Biotribology of the Natural Ankle Joint

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Ankle osteoarthritis (OA) is the most common form of arthritis and is a major cause of morbidity and disability (Buckwalter, et al., 2004), of which 4% of adult population in the UK are suffering from (Valderrabano & Horisberger, 2011). It is characterised by the progressive destruction of articular cartilage, leading to joint space narrowing, subchondral sclerosis, subchondral cyst, synovial inflammation and osteophyte formation (Buckwalter & Mankin, 1998).

The aim of the project is to investigate tribology, contact mechanics and geometry of the natural ankle joint.
A novel functional biomechanical and biotribological model for the function of tibio-talar aspect will be developed. Comparison with hip and knee biotribological studies will be made.

Geometric measurements of ankles will be made prior to carrying out any testing procedures. Dimensional indentation testing and pin-on-plate device will be used to study mechanical characterisation and tribological quantities respectively.

Currently existing clinical interventions on ankle arthritis are considered to be unsuccessful due to limited research. Hence, further research into the biotribological aspect of the natural ankle joint needs to be established in order to develop treatment options for ankle arthritis.

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Abstract Title:	**Non-invasive Image Analysis as a Process Analytical Tool (PAT) for Cell Based Therapy Manufacturing**
Author(s):	David Smith, Rob Thomas
Abstract:	Conventional pharmaceutical and biologics production has begun to embrace the concept of Quality by Design (QbD) to reduce the risk associated with poorly developed processes. A major limitation on process development using QBD is the lack of non-invasive and label free measurements of process performance. Therefore the aim of this work is to develop quantitative metrics derived from non-invasive image analysis that can be used to make process decisions that control quality within therapeutically relevant cell cultures. This is achieved using an automated live-cell phase-contrast imaging platform (Cell-IQ) to monitor clinically relevant cell lines (ESC, HSC, MSC) over time to identify key metrics using image analysis. These traits can then be validated using a variety of invasive techniques including flow cytometry and immunohistochemistry. Numerous traits have been identified during a culture period, the next stage is choosing the most informative metric(s) to be useful in manufacturing monitoring or process development that relate to cell quality.

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DTC Year:	Graduate School studentship parallel with DTC5
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Abstract Title:	**Investigation of Corneal Cell Migration in Response to Mechanical Stimulation.**
Author(s):	Preeti Puntambekar, Supervisors: Dr. Yang Liu and Dr. Pablo Ruiz
Abstract:	Cells respond to mechanical changes in their extracellular environment and these mechanical properties have a powerful influence on cell behaviour (Angelini et al. 2010). The way that cells migrate and interact with the extracellular matrix (ECM) is important in embryonic development, tissue function and repair (Aman & Piotrowski, 2010). During the process of corneal wound repair, cell migration is a critical step that occurs and the cell type involved is the corneal keratocyte, which reside in the stromal layer of the cornea. Limbal epithelial stem cells (LESCs) are found in the limbus within a stem cell niche. These stem cells are also known to migrate towards the corneal epithelium in regeneration and wound healing (Dua et al., 2005). Current research has investigated corneal keratocyte migration in 3D collagen matrices and how mechanical signals regulate cell migration and affect cell behaviour (Kim et al, 2012). Within this project a method correlating ECM displacement with cell migration in 3D will be developed through advanced imaging and image processing techniques to aid further understanding of the mechanisms underlying corneal cell migration in the damaged corneal epithelium. Following the use of a rabbit corneal epithelial cell line in initial studies, the research will potentially progress further to Human limbal stem cells to investigate how these cells respond to mechanical strain and the effects of this on cell migration. This research may have implications in corneal wound

	repair, artificial corneal design and manipulation of stem cells in a 3D environment (Stem cell niche).
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Abstract Title:	**Drug Encapsulation and Release in Electrospun Nanofibre Structures**
Author(s):	Panagiota Moutsatsou
Abstract:	Polymers that can be fabricated in nanofibre structures are of significant interest to the biomedical community as the high surface to volume ratio they offer can be exploited for localized drug delivery purposes, thus enhancing the therapeutic result. PLA (polylactide) and PCL (polycaprolactone) will be used as the model polymers to be electrospun in nanofibers. Model hydrophilic and hydrophobic drug molecules such as tetracycline hydrochloride and tocopherol will be incorporated in the nanofibre structures either by blending in the polymer solution before the electrospinning process or by surface modification after the electrospun fibers are obtained. The effect of polymer solutions rheological and surface properties (such as viscosity, surface tension and conductivity) on the fibre diameter distribution and morphology will be evaluated. The morphology will be examined by Field Emission Scanning Electron Microscopy. At a later stage, conducting polymers such as polypyrrole and Polyethylene dioxythiophene (PEDOT) will be used for fabrication of nanofibers (either by electrospinning or by coating already electrospun nanofibers) which is expected to facilitate control over the drug release mechanism. The impact of the amount and nature of the loaded drug will be discussed and finally the drug release profile will be studied in vitro in phosphate buffer solution. The aim is to obtain a controlled release profile over a predetermined amount of time that it will be possible to tune by modifying different aspects of the procedure so that the needs of specific applications will be covered.

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Abstract Title:	**Exploring the improvement of human cell cryopreservation- benchmarking the *gold standard***
Author(s):	**Tim Morris**, Chris Hewitt, Karen Coopman
Abstract:	An accepted gold standard of human cell cryopreservation is 10% (v/v) Dimethyl Sulfoxide (Me2SO) in serum. Safety concerns with Me2SO/serum mean safer and optimized cryopreservation protocols using safer alternatives are needed. The efficacy of Me2SO in serum was compared with alternative cryopreservants using HOS TE-85 cells. Alternative cryoprotectants were not as effective as Me2SO, suggesting a change in cryopreservation processes to enable these alternatives is needed. Additionally the time dependent Me2SO cytotoxicity was evaluated by overexposing HOS TE-85 cells before and after cryopreservation. These data will be used to benchmark the cytotoxicity of alternative formulations. Future work is focused on the derivation of hMSCs from fresh bone marrow, defining a baseline thereof and repeating the previous work with this more therapeutically relevant cell line. Further, a design of experiments style procedure will be applied to cryopreservation to define more parameters for the development of a new process.
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	Progress, 27(6), p.1511-1521. • Keros, V. et al., 2005. *Optimizing cryopreservation of human testicular tissue: comparison of protocols with glycerol, propanediol and dimethylsulphoxide as cryoprotectants.* Human Reproduction, 20(6), p.1676-1687. • Zampolla, T. et al., 2009. *Effect of methanol and Me2SO exposure on mitochondrial activity and distribution in stage III ovarian follicles of zebrafish (*Danio rerio*).* Cryobiology, 59(2), p.188-194.

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Abstract Title:	Small-scale skeletal muscle constructs for *in-vitro* musculoskeletal junction pre-clinical testbed.
Author(s):	Nicholas Wragg, Dr Yang Liu, Professor Mark Lewis
Abstract:	**Introduction** Currently, musculoskeletal tissue damage is surgically repaired with the aim of restoring functionality over form. Any resulting tissue damage reduces utility and can increase the likelihood of further local injury. Therefore, more effective repair and regeneration techniques are needed. By creating an in vitro musculoskeletal junction that closely replicates the form and function of the in vivo system, novel materials and chemical products could be tested for viability in a more representative manner. In order to engineer a repeatable system capable of scaling-up, a small scale model of bone and muscle must be optimised. **Materials and Methods:** 0.75mL 3D type-1 rat-tail collagen (2.20mg/mL) neutralised constructs were seeded with C2C12 murine myoblast cells at $4x10^6$ cells/mL as previous described (*Sharples et al.* 2012) and set in a gelling area of 11mm x 14mm at 37^oC, 5% CO_2 .The constructs were tethered at either end by bespoke polythene mesh floatation bars to create longitudinal lines of isometric tension. Constructs were placed in 20% FBS high glucose DMEM for 4 days and then cultured in 2% horse serum high glucose DMEM to induce differentiation.

	Results and Discussion: Immunohistochemical staining for the intermediate filament protein Desmin showed the capacity for alignment and differentiation of C2C12 myoblasts within the collagen system at this scale. This replicates at a molecular level what is seen in larger constructs (*Sharples et al.* 2012). **Conclusions:** Preliminary indications show that a small-scale tissue engineered muscle suitable for a preclinical test-bed is viable from a phenotypic standpoint. Myotubes are evident and aligned however genotypic considerations and co-cultured limitations with osteoblasts are yet to be taken into account.
References:	Sharples, A. P., Player, D. J., Martin, N. R. W., Mudera, V., Stewart, C. E., & Lewis, M. P. (2012). Modelling in vivo skeletal muscle ageing in vitro using three-dimensional bioengineered constructs. *Aging cell*, *11*(6), 986–95.

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Abstract Title:	Quantification of Sulphated Glycosaminoglycan Production During Chondrogenesis by Human Primary Chondrocytes and Stem Cells
Author(s):	T. P. Dale, N. J. Kuiper, N. R. Forsyth
Abstract:	Limited therapeutic options for the treatment of cartilage injury and degeneration have driven the development of tissue engineering and cell therapy alternatives. We have investigated the secretion of cartilage matrix-associated sulphated glycosaminoglycan (SGAG) species from primary human bone marrow mesenchymal stem cells (BMA13), chondrocytes (OK3) and embryonic stem cell derived cells (1C6) to characterise cellular chondrogenic potential. SGAGs are the sulphated polysaccharide units of cartilage proteoglycans and are responsible for maintaining cartilage tissue hydration. Cells were seeded in 2-D and chondrogenesis induced over twenty days using pro-chondrogenic media (PCM). SGAG content of the cell monolayer and media was quantified using the metachromatic dimethyl methylene blue assay to determine total and secreted SGAG, and then normalised to DNA level determined by picogreen assay. In all cases larger total quantities of SGAG were produced by cells in proliferation media than in PCM. OK3 secreted the greatest amount of SGAG (62.6 µg SGAG/µg DNA) while BMA13 secreted the least (31.4 µg/µg) where a substantial proportion of this, up to 98%, was detected in the media fraction. In the presence of PCM total SGAG secretion was reduced by approximately 2-fold in all cell types tested whereas the substrate associated fraction was significantly increased in both 1C6

	(16% to 36%) and BMA13 (4% to 15%) ($p<0.001$ at day 20) when compared to non-PCM controls. In conclusion a pro-chondrogenic influence reduced total SGAG with a concomitant significant increase in the proportion of extracellular matrix-associated SGAG. Ongoing studies will extend these observations into micromass pellet systems and determine whether the detailed structure of the SGAGs being produced is characteristic to those of articular cartilage.
References:	Sharma A, Wood LD, Richardson JB, Roberts S, Kuiper NJ. 2007. Glycosaminoglycan profiles of repair tissue formed following autologous chondrocyte implantation differ from control cartilage. *Arthritis Res Ther*, vol. 9(4), R79.

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Abstract Title:	Characterisation of human mesenchymal stem cells via extracellular markers and cytokine factors
Author(s):	Alexander Chan, Karen Coopman, Chris Hewitt
Abstract:	The application of human bone marrow derived mesenchymal stem cells (hMSCs) is a promising field of investigation for tissue engineering and regenerative medicine. Currently hMSC are defined by expression/lack of well-defined surface markers; adhesion to plastic; and ability to undergo osteo-, chondro-, and adipogenic differentiation[1]. Using the accepted panel of extracellular markers we design a multiparameter flow cytometry assay that allows simultaneous detected of multiple antigens to fully characterise an hMSC population. In addition this work has shown that CD271, considered to be a specific marker[2], is not present on multiple individual donor lines and cannot be used to isolate hMSCs from a bone marrow mononuclear fraction. Recent evidence suggests the therapeutic effects of hMSCs are due to the production of trophic factors such as growth factors and cytokines[3,4]. This work will focus on the mode of action on what is currently being treated in early-late phase clinical trials such as graft-versus-host disease and myocardial infarction. This will involve the use of ELISA assays to characterise hMSC functionality for clinical treatments.

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Development of an *in vitro*, co-culture model to evaluate competitive uptake of magnetic nanoparticles by central nervous system glial subclasses

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Both rate and extent of magnetic nanoparticle (MNP) uptake are cell-type-dependent. For example, dramatic intercellular differences are observed between central nervous system (CNS) glial subclasses, with implications for MNP-based tissue engineering applications **where mixed neural cell populations exist**, such as *in vivo*. Here, a rat glial co-culture model has been developed to test whether the rapid, extensive uptake of MNPs by microglia (CNS immune cells) serves as an 'extracellular barrier' to nanoparticle uptake by other neural cell types. The model offers unique advantages: (i) parallel derivation of all cells from a single primary source; (ii) a single cell culture medium (tested and developed for this system) for isolated- and co-cultures; (iii) stoichiometrically-defined cellular ratios, facilitating modelling of different CNS regions/disease states. Astrocytes and oligodendrocyte precursor cells (OPCs) show drastically reduced MNP uptake **when co-cultured with microglia**. This confirmation of the 'barrier hypothesis' highlights the need to consider the relative abundance of each cell type when using MNPs *in vivo*. This system can be used to test the effectiveness of MNPs designed to target/evade specific cell types, with ongoing studies assessing: (i) whether polyethylene glycol (PEG) coated 'stealth' MNPs

evade microglia (with enhanced uptake by other neural cells); (ii) if MNP uptake is altered in the immunosuppressed CNS, where microglial activity may be inhibited.

Mechanotransduction in Multipotential Mesenchymal Stromal cells

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Abstract

Background:

Mechanical stimuli have been shown to affect gene expression in a range of differentiated cell types. There have, however been limited studies of mechanotransduction in multipotential mesenchymal stromal cells (MSC), in particular in three dimensional culture.

Aims:

1. To determine the optimal strain regimen for differentiation of hMSC towards a smooth muscle lineage
2. To establish the role of the cytoskeleton, ion channels and focal adhesions (FAs) in the strain-induced differentiation of hMSC
3. To dissect the roles of downstream signalling pathways in the strain-induced differentiation of hMSC
4. To determine the local strain experienced by cells during the strain-induced differentiation of hMSC

Experimental approach:

An acellular porcine pericardium scaffold will be used to support 3D culture of MSCs and the cells will be subject to cyclic tensile and biaxial strain (2-12%) in the Tencell and Biaxcell bioreactors. Optimal strain

regimens for the differentiation of MSC towards a smooth muscle lineage will be selected for use in studies examining the mechanism of mechanotransduction in MSC.

Results:

To date studies have focussed on the preparation of the acellular pericardial scaffold. Pericardia (35) were subjected to an established decellularisation process in batches of 9 – 12, and validated using histology, DNA assays and cytotoxicity and sterility testing.

Characterisation of wear and wear debris within cervical TDR utilising Silicon Nitride Coatings under standard and adverse loading conditions.

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Cervical total disc replacement (CTDR) has been increasingly used as an alternative to fusion surgery in patients with pain or neurological symptoms in the cervical spine who do not respond to non-surgical treatment. However, current CTDRs are often associated with issues similar to those affecting other joint replacement devices, including excessive wear and wear particle-related inflammation. Currently, there is little known about the characteristics of wear debris produced by CTRD devices, and any potential adverse effects of the particles on tissues surrounding the spinal cord. Additionally, current materials and material combinations do not offer the required implant longevity and reliability. Recently a novel silicon nitride (SiN) coating has been shown to have favourable wear characteristics and to produce debris that resorb over time, thus reducing the risk of adverse biological responses to these particles. The project will investigate the characteristics of wear and debris produced by CTDR devices coated with SiN deposited utilising PVD-HIPIMS technology. Additionally, solubility and biological responses (cytotoxicity, inflammatory response) to the SiN particles will be studied. The novel SiN coating may offer more reliable CTDRs, and thereby improve the quality of life of patients, by reducing risk of failures and providing a longer lasting solution.

Mechanical Characterisation and Computational Modelling of Spinal Ligaments

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The study will focus on an often overlooked aspect of the spinal motion segment: the spinal ligaments. These ligaments provide passive stability to spine and some studies have suggested that they play a major mechanical role within the physiological range of motion. However, the existing literature on the physical and mechanical properties of spinal ligaments span a large range and depending on these characteristics the resulting mechanical effects varies dramatically. The aim of this study is to characterise the ligamentous spinal structures and identify their importance in functional spinal unit models both experimentally and computationally. The experimental aspect will involve the use of advanced imaging (MRI, microCT) and mechanical testing facilities to characterise the morphology and mechanical properties of spinal ligaments. Furthermore, the computational aspect will involve specimen-specific modelling approach making use of the Finite element analysis packages to evaluate the mechanical role of these ligaments. The work will mark a step change from the current state-of-art where ligament properties and geometry are derived from widely varying data in literature.

The Fabrication and Characterisation of Nonwoven Fibrous Scaffolds with Novel Architectures for Craniofacial Tissue Engineering

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Professor Stephen Russell, School of Design

Professor Jennifer Kirkham, Leeds Dental Institute

Professor David Wood, Leeds Dental Institute

Non-union bone defects commonly occur due to trauma and disease; these defects can be extremely debilitating and when present within the skull can leave patients' appearances altered. Globally, over 4 million operations are carried out requiring bone grafting each year[1]. Autologous bone grafts are the current gold standard but up to 30% fail due to associated problems such as pain, parasthesia and donor site morbidity[2]. The use of tissue engineered bone substitute materials as scaffolds are gaining in popularity due to their reduced risk of disease transmission and their modifiable properties. This project's focus is the fabrication of nonwoven fibrous scaffolds that promote full osseointegration within the native bone structure.

Various spinning technologies are being used to produce nonwoven collageneous fibrous meshes. In particular electrospinning and forcespinning, two fibrous manufacturing technologies that can produce biomimetic fibres at the nanoscale level and have the capabilities of being scaled up. A study is ongoing in which collagen is being spun with phosphate buffers and ethanol to improve biocompatibility. This drive to use more biocompatible substances is being continued across the entire fabrication process with novel crosslinking methods that have been proved to be less cytotoxic when compared with more traditional crosslinking methods being employed.

The long term aim of the project is to produce a nonwoven collagen scaffold with improved bone regeneration when compared with the current gold standard treatments for facial bone defects.

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Abstract Title:	A complex, 3-D Model of Spinal Cord Injury to Evaluate Interactions of Nanofabricated Polymer Scaffolds with Neural Cells
Author(s):	Alan Weightman, Mark Pickard, Ying Yang, and Divya Chari
Abstract:	**Introduction:** Polymer scaffolds are promising 'structural bridges' for the repair of spinal cord injury (SCI), but their functional testing has been limited to live animal models of neurological injury, which have several associated disadvantages, including ethical issues and technical complexity. Conversely, the 'reductionist' models described to date typically fail to replicate complex 3D tissue cytoarchitecture and pathophysiological processes found in neurological injury sites. Here, an *in vitro* model of spinal cord injury based on organotypic slice cultures was adapted, characterised and utilised to evaluate the interactions of portable, aligned nanofibre scaffolds with neural cells in injury sites. **Methods**: Mouse organotypic spinal cord slice cultures were established [1,2] and transecting lesions induced with a custom lesioning tool. A range of neural cell markers were used to identify cells within lesion sites, with and without the placement of poly-lactic acid nanofibre scaffolds (± poly-lysine and laminin coating) over lesioned slices. The extent of nerve fibre outgrowth and alignment were quantified as were the reactions of key non-neuronal cells such as astrocytes and the resident CNS immune cells, the microglia. **Results:** The *in vitro* slice lesions display cardinal features of neurological injury *in vivo* (glial cell scarring

	and microglial infiltration). Slice viability was high following placement of scaffolds over lesions. The functionalisation of nanofibres coated with proteins was found to induce significant outgrowth/alignment of nerve fibres and astrocytes within injury sites, compared with uncoated nanofibres. **Discussion:** The facile method described here utilises humane procedures, low animal usage and is relatively rapid and inexpensive. **Conclusion:** Our findings suggest that the model can provide a powerful and versatile screening tool with high predictive value for the identification of neuroregenerative materials.
References:	**1.** Gahwiler, B.H. et al. (1997) Organotypic slice cultures: a technique has come of age. Trends Neurosci. 20, pp. 471–477 **2.** Bonnici, B. & Kapfhammer, J.P. (2008) Spontaneous regeneration of intrinsic spinal cord axons in a novel spinal cord slice culture model. Eur J Neurosci. 27, pp. 2483-2492

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Abstract Title:	**Chemically diverse polyacrylate and acrylamide surfaces for human cardiomyocyte culture and their effect on phenotype.**
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Abstract:	Human pluripotent stem cell (hPSC) derived cardiomyocytes hold the potential to strengthen pharmaceutical toxicity testing and to provide disease models for development of treatment targets[1]. The maturation and maintenance of the cardiomyocyte phenotype may be controlled by the manipulation of the substrate supporting the cells[2]. However, the surfaces currently in use still fall short of producing cardiomyocytes of adult maturity. Standard culture-ware requires coating with biological substrates such as fibronectin which can be expensive and subject to poor reproducibility due to batch variation. We are exploring an alternative, combinatorial materials high throughput screening approach[3] to identify novel materials that can improve cardiomyocyte culture. Polymer microarrays comprising of 6 replicates of 116 acrylates and acrylamides are fabricated using contact printing. Cardiomyocytes derived from the HUES7 human stem cell line are seeded onto the arrays. Immunostaining of nuclei (DAPI) and the cardiomyocyte specific motor protein, sarcomeric alpha actinin is performed to visually estimate cell function and maturity and enable quantification of cell attachment in a high throughput manner using automated fluorescence microscopy and image analysis software. Surface characterisation of the arrays is performed using time of flight secondary ion mass spectrometry. Partial least squares (PLS) regression analysis allows for correlation of cell

	attachment with key molecular ions identified from mass spectrometry[4]. Successful monomers that permit cardiomyocyte attachment, spreading and contraction are identified from the first generation homopolymer microarray and are mixed pair-wise to form second generation microarrays. This diverse library of copolymers enables unique combinations of chemical moieties to be investigated. Hit monomers and combinations identified to be synergistic can be analysed for their effect on cardiomyocyte function including electrophysiology measured by patch clamping, myofibril alignment and gene expression. The lead materials generated by this approach are the first step in a discovery process for novel synthetic biomaterials capable of enhancing the culture of cardiomyocytes to move towards more reproducible, economical and defined conditions.
References:	1. Matsa E. *et al. European Heart Journal.* 2011;32(8):952-62 2. Engler A. *et al.The Journal of Cell Biology*. 2004;166(6):877–887 3. Hook A. *et al*. *Biomaterials*. 2010;31(2):187–198 4. Yang J. *et al.Biomaterials.* 2010;31(34): 8827–8838

Decellularised porcine bladder patches for bladder repair and

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Abstract:

Background

Current clinical bladder augmentation materials have limitations [1,2,3]. Previously an acellular porcine bladder biomaterial was developed [4]. Inflation of the organ enabled complete decellularisation of the thick-walled tissue, but the process is incompatible with scalable manufacturing processes.

Aims

The aim of this project is to develop a process for the decellularisation of porcine bladder which can be translated to commercial manufacture.

Experimental approach

Initial studies focussed upon understanding the physical state of the bladder required to achieve successful removal of the cellular components during the wash process**.**

Bladders from 20-26 week old pigs were transported in transport medium to the laboratory within 2 hours of slaughter. Decellularisation was performed by distending the bladders using 500 ml of sequential buffers [5]. Histological examination of the processed bladders showed the presence of cellular material. It was hypothesised that insufficient distension resulted in inadequate diffusion of the solutions into the bladder wall. Subsequent studies confirmed that the volume capacity of the bladders was substantially greater than 500ml.

Discussion/future work

Studies will progress to repeat the decellularisation process at bladder volume capacity. Data on the deformation of bladders will determine the biaxial strain at volume capacity with a view to developing a device to hold bladder patches at the biaxial stress state of fully distended bladders during a manufacturing process.

References

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Abstract Title:	Iron oxide content and application of novel oscillating magnetic fields can enhance magnetic nanoparticle labelling of neural stem cells
Author(s):	Chris Adams, Humphrey Yiu, Boris Polyak, Divya Chari
Abstract:	**Introduction:** The translation of neural stem cell (NSC) transplantation therapy, can be facilitated by labelling NSCs with magnetic nanoparticles (MNPs). This can offer several important advantages such as non-invasive cell tracking[1] and safe delivery of transplant populations to sites of pathology by magnetic cell targeting[2]. Currently however, labelling efficiencies achieved in NSCs are low (generally less than 50%) necessitating use of strategies to increase uptake such as (i) transfection agents (for example, polyethylenimine) which can be toxic to cells or (ii) functionalising MNPs with cell specific uptake enhancing molecules (such as the peptide RGD) which can lead to exocytosis of the particles and therefore loss of translational function[3]. Here, we evaluate a novel, alternative approach to enhance NSC labelling by using MNPs of varying iron oxide concentration deployed with applied static and oscillating magnetic fields. **Methods:** Four distinct polylactic acid particle types were synthesised containing different amounts of iron oxide. Chemical characterisation was performed using zetasizer, FTIR, XRD and organic/inorganic content analysis. Biological uptake in NSCs was assessed by fluorescence microscopy and safety of the procedures was analysed by examining NSC 'stemness', proliferation, viability and differentiation. **Results:** Synthesised particles were of similar size and surface charge differing only in iron oxide content. NSC uptake was significantly enhanced when using particles of higher Fe content (up to a maximum of 91%) even in the absence of applied fields. Labelling using particles with a lower Fe content could be enhanced (from 40 to

	64%) by application of oscillating magnetic fields. Proliferation, viability, 'stemness' and differentiation profiles of labelled NSCs were unaffected by the developed protocols. **Discussion:** Here we report a safe and technically simple method of enhancing MNP labelling of NSCs, a key population for cell transplantation therapies, that does not require reliance on use of transfection agents or cell targeting strategies.
References:	**[1]**Bulte JW. In vivo MRI cell tracking: clinical studies. *AJRAmerican journal of roentgenology* 2009;**193**:314–25. **[2]** Polyak B, Fishbein I, Chorny M, *et al.* High field gradient targeting of magnetic nanoparticle-loaded endothelial cells to the surfaces of steel stents. *Proceedings of the National Academy of Sciences of the United States of America* 2008;**105**:698–703. **[3]** Bakhru SH, Altiok E, Highley C, *et al.* Enhanced cellular uptake and long-term retention of chitosan-modified iron-oxide nanoparticles for MRI-based cell tracking. *Int J Nanomedicine* 2012;**7**:4613–23.

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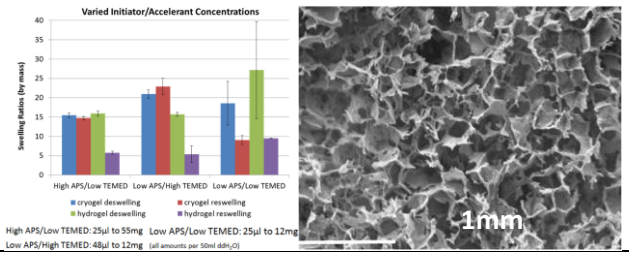
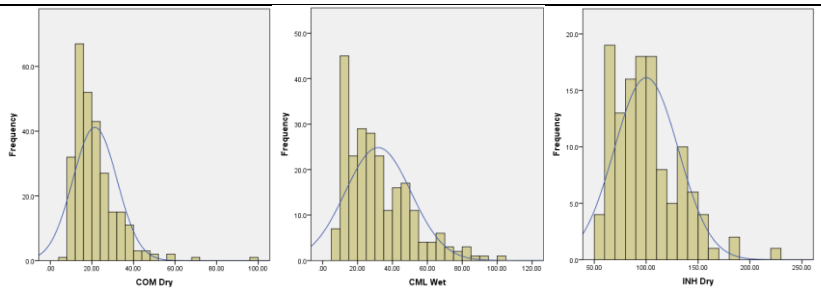
DTC Year:	DTC3
Institution:	Loughborough University

Abstract Title:	Monolithic chromatography media for the large scale separation of human therapeutic cells
Author(s):	Craig Milner
Abstract:	***Intro:*** Despite recent advances in regenerative medicine, barriers still exist for large-scale isolation of human therapeutic cells. Although magnetic separation methods have been employed extensively, there are several key disadvantages [1]. The aim of this project is to develop a new generic high-throughput cell affinity selection system based on cast polymer monolithic chromatography media featuring giant convective pores. ***Materials and Method:*** In-house-fabricated and commercially acquired (Protista Int. AB, Sweeden) polyacrylamide cryogel monoliths formed by free radical polymerisation. T-lymphocytes (Jurkat line), used for chromatography studies. Investigations included varying polymer and initiator concentrations and adding PEG. Cryogel characterisation by swelling/de-swelling rate and ratio, SEM, ESEM. Additionally, Jurkats were introduced to 1 ml Protista cryogels via a GE Akta chromatography system to analyse the 'after-flow' cell viability and performance of the cryogels. ***Results:*** Figures (below) show the (top-left) key optimisation by varying initiator conc. to improve swellability, (top-right) large pore sizes of the 'low APS/high TEMED' under SEM. and (bottom) pore size distributions. 



Pore Size	N	Mean dia.	St. Dev.	RSD%
Commercial (CM) dry	275	21.5	10.7	49.7%
CM wet	235	31.8	18.9	59.4%
In-house (INH) dry	125	100.1	30.9	30.9%

Conclusions: Optimised in-house cryogel with avg. pore size 100 µm. Commercial cryogel has much smaller pores. Difference in mechanical properties which needs to be tested.

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Toxicology of nanoscale metal and ceramic wear particles from total hip replacements

Ruth Craven, Dr. L. Jeuken, Dr. S. Williams, Dr. J.L Tipper

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Abstract

The introduction of total hip replacements (THRs) can successfully restore the mobility of diseased hip joints[1]. Hard-on-hard THRs, such as ceramic-on-ceramic and metal-on-metal were introduced to reduce wear rates and promote implant longevity. However, the biological reaction to the cobalt chromium (CoCr) wear particles produced from metal implants has raised serious concerns about implant safety. CoCr wear particles and released ions have been associated with hypersensitivity reactions, pseudotumors, and extensive necrosis[2,3]. CoCr and ceramic wear particles have been reported to be cytotoxic in a dose and time dependent manner[4,5]. However, little is known about the cellular interaction of wear particles and how this manifests to larger scale adverse reactions, especially with metal implants[6]. Furthermore, few studies have reported how the particles interact with the cell membrane and the mechanism by which they are subsequently internalised. Therefore, this project aims to determine if nanoparticles are able to damage the cell membrane with toxic consequences or whether they pass the membrane and exert toxic effects intracellularly. Thus far,

clinically relevant CoCr and ultra high molecular weight polyethylene wear particles have been generated using a pin-on-plate tribometer. Optimisation of filtration methods to isolate nanoscale ceramic nanoparticles has also been developed. Upon full particle characterisation, a unique tethered lipid bilayer membrane system will be used to assess particle and membrane interactions. The outcomes of this research will provide insights into CoCr wear particle toxicity and will have a significant impact on THR development and in nanoparticle research.

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Abstract Title:	Evaluation of electrospun gelatin/ polycaprolactone as a material suitable for use in corneal regeneration
Author(s):	**James B Rose**, David J Williams, Alicia El Haj, Harminder S Dua, Andrew Hopkinson, Felicity R.A.J Rose
Abstract:	**Background:** The advent of new surgical techniques has seen a paradigm shift in the way corneal blindness is treated[1]. These changes have called for a different approach to corneal tissue engineering. One such approach is developing component tissues of the cornea, such as partial thickness corneal stroma. In this work the authors evaluate *in vitro*, the suitability of electrospun gelatin/polycaprolactone sheets seeded with human corneal stromal cells (HCSC) as a potential candidate for preclinical development. **Methods**: Scaffolds were electrospun from blends of gelatin and polycaprolcatone at various ratios (100:0, 50:50, 25:75, 0:100 – Gelatin: PCL). Scaffolds were characterised by scanning electron microscopy (SEM), Infra-red spectroscopy, water contact angle, and histology. After seeding HCSCs on selected scaffolds, quantitative assessments of adhesion and proliferation were carried out on each of the scaffolds through the AlamarBlue© assay. Further assessment of the transparency of the cellularised scaffolds was also carried out. **Results**: The four blends of gelatin: PCL were electrospun with a range of fibre morphologies leading to significantly different fibre diameters. Significant differences in HCSC adhesion were observed between

	gelatin and PCL scaffolds; however adhesion to the electrospun blends of the materials showed no significant difference. This is proposed to be due to variations in surface presentation of the two materials in the electrospun blends. Assessment of the scaffolds after 12 days of cell culture showed that gelatin scaffolds were significantly more transparent than all others, and that HCSCs resided predominantly on the scaffold surface. **Conclusion**: This work demonstrates that gelatin: PCL blends can be electrospun with modest control and that the electrospun sheets possess good cell compatibility. Potential modification of the scaffolds may be required to more closely mimic the corneal stroma. However, the unmodified transparent gelatin scaffolds may have utility as a carrier sheets for limbal epithelial stem cell transplantation. Although far from clinical development, this work represents a good starting point for future work in the area of corneal tissue engineering
References:	Keenan, T D L et al (2011). *Br. Journal Opth, 95*(4), 468–72.

Development of Novel Tissue Constructs Using Hydrogel-3D Stem Cell Technologies

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In vivo, tissues exist in a 3D environment, being surrounded by numerous biochemical and mechanical cues, which often affect cells in a non-linear fashion. In order to mimic such a complex environment in vitro, we have designed a novel "smart" polymer hydrogel, where the gelation mechanism is precisely controlled by changes in temperature. The "smart" hydrogel of focus in this project is based on a highly branched poly (N-isopropylacrylamide) (PNIPAM) polymer (Fig 1).

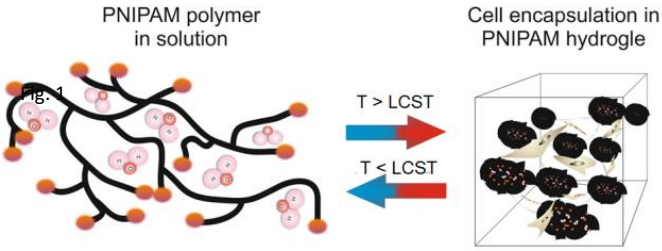

Fig. 1

A distinctive feature of this polymer gel is the rapid change of solubility at a set temperature: below 32ºC, PNIPAM exists as a highly branched system with imperfect branch shooting; above 32ºC this branched system collapses, forming a colloidal gel at a sufficient concentration, which allows cell entrapment within the gel. The gelation process of this material is reversible and reproducible.

Functionalisation of PNIPAM polymer with tri-arginine (RRR) peptide significantly improved gel properties in terms of solubility and solution-gel conversion temperature (i.e. gels at 35^{o}C). The peptide-functionalised PNIPAM was analysed by means of scanning electron microscopy to reveal highly porous structure of the material. Cone and plate viscometry was used to determine the mechanical properties of the hydrogel (with various chain-end groups) under dynamic loading conditions, allowing for comparison with natural tissue properties.

Currently, mesenchymal stem cell (MSC) spheroids and suspensions

are used for encapsulation in PNIPAM gels, which allows analysis of 3D environmental effects on cell-aggregate composition and differentiation.

The PNIPAM-based hydrogel, developed in this project, is a promising platform for studying 3D environmental, chemical and mechanical cues, which drive tissue formation *in vivo.*

Development of Acellular Xenogeneic Nerve Grafts

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Dr Stacy-Paul Wilshaw, IMCB, Biological Sciences, University of Leeds

Peripheral nerve injuries affect 1 in 1000 of the population. Microsurgical repair is the most common form of intervention, with autografts used to bridge injury gaps greater than 1-2cm [1]. However, normal sensation is rarely restored, leading to significant prolonged disability and socio-economic dependency.

Implantable nerve guides direct regenerating axons by topographic guidance, and concentrate tropic and trophic cues [1]. Synthetic nerve guides have been studied extensively for this purpose, however they are not particularly suitable as substrates for supporting either neuronal or Schwann cell growth.

Decellularised peripheral nerve scaffold offers considerable potential as an alternative nerve guides. Such a strategy would address regeneration of proximal axons at the cellular level, while simultaneously encouraging proximal regeneration towards the distal stump within a native guidance environment.

The aim of the project is to develop compatible, non-immunogenic, nerve grafts to restore sensory and motor function following injury. This will be achieved using novel, proprietary techniques to decellularise

xenogeneic nerve. It will then be used as a basis for the introduction of primary Schwann cells versus adipose-derived stem cells. [2]

Characterisation of porcine peripheral nerves through histological and immunocytochemistry analysis have shown that the nerves posses a similar morphology to that of human nerve tissue. Preliminary results have shown that decellularisation has the ability to remove cells from the tissue.

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Christopher Serna

Development of apatite-wollastonite glass-ceramic scaffolds with a trabecular bone architecture

ABSTRACT

The aim of this research is the preparation and characterisation of graded glass-ceramic scaffolds that are able to mimic the structure of natural bone tissue, formed by cortical and trabecular bone; an initial focus will be on the production of a scaffold with a trabecular bone-like architecture. Glasses will be synthesized by a conventional melting-quenching route, ground, and sieved to obtain powders of specific size (<45μm). The scaffolds will be fabricated using polyurethane (PU) sponges which will act as a template to mimic trabecular bone. A slurry of the powdered glass with inclusion of a binder (PVA) will be used to coat the sponge; once coated the the material will be fired in a furnace to specific heating regimes, resulting in burn out of the PU and PVA, forming a sintered and crystallized scaffold. Once processing conditions have been optimised, scaffolds will be characterised through morphological and crystallinity observations, density measurements, volumetric shrinkage, mechanical tests and in vitro bioactivity tests using HMBSC's.

Development of sterilisation techniques for acellular vascular grafts

Jake Milton-Barker

John Fisher, Stacy Wilshaw, Helen Berry , Eileen Ingham

iMBE

Background

Small diameter acellular porcine vascular grafts have been developed. Prior to clinical use the scaffolds must be terminally sterilised. Current sterilisation with peracetic acid is not ideal.

Aims

To test the hypothesis that acellular vascular grafts can be sterilised whilst preserving their biochemical and mechanical integrity. Initial aims were to develop methods for evaluating the effects of sterilisation on tissue properties.

Experimental approach

Acellular porcine arteries will be assessed for sterilisation induced damage/ crosslinking of the matrix proteins before and after sterilisation by gamma and E-beam radiation, ethylene oxide and super critical CO_2 using histology, biocompatibility and thrombogenicity assays, burst pressure, compliance and uniaxial testing, DSC, resistance to enzymatic digestion, multiphoton microscopy and HPLC/MS .

It was shown that it is necessary to transport porcine arteries in transport medium on ice for same day dissection and rapid freezing to prevent damage from autolysis/ ice crystals. Histology of native tissue showed alignment of elastin and collagen fibres and cell morphology through the intima to adventitia. Immunohistochemistry revealed collagen IV expression throughout the tissue.

Compliance testing methods have been developed in which arteries are kept taught to allow accurate measurements. The compliance was found to be 0.0025 mm diameter change per mmHg. Burst pressures of for Fresh/frozen and decellularised porcine carotid arteries were 3486.7

± 269.5 mmHg (n=6, ± 95% C.I.) and 3011.7 ± 164.8 mmHg (n=6, ± 95% C.I.) respectively. The grips used for uniaxial tensile testing were redesigned and this improved the frequency of failure at the centre for circumferential but not axial tissue specimens. Decellularised arteries for circumferential and axial specimens had an elastin modulus of 0.260±0.048 GPa and 0.156 ±0.045 GPa (n=6, ± 95% C.I.), a collagen modulus of 4.522±0.58 and 2.091±0.59 GPa (n=6, ± 95% C.I.), a UTStress of 3.087±0.33 MPa and 1.613±0.21 MPa (n=3, ± 95% C.I.) and UTStrain 5.702±0.23 and 1.900±0.13 (n=3, ± 95% C.I.) respectively. Initial investigations have shown that multiphoton imaging requires a higher gain to image damaged specimens.

The Development Of A Novel Human Corneal Substitute Using Decellularized Corneas

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There is currently no reliable or standardized protocol for decellularization of human corneas. The discrete integration between corneal architecture and functional integration is vital to maintaining the native (keratocyte) cell phenotype *in vivo* and this inevitably affects cell type *in vitro*. Decellularization protocols need to sufficiently eliminate cellular material with minimal disruption to tissue architecture. Additionally a technique needs to be established for successful cell infiltration.

The aim is to utilize corneal eye-bank tissue deemed unsuitable for transplantation *via* optimized human specific decellularization techniques and recellularization using novel, enriched, corneal stem cell populations which may be utilized in two key areas: (i) A more relevant human corneal substitute for drug and irritant testing in order to replace animal work and (ii) the creation of an effective engineered corneal construct for corneal transplantation.

Removal of detectable cellular and immune reactive material will be evidenced by immunofluorescence and CFSE based mixed lymphocyte

assays. Preservation of biomechanical, dynamic biomechanical analysis, optical properties and retention of corneal architecture will be assessed.

The vision is that this research will yield reproducible, reliable constructs, available on demand. From an international public health standpoint, and from the perspective of patient quality of life, there is an undeniable need to develop a reliable artificial and healthy biomimetic cornea. We endeavor to provide the underpinning research to demonstrate that a bioengineered cornea repopulated with normal stromal and epithelial cells is both possible and clinically viable.

Development of a system for the non-invasive characterisation of dental pulp stem cells by Raman spectroscopy

Adam Mitchell[1], Xuebin Yang[1], Alastair Smith[2], Jennifer Kirkham[1]

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There is need for characterisation of stem cell differentiation using non-invasive and non-destructive methods for both research applications and prior to their use in cell-based regenerative therapies. Raman spectroscopy, which uses near infra-red light to determine sample chemistry, has been used to characterise stem cells but its application has been limited due to an inability to maintain sterility. The aim of this study was to design a system to permit repeated application of Raman spectroscopy to living stem cells in long term culture. This required 1) identification of a Raman appropriate substrate, 2) development of a system to maintain culture sterility and 3) selection of an optical configuration to maximise the Raman signal. Quartz and MgF_2 were investigated as potential cell culture substrates. Dental pulp stem cells (DPSCs) were osteo-induced and their stage of differentiation assessed by qRT-PCR and alizarin red staining. Quartz cuvettes and cell culture flasks, customised to accommodate quartz coverslips were tested for culture sterility. A variety of microscope lenses were tested for optimal Raman signal acquisition and average spectra produced from 50 individual loci for each cell culture. Results demonstrated that DPSCs cultured on quartz but not MgF_2 had similar osteogenic differentiation to those cultured on tissue culture plastic. DPSCs cultured in quartz cuvettes and in customised culture flasks were successfully maintained in sterile conditions for up to 28 days. Customised culture flasks were selected as the thickness of the cuvette quartz precluded successful acquisition of Raman spectra. A 40x 0.8NA water immersion lens demonstrated reproducible spectra and the best signal intensity to noise ratio. Sterility can be maintained and Raman spectra successfully acquired using cell culture flasks customised with quartz windows and a water immersion lens. This offers a non-invasive system by which Raman spectroscopy may be used to characterise the differentiation status of stem cells.

Collagen I and N-isopropylacrylamide Hydrogels for 3D Tissue Engineering Applications

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Solubilised collagen gels are mechanically weak at physiological concentrations and prone to cellular contraction, therefore unsuitable as robust tissue scaffolds[1]. Through combining collagen I with NIPAM hydrogels we can improve the stiffness of the gel. Collagen I at 0.3wt% has a low shear modulus of 159.9 Pa, when combined with NIPAM solutions at concentrations of 2.5 wt% and 1.25 wt% the gels produced have shear moduli of 1328 and 644.5 Pa respectively. The gelation temperature of collagen I also effects the shear modulus, gels formed at 15 ^{o}C have a higher shear modulus of 501.1 Pa, compared with 159.9 Pa for gels formed at 37 ^{o} C. Thus we have been investigating the use of different blends for tissue engineering applications of mesenchymal stem cells (MSCs) for models for lymphoid stroma and cartilage, where the NIPAM content is varied to meet the mechanical needs of the application.

A 2D model of lymphoid stroma has previously been developed in our group, where MSCs treated with inflammatory cytokines upregulate lymphoid markers ICAM-1, VCAM-1 and podoplanin (unpublished results). Seeding MSCs at a high density to mimic lymphoid stroma in collagen I gels results in gel contraction. Introducing low concentrations of NIPAM (0.25 -1.25 wt%) into the gel helps to prevent this contraction and maintain the structure of the 3D model, whilst maintaining cell movement and potentially allowing matrix remodelling.

By increasing the NIPAM concentration to 2.5 -10 wt% the shear modulus of the hybrid gels is increased making the material more appropriate for load bearing applications. As the material is elastomeric, thermoresponsive and therefore injectable, and has been previously shown to be able to support chondrocytes[2] it is an ideal candidate for cartilage defect repair cartilage.

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Controlling the Delivery of Vascular Endothelial Growth Factor and Platelet Derived Growth Factor

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Statement of Purpose: Angiogenesis is the formation of new blood vessels from a pre-existing vascular network. It plays a vital role in the healing of tissues by establishing a blood supply. This process is stimulated by growth factors, particularly Vascular Endothelial Growth Factor (VEGF) and Platelet Derived Growth Factor (PDGF). These stimulate endothelial cell proliferation, migration and survival, along with recruitment of pericytes to stabilize and strengthen newly formed blood vessels.

Two sets of materials have been used to successfully release either $VEGF_{165}$ or PDGF-BB. These systems mimic heparin through electrostatically binding to the growth factor peptide sequences containing arginine and lysine amino acids. However, the aim is to produce more control over the availability of these growth factors than is possible with heparin functional materials.

Core-shell particles composed of polystyrene-co-divinyl benzene core surrounded by a polymer consisting of oleyl phenyl hydrogen phosphate (OPHP) shell crosslinked with ethylene glycol dimethacrylate (EGDMA) showed continuous release of $VEGF_{165}$ for 7 days. Polybutyl methacrylate (PBMA) particles were functionalized with either linear or hyper-branched poly(2-acrylamido-2-methyl-1-propane sulfonic acid) (PAMPS) and had the ability to release both $VEGF_{165}$ and PDGF-BB for at least 7 days.

The overall aim of this project is to produce a material capable of releasing both $VEGF_{165}$ and PDGF-BB ina controlled way over time to stimulate angiogenesis. The final intent is to develop a system in which

the patient's own growth factors can be taken up by a dressing type material and be released over time, removing the need for the delivery of external growth factors

Methods: OPHP was synthesized from oleyl alcohol (Sigma Aldrich UK) and phenyl phosphodichloridate (Sigma Aldrich UK). PS-co-DVB core OPHP-co-EGDMA shell latexes were produced in an emulsion reactor heated at 70^{o}C with stirring at 400rpm. The latexes containing glycerol methacrylate acetonide (GMAC) were produced in the same manner. PBMA latexes were made via surfactant free emulsion polymerization with PAMPS was added as a stabilizer. This produced latexes with PAMPS incorporated. Particle size, zeta potential measurements and TEM were done on all latex samples. To study protein release and binding 0.5ml 100ng/ml VEGF or PDGF solution containing 1% bovine serum albumin was added to each latex sample. The protein was left to bind for 12 hours at 4^{o}C. The protein solution was changed for PBS and samples placed in a 37^{o}C oven for varying time points. Protein release was analyzed using sandwich ELISA kits purchased from R&D systems (Minneapolis, MN 55413).

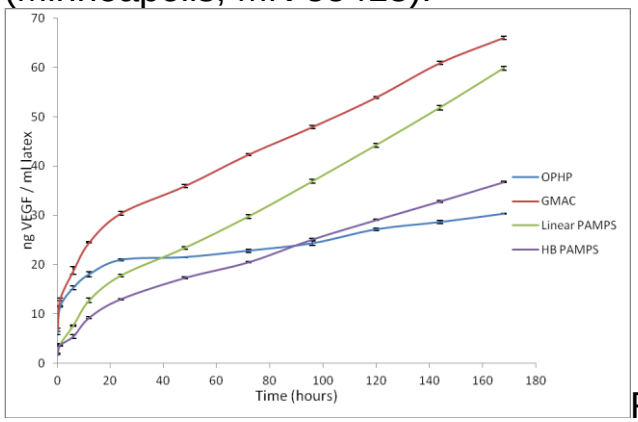

Figure 1. Release data of $VEGF_{165}$ from 50:50 OPHP:EGDMA, 50:50 GMAC:EGDMA, linear PAMPS and hyper-branched PAMPS. The phosphate based materials show a burst release profile and the sulfonic acid based materials do not. The branched PAMPS show the slowest release profile overall.

Results: OPHP and PAMPS based systems showed controlled release of pro-angiogenic growth factors over the period of 7 days. The ratio of OPHP to EGDMA can be altered to give varying release profiles of $VEGF_{165}$. The addition of GMAC to the OPHP system produces a slower release profile, without the plateau seen with OPHP-co-EGDMA. Upon deprotection of GMAC, the outer shell of the latex became larger, allowing $VEGF_{165}$ to bind deeper within the shell of the latex. This gives the steadier release profile seen with OPHP after the inclusion of GMAC. By maintaining the same quantity of OPHP the ratio of GMAC within the

latex shell can be altered to give varying release profile. The most successful release profiles were seen with 50:50 OPHP:EGDMA and 50:50 GMAC:EGDMA.

PAMPS functionalized latexes show steady growth factor release over 7 days, with no burst release. The branched PAMPS have a slower release profile compared to linear PAMPS. This is due to branched PAMPS producing a larger network structure, allowing growth factors to bind deeper within the outer shell of the particle. In contrast, linear PAMPS produced a more open structure which allows for easier diffusion.

Conclusions: Two systems have been developed that can successfully bind and release $VEGF_{165}$ and PDGF-BB by electrostatic binding of the growth factors on to or within the outer layer of polymer particles. It is next important to assess the release of $VEGF_{165}$ and PDGF-BB simultaneously along with looking at the effect on endothelial cells.

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Modelling the Mesechmyal Stem Cell Niche

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Abstract:

Mesenchymal stem cells/multipotent stromal cells (MSCs) are strong candidates for a wide range of cellular therapies, however currently little is known about the *in vivo* environment they reside. Previous research has suggested that MSCs reside within a perivascular niche due to their close association with the vasculature system. To understand niche cell-cell interrelationship, a 3D co-culture system has been developed using MSCs and endothelial cells. Previous investigations into this model have found that endothelial cells release paracrine factors that affect the differentiation and proliferation of MSCs in 2D and 3D.

Primary human MSCs were cultured with human umbilical vein endothelial cells (HUVECs) in non-adherent U-bottomed 96 well plates at different cell ratios 20:80, 35:65, 50:50, 65:35, 80:20 (MSC:HUVEC). The resulting cell aggregates or cell spheroids were analysed to look at osteogenic differentiation, proliferation and cellular organisation. Early results indicate that self-organisation occurs within the co-culture spheroids and osteogenic differentiation is affected by the ratio of MSC:HUVEC.

Title:	**Biomechanical and biotribological investigation and simulation of osteochondral substitutes in the patellar femoral joint**
Author(s):	Divya Baji, John Fisher, Eileen Ingham, Louise Jennings
Abstract:	Patellofemoral problems affect nearly a quarter of the population and are a common cause for revision of total knee replacements. Total joint replacement seems to be the final solution for improving the quality of life for patients suffering from osteoarthritis. The understanding of the joint biomechanics and the development of new cartilage repair techniques has improved the treatments for patellar chondral lesions (Nho et al., 2008). Minimally invasive interventions such as osteochondral transplantation are promising alternatives that can prevent or delay the need for such replacements (Draper et al., 2011; Fitzpatrick et al., 2011). Autologous osteochondral transplantation, though quite successful is limited to the treatment of small osteochondral defects due to donor site morbidity. Large osteochondral defects treated with the same technique would require allografts which may have the risk of immunological rejection. Development in tissue engineering is facing an exponential growth and a number of biomaterials has been developed as scaffolds for cartilage substitution (Freeman, Furey, Love, & Hampton, 2000; Ma, Xiong, Miao, Zhang, & Peng, 2010; Northwood & Fisher, 2007). Scaffolds formed from decellularized tissues are commonly utilized to enable the rapid and accurate repair of tissues such as skin, bladder and heart valves. The intact extracellular matrix remaining following the decellularization of these relatively low-matrix-density tissues is able to rapidly and accurately guide host cell repopulation. But the extraordinary density of cartilage matrix challenges both the initial decellularization of donor material as well as its subsequent repopulation (Ghanavi,

	Kabiri, & Doran, 2012). Kheir and collegues in the University of Leeds has developed an acellular porcine cartilage bone matrix that can be used as an osteochondral substitute that has the potential to be used in the treatment of large osteochondral defects using the mosaicplasty procedure. This decellularised graft has shown biocompatible in both in-vitro and in-vivo studies (Kheir et al., 2011). This project will test the biomechanical properties of these grafts in the patellar femoral joint to assess its suitability for the use in mosaicplasty for patellar femoral lesions. The research will develop and apply the methodology to investigate the biomechanics and biotribology of osteochondral substitutions in a natural patellar femoral joint.

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Abstract Title:	Identifying solutions to accelerate the adoption of MSC based therapies for simultaneous indications
Author(s):	Peter Archibald & David J Williams
Abstract:	The dominant hypothesis for the mode of action of Mesenchymal Stromal Cells (MSC) *in vivo* has been differentiation into tissue specific cells (Caplan, 1991). Recent thinking however recognises paracrine and trophic effects on host cells by biomolecule secretion into the local microenvironment to enable immune and inflammatory suppression and tissue repair (Caplan & Dennis, 2006). Also differences in sources and culture conditions make it difficult to meaningfully determine the identity of such cell populations and a variety of terminologies have been used to describe them (Corselli et al, 2010). Their surface markers and morphological characteristics have been extensively examined and summarised in the ISCT position paper defining MSCs (Dominici et al, 2006). However, to ensure that MSC-based products have the desired potency *in vivo*, they must be characterised *in vitro* in a manner reflecting their mode of action and consequent clinical benefit. Galipeau (2013) argues that surface markers, traditionally used in MSC characterisation, have little utility in determining cellular function and subsequent clinical efficacy. Practicably applicable functional assays which identify and quantify the paracrine function are therefore required. Further the multiple paracrine effects have potential for the treatment of multiple simultaneous indications including co-morbidities (Abdel Aziz et al, 2008). This does not fit with current single indication reimbursement procedures and product appraisal methods. Therefore, if such MSC-based therapies are to gain adoption and reimbursement, adaptations to these methods may be necessary and advantageous.

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Tissue Engineering of Osteochondral Grafts From Biological Scaffolds: Application at The Patellofemoral Joint

Carl Maguire

Supervisors: Prof Eileen Ingham Faculty of Biological Sciences, Prof John Fisher School of Mechanical Engineering, Dr Louise Jennings School of Mechanical Engineering

Background

Articular cartilage defects cause disability, affect the quality of life of the afflicted and are a major economic burden. With no clear gold standard treatment established there is a need to develop alternative therapies.

Aims

To generate acellular biological scaffolds for the repair and replacement of degenerate human cartilage in the patella-femoral joint.

Initial aims were to characterise natural patellar and trochlear osteochondral tissue from pigs and cows and compare to normal and degenerate human tissue biologically and mechanically in order to select the most appropriate tissue(s) for development of a decellularisation protocol

Methods

Osteochondral tissue was obtained from the patellar and trochlea of pigs [4-6 months] and cows [18 months]. Cartilage heights of osteochondral plugs were measured prior to loading in a pin-on-plate testing rig at contact pressure of 2.5 MPa for 1 hour.

The change in cartilage height was recorded and the cartilage was allowed to recover for up to 48 hours with the cartilage height measured at intervals. Osteochondral plugs were characterised using standard histological methods.

Results

In the creep and recovery tests, when data was normalised to initial cartilage heights there were no significant differences. By histology, there were no morphological differences between species or anatomical location within species.

Discussion

Little variation between species or site will allow for any or all of the tissues to be taken forward. The characteristics of the target human cartilage will be determined. The xenogeneic tissue will be decellularised and characterised with a view to development of a cartilage substitute for clinical translation.

Computationally Modelling Spinal Cord Injury

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Spinal cord injuries often result in a severe neurological deficit and permanent disability in patients. The annual incidence of traumatic spinal cord injury (SCI) ranges from 11.5 to 53.4 per million population for developed countries, young males comprise the majority of the victims [1]. The average lifetime medical expenses incurred by an individual suffering from SCI ranges from between $500,000 and $2,000,000, dependent on the severity of the injury and subsequent disability [2].

The primary damage from the initial mechanical insult is exacerbated by the secondary pathological cascade [3]. While a wide range of neuroprotective strategies have been investigated, the clinical translation of these strategies has not significantly improved patient outcomes [4, 6]. This is due in part to a lack of understanding about the biomechanics of spinal cord injuries, the distinct injury patterns produced, and how these injury patterns affect the evolution of the secondary cascade. There is a need for further investigation into the biomechanics of traumatic SCI. Computational models make it possible to acquire data that cannot be captured through animal and in vitro experiments, notably stress-strain information from inside the cord tissue. Finite element methods have been established as a useful tool for modelling the spinal cord, but have met critical limitations for this type of problem. Issues in coping with large deformations over short times, difficulty in implementing the fluid-structure interaction (FSI) between cerebrospinal fluid (CSF) and the solid tissue, and poor scalability for parallel computing systems limit the complexity and scope of FE SCI models [7].

This project aims to create more advanced coupled FSI models using the Material Point Method for the solid phases of the spinal cord and single phase Lattice Boltzmann Method for the CSF, developed with parallel capability, allowing execution on highly parallelised high-powered computing systems. These methods cope well with large deformations and their amenability to parallelisation will potentially overcome the limitations that restrict traditional FEM, providing an insight into SCI biomechanics, and establishing an alternate methodology for computationally modelling SCI going forward. The models developed for this project will be validated against existing experimental data, and will

provide an insight into the biomechanics of SCI, the relationship between the primary injury, the distributions of strain in the cord, and (in the longer-term) the possible neurological deficit.

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Biotribology of Osteochondral Grafts in the Knee

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The clinical outcome of osteochondral interventions is heavily reliant on the biomechanical and biotribological characteristics coupled with the biological structure and function of the regenerative tissue. In order to develop a successful and deliverable osteochondral graft to the patient, there is the requirement to develop robust preclinical test methods. It is anticipated that such preclinical test methods would incorporate functional biomechanical and biotribological simulations to investigate and assess the performance of the graft in the natural knee environment.

The project aims to complete the following objectives:

1. Development of the biotribological simulation model to facilitate the investigation of the biotribology and biomechanics of osteochondral grafts in the medial compartment. Initial investigations will be supported by the development and evaluation of biomechanical methods including measurements of contact pressure, graft alignment, fixation strength and structural stiffness. Methods will also be developed and applied to determine cartilage damage and wear and the release of wear products into the lubricant. Methods will be developed in an autologous graft model with appropriate positive and negative controls. The study may be further enhanced by the development of disease specific models.

2. Investigate the biotribology of osteochondral grafts in the medial compartment of the natural knee simulation model. The grafts selected and studied will include dCell ® osteochondral grafts of various species, synthetic and polymer grafts implanted into human and porcine knees. Various graft dimensions and geometries, single vs. multiple grafts and delivery methods may also be investigated.

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Abstract Title:	Precision Engineering of 3D Cell Models using Holographic Optical Tweezers
Author(s):	Emily Britchford; Kevin Shakesheff; Lee Buttery; Stephanie Allen; Glen Kirkham
Abstract:	**Introduction:** Optical manipulation is an increasingly important and established technique used to trap and move microscopic objects including cells and other biological materials and precisely position them to create very specific pre-determined 3D geometries. **Aims:** Applying this system aims to manipulate biology macroscopically, replicate cell organisation, inform basic understanding of biological micro-environments, determine localised cues in cell interactions and probe early stages of development. **Materials and Methods:** Holographic optical tweezers system utilising the dynamic forces and diffractive optical elements produced by bouncing beams of infrared laser light of a spatial light modulator focused through a high resolution and high numerical aperture microscope objective lens was used to selectively confine, trap and manipulate mouse embryonic stem cells. Cells were maintained on mitotically inactivated feeder layers supplemented with leukaemia inhibitory factor and passaged under standard protocol conditions prior to manipulation. **Results and Discussion:** The construction of mouse embryonic stem cell aggregates has demonstrated the potential of these cells to be manipulated into precise 3D structures and pre-determined cell configurations. Moreover, time-lapse imaging has provided a more in-depth understanding of cell behaviour and organisation. **Conclusion:** The enhanced capabilities of optical manipulation using computer generated holograms have demonstrated many uses in biology including the construction of complex micron-sized 3D structures. It is hoped that engineering 3D cell interactions and patterning of

	biological structures *in vitro* will allow for the generation of more complex and representative cell models with greater definable and tuneable characteristics.
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Development of an Acellular Xenogeneic Nerve Graft

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Peripheral nerve injuries affect 1 in 1000 of the population. Microsurgical repair is the most common form of intervention, with injury gaps greater than 1-2cm being bridged using autografts [1]. However, normal sensation is rarely restored, leading to significant prolonged disability and socio-economic dependency.

Implantable nerve guides direct regenerating axons by topographic guidance, and concentrate tropic and trophic cues [1]. Synthetic nerve guides have been studied extensively for this purpose, however commercially available products are not particularly suitable as substrates for supporting either neuronal or Schwann cell growth as they lack an architecture similar to that of the native ECM of the nerve.

An acellular peripheral nerve may have utility as an alternative to synthetic nerve guide conduits. Such a strategy would address regeneration of proximal axons at the cellular level, while simultaneously encouraging proximal regeneration towards the distal stump within a native guidance environment.

The aim of the project is to develop compatible, non-immunogenic, nerve grafts to restore sensory and motor function following injury. This will be achieved using novel, proprietary techniques to decellularise xenogeneic nerve. It will then be used as a basis for the introduction of primary Schwann cells versus adipose-derived stem cells. [2, 3]

Nerves that are being investigated include the sciatic, sural and femoral nerve as potential conduits. The nerves will be decellularised through a series of detergent washes which will eliminate the cellular content whilst preserving the tissue structure.

Characterisation of porcine peripheral nerves through H&E showed that the nerves possess a similar morphology to that of human nerve tissue. Immunohistochemistry using CD271 antibody showed the presence of Schwann cells whilst Oil Red O staining showed a large presence of fat surrounding the tissue.

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Electrospun Poly(lactide-co-glycolide): Hydoxyapatite Composite Membranes Intended for Bone Tissue Regeneration

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Membranes are used in dental implant and periodontal surgery to exclude soft tissues from the surgical site where bone tissue regeneration is required. However, commercially available synthetic membranes have poor bioactive properties and thus contribute little towards bone tissue regeneration beyond this barrier function. Electrospinning represents a potential method to fabricate resorbable polymer membranes or meshes with hydroxyapatite (HA) added either by a variety of methods including dipping and *in situ* titration. The aim of this research was therefore to evaluate the suitability of electrospinning for the preparation of these composites. HA is an osteoconductive and highly biocompatible material, and was synthesised via conventional wet chemical precipitation. HA was characterised using x-ray fluorescence (XRF) and x-ray diffraction (XRD). For electrospinning, p*oly(lactide-co-glycolide) (PLGA) was dissolved in dichloromethane at 20 % (w/w) and spun between 15-18 kV. Membranes were produced with an approximate fibre diameter of between 0.8 and 5 µm. In situ titration was superior in terms of homogenous distribution and mass of HA added. It was concluded that this processing route offered great potential for fabrication of osteoconductive membranes for bone tissue repair.*

Spinal cord cellular responses to wear debris from metal on metal total disc replacements.

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Motion preservation devices including metal on metal total disc replacements and fusion instrumentation (rods, plates, hooks and screws) are a valuable intervention for patients with pain and instability in the spine [1]. The longevity of these devices is thought to be compromised by wear debris and there is a growing concern within the orthopaedic community regarding the exposure of the sensitive neural tissues to both metal particles and ions from spinal devices and instrumentation. It has been hypothesised that such metal debris may impact upon and alter the functionality of periprosthetic tissues including the spinal cord. Recent research has shown that there is a link between such debris and pseudotumor formation [2].This mass of necrotic tissue may impact upon the spinal cord and in theory could cause neurological damage (neurological cell death, reduced neurite outgrowth or an inflammatory response).

This project aims to provide an insight into the responses of specific neurological cell populations (astrocytes, microglia, oligodendrocytes and primary neurons) to clinically relevant wear particles and their ions. Initially working in 2D culture with simulator generated clinically relevant Cobalt chromium debris, this project will gain an insight into the cellular reactions to nanoparticles. The use of an advanced 3D tissue engineered model will also enable investigation of more than one neurological cell type to debris and will provide information regarding the possible modes of failure of metal devices in the spine.

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How do Wear Particles for Total Joint Replacements Activate osteolytic cytokine secretion by Macrophages?

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Over 80 000 Total Hip Arthroplasties (THA's) were carried out in the UK in 2011, an increase of around 5% from 2010. These numbers are expected to continue to increase significantly over the coming years due to the aging population requiring interventions in order to remain active. In 2011 58.5% of THA's utilized a metal-on-polyethylene prosthesis, of which ≥20% are expected to require revision within 10 years. The most common mode of failure is aseptic loosening due to osteolysis surrounding the prosthesis. This is believed to be caused by the phagocytosis of ultra high molecular weight polyethylene (UHMWPE) and subsequent macrophage activation. Many studies support the role of macrophage activation and subsequent secretion of pro-inflammatory cytokines, such as tumour necrosis factor alpha (TNF-α) in response to UHMWPE wear particles. However, the molecular mechanisms underpinning this process are poorly understood. This project aims to identify serum proteins which bind to UHMWPE wear particles and dissect the role of cell surface receptors and the NALP3 inflammasome in UHMWPE induced osteolytic cytokine secretion. Further understanding of this process may enable the development of treatments to reduce the requirement for revision as well as informing the development of new materials for total joint replacement.

Development of Tissue Engineered Limbal Stem Cell Microenvironments for Corneal Repair

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INTRODUCTION

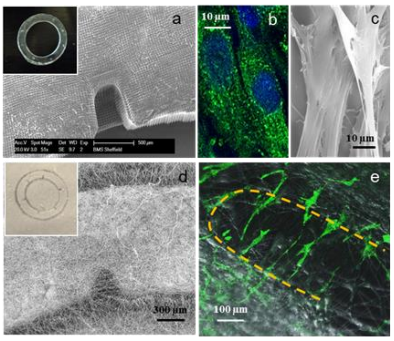

Fig 1. SEM images of PEGDA and PLGA microfeatured outer rings (a, d). SEM and fluorescence images of Rabbit limbal cells on PEGDA constructs (b, c; green:vinculin) and on PLGA scaffolds (e; green: phalloidin-FITC).

Corneal blindness occurs as a result of limbal epithelial cells (LEC) deficiency due to causes such as chemical burns, Aniridia or radiation. LEC are located in the limbus at the Palisades of Vogt in specific microenvironments or stem cell niches[1]. In some cases of corneal disease limbus and niches are destroyed. In this situation cells from the conjunctiva migrate to the cornea producing scar tissue which reduces vision[2].

Our aim is to develop experimental models of the limbus in which to study LEC activity. Specifically, we have designed two types of microfabricated corneal outer rings (one biodegradable and other non-biodegradable; fig.1a, 1d) containing micropockets to simulate LEC microenvironments.

EXPERIMENTAL METHODS

Non-biodegradable rings were made of polyethylene glycol diacrylate (PEGDA) using microstereolithography. Biodegradable rings were made

of poly (lactic-co-glycolic acid) 50:50 using a technique combination of microstereolithography and electrospinning[3]. Preliminary work on the evaluation of the constructs was performed using both limbal tissue explants and rabbit limbal epithelial and stromal cells. The potential use of the rings as cell delivery devices was evaluated using a 3D rabbit cornea model. Cells were characterized using CK3 (differentiation marker) and P63 (stem cell marker).

RESULTS AND DISCUSSION

In both cases we demonstrated that cells attach and proliferate on the constructs (fig. 1b, 1c, 1e). We specifically located cells in the artificial micropockets and for both approaches we obtained promising results regarding epithelial cell transfer and re-epithalisation of damaged corneas using a 3D rabbit model.

CONCLUSION

This work provides a technique for producing artificial niches for studying LEC behaviour using in *vitro* and *ex vivo* models. Both biodegradable and non-biodegradable rings could be potentially used as stem cell carriers for the treatment of corneal disease.

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Abstract Title:	**DEVELOPING A 3D MODEL TO MEASURE AIRWAY SMOOTH MUSCLE CONTRACTION**
Author(s):	J.C. Bridge, G.E. Morris, J. Aylott, M.P. Lewis, F.R.A.J. Rose
Abstract:	Airway smooth muscle (ASM) is the key effector cell in regulating airway contraction; previous in vitro studies into ASM contraction have largely been limited to two dimensional (2D) cell single cell cultures and collagen gel studies. Recent developments in tissue engineering have generated novel technologies for developing three dimensional (3D) multicellular models that better mimic the natural extracellular matrix (ECM). One method for producing synthetic matrices is electrospinning, where non-woven mats of polymer fibres (ranging from around 100nm to several μm in diameter) can be made by passing a polymer solution through a highly charged capillary. Aims: To assess a range of fully aligned electrospun polyethylene terephthalate (PET) scaffolds for providing the optimal topographical properties for ASM cell alignment. The optimal scaffold can then be used as a platform to model the contraction of a fully aligned sheet of ASM to various bronchoconstrictors. Methods: Solutions of PET at varying concentrations (35%, 20% and 8% w/v) were electrospun onto a rotating mandrel to produce aligned fibrous mats with a range of fibre diameters. Fibre diameter and degree of alignment were measured from scanning electron microscope (SEM) images of the scaffolds. Primary human airway smooth muscle cells were seeded onto the scaffolds and cultured for a two week period. Cell viability was monitored periodically using the alamarBlue® assay. After 2 weeks cells were fixed and immunostained to assess the degree of cell alignment, cell elongation and confluency.

	Results: Produced aligned scaffolds (>50% of fibre angle measurements within 10° of the mean angle) with average diameters 250nm (8%), 1.0µm (20%) and 5.0µm (35%). Cell alignment was shown to closely resemble that of the scaffold fibres. Cell morphology was spindle-like on all scaffolds with less apparent stress fibres when compared to 2D controls. Cell coverage increased with decreasing fibre diameter indicating that ASM is more proliferative when cultured on smaller fibres. Future Work: ASM sheets cultured on the optimised scaffolds will be challenged using bronchoconstrictors . The physical contraction of the sheets will be measured using a specifically tailored culture force monitor (CFM). This will provide novel insights into the regulation of ASM contraction in asthma and other diseases of the airways.

Tissue engineering a 3D cellular model of spinal cord injury

Authors: Jenny Smith (University of Leeds, Leeds), Prof. R. M. Hall (University of Leeds, Leeds), Dr J. Phillips (Open University, Milton Keynes), & Dr J. L. Tipper (University of Leeds, Leeds).

Spinal cord injury (SCI) causes devastating consequences such as paralysis, psychological and socioeconomic problems. SCI affects approximately 1,000 people a year in the UK and up to 12,000 in the US [1]. Treatment of SCI as yet is not ideal; following hospital discharge, less than 1% of US patients had complete neurologic recovery [2], the effect of this is the yearly increase of patients living with paralysis; in the UK and Ireland this figure is estimated at 50,000 people. Costs associated with SCI depend on the severity of injury; however it is estimated that management of SCI annually costs the UK £1 billion. The biological and biomechanical factors that might interfere with the regeneration of spinal cord tissue are not fully understood, therefore this project aims to investigate specific spinal cord populations – neurons, astrocytes, microglia and oligodendrocytes responses to tensile and compressive forces in both 2D & 3D culture in order to enable an understanding of the cellular response under normal physiological loading and loads simulating trauma. Tissue engineered constructs will be utilized within the impact loading scenarios; using tightly controlled loads within defined laboratory environments to study the cellular response; the ultimate goal is to develop a cellular model of spinal cord injury.

References:

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Enabling the Induction of Neovascularisation in Tissue-Engineered Skin

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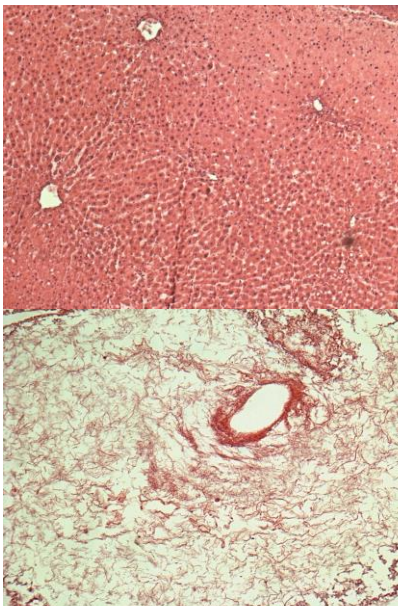
Introduction: Delays in developing new vasculature after tissue transplantation is a major problem in getting tissue-engineered (TE) skin to survive on the patient's wound bed. To overcome this we aim to induce neovascularisation in TE skin using a bioreactor and 'pre-vascularised' skin, obtained by incorporating TE skin with a re-endothelialised biological vascular network. Here we discuss the decellularisation of rat liver and jejunum to establish the networks.

Methods: Rat liver and jejunum were harvested from fresh cadavers. The inferior vena cava of the liver and main vessel of the jejunum were cannulated before flushing with heparin solution. Distilled water was perfused through the organs at 5 mL/min for 3 hours. Subsequently, 1% Triton-X 100 with 0.1% Ammonium Hydroxide was perfused for 12 hours. Distilled water was then circulated to remove residual detergent. Blue dye was injected into the organs to assess vascular patency. Low magnification and confocal microscopy along with microCT imaging were used to further examine the networks. Histochemical and DAPI staining were used to characterise organ composition.

Results: A well-defined vascular tree with multiple branching was visible from blue dye injection and imaging techniques. Histochemical analysis showed staining expected from proteinous ECM but no evidence of cells (Fig. 1). The latter point was confirmed with DAPI staining.



Conclusion: This procedure appears effective in preserving the architecture of the matrices whilst removing most of the cellular matter. Further work aims to repopulate the vascular networks with endothelial progenitor cells and understand the effects of mechanical and biochemical stimulations on the early stages of neovascularisation in a bioreactor environment.

Fig. 1 – Histochemical staining of intact liver (top) and decellularised liver (bottom)

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Abstract Title:	**Application of Quality-by-Design Principles to the Upstream Manufacture of Human Mesenchymal Stem Cells**
Author(s):	Thomas R.J. Heathman, Alexander Chan, Qasim Rafiq, Karen Coopman, Christopher J Hewitt
Abstract:	The successful translation of cell based therapies from "bench to bedside" represents a significant challenge, demanding economically scalable processes with the ability to consistently deliver safe and effective products. This will require an innovative approach to process development stepping away from a healthcare evolution and into a healthcare revolution. Current research into the scale up of human Mesenchymal Stem Cells (hMSCs) is taking an ununified approach, with little collaboration between process developers and clinical researchers which is threatening the longevity of cell therapy research. Quality-by-Design (QbD) uses a quality risk management approach to design in process quality by delivering systematic development and emphasising product and process understanding. With key regulators taking an interest in quality lead approaches to existing pharmaceutical manufacture, now is the time to apply these principles to the large scale manufacture of hMSC to maximise the significant benefits QbD has to offer. In this work, we have compiled the development history of hMSC expansion on microcarriers, highlighting gaps in our knowledge as well as demonstrating a path to apply these quality lead approaches to cell therapy manufacture. This process will facilitate growth in our process knowledge base and will help to unify the field which is critical to the successful translation of hMSC therapies.
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Abstract Title:	**Low temperature cell pausing: A short-term hypothermic preservation method for cells**
Author(s):	NJ Robinson, A Picken, CJ Hewitt & K Coopman
Abstract:	**Introduction:** Encouraging advancements in stem cell therapies have produced a requirement for an effective short-term cell preservation method, enabling time for quality assurance testing and transport to their clinical destination. Low temperature pausing of cells (>0°C) effectively bypasses cell damage caused by extreme temperature shifts during cryopreservation and using ambient temperatures in particular, will greatly reduce costs and reliability of specialist machinery. **Methods:** Cells from an adherent osteosarcoma cell line (HOS TE85) were paused at ambient temperatures (no atmospheric control) for up to 48 hours. Cell morphology was assessed using optical light microscopy and viability was evaluated by membrane integrity (Trypan Blue exclusion) and ATP-dependent metabolic activity (PrestoBlue) immediately after cell pausing and following 24 hour recovery at 37°C, 5% CO_2. **Results:** Cells appear smaller and more spherical in shape during pausing compared to the fibroblastic, adherent structure of untreated controls. After recovery, morphology returned to normal. The percentage of viable cells (Trypan Blue negative) retrieved immediately after 24 and 48 hours of storage at ambient temperature was 81% and 76% (n=3), respectively. After recovery, viable cell number increased to 170% and 88% of that measured prior to cell pausing. PrestoBlue fluorescence intensity decreased following 24 and 48 hours storage at ambient temperatures compared to untreated controls by 53% and 64% (n=3), respectively. Following recovery, fluorescence intensity increased to 151% and 144% after 24 and 48 hours of low temperature storage compared to values before hypothermic exposure. **Discussion & Conclusion:** HOS TE85 cells effectively recover in terms of morphology, membrane integrity and ATP-dependent enzyme activity from up to

	48 hours of cell pausing. Work so far is 'proof of concept' that HOS TE85 cells can be preserved for 48 hours in standard media by entering a phase of suspended animation during pausing at ambient temperatures. Successful cell pausing will create a simpler, cost effective short-term preservation method, which once applied to therapeutically relevant cells, will accelerate the progression of cellular therapies from bench to bedside.
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Abstract Title:	**Advanced bioprocess monitoring in stem cell culture**
Author(s):	Emma Neale-Edwards Supervisors: Dr Nuno Reis and Prof Chris Hewitt
Abstract:	There are many challenges faced with in the area of regenerative medicine from a biological, regulatory and financial perspective particularly when it comes to translating laboratory work successfully into the clinic [1]. Well defined and specific stem cell markers are required for multiple applications including quality control, comparing cell lines between batches and monitoring variation between culture conditions [2]. Current methods include detection of pluripotency of Embryonic stem cells by positive/negative tests on each individual cell using a panel of surface biomarkers; this is time consuming and limited in its scalability [3]. This aim of this research is to develop a microfluidic platform for monitoring and identifying stem cells quickly. Task include but are not limited to: **1.** Identifying biomarkers that correlate to specific stem cell surface biomarkers and can indicate the state of cell differentiation, including the establishing of response curves. **2.** Embed chemistry into new disposable multiplex microfluidic fabricated from fluoroplymer FEP material [4] **3.** Develop methodologies using the technology in point 2 to conduct online monitoring of cell cultures without the destruction of the cells within a bioreactor system. **4.** Investigate other potential applications for the microfluidic system particularly in terms of up

	scaling stem cell production.
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Cyclic Hydrostatic Pressure Stimulates Osteochondral Differentiation of hMSCs Seeded in 3D Collagen Hydrogels

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Mechanical stimulation of tissue engineered constructs using bioreactors may prove useful for conditioning them to physiological environments prior to clinical implementation, whilst both mechanical force and biochemical factors play an important role in regulating tissue formation. The aim of this study was to understand the combined effect of cyclic hydrostatic pressure (HP) and growth factor supplemented media on hMSCs seeded in collagen gels. Collagen hydrogels were seeded with hMSCs and cultured for 42 days in either basic unsupplemented media, osteogenic or chondrogenic media (TGFB3). A custom bioreactor (TGT, US) was used to apply 280 kPa cyclic HP, 1hr daily for 42 days. uCT analysis showed increased mineralisation in stimulated samples over unstimulated controls. ALP activity in the media was higher in stimulated samples at all time points. A significant increase in collagen autofluorescence was observed, especially when stimulated gels were cultured in osteochondral media, indicating an increase in collagen remodelling caused by HP. These results suggest that HP and biochemical factors work synergistically to promote osteochondral differentiation and matrix maturation in hMSC seeded collagen hydrogels.

Self-assembling peptide gels for articular cartilage repair

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Abstract

Background

Osteoarthritic cartilage is associated with a loss of glycosaminoglycans (GAGs) and biotribological function. There is a clinical need for early intervention treatments to restore cartilage function and delay the progression of cartilage degeneration. Previous studies have shown that the delivery of the self-assembling peptide, P_{11}-4 together with P_{11}-4 covalently linked to chondroitin sulphate may have utility in the restoration of biomechanical function to GAG depleted cartilage.

Aims

The aims of this project are to systematically evaluate a range of rationally designed SAPs for delivery of GAGs to GAG depleted cartilage with a view to restoration of biotribiological function.

Experimental approach

A range of SAPs will be selected, based upon the primary sequence. The selected SAPs will be covalently linked to chondroitin sulphate/ synthetic GAG using click chemistry. The effects of mixing different molar ratios of each SAP-GAG and SAP on the self assembling and mechanical properties of the SAP will be determined. Methods for the delivery of selected SAP-GAGs to GAG depleted cartilage will be investigated. Optimal SAP-GAGs will then be tested for their ability to self assemble within and restore biotribological function to GAG depleted cartilage in vitro.

Modelling and Simulation of Therapies for the Treatment of Human Autoimmune Disease

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Abstract

Type 1 Diabetes (T1DM) is a chronic autoimmune disease resulting from the complex interplay between the adaptive (T cells, B cells) and innate (macrophages, dendritic cells) arms of the immune system. [1{3] Agent-based modelling (ABM) enables the components of a biological system to be conceptually viewed as individualentities within a structurally de_ned spatiotemporal context. ABMs can thus elucidate behaviour that emerges consequent to the complex interplay of the systems components and their contribution to particular system response pro_les (SRPs). Diseases are SRPs that depart from homeostasis through perturbations of particular components. Using the CoSMoS process, a model is being developed to understand the potential outcome and mechanisms of action of immunomodulatory therapeutics for T1DM. Hypotheses of how disease SRPs arise and resolve are formulated, and can be tested given the re-emergence of the observed phenomena from the simulation model. [4{6]

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Surface Functionalization of electro-spun Poly(L)Lactic Acid scaffolds with Heparin to improve Tissue Integration for Surgical Treatment of Pelvic Organ Prolapse

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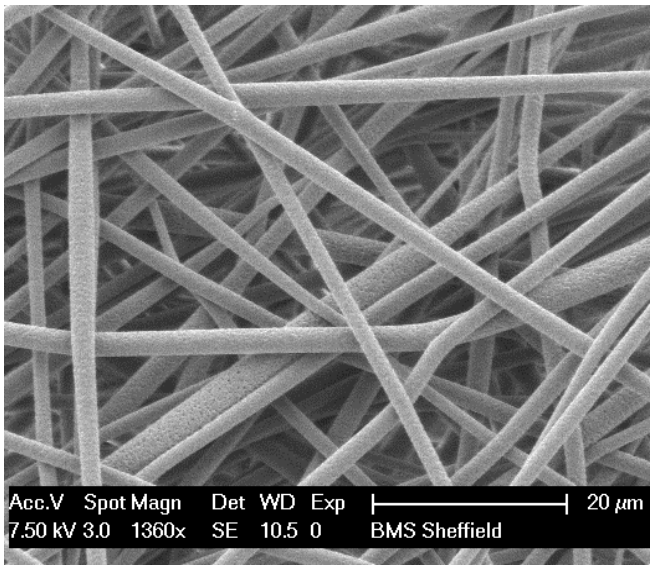
Introduction



Better biomaterials are needed to treat pelvic organ prolapse because current treatments have a high failure rate (Maher et al., 2010). Integration of the material within the host is crucial to trigger active remodelling of the new tissue and angiogenesis. It is believed that biomaterials can be specifically functionalised to improve their integration within the host. The aim of this study is to functionalize electro-spun PolyLactic Acid (PLA) scaffolds (Fig.1) to improve angiogenesis post-implantation and integration within the host.

Methods

Electrospun PLA scaffolds were firsly plasma polymerized with PolyAcrylic Acid (PAA) and coated with alternate layers of PolyEthyleneImine (PEI) and PAA or PEI and Heparin for a total of seven layers, in a layer-by-layer (LBL) coating approach. Coated scaffolds were dipped in heparin solution, dried and immersed in

Vascular Endothelial Growth Factor (VEGF) solution. Surface chemistry was evaluated by X-Ray Photon Electron Spectroscopy (XPS). ELISA was used to quantify the amount of VEGF bound onto the scaffolds.

Results

XPS showed that plasma polymerization of the scaffold with PAA was successful. Heparin bound well to LBL-coated scaffolds, compared to non-functionalised scaffolds, showing an increase in VEGF binding.

Conclusion

Heparin binding to electro-spun PLA scaffolds is greatly improved by plasma polymerization and LBL coating. As a result, VEGF binds to the fibres of the scaffold.

Future work will involve testing the pro-angiogenic effect of the heparin-coated scaffolds both *in vitro* and *in vivo.*

Key words:

Angiogenesis - electro-spun scaffold – plasma polymerization – layer-by-layer - heparin