibril scaffold. Thus LTBP-2 levels may regulate the rate and extent of elastinogenesis in some tissues.

Previously we reported that LTBP-2 strongly binds FGF-2 at a single central binding site. We have now shown that 5-fold molar excess LTBP-2 can completely block FGF-2 stimulation of fibroblast proliferation. Extensive co-localisation of LTBP-2 and FGF-2 was observed and quantitated in human hypertrophic scars and keloids. Furthermore, qPCR confirmed elevation of LTBP-2 and FGF-2 expression in these fibrotic tissue samples. The results support the concept that increased LTBP-2 expression in fibrotic disorders may act as mechanism for reducing FGF-2 activity by binding the growth factor and inhibiting normal repair processes.

LTBP-2, or a small bioactive fragment LTBP-2C F3, stimulates TGF-beta expression and secretion in MSU 1.1 skin fibroblasts. The addition of exogenous LTBP-2 or fragment F3 to the culture medium resulted in significantly increased levels of latent TGF-beta in the medium after 9h peaking at 15h. The signalling mechanism appears to involve the PI3K/Akt and p38 MAPK pathways, as incubation of cells with LTBP-2 (10µg/ml) elevated Akt 1/2/3 Ser473 and P38 D-8 phosphorylation after 30 min, as assessed by Western blot analysis.

In conclusion, these findings are consistent with LTBP-2 having novel regulatory functions in growth factor modulation and fibrogenesis which may lead to novel therapy development for fibrotic diseases.

a91954

## 03-P409 Amentoflavone, a biflavonoid, Increase Stem Cell Activity of Interfollicular Epidermal Cells by Modulating Basement Membrane

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Amentoflavone ($C_{30}H_{18}O_{10}$) is a well-known biflavonoid occurring in many natural plants. It has various bioactivities, including anti-inflammation, anti-oxidation, anti-diabetes, and anti-senescence effects. It also exhibits MMP-1 inhibitory activity in human dermal fibroblasts. Thus, it was hypothesized that it may affect skin aging by affecting epidermal stem cells. We investigated the effect of amentoflavone on epidermal cells by constructing skin equivalents. Immunohistochemical staining of cellular markers and the quantification of $\alpha 6$ and $\beta 1$ integrin expression were performed to investigate the effects of amentoflavone on skin cells. Amentoflavone increase the thickness of epidermis and the density of basal layer cells. It also increased the expression of PCNA, p63, $\alpha 6/\beta 1$ integrins, and involucrin. Our study suggests that amentoflavone may increase the number of interfollicular epidermal stem cells and regenerative potential of the epidermis by increasing the expression of $\alpha 6$ and $\beta 1$ integrin. Downregulation of miR144 was observed. It means that amentoflavone increased the expression of both integrins by decreased expression of miR144. Our study strongly suggests that amentoflavone may affect the interfollicular epidermal stem cells by modulating the basement membrane, which can provide a niche environment.

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a90189

## 03-P410 The bioactivity evaluation of basic fibroblast growth factor (bFGF) release *in vitro* from collagen/gelatin sponges

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Several studies, primarily in animals, are showing a synergistic effect when basic fibroblast growth factor (bFGF), a glycoprotein which enhances granulation and inhibits scar formation [1-7], is used together with artificial dermis [1-3, 8-12]. Our previous works on collagen/gelatin sponges (CGSs) not only showed that CGS can release bFGF for about 10 days, but also demonstrated its efficacy in the treatment of chronic ulcers in animal and clinical studies [1-2]. However, although CGSs have proved successful in improving wound repair, *in vivo* overtime bioactivity of the released bFGF has not been thoroughly investigated yet.

In this study, CGSs impregnated with bFGF at 7 mg/cm$^2$ were kept at 37° C for 1, 2, 3 , 5, 7 and 14 days. Then, after immersion in collagenase solution at 37 ° C, CGSs were completely digested within 6 hours, and the obtained mixtures were used to test the bFGF bioactivity. Indeed, to investigate the possible pro-growth effect of bFGF, the viability of fibroblasts exposed to serial dilutions of extracts from the CGSs was examined through WST-8 assay (Cell Counting Kit-8 (CCK-8), Dojindo Laboratories, Japan). The fibroblasts demonstrated viability comparable to the negative control (DMEM medium without FBS), because the presence of collagenase significantly decreases the proliferative activity. However, when the collagenase in each mixtures was neutralized by the addition of 2% FBS, a viability significantly higher than positive control (DMEM medium with 2% FBS) were detected.

Therefore, these results clearly indicate that the CGSs provide controlled release of bFGF over several weeks and preserved its bioactivity.

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a93153

## 03-P411 Extracellular Calcium in Dermal Fibroblast Conditioned Medium (DFCM) Assists Re-epithelialization via Collective Migration of Keratinocytes

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Re-epithelialization is one of the most critical phases of wound healing, involving the re-establishment of the epidermal layer through keratinocytes migration. This process is mainly regulated via paracrine signaling of cytokines, chemokines and growth factors secreted by dermal fibroblasts. Besides, extracellular calcium plays a vital role in stimulating keratinocytes differentiation during epidermis maturation. This study aimed to investigate the role of calcium along with the secretory factors of dermal fibroblasts, which was collected as dermal fibroblasts conditioned medium (DFCM) during *in vitro* culture. DFCM was prepared by culturing confluent fibroblasts with serum-free keratinocyte-specific (DFCM-KM) and fibroblast-specific (DFCM-FM) medium. DFCM collected after 3 days of incubation (DFCM-KM-3 and DFCM-FM-3) contains a higher concentration of protein compared to medium after one and two days of incubation. Besides, calcium concentration was significantly higher in DFCM-FM-3 (1.08 mmol/L) compared to DFCM-KM-3. It was also found that DFCM-KM-3 enhanced keratinocyte attachment, while DFCM-FM-3 significantly increased keratinocyte wound healing rate in a dose-dependent manner compared to other conditions. Supplementation with DFCM-FM-3 resulted in an increment of keratinocyte area and collective cell migration during healing, which was distinctly different from keratinocytes supplemented with DFCM-KM-3 or cultured in control medium. Further analysis confirmed that the presence of calcium at a higher concentration in DFCM-FM facilitated the changes. These results indicate that both extracellular calcium and fibroblast secreted proteins assist the re-epithelialization process, and potentially be used to improve wound healing efficiency.

a93169

## 03-P412 Low dose stingless bee honey increases viability of human dermal fibroblasts that could potentially promote wound healing

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Wound healing is a series of complex coordinated events involving a number of cellular and molecular components. Dermal fibroblast is one of the key player in wound healing physiology, contributing in key processes within the phases of wound healing. Data on the clinical efficacy of honey in wound healing is well established. However, majority of the research, focus on honey produced by honey bee (Apis mellifera) and little is known of stingless bee honey. This study seeks to evaluate the effect of freeze-dried stingless bee honey on the cell viability and proliferative capacity of dermal fibroblast. Dermal fibroblast viability will be evaluated via MTT assay, microscopic evaluation and cell cycle analysis. Short-term exposure of dermal fibroblasts to different honey concentrations revealed a multi peak increase in cell viability at honey concentration of 0.024, 200, and 6400 µg/ml. Long-term exposure of 0.024, 200, 6400 µg/ml honey concentrations to dermal fibroblast proves to be non-toxic under microscopic evaluation and MTT assay. The selected dose of honey also improve proliferation and did not alter normal cell cycle progression in dermal fibroblasts. The positive effect that honey have on the proliferation of dermal fibroblast suggested the capability of stingless bee honey to improve the skin regeneration.

a92884

## 03-P413 Nanochitin-nanolignin complexes to deliver bioactive molecules for skin regeneration

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### Introduction

Chitin and lignin, by-products of fishery and plant biomass, can be reused and converted to high value materials for biomedical and cosmetic applications, which are bio- and eco-compatible. On the nanoscale, chitin and lignin crystalline structure and purity can be controlled, resulting inhigh antibacterial, anti-inflammatory, cicatrizing and anti-aging effectiveness (1).Combining electropositive chitin nanofibrlis (CN) and electronegative nanolignin (NL) gives rise to microcapsules able to entrap both hydrophilic and lipophilic molecules. The aim of this study was to test the efficacy CN-NL complexes *in vitro* with human keratinocytes (HaCat cells) and human mesenchymal stromal cells (hMSCs) for the delivery of glycyrrhetinic acid (GA), as a biomolecule with antibacterial and anti-inflammatory activity.

### Materials and Methods

HaCat cells were cultured in presence of CNs, NL, CN-NL, 0.2% GA and CN-NL/0.2%GA and MTT assay was performed at 48 h to define the optimal concentration of each component. Therefore, the anti-inflammatory and immune responses of HaCat cells were evaluated by assaying the expression of pro-inflammatory cytokines IL-1 alfa, IL-1 beta, IL-6, IL-8 and TNF-alfa, anti-inflammatory cytokine TGF-beta, and antimicrobial peptide human beta defensin-2 (HBD-2) by RT-PCR at 6 h and 24 h. HMSCs isolated from the bone marrow were cultured with the abovementioned components for 1 week. AlamarBlue was used to monitor hMSC viability at higher and lower concentrations. After 1 week, hMSCs were osteoinduced for 1 week and mineralization was detected by von Kossa staining.

### Results and Discussion

By using HaCat cells, non-toxic concentrations were identified for each component. Interestingly, the presence of GA, both alone and within the CN-NL complexes, increased 1.5 time the usable concentration.

Our data indicated that GA markedly down-regulated the expression of proinflammatory cytokines IL-1 alfa, IL-1 beta, IL-6, IL-8 and TNF-alfa and up-regulated the expression of HBD-2 in HaCat cells.HMSCs were viable, with no appreciable differences using 2× concentration of CNs, NL, CN-NL, GA and CN-NL/GA. Their use did not modify osteo-differentiation capability of hMSCs.

### Conclusion

These findings demonstrate that CN-NL/GA complexes are cytocompatible and have an anti- inflammatory activity on human keratinocytes.

### Acknowledgement

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a92156

## 03-P414 Fabrication and characterization of poly(vinyl alcohol)/Red seacucumber hydrolysate hydrogel for wound dressing application

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When skin wound occur, hydrogel patches have been generally used as wound dressing for wound healing. Poly (vinyl alcohol) (PVA) is known as a commonplace material which has non-toxicity, non-carcinogenicity, biocompatibility and easy processing to fabricate hydrogel patch. Red sea cucumber (RSC) is widely found in Jeju Island in Korea and RSC decomposed by flavourzyme increased cell proliferation and collagen synthesis on neonatal normal human dermal fibroblast (NHDF) then others. So, we designed to fabricate PVA hydrogel patches blended with RSC without chemical crosslink by using freezing/thawing method. We evaluated in vitro release property of hydrogel patches (0.25, 0.5, 1.0%) then swelling property in water and mechanical property to know hydrogel patch's mechanical characterization. As a result, we observed that hydrogel patches blended with RSC have a high water absorption and mechanical properties are suitable. Although further studies, including in vivo and signaling pathway for wound healing, will be studied, the comprehensive results suggest that hydrogel patches blended with RSC are promising candidates for wound dressing and tissue engineering applications.

a90575

## 03-P415 Skin model ex-vivo of a burn induced by a Laser

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**Background :** Second degree superficial and deep burns are challenging in their diagnosis based on the clinical appearance and evolution of the lesions. Superficial second degree burns require conservative treatment while deep second degree burns need surgical treatment. Pulse dye Laser (PDL) is a method of creation of a burn, depending on the fluence and the duration of the light. Our goal is to develop a model of burn skin at different depth in order to improve therapeutic strategies.

**Study Design:** A model of burn ex vivo (1x1cm$^2$) was made on human skin by varying fluence (and pulse number, duration) of a PDL. Skin samples were then held in culture and studied at different days after the burn in order to verify the histology and the capacity of the skin to heal spontaneously.

**Results:** These results show that the ex vivo model used on human skin is specific and reproducible in order to create second degree superficial and deep burns, close to clinical reality.

**Conclusion:** Ex vivo model used on human skin will surely allow the experimentation of new treatments and the use of cosmetic products in order to improve the management of burn patients.

a90976

## 03-P416 Characterization of keratinocyte cultures and self-assembled bilayered skin substitutes produced with a new in-house developed defined medium

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Skin epithelial cell (keratinocyte) culture and self-assembled skin substitutes (SASS) have greatly improved treatments for patients with severe burns. Currently, to properly culture human keratinocytes, fetal bovine serum (FBS) must be added to the medium. However, FBS exact biological composition is undefined and variations occur from batch-to-batch. FBS is also associated with a risk of xenogeneic pathogen transmission. For these reasons, discarding FBS usage in research and clinical contexts is desirable. Although keratinocyte-specific serum-free (defined) media are commercially available, they are not effective in culturing keratinocytes for more than a few passages and are not suitable for SASS production. Therefore, we aimed to develop a defined medium optimized for keratinocyte expansion and SASS production. Three candidate molecular factors capable of substantially promoting keratinocyte growth were initially identified from an experimental screening on one epithelial cell population. Different iterations ($\alpha$, $\beta$, and $\gamma$) of our defined medium for keratinocytes (DMK), which contained these factors in variable concentrations were then ranked over their ability to sustain culture quality. Daily population doublings, number of passages, and cell size were used as proxies. Keratinocytes cultured in DMK$\beta$ were smaller and could be grown over at least as many passages as in FBS-containing medium and more passages than the other DMK iterations. Also, keratinocyte population doubling rates were greater in DMK$\beta$. Keratinocytes cultured in either DMK$\beta$ or FBS-containing medium were morphologically comparable. SASS were then produced with both DMK$\beta$ and FBS-containing medium using keratinocyte populations from four donors. SASS macroscopic and histological analyses showed no clear differences between those produced with DMK$\beta$ or FBS-containing medium. SASS similarity was further confirmed with immunolabelling of keratins 1 and 5, Ki67, and filaggrin. A portion of the amplified keratinocytes used to produce the SASS were also used to compare complete transcriptomic profiles with RNA microarrays. $R^2$ for cells cultured in both media was nearly 0.99 and no more than 0.25% of gene targets were significantly differentially expressed. In conclusion, this new defined medium is promising for keratinocyte culture and SASS production for research and clinical purposes.

a92707

## 03-P417 Physical Properties of hydrated human acellular dermal matrix

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### Introduction

Human acelluar dermal matrices (ADMs) are widely used in tissue regeneration, including the treatment of burns and chronic wounds. An ideal dermal matrix should be biocompatible with good physical properties such as tensile strength and flexibility. And it also should be able to stimulate homogeneous cell proliferation and extracellular matrix (ECM) distribution without causing any immune response. As one of safe and effective biomaterials, lyophilized ADM has been widely used in many fields as a typical skin substitute. However, the physical properties of lyophilized ADM is not good enough compared to fresh skin. In order to improve ADM's physical properties, especially the flexibility of ADM, we manufactured the raw skin materials without lyophilization process. After that, the biological and physical properties of ADM were evaluated.

### Materials and methods

Lyophilized ADM(L-ADM) was prepared as follows : removal of the epidermis, decellularization, viral inactivation, and freeze-drying.Hydrated ADM(H-ADM) was prepared in the same way except the last freeze-drying step. After the whole process was done, L-ADM and H-ADM were evaluated by histological assessment (H&E staining), stiffness and tensile strength measurement. In order to observe cell proliferation and morphology on skin samples, fibroblasts (L-929 cell) were cultured on the surfaces of L-ADM and H-ADM, respectively. After 4 and 7 days, the cell proliferation was measured by MTT assay, H&E staining, and SEM.

### Results and DiscussionIn

this study, we removed the freeze-drying step to improve the flexibility of ADMs and compared the biological&physical properties of H-ADM with traditional L-ADM. We found that both two groups were well-decellularized. The flexibility of H-ADM was higher than L-ADM, but the tensile strength did not show significant difference between two groups.In addition, we also evaluated the cell proliferation on H-ADM and L-ADM by using MTT assay, H&E staining and SEM. All results indicated that cells attached and proliferated equally well on both surfaces.In conclusion, we confirmed that H-ADM showed better physical properties than L-ADM, as well as good cell proliferation ability.

a91671

## 03-P418 Development of 3D Bioprinted Reconstructed Skin Models Using Native and Non-native Bioinks for Hair Follicle *In Vitro* Regeneration

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A variety of human skin models have been developed for applications in regenerative medicine and efficacy studies. Typically, these *in vitro* models employ matrix proteins and scaffolds that are derived from non-human sources along with human skin cells, the fibroblasts and the keratinocytes. A key limitation of these models is that they still fail in recapitulating the cellular and microenvironmental complexity, such as presence of vasculature, multiple cell types (for e.g., melanocytes, neural and immune skin cells) and adnexal structures (for e.g., hair follicles, sebaceous and sweat glands), that are representative of human physiology. The use of recombinant extracellular matrix proteins as well as introduction of other cell types can overcome these limitations. In parallel, there is a growing interest in employing 3D bioprinting platforms for tissue engineering given the possibility for precise cell positioning, flexibility, reproducibility and high throughput. Here we present our work on increasing the complexity of reconstructed skin models through the (i) evaluation of animal and non-animal derived scaffold proteins, (ii) inclusion of other cells types (e.g. melanocytes) and (iii) adnexal structures (e.g., hair follicle) using 3D bioprinting. The screening of the basal membrane proteins (collagen IV, laminin and fibronectin) demonstrated that certain protein combinations increase proliferation of keratinocytes compared to the control (no protein coating) and improve epidermal differentiation. In the investigation of the dermal components (collagen I and III, elastin and hyaluronic acid), the primary influence on the rate of fibroblast proliferation was attributed to the source of the collagen type I (rat tail, human and bovine). We will present our results on the influence of the specific bioinks on fibroblasts and keratinocytes from different donors (adult and neonatal skin origins) as well as our preliminary data on the inclusion of the dermal papilla cells for hair follicle regeneration using 3D bioprinting. Our approach highlights the relevance of the bioinks composition and the 3D bioprinting technology to the development of complex Reconstructed Skin Models that are suitable for clinical translation.

a91939

## 03-P419 3D Bioprinting of Human Skin- an In Vivo Model

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Skin is the largest organ of the human body and the first barrier to outer environment. In case of injury, for example burn wounds, the barrier is compromised and the body is more exposed to the damaging environment. For larger injuries, skin transplants might be needed. However, this cause additional pain and suffering for the patient.

3D bioprinting can be used for biofabrication of human skin grafts with autologous cells. The printed skin can be transplanted with perfect fit to the patients wound, and thereby solve the problem with lack of skin for transplantation.

In this study, we compare a bioink based on fibrin, a native human protein active in the wound healing process, mixed with nanocellulose and alginate modified with RGD to control (bioink without fibrin). Human dermal fibroblasts (HDF) were mixed with fibrin bioink or control and 3D bioprinted. Human epidermal keratinocytes (HEK) were seeded in pure fibrin on top of the constructs. The printed constructs were implanted subcutaneously in mice. At 14 and 28 days, the scaffolds were harvested for histological and immunohistochemical analysis. Cell viability was high in both groups directly after printing. Histological analysis showed both cells in the construct and mice cells infiltrating. Immunohistochemical stainings for cell proliferation, formation of basement membrane, keratinocyte detection, and neovascularisation are under investigation.

a92508

## 03-P420 Design and 3D Bioprinting of biomimetic skin grafts

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Skin wounds are a massive problem within the medical community and the current standard of care becomes increasingly complex as the size and severity of the wound increases. Currently, the gold standard of severe wound care is a split-thickness skin graft which uses a smaller portion of the patients own skin and expands it to cover the wound. However, there is a limit to the area this treatment can cover and it can leave the patient with additional discomfort from the graft site. With recent advances in 3D bioprinting it is theorized that this technology could be used to print functional tissues and organs from a patient's own cells for later transplant into that patient. If this goal can be applied to skin wounds, then the medical community could significantly reduce the cost of care and time for treatment while simultaneously increasing the maximum wound surface area that is considered treatable.

In this project, human dermal fibroblasts were suspended in a polysaccharide and fibrin based bioink and printed alongside a sacrificial ink for greater precision. The increased control over bioink distribution should allow for greater nutrient diffusion in order assess the production of extracellular matrix within the constructs. The constructs were printed and cultured for 28 days to allow for complete differentiation of cells. In order to further mimic the skin, human epidermal keratinocytes were seeded onto the constructs and differentiation was attempted to create a two-layer skin mimic. Analysis of constructs was performed via scanning electron microscopy, fluorescence microscopy, confocal microscopy, and histology.

The results from the study showed that the printing of constructs alongside sacrificial bioink can create more intricate constructs with specific architecture.

a90067

## 03-P421 Epidermis, dermis, hypodermis bioprinting: a sun tanning 3D printed skin

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What can be more important for skin than the visual aspect, even if this skin is produced *in vitro* for therapeutics or testing purposes? A lot more for sure but when all the other aspects such as multi-layered structure (hypodermis, dermis and epidermis), autologous cell content and on demand availability using 3D printing are already present, this is the final touch.

We are presenting today a full thickness skin tissue produced using medical grade 3D printing technology and having the unique capability of sun tanning. The deeper layer of the *in vitro* engineered skin is the hypodermis, containing mature adipocytes with lobules of fat. The skin have the ability to produce a strong network of cellular matrix components, including elastin and other human skin markers in its middle layer (dermis). Finally, the epidermis of this skin, containing keratinocytes and melanocytes also presents typical skin microrelief that provides a good indicator of the quality and underlying structure of skin tissue.

Proof are given through immunostaining and electronic microscopy that this bioprinted skin presents all characteristics of human skin, both at the molecular and macromolecular level.

This skin, printed directly on the patient using fully implantable medical grade bio-ink (cGMP) mixed with autologous cells from simple biopsy, will have soon a strong impact on skin surgery strategies, particularly for severe burn patients. The technique used here is taking advantage of our cold extrusion bioprinting strategy [1-2] together with a specially designed 6-axis robotic arm having the ability to print directly on 3D complex shapes such as human body.

This application will also be presented with results from our pre-clinical small animal study using *in vivo* bioprinting of dermis/epidermis constructs.

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a91655

## 03-P422 3D Bioprinted Human Skin Models with RGD Modified Alginate-Nanocellulose Bioink

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**Introduction:** The repair of skin through the grafting of autologous, allogeneic, or synthetic constructs is one of the most commonly performed medical procedures for the treatment of dermal wounds such as burns and other lesions. Recently, three-dimensional (3D) bioprinting has drawn attention as an attractive technique for the rapid and reproducible fabrication of constructs that recapitulate striated tissue structures such as skin. However, prior to development of a fully functional bioprinted skin graft, The objective of these study was to utilize bioprinting to fabricate a multilayered construct that could be utilized to evaluate size, shape, pattern, and cell viability and behavior for ultimate application as an engineered skin construct.

**Results and Discussion:** Printed constructs post-fabrication their shape and dimensions after printing and during the culture process. Cell viability was good immediately after fabrication and during the culture period. This observation is consistent with previous work from our group. After 42 days of culture, cells were well spread and formed dense layers on the surface of the construct. Keratinocytes adhered on top of the fibroblasts forming two layers. The incorporation of the void regions within the printed constructs allowed nutrient diffusion and improved cell viability.

**Conclusions:** RGD conjugated alginate-nanocellulose bioinks can be utilized to fabricate multilayered tissue constructs that are suitable for dermal tissue applications. This preliminary study showed good cell survival and biological response after 42 days of culture. Future studies will focus on the generation of more complicated dermal constructs with additional layers that contain additional cell types and growth factors to better mimic native dermal tissue.

a91871

## 03-P423 3D bioprinting of hydrogels with controlled release of rhEGF for chronic wound repair

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**Statement of Purpose:** Chronic wounds, such as venous ulcer and diabetic ulcer, have been major healthcare issues worldwide. In Chronic wounds, growth factors (GF) at the wound site generally exhibit decreased viability. Thus, GF treatment has been intensively studied in chronic wound healing process, it was found that the delivery route has much impact on the cure [1]. In this paper, 3D bioprinted wound dressing made of Gelatin methacryloyl (GelMA) and Collagen hydrogel combined with microspheres containing Recombinant human Epidermal Growth Factor (rhEGF) was developed. The results showed that the rhEGF can strongly enhance the proliferation of Human keratinocytes cell line (HaCat). Therefore, it has the potential in chronic wound healing.

**Methods:** GelMA was synthesized as described as in detail [2]. Collagen was purchased from Biosharp (Sigma C-9879). HaCat was purchased from Biovector Science Lab, Inc.. rhEGF was kindly supplied by (Wendeng Hospital, China) The combination of GelMA /Collagen solution was blended with the I-2959 as a bioink. Microspheres with different amount of rhEGF was added to the bioink prior to 3D bioprinting. Bioink combined with rhEGF loaded microspheres were printed in a layer-by-layer fashion using 3D-Bioplotter (EnvisionTEC, Germany) to produce large wound dressings.

**Results:**

By adjusting the printing process and the concentration of GelMA and collagen, a 9×9cm wound dressing was printed with predefined line spacing. Large dressing can be handled easily for wound treatment. With adjustable spatial structures, nutrition can be easily accessed by cells at wound site.

Microspheres of different amounts of rhEGF were added to the bioink, it was found at 300$\mu$l/ml of rhEGF in bioink offered the highest cell viability and proliferation, compared with 100, 300, 500 and 800$\mu$l/ml ($p<0.05$). The dose dependent effect were evident when the microspheres containing rhEGF were added in the bioinks.

**Conclusions:** In this paper, 3D bioprinted large scale wound dressing made of GelMA and Collage was developed. The dose dependent effect of rhEGF on HaCat cell proliferation was found. Wound dressing with controlled release of rhEGF by 3D bioprinting is promising in chronic wound healing.

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a91927

## 03-P424 3D Bioprinted Human Skin – a Future Replacement for Animal Testing in Cosmetics?

**Isabella Bondesson[2], Evita Ning[3], Elin Pernevik[1], Patrick Thayer[1], Erik Gatenholm[1], Hector Martinez[1], Itedale Namro Redwan[1]**

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**Objective:** 3D bioprinting is a novel technique that shows great promise for the development of translatable tissue models to be used for drug development and cosmetic research. A skin tissue model that mimics native human skin is of great interest for pharmaceutical companies, as it could be used to develop new drugs to treat for example melanoma or psoriasis. For cosmetic companies, biomimetic skin models could be used to either develop efficacious topological creams, or to minimize the risk for skin sensitization from new products.

3D bioprinting is a method of using additive manufacturing in combination with biological systems. It is a novel field within tissue engineering, enabling the complex combination of the benefits of 3D cell culture and the precision of 3D printing. The key component is the bioink, which is a mixture of biomaterials with bioactive molecules, designed to enable both good printing fidelity and cell milieu. The bioink is mixed with living cells, and the final composition and shape of the construct will determine how well the tissue model mimics the native tissue.

The objective of this ongoing study is to develop a 3D bioprinted biomimetic human skin model.

**Methods:** Several bioinks (CELLINK™, Sweden) have been evaluated for the fabrication of 3D bioprinted skin constructs, including methacrylated gelatin and nanocellulose-based CELLINK™-RGD-Fibrin. Human primary dermal fibroblasts (HDFa, Fisher) were mixed with the bioink and printed as rounded discs using an INKredible+ 3D Bioprinter (CELLINK™, Sweden). The constructs were cultured under standard cell culture conditions for one day, then human epithelial keratinocytes (HEKa, Fisher) were seeded on top of the constructs. The constructs were cultured for 28 days, and samples were collected for immunohistochemical analysis and genetic expression of Collagen I.

**Results:** After 28 days of culture a well-formed stratum corneum was present in the CELLINK™-RGD-Fibrin-based bioink, indicating terminal differentiation. Expression of Collagen Type I was also detected in the 3D bioprinted skin construct.

**Conclusions:** We have developed potent bioinks and robust bioprinting protocols to generate a functional human skin model. The methods will be further improved by incorporation of Hyaluronic Acid (HA), collagen-based bioinks, and continued development of the construct design. 3D bioprinted human skin models show great potential to provide a future replacement for animal testing in cosmetics.

a92403

## 03-P425 Skin regeneration of Biodegradable artificial dermis/SVF using 3D Bioprinters

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Skin SBU, ROKIT Inc.

Stromal vascular fraction (SVF) is a cell pellet that contains ADSCs (adipose-derived stem cells), fibroblasts, endothelial cells, and epithelial cells. SVF can be extracted easily through self-liposuction, and since it is applied immediately after extraction, there are no side effects such as immune rejection. In addition, there is no culture and expansion as is the case in other types of stem cells, and hence, an economic and efficient option. Damage to dermis is caused when 2nd degree and 3rd degree burns are treated with autograft, resulting in a scar, which then requires transplantation on damaged skin. Burn patients should use dermatologic substitutes if autograft is impossible.

The artificial dermis used in this clinical procedure is biocompatible, biodegradable, which by rapidly engaging with cells, help with hemostasis, inflammatory response, proliferation of ECM (extracellular matrix) synthesis and remodeling process. SVF acts via autocrine and paracrine effects that stimulate growth factor secretion, such as epithelial growth factor (EGF), basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), and transforming growth factor-beta (TGF-b). It is known to promote reepithelialization, collagen synthesis, and neovascularization in a full thickness wound.

After debridement of scars, 3D cell printing of artificial dermis/SVF ($3x10^6$ cells / $cm^2$) was made and the product was applied to the wound area. Artificial dermis/SVF prevented scar formation and prevented shortening of wound healing compared to SVF injection. In addition, in cases of large scar (hypertrophic scar, keloid) SVF treatment has limitations. INVIVO 3D bioprinting enables cell printing with uniform density, free lamination depending on wound dermis depth, and area depending on wound shape. 3D cell printers are not limited to wound size and joint area.

In conclusion, 3D cell-printing of artificial dermis/SVF significantly reduced wound closure time and skin contraction in scar. It is expected that the effect of cells is enhanced by inhibiting necrosis through uniform cell printing. This technique is considered to be applicable to patients who require dermal implants such as burn, diabetic ulcers.

a91242

## 03-P426 Injection molding technique for automated fabrication of bilayered skin tissue models

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Three-dimensional (3D) tissue models provide an essential improvement over 2D cultures in means to mimic the natural environment of cells and to improve our understanding of cell behavior in healthy and diseased tissues. When manually produced, the quality of these models varies in every batch and is strongly dependent on the operator. The variation influences the properties of the tissue model and complicates comparison or reproduction of experimental results. To eliminate operator caused variations and to assess cellular responses in detail, a high level of standardization of 3D tissue models is essential. This limitation of the manual process and the increasing need for standardized 3D tissue models leads to the need to develop automated production methods. In our group, we investigate the feasibility to create multilayered tissue models by injection molding. This technique is fast, requires no manual steps and parameters are easy to control. Here, we present the prototype fabrication device and show the feasibility by creating dermo-epidermal skin models from highly concentrated collagen, human primary fibroblasts and keratinocytes. We are able to fabricate functional dermal equivalents in under one minute. We analyzed the structure of the skin and cell behavior with optical, histological and immunohistochemical methods *in vitro*. The resulting bilayered skin model shows minimal tissue contractions and similarities to human skin. The cells are viable and proliferate, keratinocytes attach properly.

The injection molding technique further allows to produce different geometric shapes, use various biomaterials and cell types. These first results are promising and we continue to advance the automated fabrication of 3D models to allow us and other research groups to investigate tissue models with a declining influence of manual production.

This work is part of the SKINTEGRITY project under the umbrella of «University Medicine Zurich»

a91011

## 03-P427 *In Vitro* Development of 3D Printed Custom Shaped Nipple-Areola Skin Grafts

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As prophylactic mastectomy rates have more than tripled in the past decade[1], the demand for breast reconstruction practices and increasing nationwide desire in restoring body image represent a significant unmet clinical need. Though current breast implants provide an external contour of the natural organ, restoring the appearance of a nipple-areola complex directly on the breast remains an unresolved clinical challenge. To address this unmet need, we used a tissue engineering approach to develop an *in vitro*, 3D printed custom-shaped skin graft composed of both a cell laden bioink (CLB, containing primary human dermal fibroblasts, hdFB, and keratinocytes) and a non-degradable epoxy macroporous backbone in the desired shape of a nipple. The non-degradable material choice is key in maintaining the complex shape of the nipple as the tissue matures once incorporated *in vivo*, as a degradable backbone would cause unwanted nipple flattening over time. Material compatibility was first tested with co-printed scaffolds of a medical-grade epoxy resin (MGE) and CLB that were cultured for several days and tested in cell viability. Healthy cellular growth was demonstrated as measured by increasing DNA content throughout culture and fluorescent hdFB images displaying healthy spindle-like spread morphology around the synthetic scaffolding. Internal architecture of the domed scaffold is also greatly varied to investigate the benefits of interconnected CLB porosity, such as possible improved cell seeding and channels to guide cellular migration and tissue ingrowth, and variations of MGE print patterns, such as control over the desired tissue mechanical properties. Mechanical integrity of the varying internal architectures was tested using an Instron implementing compressive testing and corresponding young's moduli, ranging from 18.7 ± 3.95 kPa to 2.94 ± 0.921 kPa, compare to what is seen *in vivo* for human skin[2]. Future work aims to validate the formation of both dermis and epidermis using histological and immunohistochemical methods, specifically evaluating cell morphology, presence of the basal membrane, and the expression of collagen I, keratin, and tight-junction proteins. Broadly, this study investigates the critical balance needed between synthetic materials for scaffold shape maintenance and degradable materials for tissue regeneration and reconstruction. **References**:1. Kummerow K. JAMA Surg, 150, 2014 2. Pawlaczyk M. Postepy Dermatol Alergol, 30, 2013

a90562

## 03-P428 Skin grafting on 3D bioprinted cartilage constructs *in vivo*

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3D bioprinting of cartilage is a promising new technique. In order to produce e.g. an auricle with good shape the printed cartilage needs to be covered with skin that can grow on the surface of the construct.

Our primary question was to analyse if an integrated 3D bioprinted cartilage structure is a tissue that can serve as a bed for a full thickness skin graft.

3D bioprinted constructs (10 x 10 x 1.2 mm) were printed using nanofibrillated cellulose/ alginate bioink (NFC) mixed with mesenchymal stem cells and adult chondrocytes and implanted subcutaneously in 21 nude mice. After 45 days, a full thickness skin allograft was transplanted onto the constructs and the grafted construct again enclosed subcutaneously. Group 1 was sacrificed on day 60 whereas group 2, instead, had their skin-bearing construct uncovered on day 60 and were sacrificed on day 75 and the explants were analysed morphologically.

The skin transplants integrated well with the 3D bioprinted constructs. A tight connection between the fibrous, vascularized capsule surrounding the 3D bioprinted constructs and the skin graft were observed. The skin grafts survived the uncovering and exposure to the environment.

a92912

## 03-P429 Conditioned Medium of Mesenchymal Stem Cell Promotes Epithelialization and Expression of Ephrin-B2 Related to Angiogenesis in Burn Wound Healing

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Burn wounds demonstrated to cause devastating effect in functionally and cosmetically. Compared to non-burn wound, it is considered as unique due to pivotal damage on tissue and vasculature which complicates the normal wound healing. Both epithelialization and the sprouting of new blood vessels are crucial events that support wound healing. The transmembrane ligand ephrin-B2 is known as molecular marker for arterial endothelial cells and early blood vessel formation. Conditioned medium (CM) of mesenchymal stem cells (MSC) is known to rich in cytokines and growth factors that potential for tissue regeneration. **Objective**: the present study aims to investigate the effect of MSC-CM in epithelialization and neovascularization of burn wound healing. **Methods**: Male Sprague Dawley rats were randomly divided into control (C), conditioned medium (CM) and medium complete (CM) groups. Burn wounds were created by placing preheated metal plat on body site of prepared rats and received daily local application of NaCl, CM and MC respectively. Skin wound tissues were collected in time courses and processed for histological slides. The percentage of wound closure was evaluated by visitrak. Neovascularization was evaluated by counting vascular density of blood vessels that expressed ephrin-B2 in granulation tissue. **Results**: Local application of MSC-CM showed to enhance skin regeneration by promoting wound epithelialization and increased the rate of wound closure compare to control, prominently in day-18 after wounded ($p<0.05$). Moreover MSC-CM showed prominent enhanced of ephrin-B2 expression and vascular density in granulation tissue starting from day 7, 14 and 21 post wounded, compared to control ($p<0.05$). **Conclusion**: our findings suggest the benefits of MSC-CM in burn wound healing through enhances wound closure and promoting ephrin-B2 expression that represent for neovascularization. Therefore local application of MSC-CM could support burn wound healing in part through promotion of epithelialization and angiogenesis.

*Keywords*: conditioned medium, mesenchymal stem cell, epithelialization, ephrin-b2, angiogenesis, burn wound.

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**Conflict of interest**

The authors stated that there is no conflict of interest regarding this study.

a93277

## 03-P430 Epithelial keratinocyte sheets prepared by temperature-responsive dishes enhance the survival rate on artificial dermis

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**Objectives:** For severe burn injury or traumatic skin defect, cultured epithelial-keratinocyte-sheet therapy has been used in clinical practice since the 1980s. However, the survival rate of epithelial keratinocyte sheet on dermal-like tissue constructed with the artificial dermis is extremely low. Enzymatic treatment is typically used for obtaining epithelial keratinocyte sheets, but it tends to break the adhesion and basement membrane proteins, and this effect is directly linked to decrease in the survival rate of epithelial keratinocyte sheet on artificial dermis. On the other hand, a temperature-responsive culture dish require no enzymatic treatment to harvest cell sheets, and the basal membrane proteins and intercellular adhesion proteins can remain in epithelial keratinocyte sheets prepared by temperature-responsive culture dishes. This study investigated the potential to enhance the survival rate of human epithelial keratinocyte cell-sheets prepared by temperature-reducing treatment on the dermis-like tissue and compared the rate with that of the cell sheets harvested by enzymatic treatment with rat full thickness skin defect model.

**Materials and Methods:** Under inhalation anesthesia with isoflurane, a skin full-thickness defect was created in the back region of nude rats. Artificial dermis was cut to the same size as the defect and implanted by 5-0 nylon suture. The silicone sheet of artificial dermis was removed at 2 weeks after the initial operation, and the dermis-like tissue regeneration was confirmed. An epithelial keratinocyte sheet prepared from human epidermal cells in a normal culture dish by dispase treatment (DT sheets group) or a temperature-responsive culture dish (TR sheets group) was grafted on dermis-like tissue. One week after transplantation, the engrafted section was observed to measure the survival rate of epithelial keratinocyte sheets.

**Results:** The survival rate of epithelial keratinocyte sheets was 33.8% in TR sheets group, and it was significantly higher than that of DT sheets group (16.2%).

**Discussion:** This study showed that the epithelial keratinocyte sheets prepared with temperature-responsive culture dishs remarkably improved the survival rate on the dermal-like tissue after artificial dermal implantation as compared with the conventional sheet. This result suggested that the further possibility of reconstruction with artificial dermis and cultured epidermal sheet for full thickness skin defect in clinical situations.

a90611

## 03-P431 Efficacy of Cultured Epithelial Autograft after Curettage for Giant Congenital Melanocytic Nevus of the Head

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Cultured epithelial autograft (CEA) is an epithelial sheet prepared from a patient's own skin and produced using Green's technique. In japan, CEA (JACE®; Japan Tissue Engineering Co., Ltd., Gamagori, Japan) has been used for patients with severe burn since 2007, and it was approved and covered by public healthcare insurance for use in the treatment of giant congenital melanocytic nevus (GCMN) in 2016. There are several treatment options for GCMN, and one of the options is curettage. We describe a case of GCMN in the occipital region that was treated using CEA after curettage.

A 2-month-old boy had a GCMN of 21 cm × 13 cm in his occipital region. The nevus was large, so we planned two-stage surgery using the application of a CEA after curettage. We used full-thickness skin taken from the back of right auricle to prepare a CEA at four months of age. Three weeks after preparing the CEA, we performed curettage of the right half of GCMN, and the CEA was grafted onto the wound afterward. The CEA took completely, and the epithelialization was observed at 10 days after surgery. During follow up, we confirmed good result; we then performed curettage with subsequent grafting of the CEA on the left half of GCMN at seven months of age. The CEA again took completely, and epithelialization was observed in this procedure as well at eight months of age. Time of wound healing using CEA is faster than conservative treatment. Alopecia is a severe issue of concern in the treatment of GCMN in head region; however no hair loss was observed in our case. The application of CEA after curettage may be a promising option for obtaining early epithelization and cosmetically better appearance.

a91113

## 03-P432 Development of a Simple Method for Human Fibroblast Multi-Layers in Refractory Cutaneous Ulcers

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We have developed a mixed cell sheet comprising of fibroblasts and peripheral blood mononuclear cells and verified the efficacy for treating refractory cutaneous ulcers in animal models using cell sheet technology[1-3]. This study aimed to confirm the functional efficacy of cell sheets manufactured using the simple multi-layered method. Fibroblasts were isolated from tissues of healthy volunteer and incubated. In order to form multi-layered sheets, the number of fibroblasts per $cm^2$ in a dish was determined in a range from 4.5 $\times 10^5$ to 12 $\times 10^5$ cells and incubated for 3 days. The vascular endothelial growth factor (VEGF) concentration in the culture medium was measured using enzyme-linked immunosorbent assay. The VEGF concentration was the same level in supernatant of more than 6 x $10^5$ per 1 $cm^2$. The renilla luciferase gene was transfected into human fibroblasts, and the fibroblast multi-layered sheets produced using 5 x $10^5$ cells per 2 $cm^2$ in 24-well plates were transplanted onto full-thickness skin defects on NOD/scid mice backs. The luminescence was observed until 35 days post-transplantation. We compared the concentration of growth factors, such as basic fibroblast growth factor, VEGF, hepatocyte growth factor, stromal cell-derived factor 1aplha, Angiopoietin-1, transforming growth factor-beta1, and epidermal growth factor in the supernatants and the exosomes secreted from the human fibroblast multi-layered sheets produced using 2 x $10^6$ cells per 9.4 $cm^2$ in 6-well plates using ELISA. The concentration of some growth factors was higher in the exosomes than in the supernatants, and the concentration of others was higher in the supernatants than in the exosomes. Our data suggest that this multi-layered method may be useful for manufacturing cell sheets for treating refractory cutaneous ulcers.

1. Sci Rep 6, 28538, 2016.
2. Am J Transl Res 9, 2340-51, 2017.
3. Sci Rep 7, 4843, 2017.

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a90925

## 03-P433 Human Plasma-Based Matrix released factors improve epidermal substitutes production and engraftment *in vivo*

**Marina Trouillas[1], Maia Alexaline[1,2], Brice Magne[1,3], Muriel Nivet[1], Amparo Zuleta[2], Thomas Leclerc[4], Eric Bey[5], Bernard Coulomb[1], Jean-Jacques Lataillade[1]**

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Cultured Epithelial Autografts (CEAs) represent a lifesaving surgical technique in case of full-thickness skin burn covering more than 60% total body surface area. However, CEAs present numerous drawbacks, including the high fragility of keratinocyte sheets, the highly variable graft take and the immaturity of the dermal-epidermal junction (DEJ), leading to heavy cosmetic and functional *sequelae*. To overcome these weaknesses, we developed a new human epidermal substitute (ES) cultured over a human-Plasma Based Matrix (hPBM) and have showed that this substitute was able to engraft *in vivo* with a good efficiency and to reform a healthy epidermis.

In this study, we investigated the role of plasma fibrin matrix on ES formation and engraftment. Therefore, we performed a thorough analysis of hPBM as a scaffold for epidermal substitute formation compared with a matrix of fibrin from purified fibrinogen (FPF) and with a culture without any matrix. We compared the ability of hPBM, FPF and a no matrix control to support ES formation and highlighted a few mechanistic pathways involved in this process

Accordingly, we analyzed by histology and immunohistochemistry hPBM, FPF and no matrix derived ES in *an in vivo* model of acute wound. We then identified the interesting chief factors released from the two scaffold matrices *in vitro* by ELISA. The role of selected released factors on ES formation was then investigated using migration assays, WB, IF, histology and immunohistochemistry

The use of hPBM for ES production induced more proliferation, better cell organization, greater dermal-epidermal junction protein deposition and prevented their degradation. Keratinocyte differentiation was decreased using both fibrin matrices. Growth factors released from hPBM in culture medium were different from those derived from FPF, and were shown to enhance in vitro keratinocyte migration and proliferation in ES and expression of DEJ proteins. Finally, the use of hPBM as a culture support for ES allowed better engraftment directly on a NOD-SCID model of acute wound with the formation of a functional dermal-epidermal junction.

Together, these results show the positive impact of fibrin matrices and their released growth factor on ES phenotype and grafting efficiency. We hope that this new strategy could improve the current medical treatment of full-thickness burn patients in the future.

a91502

## 03-P434 Improving the Efficacy of Autologous Keratinocyte Sheets on Ovine Burn Wound Healing Using a Novel Non-enzymatic Detachment Method of Cultured Sheets

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Background: Shortage in wound cover material is a challenging problem for management of burn patients. Large burn wounds are temporarily covered with cadaver skin which will eventually be rejected requiring autologous skin grafting. The cultured autologous keratinocyte sheets have been proposed as an alternative method; however, enzymatic detachment of these sheets is associated with certain disadvantages (i.e., fragility and poor acceptance) that prevented their translation to the clinical practice. **We hypothesize that non-enzymatic detachment of keratinocyte sheets exert better effect on grafted burn wound healing compared to enzymatically detached keratinocyte sheets.** We tested our hypothesis by comparing the effects of keratinocyte sheets detached by non-enzymatic method--temperature reduction (T sheet) vs. those detached by enzymatic method (D sheet) on novel and clinically relevant ovine model of grafted burn wounds.

Methods: Six full thickness skin burns (5X5cm) were induced in sheep dorsum. 24 hrs later, burned skins were excised and wounds were grafted with ovine cadaver skin. After 3 weeks, rejected cadaver epidermis was debrided and wounds were covered with keratinocyte sheets and the wounds were assessed for two more weeks. Then sheep were euthanized for wound sample collection.

Results: Cultured T sheets were stronger and their detachment were easier compared to D sheets. T sheets were better accepted with significantly higher wound re-epithelialization percentage than D sheets at days 7 and 14 after sheet grafting (95.5 ± 1.3 T vs. 59.1 ± 5.7 D on day 7 and 98.6 ± 1.4 T vs. 81.1 ± 6.0 D on day 14). Although the epidermis thickness was comparable in wounds treated with T and D sheets, the dermal-epidermal junction was well defined in wounds covered with T sheets at 14$^{th}$ day with continuous lamina densa and high number of hemi-desmosomes. Ovine cadaver skin was rejected within 10 days, which mimics rejection time in humans (~8.4 days).

Conclusion: Ovine grafted burn wound model closely mimics the clinical situation. The novel non-enzymatic method for keratinocyte sheets detachment using temperature-responsive culture dishes provides better sheet quality and allows to overcome obstacles preventing use of cultured keratinocytes in clinical practice.

a90861

## 03-P435 Autologous Adipose-Derived Stem Cells Reduce Burn-Induced Neuropathic Pain

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Background: Burn scar pain is considered as neuropathic pain. The anti-inflammation and anti-neuroinflammation effects of adipose-derived stem cells (ASCs) were observed in several studies. We designed a study using a murine model involving the transplantation of autologous ASCs in rats subjected to burn injuries. The aim was to detect the anti-neuroinflammation effect of ASC transplantation and clarify the relationships between ASCs, scar pain, apoptosis and autophagy. Methods: We randomized 24 rats into 4 groups as followings: Group A and B, received saline injections and autologous transplantation of ASCs 4 weeks after sham burn, respectively; Group C and D, received saline injections and autologous transplantation 4 weeks after burn injuries. A designed behavior test was applied for pain evaluation. Skin tissues and dorsal horn of lumbar spinal cords were removed for biochemical analysis. Results: ASC transplantation significantly restored the mechanical threshold reduced by burn injury. It also attenuated local inflammation and central neuroinflammation and ameliorated apoptosis and autophagy in the spinal cord after the burn injury. Conclusion: In a rat model, autologous ASC subcutaneous transplantation in post-burn scars elicited anti-neuroinflammation effects locally and in the spinal cord that might be related to the relief of post-burn neuropathic pain and attenuated cell apoptosis. Thus, ASC transplantation post-burn scars shows the potential promising clinical benefits.

a91567

## 03-P436 Tissue-Engineered Skin Substitutes with Stem Cells: a Case Series of 14 Burn Patients Indicating Clinical Effectiveness

**Lucie Germain[1,2,3], Danielle Larouche[1,2,3], Bernadette Nedelec[4,5,6], Isabelle Perreault[7,8], Louise Duranceau[7,9], Patricia Bortoluzzi[8], Chanel Beaudoin-Cloutier[1,2,3,7], Amélie Dumas[10], Ariane Bussière[10], Élie Boghossian[7], Jonathan Kanevsky[11], Yannick Leclerc[10], James Lee[11], François A. Auger[1,2,3], Véronique J. Moulin[1,2,3]**

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**Background**
There is a need for organs to replace diseased or fatally wounded tissues. The LOEX center from Laval University is specialized in stem cells and the production of living tissue suitable for grafting. Our research team developed an autologous tissue-engineered skin substitute produced by the self-assembly approach of tissue engineering SASS, allowing the replacement of both dermis and epidermis in a single surgical procedure.
**Objective**
The aim of this study was to assess whether the TES could provide a permanent organ replacement. Fourteen burn patients suffering from severe full-thickness skin loss were treated with SASSs on a compassionate basis through the Health Canada's Special Access Program.
**Method**
For the first treated patient, histological analysis and keratin (K) 19 immunostaining were performed to evaluate the integrity and the persistence of stem cells within the SASS before and 21 days after surgery. After surgical debridement of allografts down to healthy tissue, SASSs were applied to full-thickness wounds. Success of the procedure was evaluated based on the presence of a stable epithelium over time. Assessment of scar quality (skin elasticity, erythema, thickness) was performed on a subset of patients.
**Results**
For the first treated patient, histological analyses revealed the presence of a tissue similar in structure with normal human skin and confirmed the presence of basal keratinocytes expressing K19 in SASS before grafting that were also detected 21 days after grafting. The mean percentage of SASS graft take was 98% (SD 5). Integrity of the SASSs persisted over time (average follow-up time: 3.2 years) without noticeable deficiency in epidermal regeneration. Minimal scarring was observed. However, non-homogeneous pigmentation was noticed in several patients. We conclude that SASSs allow permanent skin replacement. This first-in-human application of a self-assembled skin substitute could lead to the treatment of various epithelial/stroma defects.
**Conclusion:** After grafting, the SASS has very good functional characteristics: minimal contraction and visible scars as well as long-term durability and tissue regeneration.
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a91617

## 03-P437 Autologous skin cell implantation for adult patients with deep burn lesions using a xenograft scaffold

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Burns are a worldwide health problem because the extensive skin defects induced by severe burn wounds are dangerous and can be fatal. Autologous skin transplantation for the treatment is the gold standard. When burn wounds comprise more than 40% of total body surface area, which is the case of most patients in our institution, it is difficult to find healthy autologous skin for the treatment. Tissue engineering uses different skin substitutes; the best substitutes are those that combine cells. For the treatment of burn wounds, it is very important to cover the wound. For a long time porcine skin has been used as a non-cellularized scaffold (1). In this research we used a construct made of autologous fibroblasts and keratinocytes seeded onto radio-sterilized pig skin (CPS). CPS was applied in patients with an average age of 32 years. Fibroblasts were positive for the Fb protein and keratinocytes for cytokeratin 5 and 10. The CPS got 98% of viability. 60% of the patients displayed integration of the CPS to the adjacent skin. No necrosis or infections in the implanted zone were found. No patients had development hypertrophic scars. In addition, 60% of the patients showed contraction in the wounds. There was an improvement in the healing process during the 7 days following the implantation of the construct. These results point that autologous CPS is safe and accelerates the regeneration process. However, further research is needed in order to demonstrate this.

1) Yu G, Ye L, et al. Mol Med Rep.13,2570, 2016

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a92467

## 03-P438 Multi-compartment collagen devices as modulators of skin fibrosis through controlled synergistic dual delivery of anti-fibrotics

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Fibrosis is a phenomenon characterised by the formation of excessive fibrous connective tissue, which can compromise the skin's function and its mechanical properties. This can cause a huge global burden on healthcare, with millions of patients suffering from cosmetic or even functional tissue impairment, which considerably reduces their life quality [1]. In order to treat this, Il-6 antagonists and decorin have been reported as potential anti-fibrotic therapeutics [2, 3]. Therefore, in this study it was hypothesized that multi-compartment crosslinked collagen type I systems can modulate skin fibrosis through the controlled synergistic dual delivery of an Il-6 antagonist and recombinant decorin.Multi-compartment hydrogel systems were prepared in a mould as follows: solutions of dialyzed type I collagen at a concentration of 5 and 8 mg/ml, were mixed with 10x PBS, neutralised and crosslinked with different densities, creating two separate compartments, that were loaded with decorin and an Il-6 antagonist. The systems were characterised through swelling assessment, collagenase degradation assay, rheological and compression tests.The release of encapsulated drugs from the hydrogels was studied by HPLC and the effect of the delivered bioactive agents was assessed through proteomic analysis and imaging for fibrotic markers in an *in vitro* keloid model. Mechanical and biological resistance of the systems were found to be suitable for potential implantation in the skin *in vivo*. Release studies proved that the inner compartment was capable of promoting a sustained release of decorin over a long period of time (14 days), fitting the intended therapeutic release profile. Proteomic studies showed a decrease of endogenous collagen type I, TGF-$\beta$1 and $\alpha$-smooth muscle actin expression indicating reduced fibrosis.It was observed that this system is mechanically robust and stable *in vitro*, besides being an attractive dual drug delivery system for skin fibrosis, ameliorating markers of fibrosis in an *in vitro* keloid model.

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a90538

## 03-P439 Improved viability of murine skin flaps using a gelatin hydrogel sheet combined with basic fibroblast growth factor ( bFGF )

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Backgrounds: Basic fibroblast growth factor (bFGF) is involved in fibroblast and epithelial cell proliferation and angiogenesis. Gelatin hydrogel slowly releases growth factors. Recently, sheet-type gels have been developed. In the present study, we examined the extending effects of bFGF- incorporated gelatin hydrogel sheets on the flap- survival area in a mouse flap model.

Materials and methods: A flap of 1 × 3 cm was elevated on the back of a 9-week-old male C57BL/6 mouse. Gelatin hydrogel sheets incorporated various concentrations of bFGF was placed under the flap. For comparison, saline was impregnated to prepare a control group (n = 12 in each group). On the seventh day after surgery, the flap-grafted area and flap vascular network were examined by imaging. Tissue samples were subjected to HE and von Willebrand factor staining for the histological examination of skin, fat, and cutaneous muscle layer thicknesses, the number of new blood vessels, and new blood vessel areas.

Results: Macroscopically, The flap of the bFGF-incorporated gel group markedly swelled, with a flap-survival area of 195.17 ± 41.74 mm² in the normal concentrations of bFGF- incorporated gel group, being significantly larger than 147.17 ± 48.79 mm² in the control group. Vascular image analysis of the flap showed increased angiogenic-joint parameters in these group. Although the thicknesses of the dermis were not significantly different, the thicknesses of the panniculus adiposus and carnosus were significantly increased in the bFGF-incorporated gel group. Blood vessel areas and the number of blood vessels were also significantly increased in the bFGF-incorporated gel group.

Conclusions: In the mouse flap model, bFGF-containing gelatin hydrogel sheets promoted angiogenesis and increased the flap survival area. The specification of bFGF-containing gelatin hydrogel sheet may be useful for improving the flap survival rate.

a91941

## 03-P440 Development of diblock copolymer brushes-modified particles to release drug with specific stimulation and application to biomedical cosmetic materials

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Almost all of the medicinal cosmetic foundations used generally are prepared with the mixture of crude materials and drugs. It is difficult for users to control the applied dose of drug to the skin by oneself. As a means to solve the problem, we propose a controlled release material which can control the applied dose of drug.

The object of our research is development of microparticles which can be applied as a component in medicinal foundation. We design microparticles which can release drug, and develop a system which drug to treat dermatitis is released by a specific trigger such as the environmental change associated with the diaphoresis. Diblock copolymer brushes which consist of poly(carboxymethyl betaine) (PCMB) and poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA) are constructed on the surface of microparticles. And drugs for treatment of dermatitis were electrostatically bound to PDMAEMA blocks. Therefore, it is expected that electrostatically-adsorbed drugs can be released by the change of surrounding environment such as ionic strength, sult concentration and pH.

We prepared diblock copolymer brushes composed of PCMB and PDMAEMA on glass plates and silica microparticles using surface-initiated atom transfer radical polymerization. The characteristics of diblock copolymer-modified substrate were investigated with contact angle measurement, $\xi$ -potential evaluation, X-ray photoelectron spectroscopy and attenuated total reflection-infrared spectroscopy, respectively. Moreover, the compatibility between the surface of microparticles and biological matter such as proteins and cells was also investigated. Additionally, the release of drugs from diblock copolymer brushes was evaluated, resulting that drugs electrostatically bound to PDMAEMA blocks were released in aqueous solution containing salt and in the weak acid solution.

The protein adsorption to the surface modified with diblock copolymer was significantly suppressed in comparison with the surface modified with homopolymer brushes of PDMAEMA. It was considered because PCMB blocks of copolymer had the anti-biofouling property. Moreover, the surface with diblock copolymer suppressed the fibroblast adhesion. Therefore, microparticles modified with diblock copolymer are expected to be inert material for the skin.

a91134

## 03-P441 Coacervate-mediated Dual Growth Factor Delivery for Scarless Wound healing

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For the development of better substitutional medical applications for a recovery of damaged skin tissues, skin tissue engineering have been extensively investigated for years, especially for scarless wound healing. Delivery of exogenous therapeutic growth factor (GF) is one of engineering strategies for skin regeneration. Here, we have developed an exogenous GF delivery platform using coacervate (Coa), that is a tertiary complex of poly(ethylene argininyl aspartate diglyceride) (PEAD) polycation, heparin, and GFs (TGF-$\beta$3 and IL-10). Coa has advantages of high biocompatibility, facile preparation, and sustained cargo GF release. Therefore, it is speculated that dual TGF-$\beta$3/IL-10 loaded into Coa exhibit a synergistical effect for a reduction of scar formation during physiological wound healing processes. Our result indicated that administration of exogenous TGF-$\beta$3 and IL-10 enhanced *in vitro* proliferation of human neonatal dermal fibroblasts, and it was further improved by Coa encapsulation. In terms of *in vitro* gene expression profiles using RT-PCR, upregulated expression of collagen and downregulated matrix metalloproteinase expression were observed specifically at early stage wound healing. *In vivo* examination using a rat skin excisional model (2 cm $\times$ 2 cm) demonstrated that a delivery of dual GFs using Coa accelerated wound closure and skin regeneration after 3 weeks of a direct injection into around damaged skin area. Moreover, *in vivo* RT-PCR data showed a reduced expression in collagen and $\alpha$-SMA while MMP-1 expression was increased. Histological evaluation and immunohistochemical staining also demonstrated that dual GF delivery using Coa accelerated clearly epidermis formation along with facilitated angiogenesis, as compared with the administration of bolus GFs without any delivery vehicles. Based upon these results, it is concluded that polycation-mediated Coa fabrication and exogenous dual GF delivery via Coa platforms effectively enhanced both quantity and quality of regenerated skin tissues without scar formation.

a93299

## 03-P442 Comprehensive evaluation of mesoporous silica nanofibres as novel drug-eluting system

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Recently, electrospun nanofibres have attracted attention as potential matrix for controlled drug delivery due to their possible barrier effect and unique characteristics – especially small fibre diameter and high porosity as characteristics leading to the extreme surface to weight ratio. In addition to these properties held by nanofibres in general, the chemical nature, surface functionality and charge represent properties strongly affecting their biocompatibility, degradation kinetics, the material-drug interactions and loading capacity. From this point of view, silica nanofibres represent a very promising material for this application, combining traditional nanofibres ´ properties and unique properties given by their inorganic nature altered by the mesoporous structure of their surface. Silica nanofibres prepared by sol-gel method and subsequent needleless electrospinning, being thermally stabilized under specific conditions were confirmed to be fully biocompatible, nonimunogenic and biodegradable. Their stability in common solvents enables their loading by low-soluble drugs and subsequent application and sustained release *in situ*. Their loading capacity and drug release kinetics can be easily altered/ modified by surface functionalization of nanofibres via functional groups grafting. In this work we present a comprehensive overview of biodegradation kinetics, biocompatibility results against several model tissues represented by selected cell lines (3T3 fibroblast, HaCat keratinocytes, Hep G2 liver cells and THP-1 monocyte). Drug loading capacity evaluation and release kinetics are also presented.

a92094

## 03-P443 $TiO_2$ nanotubes improve soft tissue attachment on Intraosseous Transcutaneous Amputation prosthesis

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Common problems for the external prosthesis users are pressure sores, infections and unnatural gait[1]. Intraosseous Transcutaneous Amputation Prosthesis (ITAP) was developed as a new generation of limb replacements that can provide an alternative solution to amputees. ITAP is designed as one pylon osteointegrated into the bone and protruding through the skin, allowing the mechanical forces to be directly transferred to the skeleton and the external skin being freed from frictions and infections. The skin attachment to the implant is critical for the success of the ITAP, as it provides a barrier to prevent bacterial infections and stabilises the soft tissue preventing relative movement between the skin and the implant[2 3].

TiO2 nanotubes is a novel biocompatible substrate that can promote cell attachment and proliferation due to its morphology and electrical charge[4]. In this study TiO2 nanotubes were tested for cell viability and attachment using human dermal fibroblasts and keratinocytes .

The surface of pure titanium discs was modified using electrochemical deposition with $TiO_2$ nanotubes with different sizes (18-30nm, 40-60nm and 60-110nm). Human keratinocytes and human dermal fibroblasts were seeded on the top of them for three days and tested for viability and attachment. A Mann-Whitney U test was used to compare groups where p values $< 0.05$ were considered significant.

The results showed that the viability and cell attachment for keratinocytes were significantly higher after three days on controls comparing with all nanotubes ($p=0.02$), while attachment was higher on bigger nanotubes and controls. Cell viability for fibroblasts was significantly higher on nanotubes between 40 and 110nm comparing with smaller size and controls ($p=0.03$), while investigation of cell attachment is ongoing. From these early results, we can say that $TiO_2$ nanotubes can improve the soft tissue attachment on ITAP. Further *in-vitro* and *ex-vivo* experiments on cell attachment will be carried out.

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a92255

## 03-P444 The antimicrobial activity of diphlorethohydroxycarmalol (DPHC) and fabrication of polycaprolactone nanofiber mats containing DPHC by electrospinning

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Wound is a shape of injury being damaged from dangerous part such as sharp part and rough surface. One of methods for wound healing is protecting injured parts from harmful bacteria. Wound sites can be infected with harmful bacteria easily, as well as the main reason protecting bacteria is that they can cause disease or complications. In order to protect bacteria, diphlorethohydroxycarmalol (DPHC) isolated from *Ishige okamurae*, a marine brown algae, is researched. DPHC is not researched antibacterial effect yet. Also nanofiber mat is fabricated through electrospinning as fabrication to contain DPHC having antibacterial effect helping wound healing. Electrospinning is a process making fiber between nano-diameter to micro-diameter though high electric charge and voltage. Polymer solution change thin fibrous form by electrical stimulation. polycaprolactone (PCL) is widely known and mainly use to make fabrication such as scaffold and nanofiber mats. In this study, DPHC is isolated and purified by HPLC and HPCPC. Also it is identified by Q-TOF Mass. To investigate antibacterial effect of DPHC, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) are examined against *S. epidermidis*, *C. albicans*, *S. aureus* and *P. acnes*. Nanofiber mat as fabrication is containing PCL/DPHC by electrospinning. To investigate cell viability, MTT assay is proceeded to NHDF-neo cell. As a result, MIC values are about 128 $\mu$g/mL and MBC values about are 512 $\mu$g/mL. The non-toxicity of DPHC is confirmed by MTT assay. Consequently we can suggest that PCL/DPHC nanofiber mats through electrospinning have possibility of wound dressing.

a90051

## 03-P445 Combining Skin Equivalents with Perfused Vasculature in a Multi-Organ-Chip

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The skin is one of the largest organs of the human body and consists of various different cell types. Acting as the outermost barrier of the human body, it protects the inner environment from the various outer environmental stimuli. Drugs and cosmetics applied to the skin need to be screened for toxicity and efficacy. Due to ethical and regulatory reasons, animal free test methods are gaining ground. To meet the rising need, human skin equivalents composed of one or two different cell types were improved substantially and are these days widely used. However, limitations to accurately predict the human outcomes still exist due to the simplicity of these models. In particular, the implementation of a perfused vasculature would pave the way to mimic structures and physiological responses of human native skin *in vitro* more closely. Recent advances in microfluidic systems offer great potential in this context. The Two-Organ-Chip (2-OC), a variant of TissUse Multi-Organ-Chip (MOC) platform, comprises a miniaturized circulatory system with an integrated micropump. This provides pulsatile circulation of microliter-volume of medium to the tissues in a similar way to blood. Skin equivalents can be implemented either within 96-well cell culture inserts or in direct contact with the medium flow. In this study, perfusable vascular channels have been successively integrated into a skin equivalent using photopatterning technique. Following attachment of the endothelial cells to the channel walls, the vascularized skin equivalents have been cultured with continuous pulsatile flow conditions inside our 2-OC for 7 to 14 days. Histological endpoint analysis showed a regular dermal/epidermal architecture of the skin equivalents and consistency and vitality after long-term co-culture. The endothelial cells covered all walls forming a viable fluid tight layer. Further, a physiological-like elongation and orientation with the direction of flow could be demonstrated. These results suggests the MOC a useful system for engineering a vascularized skin construct promoting long-term culture and a more reliable *in vitro* evaluation of topically and systemically applied drugs and cosmetics.

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a91660

## 03-P446 Poly-e--caprolactone/ β -cyclodextrin inclusion complex scaffolds for skin regeneration

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Electrospun scaffolds of Poly-e-caprolactone/ $\beta$-cyclodextrin inclusion complex (PCL/b-CD) and Poly-e-caprolactone amino derivative inclusion complex (PCL/b-CD.$NH_2$) were prepared by electrospinning technique. The obtained mats were analyzed by by X-ray diffraction (XRD), field emission scanning electron microscopy (FESEM), Fourier transform infrared spectroscopy-attenuated total reflectance (FTIR-ATR), Differential Scanning Calorimetry (DSC) Confocal-Raman Spectroscopy, Proton Nuclear Magnetic Resonance ($^1$HNMR) and Contact Angle Measure (CA). Different mixture of solvents, such as dimethylformamide (DMF) - tetrahydrofuran (THF), dichlormethane (DCM) - dimethyl sulfoxide (DMSO) and 2,2,2-Trifluoroethanol (TFE), were tested in the fibers preparation. The results indicate that electrospun nanofibers have a pseudorotaxane structure. Epithelial growth factor (EGF) was incorporated to the PCL-$\beta$-CD.NH2. The EGF degree of conjugation with the material was measured by colorimetric ninhydrin test resulting in 142 u/$cm^2$. The protein delivery profile was determined by colorimetric methods in physiological buffer, showing the absence of protein in the media until 5 days. The material morphology was determined by scanning electronic microscopy displaying fiber (462 ± 77 nm). The hydrophilicity was measured with a contact angle almost 0° with the inclusion of the EGF. Confocal Raman images showed a homogenous protein distribution along the fibers. Complementarily, the comparative study of the epithelial regenerative capacity was developed using mouse fibroblast model. The cell proliferation was measure by MTT test and cell counting on Neubauer chamber; the results shows an increase of the fibroblast proliferation the PCL-CD.NH2 and the EGF material in contrast with the control and PCL unmodified nanofibers. Scanning electronic microscopy of the treated fibers showed cell growth with the characteristic morphology of fibroblasts. Finally, the results of the in *vivo* test agreed with the *in vitro* study showing a better and faster cicatrization with the PCL- $\beta$ -CD.NH2 and EGF. In this work a new electrospun scaffold was produced with a potential application for skin regeneration.

a92783

## 03-P447 Evaluation of Novel Keratin Templates as Dermal Equivalents for Deep Partial-Thickness Burns

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Deep partial-thickness burns damage most of the dermis and can cause severe pain, scarring and mortality if left untreated. Current treatment methods involve wound excision and skin grafting. However, autografts are limited by availability while allografts face the risk of increased immunogenicity. The use of dermal equivalents such as Integra® and Pelnac™ alleviates these issues partially, but they are costly and may not be viable for large scale burns. We had earlier developed a template consisting of human hair keratins crosslinked with alginate using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC). This hybrid template was able to form a stable and porous scaffold through the process of freeze and freeze-drying. Our previous study showed that this keratin-alginate scaffold was capable of supporting angiogenesis *in vivo*. Herein, the performance of the scaffold was tested on deep, partial thickness burns in a pig model and evaluated in comparison with allografts and Pelnac™ via wound healing parameters including extracellular matrix maturation, angiogenesis markers, and immunology markers. Similar wound recovery rates based on Collagen I, Collagen III, and fibronectin expression, as well as wound size reduction and scarring, revealed that the keratin-alginate scaffold was comparable to Pelnac™ in supporting wound healing. Further testing of the effects of the keratin-alginate templates in deep burns should be explored to fully utilise their potential as an alternative dermal equivalent for burn wound treatment.

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a91096

## 03-P449 Construction of Polyethylene Glycol (PEG)-based Three-dimensional Scaffold Materializing Microenvironment of Human Dermal Fibroblasts Using PEILDVPSTV Peptide

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Human skin is a connective tissue three-dimensionally organized by dynamic complex network with dermal fibroblasts, melanocytes, and keratinocytes. Accordingly, three-dimensional (3D) culture of each cell type constituting skin tissue has been roundly requested for artificially constructing *in-vivo*-like skin tissue. In order to construct 3D scaffold stimulating proliferation of human dermal fibroblasts (HDFs) among skin cell types, a mechanical property of 3D scaffold and a concentration of RGDSP incorporated in the 3D scaffold were optimized in our previous study as a first step. However, this 3D scaffold didn't seem to play an important role in offering 3D microenvironments of HDFs sufficiently to construct *in-vivo*-like skin tissue. Therefore, as a second step toward constructing synthetic 3D microenvironments providing advanced HDFs-friendly network, we tried to construct PEG-based 3D scaffold with addition of concentration of PEILDVPSTV in the established PEG-based 3D scaffold. For these, the presence of integrin $\alpha_4\beta_1$ on the surface of HDFs was estimated using immunocytochemistry and attachment and antibody inhibition assay. Then, effects of extracellular signaling derived from PEILDVPSTV activating integrin $\alpha_4\beta_1$ in 3D PEG-based hydrogel on stimulating proliferation of HDFs and synthesis of extracellular matrix (ECM) proteins in those was elucidated after the culture of HDFs for 14 days in 7.5% (w/v) 4-arm PEG- based hydrogels combined with 3200$\mu$M of RGDSP and each concentration of PEILDVPSTV (0, 400, 800, 1200, and 1600$\mu$M) was conducted. As the results, localization of integrin subunit $\alpha_4$ and $\beta_1$ was observed on the surface of HDFs, and the integrin heterodimers $\alpha_4\beta_1$ was present in active form. In addition, even though no significant difference in proliferation of HDFs were detected among the groups, *col1a1, elastin,* and *FN* were significantly up-regulated on the mRNA levels in HDFs cultured in the PEG-based hydrogel containing 400, and 800 $\mu$M of PEILDVPSTV, and *lam5a* was significantly expressed in the PEG-based hydrogel containing 800 $\mu$M of PEILDVPSTV. From these results, we suggest that synthetic 3D scaffold derived from 7.5% (w/v) 4-arm PEG-based hydrogel with 3200 $\mu$M RGDSP and 800uM of PEILDVPSTV can closely materialize in-vivo like skin tissue.

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a91920

## 03-P450 Construction of 3D skin models using layer-by-layer cell coating technology for international standardization of the skin model (LbL 3D Skin) as alternatives to animal testing

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In vitro generated human skin equivalents are generating interest as promising tools in basic study, as alternatives to animal testing, and for clinical applications in regenerative medicine. We recently reported a simple and unique bottom-up approach to develop 3D multilayered cell composites by the fabrication of thin films consisting of nanometer-sized layer-by-layer (LbL) fibronectin (FN)-gelatin (G) (FN-G) onto the single cell surfaces[1)]. In this study, we developed a 3D human skin models (LbL 3D Skin) by a cell coating technique using LbL assembled FN-G thin films[2)]. The constructed LbL 3D Skin models were evaluated as an alternative skin for skin irritation tests[3)]. The LbL 3D Skin models with dermis consisting of normal human dermal fibroblasts (NHDF) and epidermis consisting of human keratinocytes (KC) were easily fabricated by using this technology. Histological analysis of the constructed LbL 3D Skin models revealed four distinct layers, basal, spinous, granular, and cornified layer in the epidermis and dermis. It was confirmed that the LbL 3D Skin models are applicable to skin irritation testing as defined in the European Centre for the Validation of Alternative Methods (ECVAM) Performance Standard (OECD Test Guideline 439). We further studied the construction of the models with density-controlled blood capillary networks using human umbilical vein endothelial cells (HUVEC). The results suggest that the LbL 3D Skin models allowing incorporation of skin appendages are more promising alternatives to animal testing, and can be applied to the design of physiologically relevant in vitro skin models. From August 2018, international standardization of our developed LbL 3D Skin models as alternatives to animal testing has begun. We intend to challenge international standardization of tissue models and change the face of industry with our new "building block" method. Moreover, we challenge the development of innovative tissue models as regenerative medicine and their international standardization by utilizing academic-industrial collaboration.

a91089

## 03-P451 Construction of Polyethylene Glycol (PEG)-based Three-dimensional Scaffold Stimulating Human Dermal Fibroblasts Proliferation Using RGDSP Peptide

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Human skin is known to be a connective tissue three-dimensionally organized by dynamic complex network with dermal fibroblasts, melanocytes, and keratinocytes. Accordingly, three-dimensional (3D) culture of each cell type constituting skin tissue has been strongly required for artificially materializing *in-vivo*-like skin tissue. In order to construct 3D scaffold stimulating proliferation of human dermal fibroblasts (HDFs) among a variety of skin cell types, a mechanical property of 3D scaffold was optimized in our previous study as a first step. However, this 3D scaffold didn't seem to play a prominent part in stimulating sufficient proliferation of HDFs to construct *in-vivo*-like skin tissue. Therefore, as a second step toward constructing synthetic 3D microenvironments optimized to advanced proliferation of HDFs, we tried to construct PEG-based 3D scaffold with addition of concentration of RGDSP promoting biological process of HDFs in the PEG-based 3D scaffold with the established mechanical strength. For these, the presence of integrin $\alpha_5\beta_1$ on the surface of HDFs was investigated through immunocytochemistry and attachment and antibody inhibition assay, and effects of extracellular signaling derived from RGDSP activating integrin $\alpha_5\beta_1$ in 3D PEG-based hydrogel on stimulating proliferation of HDFs were elucidated. Localization of integrin subunit $\alpha_5$ and $\beta_1$ was observed on the surface of HDFs, and the integrin heterodimers $\alpha_5\beta_1$ was present in active form. Subsequently, the 3D scaffold based on 7.5% (w/v) 4-arm PEG hydrogels was reinforced by addition of each concentration of RGDSP peptides (0, 1600, 2400, and 3200 $\mu$M), and HDFs encapsulated in these PEG-based 3D scaffolds were cultured for 14 days. With thin and spindle morphology of HDFs, significant increase of cell proliferation was detected in HDFs cultured in the PEG-based hydrogel conjugated with 3200 $\mu$M RGDSP, compared to the others. From these results, we suggest that synthetic 3D scaffold derived from 7.5% (w/v) 4-arm PEG-based hydrogel with 3200 $\mu$M RGDSP peptides can support effectively proliferation of HDFs.

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a90155

## 03-P452 The Evaluation of Apoptosis and Necrosis of Human Skin Cell Induced By High Hydrostatic Pressure

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**Introduction:**High hydrostatic pressure (HHP) technology is reported in various fields, for example, food preservation, bacteriology, and medical science. We previously reported that HHP of 200 MPa could inactivate nevus specimens. However, the estimated cell death upon application of HHP of 180–200 MPa for a duration sufficient for the inactivation of skin cells remains unclear. In this study, we evaluated the amount of time and pressure necessary for complete skin cell inactivation as well as cell-death pathways. **Material and Methods:**We prepared six types of human cells: dermal fibroblasts (HDFa; Gibco), adipose tissue-derived stem cells (ASCs; DS Pharma), epidermal keratinocytes (HEK; Gibco), epidermal melanocytes (HEMa-LP; Gibco), malignant melanoma cells (MM; Public Health England Culture Collection), and squamous cell carcinoma cells (SCC; DS Pharma). The pressure was increased to 150, 160, 170, 180, and 190 MPa and maintained for 1 s, 2 min, and 10 min. We performed the live/dead assay, WST-8 assay, apoptosis assay using annexin V, and the apoptosis assay using zVAD-FMKof cells with HHP**.** Furthermore, we observed HDFa that was pressurized at 190 MPa and kept for 1 s, 2 min, and 10 min by transmission electron microscopy (TEM). **Results:**The results of the live/dead assay indicated cell membrane damage caused by HHP. An increase in inactivated cell was observed in the cells applied with HHP at any intensity for 10 min. The WST-8 assay showed that dehydrogenase activity was still present in five cell types (HDFa, ASCs, HEMa-LP, SCC, and MM) applied with HHP at any intensity for 1 s and 2 min. However, no dehydrogenase activity was noted in HEKa with HHP for 2 min and 10 min and in other cells that had undergone >180 MPa of HHP for 10 min. The apoptosis assay using annexin V showed that in the group that underwent HPP for 10 min, the number of cells in the late apoptotic/dead group gradually increased when the pressure was increased. The apoptosis assay using zVAD-FMK was performed on HDFa and MM. Both HDFa and MM-exposed zVAD-fmk did not proliferate. We observed the explosion of cell membrane by TEM in HDFa with HHP of 190 MPa for 10 min. **Conclusion:**The application of HHP >180 MPa for 10 min completely inactivated the six types of human cells. The sensitivity of cell viability with HHP varies by cells. Necrosis was found to be the predominant cell-death pathway.

a92317

## 03-P453 Engineering Human Dermal Fibroblasts with Impaired Migration for In Vitro Models of Aged Wound Healing: Testing of New Biomaterial-Based Therapies for Chronic Wounds

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The geriatric population is prone to chronic wounds, which cost the UK National Health System £1 billion/year. Unfortunately, current treatments are inefficient. *In vitro* models that mimic the *in vivo* scenario would be ideal to test biomaterial-based therapies for chronic wounds so their behavior when used *in vivo* could be predicted. Aged dermal fibroblasts attach normally but show deficient migration due to a significant reduction in $\alpha 2\beta 1$ integrin function, although $\alpha 2$ integrin expression is normal[1]. We hereby report the engineering of a population of cells that mimicked the behavior of aged dermal fibroblasts usually found in the clinically challenging chronic wounds that could be used in *in vitro* models of aged wound healing to test new biomaterial-based therapies. For this purpose, we used synthetic RGD peptides that bind to integrins and have been used to study cell migration or in biomaterials to promote cell attachment. The first part of our study was carried out on 3 different 2D surfaces representative of the materials used to develop dermal scaffolds: hydrophilic synthetic polymeric surface, and fibrinogen and gelatin surfaces which both contain RGD binding sites. Different variables and RGD peptide concentrations were tested. Cell attachment, migration, and integrin expression were studied. Next, RGD treated cells were seeded in commercially available, clinically used, collagen-based 3D dermal scaffolds (Integra® and Matriderm®) and their attachment and migration studied. The mechanism of action of both scaffolds is through integrin ligands. Results on 2D surfaces showed cell attachment is reduced in a concentration dependent manner. Cells cultured on gelatin surfaces looked the most spread and obtained overall the least reduction in cell migration, suggesting that the number of RGD sites of the surface may influence the effect of the peptides. Expression of integrins was not affected and the components of the migration pathway were not altered by the RGD peptides. Results in 3D scaffolds showed that the concentration of synthetic RGD peptides necessary to impair migration of dermal fibroblasts should be tailored to the number of RGD sites present in the 3D matrix. We believe that this technology could be translated to other cell types including established cells lines, thus eliminating the need for primary tissue harvest.

**References:**

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a93241

## 03-P454 Dissecting the Role of Peripheral Glia in Murine Cutaneous Wound Healing by Single-cell RNA Sequencing

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Cutaneous wound healing is a complex molecular process aiming to re-establish the structure and functions of the injured skin. Among other disorders, peripheral neuropathies are known to impair wound healing. A former study from our lab has found that PNS cells, namely peripheral glia are crucially involved in this process. Wounding of the murine back skin led to activation of peripheral glia, by inducing de-differentiation, cell-cycle re-entry and dissemination of the glial cells from the injured nerve into the wound bed[1]. Transcriptome analysis of genetically traced and isolated glial cells from the wound further showed upregulation of many secreted factors previously associated with wound healing. Those factors stimulate TGF-$\beta$ signaling pathways, which further lead to increased myofibroblast formation, a hallmark of wound healing. Depletion of the glial cells led to impaired wound contraction and healing, further proving their important role in regeneration after injury. Regarding de-differentiation, injury-activated glia upregulated the Neural Crest Stem Cell (NCSC) marker p75/NGFR pointing to a NCSC-like cell state similar to the embryonic origin of the peripheral nervous system: the Neural Crest Stem Cells. This observation made us curious about whether such NCSC-like cells in the wound derive from residual stem cells present in and around the normal nerve or whether glial cells have the capacity to undergo such a de-differentiation process to acquire progenitor fates and contribute to the healing of the tissue. In other words, whether the cells with regenerative capacity represent distinct cell types or one highly dynamic population that gets activated upon injury. To answer this intriguing question, we want to do single-cell RNA sequencing on traced glial cells (*Dhh::Cre R26R::tdTomato*) from healthy skin and wounds at different stages of the regeneration process. By doing so, we will understand how nerve-derived cells contribute to wound healing in a temporally and functionally distinct manner over the course of the healing process of the skin. Our results will be highly relevant for further research not only in the field of cutaneous wound healing but also in other tissues for which regeneration has been shown to rely on innervation, such as heart and skeletal muscle.

Reference:

[1]Parfejevs et al., *Nature Communications* 236 (2018). doi: 10.1038/s41467-017-01488-2

a92319

## 03-P455 Defining xenobiotic metabolism in human skin and tissue-engineered skin-equivalents: an *in vitro and in silico* approach

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The presence of xenobiotic metabolising enzymes in the skin is of considerable importance for the pharmaceutical industry as these enzymes can convert bioactive chemicals to toxic metabolites that may lead to toxicity or hypersensitivity. Tissue engineered skin equivalents are used for toxicity research, however, whilst gene expression of xenobiotic metabolizing enzymes in skin and skin equivalents has been detected, data on protein expression and function of these enzymes in skin equivalents is limited. Moreover, there is limited *in silico* modelling with regard to cutaneous drug delivery and metabolism. Here we characterize gene and protein expression along with functionality of a subset of enzymes in native human skin and skin equivalent models to elucidate enzymatic profiles and activity. We further use mathematical models, parameterised against *in vitro* experimental data, to inform the spatial distribution of metabolism in tissue-engineered skin.

Human skin generally expressed increased levels of phase 1 and phase 2 enzyme mRNA and protein than skin equivalents. With the exception of GSTpi, both skin and skin equivalents expressed lower levels of enzymes than liver. GST activity in tissue engineered models revealed similar levels of activity between the epidermal and dermal layers, indicating that this enzyme is uniformly distributed. For mathematical modelling a coupled system of partial differential equations was developed to describe the transport of parent compound and metabolites through a geometry representative of skin and the model was solved using finite element methodology subject to boundary conditions that resembled the *in vitro* setup. The model predicted the spatial heterogeneity in the parent compound and metabolite concentrations within the skin. The parent compound concentration was highest at the corneum and decayed through the epidermis as a consequence of cellular metabolism and this was reciprocal for the metabolites.

In summary, xenobiotic metabolising enzymes are expressed in skin equivalents and these models will provide a useful tool to investigate xenobiotic metabolism of chemicals reaching the skin. Mathematical models, parameterised by diffusion and metabolism parameters, were able to predict the spatial distribution of parent compound and metabolite within *in vitro* skin equivalents and so may aid identification of toxicity profiles for defined chemicals.

a91428

## 03-P456 Development of a 3D Human Metastatic Melanoma Model for *In Vitro* Evaluation of Targeted Therapy Efficiency

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Melanoma cell sensitivity to targeted therapy molecules is dependent on tumor microenvironment (cell-cell and cell-extracellular matrix interactions). Three dimensional (3D) *in vitro* cell culture systems better reflect the native structural architecture of tissues and are attractive to investigate cellular interactions. We have developed and compared several metastatic melanoma models: from melanoma cells (SK-MEL-28 and SK-MEL-3, BRAF V600E mutant) cultured as monolayer (2D) to a co-culture on 3D dermal equivalents with fibroblasts to better unravel factors modulating cell sensitivity to a BRAF inhibitor (BRAFi) and a BRAFi combined with a MEK inhibitor (MEKi).

Cell sensitivity to treatments was evaluated under various aspects: cell proliferation (cell counting, EdU incorporation, MTS assay), MAPK and PKB/Akt signaling pathway analysis (Western-blotting), apoptosis (TUNEL), cytokine release (ELISA) and histology (3D models).

Metastatic melanoma cell proliferation inhibition upon BRAFi treatment was assessed in both models, without apoptosis induction. In 2D melanoma cells, phosphorylated protein expressions decreased during the treatment, whereas MAPK signaling pathway was not modified in dermal fibroblasts (BRAF wt). No effect on signaling pathways was observed in the co-culture model, due to the large proportion of fibroblasts. In these 3D models, dermal fibroblasts released high levels of IL-6, IL-8 and HGF on metastatic melanoma cells, cytokines involved in cell survival. However, despite this pro-survival factor release, BRAFi treatment was efficient on metastatic melanoma cells (assessed by cell proliferation and histology). Recently, first line treatment has evolved to a combined treatment (BRAFi + MEKi) that improves patient survival. The combined treatment was also tested on *in vitro* models and it appears to improve cell response, with strong decreases in DNA synthesis and melanoma cell proliferation in both models (2D and 3D co-culture).

In the long run, this model will be established with cutaneous metastatic melanoma cells isolated from patients. These 3D predictive patient-specific models will be used to determine personalized therapy strategies, as well as to understand the resistance phenomena of melanoma cells to treatments.

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a90319

## 03-P457 The role of nerve-derived cells in human skin wound healing

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Among the many factors that influence skin wound healing, the role of glial cells derived from the peripheral nervous system have recently gained particular attention. Using a mouse wound model, we found that upon wounding the dorsal skin, de-differentiated glial cells characterized by upregulated expression of SOX10, p75, c-JUN and pERK emerge from disrupted nerves and migrate into the wound bed. Genetic depletion of peripheral glia delays wound healing, which correlates with decreased myofibroblast content in the wound. Accordingly, genetic expansion of glial cell number leads to increased myofibroblast content, although without promoting wound closure. Moreover, glial transcriptome analysis reveals that upon injury, most upregulated genes code for proteins destined for secretion, and many of which play key roles in wound healing.

To assess whether de-differentiated glia also participate in human wound healing, we performed an extensive histological analysis of human skin samples. While non-wounded skin is devoid of de-differentiated glia, such cells are found scattered throughout the area of recently-closed wounds. Quantification analysis shows that chronic wounds, particularly those with associated diabetes, have a significantly lower number of de-differentiated glia than closed wounds. Intriguingly, in contrast to closed wounds, the de-differentiated glia are restricted to the periphery of chronic wounds, suggesting that these cells fail to migrate into the chronic wound bed or the chronic wound environment does not support the survival of the cells. At the other end of the wound healing spectrum, keloids, a type of over-scarred tissues, have a high proportion of de-differentiated glia despite little myofibroblast content. This differs from our observation in mice where increased numbers of de-differentiated glia correlate with increased amount of myofibroblast. This difference may either reflect a functional difference between glial cells in wounded mouse and human skin or be intrinsic to the properties of keloids. In sum, the results suggest that nerve-derived glial cells are important contributors to human wound healing.

a91963

## 03-P458 Developments of therapeutic agent for diabetic wounds aggravated by diesel exhaust particles using ginsenoside Rg1

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**Abstract:** Diesel exhaust particles (DEP) cause serious problems in our body, one of the main causes of air pollution. In particular, diabetic wounds are delayed in wound healing due to the regulation of apoptosis and inflammatory responses due to exposure of diesel exhaust particles. Red ginseng has a good effect on improving immunity and controlling blood sugar. In particular, ginsenoside Rg1 (Rg1), a saponin component of red ginseng, has an immune function and a skin improving effect. The authors treated a full-thickness skin diabetic wound in mice to assess wound healing efficacy via oral administration of Rg1. **Materials and Methods:** In this study, adult male ICR mice (N = 36) were used. The groups were divided into three groups according to oral administration of mice: a control group treated with phosphate buffered saline (PBS), a group treated with red ginseng extract, and a group treated with Rg1. Each mouse was orally administered daily for 10 days, sacrificed on days 4, 7 and 10 after wound induction. Histologic analysis was performed with hematoxylin-eosin (H & E) staining and Immunohistochemistry (IHC). Expression of growth factors such as vascular endothelial growth factor (VEGF) and transforming growth factor $\beta$ 1 (TGF-$\beta$1) by reverse transcriptase-polymerase chain reaction (RT-PCR) and Western blotting were also investigated. In our study, we examined whether the administration of red ginseng extract and ginsenoside Rg1 affects the expression of VEGF and TGF-$\beta$1 in the diabetic wound model. **Results:** Our knowledge showed that lack of VEGF and TGF-$\beta$1 expression in the control group (diabetic wounds not treated with red ginseng) did not affect wound healing stages. These results are indicative of the wound healing in the diabetic wound model, which slows the transition to the proliferative phase due to the slower response of the inflammatory phase. Red ginseng is effective in wound healing by regulating VEGF and TGF-$\beta$ expression, as suggested by several studies. Similarly, our results suggest that treatment of red ginseng in diabetic wounds may further promote wound healing. **Conclusion:** We observed that ginsenoside Rg1 is more effective in healing wounds of diabetic wounds

a92054

## 03-P459 Engineering Wound Healing using Directional Matrix Nanotopography with Varied Sizes

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Topographic features play a crucial role in the regulation of physiologically relevant cell and tissue functions. Here, an analysis of feature-size-dependent cell–nanoarchitecture interactions is reported using an array of scaffolds in the form of uniformly spaced ridge/groove structures for engineering wound healing. On these matrices, fibroblasts demonstrate a biphasic trend of cell body and nucleus elongation showing the maximum at intermediate feature density, whereas maximum migration speed is observed at the dense case with monotonic decrease upon increasing feature size. The directional organization of cell-synthesized fibronectin fibers can be regulated differently via the nanotopographical features. It is demonstrated that the properties of repaired tissue matrices in the process of wound healing may be controlled via the feature-size-dependent cell–nanoarchitecture interactions, which can be an important consideration for designing tissue engineering scaffolds.

a90139

## 03-P460 Omega-7 increases telomerase activity and improves ovine grafted burn wound healing

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**Introduction:** Delayed healing of grafted and donor site wounds of burn patient is the challenging problem. Various factors negatively affect cell proliferation in the burn wounds. It has been shown that telomerase activity (TA) is decreased in burn wounds. We have previously reported that sea buckthorn seed (SBS) oil improved healing of grafted burn wounds. Omega-7 (ω-7) is a most abundant fatty acid in SBS and it has been shown to be critically involved in skin and mucosal tissue regeneration. The effects of omega-7 on burn wound healing are not studied. The aim of this study was to test the efficacy of omega-7 on both 3rd degree ovine grafted burn and donor site wounds with a focus on TA. **Methods:** In vitro, scratch injury was made in cultured ovine keratinocytes and various doses of ω-7 were tested at 0 and 17 h. The numbers of colonies were counted at 72 and 96 h. In vivo, total 28 full-thickness flame burn sites with size of 25 $cm^2$ were made on the both sides of ovine dorsum. 24 h after burn, escharectomy and split-thickness skin grafts were performed. The wound sites were randomly allocated to 5% ω -7 or vehicle treatment. The donor sites were also randomly allocated to daily treatment with 5% ω -7 or vehicle. Grafted site unepithelialized area, blood flow, and donor site complete epithelization day were determined $7^{th}$ and $14^{th}$ d. TA was measured in grafted wounds at $14^{th}$. **Results:** In vitro, the percent of scratch wound closure of cultured keratinocytes was significantly higher with ω-7 treatment (0.1%) vs. vehicle (p=0.002). The number of colonies in ω-7 treated group at 72 and 96 h were significantly higher than control (p=0.02 and 0.002). In vivo, the grafted site: Unephithelized area was significantly smaller in treated sites than control sites at $7^{th}$ and $14^{th}$ d (p=0.003 and <.0001). The blood flow in treated sites was significantly greater than in control sites at $7^{th}$ and $14^{th}$ d (p=0.047, respectively). TA in ω -7-treated sites was significantly higher than control (p=0.007). Donor sites: The complete epithelization time was significantly shorter in treated sites than in control sites (p=0.02). The blood flow in treated sites was significantly greater than in control sites at $7^{th}$ d (p=0.034). **Conclusions:** Topical application of 5% ω-7 increases TA and accelerates full-thickness burn wound and donor site healing. ω-7 should be considered as a cost-efficient and effective supplement therapy to boost healing of skin wounds.

a92235

## 03-P461 Transplantation of autologous cells and microcarriers to promote wound healing

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Non-healing wounds causes significant disability for patients and large costs to the health care system. Treatment to achieve healing often involves transplantation, where split-thickness skin grafts (STSGs) is considered gold standard. STSGs are associated with several drawbacks, including donor site morbidity, lack of sufficient donor tissue and poor healing outcome. To overcome these hurdles, efforts have been made to develop tissue engineered skin substitutes, usually comprised of a combination of cells and biomaterials.

The current study investigated transplantation of autologous keratinocytes and fibroblasts seeded on porous microcarriers (MC) using a porcine wound healing model. The protocol was approved by the Swedish Board of Agriculture.

Keratinocytes and fibroblasts were isolated from tissue biopsies taken from six adult female pigs and expanded in the laboratory. Cells were subsequently seeded on MCs for further expansion and transplantation, thus avoiding enzymatic detachment of cells. The MCs and cells were transplanted to a total of 120 surgical full-thickness wounds and covered with occlusive dressings. The experimental groups included wounds transplanted with MCs seeded with both cell types, MCs seeded with each cell type individually, MCs only, each cell type in suspension, and NaCl control. Wounds were allowed to heal for one or two weeks before being excised and processed for analysis.

Haematoxylin and eosin staining revealed incorporation of MCs in the healing wounds, with some degradation of the material after one week. After two weeks, MCs were to a large extent degraded. Wounds receiving MCs with keratinocytes and fibroblasts displayed a significantly thicker neoepidermis compared to control wounds ($p < 0.05$) at both time points. The neoepidermis and cells adherent to the MCs seeded with keratinocytes stained positive for pancytokeratin.

Procollagen formation was found to increase in wounds transplanted with fibroblasts compared to controls, but only at the first time point. Wounds transplanted with MCs with or without cells showed an increase in procollagen content lasting throughout the experiment.

In conclusion, the experiments performed illustrate that autologous cells seeded on MCs is a promising candidate for skin transplantation, resulting in increased epidermal thickness and collagen formation. Future studies will focus on additional outcome parameters to evaluate long-term quality of healing following transplantation.

a93078

## 03-P462 Autologous full-thickness micro skin column grafting for wound repair without donor site scarring

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Background: Autologous skin grafting is the current mainstay for wound repair, but it is limited by donor site scarcity and morbidity. We recently developed an alternative method to harvest skin - in the form of multiple small (~0.5mm diameter), full-thickness "columns" - in order to harness the benefits of full-thickness skin grafting, while minimizing donor site morbidity.

Methods: The harvesting and donor site healing of full-thickness micro skin tissue columns (MSTCs), as well as the ability of MSTCs to enhance healing, were evaluated in swine. The ability of human-derived MSTCs to restore different epidermal and dermal components was determined by engrafting human MSTCs into skin wounds on immunodeficient mice, followed by immunohistochemical analysis. Soluble factors released by MSTCs were assessed by functional assays and multiplex ELISA.

Results: In the swine model, full-thickness MSTCs could be harvested in large quantities, followed by rapid healing of donor sites with no scarring. Autologous MSTCs "scattered" (i.e. without maintaining epidermal-dermal orientation) into full-thickness porcine skin wounds led to accelerated re-epithelialization and reduced contraction. Human MSTCs engrafted into skin wounds on immunodeficient mice resulted in the restoration of many key components of normal human skin, including stratified human keratinocytes and functional melanocytes in the epidermis, human fibroblasts, hair follicles, sebaceous glands, and functional sweat glands in the dermis. MSTCs also release many soluble factors known to play important roles in the wound healing process, and these MSTC-derived factors enhanced cell migration, proliferation, and endothelial tube formation in *in vitro* functional assays.

Conclusion: Our results show that harvesting and engrafting autologous MSTCs could be a practical, bed-side therapy for wound repair, without long-term donor site morbidities. This approach was recently commercialized, and a clinical device for applying this technology is currently being utilized in wound centers in the United States.

a91747

## 03-P463 Effects of Red-wavelengths OLED and Its *in Vitro* Differential Cell Effects

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Photobiomodulation(PBM) is a safe and non-invasive method that can provide various clinical effects. Conventional PBM devices using Light-Emitting Diode and laser have various problems such as low flexibility, relatively heavy weight and non-uniform effects. In this study, we present Organic Light-Emitting Diode (OLED) PBM patch which can be attached to the human body. It will provide practical performance (> 10mW/$cm^2$) even at low voltage (< 10V). In order to study the wound healing effects, the effects of OLED PBM patch was investigated using scratch-wound healing assay model of cultured normal human fibroblasts. Results showed that red-wavelengths OLED (630, 650, 670, 690 nm) have excellent stimulatory effects on fibroblast proliferation and migration. Interestingly, there are relatively large differences according to the wavelength used. Thus, our study showed that different biologic responses can be induced according to small wavelength differences. Among the wavelengths tested, 650nm and 670 nm induced rapid proliferation of fibroblasts. In addition, all different wavelengths and energy conditions also increased fibroblast migration. However, there are also relatively large variations with different conditions. Wavelengths of 630 and 650 nm induced more migration at low energy levels while wavelengths of 670 nm and 690nm induced more migration at high energy levels. These findings indicate that different wavelengths may show different biologic responses according to the energy level. Compared to LED platform, OLED platform can provide relatively pure wavelengths. All these findings clearly show that OLED PBM patch can be expected to be beneficial in clinical application compared to LED PBM patch but their effects need to be optimized by controlling the wavelengths and energy of OLED. From these observations, we can conclude that the wearable OLED system will be used in various therapeutic applications in the future, including medical and cosmetic procedures.

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a91748

## 03-P464 Feasibility of Molecularly Targeted Therapy for Tooth Regeneration: Potential for stimulation of the formation of a third dentition

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Missing teeth is a common and frequently occurring problem in aging populations. The exploration of new strategies for tooth replacement has become a hot topic. Using the foundations of experimental embryology, developmental and molecular biology, tooth regeneration is becoming a realistic possibility. The number of teeth is usually strictly determined. Recently, many candidate molecules might be those that are involved in embryonic tooth induction, in successional tooth formation, or in the control of the number of teeth. This means that it may be possible to induce de novo tooth formation by the in situ repression or activation of a single candidate molecule. Molecular targeted therapy is a type of treatment in which drugs or other substances are used to specifically attack certain cell types by interfering with critical target molecules. Molecular targeted therapy could be used to generate teeth in patients with congenital tooth agenesis by stimulating arrested tooth germs. $Runx2^{-/-}$ mice exhibit stunted tooth formation. Recently, a patient with a unique Arg131Cys missense RUNX2 mutation was shown to have a novel dental phenotype; i.e., no supernumerary teeth but one congenitally missing tooth.We demonstrated that the deletion of Usag-1 rescued the hypoplastic and poorly differentiated molar and incisor phenotypes seen in $Runx2^{-/-}$ mice. The rescue of tooth formation in genetically defined mouse models clearly demonstrates the feasibility of inducing de novo tooth formation via the in situ repression of a single targeted gene. Our investigations and related studies clearly validate the hypothesis that the de novo repression of target genes, such as Usag-1, could be used to stimulate arrested tooth germs in order to induce new tooth formation in mammals. Furthermore, rudimentary incisors were found to survive and erupt as supernumerary teeth as a result of USAG-1 abrogation. If we extrapolate our research findings to humans, they support the suggestion that a “third dentition” of one more sets of teeth can occur in addition to the permanent dentition. In this context, stimulation of the formation of a third dentition might be a novel approach in order to achieve biological tooth replacement.

a93013

## 03-P465 Platelet lysate-based compartmentalized systems for periodontal tissue engineering

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The destruction of periodontal tissue as consequence of periodontitis, a prevalent infection of gingiva, is a major clinical issue. Current clinical treatments enable to halt the disease, but the resulting repair outcomes are fairly variable and do not restore the periodontal tissue function. Periodontal tissue engineering (TE) aims at regenerating the periodontal wound by: 1) stimulating the self-heling ability of periodontium; 2) providing a stable matrix to drive the regrowth of both soft and hard periodontal tissue; 3) preventing of soft gingival tissue collapse into the periodontal wound.

The present study proposed the use of structured compartmentalized systems for addressing such requirements and explored the application of platelet lysate (PL), as a new tool in the design of periodontal TE approaches. These bi-layered compartmentalized system was composed of 1) a PL - based construct, aiming to regenerate the root cementum and periodontal ligament, and 2) a calcium phosphate cement composite incorporated with PL-laden microspheres, aiming to promote the regeneration of alveolar bone.

The system components were shown *in vitro* to be stable, capable of supporting cell adhesion and proliferation, and released relevant growth factors for periodontal healing (namely FGF, PDGF-BB and TGF-$\beta$1). Subsequently, the developed materials were assembled as compartments of the bi-layered system, with or without cells, and implanted in 3 - wall periodontal defect in rats. Our data showed that the stabilization of platelet-origin proteins on the root surface increased the overall periodontal healing score and restricted the formation of long epithelial junctions. Finally, it was assessed the ability of a radially oriented multi-patterned device as a suitable tool for selecting the most adequate topography to be casted in PL-based constructs aiming at improve the anisotropic arrangement of periodontal ligament cells.

Overall, results obtained clearly demonstrated the versatility of PL as a source of bioactive signals prone to orchestrate the healing of periodontal wound. Moreover, the stabilization of platelet-origin proteins over the exposed root surface was proved to be of utmost importance for the predictable regeneration of periodontal tissue.

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a90707

## 03-P466 Dimethyloxalylglycine-Embedded Poly( $\varepsilon$ -caprolactone) Fiber Meshes Promote Odontoblast Differentiation of Human Dental Pulp Cells

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The *in vivo* effect of prolyl hydroxylase inhibitors on the regeneration of the pulp-dentin complex is unclear. The purpose of this study was to investigate the effect of DMOG-embedded poly( $\varepsilon$ -caprolactone) fiber (PCLF/DMOG) on odontoblastic differentiation of human dental pulp cells (hDPCs) by transplantation of the dentin slice model. hDPCs were seeded onto electrospun PCLF and PCLF/DMOG in dentin slices and then transplanted into nude mice. The surface topography was evaluated for both PCLFs, and DMOG release from the PCLF/DMOG was examined. The effects of the DMOG/PCLF were assessed by histology and RT-qPCR. The PCLF/DMOG treated dentin slices showed higher cellularity with a palisading arrangement of hDPCs and organized collagen fibers. We found that the DMOG/PCLF significantly stimulated the expression of VEGF, DSP, and BSP in the hDPCs ($P < .05$) and mVegfa, mPecam 1, and mNefl in the surrounding host cells ($P < .05$). These results show that DMOG/PCLF has potential in pulp-dentin complex regeneration by promoting odontoblast differentiation of hDPCs and by enhancing host cell recruitment, angiogenesis, and neurogenesis, through the released DMOG-mediated cell responses.

a92915

## 03-P467 The effect of four different hemo-derivative platelet rich protocols on osteoblast behaviour

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***Objective*:** The purpose of this study was to prepare and compare five different autologous blood preparations using highly standardised manufacturing methods via a clinically available PRP production machine. This enabled the assessment of the various blood product influences on primary osteoblast. Further, quantitative comparison was performed of the amount of growth factors contained within and released from the products.

***Methods*:** Five different hemo-preparations were obtained from sheep blood: Advanced Platelet Rich Fibrin (A-PRF), Injectable Platelet Rich Fibrin (i-PRF), Pure platelet rich plasma (P-PRP), Leucocyte-Platelet rich plasma (L-PRP) and thrombus (CLOT). Human primary osteoblasts were cultured with media containing the various preparations at different dilutions. Live/dead, cell proliferation, migration and mineralization assays were performed. Additionally, pH variations and the grown factor release profiles for IGF-I, PDGF-BB, VEGF and BMP-2 were determined by ELISA as well as real-time PCR for genes encoding BMP-2, RUNX-2, ALP, OCN and COL1A1.

***Results*:** Positive influences on cell behaviour were found for all experimental groups compared to the control, except for A-PRF which revealed a negative impact at 60% or higher. pH alkaline conditions were observed for all groups but they normalised to physiological levels after 10 days. Grown factors release profiles were evident for 2 weeks, especially for VEGF and BMP-2. A lower i-PRF/BMP-2 release profile (300 ng/mL) was enough to evoke high BMP-2 relative gene expression at 3 days. A significant cumulative release profile of IGF-I and PDGF-BB was noted for the A-PRF and L-PRP groups at early time points.

***Conclusion*** Advanced Platelet Rich Fibrin (A-PRF) in its pure form negatively affected cell growth, migration, mineralization and viability of primary osteoblasts. Additionally, Injectable Platelet Rich Fibrin (i-PRF) demonstrated enhancement of osteoblast performance regardless of the concentration used. A sustained growth factors release profile was a characteristic of i-PRF.

***Keywords*:** PRP, PRF, bone formation, platelets, grown factors, fibrin

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a93429

## 03-P468 IGSF10: An Old/New Factor Benchmarked to BMP2 and Implications in Bone Regeneration

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**Introduction** There is an acute need for new bone regeneration factors, given adverse effects associated with BMP2. IGSF10 was first discovered in 2004 in a single report, showing its expression in fracture callus. However, little is known whether IGSF10 ligand may or may not promote osteogenesis. Here, we explored putative osteogenesis roles of IGSF10 by molecular signaling and in an in vivo orthotopic bone defect model.

**Materials and Methods** Donor matched periodontal bone and dental pulp samples were obtained from six human subjects after IRB approval. Periodontal cells (PCs) and dental pulp cells (DPCs) were isolated. Osteogenic differentiation was assayed with alizarin red and western blot, and benchmarked to BMP2. Cignal Reporter assay probed IGSF10 signaling. Critical-size, calvarial bone defects were created in C57/BL mice, followed by IGSF10 delivery. All quantitative data were treated with statistical analysis ($p<0.05$).

**Results** IGSF10 showed ~16-fold higher expression in remodeling-active periodontal tissue than donor-matched dental pulp, which is remodeling dormant, per RNA-seq data. IGSF10 promoted proliferation and mineralization in a dose-dependent manner. IGSF10 significantly promoted PC migration, similarly to 10% FBS. Remarkably, IGSF10 promoted robust mineralization and showed synergistic effects to BMP2. JNK and other signaling pathways were activated upon 100 ng/mL IGSF10 treatment.

**Discussion and Conclusions** These findings demonstrate IGSF10's robust osteogenesis capacity, and yet in a context-dependent manner and via MAPK/JNK pathway, instead of canonical BMP signaling. IGSF10's osteogenesis potential, independent of and yet synergistic to BMP2, suggests that it may reduce BMP2 doses and/or act as a new osteogenic factor.

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a92040

## 03-P469 IGF1 regulates morphogenesis of bioengineered teeth via proliferation and differentiation of dental epithelial and mesenchymal cells

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In dentistry, studies researching regenerative therapy to replace missing teeth have been performed. Functional bioengineered teeth have been produced by the organ germ method using mouse tooth germ cells. However, these bioengineered teeth were significantly small in size and had an abnormal crown shape when compared with natural teeth. The proper size and shape of teeth contribute to their normal function. Therefore, it is necessary to develop a method to control the bioengineered tooth morphology. In the present study, we investigated whether Insulin-like growth factor 1 (IGF1) has potential to regulate the size and shape of bioengineered teeth and underlying mechanisms.

We transplanted bioengineered tooth germs, which were treated with IGF1 during organ culture, into the jawbones of living mice. The size and shape of the developed bioengineered teeth were then examined. We further analyzed the effects of IGF1 on enamel knot formation and dental epithelial and mesenchymal cells *in vitro*.

IGF1 treatment significantly increased the size of bioengineered tooth germs with the normal tooth histology. The IGF1-treated bioengineered teeth, which were developed from bioengineered tooth germs in subrenal capsules and jawbones, showed the increase in not only size but also cusp number. Moreover, IGF1 increased the number of enamel knot marker, *fibroblast growth factor* (*Fgf4*)-expressing sites in bioengineered tooth germs, and enhanced the proliferation and differentiation of dental epithelial and mesenchymal cells.

This study suggests a novel strategy for the morphological control of bioengineered teeth by the activation of signaling of IGF1.

a92988

## 03-P470 Bioengineered Tooth and Alveolar Bone Constructs

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**Objective:** Our objective is to create living, bioengineered replacement teeth as an alternative to currently used synthetic dental implants. **Methods:** Our approach uses biodegradable scaffolds, seeded with human dental pulp derived stem cells (hDPSCs), or porcine derived dental epithelial and dental mesenchymal cells, and human endothelial cells (HUVECs), for in vitro and in vivo characterizations. Scaffolds currentl being investigated include: decellularized porcine tooth bud extracellular matrix (dtb-ECM) scaffolds; GelMA hydrogels; Dental Cell Sheets; and tyrosine derived E1001(1k)-bTCP scaffolds. Optimized cell-seeding densities and in vitro culture methods have been defined. Acellular and cell seeded constructs are cultured in vitro for 7 days in osteogenic media, and then implanted and grown subcutaneously in rats for 1, 3 and 6 weeks, or intra-orally in rabbits and mini pigs for 1 and 2 months. In vivo mineralization is assessed and quantified using microCT (Bruker), and dental cell differentiation and neovascularization are assessed using immunohistological and immunohistochemical analyses. **Results:** We have defined methods to create human sized *in vivo* implanted 3D tooth bud constructs that exhibit mineralized tissue formation of specified size and shape. Histological evaluations of paraffin embedded and serial sectioned 3D biomimetic tooth bud constructs showed dental cell differentiation marker expression and neovasculature formation. **Conclusions:** We have defined novel methods and approaches to create bioengineered tooth buds that form predictably sized and shaped mineralized dental tissues when implanted in vivo. We propose our biomimetic tooth bud constructs as useful models to study dental cell differentiation and mineralized dental tissue formation, and eventually as clinically relevant alternatives for tooth replacement in humans.

a91865

## 03-P471 Surface Modification of Artificial Tooth Root for Periodontal Tissue Reconstruction

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One of problem for dental implant is the lack of periodontal ligament (PDL), which can support teeth, prevent infection, and get the feeling such as chewiness. Our objective is to develop the tissue engineered periodontal tissue unit consisting of the mandible bone, PDL and artificial tooth. We prepared the mouse decellularized mandible bone (DMB) with PDL matrix and combined it with artificial tooth fabricated by 3D printer. When the DMB with PDL matrix was implanted under the rat renal capsule, the infiltrated cells oriented along the PDL collagen fibers. However the artificial tooth was not connected enough with PDL matrix. We tried to improve the surface modification of artificial tooth root. We here used PEEK as material for artificial tooth. The PEEK plates were graft polymerized using acrylic acid under several concentration. The graft polymerization was evaluated by staining. Then PAAc-PEEK plate were coated by HAp micro particles and performed crystal growth by immersion in $Ca(NO_3)_2$ and $(NH_4)_2HPO_4$ aqueous solution. On the surface of PAAc-PEEK, needle-like HAp growing were observed under certain condition. Therefor needle-like HAp coated PEEK could be obtained. These results would indicate that surface modification on PEEK was achieved. It suggested the application possibility to the novel periodontal treatment using surface modified artificial tooth and DMB with PDL matrix.

a93194

## 03-P472 Optimization of 3D Cell Printing for Alveolar Bone Tissue engineering

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3D printing allows the fabrication of custom-made 3D structures via layer-by-layer polymeric deposition to mimic the native architecture of human tissues, and hence has been used widely for tissue engineering applications. However, recent clinical trials of 3D printed polycaprolactone (PCL) scaffolds in alveolar bone defects showed that synthetic polymers, such as PCL, lack the desired degradation properties[1]. In this context, we hypothesise that the use of a natural polymer like gelatin, combined with cell printing, will be an efficient scaffold system for alveolar bone regeneration. We successfully functionalised gelatin with a mathacrylic group (GelMa) and used it to print alveolar bone cells using a microextrusion based 3D printer. The extrusion properties of different concentrations of Gelma were studied using a rheometer. 3D printing parameters were optimized and a suitable Gelma concentration was selected for further studies. The 3D printed GelMa scaffolds were further photo crosslinked with the help of the photocrosslinkers 2-hydroxy-1-[4-(2-hydroxyethoxy)phenyl]-2-methyl-1-propanone (Irgacure 2959) and lithium acylphosphinate salt (LAP). It was confirmed that LAP achieved effective crosslinking at sufficiently low concentrations that minimised cytotoxicity. Human primary alveolar osteoblast cells were printed and the cytotoxic effects of different parameters, such as concentration of photoinitiator, UV exposure time and inner diameter of the extrusion needle, were evaluated. The optimised parameters were used for cell printing, and cell growth in the scaffold was observed over 21 days using Live-Dead imaging. This study established a 3D bioprinting system which was optimised for the printing of primary human alveolar bone cells.

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a93412

## 03-P473 Bioprinting-assistant Extracellular Matrix Composition Optimization for Periodontal Treatments

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Periodontitis is an inflammatory disease worldwide that may result in periodontal defect and even tooth loss. Stem cell-based approach combined with the usage of injectable hydrogels has been proposed as a promising strategy in periodontal treatments. Hence, it is necessary to find an injectable hydrogel to deliver stem cells into the defects and serve as the extracellular matrix (ECM) mimic during healing. Stem cells fate is related with the ECM characteristics, closely depending on the ECM composition. Therefore, stem cell-ECM interaction should be studied to optimize the ECM composition for better stem cell transplantation. Here, we developed a bioprinting-assistant strategy to study stem cell-ECM interaction and thus screen an appropriate ECM composition for *in vivo* repair of periodontal defects. Periodontal ligament stem cells (PDLSCs) were encapsulated in injectable, photocrosslinkable composite hydrogels composed of gelatin methacrylate (GelMA) and poly(ethylene glycol) (PEG) dimethacrylate. PDLSC-laden GelMA/PEG hydrogels with varying composition were efficiently fabricated via a 3D bioprinting platform by controlling the volume ratio of GelMA-to-PEG. PDLSC behaviors, including cell viability, spreading and proliferation were studied by live/dead assay, phalloidin/DAPI fluorescence staining and CCK-8 assay, respectively. Osteogenic differentiation of PDLSC in GelMA/PEG composite hydrogels with different volume ratios was characterized by ALP activity, osteogenic gene expression analysis and Alizarin red S staining. We found that PDLSC proliferation, spreading and even osteogenic differentiation increased with increasing volume ratio of GelMA-to-PEG, and the optimized ECM (the 4/1 GelMA/PEG hydrogel) was screened. For the *in vivo* study, the robust and maximal new bone formation was found in the defects treated with the PDLSC-laden hydrogels compared to the hydrogels alone and the saline. This bioprinting-based strategy may be not only helpful for screening an appropriate ECM of PDLSCs and promoting repair of alveolar bone defect, but also fit for other cell – ECM screening studies and benefit of functional tissue regeneration.

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a93372

## 03-P474 3D printed microgroove patterns for angular organizations of periodontal ligament

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**Objectives:** The periodontal ligament (PDL) has systematical force-responses as the tooth-supportive structures to remodel, preserve, or maintain tissues with anchorages between mineralized tissues; cementum and alveolar bone. For tooth-supportive functions under occlusal/masticatory loadings, controls of specific PDL orientations in micron-scaled interfaces are currently challenging in periodontal tissue engineering. Recently, we developed the 3-D PDL architectures, which can spatiotemporally organize fibrous tissues in 3-D printing system.

**Methods:** To investigate optimization of microgroove-patterns for PDL organizations, we focused on 1) additive manufacturing distance and 2) additive directions of computer-designed features for tissue orientations. After manufacturing molds, poly- ε -caprolactone was casted into 3-D printed molds and surfaces were analyzed for topological characteristics. After *in-vitro* human PDL cell cultures within scaffolds, statistical correlations were calculated with angulations which made by PDL architecture direction (reference) and cell alignments. Cell nuclei and actins in cytoskeletons were stained and angulations were measured. SEM was utilized for surface morphologies of cultured cells. In-vivo subcutaneous experiments for 4 weeks are performed to evaluate PDL cell/tissue alignments and tissue formations using immunohistochemistry.

**Results:** Controllable microgroove patterns were generated on PDL scaffolds by the additive manufacturing method. *In-vitro* cell cultures, the 25um microgroove-ridge-distant scaffolds provided more predictable cell alignments than different distances. Moreover, additive directions can significantly determine the spatiotemporal angulations of PDL constructs such as 0˚, 45˚, and 90˚ orientations to desired PDL architecture direction. In *in-vivo* experiment, fibrous connective tissues were spatially organized with specific angulations of 25um-microgroove patterns.

**Discussion**: The additive manufacturing can create various microgroove patterns on scaffold surfaces with consistence. 25 $\mu$m microgroove patterns can organize fibrous tissue constructs with specific cell orientations in periodontal tissue engineering in *in-vitro* and *in-vivo*. The 3-D printing technique will facilitate PDL regeneration in PDL interfaces with the controls of oblique or perpendicular orientations to tooth-root surface.

a91118

## 03-P475 The development of 3d-printed strontium-doped calcium silicate scaffold for alveolar bone regeneration

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Periodontal disease is the most easily overlooked disease of oral cavity disease and often causes irreversible loss of periodontal tissue when it is found to have periodontal disease. Periodontal disease treatment can only remove the pathogen, can not make the periodontal tissue returned to its original state. There are two ways to treat defects in periodontal tissue, root-coverage surgery and guided bone regeneration and guided tissue regeneration. Root-coverage surgery can only be used in mild periodontal disease while severe periodontal disease is used to guide bone regeneration and guidance tissue regenerative surgery, but tissue growth is very slow, in order to accelerate the recovery of periodontal tissue, we use 3d printed scaffold to control the hole size and low material cost. There are many literatures shows that periodontal disease is closely related to osteoporosis. Therefore, we used a mixture of strontium and calcium silicate in this experiment because strontium and calcium have similar chemical properties, and strontium can enhance the osteogenic ability of osteoblasts, at the same time inhibit the differentiation of osteoclasts, but also can improve osteoporosis, and avoid cause the body's allergic reaction. Calcium silicate shows good osteoconductivity and promotes the growth of vascularized bone and human dental pulp cells (hDPCs). In addition, soft tissues in oral cavity can affect the growth of dental pulp cells. We use hyaluronic acid mixed with methyl cellulose as a preventive measure to prevent the effects of gingival tissue alveolar bone growing film covered the top of scaffold. Observation of the above test data hope that the scaffold has sufficient mechanical strength, but also have batter bone regeneration to facilitate future clinical applications.

a91252

## 03-P476 Effects of amniotic membrane as a substrate for human periodontal ligament fibroblast cell sheet on growth factor production

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**Objective:** The objective of the study was to develop a new cultured cell sheet effective for periodontal tissue regeneration. Amniotic membrane (AM) is a biomaterial with anti-inflammatory and infection-control effects. Its utility and effectiveness as a culture substrate for various cells has attracted attention. We have prepared human periodontal ligament fibroblast (hPdLF) cell sheets cultured on AM substrate and demonstrated that they could regenerate new bones (periodontal tissue) when transplanted into an experimental animal. In addition, the production of chemokines SDF-1/CXCL12 and CXCL14 are promoted using an AM substrate for hPdLF cell culture. In the present study, the effects of AM on the production of hPdLF cell proliferation/differentiation-inducing factors were examined.

**Materials and Methods:** The present study was conducted with the approval of the Medical Ethics Committee of our university (RBMR-C-1111-2). Human AM and hPdLF cells (Lonza) were used. HPdLF cells were cultured for three weeks on the AM with epithelial cells removed. RNA was extracted from the AM-cultured hPdLF cell sheet for global microarray analysis of the growth factor genes. In addition, the growth factors in the culture supernatant were immunologically analyzed. HPdLF alone was cultured as a control.

**Results:** Microarray analysis revealed high expression levels of IGF-1 and VEGF-A genes in the AM-cultured hPdLF cell sheet, compared with the control group. Dkk-1 gene expression was decreased. IGF-1, VEGF-A, BNDF, and NGF in the culture supernatant were significantly increased, while Dkk-1 was significantly decreased.

**Discussion:** The AM-cultured hPdLF cell sheet not only induced differentiation into bone tissue, but also promoted the production of growth factors and chemokines, and, therefore, should facilitate regeneration of the surrounding tissues by inducing migration and differentiation. The IGF-1 concentration in the supernatant of the cultured cell sheet was maintained for about three weeks, although it continuously decreased in the control group. Furthermore, the cultured cell sheet inhibited the production of Dkk-1, which inhibits the differentiation of mesenchymal stem cells into osteoblasts, potentially facilitating osteoblast differentiation.

**Conclusion:** AM is a useful substrate for culturing hPdLF, and the AM-cultured hPdLF cell sheet may be effective for periodontal tissue regeneration.

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a92933

## 03-P477 Regeneration of full size teeth using decellularized tooth bud scaffolds

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**Introduction:** At the present time, one of the major obstacles of biological tooth regeneration approaches is the inability to bioengineer full sized teeth. It has recently been shown that decellularized organ scaffolds can support the regeneration of full size organs, such as heart, bladder and lung. Our preliminary studies in a mini-pig jaw implant model showed guided dental tissue formation by dental cell-seeded decellularized porcine tooth bud scaffolds (dTB).

**Materials and Methods:** Minipig jaw tooth bud constructs were prepared as follows. Unerupted molar tooth buds (TB) were harvested from discarded 6 month-old pig jaws and decellularized by successive SDS/Triton-X cycles. Three types of replicate tooth implants (n=3) were prepared: 1) dTB seeded with three types of cells (Recell-dTB) including porcine dental epithelial (pDE) cells, human dental pulp (hDP) cells and human Umbilical Vein Endothelial Cells (HUVEC); 2) acellular dTB scaffolds alone; and 3) freshly isolated non-decellularized natural TB (nTB). Replicate implants were implanted in fresh tooth extraction sockets of adult (2Yr) Yucatan mini-pig hosts and grown for 2 or 4 months. Each animal received 2 implants in each hemi-mandible. For each time point, seven minipigs were used, including two empty socket controls.

Staged Xylene orange and calcein dye injections were performed to label newly formed calcified tissues. At harvest, mandibles were fixed in formalin via perfusion and examined using Micro-CT. Imaged mandibles were then either embedded in PMMA for hard tissue sectioning, or decalcified, embedded in paraffin, and sectioned for histological and immunohistochemical analyses.

**Results and Discussion:** Preliminary Micro-CT results indicated the formation of tooth-like structures of comparable size to natural human teeth (Figure 1). Bright field imaging of sectioned mineralized PMMA embedded, and paraffin embedded and sectioned demineralized constructs both showed the formation of tooth-like structures predominantly in Recell-dTB. Immunostaining was used to confirm mineralized dental tissue formation.

**Conclusions:** Our results demonstrate that dTB scaffolds may provide the potential for functional human whole tooth regeneration.

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a92667

## 03-P478 BMP-2/7 heterodimer produced gene therapy for alveolar bone regeneration

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Background: The alveolar bone is very important for tooth retention. However, periodontitis or trauma cause loss of the alveolar bone, which is not spontaneously regenerated.

Methods: We constructed BMP-2/7 heterodimer produced gene expression plasmid vector, pCAGGS-BMP-2/7. BMP-2/7 heterodimer could induce the ectopic bone formation more rapidly than BMP-2 or BMP-7 homodimer. We injected pCAGGS-BMP-2/7 into the periodontal tissues of the first molar in the maxilla of 9-week-old male Wistar rats and immediately electroporated in the condition of 50V., 50msec., 32 pulses. In the histological analyses, we obtained time course samples and conducted hematoxylin-eosin staining and immunohistochemical staining. To investigate the mineral apposition rate (MAR) of alveolar bone in the target site, we performed double bone staining using calcein and tetracycline.

Results: Five days after the BMP-2/7 gene transfer, the new alveolar bones were formed in the target areas. MAR of the target areas were rapid than that of the control site.

Conclusions: We constructed the animal model of the gene therapy for alveolar bone regeneration in rats. Therefore, it could be expected to be applied this system clinically.

a90735

## 03-P479 Interleukin 4-loading mesoporous silica nano particles facilitate dental pulp regeneration by functional immunomodulation

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To date, dental pulp regeneration remains to be a major challenge in modern dentistry, which results in permanent tooth devitalization and then the consequent complication such as tooth fracture. This creates the needs for tissue engineered substitutes to facilitate the regeneration of damaged pulp tissues—which are mainly due to pathogen infection and the related sustained inflammatory response. Macrophage—the key player of the innate immune response—is activated by pathogens and then polarized towards its pro-inflammatory phenotype, which leads to a microenvironment unsuitable for dentin-genesis; while this polarization could be reversed by the cytokine interleukin 4 (IL-4) and therefore benefits the pulp tissue repair. The current study aims to develop the customized mesoporous silica nano particles (MSNs) to deliverer IL-4 in a controlled manner, effectively converts macrophages from the pro-inflammatory phenotype (M1) towards the tissue regenerative one (M2), therefore creates a beneficial microenvironment for pulp regeneration. Out results showed that MSNs facilitated the long-term controlled release of IL-4; the IL-4-loading MSNs (I-MSNs) effectively drove macrophages polarizing towards the M2 phenotype in infectious conditions. Moreover, the I-MSNs treated macrophage-derived secreted factors were found to improve the odontoblast differentiation of dental pulp stromal cells (DPSCs) *in vitro*, as shown in upregulated odontogenic markers such as dentin sialophosphoprotein (DSPP) and dentin matrix protein 1 (DMP-1); as well as induced mineral deposition. Therefore, our study provides a promising therapeutic approach for pulp tissue regeneration in endodontics.

a93436

## 03-P480 Development of Novel Chitosan Based Nanosphere to Prolong PRP Growth Factors Release in Dental Tissue Wound Healing

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In this study, PRP was encapsulated in chitosan to form a nanosphere with size below 100 nm with an idea to prolong growth factors release. Chitosan nanosphere was prepared by ionic gelation method, while PRP was encapsulated by inclusion method. Results showed that PRP was encapsulated efficiently in the chitosan matrix with stable colloid, having average size of 51.27±33.75 nm; suitable for biomaterials used in body. The protein release assay showed an initial burst, followed by a steady-release, then "quasi-plateau" after 96 hours (at around 60%). The nanosphere also showed an excellent antibacterial activity against S.*mutans* (90.63% bacteria inhibition). The results showed that chitosan – PRP nanosphere could be used as a novel approach for complex wound healing and tissue regeneration following periodontitis treatment or tooth extraction.

a93115

## 03-P481 EGCG promotes osteogenic differentiation along with anti-inflammatory effects in dental implant surgery: in-vitro study

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Most time in dental implant surgeries, bone substitutes are needed and widely used for the successful outcomes. Regardless of many clinically available bone substitutes, the risk of inflammation cannot be avoided. This in-vitro study aimed to investigate the effect of Epigallocatechin gallate (EGCG) which can be derived from plants and is known to be effective on preventing or treating inflammation. Moreover, the effects of EGCG on differentiation of stem cells (human mesenchymal stem cells) were also studied as osteogenic differential environments are critical to satisfactory outcomes.

A commercially available bone substitute (BoneD bone-xp®, MedPark, Korea), derived from porcine spines, was used. For the initiation of inflammation LPS(100ng/ml) was used. Five groups were set: (1) cultured in basal media (G1), (2) cultured in osteogenic media (G2), (3) addition of LPS to group G2 (G3), (4) treated with EGCG in group G3 (G4), and (5) application of hydrostatic pressure (HP) to group G4 (G5). Cell viability was accessed by SEM and DNA assay. All the markers related to inflammation and osteogenic differentiation were measured by RT-PCR at day 6 and 8. ALP assay was also performed.

All the groups showed good viability with no significant difference among them, which confirmed the harmless to the cells. The inflammation related makers were highly expressed at 6 hours after LPS was added. However, their expressions were remarkably decreased thereafter when EGCG was engaged. The osteogenic markers in group G4 were highly expressed than those in group G3, and even higher than those in group G2. However, the current study did not show positive effect of HP as only one pattern of HP was engaged. Through this in-vitro study combined with bone substitute, not just cell study, showed another potential of EGCG, osteogenic differentiation capability, in dental implant surgery. Further studies on long term, various patterns of HP and animal experiments are recommended.

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a91247

## 03-P482 A NOVEL BIPHASIC BIORESORBABLE SCAFFOLD FOR GUIDED TISSUE REGENERATION

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**BACKGROUND:** Bone grafts are often inserted into defect sites in the jaw to encourage bone formation prior to inserting periodontal implants. As bone is relatively slower growing than the surrounding gum tissue then a barrier membrane (BM) is often used to prevent soft tissue overgrowing the site on grafts where bone formation is required. While collagen membranes are most commonly used they cannot preserve their physical barrier function for more than a month and with bovine collagen one needs always to consider the risk of disease transmission.

**AIM:** Our aim was to develop a synthetic biodegradable BM that would act as a physical barrier for preventing epithelial invasion for up to 4 weeks without limiting the diffusion of waste and nutrients, and which would guide bone formation by ingrowth into a highly porous and interconnected bone-like structure.

**EXPERIMENTAL:** A pre-polymer of PCL was synthesized and methacrylated to obtain a photocurable polymer (PCLM). The first layer (L1) was produced by casting of PCLM polyHIPE under UV light. To produce a barrier layer to prevent soft tissue entry, we have previously reported a bilayer membrane consisting of nano and microfibres that can support both bone-forming cells and soft tissue without allowing intermingling of cells for several weeks (Puwanun et al. 2016). Accordingly to provide barrier function by preventing epithelial cell infiltration while allowing cell attachment and proliferation, we first electrospun PCL dissolved in 4 separate solvent compositions to find the best solute to manufacture random nanofibers for this second layer (L2). We compared fibre diameters and bead formation on SEM images. Fibroblasts and human embryonic stem cell-derived mesenchymal progenitors (hES-MPs)-capable of forming bone- were labelled with different fluorescent dyes and they were cultured over 4 weeks on L1 and L2 respectively. A resazurin reduction assay was performed for assessment of cell viability on each side. Fluorescent and histological images showed that fibroblasts were not able to penetrate into the L2, while L1 encouraged the penetration and ingrowth of hES-MPs.

**CONCLUSION:** A bilayer membrane was successfully produced by combining PCLM polyHIPE and electrospun PCL nanofibers. This novel bioresorbable membrane has potential to be used in GTR applications by acting as a barrier for epithelial cells for up to 4 weeks while allowing bone cells to grow in the implant zone.

a92008

## 03-P483 Isolation, Characterisation and Osteogenic Differentiation of Human Periodontal Ligament Stromal Cells in vitro

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**Introduction:**

Bone replacement is a necessary steps for management of oral pathological and traumatic defects.The current study aims to investigate the osteogenic potential of human periodontal ligament stromal cells (hPDLSCs) for bone tissue regeneration *in vitro*.

**Methods:**

Human periodontal ligament tissues were isolated from sound molar teeth obtained from Leeds Dental Research Tissue Bank.The presences of progenitor cells were confirmed using Colony Forming Unit- fibroblast (CFU-F) Assay. Cells from three different donors were cultured at a density of 100 cells/35 mm diameter petri dish in basal medium for ten days. Flow cytometry was used to measure the expression levels of mesenchymal stem cell markers (CD29, CD73 and STRO-1) and haematopoietic cells markers (CD34 and CD45). Osteogenic differentiation of hPDLCs was induced by culturing in StemMACS OsteoDiff Medium, with cells cultured in a basal medium were used as the control group. Alkaline phosphatase (ALP) activity of these cells was examined with ALP stain and activity assay. Immunocytochemical stains were used to examine the expression of osteogenic markers (collagen type-I, osteopontin, osteocalcin). Extracellular deposition of Calcium and phosphate was detected by Alizarin red and Von Kossa stains.

**Results:**

The isolated hPDLCs demonstrated a fibroblast-like spindle shape with a prominent nucleus. Among the three donors, the CFU-F data illustrated that the percentage of colonies containing more than 50 cells each was 42.2 ± 12.6. this reflect the donor dependency donor dependent. Moreover, these cells expressed high levels of both CD29 and CD73 markers with a lower level of the STRO-1, while, haematopoietic markers were shown at lower levels. After 21 days of culturing in osteogenic differentiation medium, the cell morphology demonstrated more cuboidal-like shaped, with a large nucleus and numerous cytoplasmic processes. Furthermore, those cells showed a deeper ALP stain compared to control group. This result was confirmed by ALP assay. Immunocytochemical stains proved the presence of higher levels of bone-related proteins. Similarly, these cells demonstrated stronger red and black stains for Von Kossa and Alizarin red stains respectively.

**Conclusion:**

Human periodontal ligament tissue contains mesenchymal stem cells that can differentiate into osteogenic lineage with the extracellular mineral deposition capacity. This indicates the potential of using hPDLCs for bone tissue regeneration.

a93030

## 03-P484 Plasminogen activator inhibitor-1 promotes cementogenesis by human periodontal ligament stem cells under 3-D culture conditions

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**Background:** Cementogenesis, using human periodontal ligament stem cells (HPLSC) that have multilineage differentiation abilities, would be highly beneficial to periodontal tissue repair. Three-dimensional (3D) culture systems have been applied in regenerative medicine strategies because they provide a convenient in vitro model for cell-cell and cell-matrix interaction. In this study, we examined whether the cementogenic differentiation HPLSCs can be accelerated using 3D culture systems.

**Methods:** Confluented HPLSCs were treated either with growth medium (GM; DMEM+10% FBS) , osteogenesis-induced media (OIM; GM containing ascorbic acid, glycerophosphate, and dexamethasone), or cementogenesis-induced media (PAI; OIM containing 100 ng/ml recombinant human plasminogen activator inhibitor-1[rhPAI-1]) for 7, 14, 21, and 28 days. HPLSCs cultured for 14 days, were formed into spheroids using the ultra-low binding plates with 10,000 cells per well. As a control, cells were plated as adherent monolayers. To enhance the cementogenesis of HPLSCs, we performed that HPLSC spheroids, treated either with GM, OIM, or PAI, were entrapped in collagen hydrogels for 5 days. We examined that the effect of OIM and PAI on an induction of both osteogenesis and cementogenesis in HPLSCs via western blotting and immunocytochemical assays, and alkaline phosphatase (ALP) and alizarin red (AR) staining.

**Results:** HPLSC spheroids treated with OIM and PAI showed increased expression of osteogenic factors (Runx2 and osterix), compared with monolayers cells treated with them. Both factors were localized immunocytochemically in the intranuclear portion. PAI induced unregulated expression of cementogenic factors, cementum protein 1 (CEMP1) and cementum attachment protein (CAP), in HPLSC spheroids. In contrast to cytoplasmic expression in monolayer cells, HPLSC spheroid-derived cells treated with PAI showed intranuclear expression of CEMP1. Intensity of ALP and AR staining was observed in HPLSC spheroid-derived cells treated with PAI, compared with monolayer cells. Furthermore, sections of collagen hydrogels containing HPLSC spheroid treated with PAI revealed a formation of cementum-like extracellular matrix, showing collagen type I and CEMP1 staining, and AR-positive mineralized structure.

**Conclusion:** Our data show that HPLSC in 3D culture with the addition of PAI has the potential for the regenerative reconstruction of periodontal tissues.

a90900

## 03-P485 3D human cell-based *in vitro* gingival tissue model

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The development of three-dimensional tissue engineered gingival tissue with physiological phenotypic is critical to better understand the underlying mechanisms for gum disease, plaque progression, oral cancer, and gingivitis, amongst other oral diseases. Current gingival tissue engineering strategies are mainly based on planar, two-dimensional culture systems on a support membrane (i.e., collagen, polycarbonate) to grow immortalized cells into a stratified epithelium at air-liquid interface. Here, we developed a high-fidelity, 3D gingival tissue, based on a lyophilized silk protein sponge scaffolding, seeded with human primary gingival fibroblasts and keratinocytes for the development of a novel and human-relevant in vitro tissue system. The use of silk scaffolds provides biocompatibility, porous features for transport, robust mechanical properties, retention of size, shape and open porous structure for extended time frames in culture, and no chemical crosslinking is required to establish the stable matrix. We demonstrated that the relevant 3D human cell-based *in vitro* gingival tissue model showed physiological tissue organization and cellular composition, as well as differentiated epithelium organization, based on trans epithelium resistance and markers of differentiation. In addition, the design of a physiologically relevant gingival pocket allows the formation of an anaerobic area deep in the sulcus to foster the proliferation of oral pathogens. The sustainability of such cultures (for at least 6 weeks) should support future studies of long term chronic infections. This tissue model should provide new information on how bacteria aggregate in association with living tissues, as well as on the growth of the organisms and the onset of virulence.

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a92393

## 03-P486 Tissue engineering a model of bisphosphonate-related osteonecrosis of the jaw

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### Introduction

Bisphosphonate-related osteonecrosis of the jaw (BRONJ) is a disease found in patients taking bisphosphonates (BPs), a group of drugs widely used to treat osteoporosis and bone metastases. BRONJ often follows dental surgery, and presents as exposed, necrotic sections of the jaw where the overlying soft tissue fails to heal. This study aims to model the soft tissue effects of BRONJ in vitro using a 3D oral mucosa model.

### Methods

Human oral fibroblasts and keratinocytes were cultured onto de-epidermised dermis for 3 days before models were lifted to air liquid interface (ALI). Models were then cultured at ALI for up to 14 days to allow keratinocytes to form a stratified epithelium. Different wounding methods were tested to optimise the model. After growth at ALI, the models were dosed with pamidronic or zoledronic acid, two of the most commonly prescribed BPs worldwide. Resazurin assays were used to quantitatively assess the effects of wounding and BPs, with histology used for qualitative assessment of growth and healing. Cells were also seeded in 2D to further investigate BP effects.

### Results

Tissue comparable to native oral mucosa was cultured, with histology showing a stratified squamous epithelium. Wounding caused a drop in cell viability which was recovered over time. Biopsy punch wounds to the epithelium healed completely over 10 days. Cell viability was lowered in the presence of physiologically relevant concentrations of BPs in a dose dependent response, in both 2D and 3D. Assessment of the oral mucosa model's response to wounding in the presence of BPs is ongoing.

### Discussion

BRONJ is a complex condition, with several processes involved in its development. The bone must be exposed through the oral mucosa for a BRONJ diagnosis, and the impairment of soft tissue wound healing is thought to be instrumental, with the majority of BRONJ cases preceded by tooth extraction or other dental surgery. An *in vitro* model of the condition will allow for a much greater understanding of the disease and its pathophysiology, and present a method by which to study potential treatments.

a93521

## 03-P487 A Clinical Report of Periodontal Regeneration Therapy by Gelatin Hydrogels lncorporating bFGF

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Gelatin hydrogels have been demonstrated to effectively achieve the regeneration repairing of various tissues by basic fibroblast growth factor (bFGF). This paper reports a clinical study of periodontal regeneration therapy by utilizing the gelatin hydrogels incorporating bFGF. bFGF is released from the gelatin hydrogels incorporating bFGF for 2 weeks through the water-solubilization of gelatin by the enzymatic degradation of hydrogels in vivo. A film of gelatin hydrogel incorporating bFGF( 0.3% averagel00ug ) was applied to the alveolar bone defect of 5 patients with periodontal diseases without any additional treatments. The X-ray examinations 6 months after the operation revealed that an attachment gain of 3 mm in the periodontal pockets was defected for 3 patients while their pathological motion of tooth treated disappeared. The patients showed the regeneration of alveolar bones clinically acceptable and the regeneration of healthy and thick gingiva was observed in 2 cases among them. For all 5 patients, no adverse effects were detected while their periodontal inflammation was clearly relieved. lt is concluded that the gelatin hydrogel incorporating bFGF was a promising therapeutic method to induce the regeneration repairing of periodontal tissues damaged.

a91773

## 03-P488 Fluorination enhances the osteogenic capacity of porcine hydroxyapatite

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**Introduction:** In a previous study, we successfully prepared fluorinated porcine hydroxyapatite (FPHA) by immersing porcine hydroxyapatite (PHA) in an aqueous solution of 0.25 *M* sodium fluoride (NaF) under thermal treatment, and the resulting FPHA showed better physicochemical and biological properties than PHA. The purpose of this study was to further investigate how fluorine incorporation influenced the biocompatibility and osteogenic capacity of PHA.

**Materials and methods:** The concentrations of Ca, P, F, and *M*g ions in PHA and FPHA extracts were detected by ICP-OES. Rat bone marrow stromal cells (rBMSCs) were treated with PHA and FPHA extracts, and the effects of these extracts on cell proliferation and osteoblastic differentiation were evaluated *via* CCK-8 assay, alkaline phosphatase assay, and RT-qPCR. For the *in vivo* assessment, PHA and FPHA were implanted into subcutaneous pockets (n = 6) and rat calvarial defects (diameter = 5 mm, n = 14) for 12 weeks to determine their biocompatibility and osteogenic capacity using micro-CT and histological analysis.

**Results:** FPHA extracts, which release higher concentrations of F and *M*g ions, better promoted the osteoblastic differentiation of rBMSCs *in vitro*. The result of biocompatibility evaluation confirmed the host response and chronic inflammation cells infiltration degree around PHA and FPHA granules were similar. Micro-CT and histological analysis showed more new mineralized bone formation in rats with FPHA-treated defects than in rats with PHA-treated defects.

**Conclusion:** The results of *in vitro* and *in vivo* tests consistently indicate that fluorine incorporation effectively enhanced the osteogenic capacity of PHA.

a92670

## 03-P489 ALK5 is essential for tooth germ differentiation during tooth development

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The TGF$\beta$ superfamily plays an important role during tooth development, with TGF$\beta$1 and TGF$\beta$3 regulating odontoblast differentiation and dentin extracellular matrix synthesis. However, the expression of TGF$\beta$ family member ligands is well-characterized during mammalian tooth development, less is known about the functions of TGF$\beta$3 receptor, a heteromeric complex consisting of a type I and type II receptor. As a key member of the TGF$\beta$ superfamily, the molecular mechanism of ALK5 (also named TGFBR1) in the dental mesenchyme is not clear. In the present study, we investigated the role of ALK5 in dental mesenchymal cells from the lower first molar tooth germs of E15.5/16.5 mice. Human recombinant TGF$\beta$3 protein or an ALK5 inhibitor (SD208) was exogenously added to the cells. Cell proliferation was inhibited by SD208 and promoted by TGF$\beta$3 protein. Next, we found that SD208 inhibited osteo/dentinogenesis of the cells. Both canonical and non-canonical TGF$\beta$ signaling pathways took part in the process, with TAK1, P-TAK1, p38, and P-p38 expressed at higher levels and SMAD4 at lower levels when ALK5 was inhibited. In addition, Sonic hedgehog (SHH) signal, another important pathway during tooth development, may be downstream of TGF$\beta$ signaling. These results provide insights into the role of TGF$\beta$ signaling in the differentiation of mesenchymal stem cells derived from dental germ and suggest possible targets for optimizing the use of stem cells of dental origin for tissue regeneration.

a90096

## 03-P490 HOMOGENEOUS BIODEGRADABLE URETERAL STENT: *IN VIVO* EVALUATION IN A PORCINE MODEL

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Ureteral stents are routinely used in daily urological practice and it is widely recognized that can produce significant symptoms like infection, encrustation, patient discomfort. Several biodegradable stents have been reported but all of them have failed. The main challenge remains to have a homogenous degradation preventing the formation of fragments that can cause obstruction. We recently described a biodegradable ureteral stent (BUS) produced with natural-based polymers. In the present study, we assessed in-vivo (porcine model) this newly developed BUS, by comparing its degradation mechanical properties drainage physiological and histological features with those of a commercially available ureteral stent. To perform an in-vivo assessment of a newly developed BUS produced with natural based polymers. The BUS is based on a patented technology combining the injection process with the use of supercritical fluid technology. The study was conducted with a total of 10 domestic pigs were used. In 7 animals the experimental BUS stent was inserted, whereas in the remaining a commercially available stent was used. Post-stenting intravenous pyelogram was used to evaluate the degree of hydronephrosis. The in-vivo stent degradation was measured as a function of the weight loss. Moreover, the tensile properties of the BUS were tested during in vivo degradation. After maximum 10 days, animals were sacrificed and necropsy was performed. Tissues were compared between the stented groups as well as between the non-stented contralateral ureters and stented ureters in each group. Biocompatibility was assessed by histopathological grading. In all cases, the BUS was only visible during the first 24 hours on X-ray, and in all cases, the BUS was completely degraded in urine after 10 days, as confirmed on necropsy. During the degradation process, the mechanical properties of the BUS decreased, while the commercial ureteral stents remained constant. At all time-points after stent insertion, the level of hydronephrosis was minimal. Overall, animals stented with BUS had an average grade of hydronephrosis which was lower compared to the controls. The BUS showed better pathological conditions, and hence better biocompatibility when compared with commercial stents. Notwithstanding the limitations of the present study, the in vivo testing of our novel natural origin polymer-based BUS suggests this device to feature homogeneous degradation, good urine drainage, and high biocompatibility

a90778

## 03-P491 Non-ischemia-reperfusion and dorsal slit approach for kidney surgery in a mouse model

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Kidney ischemia-reperfusion (IR) through laparotomy is a conventional method for kidney surgery in a mouse model. However, IR causes serious acute and chronic renal injury through apoptotic and inflammatory pathway, and laparotomy is an invasive procedure. To avoid these adverse responses, a non-IR and dorsal slit approach was designed for kidney surgery. The effects of this method for renal treatment were verified with animal viability, renal function, apoptosis, inflammation, fibrosis, renal regeneration and systemic response using histology, immunohistochemistry, real-time PCR, serum chemistry, TUNEL staining and Masson's trichrome staining. The non-IR/dorsal group showed 100% viability with mild BUN and serum creatinine values at day 1 after surgery, while, the IR/ventral group showed 20% viability and lethal functional abnormality. Histologically, renal tubule epithelial cells' injury was considerably emerged at day 1 in the IR/ventral group, and cellular apoptosis enhanced TUNEL-positive cell number, Fas/Caspase-3 and KIM-1/NGAL expression. Inflammation and fibrosis were serious in IR/ventral group with enhanced CD4/CD8 positive T cell infiltration, inflammatory cytokines secretion, and Masson's trichrome stain positive cell numbers. The non-IR/dorsal group showed a suitable microenvironment for renal regeneration presenting enhanced host cells migration, reduced immune cells influx, and increased renal differentiation genes and anti-inflammatory cytokines expression. The local renal IR influenced distal organs' apoptosis and inflammation through release circulating pro-inflammatory cytokines. Thus, the non-ischemia-reperfusion and dorsal slit method for kidney surgery in a mouse model can be an alternative surgical approach to researchers who do not want adverse reactions such as apoptosis, inflammation, fibrosis, functional impairment and systemic reactions. (2014M3A9D3034164), (2015R1C1A1A01053509) (2016R1C1B1011180), (2015R1D1A3A03020378), (R0005886).

a93240

## 03-P492 Angiogenic and neurotrophic effects of human bone marrow mesenchymal stem cell derived conditioned medium for erectile dysfunction

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The development of erectile dysfunction (ED) is caused by multiple factors including endothelial dysfunction and nerve damage. Therefore, two strategies for angiogenesis and neural regeneration should be considered to handle the disease at the same time. Here, we investigated a therapeutic effect of conditioned medium (CM) for ED, which was harvested from human bone marrow-derived mesenchymal stem cells (hBM-MSCs). Parameter studies considering culture condition, incubation and centrifugation times were conducted to improve protocols for CM harvesting. The harvested cytokines were quantified through cytokine and enzyme linked immunosorbent assay. Then tube formation and neurite outgrowth assay were conducted with endothelial cell (EC) and mouse primary neuron to investigate its functionality. The results revealed that hBM-MSCs cultured in three dimensional (3D) culture secreted a higher amount of proteins in comparison with monolayer culture. The concentration of cytokines in CM was linearly increased with the centrifugation time. Factors such as angiogenin, vascular endothelial growth factor (VEGF), glial cell-derived neurotrophic factor (GDNF), nerve growth factor (NGF), and brain-derived neurotrophic factor (BDNF) which affect angiogenesis and neural regeneration were also identified through the assay. In addition, the functionality test showed that CM treatment enhanced not only EC viability but also the formation of tubes, nodes and meshes. And the CM also improved neurotrophic effects in differentiation. These results demonstrated that CM had angiogenic and neurotrophic factors in concentration-dependent manner. CM is expected to be one of promising methods as regenerative medicine to handle ED disease.

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a93294

## 03-P493 Dose-dependent barrier function properties of bladder urothelium in an in-vitro model

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Bladder urothelium plays a critical role as a permeability barrier to urine. Recently the study of bladder permeability barrier (BPB) has attracted great deal of attention, and different methods have been developed. Whilst tight urothelial barrier is critical to normal bladder function and metabolic homeostasis, functional intravesicular drug delivery (IDD) systems for treating genitourinary conditions such as bladder cancer rely on increased bladder permeability to allow drugs cross the barrier of urothelium. With regard to importance of precise quantification of BPB we have developed a system to study permeability of bladder urothelium in an in-vitro urothelium.

To build up the multilayered urothelium in vitro, we seeded porcine minced urothelium onto a hybrid scaffold of weft-knitted silk fibroin (SF)-compressed collagen . In brief SF knitted fabric was placed into circular shaped molds, was covered by a collagen hydrogel, and incubated for 10 min to undergo collagen hydrogel gelation. Then minced mucosa of porcine bladder was seeded on top, and the whole construct underwent plastic compression (PC), and was incubated for 6 weeks *in vitro* until a multilayered urothelium covered the scaffold. Permeability studies were performed using a home-made device. The urothelium model (the seeded scaffold) was in contact with synthetic urine (with added Selenium) on one side, and synthetic urine (without Selenium) on the other side. After 1 hour, the precise amount of Se passed was measured using Inductively Coupled Plasma Mass Spectrometry (ICPMS). We used porcine bladder (within 6 hours from slaughter) as control and applied different concentrations of Selenium salt in urine solutions (around 12 µg/L to 14 mg/L) to study the effect of selenium salt concentration onto permeability of the urothelium. We observed similar permeability for porcine bladder wall (11%) and our in-vitro urothelium model (12%) based on weight percentage of passed Se (innitial Se concentration:12 µg/L ). Moreover, increasing the initial concentration of Se salt in synthetic urine solution decreased the relative permeability of the urothelium layer so that it reached around 1.5% (initial Se salt concentration of 14 mg/L).

The method introduced here can be applied for both tissue regeneration studies, and optimizations in drug delivery (IDD) systems.

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a92075

## 03-P494 STEM CELL-ENRICHED COLLAGEN SCAFFOLD PROMOTES SMOOTH MUSCLE REGENERATION, VASCULARIZATION AND INNERVATION IN RAT BLADDERS AFTER PARTIAL DETRUSORECTOMY

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### PURPOUSE

The use of intestinal tissue as a gold standard for bladder repair in children and adults suffering from an end-stage bladder disease is associated with significant long-term complications. Therefore, there is a strong clinical need for alternative sources of stable and reliable bladder tissue. Our study aims to bioengineer functional detrusor muscle using cells in hydrogel scaffolds.

### METHODS

Polyethylene glycol (PEG) and compressed collagen (CC) scaffolds were seeded with primary bladder smooth muscle cells (SMC) and pre-differentiated smooth muscle-like adipose-derived stem cells (pADSC) in various ratios (1:1, 1:2, 1:3 of SMC:pADSC). Their viability, phenotype and morphology were assessed over three weeks of culture.

After defining the best performing cell ratio (1:1), the hydrogel scaffolds were either left unseeded or pre-seeded with the SMC:pADSC co-culture and implantated into rat bladders after partial detrusorectomy. Untreated detrusorectomized rats were used as controls. Bladders were harvested after four weeks, embedded in paraffin, sliced and analyzed for tissue morphology, cell phenotype, vascularization and innervation.

### RESULTS

Cells in both scaffolds exhibited native SMC-like morphology, high viability and strong SMC marker expression. Cells grown in PEG formed organoid structures already after one week that became macroscopically visible after three weeks of culture. Contrary, cells cultured in CC formed well-distributed and oriented layers and networks throughout the hydrogel. Functionality of the cells grown in CC scaffolds was confirmed. Two-week timepoint was chosen for scaffold implantation into the partially detrusorectomized rats.

Morphologic analysis of the bladder wall after detrusorectomy showed a successful detrusor removal, an intact urothelium and a remaining, vascularized lamina propria. The defect was then covered with the PEG or CC scaffold. After four weeks PEG+cells was mostly degraded while PEG-cells was encapsulated. Strong inflammation, but no muscle regeneration was detected. Contrary, CC ± cells did not cause any inflammation and fully integrated into the bladder wall with CC+cells exhibiting well distributed vascularization, smooth muscle formation and neuronal ingrowth.

### CONCLUSION

We have shown that CC seeded with SMC-pADSC co-culture in a 1:1 ratio is a promising scaffold for functional detrusor bioengineering and see this as a first step for future clinical detrusor repair and whole bladder wall reconstruction.

a93473

## 03-P495 Exploring the Role of Modified Adipose Derived Stem Cell-based Self assembled Scaffold in the Engineering of Full Thickness Human Urethra

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The urethral stricture, which develops due to scarring process, is treated by urethral dilation and urethrotomy; but in complex and long urethral strictures, substitution urethroplasty is the only option. Autologous genital skin and buccal mucosa grafts are currently used in urethral reconstruction; however risk of complications such as hair ingrowth in the urethra, stone formation and donor site morbidity, restrict the application of these grafts. Therefore, an alternative to these grafts is required. The project aims to construct a full thickness, human urethra consisting urothelium, submucosa and smooth muscle layers from adipose derived stem cells (ASCs). ASCs were isolated from human adipose tissue and were cultured in growth media containing ascorbic acid to stimulate extra cellular matrix secretion and production of self-assembled scaffold (cell sheet/submucosa layer). To obtain a thick cell sheet, the effect of cell seeding density, $O_2$ level concentration, and ascorbic acid concentration were examined under static and dynamic culture conditions. As a control, cell sheet produced from human dermal fibroblast (DFs) was produced alongside. The cell sheet was stained with collagen type I antibody and the thickness was measured under a confocal microscope with image analysis software. Our results showed that in a static culture condition and with a seeding density of 1.5 x $10^4$ cells/$cm^2$ , ASCs under hypoxic (1% $O_2$) condition, produced thicker cell sheet (22.50±3.90 um) compared with those under normoxic (21% $O_2$) condition (18.86 ± 1.68 um). Similarly, at a higher cell seeding density (3.0 x $10^4$ cells/$cm^2$), cell sheet was thicker for ASCs under hypoxic (23.00±1.20 um) and compared with normoxic (17.26±3.34 um). However, thickness of cell sheet produced at 1.5 x$10^4$ and 3.0 x $10^4$ cells/$cm^2$ under hypoxic condition were not statistically significant ($p < 0.05$). This showed that altering the oxygen level but not the seeding density, during ASCs culture affects the resulted cell sheet thickness. Comparatively, decreasing oxygen level and increasing cell seeding density increase cell sheet thickness produced by DFs. In conclusion, ASCs cultured in hypoxic condition with 1.5 x $10^4$ cells/$cm^2$ of cell seeding density resulted in the best of cell sheet in term of thickness.

The authors declare that there is no conflict of interests regarding this work.

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a92307

## 03-P496 The composition of GMP-compliant expansion medium regulates the expression levels of CD146 and SUSD2 and the differentiation capacity of human placenta-derived mesenchymal stromal cells in vitro

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Human placenta-derived mesenchymal stromal cells (MSCs) are investigated in vitro and in preclinical in vivo models manifold to serve as cellular therapy in different clinical situations such as regeneration of muscular tissues eventually. For production of MSCs in vitro standard media often include fetal bovine serum (FBS) as source of growth factors. For use of MSCs in a real clinical situation xenogenic components such as FBS must be avoided in many countries. Therefore, xeno-free MSC expansion media were developed and FBS was replaced e.g. by human serum (hS), human plasma (hP) and/or platelet lysate (hPL). However, differences in these medium complements could influence the phenotype and characteristics of MSCs. We therefore compared the expression of cell surface markers, the differentiation capacity and the expression of key cytokines involved in muscular regeneration in MSCs expanded in different xeno-free and GMP-compliant expansion media.

We report that addition of 5% hPL to either commercial MSC expansion medium (medium with FBS) or to GMP-compliant MSC expansion medium (DMEM + 5% hS + 5% hPL) significantly increased the MSC proliferation ($p<0.05$), and reduced the expression of CD146 ($p<0.05$), whereas CD73 and CD90 and other markers remained unchanged, on both transcript and protein levels. Expression of stem cell marker SUSD2 was not significantly regulated by hPL. The osteogenic differentiation capacity was reduced in MSCs expanded in DMEM medium complemented with 5% hP + 5% hPL in comparison to the same cells expanded in medium complemented with FBS without hPL, but not in DMEM + hS compared to DMEM+ hS + hPL. Significant changes in the expression of neuro- or myo-regenerative cytokines including BDNF, bFGF, GDNF, HGF, IGF-1, IGF-2, TGFß, or VEGF in MSCs was not influenced by the composition of the GMP-compliant expansion media. However, some interindividual differences between MSCs from individual donors and between batches of hPL were noted. We conclude that the expression of CD146 and the osteogenic differentiation potential of human term placenta-derived MSCs is influenced by the expansion medium. The expression of the regenerative cytokines investigated was not biased by hPL in the expansion medium. Expansion of MSCs in GMP-compliant medium enriched by hP + hPL could therefore be an advantage when bone formation by the MSCs applied should be avoided in clinical situations.

a91528

## 03-P497 Functional nephron progenitors from pluripotent cells: Transition from transient phase to long-term *in vitro* maintenance

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Nephron progenitor cells (NPCs), the precursors of nephrons, can be generated from pluripotent stem cells. In conventional renal differentiation protocols, formation of nephron progenitors is a transient phase of differentiation and hence these cells cannot be maintained in progenitor state in culture. In this study, we established protocols for two-dimensional expansion of NPCs derived from human embryonic stem cells and induced pluripotent stem cells. After 15-fold expansion, NPCs retained the expression of NPC specific markers SIX2, CITED1, SALL1, as confirmed by immunocytochemistry. Further we showed that NPCs after cryopreservation can be culture expanded and retain the expression of NPC specific markers. Both culture expanded and cryopreserved NPCs are capable of nephrogenesis in organotypic cultures. Long-term expansion of NPCs will provide a platform to get sufficient number of cells for kidney regeneration, drug screening and disease modeling of kidney disorders.

a91413

## 03-P498 GMP: the challenging milestone on the way towards clinical application of hMPCs for incontinence treatment

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Recent advances in cell-based therapies have provided a variety of opportunities to seek alternative solutions to restore damaged sphincter function in patients with urinary incontinence. Various pre-clinical studies have shown promising results towards successful skeletal muscle regeneration using autologous injection of patients muscle progenitor cells (MPCs). During the production of MPCs a tightly regulated network ensures highest product quality (good manufacturing practice, GMP) to rule out any chance of contamination.
Especially in the interface, where GMP regulations meet the clinics, challenges may arise while implementing the MPC therapy and turn into bottlenecks for the clinical application.

For the first time, we will combine autologous cell injection of MPCs with early neuro-muscular electromagnetic stimulation in patients suffering from stress urinary incontinence and evaluate the efficiency of muscle regeneration. Within the setup of the GMP-compliant production, the transport of the biopsy material and the final product became the interface of the GMP and good clinical practice regulatory network and rose several specific questions.

During the transport, responsibilities between both parties become unclear and a well-developed scheme has to identify accountabilities. The release of the final product has to meet certain release criteria, especially with regards to the use of collagen from animal origin. But, the release of the production documents of the final product can take days which collides with the shelf life of the final product. To prevent premature formation of gels of the collagen, constant cooling needs to be provided. However, cooling tools have to be validated; same as all other machines and materials involved in the production of MPCs. Temperature loggers whose data need to meet a pre-defined range tightly control this transport under constant cooling.
Hence, to solve all related questions regarding the transport, a complex network of documentations and responsibilities is built which is the key stone for a smooth and compliant trial conduct.

A careful navigation through already established regulations is necessary to lead this complex project for the application of MPCs as a novel cell-based therapy. All involved parties have different primary goals which need to be taken into consideration while being compliant with the regulatory network on the way to the common goal: The improvement of the Quality of life of the patients.

a92341

## 03-P499 ADIPOSE-DERIVED STEM CELL AND BLADDER SMOOTH MUSCLE CELL CO-CULTURE IMPROVES FUNCTIONAL SMOOTH MUSCLE FORMATION FOR DETRUSOR BIOENGINEERING

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**PURPOSE**

Patients suffering from an end-stage bladder disease often exhibit fibrotic detrusor muscle tissue that rarely offers a high number of healthy, viable smooth muscle cells (SMC) for therapeutic applications. Hence, in order to develop a functional detrusor muscle, we decided to replace a part of the SMC used for its future reconstruction with the highly abundant adipose-derived stem cells (ADSC) and determine their optimal, clinically relevant ratio.

**METHODS**

Primary bladder SMC and ADSC were isolated from female Lewis rats and characterized. ADSC were pre-differentiated into SMC-like cells (pADSC) and co-cultured with SMC in various ratios (3:1, 1:1, 1:3, 1:5 of SMC:pADSC). Morphologic, phenotypic, proliferative and functional differences between the various cell ratios were assessed. After defining the best performing ratio (1:1) a proteomic analysis of the secretome and the cell pellets of a directly mixed co-culture vs co-culture using transwell inserts vs the monoculture controls was performed.

**RESULTS**

We observed significant, ratio-dependent morphological changes, with the 1:1 cell ratio generating a the highest number of organoids after 2 weeks and the largest organoids after three weeks compared to other cell ratios and the monocultures. Further, the 1:1 cell ratio exhibited a particularly similar phenotype to the SMC controls that almost reached the SMC-marker expression levels later during cultivation. Moreover, the 1:1 cell ratio showed a most consistent increase in cell proliferation and functionality over time compared to all other conditions. Transwell experiments indicated the involvement of paracrine signaling in these processes.

Proteomic analysis showed an increased presence of angiogenin and VEGF-A, as well as neudesin neurotrophic factor and glia maturation factor-$\beta$ in the 1:1 co-culture compared to the SMC monoculture. Further, connective tissue growth factor, SDF-1 and TGF-$\beta$1, 2 and 3 were also increased. ECM proteins, such as collagen type I, II, III, V and XI and integrins important in cell-cell and cell-ECM interaction were also elevated in the 1:1 co-culture.

**CONCLUSION**

We have shown that a SMC–pADSC co-culture in a 1:1 ratio increases cell proliferation, organoid formation and leads to the expression of various factors important in tissue regeneration, while retaining a strong, functional SMC phenotype. We envision this cell combination to help us engineer a stable, functional detrusor muscle for future clinical applications.

a92513

## 03-P500 An experimental study of topical insulin-like growth factor-1 sustained release to improve urethral wound healing in a rabbit model

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**Background:** Urethral stricture is characterized by a narrowing of the urethra with formation of scar tissue, and few anti-fibrotic agents have been used to prevent urethral stricture formation after urethral injury. Insulin-like growth factor-1 (IGF-1) has emerged as an anti-fibrotic agent and we evaluated the effect of topical IGF-1 sustained release on urethral wound healing after urethral injury.

**Methods:** We made urethral catheters coated either with IGF-1 impregnated collagen hydrogel or with only collagen hydrogel. A total of 31 male Japanese white rabbits (2.5 to 3.0 kg) were divided into 3 groups after their urethras were injured by electrocoaglation using spherical monopolar electrocautery. Group 1 (n = 5, control group) received no additional treatment after urethral injury. Group 2 (n = 6, collagen hydrogel group) had a urethral catheter coated with only collagen hydrogel inserted immediately after urethral injury. Group 3 (n = 10, IGF-group) had a urethral catheter coated with IGF-1 impregnated collagen hydrogel inserted immediately after urethral injury. Fourteen days after urethral injury, retrograde urethrography and urethroscopy (diameter 2.7 mm) were performed to evaluate the injured urethra, and the injured segments were also histologically evaluated. In addition, the sustained release of indocyanine green labeled IGF-1 from collagen hydrogel was evaluated by in vivo fluorescence imaging.

**Results:** The mean urethral diameter on urethrography in group 3 (5.6 mm) was the largest among the three groups (1.9 mm in group 1 and 4.1 mm in group 2, $p < 0.01$). The ratio of urethral lumen in injured urethra to that in normal urethra in group 3 (56.0%) was the largest among the 3 groups (21.0 % in group 1 and 37.6 % in group 2, $p < 0.01$). Nine of 10 (90.0%) had a urethral lumen large enough for passage of a urethroscope, which is higher than the fraction in the other groups (1 of 5, 20.0%, in group 1 and 4 of 6, 66.7%, in group 2). The mean injured area not covered with urethral epithelium in group 3 (9.45 $mm^2$) was the smallest among the three groups (36.26 $mm^2$ in group 1 and 26.24 $mm^2$ in group 2, $p < 0.05$). The sustained release of IGF-1 from collagen hydrogel was observed for a week.

**Conclusions:** Topical IGF-1 sustained release with collagen hydrogel significantly improved urethral wound healing and prevented urethral narrowing. Thus it might be useful in the prevention of urethral stricture after internal urethrotomy or urethral dilation.

a93225

## 03-P501 Development of nano-functionalised electrospun meshes for the management of incontinence-related problems

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Pelvic organ prolapse (POP) is a highly prevalent disease, occurring in up to 50% of women above the age of 50, and with a lifetime need for intervention approaching 20%[1]. Considering the limitations in conventional approaches and materials (poor biocompatibility, low mechanical properties, and high risk of infection), the development of an implant that can address the current challenges is of paramount importance[2].

This work aimed to develop a biodegradable mesh that, by mimicking the three-dimensional (3D) architecture of the pelvic floor, can provide better biomechanical integration into the host tissue, promote its repair, and exert an antibacterial action in-site. Polycaprolactone (PCL) meshes were produced by electrospinning, with fibre diameter in the range 0.7-0.9μm (by Scanning Electron Microscopy, SEM). The meshes were aminolysed to obtain a positive charge on the surface by -NH2-grafting. Subsequently, a layer-by-layer (LbL) assembly was applied to obtain a multilayered electrostatic nanocoating (18 nanolayers), consisting of honey as polyanion and poly-(allylamine hydrochloride) (PAH) as polycation. SEM analysis, infrared (FTIR/ATR) and X-Ray photoelectron spectroscopy (XPS) demonstrated the successful functionalisation at the nanoscale. In particular, SEM showed rougher fibre surface compared to unfunctionalised meshes, revealing the formation of a regular nanocoating, while FTIR-ATR showed the typical bands and peaks of the polyelectrolytes. XPS analysis on un-coated samples showed three peaks equivalent to the different carbon oxidation states ( – C – H- or – C – C – bonds, – C – O-bond and -N-C=O bond). For the coated samples, the atomic concentration of C – C bonds increased whereas the C – O bond one decreased.

The biocompatibility of the electrospun functionalised meshes was assessed in terms of adhesion and 7 days proliferation of human endometrial stromal (ThESC) cells, showing cytoskeletal organization and viability.

Considered together, the results of this work are of interest for pelvic floor repair, since they demonstrate the possibility to successfully produce novel bioartificial surgical meshes as potential treatments for POP.

**References: 1.** *Fialkow et al., Intl Urog Journal, 19(3):437, 2008;* **2.** *Gigliobianco et al., BioMed Res Int, paper ID 968087, 2015.*

**ACKNOWLEDGMENTS**

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a90012

## 03-P502 High biocompatibility Hyaluronic Acid/PLCL core-shell structured nanofibers scaffold for bladder regeneration

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Hyaluronan (HA) is the important component of ECM, electrospinning scaffold has the similar bionic characteristic with autologous ECM. However fabrication of pure HA electrospinning scaffold is difficult. This study reports the fabrication of hyaluronan- (HA) coating biomimetic nanofibrous scaffolds via coxial electrospinning of Poly(l-lactide)/Poly(e-caprolactone) (PLCL) and HA in modulating proliferation and immigration of smooth muscle cells (SMCs) for bladder regeneration. Highly uniform HA/PLLA nanofibers with core-shell structure was verified by SEM and TEM. HA-PLCL electrospinning were composed of randomly-oriented nanofibers and had a 3D porous network structure. Formation of the HA-coating layer atop each PLCL nanofiber surface endowed the nano-fibrous mats with increased anisotropic wettability and welling. The HA/PLCL nanofibers significantly promoted SMCs to proliferate and immigrate in vitro. 4 or 8 weeks of in vivo implantation in rat bladder augmentation showed that bladder wall made of dome HA/PLLA nanofibers mesh could integrated with the host tissue and supported de novo formation of smooth muscle layers with contractile protein expression a-smooth muscle actin (a- SMA). In addition, bladder capacity was significant augments improved by HA/PLCL nanofibers mesh.

This study demonstrated that the bilayer HA/PLCL scaffold may be a promising scaffold with good biocompatibility for for engineering of bladder.

a90782

## 03-P503 Bladder regeneration using a PCL scaffold with growth factors EGF, VEGF and bFGF in a partial cystectomized rat model

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Tissue engineering is an alternative method for bladder augmentation. Conventional scaffolds result in fibrosis and graft shrinkage. To solve these problems, this study designed PCL scaffold (diameter 5 mm) with growth factors (GFs) EGF, VEGF and bFGF to enhance bladder tissue regeneration and volume recovery in a rat model. A partial cystectomized rat (Slc:SD, male, 5 weeks) was reconstructed by scaffold (Scaffold group, n=25) or GFs combined scaffold (GF group, n=25), and compared to a sham operated (control group) and an untreated rat (partial cystectomy group). Bladder volume, histological, immunohistochemical (IHC) and molecular evaluation were performed 4, 8 and 12 weeks after bladder operation. Bladder volume of the Scaffold and GF group were recovered to normal range, and the GF group showed more enhanced augmentation than the Scaffold group. In histological evaluation, the GF group showed organized urothelial lining, dense extracellular matrix, frequent angiogenesis, and enhanced smooth muscle bundles compared to the Scaffold group. In IHC with aSMA and Pan-cytokerain Ab, the GF group showed relatively enhanced smooth muscle and urotherium formation compared to the Scaffold group. Those results were confirmed with smooth muscle and urothelium differentiation related genes expression. In long term (6 months) in vivo safety analysis, graft groups did not show abnormal morphology and histology. Therefore, bladder regeneration using the PCL scaffold with EGF, VEGF and bFGF improved functional and histological bladder regeneration in a partial cystectomized rat model. (2014M3A9D3034164), (2015R1C1A1A01053509), (2016R1C1B1011180), (2015R1D1A3A03020378), (R0005886)

a91719

## 03-P504 Synergy effect of stem cells into nerve differentiation with nanofibrous meshes around the injured cavernous nerve in rats

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**Introduction:** Injection of mesenchymal stem cells (MSCs) into corpora cavernosa have been used for the regeneration of the damaged cavernosa in the cavernous nerve (CN) injured rat model. However, it is not sure that transplanted MSCs around injured CN can enhance regeneration of injured CN. Electrospun nanofibrous meshes show morphologies resembling natural extracellular matrices and show the up-regulation of myelination markers and the axonal outgrowth of peripheral neurons. This study was performed to examine the differentiation of transplanted MSCs into neuron-like cells and synergy of nanofibrous meshes with MSCs after transplantation around the injured CN of rats.

**Materials and Methods:** The synthesized polymer was electrospun in a rotating drum to prepare nanofibrous meshes. Human MSCs were prepared and confirmed. Eight week old male Sprague-Dawley rats were divided into 5 groups of 6 each, including group 1=sham operation, group 2= CN injury, group 3= human MSCs treatment after CN injury, group 4= nanofibrous meshes treatment after CN injury and group 5= nanofibrous meshes -MSCs treatment after CN injury. Immediately after the CN injury in group 5, nanofibrous meshes containing MSCs encircled the injured CN. Erectile response was assessed by CN stimulation at 2, 4 weeks. Thereafter, penile tissue samples were harvested and examined using morphological analysis and immuno-histochemical stain against nerve, endothelium and smooth muscle.

**Results:** The group transplanted with nanofibrous meshes-MSCs showed higher erectile function than the group with MSCs alone. At 4 weeks, immunoreaction-positive for neural cells for NF, Tuj1 as neuron was demonstrated for neuronal differentiation of MSCs or nanofibrous meshes-MSCs at MPG after transplantation. Immunoreaction-positive for neural cells for NF, Tuj1 as neuron, MAP2, MBP, peripherin was demonstrated at injured cavernous nerves after transplantation of MSCs. Nanofibrous meshes-MSCs showed more increased expression of neuronal differentiation than MSCs alone.

**Conclusions:** Synergy of transplanted stem cells into neuron-like cell differentiation was found with nanofiber meshes around the injured nerves in rats with cavernous nerve injury. Nanofibrous meshes containing MSCs encircled the injured CN during the operation of radical prostatectomy can enhance regeneration of injured CN for the regeneration of erectile dysfunction.

a92445

## 03-P505 Gel casting as an approach for tissue engineering of multilayered tubular structures: application for urethral reconstruction

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Introduction: Currently there is a lack of tissue-engineered solutions for replacement of urological tissues, like ureters and the urethra/CS. As the corpus spongiosum (CS) is an integral part of the urethra and important in supporting the function of the urethra, tissue engineering of the urethra should be combined with reconstruction of the CS [1]. The CS is a three-layered, highly vascularized structure with distinct distribution of extracellular matrix components. Here we propose an innovative casting approach to engineer multilayered tubular constructs to mimic the organization of the native CS.

Materials and Methods: A mold with three chambers, representing the three layers of the CS, was designed and fabricated. The chambers were loaded with gelatin-based hydrogels containing a coculture of endothelial cells and pericytes (chamber 1 and 3) and smooth muscle cells (chamber 2). A fiber mesh was placed at the base of the construct to serve as a porous support for the gels and to roll the construct into a multilayered tubular construct. The gels were mechanically tested and compared to native tissue.

Results: The gel can be casted and rolled into multilayered tubular constructs. The encapsulated cells formed little capillary-like structures (chamber 1 and 3) and produced elastin (chamber 2) within two weeks of culturing. The compressive modulus of the construct was comparable to that of the native tissue.

Conclusion: Our approach enables to construct tubular structures with distinct composition in the different layers. Cell survival and functionality up to 14 days has been achieved. The compressive modulus of the gelatin gels within the same range as native tissue. This approach towards tissue engineering of multilayered tubular structures may be applicable to the urological field as well as in other fields of soft tissue engineering.

a90973

## 03-P506 *Molecular studies in bladder wound healing*

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### Molecular studies in bladder wound healing

*Several conditions, such as chronic inflammation, cancer or trauma may disturb the anatomy and physiology of the urinary bladder. Efficient wound healing after surgical intervention or disease, is of high importance to protect the upper urinary system and to minimize the risk of urinary incontinence. The healing process in the urinary bladder is today not well characterized. Given the anatomical and functional similarities of the urinary system between human and rat, rodent models are useful for studying the molecular mechanisms involved in the healing process when injury occurs. In this study, we aimed at characterizing wound healing in the urinary bladder at a molecular level, using a rodent model of incisional wounding.*

*Materials and Methods: A rodent model of bladder healing (n=88) was used for molecular characterization of the healing process up to 28 days post incisional wounding. The samples were collected at different time points for RNA extraction. Genes expressed in wound healing was analyzed using a rat RT2-PCR wound healing array (Qiagen). Shams were used as controls.*

*Results: Eighty-eight different genes were analyzed in the array. The significant up-regulated genes included genes related to inflammation, such as Il1beta, Interleukin 6 and members of the chemokine CXC motif family. Several growth factors from both the epidermal and the fibroblast family were represented in the set of significantly up-regulated genes. Genes of the extracellular matrix receptor, such as integrins, were expressed differently at specific times of the healing process. Furthermore, we observed a time dependent up-regulation of matrix remodeling enzymes and inhibitors of proteases. Differential expression of members of collagens genes was an early event and then maintained between 2 and 4 weeks post wounding.*

*In conclusion, we identified genes expressed at each phase of the healing process: inflammation, proliferation and tissue remodeling. Further studies will analyze whether these findings can be extrapolated to humans.*

a91549

## 03-P507 Cell synchronization and a novel kidney extracellular matrix based hydrogel to promote efficient derivation of renal vesicles from pluripotent stem cells

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Pluripotent stem cells (PSCs) represent an important cell type for tissue engineering strategies aimed at kidney regeneration. Intermediate mesoderm is a lineage derived from the mesoderm, one of the germ layers from which kidney develops. Efficient initiation of differentiation of pluripotent stem cells (PSCs) is dependent on the responsiveness of the stem cells to the external induction cues, which is a prerequisite for deriving a homogeneous population of nephron progenitor cells (NPCs), the renal precursors. In this study, we established protocols for driving efficient differentiation of human embryonic stem cells and induced pluripotent stem cells into NPCs and developed an extracellular matrix (ECM) based hydrogel to improve the further differentiation of NPCs into renal vesicles. We showed that chemically induced reversible arrest of the PSCs in G1 phase of the cell cycle by dimethyl sulfoxide synchronizes the cell cycle of the pluripotent stem cells and their response to WNT agonist CHIR99021, thus efficiently stimulating the directed differentiation of PSCs to a homogenous population of NPCs. The cell cycle synchronization was validated by the expression of Retinoblastoma protein 1. The NPCs were characterized by the gene expression of HOXD11, CITED1, SIX2 and SALL1. The homogeneity was also validated by the expression of SIX2 and SALL1 by immunocytochemistry and flow cytometric analysis. NPCs when synchronized gave a higher yield of renal vesicles, which eventually would give higher number of nephrons. To further improve the generation of renal vesicles from NPCs, we have also developed successfully a hydrogel based on the ECM derived from sacrificial kidney tissue. Upon induction with exogenous Activin A and FGF9 in kidney derived sacrificial ECM hydrogel, NPCs successfully formed renal vesicles. The renal vesicles were characterized by the expression of flurochrome conjugated LTL. This strategy will provide a platform to get sufficient and homogenous number of NPCs and renal vesicles for kidney regeneration, drug screening and in vitro disease modeling of kidney disorders.

a90219

## 03-P508 Off the shelf graft for urethral reconstruction surgery,a step closer to clinical translation

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**Introduction:** There is a need for a surgically efficient and cost effective graft in urethral surgery. Current available surgical techniques require harvesting of grafts from autologous sites. This increases the risk of complications from 2 operative areas, lack of tissue availability and added patient discomfort. Cell based approaches have shown progress in this area, however, are associated with high costs and regulatory hurdles. An acellular graft with good regenerative potential has a better chance to be translated into clinical practice.

**Material & Methods** We have worked on four versions of urethral grafts over a period of 3 years resulting in the engineering of a final relatively easy to fabricate acellular bovine collagen graft with enhanced mechanical property, allowing better surgical and regenerative performance. This was achieved, by varying the collagen density and fibre distribution. The tubular grafts were used as 2cm urethral grafts in New Zealand white rabbits. This procedure was done in 49 male rabbits in two different centres in the world. At 1, 3, 6, 9 and 11 months the animals were macroscopically evaluated and contrast voiding cysto-urethrography was performed. After sacrifice histological examination was done on the implanted sections. A clinically relevant 4cm urethral defect study was initiated utilizing the dog model in 2017. A total of 8 dogs have been implanted with a 4cm acellular tubular graft. An endoscopic examination of the urethra and a contrast voiding cysto-urethrography as preformed in clinically practice will be done at the time of euthanasia. The study duration is for 1 year.

**Results** The multi-centric rabbit study revealed from the contrast voiding cysto-urethrography that the initial produced acellular collagen tube had a 40% failure rate, while the final version had a 10% failure rate. Spontaneous urothelial coverage of the grafts and time-dependent smooth muscle cell migration could be observed in all grafts on histology and immunohistochemistry. The initial 2 dogs from the pilot study were euthanized at 2 months post-surgery and the histology showed regeneration of the implanted site.

**Conclusions** A graft that is off the shelf which is easy for the surgeon to work with, regenerative efficient, safe and cost effective is ideal for urethroplasty. We have shown that this is possible using an acellular platform. The data generated from this study will be used for future submission for clinical trial application.

a91705

## 03-P509 Novel sclerosant foam for cyst ablation through two-photon laser for polycystic kidney treatment

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Polycystic kidney disease (PKD) is a systematic genetic disorder and most common hereditary disease that ultimately leads to end-stage renal disease. Innumerable cyst development in the kidney is dominant trait of PKD progression. Especially, progressive enlargement of renal cyst frequently causes pain and complication related to cyst burden. Treatment options are limited to analgesics or invasive open or laparoscopic deroofing of cysts. Cyst aspiration and sclerotherapy are relatively recent treatment methods, but complication such as cyst refilling and infection remains to be overcome. Here, we studied novel foam sclerosant with rose bengal to both reduce cyst burden and induce photochemical tissue bonding by two-photon laser for better cyst ablation. Foam sclerosant is more efficient than a solution as it is retained in the injected area and covers the larger surface. We tested if the addition of glycerol could enhance foam retention time to augment foam efficacy. We found that adding glycerol to foam constituent not only improved foam retention time but also its effectiveness in destroying cyst epithelial cells in vitro. Using this enhanced foam, we also added rose bengal to compress cyst membrane by crosslinking with photochemical tissue bonding to avoid treatment complication such as reaccumulation of cyst fluid and infection. Delivery of rose bengal in foam resulted in the homogenous dyeing of cyst inner membrane epithelium in vitro and in vivo. Membrane crosslinking by photochemical tissue bonding was tested in vitro, and in vivo by two-photon laser for deep tissue penetration. We also show that the crosslinked cyst by the treatment of novel foam is much less subject to refilling and infection, suggesting better cyst ablation method to improve the life of the PKD patients.

a91197

## 03-P510 Underwater Tissue Adhesive using Coacervated Mussel Adhesive Protein for Repair of Vesicovaginal Fistulae

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Vesicovaginal fistula (VVF) is an abnormal opening between the bladder and the vagina that results in continuous and unimpeded leakage of urine. It has profound effects on physical and psychological health of the patients. Particularly in underdeveloped countries where urinary fistulas devastate millions of young lives, conventional management strategies are not satisfactory. For more effective and non-invasive repair, fluid-type tissue adhesives or sealants have been suggested. However, conventional products do not provide a suitable solution due to safety problems and poor adhesion under physiological conditions. Herein, we proposed a unique water-immiscible mussel protein-based bioadhesive (WIMBA) exhibiting strong underwater adhesion which was employed by two adhesion strategies of marine organisms; 3,4-dihydroxy-L-phenylalanine (DOPA)-mediated strong adhesion and water-immiscible coacervation. Good performances of the WIMBA on the management of VVF was confirmed *ex vivo* and *in vivo*. The WIMBA with strong adhesion and good durability repaired the fistulas in pig bladders with a 75% success rate which is comparable to that obtained with suturing (50%). Additionally, WIMBA can be applied to the fistula site by cystoscopy because it is fluid and water-immiscible. Collectively, WIMBA could be a promising sealant for urinary fistula with further expansion to diverse internal body applications.

a80000

## 03-P511 Isolation of osteotropic prostate cancer cells with an osteomimetic microfluidic device

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Paget's soil-seed theory suggests the bone marrow provides a favourable metastatic "soil" for the circulating metastatic "seeds", leading to the development of fatal bone metastatic disease (BMD) in prostate cancer. The study of early events in metastasis is thus important for the development of novel therapeutics targeting BMD; such efforts, however, are limited by the lack of relevant disease models. Here, we developed a biomimetic microfluidic device to replicate osteotropism of prostate cancer cells in vitro. Soft lithography methods were used to generate a three channel device from PDMS. Our device comprises type I collagen matrix in the middle channel, flanked by mesenchymal stromal cells (MSC) and prostate cancer (PCa) cells on either side. The MSC represent a simple bone stromal niche, used to attract PCa from the opposing channel. Our results showed migration of PCa cells into the gel region over a course of 3 days. Migration could also be elicited when chemokines was applied in place of MSC. However, this migration was not observed when MSC was replaced with MSC-conditioned media, suggesting a role for crosstalk between MSC and PC3. Across different cell lines, PC3, an aggressive bone metastatic prostate cancer cell line, was shown to have higher migration rates than LNCaP, a prostate cancer cell line with lower metastatic potential (14.0 ± 8.2 captured PC3 vs 0 LNCaP, N=6). Migratory PC3 within the collagen matrix were recovered from the device for subsequent expansion and characterisation. Finally, to evaluate the role of two dimensional versus three dimensional culture, MSC spheroids were used in place of the MSC monolayer, with increased PC3 invasion. In conclusion, we have developed a device that can sequester bone metastatic cancer cells in the early stages of metastasis, with potential applications in drug testing, prognostic testing or as a disease model for basic research.

a93491

## 03-P512 The curative effect of collagen-gelatin sponge sustained release of bFGF for tympanic membrane perforation on animal model

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The purpose of this study was to evaluate the efficacy of regenerative treatment using collagen-gelatin sponge (CGS) in sustaining the release of basic fibroblast growth factor (bFGF) with tympanic membrane perforation (TMP). The current study, the effects on epithelialization, granulation, and vascularization of wound healing demonstrated that, as a scaffold, CGSs are equal or superior to conventional gelatin sponge. In addition, the efficacy of CGS combined with bFGF in wound healing was evaluated. Therefore, in this study, GCS immersed with bFGF was used to enhance the healing process of chronic TMP in a guinea pig model. Female guinea pigs were divided into three groups: CGS alone (CGS group); CGS with bFGF (200μg/ml; CGS+bFGF group); or saline (a sham-treated group). Chronic TMP were created by electrocautery myringotomy. GCS or GCS immersed with bFGF was applied to the right ears and saline to the left ones. Then GCS immersed with bFGF was placed over the perforation during about two weeks. In all groups, on the 3rd, 7th, 14 days, macroscopic examinations of TMP and the expressions of bFGF in TM epithelia, fibroblasts, and macrophages were performed by histological examination and compared among the groups. Application of CGS combined with bFGF to chronic TMP has early closure and has significant effects on growth factor for certain durations.

a92816

## 03-P513 Corneal endothelial cells culture methods in vitro for cornea regeneration

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Corneal endothelial cells (CECs) have limited proliferation capacity, there exists constant demand of expansion of CECs in vitro for cornea regeneration in the clinical field. There are many studies to increase the proliferation of CECs in vitro. The addition of Rho-associated kinase (ROCK) inhibitor is known as an effective method. Conditioned medium derived from mesenchymal stem cells (MSC-CM) has been employed as a cell proliferation supplement. In this study, the effect of combined treatment during CEC culture was observed. The combinations of ROCK-inhibitor and MSC-CM on the proliferation of CECs were investigated. MSC-CM was prepared using primary cultured MSCs from Wharton's jelly of human umbilical cord. CECs were isolated from rabbit cornea and cultured in the ROCK-inhibitor, Y-27632 (10$\mu$M) and MSC-CM containing culture medium. The ability of CEC proliferation was confirmed through AlamarBlue assay, and it was confirmed that cells grown under MSC-CM showed a 2.5-fold higher proliferation than the control group. However, when treated with Y-27632, the cell proliferation rate was rather reduced. From these results, it was concluded that ROCK-inhibitor does not enhance CEC proliferation but is important for maintaining cell size and morphology, and that the addition of MSC-CM contributes to cell proliferation. RNA sequencing further confirmed that MSC-CM and ROCK-inhibitor combination, maintained the highest similarity to the initial stage of CECs with low passage number.

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a92552

## 03-P514 Self-assembling collagen-like-peptide nano-implants as stem cell loaded substitutes to human cornea transplantation

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**PURPOSE:** The consistent global shortage in donor corneas, indicate the need for lab-grown alternatives. Collagen-like-peptides (CLP)[1] have been proposed as alternatives to full-length extracellular matrix proteins for tissue regeneration. The aim of this study is to investigate CLP[1] as a corneal substitute and nano-refine it for corneal epithelial stem cell loading.

**METHODS:** CLP hydrogels were tested for physical properties (DMA – collagenase challenge), optical properties (Transparency), microbial susceptibility (P. aeruginosa challenge) and genotoxicity (VITOTOX). CLP hydrogels were nano-surface modified with 3D topographic or fibronectin micro-contactprinted patterns[2]. Cell viability and cell proliferation of immortalized corneal epithelial cells cultivated on the hydrogel were investigated using BrdU, and Live cell imaging assays. Composite grafts with cultivated primary limbal epithelial stem cells (LESC) were characterized using SEM, TEM, qPCR and IHC analysis. Acellular hydrogels were implanted in the cornea of mini-pigs to test for in vivo biocompatibility (follow-up 12months)[1].

**RESULTS:** CLP hydrogels form transparent hydrogels that are mechanically stable and strong. CLP shows no genotoxic effect, and hydrogels are 100-fold more resistant to microbial growth when compared to amnion. No difference in cell viability and active proliferation rates were noted when compared to tissue culture plastic. Cultivated primary LESC preferentially first attach to nano- modified patterns before spreading over the rest of the hydrogel. Cultivated LESC keep expressing their adherent stem cell phenotype (low expression CK3/12 & GJA1 – high expression $\Delta$Np63$\alpha$, ITGA6 & ITGB1). *In vivo*, CLP hydrogels showed stable and functional corneal integration promoting regeneration of corneal epithelial and stromal tissue[1].

**CONCLUSION:** The favorable optical characteristics, relative microbial resistance, successful composite graft generation and *in vivo* biocompatibility prove that CLP is a highly promising alternative for human donor corneas. Furthermore, the benefits of surface nano-surface modification will enable production of custom-built cell loaded cornea grafts.

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a90278

## 03-P515 Engineered corneas from decellularized xenogenic tissues

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### Introduction

To overcome donor shortage for corneal transplantation, alternatives based on tissue engineering and cell therapy are being researched. Biomaterials used as corneal substitutes need to be transparent, allow for endogenous cell repopulation and have enough mechanical strength to be sutured. The use of decellularized corneas offers great potential as they match native mechanical strength and maintain the micro architecture of the native tissue. In this study we evaluated the use of decellularized porcine corneas as scaffolds for tissue engineering a corneal substitute.

### Materials and methods

Corneas were decellularized with SDS, Triton X-100, and DNAse and RNAse [1]. DNA, sGAG and collagen were analysed using histology stainings and biochemical assays. Scaffolds were seeded with human corneal fibroblasts for 2 weeks with serum-supplemented medium (Expansion) and for another 2 weeks with Expansion medium or serum-free Keratocyte medium. Penetration depth of migrated cells was measured by sectioning the scaffolds and staining with DAPI and H&E. A second method, named the sandwich method, based on the stacking of thin sheets of decellularized cornea with cell-laden collagen gels was compared in terms of repopulation potential to the larger tissue pieces. Furthermore, scaffolds were seeded with limbal epithelial stem cells for 2 weeks. Immunostaining was performed to assess cell phenotype.

### Results

Decellularization was confirmed by the absence of cell nuclei and remaining DNA levels of 35 ng/mg scaffold. Sulphated glucosaminoglycans were significantly reduced, while collagen levels were maintained. Scaffolds showed >40% of light transmittance and became fully transparent upon immersion in glycerol. Scaffolds were effectively infiltrated with human corneal fibroblasts. There was no statistical difference between the 3 media conditions tested in terms of distance migrated by the cells. However, when cells were cultured in Keratocyte medium they regained expression of ALDH3A1 as they would *in vivo*. The sandwich method allowed for homogeneous cell distribution throughout the constructs, in a more native-like way. Scaffolds were rapidly repopulated by a tight, stratified and $CK3^+$ epithelium.

### Discussion

These scaffolds showed promising *in vitro* results and therefore *in vivo* studies in a rabbit DALK model will follow.

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a90580

## 03-P516 Corneal Bioprinting Utilizing Hydrogels and Corneal Stromal Keratocytes

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In modern medicine corneal transplantation is the cornerstone of treatment for advanced corneal diseases. Still many factors hinder its long-term success including limited graft survival, allogeneic graft rejection, use of immunosuppressants and most importantly a global donor material shortage. Corneal stromal keratocytes (CSK)-loaded tissue-engineered cornea substitutes could substantially reduce donor material shortage and graft rejection. The main goal of this study is the bioprinting of CSK-loaded hydrogels that can be used in the future either as *in vitro* testing platforms for drug screening in personalized medicine, or as implants in corneal transplantation.

This study focused on the production of structurally sound, translucent, tissue-engineered artificial corneas by bioprinting of CSK-loaded hydrogels. Firstly, hydrogel-based cornea substitutes were designed with CAD and printed using a drop-on-demand custom-made bioprinter. After successful isolation and culture of human primary CSK, cells were embedded in printable collagen-based bioinks and printed as constructs with a dome shape. CSK viability after bioprinting was evaluated with live/dead staining and keratocyte phenotype was analyzed by histology and immunohistochemistry (Kera, ALDH3A1, SMA and Lum) and ELISA (Kera). The thickness and translucency of bioprinted artificial corneas was measured by optical coherence tomography.

Live/dead staining showed viable CSK in bioprinted hydrogels one and seven days after the bioprinting process. Moreover, drop-on-demand bioprinting of living CSK seemed not to affect their original keratocyte phenotype, as proved by immunohistochemical stainings and ELISA. Bioprinted constructs also showed satisfactory levels of translucency in the light of their application as implants for corneal transplantation.

This study reveals the potential of applying 3D bioprinting technologies in ophthalmological research for obtaining individualized solutions for patients in need for corneal cell therapies.

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a92192

## 03-P517 Functionally designed nano-scaffolds for the regeneration of chronic tympanic membrane perforations

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Various patches releasing growth factors have been developed to treat chronic tympanic membrane (TM) perforations. However, nanostructures influencing cellular functions have never been applied to patches before. Aligned anisotropical nanostructures, directed toward the center, are anticipated to help the wound healing of TM. Thus, we developed a radially aligned electrospun patch scaffold that releases epidermal growth factor (EGF) and applied it to animal models. EGF-releasing radially aligned patch scaffolds (EGF-A) were fabricated by electrospinning and compared with electrospun EGF-releasing random patch scaffolds (EGF-R). Both patches were observed by scanning electron microscopy (SEM), and their alignments were analyzed by Image J. Also, the incorporation of EGF on scaffolds was confirmed by Fourier transform infrared spectroscopy (FTIR) and their release efficacies were evaluated by enzyme-linked immunosorbent assay (ELISA). The cell viability of all the patches was assessed with primary TM cells isolated from Sprague-Dawley (SD) female rats (4 weeks, 50-100g). In *in vivo*, SD female rats (7 weeks, 200-250g) were used, and patches were applied to the perforated ears. Then, their healing status were checked and imaged once a week for 8 weeks. Non-treated group which induced spontaneous healing was set as the control group. For histological analysis, TMs to which patches were applied for 8 weeks were obtained and were analyzed with hematoxylin and eosin staining.

a92424

## 03-P518 Decellularized ECM Bio-inks Manufactured from Cell Sheet Technology

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The purpose of this study is to make the collagenous scaffold obtained from decellularized ECM which is made by cell sheet method into 3D bio printer ink. Basically, the main material of bio-ink is used as hydrogel such as collagen, gelatin, hyaluronic acid and so on. However, the fundamental problem of these inks isn't directly related to the cells of the scaffold that they are trying to make, so there could be a difference in cell viability. In order to solve such problems, we were able to check biocompatibility and cell proliferation by cultivating cell sheets of various cells on biocompatible scaffold and bio ink. The output was made using an INVIVO bio-printer and a 10 x 10 x 3 mm^3 scaffold was printed. The comparative groups were gelatin-based in-vivo gel, medical collagen and dH-ECM Gel. The uniformity of the output scaffold was observed through a microscope. The printability was similar to that of gelatin. The rheometer was used to measure the viscoelasticity of each scaffold. Confocal image was confirmed after live and dead treatment at 1, 7, and 14 days intervals for cell proliferation after seeding of fibroblast cells, and cell proliferation of dH-ECM gel was confirmed to be higher than other scaffolds. The described dH-ECM Gel is expected to provide a new technology for bio-inks, customized for use in 3D cultures of a variety of cell types, with ease of production and biocompatibility.

a93021

## 03-P519 Magnetic Iron Oxide Nanoparticles for MRI Tracking of Human Embryonic Stem Cell-Derived Photoreceptor Precursors Cells

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*In vivo* cell tracking is important to obtain a better understanding of the biodistribution of cells applied as therapeutic agents. Magnetic resonance imaging (MRI) provides an opportunity to visualize transplanted cells labeled with superparamagnetic iron oxide (SPIO) particles and the surrounding tissues at the same time. However, the applicability of those techniques for the living retina has not been investigated. The goal of this study was to assess the effectiveness of MRI tracking of SPIO-labeled cells that transplanted into subretinal space.

We labeled photoreceptor precursors cells derived from human embryonic stem cell with SPIO using the FeraTrack labeling kit (Miltenyi Biotec, Bergisch Gladbach, Germany). The next day, SPIO-labeled cells were harvest for transplantation to Royal College of Surgeons (RCS) rat model. As a control, SPIO particles containing media was also injected into subretinal space of RCS rat. Animals were examined by MRI with T2* weighted sequences immediately after cell injection, as well as 4, 8, 12, 16, and 20 weeks later. Histologic analyses were taken at week 12 or 20 and compared to MRI results.

Hypointense artifacts corresponding to the transplanted cells were distinctively visible at the injection site. They decreased over time, but still remained visible over the whole follow-up period. Histology showed that SPIO-labeled cells were distributed in the subretinal space that corresponded to the hypointense artifacts in MRI images. However, hypointense artifacts were decreased over time much faster in controls with SPIO particles containing media and undetectable since week 12.

This study demonstrates the feasibility of MRI tracking of SPIO-labeled cells after the subretinal injection. MRI cell tracking is a convenient and effective method for the detection of SPIO-labeled cells in subretinal space and can be appropriate for monitoring distribution of cells in cell therapy of various retinal diseases.

a92849

## 03-P520 Human Sclera Maintains Common Characteristics with Cartilage throughout Evolution

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Background: The sclera maintains and protects the eye ball, which receives visual inputs. Although the sclera does not contribute significantly to visual perception, scleral diseases such as refractory scleritis and deformity of the eyeball are considered incurable or difficult to cure. The aim of this study is to identify characteristics of the human sclera and the anteroposterior spatial gene expression hierarchy in the human sclera to develop a hypothesis for deformity of the eyeball.

Methodology/Principal Findings: We analyzed the global gene expression of human scleral cells of the human infant sclera obtained from surgically enucleated eyes with retinoblastoma, using Affymetrix GeneChip oligonucleotide arrays. Hierarchical clustering analysis showed similarity between scleral cells and auricular cartilage-derived cells. Cultured micromasses of scleral cells exposed to TGF-bs and BMP2 produced an abundant matrix. The expression of cartilage-associated genes, such as Indian hedge hog, type X collagen, and MMP13, was up-regulated within 3 weeks in vitro. After human scleral cells labeled with DiI were implanted into a rat cartilage defect, the cells expressed type II collagen. These results demonstrated that human scleral cells retained chondrogenic potential both in vitro and in vivo. K-means clustering analysis of gene expression revealed that expression levels of cartilage-associated genes increased from the anterior to the posterior part of the sclera. Microarray analyses and RT-PCR data showed that the expression levels of *MGP*, *COLXIA*, *BMP4* and *WNT2B* were significantly higher in the posterior than in the anterior sclera. The results of luciferase reporter assays suggested that a GSK-3$\beta$ inhibitor stimulated Wnt/$\beta$-catenin signaling particularly strongly in the posterior sclera.

Conclusion: Our study showed chondrogenic potential of human sclera. Interestingly, the sclera of certain vertebrates, such as birds and fish, is composed of hyaline cartilage. Although the human sclera is not a cartilaginous tissue, the human sclera maintains chondrogenic potential throughout evolution. Furthermore, chondrogenic potential was higher and Wnt/$\beta$-catenin signaling was more potently activated by a GSK-3$\beta$ inhibitor in the posterior than in the anterior part of the human infant sclera. This anteroposterior hierarchy in the sclera might contribute to disorders involving deformity of the eyeball. (Seko et al. PLoSOne, 2008; Cur Eye Res, 2016)

a91041

## 03-P521 Cell culture simulation based on Multi-agent computational model describing behavior of cells cultured in production of a corneal epithelial cell sheet to predict its quality

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Regenerative medicine using a person's own cell, generally an autologous cell takes a procedure that a doctor harvests cells from a patient which cells are cultured on a plate to make a target tissue, and then transplants the tissue to the same patient to cure the disease. In order to provide high quality cells to patients, it needs a technology to predict cell proliferation, differentiation and etc. at the most effective transplantation based on observation and analysis of cell behaviors, tissue structures, culturing medium profiles and etc.

We focus on culturing corneal epithelial cells which sheet is transplanted to treat a disease of corneal epithelial stem cell impoverishment symptom, and have developed a cell culture simulator. We designed the simulator using a Multi-agent computational model that enables an agent to mimic a cell and probabilistically interact each together with its own decision-making rules defined in functional modules of migration, division, differentiation, cell death and etc. Our objective of this research is to suggest which factors of the cultured cell property would effect or affect quality of the final cell sheet to be transplanted.

We present the simulator represented qualitatively cell behaviors in proliferation and differentiation to be structured along the culture process time to predict cell quality. We verified the simulator with experimental data of 3D positions of p63-marked epithelial stem cells, populations of cells structured in a cell sheet and etc. measured in cultures of the corneal epithelial cell using different cell lines in quality. We also studied which parameters used in the simulation model could relate more to the cell sheet quality; a probability in cell differentiation vs. a population of proliferative cells, a life time of cell connections vs. a population of cells in each layer of a structured cell sheet and etc.

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a91976

## 03-P522 Influence of Material Stiffness on a Corneal Epithelial Cell Line

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### Introduction

Corneal blindness is one of the most common causes of blindness worldwide, shortage of donor tissue and increased graft failure makes treatment options limited [1]. The corneal epithelium, the outer layer of the cornea, can be damaged by injury or disease causing pain and loss of vision.

Tissue engineering techniques to grow corneal epithelium could aid in the shortage of available tissue. To do this, the factors that control corneal epithelial behaviour must be first understood. Mechanical stress on cells can affect their activity[2], however, little is known about its effect on the corneal epithelium. This study examines how material stiffness regulates epithelial cell behaviour using a human telomerase immortalised corneal epithelial cell line (hTCEpi) to determine optimal conditions for generating new tissue.

### Materials and Methods

Polydimethylsiloxane (PDMS), was used to study the effect of material stiffness on corneal epithelial cells. Blends of PDMS were prepared and tensile tested. An elastic modulus from 12kPa to 2.24MPa was achieved. PDMS was cast into cell culture dishes and seeded at a density of 5,000 cells/cm$^2$ for 7 days. Cell morphology, viability, protein expression of a proliferation marker phosphorylated extracellular regulated kinase (pERK), mature corneal epithelial marker cytokeratin 3 (CK3) and mRNA expression of CK3 was carried out.

### Results

Preliminary data show that stiffer substrates promote the 'cobblestone' morphology observed in these cells. Stiffer substrates had a significant increase in cell viability and an increase in pERK protein expression. CK3 was not expressed in any groups. Therefore, RT-PCR was performed to see if differences in mRNA expression for CK3 was observed, stiffer substrates expressed increased CK3 expression. Further replications of these experiments will be performed to confirm these findings.

### Discussion

Our data shows that stiffer substrates increase cellular viability and proliferation as well as increased mRNA expression of the mature corneal epithelial marker CK3. In addition to this, stiffer substrates produce the typical 'cobblestone' cell morphology that is observed in corneal epithelial cells. This information may be used in the design of biomaterials of particular stiffnesses for corneal tissue regeneration.

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a90393

## 03-P523 Mechanical stimulation and inducing medium synergistically promote the differentiation of PDLSCs into corneal keratocytes

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Our previous study has reported that corneal-shaped static mechanical stimulation promotes the expression of keratocyte markers in cultured human keratocytes. This study aims to explore the role of corneal-shaped static mechanical strain on the differentiation of human periodontal ligament stem cells (PDLSCs) into keratocytes, and the possible synergistic effects of mechanics and inducing medium. Human PDLSCs were exposed to 3% static dome-shaped mechanical strain in a Flexcell® Tension System for 3 and 7 days. Up-regulation of genes for aldehyde dehydrogenase 3A1 (ALDH3A1), CD34, lumican, and collagen type I and V was seen, as well as for integrin alpha 1, alpha 2 and beta 1, and non-muscle myosin II B. Keratocyte differentiation (inducing) medium was introduced in the Flexcell® system, either continuously or intermittently combined with mechanical stimulation. A synergistic effect of mechanics and inducing medium was found on keratocyte differentiation by evaluating gene and protein expression of keratocyte markers. Finally, a multi-lamellar cell-sheet was assembled under the treatment of mechanics and inducing medium simultaneously. The cell-sheets were transparent, multi-lamellar and expressed typical markers of corneal stroma, which show potential for future clinical applications.

a92551

## 03-P524 Nano-Surface modified Recombinant Human Collagen type I Hydrogels as Superior Carriers for Corneal Epithelial Stem Cells

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**PURPOSE:** Cultivated limbal epithelial transplantation (CLET) can successfully regenerate the anterior cornea in Limbal Stem Cell Deficiency. The cell carrier most commonly used in CLET is the human amnion (HAM). The aim of this study is to investigate recombinant human collagen type I (RHCI) hydrogels[1] as a replacement to HAM for cultivating Corneal Epithelial Stem Cells (LESC).

**METHODS:** Ultrathin (<100µm) RHCI hydrogels[1] and HAM were tested for ultrastructure (SEM, TEM, AFM), optical properties (Refractometry - Transparency), bulk mechanical properties (Rheology, DMA), microbial susceptibility (P. aeruginosa challenge) and genotoxicity (VITOTOX). Cell viability and cell proliferation of cultivated immortalized corneal epithelial cells were investigated using PrestoBlue and BrdU. For primary cell cultivation, fibronectin nano-stripes were printed on the hydrogel's surface. Primary composite grafts were characterized using qPCR and IHC analysis. Acellular hydrogels were implanted subcutaneously in rats to test for bio-integration and degradation (follow-up 90 days). To validate hydrogels' effectiveness in ophthalmology, RHCI hydrogels were implanted in pig corneas (follow-up 1year).

**RESULTS:** RHCI hydrogels consist of randomly arranged collagen fibrils, contributing to its inherent mechanical strength. RHCI is more transparent than HAM. Collagen scaffolds show no genotoxic effect, and are 100-fold more resistant to microbial growth. No significant difference in cell viability and active proliferation rates were noted between the scaffolds. When cultivated on RHCI, primary LESC keep expressing their undifferentiated adherent stem cell phenotype. In rats, RHCI hydrogels show good biocompatibility as hydrogels were relatively intact and showed minimal tissue irritation.

**CONCLUSION:** Primary LESC can successfully be cultivated on RHCI using standardized xeno-free GMP-conditions. The favorable optical & mechanical characteristics, relative microbial resistance, successful composite graft generation, and biocompatibility prove that RHCI is a highly promising scaffold for ocular application. Nano-lithography improves cell adhesion. Verification of RHCI corneas in pigs is ongoing.

**REFERENCE:** [1]Yaari A. et al., Tissue eng part A. 19, 1502-6, 2013

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a93136

## 03-P525 Development of retinal degeneration model in vitro and identification of retinal regeneration inducing factors

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Adult mammalian retina is incapable of regeneration in retinal dysfunctions, which results in further retinal degeneration and permanent vision loss. There is currently no treatment for total blindness. Lower vertebrate can restore the vision by inducing retinal regeneration after severe retinal damage. After traumatic lesion, Müller glia can start to proliferate and differentiate into neuronal cells in zebrafish retina. Therefore, we hypothesized that application of a principle of retinal regeneration in zebrafish to mouse retina by gene transfection allows to us to induce retinal regeneration. For the cure of impaired vision in human disease, the objectives of the study were 1) to identify the proliferation and neuronal differentiation inducing genes, 2) to develop a new retinal degeneration model in vitro mouse retina, and 3) to determine whether retinal degeneration is rescued by gene transfection.

We have performed in vitro assay for screening of transcription factors and found that Ascl1-NICD3 cotransfection induces Müller glia proliferation. However, neuronal differentiation was not observed in Ascl1-NICD3 cotransfected cells. On the other hand, additional transfection of Sox4 to Ascl1-NICD3 induced neuronal morphology and the expression of retinal ganglion cell markers in a few transfected cells. To increase the efficiency of neuronal differentiation, we attempted to construct a novel gain-of-function system which induces expression of transcription factors via a two-step manner. This system is possible that we introduce proliferation inducing genes in the first step and differentiation inducing genes in the second step by mimicking retinal stem cells in zebrafish. In preliminary studies, we found that DsRed and EGFP expression are induced in two-step with high efficiency.

To develop a new retinal degeneration model in vitro, we used a phototoxic red fluorescent protein KillerRed that produces reactive oxygen species (ROS) and results in cell death. Carrying out a series of staining of various markers for retinal degeneration by immunohistochemistry, we confirmed that active caspase-3 was specifically detected in rod photoreceptors by Nrl-drived KillerRed transfection, phagocytic microglia was observed in the ONL, and GFAP expression was induced in Müller glia. These results demonstrate that KillerRed-based retinal degeneration can be used as a new model in vitro.

a93470

## 03-P526 Three-dimensional Stem Cell Niche for Cultivated Oral Mucosal Epithelial Transplantation and its Application for Corneal Reconstruction

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Most protocols for cultivating keratinocytes for clinical transplantation requires 3T3 cells as feeder layer to promote ex vivo expansion of progenitor cells. Due to the concern of xenogenic infection such as prion or reverse transcription viruses, regulation on using animal supplements during cell culture are strict. On the other hand, the standard trypsin/EDTA treatment on tissue often results in massive exfoliation and death of keratinocytes, leaving only a tiny portion of progenitor cells survive. For this, recently we have developed a cell culture technique that doesn't require animal ingredient for cultivating human oral mucosal epithelial cells (OMECs) for transplantation. Briefly, oral mucosal tissue taken from biopsy was treated overnight with collagenase A at 37 ℃, then cultivated with SHEM medium containing artificial serum PLTMax. Denuded human amniotic membrane was used as a cell culture substrate. We found that compared with OMEC sheet isolated by trypsin/EDTA and fed with fetal calf serum, cell density, BrdU label retention capacity, colony formation efficiency were all significantly higher in the collagenase group. Expression of stem cell markers p63 and p75 were also higher. Pathway analysis revealed that genes related to integrin-linked kinase (ILK) were selectively up-regulated. We therefore hypothesize that following collagenase treatment, niche micostructure, namely the basement membrane protein components were maintained, which elicits ILK activation and in turn up-regulates Wnt pathway, resulting in proliferation of OMEC progenitor cells. Cultivated oral mucosal epithelial transplantation (COMET) using the aforementioned technique has been approved by TFDA as a phase Ib clinical trial for treating severe ocular surface diseases with limbal stem cell deficiency. To date, three patients have successfully received COMET. A 42-year-old male with severe alkaline burn OD received COMET, and after the corneal surface became stable, he received corneal transplantation, resulting in significant improvement in vision. The removed corneal button was subject to immunoconfocal microscopic study, and the epithelial layer was positive for keratin 3 and keratin 4 but negative for keratin 8 staining, compatible with the phenotype of OMEC, and positive staining for p63 and p75, imply the engraftment of OMEC progenitor cells in the cornea. The initial result is promising; yet more cases are needed to justify the efficacy and safety of the technique.

a90927

## 03-P527 A Novel Preservation Technique for Long-Term Storage and Ambient Temperature Distribution of Transplantable Human Corneas

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Worldwide, only 100,000 corneal transplants are performed annually, despite the fact that there are 1.5 million cases of corneal blindness diagnosed each year. One of the biggest limitations to corneal transplantation is access to quality donor tissue due to inadequate eye donation services and infrastructure in developing countries. This is compounded by the fact that there is no long-term storage solutions for effectively preserving spare donor corneas collected in countries with a surplus. Eye banking infrastructure requires large amounts of local investment and labour to put in place, however, increased access to tissue can be achieved by development of preservation techniques to increase corneal storage times and allow for global shipping at ambient temperature. This preservation will also allow storage on hospital shelves, for use in emergencies, where waiting for donor corneas is not possible.

In this study, we used a novel drying technique to preserve human corneas collected in the US and UK, that were deemed unsuitable for transplantation. We assessed weight, thickness, transparency, cell viability, cell membrane permeabilisation, ECM content and structure, comparing to non-dried donor corneas. A subcutaneous implantation model was performed in rats to assess biocompatibility and cell integration of the dry corneas. Clinical suitability was assessed through market access research targeting corneal consultants in the UK.

The dried corneas were comparable to non-dried donor corneas in all investigated aspects except cellular viability. When implanted subcutaneously in rats, the dried cornea was well tolerated, with cellular migration into the matrix and no visible immune rejection. We spoke to 12 corneal consultants, at 7 different hospitals, all gave positive feedback regarding future use and potential clinical indications.

Our preservation technique provides an easy-to-manufacture, non-viable, dehydrated, cornea suitable for a range of clinical indications and tectonic support in emergency situations. It can be stored on the shelf in hospitals for over 2 years and can be shipped at ambient temperatures worldwide, relieving the global shortage of corneal tissue.

a92953

## 03-P528 Bone Marrow Endothelial Stem Cell Therapy in Ischemic Diabetic foot

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Diabetes is one of the main and life threatining disease in human life which consists multi-organ distribution of the macrovascular complications. Diabetic foot is seen with disability- long time hospitalization, treatment with wide ranged antibiotherapy and finally an amputation could be performed to the limb. In our study we discuss how can we avoid this major vascular complication, by inducing the tissue repair, regeneration and neovascularization to the ischemic area, using the stem cell therapy. Bone marrow endothelial progenitor cells (BMEC) are adult stem cells located in bone marrow, can be differentiated in blood vessels by angionesis process which promotes tissue oxygenization and also regeneration. Due to their capacity to contribute in neovascularization in the ischemic tissues this remedy have been commonly used in modern medicine. We have treated 6 patients who have diabetic foots whose limbs is in infectious-neuropathic-ulcerated state . After a series of debridment to necrotic tissue, we injects BMEC intramuscularly around the limb muscles. Later we follow up them approximately three months with blood glucose regulation next to the discharge from hospital and eventually see the change for the ischemia to better: the demarcation line is decreased by vision , microcirculation and superficial blood flow is rised depending upon the results of ICG anjiograpy(SPY) system. We suggest BMEC raise the prospect of extending regeneration to the treatment of diabetic foot, with adding the prolonging amputation free survival period. Also we come up with the data that BMEC are the beneficial candidates to manage promoting the considerable wound healing in the diabetic patients, who have peripheral ischemic complications.

a92118

## 03-P529 Modeling vascularized glomeruli in vitro using a microfluidics chips based approach

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Introduction:
Many kidney diseases are caused by failure of glomerular filtration function, loss of podocytes and increasing glomerular fibrosis. Regenerative therapies may provide means to delay the loss of podocytes, replace damaged cells or repair and regenerate glomeruli. Renal precursor cells and mature glomerular cells are needed to study these regenerative approaches in vitro and in vivo. However, existing in vitro models of glomerular cell types and of the glomerulus are not well suited for such investigations. One obstacle for glomerular cultivation is the lack of a vasculature, an essential part for its function and maintenance. In this study, co-culture system of different types of cells will be introduced, which may be suitable for the of the various glomerular cell types.
Material & Methods:
We have used a vascularized microfluidics chip - based approach to culture and vascularize glomeruli isolated from human kidney of nephrectomy patients. Glomeruli were co-cultured with human umbilical cord vascular endothelial cells (HUVEC). The formation of vascular connectivity was confirmed by flowing of fluorescent polystyrene beads. Functional test of glomeruli was performed by filtration of dextran.
Results:
The glomeruli survive in the chip and connect to the vascular network formed by HUVEC. Perfusion of the vascular network together with the glomeruli was shown and allows functional analysis.
Conclusion:
The established microfluidics chip-based culture system is suitable to assay the potency of renal cells to integrate into healthy and damaged glomeruli. In addition, the system is adaptable for the co-culture of various cell types of the kidney and the analysis of inductive events.
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a91915

## 03-P530 Development of large-scale single-use bioreactor system for a megakaryocytic progenitor cell line

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The increasing application of regenerative medicine has generated a growing demand for stem cells and their derivatives. A scalable production method is crucial for industrialization of regenerative medicines. Single-use bioreactors offer an attractive platform for stem cell expansion owing to their scalability for large-scale production and feasibility of meeting clinical-grade standards.

We evaluated the capacity of a single-use bioreactor system (1L working volume, cylindrical, orbital shaking) for expanding Meg01 cells, a megakaryocytic (MK) progenitor cell line. It is well known the shaking speed is a critical factor in a bioreactor system in terms of oxygen supply and physical stress. First, we evaluated correlation between shaking speed and Oxygen transfer rates ($k_La$). The $k_La$ of shaking speed, 50, 100, 125 rpm were estimated to be 0.39, 1.12, and 10.45 $h^{-1}$, respectively. Second, Meg01 cells were cultured in each shaking speed condition. At 50 rpm, cell growth was limited an insufficient mixing. Cell density was gradually decreased at 125 rpm, due to high hydrodynamic shear stress. The bioreactor culture achieved the highest growth profile when shaken at 100 rpm, achieving a total expansion rate up to 5.7-fold with a total cell number of $1.2 \pm 0.2 \times 10^9$ cells $L^{-1}$. Finally, differentiation potential to MK lineage was evaluated. MK specific surface protein and morphological character were comparable to conventional culturing system. In this study, we developed single-use bioreactor system for megakaryocyte differentiation from progenitor cell line.

a92276

## 03-P531 Development of Waterproof Humidifying Bag Type Container using a Polyethersulfone Nanofiber Sheet Membranes

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Base on the urgent need to prevent cross contamination, especially in the management of $CO_2$ cell culture incubators in aseptically controlled cell processing facility, we designed closed bag type humidification container comprised with nano fiber sheet (Aquavator), which we have newly developed and exhibited superior functions of both moisture permeability and waterproofing performance. Here we evaluated practical usefulness of NF using cell culture assay. The nano fiber was made of polyethersulfone as $\phi$ 350nm in diameter, and constructed into a nonwoven fabric sheet with an average pore diameter of 450nm by electrospinning method. The thickness of nano fiber sheets are adjustable between 20 to 90 $\mu$m. Aquavator was configured as a bag type container having an nano fiber membrane on its one side; 27 x 31 cm square. Performance comparison with the other moisture-permeable waterproofing materials (Tyvek®, Beauty-ace®) are conducted. By the comparison with water bat (Conventional), a practical usability equipped in CO2 incubator and biological influence using cell culture assay are examined. Assessed parameters are %Room humidity, temperature, cultured cell number and viability, apoptosis. The results are the follows; 1) Aquavator exhibited superior performance both in moisture permeability and waterproofing by approximately 10 times higher than conventional products. 2) The adjustment ability of humidity by Aquavator showed equivalent to Conventional apparatus. 3) Cell culture analysis revealed no significant differences in cellular growth, cell viability and apoptosis after 6 days Jurkat cell culture between Aquavator and Conventional apparatus. We concluded that Aquavator exhibit equivalent performance of humidification as conventional water bat apparatus and contribute remarkable benefit to prevent cross contamination in $CO_2$ cell culture incubator management.

a92387

## 03-P532 Validation of Analytical Procedures using BacT/ALERT 3D Microbial Detection System

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Recent innovative technology of Rapid Microbiological Method (RMM) is getting recognized for its usefulness as alternative method for its rapidness, accuracy, selectivity and sensitivity, but for clinical application, it is still required for its method validation based on Japanese Pharmacopeia (JP) regulation. BacT/ALERT 3D microbial detection system (BacT) (Bio Merieux) is one of the well-evaluated devices in clinical fields, which automatically detect bacterial growth based on $CO_2$ colorimetric detection. In this study, we designed and assessed the bioanalytical method validation of RMM as alternative way for conventional standardized sterility test. Firstly, we evaluated "selectivity" and "sensitivity" to be compared for cultured microbial strains growth promotion between BacT and conventional method. Secondly, we performed method validation procedures using NKT cells as cell-based medical product for clinical trial, in which AIM V medium (Life Technologies) contained antibiotic agent being used as culture medium. Due to exsistence of antiotics, the BacT culture bottles contained adsorbent polymeric beads neutralizer were applied following manufacturer's manual for *growth promotion testing* of the media. In growth promotion test, all microbial strains described (according to) on JP were tested and consistently detectable within 1 days (bacteria), in both methods, suggesting that "selectivity" of BacT is equivalent to conventional method. As evaluation of "sensitivity", a single strain was tested for each species at target inoculum levels of approximately 30 CFU/bottle and compared each time duration of appearance of bacterial growth colony, and the result suggests that "sensitivity" of BacT is equivalent to conventional method (both within 1 days for bacteria, 2 days v.s. 2.7days for fungus ) . In method validation, all species tested were detectable by BacT method, whereas *A.brasiliensis* and *C.albicans* were undetectable by conventional method. These results suggest that beads neutralizer might contribute to efficiency of fungal detection even under antibiotics usage. In conclusion, systematic method validation procedure is indispensable before applying recent technology RMM as sterility testing of cell-based medical products to approve selectivity and specificity of the method. BacT/ALERT 3D is usable as alternative method of conventional sterility test under the proper validation.

a92928

## 03-P533 Development of hybrid cell sheets using peritoneum and MSC for prevention of anastomotic leakage after operation of rectal cancer in a rat model

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Introduction: Anastomotic leakage is a major complication in rectal cancer surgery. Although there are preoperative procedures and ingenuity of sutures, and progress of surgical techniques and devices, the incidence is still high at 3-14%. We attempted to introduce a cell sheet, which is a regenerative medical technique, to prevention of anastomotic leakage.

Objective: Hybrid adipose-derived stem cell sheet combining adipose-derived stem cell sheet and peritoneum was prepared and examined for histological evaluation and graft survival.

Method: Adipose tissue was collected from the inguinal part of GFP rat, and anadipose-derived stem cell sheet was prepared. The peritoneum of SD rat was collected, and the fascia in the median back of the rat was removed and the muscle was exposed. Hybrid adipose-derived stem cell sheet was transplanted to the back muscle, and histological findings (HE, GFP, immunostaining) and engraftment were evaluated (day 3, day 7).

Result: Hybrid adipose-derived stem cell sheets could be confirmed in a relatively stable state macroscopically and histologically.

Conclusion: Hybrid adipose-derived stem cell sheets may be engrafted in a stable state and may be useful for prevention of rectal rectal anastomotic leakage.

a93383

## 03-P534 Surfactant-free solubilization and systemic delivery of anti-cancer drug using low molecular weight methylcellulose

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Docetaxel, an advanced taxoid, has been widely used as an anti-mitotic agent, but further augmentation of its properties is still required, including improvement in low aqueous solubility[1]. In this study, we report the development of bio-eliminable low molecular weight methylcellulose-based surfactant-free injectable formulation for the delivery of docetaxel. Methylcellulose, a hydrophobically modified cellulose derivative, was hydrolyzed by an enzymatic degradation method to obtain low molecular weight methylcellulose (LMwMC)[2]. The current study pursued to evaluate the potential of the surfactant-free polymeric micellar aggregates. A biocompatible, bio-eliminable, and natural polysaccharide methylcellulose was prepared by enzymatic degradation to produce LMwMC. DTX administered intravenously, has high hydrophobicity, captivating the utilization of surfactants or organic solvents limiting the efficacy of DTX as they lead to low bio-availability. DTX-loaded LMwMC (DTX-LMwMC) micelles were prepared by the thin-film hydration method, and their various physicochemical properties, including critical micelle concentration, morphology, size determined by transmission electron microscopy (TEM), and *in vitro* release behavior, were evaluated. We further evaluated the *in vivo* anti-cancer effects against a B16F10 melanoma cancer cell-xenograft model following intravenous injection of the DTX-LMwMC micelles. This study considers the applicability of surfactant-free micelles for hydrophobic anti-cancer drugs.

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a91966

## 03-P535 First in human clinical trial of treatment of familial LCAT deficiency syndrome by self-transplantation of therapeutic-enzyme secreting adipocytes

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Familial lecithin:cholesterol acyltransferase (LCAT) deficiency syndrome is an autosomal recessive disease characterized by severe dysfunction of HDL with subsequent generation of abnormal LDL. Patients often develop life-threatening complications such as renal failure and corneal opacity. Long-lasting LCAT protein replacement is one of the therapeutic approaches to prevent the LCAT-deficient patients from progressive tissue damages. However, such treatment has not been developed since it is difficult to provide stable recombinant enzyme due to the cost problems and the rareness of disease. Our previous basic experimental data together with clinical experience in transplantation therapy strongly suggested that autologous adipocyte transplantation is an effective maneuver to perform *ex vivo* gene therapy enabling sustained secretion of therapeutic enzymes. We have reported that ceiling culture-derived proliferative adipocytes (ccdPAs) are useful for an effective LCAT production using the retroviral gene transfection. The *lcat* gene-transduced ccdPAs secreted functional LCAT protein, which caused maturation of HDL derived from LCAT-deficiency patients and reduced renal-damaging LDL poor in esterified-cholesterol, *in vitro*. Furthermore, subcutaneous transplantation of the ccdPAs ameliorated the circulating abnormal lipoproteins in *lcat*-deficient mice. Thus, ccdPAs would provide an excellent platform for developing a protein replacement therapy not only for LCAT deficiency but also other disorders caused by deficiency in serum protein requiring long-term therapeutic protein supplementation. In addition, fibrin glue, which is clinically available scaffold, increased the survival of transplanted *lcat*-gene transduced ccdPAs in mice. Based on the accumulated pre-clinical data, the *ex vivo* gene and cell therapy protocol has been approved under the Act on Securement of Safety of Regenerative Medicine in Japan. First patient was enrolled last year and the safety as well as efficacy is now under investigation.

a91909

## 03-P536 A novel technology for lifelong treatment of intractable plasma protein deficiency: *Ex vivo*-manipulated adipocytes for sustained secretion of therapeutic proteins

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Despite the critical need for lifelong treatment of inherited and genetic diseases, there are no developmental efforts for most such diseases due to their rarity. Based on much experiences of clinical transplantation therapy in cosmetic and reconstructive surgery, adipose tissue is now recognized as a source of proliferative cells for cell-based therapy. Most commonly used adipose tissue-derived cells are stromal vascular fractions (SVF), which is the sediment after collagenase digestion followed by centrifugation. Many studies demonstrated that SVF exhibits multi-lineage differentiation potential, suggesting a suitable source for regenerative medicine. However, accumulated data indicate that SVF are heterogeneous in nature and may not be a stable therapeutic gene vehicle from the GMP point of view and also from the safety concern. To solve this problem, we have developed the method to obtain the proliferative cells from the mature adipocytes via ceiling culture. The propagated cells, designated as ceiling culture-derived proliferative adipocytes (ccdPAs), were subjected to retroviral/lentiviral gene transduction which resulted in good gene transduction efficiency with stable secretion of the transgene products. GMP production procedure, by which the gene-transduced ccdPAs could be expanded up to $10^{12}$ cells from 1 g of fat tissue within one month after fat tissue resection, has been developed. Our platform technology has been applied for clinical development of *ex vivo* gene therapy of familial lecithin:cholesterol acyltransferase (LCAT) deficiency. Abnormal lipid profiles in serum of LCAT deficiency patients caused by LCAT dysfunction were ameliorated by addition of LCAT produced by human *lcat* gene-transduced ccdPAs *in vitro*. Fibrin glue, clinically approved tissue sealant, worked well as a scaffold to enhance transplanted cells to survive in mice and secret LCAT into the blood stream. First in human clinical trial has been started in Chiba University under the Act on Securement of Safety of Regenerative Medicine in Japan. We further continue our research and development to obtain the approval as gene therapy medicine. We are also developing protein replacement therapy of hemophilia, lysosomal storage diseases, and neurological diseases.

a90705

## 03-P537 Effect on Cell culture environment from Earthquake

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The time taken to commercialize regenerative medical products depends largely on cell proliferation and is longer than that of general products. For this reason, large earthquakes occurring during cell culture have a great influence on cell production. That not only increases the manufacturing cost of the manufacturer due to the manufacturing process being redone, but also makes it impossible for the patient to receive the product when necessary. When such a situation occurs, it affects the life of the patient, so it is very important for facilities that manufacture regenerative medical products to take measures that can continue culturing even when an earthquake occurs. However, it has not been clarified how much the earthquake can continue culturing. Therefore, a CO 2 incubator was actually installed in the space assumed for the culture room, and the excitation experiment was carried out to evaluate the maintenance of sterility. As a result, in a building without a base isolation function, it becomes difficult to secure sterility at a seismic intensity of 5 or more, but it became clear that in a building with a base isolation function, sterility is secured even at a strong seismic intensity of 6 It was. Therefore, the acceleration received by the culture vessel and the cells by each excitation was measured, and the influence on the cultured cells was evaluated. Report the results.

a92103

## 03-P538 Development of a novel modular system for cell production: an assessment for improvement of production efficiency by changing the operation method

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The main steps of the cell production, such as medium exchange or passaging operations, must work at defined intervals to grow the cells. Therefore, general assembly line operation is not suitable for cell production. When the production of cell-based products in the container handling which is handled by opening the vessels, aseptic cell processing areas in facility, which install commercial isolator systems, are difficult to be managed with high usage rate. It is reason that isolator systems are easy to maintain aseptically whereas they are not suitable for production methods sharing the chamber as a common processing area between multi-manufacturing lines.

If common apparatus could be shared between multi-manufacturing lines parallel in an isolator system, we think it would obtain an improved production management and contribute to significant cost-saving. Therefore a novel process assembly method, called a flexible modular platform (fMP), has been developed by combining modules composed the individual aseptic isolator. A key technology of fMP is the aseptic connection interface for transfer between modules which is decontaminated, and the standardization of aseptic transfer interface has been discussed in the working group of ISO/TC 198/WG9.

In this study, first, the production capacity of a facility model adopting the isolator system of fMP was compared with that of the facility adopting the conventional isolator system. The results showed that shorten of changeover process, which contains cleaning process time at the end of operation and connection time between modules on the eve of operation, was one of the critical issues. Then, the effects of improving production efficiency were evaluated by calculating the cost of the structural facilities, such as facilities or equipment, by using the manufacturing process of a model product which derived from allogenic iPS cells. It was found that the structural facility cost of the model adopting fMP increases much more slowly than the model adopting conventional isolator system as the number of production increases.

a90192

## 03-P539 Nano-Cucumis: Anti-microbial and Anti-biofilm Nanocomplex

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In this study, we constructed cucumber-like nanocomposites. The nanocomposite has grown to form copper nanoparticles on the surface of CNTs, resulting in a combination of particle shape (zero dimesion) and rod shape (one dimenstion). These cucumber-shaped nanocomposites have proven to have strong bactericidal activity and also to remove biofilms. However, at the lowest concentration with bactericidal activity, it was able to prove to be harmless to human cells. These cucumber nanocomposites are expected to be applicable to implants and medical devices where biofilms are easily formed.

a91224

## 03-P540 An Industrially Scalable Small Molecule Gelator with Applications in Tissue Engineering and Regenerative Medicine

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Recently, a lot of research has been invested in the use of hydrogels in tissue engineering and regenerative medicine (TERM) as scaffolds mimicking the extracellular matrix (ECM).[1] Polymer based hydrogels are extensively being investigated in the field;[2] however, in the class of low molecular weight gelators (LMWGs), mainly peptide based materials are used.[3] These are very interesting because of the functionality of the peptides as well as the smart material capabilities, well-defined structure, and ability to form dynamic systems of the LMWGs. Because of the many advantages of LMWGs but the costly and elaborate synthesis of peptides, this work focuses on the development of non-peptide based low molecular weight hydrogelators with applications in TERM. These gelators were synthesized in quantitative yields, and the scalability of their synthesis was shown using the green and scalable technique of ball milling. Gelation tests showed that these compounds could form hydrogels in water, PBS, DMEM, and cell culture medium. Assessment of the mechanical properties of the hydrogels, using shear rheology, showed that the materials have thixotropic properties, indicating that these materials are suitable for delivery via injection. SEM and AFM studies showed that the gelator network consisted of ribbon-like nanofibers. Cytocompatibility studies, performed by encapsulating L929 fibroblast cells, demonstrated that these cells remained viable in the hydrogels and proliferated over several days. In conclusion, we believe this material could provide a low cost and cytocompatible hydrogel scaffold for cell expansion and minimally invasive delivery.

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a91721

## 03-P541 Phase Transformation of Graphene Oxide and its Biomedical Applications

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Graphene oxide (GO) has attracted significant interest as a template material for multiple applications due to its two-dimensional nature and established functionalization chemistries. Currently, as-synthesized GO nanosheets are employed directly to this end with no structural modifications. Here, we induce a phase transformation (oxygen clustering) in GO (also named as *treated*-GO) and demonstrate its benefits for several bioapplications. We utilize the modified form of *treated*-GO to construct a highly sensitive and planar device that allows for quick and efficient capture of murine class-II $MHC^{+}$ cells from blood sample. Our approach leads to a capture efficiency of ~92% at room temperature. Besides, we demonstrate the capability of *treated*-GO to play a dual-role simultaneously as a: (i) cellular imaging agent, and (ii) drug delivery agent in CT26 cancer cells, without any need for additional fluorescent protein labeling. This extraordinary surface also allowed to carry out better molecular absorptions, chemical conjugations, within the coexistence of abundant oxidized and graphitic domains, which are capable of accelerating human mesenchymal stem cell towards the osteogenic lineage. Overall, our work highlights the impact of phase transformation in GO for applications in biomolecule detection, cellular imaging, drug delivery and stem cell differentiation, hence lead material science a brand new era.

a92662

## 03-P542 Synthesis of sub 3 nm-sized uniform magnetite nanoparticles using reverse micelle method and examination of its biological compatibility

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Nanoparticles play important role in diagnosis and therapy of many diseases. However, the irregularity of nanoparticle size and the use of detrimental solvent during produce of nanoparticles are obstacles in use of nanoparticles for clinics. To solve these problems, we report a novel and facile method for the synthesis of small and uniform magnetite ($Fe_3O_4$) nanoparticles. Magnetite nanoparticles were synthesized via a reaction of iron(III)-stearate with hydrazine in reverse micelles composed of oleylamine, F127, xylene, and water at the reaction temperature of 90 °C in air atmosphere. The synthesized 3 nm-sized spherical magnetite nanoparticles exhibited good size uniformity. By controlling the experimental conditions, we could easily control the size and size uniformity of the magnetite nanoparticles. The biological compatibility of synthesized nanoparticles were tested *in vitro*. Also the use of these synthesized nanoparticles as a $T_2$ MRI contrast agent is now examined. These small and uniform magnetic nanoparticles are usefull in the various biomedical applications, for example, sensing and drug targeting.

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a92739

## 03-P543 Cartridge type microfluidic system for Body on a Chip applications

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Microfluidic chip is a strong tool to conduct biological experiments for analyzing, stimulating and manipulating cells by controlling surrounding micro-environments. By taking advantage of the controllability of fluid flow in microfluidic chip, the system resembling organ or human body in a chip, so called organ on a chip or body on a chip, are rapidly developing in the world. They are expected to be used as a new experimental model alternating an animal experiment for biological applications such as drug evaluation and analysis of immune systems. The ultimate goal of the body on a chip is imitating the circulation system of human body in a chip by including multiple organs and producing cell-tissue or tissue-tissue interactions in a chip. However, the more system is complicated with including multiple organs, the operation procedure becomes too complicated for practical use. Especially, the application of the microfluidic chip for 3D cell culture is still limited due to the complexity of the experimental procedure. The time consuming protocol deteriorates cell viability and it causes variation of the experimental results, which are frequent problems in 3D culture. Here I present the cartridge type microfluidic device to facilitate the procedure of 3D culture in a chip. Hybrid gel cube (HGC) device was employed to separate cultivation system for 3D tissue and control system with microfluidic chip. It allows us to insert and release the cultured tissue in a few seconds to/from microfluidic chip at desired timing as if we change an ink cartridge from printer. Thus, the cell damage due to the experimental procedure can be minimized. In additions, the large-sized tissue can be imaged with high resolutions by rotating the sample with HGC for multi-scanning via laser microscopy system after releasing from the chip. In order to present the capability of proposed system, I have conducted analyzing side effect of doxorubicin on cardiac myocytes. The proposed cartridge type microfluidic system can supply simplicity for the growing Body on a Chip system to be practically used.

a92475

## 03-P544 Development of Comprehension Evaluation System for Laws and Regulations of Regenerative Medicine

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The revision of the Pharmaceutical Affairs Law was conducted and renamed as the Pharmaceuticals, Medical Devices, and Other Therapeutic Products Act came into force in November 2014 in Japan, and the category of regenerative medicinal products were defined independently. As the regulatory frameworks of cell & gene medical products in Europe and the United States also has been getting promoted, specialized education and better understanding of this new law and relative guidelines are getting more indispensable for those handling regenerative medicine used for academic clinical trials and therapeutic applications of pharmaceutical industries.

While continuous education for facility management staffs and operators engaged in aseptic cell processing facility are commonly accepted to be important for ensuring safety and quality management of products, there seldom established on the comprehensive evaluation method. To resolve these requirements on the field of cell & gene based medical products, we have developed a new system to evaluate the comprehensive degree for the content of the new law, and constructed as cloud-operative system for better application method: “e-COMPASS”.

The quantitative evaluation system “e-COMPASS” consisted of the questions based on of this law and also included some contents from “Good Manufacturing Practice” and “the Act on Securement of Safety of Regenerative Medicine, etc.”. These questions were classified into twenty categories based on the chapter of the law and relative guidelines, and the test was constructed twenty questions selected randomly from the pooled questions of each categories. The test was conducted in three groups; Group A: undergraduate students in Biomedical engineering, not having GMP education, Group B: Medical undergraduate students, having GMP education, and Group C: Experts of GMP, practically working in Cell Processing Facility in Japan.

The “e-COMPASS” successfully extracted some specific categories among the Groups which include risk factors for manufacturing and specific features to regenerative medical products not found in conventional medicines, providing the important target categories to be required for advanced and more intensive education content.

In conclusion, the “e-COMPASS” system is useful for evaluating the degree of comprehension of law concerning regenerative medicine.

a90288

## 03-P545 DIFFERENTIATION OF HUMAN AMNIOTIC FLUID STEM CELLS CULTURED ON BIOMATERIALS HAVING NANOSEGMENTS

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Human amniotic fluid stem cells (hAFSCs) are pluripotent fetal cells, which are capable to differentiate into multiple lineages of the cells containing three embryonic germ layers. Thus, hAFSCs may become a more suitable source of stem cells in tissue engineering and regenerative medicine in future. Retinal pigment epithelium (RPE) is the pigmented cell layer just outside the neurosensory retina, which nourishes retinal visual cells. Retinal pigment epithelial cells can differentiate into many other ocular tissues. In human clinical trials, transplantation of RPE derived from human embryonic stem cells (hESCs-RPE) resulted in extensive photoreceptor rescue and improvement in visual function. In clinical applications, it has been shown that RPE derived from stem cells is valuable for the recovery of the disease of eyes, such as Age – related macular degeneration (AMD) and Stargardt's macular dystrophy. However, there has been no study of RPE derived from mesenchymal stem cells, which was used for clinical trials and animal experiments. Therefore, we have investigated the differentiation of hAFSCs into RPEs in this study. We designed the culture method of hAFSCs where the different substrates were coated on tissue culture polystyrene (TCPS) dishes, which were used to culture hAFSCs such as (1) TCPS coated with Synthemax II (oligo-vitronectin based substrate), (2) TCPS coated with human recombinant-vitronectin and (3) TCPS coated with poly(N-isopropylacrylamide-co-butylacrylate) (ploy-Nippam-co-BA). We found that the coating of these substrates on TCPS supports strong attachment of hAFSCs on the dishes, which is useful for the differentiation of hAFSCs into RPE.

a90792

## 03-P547 EGF and 3D alginate scaffold improved oocyte-like cells differentiation

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Three-dimensional (3D) culture matrices based on biomaterials are porous substrates that can support cell proliferation, differentiation and regeneration. Some growth factors, such as epidermal growth factor (EGF) also stimulate normal meiosis during oocyte maturation in vivo. A 3D culture system using calcium alginate encapsulation was applied to induce oocyte differentiation from mouse embryonic stem cells (mESCs). Here we report that mouse embryonic stem cells can be induced to differentiate into oocyte-like cells using embryoid body (EB) protocol culturing in two-dimensional (monolayer) or three-dimensional microenvironment in vitro. For the effective differentiation of oocyte-like cells we employed coculture system using ovarian granulosa cells in the presence or absence of EGF (+EGF or –EGF) for 14 days.

In the cells exposed to EGF in the three-dimensional microenvironment, the gene expression levels of premeiotic (Stra8, oct4), meiotic (SCP1, SCP3, Rec8) and oocyte maturation (GDF9, CX37, ZP3) were upregulated significantly. The high efficiency of differentiation into oocyte-like cells from mESCs reflects the culture method employed in the 3D coculture system. These results showed that this culture system along with EGF improved the rate of in vitro oocyte differentiation.

a92567

## 03-P548 Enhancing the Study of Wound Healing through a Multiplexing Approach of an *In vivo* Negative Pressure Wound Therapy Porcine Model

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Negative pressure wound therapy (NPWT) has been used as an effective surgical adjunct technique for accelerating the closure of acute and chronic wounds for nearly two decades. NPWT is used in battlefield trauma and routinely in the operating room to close a variety of full thickness and partial thickness wounds. Considerable efforts have been invested into elucidating the cellular and molecular mechanisms behind the efficacy of NPWT; however, these mechanisms are still not fully understood due to challenges in directly observing NPWT histologically, in real-time. The ability to observe how the polyurethane foam dressing interfaces with the wound bed and surrounding tissue over time, would provide significant insights into how NPWT has been proposed to aid the body in wound healing and wound closure. In the work presented, a series of full thickness wounds were made in a porcine model, and the character of the wound bed was assessed over nine days when exposed to NPWT and without NPWT. A specialized multiplex format was used to execute multiple analyses on each wound in parallel to maximize the data collected for each sample while minimizing discomfort to the animal. Our findings are the first to histologically capture the in-growth of the granulation tissue as well as infiltration of dermal fibroblasts into the polyurethane foam dressing while wound sites are under negative pressure. Furthermore, our findings raise new questions regarding the direct and indirect effects of how NPWT stimulates cell proliferation and extracellular matrix remodeling, which may have implications for enhancing repair of other critical defects in soft tissues using deformable biomaterials that alter the surrounding tissue.

a91995

## 03-P549 Modulatory effects of vitamin C on microRNA expression on murine ovarian follicles grown *in vitro*

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The regulatory effects of vitamin-C (vit-C) were reported in the *in vitro* murine ovarian follicular growth model (Yong Jin Kim et al., Reprod Sci 2010). MicroRNAs (miRs) are known to suppress target gene expression post-transcriptionally in the ovarian tissue. Currently, miR expression profile during follicular growth has not been well-established. We attempted to evaluate the miR expression pattern changes after vit-C treatment.

Pre-antral ovarian follicles were retrieved from 13-day-old C57BL/6 female mice and cultured under treatment with follicle-stimulating-hormone and luteinizing-hormone. Fifty and 100 μM of vit-C was added for 10 days during *in-vitro* culture. At full maturation, follicles were treated with human chorionic gonadotropin and epidermal growth factor. qRT-PCR was conducted to evaluate the miRs that are expressed mainly in murine ovaries.

SA-$\beta$-gal (senescence-specific staining)-positive population was significantly reduced in the treatment group compared to control. The expression of oocyte- and follicle-specific genes such as Oct 4 was up-regulated in the vitamin treatment group. The expression of apoptosis-specific miRs was down-regulated by vit-C treatment.

Taken together, vit-C treatment at tested doses altered the miR expression pattern of oocytes and granulosa cells during *in-vitro* growth of murine ovarian follicles, probably via retarding senescence (2016R1E1A1A01943455).

a93182

## 03-P550 Use of hyperpolarized magnetic resonance spectroscopy (HP-MRS) in cell and tissue engineering

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Herein, hyperpolarized (HP) magnetic resonance spectroscopy (MRS) was used for direct monitoring of the metabolic alterations induced by the spheroidal culture of tumor cells. HP-MRS is a powerful method to increase the sensitivity of nuclear magnetic resonance (NMR) over several orders of magnitude (> 10,000 times), which enables noninvasive and quantitative assessment of metabolic process in living systems by the use of functional chemical probes labeled with non-radioactive nuclei such as $^{13}C$ as a tracer. In this work, increase in the conversion of HP [1-$^{13}C$]pyruvate to [1-$^{13}C$]lactate, which reflects the enhanced aerobic glycolysis known as the Warburg effect, could be directly monitored in tiny multicellular tumor spheroids (~300 μm) put in an NMR tube, which was compared with those of normal cells from flat-bottomed culture. These findings suggest that HP [1-$^{13}C$]pyruvate MRS can be used for the noninvasive evaluation of the functions of biological mimetics. Furthermore, increase in the aerobic glycolysis in early tumorigenesis, which seems to be ahead of malignant transformation (*e.g.* hypoxia, necrosis or vascular formation), can be an ultra early diagnostic marker on HP-MRI. Given the functional HP chemical probes of interest, noninvasive evaluation of biological functions, and exploration of biological markers in various biological mimetics may be possible by the use of HP-NMR/MRI.

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a92047

## 03-P551 Lectin profile variation in mesenchymal stem cells derived from different sources

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**Background**: Human mesenchymal stem cells (MSCs), as a good source of stem cells for regenerative medicine, have different morphological and functional features. Carbohydrate moieties on the cell surface have important roles including cell-cell interaction and cell recognition. The objective of this study was to find the variation in glycoconjugate distribution pattern of MSCs derived from various sources.

**Material and methods:** MSCs were isolated from the adipose tissue, bone marrow, Wharton's jelly, and cord blood. Then, they were stained with FITC-conjugated wheat germ agglutinin (WGA), peanut agglutinin (PNA), concanavalin A (Con A), *Ulex europaeus* (UEA), *Dolichos biflorus* (DBA) and atto-488 conjugated *Phytolacca americana* (PWM) lectins. The intensity of the reactions was scored by ImageJ software. Flow cytometry was performed to detect the expression of endothelial marker, CD144. The data were analyzed by ANOVA and LSD.

**Results**: Lectin reactivity of MSCs from various sources had different distribution patterns. Cord blood derived-MSCs showed the significant highest intensity of the reaction with all lectins. All MSCs were also stained with PNA "moderately. Bone marrow-derived MSCs failed to react with UEA, DBA and Con A. Wharton's jelly derived MSCs also could not be stained with Con A. Cord blood-derived MSCs contained subpopulations with different lectin reactivates and none of them expressed CD144.

**Conclusion**: It seems that the MSCs derived from various sources had different lectin reactivity.

a92236

## 03-P552 Innovative Gas Phase Sterilization System for Aseptic Cleanroom Operation

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Recently we have developed innovative technology as gas phase sterilization system based on a catalytic reaction mechanism by use of methanol to generate mixed biogas, which simultaneously achieve remarkable performance of nucleic acid decomposition, as well as sterilization effect (pat. 4292234, 5463378). We report here 1) overall view of the system, 2) results of both sterile assurance level and nucleic acids (DNA/RNA) decomposition ability. This system consists of a mixed biogas generator, a small experimental chamber and a scrub exhaust device for detoxification equipped with catalyst detoxification apparatus for safety. The system enables to make continuous monitoring of temperature and humidity as well as the hydrogen concentration and other biogas elements. Sterility assurance level (SAL) has been accomplished $10^{-6}$ within up to 5 minutes biogas exposure under 37℃ environmental temperature. This system shows a sterilization effect to cover a wider spectrum including *Bacillus atrophaeus, Geobatillus stearothermophilus* and *B.subtilis var globigli*. ii) The system achieved complete DNA/RNA decomposition performance even at 37℃, resulting in less than 10 base pairs, within up to 5 minutes gas exposure for dsDNA, ssDNA and RNA, exhibiting time-, temperature-, dose-, and phase- dependency. For safety evaluation, composition analysis of biogas samplings components was performed before and after catalyst detoxification treatment, and identified the concentration of several major components such as methanol (54,000 / <0.1; ppm), formaldehyde (98 / 2.3; ppm), carbon dioxide (3 / 8.7; ppm), oxygen (25 / 14; ppm), hydrogen (>2.0 / <0.5; ppm), etc, resulting that most exhausted biogas components have been eliminated through catalytic process, moreover, they reached into undetectable levels after scrubber process, suggesting no harmful gas components are diffused into environment by appropriate treatment for exhausted biogas. Therefore, this innovational gas phase sterilization system revealed to exhibit remarkable nucleic acid decomposition performance as well as rapid aseptic effect as sterility assurance level, $10^{-6}$, and will be highly useful for managing aseptic and virus free clearoom, contributing safety and quality management of manufacturing cell and gene medical products.

a92331

## 03-P553 Lectin histochemisry showed a heterogeneous population of cells among human mesenchymal stem cells isolated from adipose tissue

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**Background**: Adipose tissue as an appropriate source of mesenchymal stem cells (MSCs) has the potential to differentiate into multiple lineages. Glycoconjugates content of the MSCs can be considered as biomarkers in self-renewal, pluripotency and differentiation processes. In this study, the lectin profile of MSCs isolated from adipose tissue was detected and according to that, a subpopulation was determined.

**Materials and Method**: MSCs were isolated from adipose tissue by explanting of the tissue pieces. The FITC-conjugated lectins, WGA, UEA, PNA, BSA and PWM were used to detect the terminal sugar residues. The cells were then counterstained with DAPI. The intensity of the reaction was evaluated by ImageJ software. The cells were also stained with PAS method.

**Results**: MSCs were reacted with all lectins with different intensity of the reactions. The cells reacted with WGA, UEA, and BSA "strongly "and with PWM "moderately" and with PNA with "weak" intensity. The morphological analysis of the isolated MSCs revealed the existence of the two different cell types in the cultures. Two types of cells were detected according to nucleus size and lectin reactivity. The cells with large nuclei constitute 20.62% of the total cells and stained with significant more intensity by UEA and less intense with PWM (both P=0.014) and PNA (P=0.044). Flow cytometry with CD34 shows that these large cells were not endothelial cells.

**Conclusion**: The MSCs derived from adipose tissue seem to be a heterogeneous populations and lectin profile of the cells showed they are different in the expression of the glycoconjugates.

a90874

## 03-P554 Delayed fraction injection of PRP promotes autologous fat graft regeneration

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Background: The long-term retention rate of autologous fat grafts is a common problem in cosmetic surgery. The main reason is that the autologous adipose tissue after injection, early local inflammatory response and hypoxia-induced adipocytes and adipose-derived stem cells (ADSC) survival rate is not high.Then the number of newborn adipose tissue is insufficient, it is difficult to achieve long-term retention. After early inflammatory response, and then injection PRP into the grafted fat, which may be able to achieve a better effect of the regeneration of fat transplantation.

OBJECTIVE: To observe whether delayed fraction injection of PRP can increase the survival rate of fat transplantation. Compared with traditional single PRP implantation, is it advantageous to promote fat regeneration?

METHODS: The following four groups of fat grafts were injected into the back of 16 nude mice respectively: 1, saline mixed group (0.35ml fat + 0.15ml saline); 2, PRP mixed group (0.35ml fat + 0.15ml PRP); 3, delaying the 7-day injection group (0.35ml fat + 0.15ml PRP day7); 4, delayed fraction injection group (0.35ml fat + 0.075ml PRP (day7) + 0.075ml PRP (day14) The animals were sacrificed at one month and three months after transplantation . The volume, weight and morphology of transplanted adipose tissue were compared. HE, CD31, perilipin and MAC1 / 2 were also observed to compare the structures, blood vessels, inflammatory reaction, newborn adipose tissue.

Results: Compared with the saline injection group, the volume and weight of the transplanted fat in the PRP mixed group, the delayed injection group for 7 days and the delayed fraction injection group for 7 days were significantly higher than those in the saline mixed group , But there was no significant difference among the three groups. The degree of inflammatory reaction: MAC1 / 2 staining showed that the number of macrophages around the fat-mixed group was the highest, suggesting the highest degree of inflammation; vascularization: CD31 results showed that the number of neovascularization in the delayed fraction injection group was the highest. The newborn adipocytes: perilipin staining showed that the number of surviving and new born adipocytes was highest in the delayed fraction injection group.

Conclusion: Delayed fraction injection of PRP can improve the survival rate of fat transplantation; and compared with a single injection of PRP, can promote the regeneration of adipocytes.