“The Life Cycle of Regenerative Medicine”

EDITED BY:
RICHARD P HARRISON
HELEN E JESSON
INES MORENO
Preface

The Future Investigators of Regenerative Medicine (FIRM) is a society established by 5 members of the EPSRC Doctoral Training Centre (DTC) in Regenerative Medicine based between Loughborough, Keele and Nottingham Universities. Recognising the ever growing regenerative medicine community, we decided to bring young researcher from European institutions and further afield together. After another successful symposium in 2014, we decided to hold the 3rd FIRM Symposium.

This symposium is organised by early career researchers for early career researchers. One of the main aims of the symposium is to encourage young researchers such as ourselves to get to know each other on a friendly and informal level in this way establishing an international network from early on in our careers. The FIRM PhD Symposium will showcase the latest advances in regenerative medicine research undertaken worldwide and create a platform on which to build collaborations.

The EPSRC Doctoral Training Centre (DTC) in Regenerative Medicine is an internationally competitive consortium of key academic, clinical and industrial partners in the fields of Engineering, Tissue Engineering and Regenerative Medicine in the UK. The centre has developed a capacity of ~80 PhD students supported by a large number of post-doctoral researchers and academics. The centre conducts research covering the translational pipeline from basic biology, biological manufacturing, biomaterials, pre-clinical modelling and the commercial translation of regenerative medicine.

We are thankful and acknowledge the help and guidance along with all our sponsors in the organisation of this symposium.
Committee

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Area Map and Directions

The hotel Cap Roig is split over several levels and is highlighted in yellow on the map.

To get to the town centre follow the blue route on the map below. This is a 15-20 minute walk although there are bars and restaurants closer.

To get to the beaches, when facing reception, turn left and follow the corridor until there are stairs ahead and a lift on your right. Take the stairs or lift down to the bottom level. From here you can take the green path (right) to the closer beach of the red path (left) to the larger beach.

Map adapted from Google Maps for non-commercial use.
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Day 1

Sessions

1a “Fundamental Biology”

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Workshops

“Publish or Perish”

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<td>10:30-11:15</td>
<td>Wnt Pathway Activating Polymeric Nanoparticles for Fracture Localisation</td>
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<td>11:15-11:30</td>
<td>Osteogenic Acceleration: Blocking mir-31 with Antisense RNA Conjugated Gold Nanoparticles Increases Osteogenic Markers</td>
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<td>Effect of Bioimprinted Cell Topography on Skeletal Stem Cell Differentiation</td>
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<td>Improved Schwann Cell Isolation and Culture After Extracorporeal Shockwave Treatment</td>
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<td>The Effect of Extracellular Matrix Stiffness and Dimensions in Collective Cellular Mechano sensing</td>
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<td>Exploring the Role of Metal Ions in Wound Healing</td>
<td>Artificial HIF-1α Stabilisation Can Enhance Cell and Tissue Construct Survival</td>
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<td>Hydrogel Scaffold Engineering for Osteochondral Defects</td>
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<td>Perfluorocarbons – Potential for Successful Expansion of Bone Marrow-derived Human Mesenchymal Stem Cells at Liquid/Liquid Interface</td>
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Monday 26th September
Keynote Profile
Robert J. Hariri, MD, PhD

“Genomics, Stem Cells, Regenerative Medicine...etc”

The Chairman, Founder & Chief Scientific Officer, and former Chief Executive Officer of Celgene Cellular Therapeutics, one of the world’s largest human cellular therapeutics companies, Dr. Hariri has pioneered the use of stem cells to treat a range of life threatening diseases and has made transformative contributions in the field of tissue engineering. His activities and experience includes academic neurosurgeon at Cornell, executive, military and defense scientist, surgeon, aviator and aerospace innovator. Dr. Hariri has 100 issued and pending patents, has authored over 100 published chapters, articles and abstracts and is most recognized for his discovery of pluripotent stem cells from the placenta and as a member of the team which discovered TNF (tumor necrosis factor).

Dr. Hariri was recipient of the Thomas Alva Edison Award in 2007 and 2011, The Fred J. Epstein Lifetime Achievement Award and has received numerous other honors for his many contributions to biomedicine and aviation.

Dr. Hariri is also the Founder and Chairman of Myos Corporation, a bionutrition and biotherapeutics company and Co-Founder of Human Longevity, a genomics and cell-therapy company. Dr. Hariri also serves on numerous Boards of Directors including Myos Corporation and Provista Diagnostics.

Dr. Hariri is an Adjunct Associate Professor of Pathology at the Mount Sinai School of Medicine and a member of the Board of Visitors of the Columbia University School of Engineering & Applied Sciences and the Science & Technology Council of the College of Physicians and Surgeons, and is a member of the scientific advisory board for the Archon X PRIZE for Genomics, which is awarded by the X PRIZE Foundation. Dr. Hariri is also a Trustee of the Liberty Science Center and has been appointed to the New Jersey Commission on Cancer Research by Governor Chris Christie. Dr. Hariri is also a member of the Board of Trustees of the J. Craig Venter Institute.

Dr. Hariri received his undergraduate training at Columbia College and Columbia University School of Engineering and Applied Sciences and was awarded his M.D. and Ph.D. degrees from Cornell University Medical College. Dr. Hariri received his surgical training at The New York Hospital-Cornell Medical Center where he also directed the Aitken Neurosurgery Laboratory and the Center for Trauma Research.
Wnt pathway activating polymeric nanoparticles for fracture localisation

Edoardo Scarpa¹, Agnieszka A. Janeczek¹, Alethia R. Hoad¹, Richard O.C. Oreffo¹, Tracey A. Newman², Nicholas D. Evans¹

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Keywords: Nanoparticles, bone, fracture, regeneration, drug delivery

An osteoporotic fracture is diagnosed every 3 seconds costing to the European economy ~€100 million per day. 10% of bone fractures will not heal adequately and will require surgical intervention, as yet, there is no approved systemic drug enhancing fracture healing. Activation of Wnt signalling offers a promising therapeutic strategy for promoting bone repair, but can have both positive and negative effects on bone cell depending on the timing and site of delivery. We propose that controlled activation of the Wnt signalling pathway may be achieved using polymersome nanoparticles (PMs) loaded with the Wnt agonist BIO. Here we examine whether loaded PMs induce Wnt signalling activity in human skeletal stem cells (hSSCs), and their biodistribution in a mouse model of bone injury. BIO-loaded PMs were produced using polyethylene glycol-b-polycaprolactone block-copolymer. Their ability to activate the Wnt signalling was by measured qPCR in hSSCs. In vivo distribution in mice with a femoral drill defect was assessed 0-10 days after intravenous injection of fluorescent PMs using IVIS and histological analysis. BIO-PMs induced a significant increase in the expression of both the Wnt target gene AXIN2 (p<0.05 n=3) and the early osteogenic marker RUNX2 (p<0.05 n=3) in hSSCs. PMs injected intravenously localised in the fractured bone, the maximum accumulation of PMs in the injury was observed after 48 hours (73.55±17.74 fold increase over background, n=5) and could be detected by histology. We conclude that the spatio-temporal controlled localisation of BIO-PMs could represent an effective pharmacological treatment to promote bone regeneration after fracture.
Osteogenic acceleration: Blocking mir-31 with antisense RNA conjugated gold nanoparticles increases osteogenic markers

Dr. Mark McCully¹ João Conde², Pedro Baptista³, Val Olive⁴, Margaret Mullin⁵, Matthew J. Dalby⁵, Catherine C. Berry⁵

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²Massachusetts Institute of Technology, Institute for Medical Engineering and Science, Harvard-MIT Division for Health Sciences and Technology, Cambridge, Massachusetts, USA.
³Istituto di Ci insetica “E. Caianiello”, Faculdade de Ciencias e Tecnologia, Universidade Nova de Lisboa, Campus de Caparica, 2829-516 Caparica, Portugal.
⁴Scottish Universities Environmental Research Centre, Rankine Avenue, Scottish Enterprise Technology Park, East Kilbride, UK, G75 0QF
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Keywords: Nanoparticles, MiRNA, stem cells, osteogenesis, bone

MiRNAs are major regulators within cells and have become the subject of many investigations trying to harness their dynamic potential for therapeutics. These short single stranded RNA molecules (~20 nucleotides) are able to alter gene expression and suppress disease phenotypes. However as with all RNAi technologies, miRNA therapeutics suffers from delivery issues. Mir-31 has long been associated with tumours and disease phenotypes. However a new role has been identified, linking mir-31 with osteogenic suppression and the master osteogenic transcription factor Osterix (OSX). Mir-31s role in bone regulation could be a potential target to treat osteoporosis. Every 3 seconds an osteoporotic fracture occurs somewhere in the world, producing more than 8.9 million fractures annually. Here we report that a thiolated antagonist of mir-31 (antagomir-31) can increase OSX levels, using Gold Nanoparticles (AuNPs) as a delivery vector. The AuNPs were delivered to the OSX suppressed cell line MG63 and to bone marrow derived human mesenchymal stem cells (HMSC). Treatment with antagonists of mir-31, in HMSC rapidly accelerated osteogenesis, and bone nodule formation. HMSC treated with the AuNPs produced numerous nodules 3 weeks after treatment, in contrast to control HMSC that only began producing nodules after week 5.
Effect of bioimprinted cell topography on skeletal stem cell differentiation

Shona Waddell1, María C. de Andrés1, Isha Mutreja2, Ben Schon, Khoon Lim2, Tim Woodfield2, Richard OC Oreffo1

1Bone and Joint Research Group, Institute of Developmental Sciences, Faculty of Medicine, University of Southampton, Southampton, UK, SO16 6YD
2The MacDiarmid Institute of Advanced Materials and Nanotechnology, Department of Orthopaedic Surgery and Centre for Bioengineering and Nanomedicine, University of Otago, Christchurch, New Zealand

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Keywords: stem cell differentiation topography bone

Background: Topographical features on cell culture surfaces have been shown to alter the mechanism of stem cell adherence resulting in cytoskeletal changes, culminating in altered gene expression. Bioimprinting technology has been developed to create a plastic cell culture surface which replicates the phenotypic features of a fixed cell template. These topographical features can, potentially, alter stem cell fate. In the current study, skeletal stem cells (SSC) were cultured on bioimprints of osteoblast-like cells and their potential for osteogenesis studied.

Hypothesis: Surfaces bioimprinted with osteoblast-like cells can promote osteogenesis of SSC.

Methods: SSC were cultured under osteogenic conditions for 7 and 21 days, producing an early and late osteoblast phenotype. The relief profile of these differentiated cells was replicated into polydimethylsiloxane (PDMS). Subsequently, a polystyrene surface was created from the PDMS mould, producing a surface bioimprinted with osteo-induced SSC. SSC were cultured in non-osteogenic media on bioimprinted surfaces and non-imprinted tissue culture polystyrene (TCP) controls, and their potential osteogenesis studied through gene expression, morphological changes and cell proliferation.

Results and discussion: Preliminary gene expression results indicate an increase in expression of osteogenic marker COL1A1 in SSC cultured on late osteoblast bioimprints (238.7±152.4) compared to early (148.3±91.29) relative to TCP at 21 days. Variation in expression of COL1A1 between samples resulted in no significant difference in expression (p<0.05). Increasing sample number would reduce variation. Image analysis of initial cell morphology indicated a decrease in cell spreading and cell proliferation following culture on early and late osteoblast bioimprints compared to TCP. To investigate this further, proliferation will be measured using DNA quantification, and changes in cell morphology will be examined using cell area and axial ratio measurements.

Conclusion: Initial results indicate bioimprinting affects SSC behaviour. Bioimprinting offers an innovative approach to study cell morphology and differentiation.
Improved Schwann cell isolation and culture after extracorporeal shockwave treatment

Christina M.A.P. Schuh¹, David Hercher¹, Michaela Stainer¹, Andreas Teuschl², Robert Schmidhammer¹, Heinz Redl¹

¹Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, and Austrian Cluster for Tissue Regeneration, Vienna AT
²University of Applied Sciences Technikum Wien, and Austrian Cluster for Tissue Regeneration, Vienna AT

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Keywords: Extracorporeal Shockwave Treatment, Schwann cells, Peripheral Nerve

As new approaches for peripheral nerve regeneration are sought, there is an increasing demand for native Schwann cells for in vitro testing and/or reimplantation. Recently, Hausner et al. showed that extracorporeal shockwave treatment accelerated regeneration after peripheral nerve injury. The mechanisms underlying this effect remain widely unknown, but it was hypothesized that Schwann cells react to ESWT. Based on this in vivo findings we investigated behaviour of Schwann cells after ESWT.

Sciatic nerves from adult rats were dissected and treated ex vivo with ESWT. Subsequently, Schwann cells were isolated and cultured for 15 passages. ATP release in combination with LDH release was assessed directly after ESWT. Schwann cells were evaluated concerning morphology as well as expression of Schwann cell specific marker S100b and markers indicating regenerative phenotype (P75+, cJun+, GFAP+, P0-).

Single treatment led to significantly increased extracellular ATP release (but not LDH) as an immediate consequence, and subsequently a number of effects on the culture were observed, starting with a significantly increased Schwann cell yield after isolation. In the ESWT group quality of culture, reflected in consistently higher purity, proliferation rate (BrdU, population doublings per passage) and expression of regenerative phenotype-associated markers was significantly improved. In contrast, the control group exhibited progressively senescent behaviour (decrease of proliferation, loss of specific markers, increase in P16INK4A expression).

Summarizing, Schwann cells treated with ESWT revealed a higher proliferative activity without phenotype commitment, increased purity of the culture and reduced expression of senescence-associated marker even after long cultivation periods. These improved characteristics may be utilized in future tissue-engineering experiments.
The effect of extracellular matrix stiffness and dimensions in collective cellular mechanosensing

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1Centre for Human Development, Stem Cells and Regeneration, University of Southampton, SO16 6YD, UK
2Craniofacial Development & Stem Cell Biology, King’s College London, SE1 9RT, UK
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Keywords: Mechanosensing, Extracellular matrix, Colonies of cells, Polyacrylamide

Regenerative medicine aims to restore damaged tissues and organs. Aside from chemical cues, cells can sense the mechanical properties of extracellular matrix (ECM). An increase in stiffness is known to affect the growth and differentiation of individual cells, but the mechanics by which groups of cells respond to stiffness is poorly understood. Here we aim to test the hypothesis that groups of cells are able to sense ECM stiffness, which is proportional not only to the elastic modulus of the material, but also to its dimensions.

To develop ECMs that vary in stiffness and in thickness, we bound collagen and fibronectin to polyacrylamide (PA) hydrogels at concentrations ranging from 0.001 to 1 mg/ml. MDCK epithelial cells and MG63 mesenchymal cells were seeded on PA gels with variable stiffness (0.5 and 40 kPa) and thickness (20 - 150 µm) and allowed to form colonies for 6 days. Cells were imaged using an inverted microscope and cell characteristics such as cell number and cell spreading area, were obtained from image analysis.

An increase in cell spreading and adhesion was observed on stiffer hydrogels (40 kPa) compared to soft ones (0.5 kPa). We found that single cells start to sense an underlying rigid surface at about 15 µm (MG63) and 10 µm (MDCK) gel thickness. Cell spreading was maximal on intermediate concentrations of collagen (0.01 mg/ml) but was reduced at high concentration (by 20 %, p < 0.05). In contrast cell spreading was increased as a function of fibronectin concentration. Colony spreading was significant enhanced (p < 0.001) on thick (MG63: 58 560 µm²) versus thin gels (6 880 µm²).

Overall, the nature of ECM protein, elastic modulus and dimension induce a biomechanical response to cells phenotype. ECM-cells interactions may have important consequences in the study of embryogenesis, skin repair and regenerative processes.
Exploring The Role of Metal Ions in Wound Healing

Holly Wilkinson¹, Karen Theis¹,², Paul Lythgoe², Kimberly Mace¹, Matthew Hardman³

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Keywords: Wound healing, metals, chronic wounds, cells, scaffold

Our skin has evolved a tightly co-ordinated and efficient ability to heal wounds. As we age, or in conditions such as diabetes, this ability is attenuated. In fact, age and diabetes are major risk factors for developing chronic skin wounds that fail to heal entirely. While numerous studies have identified genes and proteins important for efficient wound healing, much less is known about the role of metals in normal and pathological repair. Here, we hypothesise that changes in the skin metallome (the global profile of metal ions) are functionally important for pathological wound healing. To test this we have used techniques such as ICP-MS to carefully profile the metal levels in skin and wounds from normal and pathological healing models. Our pilot data now suggest, for the first time, that alterations in the metallome are linked to pathological healing. Current studies are: i) testing the mechanistic role of specific metals in vitro and in vivo and; ii) exploring methods to restore metallome defects in pathological healing. Ultimately, this work will reveal new insights into the underlying causes of chronic wounds, and provide new avenues for future therapeutic exploitation.
Artificial HIF-1α Stabilisation Can Enhance Cell and Tissue Construct Survival

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Keywords: Cell Therapy and Tissue Engineering

Stem cell therapy is the promising technology that has the potential to revolutionise modern medicine. However, there are challenges associated with cell survival and phenotype stability during cryopreservation, transportation and following implantation. Strategies would enhance the translation potential of cell therapy. Current researches have demonstrated that placing cells in mild hypoxia insult can enhance cellular adaptation to adverse environments. The success of hypoxia preconditioning is, however, curtailed by the effects of a lack of oxygen on cell growth, phenotype stability and reperfusion injury. Cell survival may be artificially increased by stabilising HIF-1α through the use of HIF stabilising mimetics, in normoxic conditions prior to implantation or storage. Stabilising HIF in normoxia may activate a number of pro-survival factors whilst preventing the oxidative stress associated with hypoxia-reoxygenation reperfusion injury.

Liver cancerous cells (HepG2) preserved at cold ischemia by replacing 37oC culture media with 4oC perfusion liquid (Saltron) for different time-points with/without a range of hypoxia mimicking agents (CoCl and DMOG, then perfusion liquid was replaced with 37oC culture media (reperfusion). Cell survival was determined by cell number/DNA content (day 1, 3, 7) with Cyquant assay. Proliferation rate is normalized by day 1 in percentage. Cytotoxicity study was determined by CellToxTM green cytotoxicity assay following resuscitation.

These results have demonstrated that preconditioned cells in hypoxia mimicetic agents, 100uM Cobalt ions and 500uM DMOG can increase cell survival in adverse conditions e.g. Cold Storage 4oC, respectively. Current Studies are underway to understand the pro-survival factors expressed in HIF stabilised cells. The incorporation of HIF mimetics into tissue scaffolds may provide a means to increase cell survival following implantation.
Hydrogel Scaffold Engineering for Osteochondral Defects

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**Keywords:** scaffolds for osteochondral tissue regeneration

**Introduction:**

Osteochondral damage is one of the growing problems all over the world, causing disabling conditions especially for elderly people. Current surgical interventions offer a degree of success. However, there are limitations to these procedures with clinical trials underway to understand the potential for cell transplantation and the role of biomaterials to support tissue regrowth. Tissue engineering often makes use of specifically designed biomaterials in order to control cellular attachment and the organisation of developed tissues. Biomimetic scaffolds are designed to (bio)chemically and topographically mimic natural tissue. Many of the scaffolds currently used to repair osteochondral defects are laminates of several separate scaffolds, each designed to promote different cellular support. The present work focuses on testing selected biomaterial hydrogels which are N-isopropylacrylamide (NIPAM) and N-tert-butylacrylamide (NTBA) to identify the effects of specific features of the gels and their architecture on bone cell line behavior, activity, and osteogenic potential after specific time of culturing. Both of the gels present variable surface charges density which makes them differs in terms of the degree of hydrophilicity and wettability.

**Materials and methods:**

Gels have been characterized to identify the specific IR spectral pattern and functional groups, scanning electron microscopy images of the gels were used to clarify internal gel architecture and pore size. Biochemical tests including cell cytotoxicity and alkaline phosphatase activity have been performed to identify cellular viability of MG63 as well as osteogenic potential. Cytoskeletal fibrin has also been examined using phalloidin staining to identify specific MG63 cells behavior and morphology during interaction with these scaffolds. Alizarin red staining tests support the findings for osteogenic activity through verification of calcium deposits produced by the cells.

**Conclusion:**

Results showed the capability of NIPAM gel of being a good carrier for MG63 cells in supporting cell adhesion and proliferation, and further NIPAM demonstrated greater osteogenic potential compared to NTBA.
Perfluorocarbons – potential for successful expansion of bone marrow-derived human mesenchymal stem cells at liquid/liquid interface

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Keywords: hMSCs, liquid/liquid system, expansion, perfluorocarbons, mesenspheroids

The traditional in vitro culture systems of adherent cells typically make use of solid surfaces (i.e. polystyrene flasks or solid microcarriers). However, that approach generates surface-attached monolayers that require the use of harsh enzymatic treatments for cell harvest which could result in the long term damage of cell adhesion proteins. Our solution is using a non-toxic, biocompatible, cost effective and simple liquid/liquid system as an alternative to the complicated, polymer-based systems currently available on the market. Our system uses as a cell culture platform the „flexible surface” provided by the interfacial area of two immiscible liquids: a perfluorocarbon liquid (hydrophobic; Fluorinert FC40) and cell culture medium (aqueous; DMEM). The benefits of using perfluorocarbon liquids to cell culture systems include: easy sterilisation, recoverable and recyclable and increased solubility for respiratory gases. In this piece of work, we showed that two hMSC lines derived from different donors successfully attached and proliferated on the flexible liquid/liquid interface provided, while exhibiting cell morphologies similar to those on typical solid culture surfaces. The cells were expanded at the liquid/liquid interface with the option of harvesting as intact cell sheets without the use of proteolytic enzymes by simply aspirating the interface. After 10 days in culture on the liquid/liquid interface, harvested hMSCs could be successfully sub-cultured on TCPS and exhibited the typical hMSC morphology. In addition, the harvested cells maintained their identity and multipotency.
Day 2

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2b “Biomaterials”

3a “Preclinical Foundation”

3b “Preclinical Foundation”

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Keynote Profile
Liam M Grover
BMedSc(Hons), PhD, FIMMM

“Biomaterials for Tissue Regeneration”

Prof. Grover is a biomaterials scientist whose research focuses on designing materials that enhance the tissue regeneration process. He studied for both his undergraduate degree (biomedical materials science) and his PhD on the development of a novel bioresponsive ceramic (under the supervision of Jake Barralet) at the University of Birmingham, UK. On completion of his PhD, Prof. Grover spent two years working in the labs of Jake Barralet and Marc McKee at McGill University, Montreal, where he was awarded a CIHR Skeletal Health Scholarship to study the role of a range of proteins and condensed phosphates in the formation of minerals. He returned to the University of Birmingham in 2006 to establish a research group in the School of Chemical Engineering. In the time since, Prof. Grover has held funding from a multitude of funding bodies, including: the EPSRC, BBSRC, MRC, NC3Rs, the Wellcome Trust, Orthopaedics Research UK, the MoD, the EU, the Drummond Foundation and the NIHR, as well as numerous industrial partners. Since 2006, he has been involved in raising more than £20m of research funding that has provided the University of Birmingham with an exceptional infrastructure for the development of medical technologies. He has published in excess of 100 peer reviewed publications, more than 200 conference contributions, 4 book chapters, has been an inventor on five patent applications and has made more than 35 invited presentations. He was made a Fellow of the Institute of Materials at 30 and was made one of the youngest full Professors in the history of the University of Birmingham at 32. He is a visiting Professor at the University of Sao Paolo.

Prof. Grover’s research currently focuses on the tailoring the interactions that occur between synthetic materials and biological systems. In this talk he will describe how he is i) using biological pathways to initiate the degradation of materials (bone graft replacements); ii) using populations of cells to generate heterogenous structures designed for augmenting the hard/soft tissue interface and iii) developing materials to influence collagen fibril formation and reduce the severity of scarring. He will also talk about how he is studying the progression of pathological ossification as a means to solve the problem of fracture non-union.
Synthesis of enzyme responsive surfaces – towards a smart cell-material interface

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Keywords: enzyme responsive materials

Materials which undergo a function in response to a trigger encountered in a biological system e.g. enzyme, glucose, pH, are particularly advantageous for applications in tissue engineering, biosensors drug delivery. Enzyme responsive materials (ERMs) are a promising new class of stimuli responsive materials. Recent progress includes the use of enzymes as triggers in molecular self-assembly¹ and in enzyme-mediated release of therapeutics².

We aim to design reversible enzyme responsive surfaces that undergo an optically monitorable conformational change on the action of cell secreted enzymes. This response will demonstrate dynamic interactions at the bio-interface, with future potential to modulate cell behaviour. N-carboxy anhydride ring opening polymerisation (NCA ROP) is a versatile facile technique to synthesise polymers of long chain lengths. Here in, homo and copolymers of Glutamic acid and Serine with tuneable conformations have been synthesised via ROP. Composition/conformation relationships in solution have been identified and reaction kinetics have been used to gain insight into how polypeptide properties can be controlled. This has been translated to the preparation of enzyme responsive polypeptide planar (glass coverslips) and non-planar (silica nanoparticles)surfaces. The former have been analysed and tested for suitability to support the culture of two cell types. Reversible enzyme responsive behaviour was demonstrated by measuring casein kinase II induced surface phosphorylation and alkaline phosphatase induced dephosphorylation with FTIR and time of flight secondary ion mass spectrometry (ToF-SIMS).

Stable encapsulation of active VEGF in PLGA nanoparticles

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Keywords: Growth factor, nanoparticles, cells, bone, regeneration

The intrinsic healing capacity of bone is compromised in cases of large segmental bone defects, avascular necrosis and non-unions, typically as a consequence of a loss of vasculature and subsequent inability to restore tissue vasculature. Therefore to enhance fracture healing it is pivotal that stimulation of the angiogenic process within the defect site occurs. The current study is focused on the development and analysis of a novel strategy for the temporal delivery of bioactive molecules, including VEGF, encapsulated within biodegradable poly(lactic-co-glycolic acid) (PLGA) nanoparticles to develop smart materials to direct the differentiation of cells during bone regeneration. VEGF165 was encapsulated within poly(lactic-co-glycolic acid) (PLGA) nanoparticles (NP; 500-700nm diameter) by a double emulsion process. VEGF release from nanoparticles was analyzed using ELISA, while the bioactivity of the released growth factor was analyzed by its capacity to stimulate human endothelial cell proliferation and expression of the VEGF receptor, KDR. A 50% increase in KDR gene expression in cells cultured in medium with the addition of released VEGF compared to no treatment control was observed after 18 hours. These results indicate that VEGF retains its bioactivity when encapsulated and released from PLGA nanoparticles. Delivery of NP-encapsulated growth factors offers the potential of reduced growth factor application during treatment and concurrent reduced therapeutic costs compared to other strategies. Furthermore, our overall approach for the development and delivery of multiple growth factors within PLGA nanoparticles, with different degradation rates, could provide a powerful strategy to enhance bone regeneration processes.
3D Bioprinting of mechanically strong chondrocyte-laden constructs for major nose reconstruction

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Keywords: 3D Bioprinting

Current gold standard for nasal reconstruction after rhinectomy or severe trauma includes transposition of autologous cartilage grafts in conjunction with coverage using an autologous skin flap. Harvest of autologous cartilage requires a major additional procedure which creates donor site morbidity. Major nasal reconstruction also requires sculpting autologous cartilages to form a cartilage framework, which is complex and time-consuming. The reconstructed nose has a risk of deformity due to the force exerted from scar tissue formation in the first 6 months after surgery and the lack of structural integrity of the reconstructed nose. To address these clinical challenges, we aim to fabricate a mechanically robust cartilaginous constructs by using bioprinting of a polymer scaffold for structural support and a chondrocyte-laden hydrogel for cartilage regeneration.

Personalised nose scaffolds were designed using the CT scan of the patient and MIMICS software. Then they were 3D printed using two different thermoplastic polymers including polycaprolactone and poly (lactic-co-glycolic acid). The hydrogel gelatin methacrylate was used as a cell carrier during the printing process. A porous scaffold was obtained after the co-printing of PCL and GelMA/chondrocytes. Cells remained viable after the deposition process. The encapsulated bioprinted chondrocytes proliferated and secreted a cartilage-relevant matrix during 45 days in culture.

We have demonstrated the feasibility of 3D printing personalised nasal scaffolds using PCL and PLGA. Further to this, we have demonstrated that co-printing facilitates the formation of scaffolds with a mechanically supportive component and a biological component (chondrocyte-laden GelMA). Formation of a cartilage-relevant matrix in GelMA has been observed during long-term culturing.
Chemical functionalization of urethane pre-polymer to enhance endothelial cell fate

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Keywords: Vascular, Polymer, Chemistry, Endothelialization, Coatings

New biomaterial platforms that encourage endothelisation are highly desirable in the field of regenerative medicine. Urethane based pre-polymers are commercially used as moisture cure coatings and adhesives. However, their application to promote cell behaviour has yet to be explored. The pre-polymer chains are typically terminated by reactive isocyanate (-NCO) groups, hence we hypothesise that -NCO groups could be utilised for further reactions to yield a range of surface chemistries to influence cell behaviour. The aim of this study is to develop novel pre-polymer coatings for cardiovascular devices to enhance in situ endothelialisation.

NCO-terminated polycarbonate-urethane pre-polymer was spin-coated onto 316L grade stainless steel discs followed by chemical treatment using ethylenediamine (EDA) and 3-mercaptopropionic acid (MPA) with varying concentration (25mM-500mM). It is envisaged that -NCO groups react with EDA or MPA giving rise to amine (-NH₂) and carboxylic acid (-COOH) functionality, respectively.

Electron microscopy revealed formation of both micro-ridges and nano-features at lower concentration EDA-treated (25-250mM) samples, which diminished to exhibit a flat morphology at 500mM EDA. For MPA-treated samples, nano-ridges was observed on higher MPA concentration (100-500mM), but became less observable and increasingly similar to untreated controls with decreasing concentration. Surface –NH₂ and –COOH were detected via colorimetric assays on all EDA and MPA treated sample respectively, and was further supported by water contact angle measurements. Both EDA and MPA modified coating systems were shown to enhance human umbilical vein endothelial cells (HUVEC) metabolic activity, growth and proliferation with changes in morphology when compared with controls.

Hence, we have developed a novel and simple chemical-curing approach using urethane based pre-polymers, incorporating surface functionality and micro- and nano-scale features to enhance endothelial cell fate. Such coatings can be used for further bio-conjugation of pro-endothelialising biomolecules to promote in situ endothelialisation, creating a new generation of pro-healing coating material platform.
Introduction

Articular cartilage has an anisotropic, zone-specific structure, extending from the articular surface to the subchondral bone.

The aim of this project is to fabricate and utilize nanofibrous scaffolds with different fiber organizations to mimic the native variations of ECM between zones, enabling a better cartilage tissue formation.

Methods and materials

Bovine chondrocytes were used. Electrospinning technique established in the lab has been used to produce nanofibrous zonal-specific scaffolds. 2% poly (lactic acid) solution has been used to obtain nanofibers.

Agarose gel as the base has been used to support nanofibers’ stability. Various characterisation assays including live images to assess cell morphology, DMMB assay to quantify GAG production, MTT assay, DNA content assay and live-dead staining for cell viability have been conducted.

Results & Discussion

The seeded chondrocytes on nanofiber scaffolds shown different morphologies, viability, proliferation rate and GAG contents.

This study has confirmed that aligned nanofiber mesh placing on agarose gel induced chondrocyte alignment and generated ECM as found in the superficial zone, evidenced by the elongated cell morphology, low GAG production and higher cell proliferation rate; the nanofiber mesh with randomly alignment placing on agarose gel induced chondrocyte aggregation and generated ECM as found in the middle zone; and the aligned nanofiber mesh with bundle arrangement placing on agarose gel induced chondrocyte into aligned aggregates and generated ECM as found in the deep zone, which produced the highest GAG quantity with low cell proliferation rate.
Matrix-stiffness driven osteogenic differentiation of human adipose derived stem cells

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Keywords: hydrogels, biomechanics, stem cells, urinary bladder, scaffolds

The phenotypic signature and function of stem cells are governed by their ability to integrate local micro-environmental signals, among matrix-mediated triggers, such as matrix biomechanics, were shown to influence the lineage specification of stem cells. The interplay between cells and matrix becomes relevant when designing tunable extracellular matrix (ECM)-like platforms to mimic the regenerative cellular niche. Herein, we introduce a unique class of ECM-like substitutes that resemble the stress-stiffening behavior of biological networks. These matrices are based on a highly versatile, thermoresponsive, synthetic polyisocyanopeptide (PIC) coupled with cell-adhesive molecules (RGD). We investigated the putative effect of PIC-RGD-based matrix-stiffness over stem cell behavior and differentiation potential towards the osteogenic lineage.

PIC-RGD hydrogels with low (20-30Pa=soft) and high (200-300Pa=hard) storage moduli (G') were loaded with human adipose derived stem cells (hASCs) and cultured in either expansion or osteogenic differentiation conditions. The surface marker profile of hASCs, as well as their morphology, proliferation rate and protein deposition, were evaluated prior (day 0) and after encapsulation (day 7). The osteogenic committment was confirmed by calcium quantification (day 28).

We show that soft PIC-RGD sustained the immediate proliferation and arrangement of hASCs in 3D-like networks and reduced spontaneous differentiation compared to hard PIC-RGD where cellular spouting was delayed up to 7 days. Under such conditions, cells were privileged towards a strong osteogenic phenotype, characterized by a collagen-enriched matrix and subsequent strong mineralization.

Our results recognize the role of matrix-stiffness in lineage assignment and introduce PIC-RGD-based 3D platforms with tunable mechanical properties for tissue engineering applications.
Keynote Profile
Jillian Cornish
PhD

“Biological Strategies for Promoting Bone and Tendon Healing for Translational Regenerative Medicine”

Professor Cornish leads the Skeletal Biology Research Group in University of Auckland, New Zealand. Dr Cornish’s group investigates factors that are anabolic to bone cells, cartilage and tendon cells for which they hold international patents. The group has established numerous in vitro and in vivo models in skeletal biology and developed a keen interest in skeletal regenerative medicine. She has received prestigious awards, including the 2014 Paula Stern Achievement Award, (American Society of Bone and Mineral Research) and 2014 Career Achievement Award, (Australia and New Zealand Bone and Mineral Society) and she has served on editorial boards and boards of the International Bone and Mineral Society, International Society of Bone Morphometry and is a past-president of Australia and New Zealand Bone and Mineral Society.
Keynote Profile
Katie Lidster
PhD, FIMMM Programme Manager – Animal Welfare, NC3Rs.

“NC3Rs Workshop: How to apply the 3Rs to your research”

Dr Katie Lidster is a Programme Manager for Animal Welfare at the UK National Centre for the 3Rs (NC3Rs). Katie obtained her BSc in Biological Sciences at the University of Edinburgh, followed by a PhD in Neuroimmunology at Queen Mary University of London and she completed a post-doctoral project funded by the NC3Rs. In 2013, Katie joined the NC3Rs and she currently leads the animal welfare programme of work to develop and promote refinement opportunities in laboratory animals.
Translation of Novel Strategies for Mechanical Conditioning for Functional Bone Tissue Engineering

Hariklea Markides\textsuperscript{1}, Jane McLaren\textsuperscript{2}, James R. Henstock\textsuperscript{1}, Bridgitte Scammell\textsuperscript{2}, Kevin Shakesheff\textsuperscript{2}, Alicia J. El Haj\textsuperscript{1}.

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\textbf{Keywords:} Magnetic Nanoparticles Tissue Engineering, Animal Models

We aim to develop a cell-based injectable solution for treatment of non-union bone fractures. Our approach is based on the use of functionalised magnetic particles (MNPs) targeted to the TREK-1 mecho-sensative receptor on the mesenchymal stem cell (MSC) membrane. Attached MNPs respond to the application of an oscillating external magnetic field resulting in remote receptor activation and enhanced osteogenic differentiation. In this way, we can deliver functional mechanical stimuli to therapeutic cell populations in vivo following injection either systemically or to the repair site. This concept has been validated through a series of in vitro, ex vivo and small animal studies and is currently being tested in a pre-clinical sheep model for bone repair. 5x10\textsuperscript{6} autologous MNP-labelled STRO-4 positive MSCs were encapsulated within a naturally derived bone extracellular matrix gel and implanted within a critical sized defect (0.8x1.5cm) in the medial femoral condyle of a sheep. Implanted cell populations were stimulated over 13 weeks by custom built magnetic array housed within a standard sheep harness. Bone fill was assessed by \textmu CT and validated histologically. Preliminary data suggests enhanced targeted bone fill in MNP-magnet stimulated cell groups over non-stimulated cell groups. Variability in donor response was further evaluated in vitro in 3D hydrogel systems by encapsulating MNP-labelled MSCs from 17 donors within a 2.5mg/ml collagen hydrogel, magnetically stimulating (MICATM bioreactor) for 1hr/day over 28 days and mineralisation levels analysed by \textmu CT. Our novel results demonstrate the feasibility of a remote magnetic nanoparticle approach for cell therapy applications.
Novel delivery of proangiogenic VEGF using clay biomaterial to augment the recovery of chronic skin wounds

Daniel Page¹, Jonathan Dawson², Raj Mani³, Claire Clarkin⁴, Nicholas Evans¹

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Keywords: clay, biomaterial, angiogenesis, wound, ulcer

People that suffer with diabetes have a high risk of developing diabetic foot ulcers, a type of chronic wound associated with hyperglycaemia. Biological agents, including vascular endothelial growth factor (VEGF), have been investigated as agents that improve wound healing, yet delivery in an active economical form remains a significant clinical challenge. We tested the hypothesis that Laponite, a synthetic smectite clay biomaterial, can (1) retain VEGF in an active form and (2) localise bioactive VEGF to skin injuries to increase the rate and quality of wound healing. To investigate retention of bioactive VEGF, aqueous rhVEGF165 was pre-mixed within 3% Laponite hydrogels and coated on cell culture surfaces. Human umbilical vein endothelial cells (HUVECs) were seeded on Laponite-coated surfaces and incubated at 37°C for 18 hours. Tubule networks were quantified by microscope image analysis. Laponite hydrogels (∼± 1 µg/ml VEGF) were then administered to circular wounds made in back skin of healthy mice; wound healing was assessed by measuring the rate of wound closure and by histological analysis of blood vessel formation. HUVEC tubule formation was significantly increased when cultured on Laponite hydrogels containing 1.00-5.00 µg/ml VEGF (p = <0.01 - <0.0001). Laponite hydrogels were successfully retained ≤11 days in vivo; visually an increase in blood vessel staining in VEGF-Laponite treated wounds was detected, however no significant differences were measured. Results indicate that clays could have significant potential to localise proangiogenic VEGF to wounds. Future work will address whether Laponite-incorporated VEGF augments wound recovery in a chronic wound model.
3-D in vitro models of osteosarcoma: identifying different patterns of invasion

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Keywords: 3-D, osteosarcoma, microenvironment, biomimicry, invasion

Osteosarcoma is the predominant type of primary bone cancer in children and adolescents, occurring mainly at the intramedullary cavity of long bones. 68% of patients have an overall survival of 5-years, while poor responders to standard therapy die within that same time. The disappointing results of the EURAMOS-1 study stress the urgent need for alternative regimens. Heterogeneity, rarity and the lack of a flexible clinically relevant disease model, pose key limitations to research. As such, there is a requirement for a customisable model of osteosarcoma to reproduce and characterise patient tumours. It is hypothesised herein that a modular tissue engineering approach to develop a 3-D in vitro model of osteosarcoma, which mimics the in vivo tumour microenvironment, could deliver a platform for assessing personalised hallmarks of this cancer. Osteosarcoma cues were dissected into semi-autonomous modules; a 3-D cellular Artificial Cancer Mass (ACM) nested inside a 3-D acellular Bone-Marrow-like Stroma (BMS), overall termed ‘tumoroid’.

Complexity was introduced gradually, in the form of spatiotemporal matrix composition and stiffness. Following the generation of metastatic (HOS-143B) and non-metastatic (MG-63) tumoroids, a difference in invasion pattern was observed. Metastatic tumoroids invaded mainly as cell sheets while non-metastatic tumoroids invaded mainly as cell spheroid masses. Furthermore, metastatic tumoroids achieved a 45% (p<0.01) greater surface area (μm2) of invasion compared to non-metastatic. By adding the structural protein Fibronectin to the BMS of metastatic and non-metastatic tumoroids to further mimic the bone intramedullary environment, there was a 30% (p<0.001) increase of sheet-like invasion in metastatic tumoroids, but no significant changes with non-metastatic. The controlled increase in matrix complexity and biomimicry resulted in the faithful reproduction of the known in vivo characteristics of the cells used to generate 3-D tumoroids, demonstrating the capacity of this model to reproduce native tumour features in vitro.
Day 3

Sessions

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4b “Enabling Technologies”
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Workshops

“Additive Manufacturing”

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Keynote Profile
Alvaro Mata
PhD,

“Enabling Technologies for Biofabrication of Functional Materials and Biomimetic Environments”

Alvaro Mata’s research is focused on the bioengineering of novel biofabrication strategies and biomaterials for tissue engineering, regenerative medicine, and in vitro models. He holds a Bachelor’s Degree from the University of Kansas, a Master's Degree from the University of Strathclyde, and a Doctor of Engineering Degree from Cleveland State University. During his doctorate he worked at The Cleveland Clinic with Prof. Shuvo Roy and as a Postdoctoral Fellow with Prof. Samuel Stupp at Northwestern University. From 2008-2013 he was Head of the Nanotechnology Platform at Parc Científic Barcelona in Spain and is currently Reader in Biomedical Engineering and Director of the Institute of Bioengineering at Queen Mary University of London. He holds seven patents or patent applications and publications in journals including Science, Nature Chemistry, and Nature Materials.

More information can be found here:

www.matabioengineering.com
twitter.com/mata_lab.
Characterising the enhanced intracellular delivery of Magnetic Nanoparticles by GET

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Keywords: Nanoparticles, GET, delivery, cells and characterization

Magnetic nanoparticles (MNP) small size enables them to interact at cellular and molecular levels, furthermore their paramagnetic properties makes them suitable for manipulation through a magnetic field opening a whole range of different applications in biomedicine including cell tracking, cell labelling, hyperthermia or drug delivery. GET (GAG-binding enhanced transduction) system is based on a novel fusion protein that couples a membrane docking peptide to heparan sulfate glycosaminoglycans (GAGs) with a Protein Transduction Domain (PTD) and has been previously reported to improve delivery of different cargoes into cells. The aim of this work was to develop and characterize a delivery system for MNP to cells. We have demonstrated that GET enhances MNP uptake on cells in vitro and its delivery efficiency is not impaired by the presence of serum in the media.
Enhanced and sustained chondrogenic differentiation using graphene oxide for pre-distributing factor TGF-beta3 in a 3-dimensional collagen hydrogel

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Keywords: hydrogel, nanoparticles, drug delivery, stem cell, chondrogenesis

The use of graphene (G) and its oxides (GO) to form cell substrates and guide appropriate cell function is a relatively new topic. Formats of 2D G/GO-coated surfaces, 3D chemical-vapour deposited foams and GO dispersions within cell pellets have generated fascinating results particularly in the area of directing stem cell differentiation into specific lineages. Here we report the use of GO flakes dispersed in collagen hydrogels to deliver a chondrogenic factor within a 3-dimensional collagen hydrogel for inducing chondrogenic differentiation of human mesenchymal stem cells (hMSCs). GO with TGF-beta3 pre-adsorbed on their surface were dispersed in the collagen hydrogel with hMSCs simultaneously encapsulated. Interestingly, the flakes can adsorb >99% TGF-β3 with <5% released over 28 days. The adsorbed TGF-beta3 had conformation mimicking its native (active) conformation, and this conformation can be retained for longer when adsorbed on GO, compared to free TGF-beta3 in culture medium. For the encapsulated cells within GO-containing gels, over 99% remained alive indicating the system’s excellent biocompatibility and uptake of the nano-flakes into cells was minimal. TGF-beta3 pre-adsorbed on GO were able to efficiently guide cell differentiation into a chondrocytic phenotype. The expression of chondrogenic genes in gels with TGF-beta3 preloaded on GO exceeded the expression in gels with TGF exogenously supplied; also, the deposition of cartilage-specific extracellular matrix were further enhanced with TGF-beta3 preloaded. Furthermore, the enhancement of chondrogenic genes and of subsequent ECM formation sustained longer in gels with preloaded TGF-beta3. Therefore, our results showed that GO acts as an efficient growth factor delivery carrier to supply a matrix-bound growth factor effectively. This then creates a platform for potentially loading multiple factors to construct bioactive 3D cell-scaffolds for various tissue engineering purposes.
Imaging of microfluidically arrayed immunolabelled bone marrow cells

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Keywords: Microfluidics, SSCs, Immunostaining, Bone, Differentiation

Skeletal stem cells (SSCs) can be isolated from the bone marrow and may be used for regenerative medicine applications because they can differentiate into bone, cartilage and fat cells. However, SSCs constitute less than 1% of the human bone marrow mononuclear cell (BMMNC) population, which presents challenges for their isolation and characterization. Moreover, they are heterogeneous and have variable potential for proliferation and differentiation. Cell surface markers have been shown to mark putative stem cell or lineage progenitors, but methods such as flow cytometry limit analyses that can be performed on isolated cells.

We present a microfluidic device that is capable of arraying hundreds to thousands of BMMNCs, including SSCs. The device enables SSC microscopy studies without the requirement for a specific SSC purification step. This is a two-height microfluidic device consisting of eight chambers, each with a winding main flow channel. A hydrodynamic flow guides cells from the winding channel into height-restricted cell trap pockets sited at the side of the main channel and the cell trap sites result sequentially filled. BMMNCs are isolated from bone marrow samples using buffers, lysis reagents and density centrifugation. These cells are, then, labelled with DAPI and two membrane antibodies: STRO-1 (binding SSCs and erythroid progenitor cells) and glyco phosphorin A (binding erythroid progenitors only). Once the sample is flown in the microfluidic device, cells are trapped and can be identified by fluorescence and brightfield microscopy – useful also for qualitative flow cytometry studies. Microfluidic cell arrays coupled with microscopy of the immunostained sample allowed us to image SSCs.
Aptamers: harnessing a synthetic nucleic acid platform technology for skeletal stem cells biomarker discovery

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Keywords: aptamer, biomarker, skeletal stem cell

Skeletal stem cells (SSCs) comprise a rare cell population present within bone marrow that can differentiate into the principal stromal lineages; bone, fat, and cartilage, with significant therapeutic potential. Crucial to studying and utilising SSCs is the ability to reproducibly isolate a homogenous population from the wider bone marrow populations. However, to date, in the absence of specific SSC markers, no consensus on effective separation methodology has been reached. As a consequence, numerous methodologies exist with variable results indicated for stromal differentiation and therapeutic efficacy.

We hypothesise that Aptamers, single-stranded nucleic acid chains that can bind to a wide variety of targets with high affinity and specificity, offer an innovative approach to aid SSC biomarker discovery. Aptamers are synthetically-produced and selected through a cyclical process called systematic evolution of ligands by exponential enrichment (SELEX), refining a random population to a specialised subset, and offers the unique approach that the target does not need to be known beforehand. This study aims to isolate aptamers selected against human SSCs and examine ligands for new biomarkers, enabling a better understanding of SSCs, their niche and expression, as well as facilitating high purity isolation.

We have developed a robust and reproducible methodology using the immortalised cell lines; SAOS-2, and Raji. An aptamer library of 1015 molecules was incubated with SAOS-2 cells, binding sequences are recovered and amplified. The cycles introduced more stringent selection pressures to isolate sequences specific to SAOS-2, while removing those that bind to Raji cells. Now developed, this methodology can be applied to the more finite resource of bone marrow tissue, identifying aptamers which bind specifically to SSC markers. These markers can be isolated and analysed using protein and mRNA sequencing to identify biomarkers on the cell surface, facilitating a greater understanding and separation of the SSC population for clinical applications.
A predictive model for microscale 3D geometry in tissue engineering scaffolds fabricated by extrusion-based 3D printing

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Keywords: Bioprinting, computer simulation, scaffold, FEA,

Extrusion-based 3D printing is widely used for tissue engineering scaffolds. It has the ability to create intricate microarchitectures, which are dictated by the geometries of individual 3D printed filaments. Filament geometries depend on complex interactions with other filaments. In particular, they often widen at crossover points. Therefore, there may be significant discrepancies between the designed and manufactured 3D structures. No models currently exist that can predict these discrepancies. Existing computer models consider individual aspects of the bioprinting process but are highly computationally demanding and cannot consider a full tissue engineering scaffold. Therefore, expensive time-consuming trial and error approaches to scaffold design are currently necessary.

We present a new 3D modelling technique that can predict the final 3D printed microscale geometry of tissue engineering scaffolds. The physical extrusion of material is simulated in a virtual environment according to the planned print path and the bioprinter setup including nozzle diameter, extrusion rate and layer height. The modelling technique generates a 3D geometry model of the predicted scaffold.

The outputted 3D geometry model was used to analyse the expected pore size, pore shape and pore distribution, and was validated against experimental bioprinted samples. The model gave an effective prediction of porosity ranging from 58% to 77% as the height increase between bioprinted layers was increased in the bioprinter control software. As the height increase between layers was reduced, experimental scaffolds densified and compressive modulus increased 5.5 fold. The model effectively predicted a 5.9 fold increase through finite element analysis (FEA) simulations of compressive moduli.

The model enables scaffold designs to be validated and optimised prior to manufacture. Integration of the model into 3D printer control software may ultimately enable automatic optimisation of scaffold designs.
Using holographic optical tweezers as a tool to investigate cellular interactions

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Keywords: Patterning, High-resolution, Cells, Microparticles, Niche

Biological, chemical and physical cues all play major roles in the differentiation of stem cells into terminally differentiated cells. However, we know relatively little about how cells interact with each other on the very small scale. The investigation of cellular microenvironments is currently limited by the technologies available to manipulate cells in 3D at a high enough resolution. Here we demonstrate the application of holographic optical tweezers to precisely control and manipulate cells and microparticles in 3D on a microscopic scale. The holographic optical tweezers provide a high resolution tool for investigating cellular interactions with other cells and their environment. Using this technology we aim to improve our knowledge of fundamental stem cell biology to help guide future tissue engineering research on a larger scale.
Keynote Profile
Maria Pereira
PhD, Head of Research at Gecko Biomedical

“Nature-Inspired Biomaterials for Tissue Repair in Cardiovascular Applications: A Translational Story”

Maria PEREIRA, Ph.D., Head of Research at Gecko Biomedical, is responsible for the research activities and for the translation of the core technologies from the academic to the industrial settings. Maria holds a PhD in Bioengineering systems under the scope of the MIT-Portugal program, where she focused on the development of biomaterial-based solutions for unmet medical needs in the fields of tissue adhesion and drug delivery. Maria managed several multien-center collaborations leading to multiple high impact publications. In 2014, she integrated MIT’s Technology Review Magazine list of Innovators Under 35 Award and in 2015, she was recognized by Forbes Magazine as part of the 30 under 30 list in Healthcare and by TIME Magazine as a Next Generation Leader.
Magnetic Iron Nanoparticle Intracellular Kinetics and Metabolism

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Keywords: Nanosensors, on-line monitoring, enabling technologies

Super paramagnetic iron particles represent an advanced technology capable of both targeted delivery of essential therapeutics as well as diagnosis and prognosis of disease. This is due to their augmented capabilities of encapsulating and targeted controlled release of active pharmaceutical ingredients (API), as well as their innate magnetic properties that permit their use as contrast agents using magnetic resonance imaging (MRI). However, in order to realise their full clinical potential it is important to understand their intracellular fate and mechanisms of particle degradation. In this article, we correlate simulated lysosomal degradation of the silica iron paramagnetic (SiMAG) particles to the intracellular mechanisms of particle degradation in human mesenchymal stem cells (hMSCs) to determine their intracellular kinetics and metabolism, using the analytical techniques of super resolution microscopy and flow cytometry. Super resolution microscopy showed SiMAG nanoparticles are endocytosed and are co-localised with lysosomes. Suspension of SiMAG particles in simulated lysosomal conditions revealed particle degradation >50%, as indicated by loss of fluorescence intensity, over a period of 7 days. The intracellular metabolism of SiMAG particles was monitored in hMSCs using extended dynamic range pH-sensitive nanosensors. Intracellular pH mapping elucidated SiMAG particles are degraded intracellularly over a period of 4 days which corresponds to a drop in lysosomal pH from 5.43 ± 0.06 to 4.81 ± 0.14 and is followed by a pH recovery to 5.33 ± 0.17 on days 5 and 6. This observation corresponds to a drop in fluorescence, indicating SiMAG degradation, for the first 4 days. These results were mirrored in macrophages, which are thought to be the major in vivo sink for SiMAG degradation. We anticipate our methods will pave the way forward to determine in vivo particle cellular fate.
Biologic scaffolds derived from human and porcine extracellular matrix for spinal cord injury

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Keywords: stem cells, scaffold, extracellular matrix, hydrogels, neural regeneration

Hydrogel scaffolds that aim to restore neuronal function after spinal cord injury (SCI) should temporarily fill the lesion, promote cell adhesion and provide mechanical support for new tissue growth. To bridge the lesion and re-establish connections after SCI, extracellular matrix (ECM) hydrogels were prepared from human and porcine tissue. The hydrogels were characterized in vitro with respect to their composition and their effects on neurite outgrowth and proliferation and migration of human mesenchymal stromal cells (hMSCs) from bone marrow, adipose tissue and Wharton jelly.

The ECM were extracted from human umbilical cord (UC-ECM), and porcine tissue: urinary bladder (UB-ECM); spinal cord (SC-ECM) and brain (B-ECM). DNA concentration, collagen and glycosaminoglycan content were evaluated for each hydrogel. Fibronectin and laminin were assessed immunohistochemically. Cell viability and migration were evaluated using a WST assay and xCELLigence system. The neurotrophic properties of ECM hydrogels were assessed on dorsal root ganglion (DRG) explant cultures. FlowCytomix bead assay was used to determine the amount of growth factors.

After decellularization, DNA amount was <50 ng/mg in all ECM hydrogels. The highest amount of collagen and glycosaminoglycans was found in UC-ECM. All ECM hydrogels promoted proliferation and migration of hMSCs, as well as massive neurite sprouting out of DRG explants, with the largest neurites found on UC-ECM. The protein analysis revealed the highest amount of HGF, VEGF-A, PDGF-BB and TNF-α in UC-ECM. When seeded in 3D culture rapid hydrogel contraction was apparent after 4 and 24 hours.

ECM hydrogels are injectable, biocompatible, promote cell growth and reveal in vitro chemotactic and neurotrophic properties. UC-ECM displays better biological functions than porcine ECM hydrogels, thus represents a promising material for the repair of injured neural tissue. However, when combined with hMSCs rapid hydrogel contraction might be a limiting factor for the use of ECM hydrogels in SCI repair.
Using 3-D 'smart' biomaterials for protective delivery of genetically enhanced olfactory mucosal cells for chronic spinal cord injury.

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Keywords: Spinal cord injury, hydrogel, nanoparticle, OEC, translation

A recent clinical trial demonstrated improved motor function in companion dogs with chronic spinal injury following autologous olfactory mucosal cell (OMC) transplantation (Granger et al, 2012). The utility of this therapy in canine subjects offers considerable promise to human translation as companion dogs are highly comparable to human patients in terms of spinal lesion heterogeneity, genetic/environmental variation and management strategies. However, major translational challenges still exist: (1) Cell loss during transplantation due to mechanical stress, (2) Incomplete corticospinal tract regeneration. To address this, we combine two next-generation nanotechnologies: Advanced 3-D hydrogels could function as protective cell delivery systems, but their utility for OMC delivery has never been investigated. We demonstrate the feasibility of safely encapsulating canine OMCs within a 3-D implantable hydrogel matrix. We also demonstrate the ability to safely genetically engineer this population to express a major neurotrophic factor, to augment therapeutic capacity, using magnetic nanoparticles combined with Magnetofection Technology (magnetically-assisted transfection) and novel DNA minicircle vectors. Our results suggest this combination of technologies could enable delivery of a therapeutic “plug” of nano-engineered cells within an implantable matrix into sites of neurological injury, a strategy with considerable clinical potential.

Regenerating nerves with fat: harnessing the differentiation potential of adipose derived stem cells

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Keywords: Adult Stem Cells, Nerve Regeneration, Biomaterials

Injuries to peripheral nerves are common, vastly debilitating and affect a young and working population, with significant socio-economic impact. Current therapeutic strategies consist in end-to-end nerve repair or nerve grafting when a gap injury is present; surgical outcomes remain poor, therefore alternatives are sought. Our team of clinicians/biologists/materials specialists are working to develop a new strategy for peripheral nerve repair by combining the potential of stem cells with a novel synthetic nerve guide.

We propose a novel bioartificial nerve graft which is polymer-based with micro-topographic cues (Polynerve) to replace autologous nerve grafting. With extensive in vitro and in vivo studies, we have demonstrated the efficacy of Polynerve as nerve guidance tube, and a safety clinical trial will commence in January 2017.

Polynerve has the potential to deliver cell therapy which can enhance the regenerative effect. Schwann Cells (SC), the regenerative cells of the peripheral nervous system, cannot be used for this purpose due to difficulties in culture and expansion. Our solution is to differentiate human adipose-derived stem cells (hASC) into Schwann-like cell (dhASC) which display morphological, molecular and functional similarities to SC. dhASC were characterized by qRT-PCR, ELISA, Western Blot and showed increased neurotrophic potential when compared to hASC, however, our current protocol does not produce terminally differentiated SC and requires further refinement. Recently, we performed high-throughput RNA-sequencing and LC-MS proteomic analyses to achieve deeper understanding of the differences/similarities between fat-derived hASC/dhASC, compared to nerve-derived human SC. These studies allowed us to identify specific signalling pathways that might be the key for obtaining terminally differentiated SC. To our knowledge, this is the first study providing extensive high-throughput characterisation of hASC/dhASC, in comparison to human SC. Using this knowledge, we will optimise our stem cell strategy for Polynerve scaffold delivery towards future clinical trials on peripheral nerve injured patients.
Computational techniques informing surface engineering for in vitro neural stem cell control

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Keywords: Computational, surface engineering, neural cell

The interest in the clinical use of stem cell therapies is increasing rapidly, with a need for more control over cell populations cultured/expanded in vitro. This is particularly relevant for the treatment of neurological disorders such as Parkinson's disease where positive outcome measures of clinical trials will be limited by the number of derived neurons and their specific sub-types. The aim is to generate enhanced neural cell populations from stem cells through the design of the cell-material interface.

The niche micro-environment is complex, being responsible for cell attachment, proliferation and differentiation. Material engineering approaches to better control cell responses have looked towards surface chemical, topographical and mechanical cues. The many permutations of these factors poses a major challenge in the optimisation of biomaterial design. Machine learning techniques will be used to assess the impact of surface properties on the biological micro-environment.

Cell interaction/response provides computational outputs, with input variables being derived from material properties such as surface chemical characteristics (logP, charge, density, wettability, etc.) and topography (nano- and micro-scale, aspect ratio, etc). The aim is to uncover the relationship between cells and biomaterial surface of in vitro cell culture. In vitro experiments and in silico modelling will continually inform each other towards the optimisation of neural cell characteristic responses.
Wnt pathway regulate endothelial to mesenchymal transdifferentiation in osteoarthritis.

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Keywords: cell biology, cartilage

The pathogenesis of osteoarthritis (OA) in relation to cartilage-subchondral bone interaction is a complex process. Despite extensive studies, the exact mechanism involved is still not clearly understood. Under normal physiological circumstances, endothelial cells appear to be quiescent. It is not clear what stimulates endothelial cells to migrate towards articular cartilage in OA. These alterations result in an increase of endothelial and stromal tissues in the subchondral bone. This in turn results in vascular and stromal tissues migration, and accumulation in the non-calcified articular cartilage all of which alters the architecture of articular cartilage. The aim of this study is to investigate possible mechanism behind endothelial cells invasion of articular cartilage.

Histopathological analysis of osteoarthritic human showed aberrant stromal and endothelial cells proliferation in the subchondral bones of OA tissues investigated, with endothelial and bone marrow stromal cells extending into the non-calcified articular cartilage. Immunohistochemical staining of VE-cadherin and β-catenin showed down-regulation of VE-cadherin and nuclear translocation of β-catenin indicative of endothelial-mesenchymal transformation. Vascular endothelial cells extending into the articular cartilage positively expressed both α-SMA and vimentin whilst some cells retained CD-34 and Dkk-1 expression. In addition, invading stromal and endothelial cells expressed Snail. In vitro co-culture assay showed endothelial cells undergoing EnMT changes when co-cultured with chondrocytes marked by upregulation of α-smooth muscle actin, F-actin and Snail and downregulation of VE-cadherin, β-Catenin.

This study suggests that endothelial to mesenchymal transition may be activated during osteoarthritis. This phenomenon can in part be explained by aberrant Wnt pathway activation in OA. It is yet to be determined what stimulate cellular migration and EnMT activation from subchondral bone. One possible hypothesis is that changes occurring at the non-calcified articular cartilage, marked by superficial zone fibrillation, expose subchondral bone cells to mitogens therefore attracting endothelial cell migration and mesenchymal transformation.
Day 4

Sessions

6a “Translation and Commercialisation”

6b “Translation and Commercialisation”

5a “Clinical Application”

5b “Clinical Application”

Panel Discussion

“Choose the Right Career Path for You”
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Keynote Profile
Erik Shapiro
BS, MS, PhD

“Molecular Imaging Strategies for Biomaterials and Regenerative Medicine”

The research emphasis of the Molecular and Cellular Imaging Laboratory (MCIL) is generally focused on developing and using magnetic resonance imaging (MRI) and X-ray computed tomography (CT) for molecular and cellular imaging of biological phenomena, regenerative medicine and early detection of disease. Molecular and cellular imaging allows scientists and doctors to move past simple anatomic imaging, enabling the observation and measurement of metabolic, physiological and functional processes in living subjects, non-invasively. Working at the intersection of chemistry, physics and biology, my laboratory has three main cores. The first is the development of novel nanoparticle contrast agents. For MRI, the focus is the construction of high relaxivity superparamagnetic nano- and micro-particles whose MRI properties can be made sensitive to various stimuli, gene expression, for example. For CT, the intent is to efficiently deliver large payloads of high-z metals such as bismuth, gold or gadolinium in a safe polymer construct. The second core is the use of molecular and cellular imaging for monitoring cell migration. Cells can be loaded with MRI contrast agents and observed using tailored experimental conditions. This can be accomplished both in transplant models and in the detection of endogenous cells, with the ability to detect single cells, in vivo. The third focus of the laboratory is the use of targeted contrast agents to detect specific molecular epitopes. The strategy here is to selectively target contrast agents to precise tissues or cells of interest by way of antibody- or receptor-mediated affinity. This could be particularly useful in detecting cancer and for identifying unique cellular populations.

For More information please visit: www.facebook.com/msumcil
To those of us working in the field of regenerative medicine, the battle cries of “Translation” and “Bench to Bedside- cutting edge research” are motivating and yet frustrating.

Early into our project, we defined a cell population that proved to have a high angiogenic and osteogenic potential and strong immunomodulatory capacities, which are undoubtedly of crucial importance to healthy tissue regeneration. We could further verify our results in vivo, using an animal model that had previously been declared as clinically relevant and functional.

At the height of scientific creativity we were traditionally confronted with the urgency to publish, create IP, present at conferences and raise funding to keep the project flowing. At the same time, one of the major industrial partners closed down financial support for its musculoskeletal research division, evidence accumulated that results from the animal model could not so easily be translated into a human clinical set-up (promising as they might be), that the cells might not be minimally manipulated by our enrichment strategy after all, that alternatives are rare and costly, that the cell carrier material is of particular interest to the regulatory agencies, and that – of course - development takes time.

While research seems eternally determined to be forwards only, the modus operandi is not necessarily unidirectional but requires circular movements and the phrase “from bench to bedside” should be ideally completed with “…and back” to be reasonable.

By addressing a presumably highly translational question we were relentlessly suck into a maelstrom, having to repeatedly scrutinize results, strategies and trajectories alike. By now, the project is flourishing, keeping many minds busy and, last but not least, it has reached the status of a multilevel translational topic, including aspects of molecular biology, biomaterials, diagnostics and marketing. The life cycle of the cell therapy has only just started!
The Power of Bespoke Biocompatible and Biodegradable Hydrogels

Ayeesha Mujeeb¹

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Keywords: Hydrogel, Cell engineering, Tissue Engineering, Medical device coating, Drug delivery systems

PeptiGelDesign Technologies commercialises a family of self-assembling peptide based hydrogel that mimics the cell microenvironment and provides a natural physiological environment for 3-dimensional (3D) cell culture. In addition to its standard formulation PeptiGelDesign also offer a design service, which allows it to deliver hydrogel with tailored properties. These systems have tunable mechanical strength to suit a range of different cell types and can be functionalization with biological epitopes or formulated with small or large molecules such as growth factors. Example areas of use include 3D cell culture, stem cell culture and directed differentiation. These products have also great potential for applications in the regenerative and medical field as they are animal free, biocompatible and biodegradable. They can therefore potentially be used as primary packaging for the in-vivo delivery of drugs, cell or other biological factors. Our hydrogels can be designed to be injectable, sprayable and are naturally mucoadhesive. To summarize the material design platform developed by PeptiGelDesign Technologies allows us to offer bespoke hydrogels with tailored properties to suit the needs of our customers.
Designing an in vitro functional model of the basal ganglia for pre-clinical study

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Keywords: Brain, Parkinson's, model, microfluidics, electrode

Cell damage or dysfunction of neurons within a midbrain area named the basal ganglia can lead to Parkinson’s and Huntington’s diseases. However, the basal ganglia is difficult to access (located in the centre of the midbrain) and the neurons within are densely interconnected, meaning neural study in vivo is very challenging. By re-creating the basal ganglia in vitro, it will be significantly easier to study, but can a complex functioning neural circuit be created in vitro?

In this study, a microfluidic model has been developed with five separate areas for culture of primary cells from the five main functional areas of the basal ganglia; namely the cortex, striatum, substantia nigra (pars compacta and pars reticulata) and the globus pallidus. These areas (or ports) are connected by microchannels of varying widths through which axons can grow, connecting the cell populations and creating a circuit, mimicking the in vivo basal ganglia. In addition, the whole circuit is cultured on a multi-electrode array (MEA), so that the external electrical signals of the cells can be measured to ensure a functional connection between neural cell populations. Once a complete mimic of the basal ganglia is completed, it can be specifically damaged to mimic the Parkinson's or Huntington's disease states. Data can be gathered concerning Parkinson’s and Huntington’s by testing pharmaceuticals, neuroprotective factors and stem cell therapies with this novel in vitro neural model.
Dynamic hydrostatic pressure – an osteogenic stimulus for mesenchymal stem cells in 3D hydrogel culture

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Keywords: Bioreactors, mechanotransduction, hMSC, 3D hydrogels

Objectives: Hydrogel scaffolds provide an ideal culture environment for many cell types, being able to approximate some of the biochemical and structural elements of tissues, yet it can be challenging to provide an appropriate mechanical stimulus to cells in such a soft matrix. These physiological forces are known to be crucial in mediating critical aspects of tissue formation, function, growth and repair.

Methods: Dynamic hydrostatic pressure is an important mechanical stimulus for cells throughout the body, transducing the compressive loading of tissues into a fluctuating change of pressure in the interstitial fluid which can then be detected by cells. In this study, a range of hydrostatic pressure regimes were applied to human mesenchymal stem cells in 3D hydrogel cultures using a bioreactor.

Results: Stimulation of the constructs with 280kPa cyclic hydrostatic pressure at 1Hz (matched to normal physiological exercise) resulted in up to 75% mineralisation in the hydrogel, whilst standard incubator culture, constant high pressure or either low-frequency or low-magnitude stimulation had no effect (<2% mineralisation). These results suggest that MSCs may be primed to respond to pressures within a specific physiological range.

Conclusions: As tissue engineering strategies are now entering clinical translation, understanding how physiological loading interacts with, drives and directs tissue growth will be fundamental to optimising treatments. Mechanical signals are therefore an essential component in recreating tissue environments and translating in vitro regenerative medicine into clinical treatments.
Development of a microcarrier-based culture process for the expansion of human mesenchymal stem cells

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Keywords: Cell therapy manufacturing, mesenchymal stem cells, microcarriers, bioreactors.

As the number of mesenchymal stem cell based therapies proceeding through clinical trials increases so does the demand for well characterised, scalable expansion technologies that can yield the estimated number of cells required. Microcarriers used in conjunction with stirred tank bioreactors provide a suitable platform for this large scale expansion.

The overall objective of this project is to characterise and develop the key unit operations for this type of expansion process. The scope of these selected unit operations encompasses the span of the process, from working cell bank formulation through to the final cell harvest.

The results presented here are extracts from two distinct series of experiments. The first of which explores the exploitation of the bead-to-bead cell migration mechanism to streamline the bioreactor inoculation phase of the expansion process. Data here confirms the feasibility of inoculating a bioreactor directly from the cryopreserved working cell bank, then shows how this operation can be improved by cryopreserving the cells whilst attached to the microcarriers. This inoculation via bead-to-bead transfer method minimises the lag phase, reducing the culture time by 24-hours whilst achieving the same cell yield.

The second series of experiments identifies and systematically addresses the limiting factors that result in the termination of the growth phase. Data here highlights the 20% improvement to cell yield that can be achieved by reducing microcarrier aggregation and managing nutrient exhaustion and metabolite build-up by adopting a suitable agitation and medium exchange strategy.

This project is supported by Pall Life Sciences and the EPSRC.
Bioinspired Protein Scaffolds as Xeno-Free Substrates for Embryonic Stem Cell Self-Renewal

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Keywords: Biomaterials, hPSCs, chondrogenesis, protein fibres

Human pluripotent stem cells (hPSCs) have enormous potential to treat various degenerative diseases. However, the therapeutic application of hPSCs is hindered by the risk of pathogenic or immunoreactive transmissions from animal-derived culture components. Natural polypeptides of the extracellular matrix are routinely used as substrates for hPSC self-renewal and maintenance of pluripotency. However, heterogeneous sampling and potential zoonotic contamination make such substrates a poor choice for hPSC scale-up and clinical use. Here, we introduce a recombinant protein scaffold containing a genetically-encoded cell adhesive moiety and assess its potential to support hPSC self-renewal. Protein-composed materials offer clear benefits over polymer and peptide-derived counterparts including an increased capacity for bottom-up functionalization and display of bioactive motifs with near-native stoichiometries. Additionally, the spatial density and topographical dispersion of functional moieties are controllable at the nanoscale.

We show that human embryonic stem cell line HUES7 cells attach and spread on the recombinant substrate at a rate comparable to Matrigel and fibronectin controls. Cells retain an undifferentiated phenotype over multiple passages as demonstrated by immunocytochemical staining and RT-qPCR analysis of key pluripotency markers. Intriguingly, initial cell attachment to the adhesive moiety is initiated by both α5β1 and αvβ5 integrin heterodimers, whilst attachment to control substrates is mediated by alternative integrin engagement. Our results suggest that this novel scaffold holds great promise as a xeno-free culture component for feeder-free expansion of hPSCs.
Keynote Profile
Bo Kara,
Head Process Development, Cell Gene Therapy Platform CMC, GSK

“Commercialisation of Ex Vivo Cell Gene Therapy Products – Establishing Processes, Manufacturing and Supply Chains”

Bo Kara leads the development of manufacturing processes for GSK’s cell gene therapy vector and autologous cell processing platforms. Bo has nearly 30 years experience in designing research and development programmes to develop scalable and validatable manufacturing processes for biologics products. He has published widely and is primary inventor on a range of process/stem cell patents. He has developed a keen understanding of the technical, regulatory and commercial issues faced at each stage of the development of manufacturing processes.
Poster Session A

Posters A1 – A12
Development of Chandler Loop for examining thrombus formation

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Poster Number: A1

Keywords: Chandler Loop development, Microvesicles, coagulation

Introduction: Haemostasis is the physiological process that serves to maintain the integrity of the circulatory system and requires a delicate balance between procoagulant activity and anticoagulant activity. Any significant disruption to this system may lead to severe complications such as bleeding or thrombosis.

Microvesicles (MVs) are small plasma membrane fragments (0.1-1μm) released into the circulation by blood and vascular cells after physical stress or stimuli.

MVs promote coagulant activity and are also involved in anticoagulant pathways, due to the proteins and lipids they carry. Despite this, we do not know whether MVs are involved in thrombus formation or breakdown. Many studies used static thrombi which have no discernible structure. Developing Chandler Loop (CL) methodology mimics clinical thrombi

Aim: To develop a method to allow examination of thrombus formation

Method: The Chandler Loop method forms a thrombus similar to in vivo thrombi. Whole blood was collected from healthy donors into trisodium citrate. Blood was re-calcified with 0.25M CaCl2 and placed in the tubing before rotation for 90 minutes at 30rpm. Each thrombus was then measured in terms of weight and length, recording head and tail sections.

Results: The CL produced consistently sized and shaped thrombi consisting of head and tail regions of 3mm±0.2mm and 7mm ± 0.5mm length and 1mg±0.1mg and 3mg± 0.2mg weight respectively.

Conclusion: We have developed and optimised a system for investigating thrombus formation and will now use this to research where MVs locate within the thrombus, and to study clot formation and dissolution kinetics.
Effect Of Variable Fibre Size Electrospun PET Scaffolds On Macrophages

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Poster Number: A2

Keywords: Scaffold, Tissue Engineering, Biomaterial

Macrophages are one of the most important cell types in immune system. Macrophages broadly have a pro-inflammatory (M1) and anti-inflammatory, pro-healing (M2) phenotype. For biomaterial applications, immune response is the key part of the failure or acceptance of the application. Controlling M2 phenotype differentiation provides an opportunity to reduce inflammation and increase wound healing efficiency. A lot of studies have shown that implant topography significantly influences cellular response. THP-1 monocyte-like cells were activated with PMA and cultured on different fibre size polyethylene terephthalate (PET) to show the effect of fibre size and random vs aligned fibres on the phenotype of macrophages.
Novel 3D scaffolds for skeletal regeneration and cell printing

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Poster Number: A3

Keywords: Bone Tissue Engineering, Bioprinting, Biofabrication

INTRODUCTION: In recent years, Additive Manufacturing (AM) has come to the fore in engineering tissues such as bone, due to the ability of AM to deposit spatially precise fibres and print complex geometry scaffolds [1]. We seek to engineer a robust, reliable 3D print bioconstruct capable of integration with the damaged tissue and able to aid skeletal regeneration through precise spatial deposition of skeletal stem cells and biomaterial simultaneously.

METHODS: Our biofabrication system produces accurate and high speed movements as a consequence of specific control over micro-stepper motors and physical motion components [2]. Biologically degradable clay hydrogels can be plotted to create high definition fibre deposition because of the tuneable shear-thinning properties of the clay gel.

RESULTS: Rheological properties of the synthetic smectite clay allowed the creation of gels enabling uniform and viable cell encapsulation. Live/Dead assay demonstrated cell viability within polymeric clay blends. A range of polymer-clay materials were found to support skeletal cell compatibility as well as retaining thixotropic – shape retention properties that facilitated material printing.

DISCUSSION: Current study demonstrates efficacy of cell encapsulation within clay-biopolymer constructs with observed high cell viability and accurate spatial 3D cell distribution. Additional work is centred on engineering large scale, viable and reproducible biologically relevant constructs for skeletal evaluation.

Differentiation Protocols for Osteoblast and Adipocyte Formation from Human Bone Marrow Stromal Cells (HBMSCs)

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Poster Number: A4

Keywords: Stem Cells, Differentiation, Osteoblasts, Adipocytes

Medical advances have led to a welcome increase in the aging population although, as a consequence, diseases that lead to bone and joint injuries (e.g. osteoporosis, osteoarthritis) are more prevalent. Stem cell based therapies have the potential to treat these diseases. However, one of the limiting factors for improved skeleton healing remains the paucity of sufficient skeletal stem/progenitor cell populations for bone tissue repair.

Skeletal stem cells (SSCs), commonly referred to as mesenchymal stem cells (MSCs), have the potential to give rise to several stromal tissues (cartilage, fat, bone) but remain inefficient in clinical therapeutic application. Our ultimate aim is to generate induced SSCs (iSSCs) from induced pluripotent stem cells (iPSCs), investigate epigenetic changes and compare the potential for osteoblast/adipocyte formation with HBMSCs. We hypothesised that iSSCs will resemble an earlier bone phenotype rather than the aged parent. We isolated SSCs from the bone marrow of patients undergoing hip-replacement surgery and differentiated the cells using osteogenic (Dexamethasone, Ascorbic Acid, Vitamin D3) and adipogenic (Dexamethasone, Rosiglitazone, IBMX, ITS) media for 21 days. Molecular analysis showed the specific expression of key marker genes under osteogenic/adipogenic conditions compared to control: PPARγ (peroxisome proliferator-activated receptor gamma), ALP (alkaline phosphatase) and RUNX2 (Runt-related transcription factor 2) up to 10-fold higher expressed. Staining for ALP and lipids confirmed these findings. In conclusion, we were able to demonstrate the efficient differentiation of osteoblasts/adipocytes from HBMSCs. Future work will focus on the generation of a tailored protocol for iSSCs and osteoblasts/adipocytes differentiation from iPSCs as well as in depth analysis of the epigenetic state at the different differentiation stages.
Applying physiological forces to cells in culture using commonly available lab equipment.

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Poster Number: A5

Keywords: Cartilage, hydrogel, bioreactor, shear, perfusion

INTRODUCTION: Dynamic 3D culture using bioreactors helps to mimic physiological conditions and close the gap between in vitro and in vivo research. Many bioreactors are highly specialised apparatus and unavailable in most labs, but in this investigation we aimed to determine if commonly available equipment such as a plate rocker could be used to provide measurable dynamic flow conditions, nutrient perfusion and shear forces to cells in 3D hydrogel culture.

METHODS: We used a plate rocker to provide a basic, easily-parameterised flow/shear environment to bovine chondrocytes cultured in an agarose hydrogel as a simplified model of tissue engineered cartilage. The hydrogels were placed on the rocker set at a 10° tilt, 60rpm for 1 hour, 5 days per week for 4 weeks in 6ml media.

RESULTS: Under rocking conditions, the cells increased production of glycosaminoglycans and collagen, but much of this was found in the media, with reduced amounts retained in the hydrogels. Total protein synthesis was raised in both the media and within the hydrogels. Live-dead staining at 4 weeks showed a greater proportion of live cells in the experimental group.

CONCLUSION: The findings of this study suggest that a plate rocker can be used to generate preliminary data on how cells respond to flow, perfusion and shear forces with universal lab equipment. Coupled with 3D cell culture, this allows relatively large tissue-like constructs to be engineered and tested in the lab, with applications in both regenerative medicine and pre-clinical screening to refine animal experiments.
Therapeutic potential of mesenchymal stem cells for the treatment of ocular surface disorders

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Poster Number: A6

Keywords: Mesenchymal stem cells, ocular surface damage, nanofiber scaffold, immunosuppression, cell-based therapy.

Ocular surface injuries or diseases represent serious health problem causing a decreased quality of vision or even to blindness. If the corneal disorders are associated with limbal stem cell (LSC) deficiency, the only effective treatment protocols involve transplantation of limbal tissue or a transfer of stem cells. If autologous LSCs are absent or unavailable in a sufficient amount, mesenchymal stem cells (MSCs) represent an alternative and promising stem cell source. MSCs represent a population of adult progenitor cells with multipotent differentiation potential. In addition, MSCs possess a potent immunoregulatory properties and are a source of numerous trophic and growth factors. In this study we analysed the effects of bone marrow-derived MSCs on ocular surface reconstruction in a rabbit experimental model. The injury of the cornea was induced by alkali which caused the damage of corneal epithelium and a strong infiltration with immune cells. MSCs were phenotypically and functionally characterized in vitro and were grown on nanofiber scaffold, transferred onto alkali-injured ocular surface and the healing process and suppression of a local inflammatory reaction were determined. The treatment of injured eyes with nanofiber scaffolds seeded with MSCs significantly decreased a harmful local inflammatory reaction and supported healing of damaged ocular surface. The results suggest that MSCs can replace LSCs for the treatment of damaged ocular surface and that the main role of MSCs in the healing process is mediated by the suppression of a local inflammatory reaction, production of growth and trophic factors and by a direct transdifferentiating into corneal epithelial cells.
Nanofabricated cell culture surfaces to control self-renewal and differentiation of mesenchymal stem cells

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Poster Number: A7

Keywords: MSCs, cartilage, biomaterials, nanotopography,

Mesenchymal stem cells (MSCs) have been proposed as a promising cell source for cartilage tissue engineering as they can differentiate towards the chondrogenic lineage. Up until now, MSC chondrogenesis has been achieved by using 3D cultures supplemented with bioactive substances (e.g. TGF-β), which can also act as limiting factors when larger cultures are needed. With these limitations in mind, material induced control of cell responses, via control of initial cell adhesion is a promising tool.

The aim of this work is to generate surfaces with defined chemical features and associated nanotopographical profiles by varying the chain length of a silane used in the production of a self-assembled monolayer (SAM) and, assess their effect on MSC self-renewal and differentiation.

SAM characterisation demonstrated subtle differences in dynamic contact angle and surface roughness. Although surfaces did not show any statistical significance in cell viability or morphology, PCR results demonstrated that ODDMCS induced the highest chondrogenic marker gene expression. Morphology wise, chondrocytes exhibited differences in cell shape depending on which SAM they were cultured on.

While an optimal surface chemistry has not been identified yet, the cell compatibility and influence on phenotype of the surfaces allows experiments to be initiated to assess how OH and NH2 SAMs affect MSC responses.
The comparison of multiple bioreactor technologies for the xeno-free expansion of human mesenchymal stem cells

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Poster Number: A8

Keywords: Bioreactors, stem cells, xeno free, microcarriers

Due to their multipotency and immunomodulatory properties human mesenchymal stem cells (hMSCs) have emerged as a therapeutic intervention for many diseases. However, the use of hMSCs in clinical applications remains limited due to the lack of advanced, automated, reproducible methods for the isolation and large scale expansion of stem cells in a cost efficient manner to meet the quality and quantity for clinical need.

The work presented here describes the xeno-free isolation and expansion of bone marrow (BM) derived hMSCs using novel antibodies to isolate more functionally relevant BM-hMSCs which will enable integration into the automated stem cell factory (Autostem). The work focuses on small scale experimental studies using microcarriers in both the stirred tank and Wave® bioreactor system. Studies have focused on identifying the optimal microcarrier type, dO2, dCO2, pH, microcarrier and cell concentrations, medium exchange regime and mode of operation. Measurements on cell viability, functionality, cell quality and consumption/production of key metabolites were obtained in response to changes in cell culture regime. Initial studies compared growth kinetics in monolayer cultures for both xeno free and serum containing media using multiple BM-hMSC donor cell lines. These studies will establish a standardised set of process conditions for the expansion and harvest of the BM-hMSCs, which will be used to inform larger-scale single use stirred-tank bioreactor runs (>3L) to reduce the potential regulatory burden of validating SIP/CIP procedures and to develop a GMP-compatible bioprocess.
The in vitro effect of syndecan-3 gene knockout on characterisation of bone marrow derived mesenchymal stem cells

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Poster Number: A9

Keywords: murine MSCs, syndecan-3

Introduction:
Syndecans (1-4) are a family of single transmembrane heparan sulfate proteoglycans that feature functional diversity, from cell proliferation, differentiation, to adhesion and migration. The immunomodulatory effects of bone marrow derived mesenchymal stem cells (MSCs) has been widely studied and the recent observation that syndecan-3 (SDC-3) was selectively pro-inflammatory in the rheumatoid joint led us to speculate that SDC-3 might have a role in MSCs biology. To investigate this we performed a side-by-side analysis of MSCs derived from wild type (WT) and syndecan-3 knockout (SDC-3-/-) mice.

Materials and Methods:
MSCs were isolated from bone marrow of C57Bl/6 WT (n=6) and SDC-3-/- (n=6) mice. Cells between passage 3 and 5 were used to assess the following: flow cytometry (immunophenotype, size and complexity analysis); population doubling time (PDT); colony-forming units (CFU-F); osteogenic, adipogenic and chondrogenic differentiation; and adhesion properties to laminin.

Results:
Immunophenotypic analysis indicated that both MSCs types were positive for Sca-1 and CD44. Notably, half of the SDC-3-/- MSCs samples were positive for CD105, while only one SDC-3-/- sample was slightly positive. Flow cytometry showed similar mean values for cell size (forward scatter) for both cell types. In terms of complexity (SSC, side scatter) the WT MSCs showed significantly higher values then the SDC-3-/- sample. Population doubling time, colony-forming units and osteogenic, adipogenic and chondrogenic differentiation were equivalent for both cell types. Laminin significantly increased the adhesion of WT MSCs, but not for the SDC-3-/-.

Conclusions:
Our results suggest strong similarities between WT and SDC-3-/- MSCs, but also notable differences in terms of CD105 expression, cell complexity and adhesion to laminin, which indicate the alteration of bone marrow derived MSCs biology. Future work will include testing the adhesion properties of WT and SDC-3-/- MSCs to collagen and fibronectin.
Developing three-dimensional muscle-nerve constructs using induced pluripotent stem cells

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Poster Number: A10

Keywords: iPSCs, musculoskeletal, neuromuscular junction, tissue engineering

Motor neurons (MNs) are specialised cells of the nervous system which innervate skeletal muscles and allow locomotion. These cells undergo deterioration in diseases such as Amyotrophic Lateral Sclerosis (ALS) and Spinal Muscular Atrophy (SMA). As a consequence, patients experience progressive paralysis due to malfunction of the neuromuscular junction (NMJ) and associated atrophy of the muscle. Developing a human in vitro model of the NMJ is a challenge yet to overcome. Previous work based on animal-derived cells or human embryonic stem cells was done in monolayer, lacking the three-dimensional in vivo features. Deriving MNs from human induced pluripotent stem cells (hiPSCs) and co-culturing them with human skeletal muscle cells would represent an innovative disease model. It would allow drug screening and understanding of the cellular and molecular mechanisms that contribute to disease aetiology and pathology. This model will be associated with tissue engineering constructs which are suitable for physiology studies, mechanical stimulation of the cells and analysis of their functional properties. Creating a fully human model will reproduce a functional 3D environment. Along the advantages of the use of hiPSCs we acknowledge higher availability and reduced ethical problems which are typically related to animal-derived or embryonic stem cells.
Mathematical modelling as a tool to inform the design of spray systems for cell-based therapies

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Poster Number: A11

Keywords: Cell spraying therapies, mathematical modelling

Chronic degenerative diseases such as cancer and Parkinson’s disease are the leading cause of mortality worldwide, and can cause permanent loss of cells and organ dysfunction. Spraying of cells directly to the affected area is one delivery mechanism that has been shown to promote cell proliferation and cell viability, as cells are delivered on a layer-by-layer basis in a fast and controlled manner, thus accelerating the healing process. The requirements of cell spraying are specific to the tissue being treated, especially due to geometric variability between tissues. Here we present a case study based on spraying cells to the back a human eye, a therapeutic option for treating blindness.

The CFD solver STAR-CCM+®, based on finite element methods, was used to enable spatial simulations of spraying, using realistic clinical geometries and parameters from commercially available cell-delivery spraying systems. A continuum mechanics approach was used, where the cellular suspension drops are described using a multi-phase model. The cells were sprayed into the eye through an injector with known dimensions and position.

We were able to predict the thickness and spatial distribution of the cellular over the time course of one spraying event. The thickness and variability of this layer thickness is determined by the flow rate of delivery and spraying pressure. The section of the eye that is covered, is determined by the spraying direction and nozzle geometry, and the model enables these parameters to be varied to inform spray operation.

The computational models developed provide insight into the link between spraying pressure and flow rate, and the spatial distribution of delivered cells. The results provide a starting point for building standardised computational models relevant to more complex geometries and spraying scenarios, with a view to informing cell delivery parameters in a way that minimises costly and time consuming experimentation.
Self-assembling peptides for Cartilage Regeneration

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Poster Number: A12

Keywords: Self-assembling peptides, Cartilage Regeneration, Biomaterials

The poor regenerative capacity of human articular cartilage contributes to development of debilitating osteoarthritis and remains a clinical challenge to date. A net depletion of glycosaminoglycan’s (GAGs) in osteoarthritic (OA) cartilage has been reported in the literature. This has been shown to result in the loss of mechanical properties and function in vitro, and is considered to be a major contributor to the disease progression. This has led to the development of novel cartilage treatment strategies including reconstructive or reparative methods in an attempt to delay or “bridge the gap” to joint replacement. Further development of tissue engineering-based cartilage repair methods have been pursued which may restore GAG levels and maintain functional cartilage material properties. Initial studies, which were carried out to restore GAGs to GAG depleted articular cartilage with self-assembling peptides (SAPs) and chondroitin sulphate (CS), have shown promising results in the restoration of cartilage properties. The goal now is to pursue these findings further in a systematic manner via:

1) Rational selection of a panel of self-assembling peptides (SAPs)

2) Investigation of the effects mixing the SAPs and GAGs at varying molar ratios on the self-assembly, biomechanical and biophysical properties of the material.

3) Optimisation of the mode of delivery of the combinations of SAPs and GAGs to the GAG depleted articular cartilage.

4) Detailed investigation of the biological and mechanical properties of the treated GAG depleted articular cartilage treated with SAPs/GAGs via indentation testing, tensile testing and friction testing.

These studies will increase the knowledge of the potential for the therapeutic use of SAPs/GAGs.
Poster Session B

Posters B1 - B15
Mimicking extracellular matrix (ECM) to promote nerve growth for spinal cord injury (SCI) repair

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Poster Number: B1

Keywords: Scaffold, ECM, self-assembling peptides, hydrogel

Clinical need
Spinal cord injury (SCI) affects around 1000 people in the UK yearly (NHS England, 2013) with treatment cost approximately £1 billion (Spinal Research, 2011). Symptoms stretch beyond impaired bodily functions to poor mental health (WHO, 2013) and currently no recognised treatment to regenerate damaged spinal cord exists.

SCI is characterised by initial trauma and secondary scar formation. Being both fibrotic and glial (Yuan & He, 2013), axons are chemically inhibited due to proteoglycan overexpression as well as being physically blocked (Jones et al., 2001). Attempting to control the mechanical environment using material-based regenerative therapies may lead to enhanced repair. These could be introduced during routine spinal stabilisation surgery.

Project description
Recent research has shown the self-assembling properties of the peptide P11-8 can operate during electrospinning alongside polycaprolactone (Gharaei et al., 2016). This gives rise to a nonwoven fabric with a submicron network and tunable nanoscale network that are connected and visually resemble native extracellular matrix (ECM). Introduction into a hydrogel will produce a hydrated, mechanical environment with control at the nano-, micro- and macroscale.

Research challenges include:
- Optimising the nano-/microfibre manufacturing process to create a porous, fibrous architecture with appropriate fibre orientation and morphology
- Integration into a novel hydrogel for superior mechanical performance and tissue-like behaviour
- Characterising the system to elucidate structure-function relationships and tailoring for human spinal cord

The target outcome is a mechanically tunable structural support within a hydrated environment. The research involves development of a new sub-micron fibre spinning technology capable of supporting peptide self-assembly, materials characterisation and investigation of neural cell responses.
Usage of Rapid Prototyping for Transferring Batch to Microfluidic Processes in Cell Downstream Process Development

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Poster Number: B2

Keywords: cell separation, ATPS, process development, rapid prototyping

Transferring early scientific findings from high-throughput experimentation into an industrially relevant process is a major issue and it is often related with high investment costs. Especially emerging technologies often lack in process knowledge and longstanding experience. Thus, a flexible process development with low financial risk is beneficial.

These challenges can be managed by using rapid prototyping techniques being suitable for such a sophisticated task. Due to a short workflow, a fast optimization and adaption can be performed providing even new possibilities to manufacture complex geometries. In the field of life science technology, standard laboratory equipment can be combined with 3D printed biocompatible polymer devices in order to create innovative systems, where fabrication of tailor-made process units by traditional production methods fail. In many cases, rapid prototyping can be easily implemented by inexpensive benchtop 3D printers.

In the presented study, the transfer of batch data for cell partitioning in aqueous two-phase systems (ATPS) into a microfluidic process using rapid prototyping techniques is presented. Cell separation using ATPS is a promising alternative, label-free method in cell downstream processing. Currently, the major drawbacks of the method are the multiple parameters influencing the cell separation which makes separation predictions highly empirical. Our in house high throughput screening (HTS) platform was used to optimize cell partitioning in ATPS for a model cell line. The batch optimized ATPS conditions were then directly transferred into a flow process in microfluidic format using the rapid prototyping approach. For this purpose, we have designed and 3D printed devices to connect the microfluidic device, pumps and a microscope, as an analytical tool, in order to create a tailor-made and flexible process setup.

Our work demonstrates the usefulness of rapid prototyping techniques in early process development by highlighting the advantage of a short workflow and a high degree of freedom in the design of newly created devices.
In vitro models of the eye for therapeutic delivery evaluation in the treatment of retinal pathologies

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Poster Number: B3

Keywords: Modelling, CFD, Eye, Cells, Flow

Injections into the vitreous are currently used in the treatment of conditions such as diabetic retinopathy. Incorporating therapeutic agents into a silicone oil tamponade and utilising this as a controlled release delivery system to the back of the eye could provide an improved clinical approach compared to intravitreal injections. It is however, important that local concentrations and the release rate of the therapeutic agents are predictable in order to avoid either toxic or ineffective treatment. The aim of this study was to develop equivalent in vitro and computer models to evaluate the design of these systems.

Using in vitro eye models, aqueous outflow movements in the posterior segment can be simulated to assess the flow patterns and agent concentrations within the model, in an attempt to estimate clearance from the silicone oil tamponade. One system modifies the Kirkstall QV600 culture chamber to incorporate a physiological barrier at the interface of flowing culture media and a silicone oil tamponade for permeability studies. The other system provides a more representative geometry, comparable with the ocular globe, to assess realistic flow patterns. Using COMSOL Multiphysics computational fluid dynamics software, corresponding computer models have been developed which have been validated using the in vitro models to accelerate the design process.

Using image analysis, qualitative data indicated the computer model was an accurate representation of the in vitro model. Currently work is being done to confirm this quantitatively using pressure and velocity measurements.

The combined use of in vitro and computer modelling could be widely used in the development of many regenerative medicine approaches for retinal pathologies.
Functionalised graphene layer-by-layer constructs as support for NIH-3T3 fibroblasts and implications in wound-healing applications.

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Poster Number: B4

Keywords: Graphene, biocompatibility, wound-healing, nano, layer-by-layer

We present a novel synthesis of graphene sulfonate (G-SO3) and its incorporation in a layer-by-layer (LbL) construct, for thin, water-permeable films for use in dressing of superficial wounds.

Biocompatibility was assessed using NIH-3T3 cells, and compared to LbLs containing GO and GO-SO3, using a LIVE/DEAD® Viability Assay. DNA quantification was performed using Quant-iT™ PicoGreen® dsDNA Assay and metabolic activity assessed using the AlamarBlue® assay. The adsorption of extracellular matrix proteins (fibronectin and bovine serum albumin) onto the LbLs was profiled using a NanoOrange® Protein Quantitation assay.

GO was produced by a modified Hummers’ method. G-SO3 and GO-SO3 were produced via electrophilic aromatic substitution of graphene and GO, respectively. LbL constructs were made by alternating layers of negatively charged GO, GO-SO3 or G-SO3, with positively charged poly(ethyleneimine) (PEI) onto glass coverslips. Characterisation of GO, GO-SO3 and G-SO3 and LbL constructs was achieved using XPS, Raman mapping, FTIR, UV-vis, Zeta potential, CHNS and bright field imaging.

Successful NIH-3T3 proliferation was observed on PEI/GO, PEI/G-SO3 and PEI/GO-SO3 over the three time points, for all assays. Metabolic activity and proliferation of NIH-3T3 cells was lower on PEI/G-SO3 than on PEI/GO-SO3 PEI/GO constructs. Fibronectin adsorption was also significantly lower on PEI/G-SO3 than on PEI/GO-SO3 or PEI/GO. No significant differences in BSA adsorption were observed between the graphene-containing LbL constructs.

Good biocompatibility for all the graphene-containing LbL constructs has been demonstrated, making them suitable candidates for wound-healing applications. Protein adsorption and conformation appear to be influenced by the charge and composition of graphene, causing subsequent affects on cell behaviour.


ACKNOWLEDGEMENTS: BBSRC
Rapid label-free separation of specific stromal cell populations for autologous cell therapy in musculoskeletal disease

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Poster Number: B5

Keywords: Stem cells, dielectrophoresis, acoustic waves, bone, cell separation

Musculoskeletal diseases represent the second greatest cause of disability worldwide. Current strategies include the use of stem cells to coat engineered scaffolds in order to repair the damaged tissues. For this, the patients’ autologous cells are harvested and manipulated in the laboratory to obtain a clinically relevant number of cells to seed on the scaffolds. This process requires time and cell manipulation which can have an impact on the patients’ safety and on the final cost of the therapy. In order to make this approach easier and more cost-effective, there is a need for a device enabling label free separation of stem cells in a real intra-operative time. By using remote dielectrophoresis, where an electric field is coupled into a microfluidic channel using surface acoustic waves, cells can be separated with minimal manipulation. Proof of concept of this technology has been already done on yeast cells and dental pulp cells. The aim of this project is to scale up this device in order to deliver the cell separation in intra-operative times while maintaining their osteogenic potential. The device will be first optimised with the use of finite element modelling of microfluidics and tested with a complex mixture of cells. After separation, the cells will be tested by seeding them on scaffolds and staining for osteogenic potential markers. After in vitro validations, cell seeded scaffolds will be implanted into athymic rats with calvarial defects. The performance of the cell separation will be compared to that obtained with magnetic activated cell sorting.
Click-crosslinkable Collagen Hydrogels For Cytocompatible 3D Culture In Regenerative Medicine

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Poster Number: B6

Keywords: Collagen hydrogels, injectable device, biomaterials

Hydrogels provide a three-dimensional (3D) framework with tissue-like elasticity for culturing cells in regenerative medicine. Injectable materials delivered in aqueous solution are considered ideal for the introduction of cells and bioactive factors through minimally invasive methods to fill complex 3D shapes. A novel injectable click-crosslinkable collagen-based hydrogel has been created capable of cell encapsulation in vitro/in vivo.

The objectives were to prepare a novel cross-linking strategy for the formation of hydrogels with varied mechanical and physical properties to examine differentiation and proliferation with dental pulp stem cells (DPSCs).

Collagen was thiolated by a substitution reaction using Traut’s reagent at pH 7.4 without the need of an additional base. The degree of functionalization was quantified using a TNBS assay and circular dichroism was used to confirm the ratio of positive to negative peaks (RPN) was comparable to native collagen. The hydrogels were formed in 10 minutes by a thiol-ene photo-click reaction using violet light (405 nm) between the thiolated collagen and poly(ethylene glycol) norbornene. Varied mechanical and physical properties of the gels have been made by varying the molar ratio of SH: norbornene and the degree of thiol functionalization. The effect of degradation on the mechanical and physical properties were studied using both simulated body fluid and collagenase. The prepolymer solution was prepared in PBS and DPSCs and G292 cells were encapsulated in the gel in vitro. Cell spreading, cell migration and cell survival were analysed using ATP assay and LIVE/DEAD staining.
Rapid label-free separation of specific stem cell populations for autologous cell therapies

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Poster Number: \textbf{B7}

\textbf{Keywords:} Stem cells, minimal manipulation, autologous, cell sorting, regenerative therapies

There are vast potential uses for stem cells in regenerative therapies addressing clinical challenges in oncology, Parkinson’s disease, spinal cord injuries, bone, muscle or cartilage defects and diabetes. Using autologous stem cells is advantageous due to their accessibility and safety but a major limitation is the isolation of rare stem cell populations in adult tissues containing many other different cell-types. The aim of this project is to develop a working prototype device capable of selecting specific stem cells from clinical samples with minimal manipulation and in real intraoperative times for immediate clinical use. This would avoid cell manipulation and costly cell expansion, providing an accelerated translation pathway. The device will use remote dielectrophoresis, where an electric field is coupled into a microfluidic channel using surface acoustic waves, combined with biosensor-based technology using protein-binding proteins (Adhirons). Specifically, the project will: identify markers for stem cell selection; optimize surface chemistry for cell capture and release; determine specificity and sensitivity of the methodology and determine the multi-lineage potential of the cells post-separation. Nanofabrication techniques will be used to manufacture the device whilst biological techniques will determine the specificity and selectivity and demonstrate that cell phenotype is unaffected. The project will impact researchers, clinicians and patients alike, as isolation of specific stem cell populations will assist in the elucidation of regenerative mechanisms as well as delivering a minimally manipulated, enriched population of specific stem cells for therapeutic purposes.
Tissue Repair Capacity of Prototype Antimicrobial-Releasing Scaffold

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Poster Number: B8

Keywords: Biomaterial, Regenerative, Antimicrobial, Soft Tissue

Advanced biomaterials have been designed to aid the regeneration of soft tissue lost during surgery, disease or infection. They are expected to play key roles in the future of periodontology, oral surgery and implantology. Bacterial infection is a common complication which can prevent successful tissue integration and cause failure of medical implants. Misuse of antibiotics has led to the concerning spread of antimicrobial resistance (AMR) so alternative approaches are required to minimise the risk of bacterial contamination.

PhotoTherix is an advanced bioresorbable polymer scaffold equipped with photodynamic therapy (PDT) technology aimed for use in maxillofacial applications. Typically, an antimicrobial PDT agent is loaded in its inert form and then activated locally through a light source if infection should occur. To enable translation to clinical use, this study aims to investigate how the scaffold architecture and antimicrobial functionality are affected by its chemical, physical and mechanical properties. The incorporation and controlled release of various PDT agents will be studied for their bactericidal effectiveness through various biochemical assays. Evaluation of the cell viability of the scaffold will be performed to determine tissue regenerating capabilities and cell-scaffold relationships.

If the technology is established, it could also be extended to the treatment of chronic wounds. The resulting prototype would have a great impact on the health industry by improving patient outcomes, reducing the health economic burden and controlling the spread of AMR.
Comparison of Human Bone Marrow Mesenchymal Stromal Cells, Adipose Tissue-Derived Stromal Cells and Bone Marrow Mononuclear Fraction in a Diabetic Wound Healing Model

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Poster Number: B9

Keywords: stem cells, scaffolds, hydrogel, spinal cord injury, extracellular matrix

Wound healing impairment is one of the diabetic complications associated with neuropathy, immune system dysfunction and ischaemia. Mesenchymal stromal cells (MSCs) might provide a supportive environment for angiogenesis in wound healing in diabetic patients. We compared the process of full-thickness wound healing in diabetic rats treated with human MSCs isolated from bone marrow (BM-MSCs), adipose tissue (ASCs), or bone marrow mononuclear cells (BMC) obtained from diabetic (DM) and healthy donors (nonDM).

BMC were isolated using Gelofusine separation, BM-MSCs and ASCs were isolated and cultured according to standard protocols. Diabetes was induced in 56 Wistar rats by an intraperitoneal injection of streptozocin 64 mg/kg and confirmed 48 h later by a glucose level higher than 15 mmol/l. Two full-thickness skin wounds, 0.8 cm in diameter, were created by a punch biopsy at the back of each animal. The cell suspension (0.2 mil. hBMC, 2 mil. hBM-MSCs and hASCs) was injected into the base of the wound, and its close surroundings. The speed of wound closure and neovascularization performed by Vessel Index (VI) counted as the ratio of CD31+ cell number to wound area was assessed after 3, 7 and 14 days.

A significant acceleration of wound healing was seen in cell treated groups in comparison with controls after 7 and 14 days (BMC p<0.001; BM-MSCs p<0.01; ASCs p<0.05). No difference between diabetic cell- and nondiabetic cell-treated groups was observed. VI was significantly higher in cell-treated groups in comparison to controls (p<0.05). Lower VI was found after treatment with diabetic BMC in comparison to the nondiabetic BMC and nondiabetic BM-MSCs, (p<0.05).

Both diabetic and nondiabetic cells improved healing of full-thickness wounds in diabetic rats. BMC, BM-MSCs and ASCs represent a safe and efficient method for allogeneic as well as autologous wound treatment in diabetic patients.
Improved (Bio)mechanical Performance of Tubular Collagen Type 1 Sponges

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Poster Number: B10

Keywords: tubular scaffolds, biomechanics, urinary tract, collagen

Stricture formation up to occlusion is a main problem in reconstruction of tubular organs. Thus, the ideal tubular template should have adequate (bio)mechanical properties matching the native tissue to avoid compliance mismatch and be able to function as a stent during regeneration. In this study we created and mechanically tested a hybrid tubular template composed of a tubular collagen sponge reinforced with a coupled helical coil (CHCs) -a promising tool inspired by nature, giving sufficient strength and functionality to a structurally weak biomaterial.

CHCs were prepared by winding a resorbable surgical suture filament in a clockwise- and anticlockwise helix, resulting in tubular structures with highly organized crosswise pattern. Fiber crossover points were fused together in a “joint-like” structure by heating. To create a biohybrid, CHCs were embedded in 0.5%(w/v) type I collagen in tubular molds and subsequently frozen, lyophilized and cross-linked. To evaluate the (bio)mechanical performance of created constructs, compression test, 3point bending and tensile ring tests were performed. Ring specimen of native ureteral tissue and collagen alone were used as reference.

Fabrication of the biohybrid resulted in a seamless tubular structure. Wetted CHC constructs maintained an open lumen and showed increased resistance against compressive loads, whereas the collagen-only templates collapsed. Flexibility of CHCs reinforced scaffolds decreases with increasing CHCs thickness. SEM revealed a porous structure with CHCs incorporated within the scaffold. The hybrid scaffolds and native tissues showed a typical “J” shaped stress/strain curve beginning with an initial high elasticity up to an uprising and significantly stiffer region. The (bio)mechanical behavior of tubular templates prepared from collagen alone significantly differed in both initial and upswing modulus.

In conclusion, the reinforcement with CHCs, a structure similar to the arrangement to the collagen fibers within the submucosa of various tubular organs, prevented their collapse. CHCs can be produced from different (biodegradable) materials and different fiber arrangements can tune the mechanical properties.
An in vitro co-culture model of the retinal vasculature for use in the development of regenerative medicine strategies for diabetic retinopathy

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Poster Number: B11

Keywords: biomaterials for regenerating retinal microvasculature

Diabetic retinopathy (DR) results in vision loss and is characterised in the early stages by the breakdown of the blood retinal barrier and loss of capillary viability. Endothelial progenitor cells (EPCs) play an important role in tissue regeneration by promoting blood vessel repair. The aim was to develop a co-culture model using primary human retinal cells to allow us to explore the response to stresses such as high glucose and low oxygen, mimicking the environment of the retinal microvasculature during DR. Greater understanding of the signalling pathways and cellular changes occurring at early stage DR, will promote the development of regenerative medicine strategies for earlier intervention to prevent vision loss.

Human retinal pericytes (hRP) and human retinal microvascular endothelial cells (hRECs) were characterised by immunocytochemistry (ICC). Cells were cultured in various concentrations of serum and glucose over time for optimisation of the model. Cells were grown on either side of PET transwell inserts to mimic a healthy retinal microvasculature. Cell morphology and metabolic activity was monitored over time.

ICC of hRP and hRECs confirmed the maintenance of their pericyte and endothelial phenotypes respectively, in vitro. There was no difference to hRP cell number and metabolic activity when cultured in 5% or 20% serum in a culture dish, however 10% serum was required for attachment of the hRP on the underside of the transwell insert. More than 10 days of high glucose conditions was required to affect hRP cell number and metabolic activity. hRECs can survive in the hRP specific medium for up to 4 days, suggesting they can be grown successfully in non-optimal conditions although the metabolic activity is reduced.

The next step is to grow the hRP and hRECs together on either side of the transwells and confirm their presence and phenotype using ICC.
Cell microfactories: Manufacturing cell-based therapeutics

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Poster Number: B12

Keywords: Cell Manufacturing, Re-Distributed Manufacturing, Cell Therapy Cost of Goods Modelling

Cell-based therapies may offer solutions for many of the world's health problems. Early Biotech-led approaches are supporting novel cell and tissue-based therapeutics (CATBTs) through biomedical trials. However, potential benefits are currently curtailed by high cost of goods (COGs) linked to high cell dose requirements which pose availability and manufacturing challenges. Although efficiency can be improved on the delivery side, successful commercialisation of autologous products has remained challenging. To this end, we have developed a cost model examining business strategies for CATBTs with a particular focus on redistributed manufacturing (RDM) to identify those which present the highest probability of success for future business.

A detailed cost model was developed using guidance of several key stakeholders in the regenerative medicine field. Input variation caused by biological variability were provided through culture of multiple cell lines known to display a spectrum of growth kinetics. Variation between manual and automated culture conditions were examined using the Compact SELECT automated culture platform. Accurate values for all direct and indirect costs as well as business strategies were supplied through research, quotes or personal communications.

The model created here is based on real cell culture data from both manual operators and automated culture platforms across a range of donor cell lines known to have variable growth kinetics. This provides a robust base on which to extrapolate theoretical cell expansion potentials in various manufacturing scenarios. This has been further augmented by a range of data on current business practices for not only manufacturing, but delivery, and adoption of therapeutic products. Furthermore, detailed costs and usage patterns for GMP-grade equipment and consumables have been obtained. This has allowed the theoretical costs for various manufacturing scenarios to be calculated over whole production runs.

A range of different business models were examined for their potential to provide cost-effective and efficacious manufacturing of CATBTs. We present a sample of potential data outputs from the model. The overall goal of this model is to provide a tool with which stakeholders in the industry can identify winning business strategies at an early stage, reducing the risk of building large facilities with high sunk capital costs for poor business ventures.
Bone healing and regeneration in an ex vivo bioreactor – evaluation of angiogenesis and reparation for clinical application

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Poster Number: B13

Keywords: Bone Tissue Engineering, Ex Vivo, Clinical Translation

BACKGROUND

Bone fracture treatment often requires the use of bone filling materials to aid tissue regeneration. Many materials have been developed in the search for the ideal bone inductive material which also mimic the properties of natural bone. However, these materials require extensive testing, using animal models prior to clinical application.

RESULTS

As an alternative, we have collected waste human bone tissue after hip operations and cultured the bone using the membrane from a developing chick embryo rich in blood vessels and with no nerve supply. We show that the bone tissue is nourished by the chick blood supply, maintained the viability of the human tissue and resulted in the formation of progenitor cells. In addition, we used this model to test two different materials containing growth factors and implemented X-ray approaches to show significant changes in bone formation between treatments.

CONCLUSIONS

In conclusion, we have shown that using the chick embryo membrane as a bioreactor for the human bone provides a less invasive and a more clinically relevant approach to study bone regeneration. This model offers a step towards a less sentient animal model, able to reproduce the clinical response as well as providing a screening platform for novel materials for the clinic.

ACKNOWLEDGMENTS

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Biopreservation Requirements for Cell Therapy Commercialisation: A T-cell therapy case study

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Poster Number: B14

Keywords: Biopreservation, Distribution, T-cell therapies, Business Models, Commercialisation

One of the biggest challenges for the commercialisation of cellular therapies/regenerative medicine products is securing the therapeutic potential of the cells. This requires cellular preservation to be managed throughout the manufacturing and distribution process in order to ensure a quality and efficacious product is delivered to the patient.

The project aims to provide effective modes of production and supply for cellular therapies by improving the reproducibility and reducing the constraints on the preservation steps and the successful integration with a theoretical model of production for industry-friendly practice, whilst remaining compliant with the evolving regulatory landscape.

Since the turn of the millennium, clinical interest in immunotherapies has risen, with an 8-fold increase in the number of trials using T-cells in the last decade. With more than 10 categories of T cells for at least nine clinical indications, a number of manufacturing challenges must be addressed in order to facilitate the introduction of these therapies to market.

Using T-cell therapies as a case study, the manufacturing processes were mapped, as unit operations. The units that require biopreservation were identified and then divided into three categories, namely: i) cryopreserved cell banks, ii) inter-process pooling, iii) cell therapy distribution. With both autologous and allogeneic therapies currently being investigated, encompassing these areas into flexible business models would highlight critical aspects to consider and consequently, control.

During commercialisation, the storage, preservation and delivery requirements of T-cell therapies can be managed by focusing on i) the scale at which the processes is run, ii) the distribution logistics and iii) the final processing requirements at the clinical site. By examining the steps as individual components, then as a whole process, the suitability of process automation, scalability and the successful integration into manufacturing facilities and the supporting distribution network can be determined.
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For further information, please visit: www.nc3rs.org.uk
The EPSRC/MRC Loughborough-led CDT in Regenerative Medicine brings together Midlands universities to equip cohorts of post-graduate researchers with the tools and skills needed to translate the potential of cell or tissue based therapies from bench to bedside. Loughborough University, The University of Nottingham and Keele University provide environments to gain new technical skills and also learn about the research environment in three very different institutions.

We believe that building a strong cohort of students so they develop ‘links for life’ is important and we start this by holding a team building weekend at the start of every year which brings together new and existing students as well as some of our staff. We also provide opportunities for students to build up transferrable skills essential for todays highly competitive and increasingly international field. Dedicated young-researcher led public engagement is carried out via the HEART team which runs an online blog and visits schools as well as attending big science fairs.

Young researchers from the Loughborough CDT also established the Future Investigators of Regenerative Medicine (FIRM) Symposium Network and have run multiple symposia for young researchers, creating their own scientific programmes, inviting international speakers and seeking sponsorship to fund the event.
The EPSRC Centre for Doctoral Training in Tissue Engineering and Regenerative Medicine - Innovation in Medical and Biological Engineering provides postgraduate research and training to research, develop and deliver regenerative therapies and devices, which can repair or replace diseased tissues and restore normal tissue function.

By using novel scaffolds in conjunction with the patient's own (autologous) cells, effective acellular regenerative therapies for tissue repair can be developed at a lower cost, reduced time and reduced risk, compared to alternative and more complex cell therapy approaches.

Regenerative medicine has been identified by the government as one of “8 great technologies” vital to driving UK economic growth and our centre confirms Leeds' place as a leader in the discipline. Research projects already underway include creating biological scaffolds to repair tissues by regenerating a patient's own cells and developing practical stem cell-based therapies for musculoskeletal, cardiovascular and neural problems.

Our expertise is in the area of musculoskeletal and cardiovascular systems with our research focusing on three main areas - Joint Replacement, Tissue Re-engineering and Functional Spinal Interventions.

Multidisciplinary research projects are available in a number of faculties and departments across the university including in the School of Mechanical Engineering, School of Biomedical Sciences, Medicine, Dentistry, Design and Chemistry.

The Centre for Doctoral Training in Tissue Engineering and Regenerative Medicine - Innovation in Medical and Biological Engineering comprises of a blended programme of specialised subject modules, along with professional development and skills training, integrated with research over two years. These will form an integrated MSc in Tissue Engineering and Regenerative Medicine.
Our Vision: Translating pioneering developmental and stem cell science for patient benefit

The Centre for Human Development, Stem Cells and Regeneration (CHDSCR) was founded in 2004 as a cross-disciplinary research and translational programme within the Faculty of Medicine at the University of Southampton.

The Centre undertakes fundamental research into early development and stem cells together with applied translational research targeting the NHS and patient benefit.

The Centre has vibrant and thriving interdisciplinary research programmes that harness the translational strength of the University together with an innovative Stem Cell PhD programme, outstanding clinical infrastructure and enterprise to deliver on this vision.

The Centre comprises a grouping of scientists and clinician scientists with a shared interest in Stem Cells, Development and Regenerative Medicine within excellent core space in the Institute of Developmental Sciences and units across Southampton General Hospital and Institute for Life Sciences.

Our Mission: Our aim is to understand early human development and fundamental stem cell biology to inform and aid our translation and development of cell therapies for regenerative medicine.
The University of Nottingham has formally recognised “Regenerative Medicine and Stem Cells” as one of the core Research Priority Areas at the University of Nottingham 2020 strategy. There has been considerable investment in people of spread across numerous departments.

The EPSRC/MRC CDT in Regenerative Medicine partnered with Loughborough University, is led by Lee Buttery at The University of Nottingham. With links to the Drug Delivery and Cancer and Stem Cells groups as well as the Division of Regenerative Medicine and Cellular Therapies led by Felicity Rose, this environment provides a diverse array of research themes related to Regenerative Medicine.

In addition to a strong research focussed environment, Nottingham has a number of public engagement programmes which researchers have participated in. These include the Catalyst working with schools programmes, May Fest, Big Bang Fair as well as contributing to the CDT outreach programme. The Division of Regenerative Medicine and Cellular Therapies also recently had a stand at the Royal Society Festival called Biology Builders.
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Best thing about FIRM:

Worst thing about FIRM:
<table>
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<th>Presentation Title</th>
<th>Best Poster Presentation</th>
<th>Poster Title</th>
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future investigators of regenerative medicine
Ask any questions you would like to get the panel discussion started on Thursday.