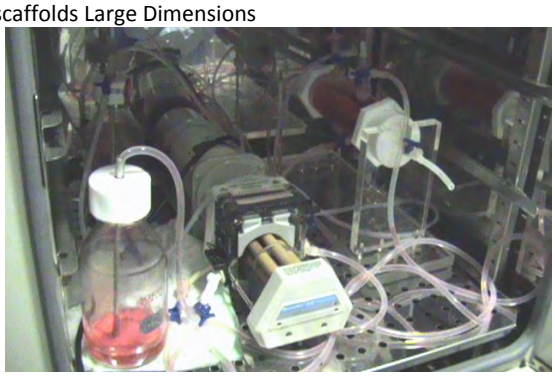
environment that favours the access to nutrients and removal of metabolic wastes of the cells located in the inner regions. Starch/Polycaprolactone (SPCL) fibbers mesh scaffolds (14 samples with 16mm x 4mm thickness with a concentric hole of 6mm) were seeded with 1x106 goat marrow stromal cells and stacked, completing a 48 mm thick construct. After 14 and 21 days of culture in the bioreactor at a flow rate of 1 ml/min, the samples were collected for DNA/ALP concentration, and SEM. Static cultured constructs were used as controls.

The results showed higher ALP activity levels in dynamic cultures than those obtained under static conditions. However, the number of cells (obtained from DNA amounts) in constructs cultured in the bioreactor showed lower values compared to static cultures, showing that static conditions tend to privilege the metabolic way for cellular proliferation while dynamic conditions tend to privilege the metabolic way for osteogenic differentiation. The lower values of the DNA amount of the constructs in the bioreactor could be explained by shear forces in the constructs, thereby hampering cell proliferation but enhancing cell differentiation. The BCFB can be used for enhancing cellular differentiation and proliferation by applying flow perfusion. Therefore, this bioreactor could be applicable to generate large-sized 3D scaffolds.

Keywords. Bioreactors; Bone Tissue Engineering; 3D scaffolds Large Dimensions



(7.O10) A PERFUSION BIOREACTOR SYSTEM FOR THE DEVELOPMENT OF TISSUE-ENGINEERED BONE CONSTRUCTS

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Introduction. The development of tissue engineered bone constructs is of considerable importance to fill defects associated with segmental bone replacement in bone cancer or spinal fusions.

Aim. To culture mesenchymal stem cells (MSCs) on porous and granulated scaffolds using a perfusion bioreactor system (PBRS) and study their proliferation, osteogenic differentiation and distribution compared to statically cultured constructs.

Hypothesis. A PBRS will provide an even distribution of MSCs throughout porous and granulated scaffolds and will enhance MSCs proliferation and osteogenic differentiation compared to statically cultured scaffolds.

Methods. An easily sterilised and assembled PBRS was designed and implemented. The scaffolds were Silicon substituted hydroxyapatite granules (Si-HA) and calcium-phosphate coated Ti6Al4V porous cylinders (CaP-Ti). Ovine MSCs were isolated from bone marrow aspirates, expanded in DMEM with 1%antibiotics-10%fetal calf serum (DMEM+) and characterised by differentiating them down the adipogenic and osteogenic lineages. Seeding studies were conducted for both scaffolds. Seeded scaffolds were either statically cultured in well plates or in the PBRS with a flow rate of 0.75mL/min, both with DMEM+. At days 4, 7 and 14 cell proliferation (AlamarBlue and DNA assays, n=3), osteogenic differentiation (ALP assay, n=3) and cell distribution (histology) were analysed. Constructs were visualised by SEM.

Results. Statistically significant increased cell proliferation ($p \leq 0.05$) was seen in samples cultured under flow perfusion conditions for both scaffolds at all times. ALP activity was significantly higher ($p \leq 0.05$) in the bioreactor constructs at all times points for both scaffolds. Histological analysis revealed a more even cellular distribution in the constructs cultured in the PBRS. The development of a cell layer over time was observed by SEM.

Conclusions. The PBRS used in this study increases cell proliferation and osteogenic differentiation and improves cell distribution throughout the scaffolds. We conclude that the development of constructs for bone tissue-engineering purposes can be achieved by using a PBRS.

Keywords. flow perfusion bone

SEM photo at day 4 of flow perfusion culture of CaP-Ti cylinder where cells have proliferated.

(7.O11) THE IMPORTANCE OF GRADIENTS IN ARTICULAR CARTILAGE

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It is hypothesized that gradients of growth factors (GFs) and GF antagonists exist in articular cartilage and play an important role in the balance between anabolic and catabolic processes. It is believed that such gradients are, at least partially, responsible for the zonal organization of articular cartilage. Despite their importance, current bioreactor designs for articular cartilage tissue engineering have limited options for introducing GF and GF-antagonist gradients. To address this issue we have developed a dual flow bioreactor which can accommodate four articular cartilage cubes (4.5x4.5x3mm) between two medium compartments. The reactor was designed in such a way that it mimics the knee joint as good as possible. The top and bottom compartment are mimicking the synovial fluid and subchondral bone respectively. The bioreactor was

complemented with a plunger that was attached to a compression insert. In this way load can be applied from a vertical position (Figure 1A).
Computational fluid dynamics was used to predict the occurrence of an oxygen gradient, which is shown in figure 1B. The model was then evaluated with a cell line containing a reporter system consisting of a HRE element controlling GFP expression. Medium in the top and bottom compartment were saturated with a different oxygen concentration. Quantification of the GFP expression showed the occurrence of an oxygen gradient (Figure 1C+D).
In conclusion, this unique bioreactor design assists in creating gradients, as shown for oxygen, and it will be used for creating gradients of growth factors and regulatory molecules. The ability to manipulate these gradients can aid in creating an ex vivo environment which may support the engineering of the native structure of articular cartilage.
Keywords. bioreactor, gradient, oxygen

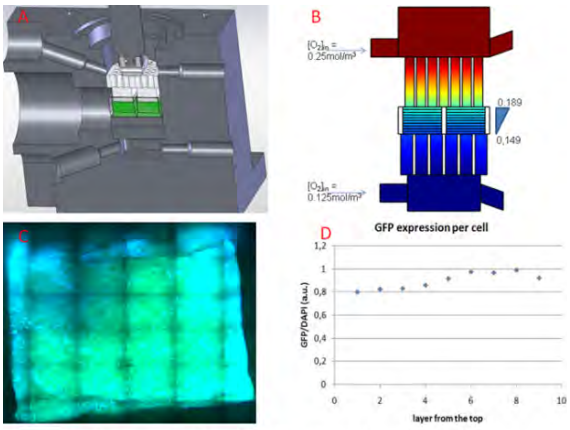

Figure 1: (A) cross section of the bioreactor chamber, (B) computational model of the oxygen gradient within the bioreactor chamber, (C) agarose-embedded cells stained with DAPI and expressing GFP (green) and (D) quantification of different layers in the gel from top to bottom. GFP expression increases when oxygen tension decreases.

(7.O12) NUMERICAL ANALYSIS OF NUTRIENTS TRANSPORT IN CONVECTION-ENHANCED HFMBS FOR LONG BONE TISSUE ENGINEERING

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Introduction. Recent experimental evidence shows that delocalized and distributed nutrients supply and high spontaneous Starling flows in hollow fibre membrane bioreactors (HFMBs) yield cm-scale BMSC aggregates, possibly by relieving nutrients limitations typical of other bioreactors for bone tissue engineering (BTE). The difficult non-intrusive measurement of nutrients and cell concentrations during culture makes mathematical modelling of mass transport, cell growth and metabolic reaction kinetics very attractive: to analyze the effects on cell organization and growth of nutrients transport, cell seeding and bioreactor geometry and operation; and to optimize bioreactor design and operation. Unfortunately, the non-uniform cell distribution observed in culture experiments and high Starling flows render most proposed models inadequate to the purpose. This paper presents mathematical models of HFMBs operated in close shell mode covering the range from diffusion-limited to convection-dominant nutrients transport conditions for both uniform cell distribution and the actual non-uniform cell distribution observed in experiments with BMSCs at different culture times.
Methods. Models are based on a multi-compartment description of HFMBs based on the Krogh cylinder assumption, and on a quasi-steady state analysis of evolution of nutrients and cell concentration profiles. Relevant non-dimensional parameters were identified, and governing momentum and mass transport equations were numerically solved with a finite element commercial code with particular reference to oxygen and glucose. Where possible, parameters assessed from culture experiments were used.
Results and conclusions. Simulation results demonstrate the importance of convective nutrient transport, membrane permeability and packing density in the cell compartment. They also suggest that bioreactor operation should be changed during culture to adapt to the variable nutrients demand of cells in the HFMB shell, as they proliferate and aggregate in 3D structures slowly filling up the shell space and exhibiting a Darcy permeability increasing in time.
Keywords. Nutrient transport; Bone tissue; Hollow fibre membrane bioreactor

(7.O13) VESSEL METABOLISM UNDER MECHANICAL LOAD - IMPLICATIONS FOR VASCULAR TISSUE ENGINEERING

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Introduction. Tissue engineered prostheses like vascular grafts or heart valves are usually generated in perfusion bioreactors which provide mechanical stimuli to condition the constructs. To assess whether conditioning alters nutritional requirements, we investigated the effects of shear forces and luminal pressure in a vessel model.
Methods. Bovine saphenous veins were perfused in mock circulations for 4 days. Group 1 vessels were perfused with M199 at 40ml/min. Group 2 vessels were subjected to increased shear forces (+12% dextran). Group 3 vessels were additionally challenged by increased luminal pressure (+20mm Hg). The corresponding groups 1', 2', and 3' were endothelium-denuded before perfusion. Substrate conversion was calculated from glucose and lactate levels. Blood gases were measured upstream and downstream of the samples. Contractile function and tetrazolium dye reduction were determined before and after perfusion.
Results. Noradrenaline-induced contractions after perfusion were significantly stronger in group 3 vessels and significantly lower in denuded vessels. Tetrazolium dye reduction was attenuated in groups 1'-3'. Glucose was converted stoichiometrically to lactate except groups 3, 1', and 3' which produced more lactate than glucose could supply. Oxygen concentrations were unaltered between vessel inlet and outlet except in group 2.