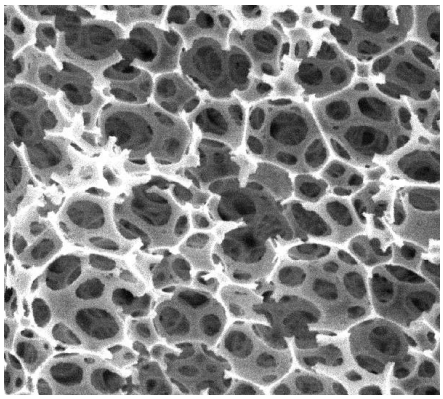
Project Title: Development of Novel Bioactive Porous Scaffolds and Gels for Cartilage Repair
Supervisors: Prof. Tim Hardingham and Dr Nicola Tirelli (Manchester University)
Drs Richard A. Black and Andrew I. Cooper (Liverpool University)

Background

Research in the field of Tissue Engineering continues to focus on the use of biodegradable materials that can be used as temporary scaffolds to deliver cells, antibiotics, growth factors etc., to an implantation site. Examples include bioresorbable poly(ester)s such as polylactide (PLA), polyglycolide (PGA) and their co-polymers, and inorganic materials such as tri-calcium phosphate (TCP), coral, bioactive glasses and glass ceramics. Resorbable polymeric biomaterials are traditionally processed in organic solvents such as chloroform or methylene chloride, to which a porogen is added to make the scaffold. Supercritical fluids, on the other hand, are inexpensive, non-toxic and non-flammable, and can be removed from the polymer matrix upon de-pressurisation with ease, making them especially attractive solvents to use in the context of Tissue Engineering. Unlike conventional liquid solvents, which are essentially incompressible, the density of supercritical carbon dioxide ($scCO_2$), for example, can be varied over a wide range at a constant temperature of 35ºC. As its critical pressure, Pc, of 73.8 bar is approached, there is a sudden increase in density. By fine tuning the density (and therefore the solvent properties) of the $scCO_2$, a number of variables, including particle size and pore structure, can be readily controlled.

The Cooper group in the Department of Chemistry has successfully used this method to fabricate macroporous cross-linked polymer monoliths with a wide range of pore sizes, but which have a very narrow pore size distributions.[1,2] The materials are removed from the reaction vessel as continuous monoliths, which conform to the shape of the reaction vessel. Since the template phase is CO_2, no organic solvent residues are left in the materials at the end of the reaction. Furthermore, the process is not limited to materials that can be foamed (i.e., rigid inorganic frameworks are also possible). The technique has also been used to prepare macroporous polymer beads by dispersion polymerisation.[3] Most recently, the Group has developed a method for the preparation of highly porous materials by emulsion templating.[4] An electron micrograph of one of these materials is shown on the right. Because the process is carried out at low temperatures, it should therefore be possible to incorporate appropriate proteins and growth factors into the structure during the fabrication process without denaturing them.



Research Plan

The proposed studentship project will investigate each of the above methods for processing medical grade polymeric materials into 3-dimensional scaffolds. The student will make porous scaffolds in a number of biodegradable polymers and determine the processing parameters associated with each polymer. This work will involve a number of different physical techniques. In addition to scanning electron microscopy, we will use mercury intrusion porosimetry to determine the internal structure and quantify the size and distribution of the pores, and the total internal surface area in each case. Samples will also be examined by differential scanning calorimetry (DSC) to determine the effect of processing on the polymer structure. Chromatography (GPC and HPLC) will be use in polymer degradation studies and to quantify the release kinetics of various soluble factors incorporated during the fabrication process. The release kinetics associated with different structures will be determined under both static and dynamic culture conditions in different bioreactor configurations.

The project will also involve work to characterise the mechanical properties of these scaffolds once these have been impregnated with a mixture of cells and hydrated biopolymers such as collagen or hyaluronan. The viscoelastic properties of these constructs will be determined, and monitored over time as cells infiltrate, proliferate and secrete their own extracellular matrix within the interstices, and as the polymer(s) degrade. The efficiency of cell seeding will be determined in terms of the viability and proliferation of cells with the aid of confocal laser micrcroscopy; the presence of proteoglycan and collagen, for example, will be determined by immunostaining following embedding and sectioning of the samples. Total matrix formation per cell will be determined using a DMB assay for GAG, hydroxyproline assay for collagen and DNA assay for cell content.

The above project would form the basis for a new collaboration between established groups at the University of Liverpool. The student, having a background in polymer chemistry, materials science or engineering, will work alongside those PDRAs in Liverpool involved in the synthesis of biodegradable polymers and in the fabrication of 3D scaffolds by other means. The work programme will also be developed in close collaboration with those working on Clinical Components in Manchester, including CCI (Skin/Wound Healing) and CCII (Cartilage).

References

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Hyaluronic acid-based matrices for growth factor release to enhance cartilage engineering
Proposal for PhD funding from Prof. Tim Hardingham and Dr Nicola Tirelli (Dept Pharmacy, Manchester)

Summary: This project will develop novel hyaluronic acid (HA)-based gels characterized by the following features: 1) prepared as injectable precursors for *in situ* gelation (compatible with minimally invasive surgery techniques); 2) controlled release of growth factors for promoting cartilage regeneration and for maintaining chondrocyte differentiation, 3) with adjustable biomechanical properties (for matching the mechanical properties of the surrounding tissues).

Matrix: HA is chosen due to the many favourable properties of this material (degradable, non-inflammatory, gel-forming), suitable for cartilage substitution as HA gels and it can be easily degraded (remodelled) by chondrocytes.

Growth factor: IGF-1 (insulin-like growth factor) will be used in this project as it promotes cartilage repair[1], as up-regulates HA production[2] and inhibits matrix degradation[3]. Many other growth factors (GFs) bind initially to the extracellular matrix (ECM) proteoglycans before binding to their cognate receptors and this enhances the stability of the otherwise labile GFs. HA or other GAGs can therefore be used to provide appropriate matrix docking sites to attract endogenous growth factors.

Loading and release: IGF-1 release will be monitored in three different loading strategies:

A) Use of the weak heparin-like properties of HA (heparin binds to HA receptors like RHAMM[4]; it also inhibits hyaluronidase[5]) for complexing GFs displaying heparin-binding domains; stronger binding by inclusion of heparan sulphate in the gel will also be tested[6].

B) direct covalent binding of IGF-1 to the HA structure through hydrolizable bonds.

C) covalent binding of IGF-1 to a third component, which shows a protection action towards IGF and displays a labile bond.

Approach C will be investigated in greater detail: if an appropriate polymeric structure is developed which allows the insertion of a stimuli-dependent gelling group (for *in situ* sol-gel processes -> injectability), potentially controlling the mechanical properties of the gel.

Gel formation and structure: In a similar, but less complex approach, Dr Tirelli has shown the possibility to obtain photopolymerized HA gels decorated with adhesion peptides and tunable mechanical properties[7].

In the present project the stimuli-responsive polymer will be chemically linked to carboxy groups of the HA structure and will contain reactive groups for IGF-1 binding. Two synthetic structures will be used, both in the three strategies above (no bonding, bonding to HA, bonding to the synthetic polymer):

I) temperature-sensitive polyethers (Pluronic-like polymers): gelation will be caused by an increase in temperature (10->37°C); moduli up to 100 kPa

II) polyethers end-functionalized with peptides containing lysine and glutamine residues: by mixing the matrix polymer with thrombin and factor XIII a fibrin-like gelation will occur; moduli up to 1 MPa

Cell response: chondrocytes will be either dispersed as isolated cells in the HA precursors before gelation or loaded onto preformed gels. Therefore there will be 12 systems under study (3 loading strategies * 2 polymeric structures * 2 cell-gel formulations). The cell phenotype will be monitored and the cells grown on gel films will be also analyzed for the expression of mRNA encoding ECM proteins to characterise the chondrogenic phenotype to distinguish it from chondrocyte hypertrophy and fibroblastic de-differentiation .

References

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