lower temperature were higher than those prepared at the higher temperature. Upon coating, the pore sizes of the foams decreased from 50-200 µm to 25-170 µm while those of the foams prepared at -80 oC decreased from 30-80 µm to 15-60 µm. CLSM and SEM analysis of the cell seeded scaffolds show that coating the foams with alginate enhanced cell attachment and ECM production.

**Conclusion.** Surface and mechanical properties of the alginic acid-coated PLLA-PLGA foams were significantly better than those of the uncoated ones, however, porosity decreased upon coating. The results show that the foams prepared at -20 oC have properties comparable with those of the native meniscus.

**Keywords.** Mechanical characterization, scaffold, meniscus, biodegradable polymers

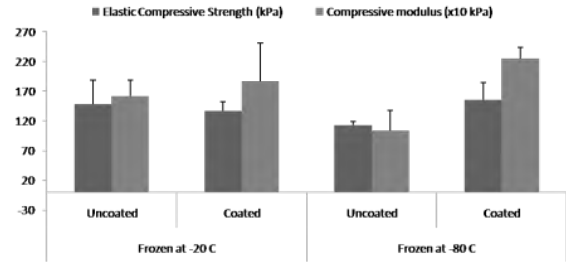


**Figure 1.** Influence of coating with alginic acid and praparation condition (freezing temperature) on compressive properties of the PLLA-PLGA foams.

## (27.O17) FABRICATION AND MECHANICAL CHARACTERIZATION OF A NOVEL THREE-DIMENSIONAL CELL-SEEDED COLLAGEN/HYDROXYAPATITE OSTEOCHONDRAL SUBSTITUTE

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**Introduction.** When a cartilage injury occurs, the underlined bone changes, thickens and becomes pathologically altered. In these conditions, a successful procedure for cartilage regeneration should not involve only the cartilaginous tissue but the whole ostechondral compound. Nowadays, in the clinical practise, only cell-free composites are available. However, although the bone tissue is able to colonize an osteocompatible scaffold, the cartilage cannot. We believe that seeding cells into the chondral part of a biphasic substitute, could represent a promising solution for the ostechondral tissue repair. In this study, a novel three-dimensional collagen/hydroxyapatite ostechondral substitute was developed, cell-seeded and mechanically characterized.

**Methods.** A hydroxyapatite macrochanneled porous scaffold was produced by polymer sponge templating method using a reactive sub-micron powder synthesized by hydroxide precipitation sol-gel route. The ceramic scaffold was then integrated to a collagen part obtained by a freeze drying technique. Morphology and mechanical properties of scaffolds was analysed by scanning electron microscopy and compression test. Expanded swine chondrocytes were suspended within the collagen part. Samples were retrieved from culture after 1, 3, 5 weeks. All samples were processed for histological evaluation and mechanically tested to evaluate the engineered constructs stiffness.

**Results.** The ceramic part of the scaffold had high mechanical performance (compressive strength ~ 0.51 MPa) compared to literature data. The collagen scaffolds showed a regular structure and homogeneous porosity (~ 100 µm). The histological analysis showed cell survival and matrix production within the scaffolds' fibres. The mechanical strength of the tissue-engineered construct increased significantly after 3 weeks of culture (from 7 to 55KPa).

**Conclusions.** Three-dimensional collagen/hydroxyaptite scaffolds could be properly fabricated and mechanically characterized. An in vivo study, to evaluate the efficacy of the scaffolds for repairing osteochondral defects is in progress in a pig model.

**Keywords.** scaffold, engineered construct, compression test

## (27.O18) EFFECT OF SUBSTRATE STIFFNESS AND PHYSICOCHEMICAL PROPERTIES ON HUMAN MESENCHYMAL STEM CELL ACTIVITY IN 3D SCAFFOLDS

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**Introduction:** Developing scaffolds with instructive properties to control stem cell behavior is an emerging strategy in tissue engineering. With the aid of mathematical models, theories were proposed to understand the influence of mechanical stress and strain on cellular behavior, while substrate elasticity was experimentally shown to direct stem cell differentiation. Yet, it is not known whether cells adhered to the surface of scaffolds perceive a different magnitude of physical stimuli compared to cells embedded in synthetic or natural extracellular matrix (ECM). We developed finite element models (FEMs) to predict the distribution of stresses and strains in 3D scaffolds displaying variable stiffness and physicochemical properties. Assuming that different stress/strain regimes result into different cell activity, we also initiated the experimental evaluation of the influence of scaffold stiffness and physicochemical properties on human mesenchymal stem cells (hMSCs).

**Method:** 3D scaffolds from different biomaterials (300PEOT55PBT45, 1000PEOT70PBT30, PCL, PLDLA) with equal pore architecture were fabricated by rapid prototyping. Scaffolds were imaged through microcomputed tomography and converted to FEMs to

calculate the surface and volume strain distribution and fluid shear stresses upon compression and perfusion. The influence of substrate bulk and apparent stiffness on cellular activity was investigated. Static culture experiments were performed on 3D scaffolds seeded with hMSCs exposed to basic, osteogenic, chondrogenic and adipogenic medium.

**Results:** Scaffolds consistently supported a lower surface strain compared to the volume strain. This implies that the mechanical stimuli perceived by cells on a 3D scaffold are of different magnitude than cells embedded in a synthetic or natural ECM. Static culture experiments showed hMSCs adhesion and distribution on 3D scaffolds varied by biomaterial. hMSCs adhered and distributed better on 300PEOT55PBT45 followed by PCL, PLDLA, and 1000PEOT70PBT30. Further experiments will aim at correlating substrate physicochemical properties and stiffness with hMSC differentiation in 3D scaffolds in a dynamic environment.

**Keywords.** 3D scaffolds, substrate stiffness, physicochemical properties

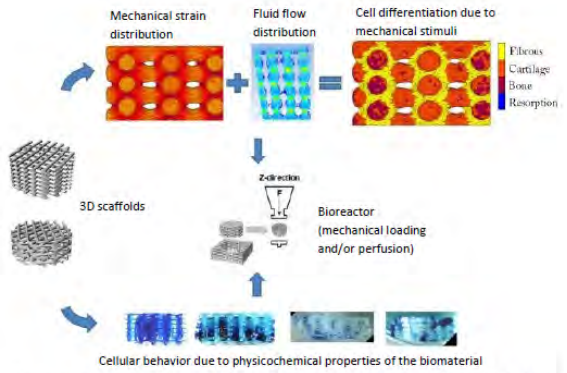


Figure 1: Top track: from uCT, Finite Element Models are developed in which the strain distribution and fluid flow can be calculated and the influence of bulk stiffness and apparent stiffness can be investigated. Taken together, these mechanical stimuli could predict cell differentiation due to the loading regime in a bioreactor. Lower track: the physicochemical properties of the scaffold have an influence on cellular behavior, which might be of interest in a dynamic loading regime as well.

## (27.O19) VALIDATION OF THE EFFECT OF WALL SHEAR STRESS ON CELL ADHESION FOR A PERFUSION BIOREACTOR MATHEMATICAL MODEL

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**Introduction.** Mathematical modelling of tissue engineering bioreactors has been used to determine the best operational conditions. The lattice Boltzman (lB) method allows the inclusion of all the parameters into a model for a perfusion bioreactor. The effects of fluid shear stress in cell seeding and cell differentiation in such systems have been studied previously; however a math model that allows the optimization of flow rate and cell concentration, among others parameters, have not yet been developed.

**Materials and Methods.** The lB model included velocity and concentration evolution equations to model the behaviour of cells when seeded into a scaffold; wall shear stress and attachment were also modelled. The simulations were done in straight and circular channels. For the validation, protein-coated and uncoated tygon tubings were seeded with hMSC and cultured for 24 hours. Non-stained samples were CT imaged including sections at 0* and 180* to mimic the flow of cells through a porous scaffold.

**Results.** The mathematical model showed cell alignment as well as concentration gradients in the case of the curved channel; experimental work confirmed cell deposition difference between uncoated and protein-coated tubing as well as in different areas of the same tubing.

**Discussion and Conclusions.** The lB code produced cell attachment and wall shear stress models depending on cell concentration and flow. Although work is still ongoing to improve the resolution of some images, current CT data has produced useful information for the math model.

**Acknowledgements.** We want to acknowledge the Henry Moseley X-Ray Imaging Facility for the assistance on the CT scans and image analysis. This project has been supported by the BBRSC research grants BB/F013892/1 and BB/F013744/1.

**Keywords.** mathematical model, Lattice Boltzman, perfusion bioreactor, wall shear stress, microCT

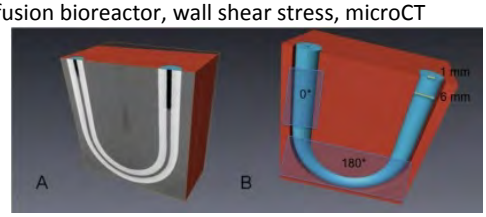


Fig. 1. (A) Reconstructed image of tubing containing cells (B) 0* and 180* sections for the model validation.

## (27.O20) MECHANICAL PROPERTIES EVOLUTION OF A PLGA/PLCL KNITTED SCAFFOLD IN CULTURE CONDITIONS

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**Introduction.** The mechanical properties of a scaffold during its degradation are of particular importance in tissue engineering. Indeed, the scaffold must ensure the transmission of mechanical efforts until a neo-tissue is formed. In this study, we studied the evolution of mechanical properties of a scaffold designed for ligament tissue engineering under different culture conditions.

**Methods.** The evolution of the mechanical properties of a knitted scaffold in PLGA with an electrospun PLCL fiber membrane was studied in three culture conditions: (I) static without cells; (II) static with 250 000 rMSC seeded at D0 in normoxia conditions from D0 to D28 and (III) cyclic traction-torsion (stretching: 10%, torsion: 90°) at 0.33Hz 2 hours per day in a bioreactor from D0 to D14 at 37°C (Medium was changed twice a week). Mechanical tests of uniaxial traction were made at D0, D7, D10, D14, D21 and D28 for group I and II and until D14 for group III (n=3). Biocompatibility test (AlamarBlue) was made for group II and III (n=3) and cell colonization was investigated by staining cell nuclei (TO PRO3) under confocal microscope.

**Results.** The scaffold immerged into culture medium without cells kept its mechanical properties during the 21st days and then lost rapidly its mechanical quality