P-74: Comparison of 3D Micro-Printed Polycaprolactone Conduits and Fibrin Conduits for Peripheral Nerve Repair

Mallappa K Kolar[1, 2], Christopher J Pateman[3], Lev N Novikov[1], Mikael Wiberg[1,2], Frederik Claeyssens[3], John W Haycock[3] & Paul J Kingham[1]

[1]Department of Integrative Medical Biology and [2]Department of Surgical and Perioperative Sciences, Umeå University, Umeå, Sweden, [3]Department of Material Science and Engineering, University of Sheffield, UK

mallappa.kolar@umu.se / mncjp@leeds.ac.uk

INTRODUCTION: Peripheral nerve injuries affect 3% of trauma patients[1]. In cases of gap injuries, an autologous nerve graft is the gold standard technique used to repair the defect. Artificial nerve conduits constructed from either natural or synthetic materials have been developed but their ability to promote regeneration is inferior to nerve grafts[2]. Advanced fabrication techniques might enable the production of more precisely designed conduits with suitable chemical properties to better support axon growth and Schwann cell biocompatibility. In this study we have compared a new 3D-structured polycaprolactone (PCL) conduit with simple tubular constructs made from fibrin glue.

METHODS: Caprolactone pre-polymer was microwave synthesised, methcrylate functionalised and then UV cured into 3D structures via stereolithography (PCL conduit)[3]. Tubular fibrin conduits were moulded from two-compound fibrin glue (Tisseel™ Duo Quick; Baxter)[4]. The constructs were used to repair a 10mm rat sciatic nerve gap. Regeneration was compared with autologous reverse nerve grafts at 3 weeks.

RESULTS: *In vitro* testing demonstrated cellular adhesions and neurite outgrowth on the caprolactone material. *In vivo*, the 3D structured PCL conduits supported a 6-fold higher level of axon regeneration into the distal stump, compared with the fibrin conduit. Numerous regenerating axons were adherent to the wall of the PCL conduit, often in close association to the infiltrating Schwann cells (Figure 1). The Schwann cell ingrowth was more extensive in the PCL conduit compared with the fibrin conduit. Both conduits supported early vascularisation and RECA-1 positive endothelial cells also attached to the walls of the PCL conduit.

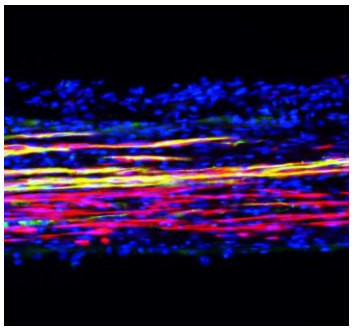


Figure 1. Beta-III-tubulin immunostaining for regenerating axons (green) and S100 staining for Schwann cells (red) on the wall of the PCL conduit. Nuclei are shown by DAPI staining (blue).

DISCUSSION & CONCLUSIONS: In summary, the 3D micro-printed PCL conduit demonstrated the usefulness of photocurable, degradable polymers as a tool to manufacture a new generation of conduits in peripheral nerve repair.

REFERENCES:

1. Pfister BJ *et al*. (2011) **Critical reviews in biomedical engineering.** 39:81-124.
2. Bell JHA, Haycock JW (2012). **Tissue engineering.** 18(2): 116-128.
3. Koroleva A *et al*. (2012) **Biofabrication.** 23; 4(2):025005.
4. Kingham PJ *et al*. (2014) **Stem cells and Development.** 1;23(7):741-54

ACKNOWLEDGMENTS: This study was supported by the Swedish Research Council, European Union, Umeå University, the County of Vasterbotten, the NIHR i4i and the EPSRC, UK.

P:77: Cross-flow Membrane Emulsification for Tunable-sized Monodisperse Microparticles

Sanchita Sharmin Chowdhury[1*] Abdulrahman Baki, Omar Qutachi, Felicity RAJ Rose, Kevin M Shakesheff and Cheryl V Rahman

Wolfson Centre for Stem Cells, Tissue Engineering, and Modelling (STEM), Centre for Biomolecular Sciences, School of Pharmacy, University of Nottingham, Nottingham, UK
paxsc@nottingham.ac.uk

INTRODUCTION:

In the field of tissue engineering (TE) and regenerative medicine, polymeric microparticles play distinct role, according to size[1]. Microparticles of 50µm and above sizes have been used as building blocks to construct injectable scaffold *in vitro*[2] or as cell carrier systems to form scaffold *in situ* [3]. To enhance efficiency of the scaffold in terms of repeatable initial cell numbers per scaffold and eventually to have better control over the interpretation of experimental data, monodispersity in the microparticle size distribution is crucial. Cross-flow membrane (CFM) emulsification is a robust technique that has been used to fabricate monodisperse microparticles but mostly of mean size 36µm or below[4]. This study therefore aimed at preparing monodisperse microparticles of larger sizes that can be used as scaffold building blocks in TE applications using CFM emulsification technique.

METHODS:

Polycaprolactone (PCL) solution in dichlomethane was passed through the membrane under N_2 pressure into PVA solutions flowing across the membrane and emulsion droplets were generated into the PVA solution (Fig.1). Subsequent solvent evaporation yielded solid PCL microparticles.

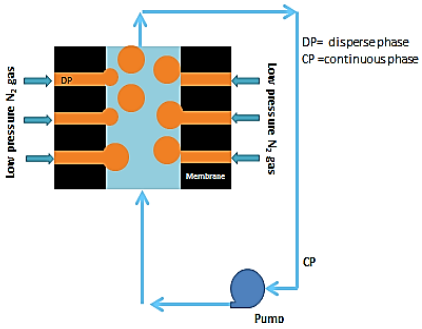


Fig.1: Schematic illustrates CFM emulsification process.

RESULTS:

PCL microparticles of uniform shape and narrower size distribution were obtained by the technique in comparison to that of microparticles prepared by conventional high speed homogenization method (Fig.2).

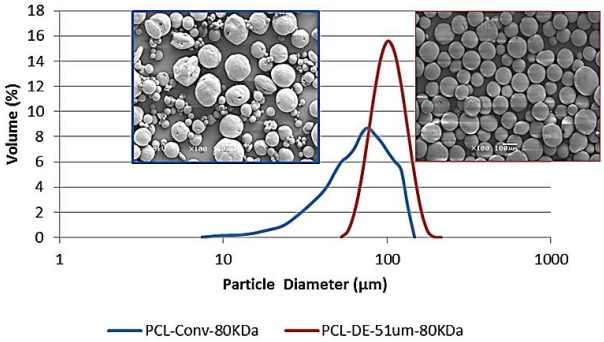


Fig.2: Particle size distribution of PCL microparticles prepared by conventional (blue curve and blue-bordered SEM image) and cross-flow membrane emulsification **(red curve and red-bordered SEM image).**

DISCUSSION & CONCLUSIONS:

In this study, we demonstrate the potential of cross-flow membrane emulsification, to prepare larger sizes monodisperse microparticles. The sizes were mainly controlled by selecting appropriate membrane pore size, working pressure and PVA concentration.

REFERENCES:

1. M. B. Oliveira *et at* (2011), Biotechnol prog **27**: 897-912
2. J. M. Curran *et al* (2013), *Biomaterials* **34**: 9352-9364
3. E. Bible *et al* (2009), *Biomaterials* **30**:2985-2994
4. F. Spyropoulos *et al* (2014), *J Sci Food Agric* **94**: 613–627

ACKNOWLEDGMENTS:

The authors would like to thank Islamic Development Bank for funding to this project.

P-78: Human osteoblasts within soft peptide hydrogels promote mineralisation *in vitro*

Luis A Castillo,[1] Julie E Gough[2], Alberto Saiani[2], Aline F Miller[1]
luis.castillodiaz@postgrad.manchester.ac.uk
[1]Manchester Institute of Biotechnology, and [2]The School of Materials, Manchester, UK

INTRODUCTION:

Biomaterials with cell culture and tissue engineering applications are a promising alternative to treat degenerative diseases such as periodontitis[1]. The octa-peptide FEFEFKFK, where F is phenylalanine, E glutamic acid and K lysine, spontaneously self-assemble into □-sheet rich fibres that self associate to form a rigid hydrogel when above a critical concentration[2,3]. Here the mechanical properties and the ability of the gel to support the 3D culture of human osteoblasts (HOBs), the production of bone proteins and bone mineralisation are explored.

METHODS:

3% wt FEFEFKFK hydrogels were used to culture HOBs. The elastic (*G'*) and viscous (*G''*) moduli of gels were recorded as function strain (0.01-100%) at 1 Hz and oscillatory frequency (0.1-100 Hz) at 1% strain using an ARG2 rheometer. HOBs were grown under standard cell culture conditions. Live/dead and Picogreen assays were carried out following manufacture's instructions. Detection of collagen I (Col I), was carried out using ICC staining. Quantification of bone proteins as well as the presence of calcium deposits were determined using colorimetric and Alizarin red assays respectively.

RESULTS:

HOBs within gels, presented good viability (Figure 1A), and proliferation (Figure 1B), increases and remain steady over 14.

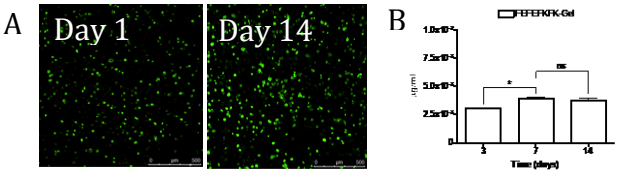


Fig. 1A. HOBs viability. 1B. HOBs DNA content.

FEFEFKFK G' increased in the presence of cells probably due to deposition of extracellular matrix (Figure 2).

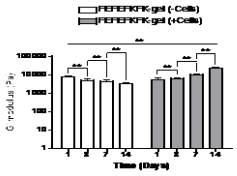


Fig. *2. FEFEFKFK mechanical properties with and without cells as control.*

HOBs produced Col I, osteocalcin (OCN) and alkaline phosphatase (ALP) inside gels (Figure 3), over 14 days of culture (Figure 4 A, B & C).

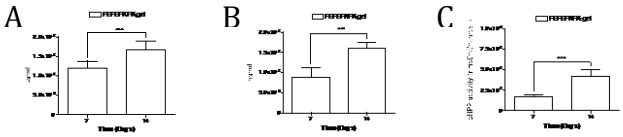


Fig. 3 A, B and C. production of Col I, OCN and ALP within gels respectively.

Calcium deposits were detected inside gels up to the 14 days explored here (Figure 5).

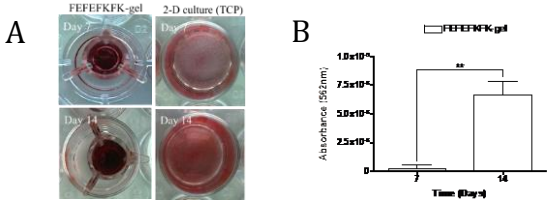


Fig. 4 A and B. Presence and absorbance values obtained from calcium ions deposited within gels and TCP respectively.

DISCUSSION & CONCLUSIONS:

The FEFEFKFK gel supports the viability and proliferation of HOBs for 14 days. During this time HOBs express osteogenic proteins, and the gel mechanical properties increases. Thus, the FEFEFKFK system has potential to be used as 3D scaffold to culture HOBs with regenerative applications.

REFERENCES:

1. Feng X. *et al*., ARPMD (2011), 6:121-45.
2. Saiani A. *et al*., SM (2009), 5:193-202.
3. Mujeeb, A. *et al*., AB (2012), 9:4609-4617.

ACKNOWLEDGMENTS:

To CONACYT-Mexico for financial support.

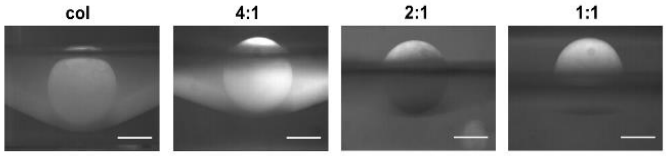
P-79: Mechanical and Viscoelastic Properties of Collagen-Elastin Hydrogels for Lung Tissue Engineering

Jessica Bratt[1], Siobhán Dunphy[1], Nicholas Forsyth[1] & Alicia El Haj[1]
[1]Institue for Science and Technology in Medicine, Keele University, UK
j.bratt@keele.ac.uk

INTRODUCTION: The aim of this study was to produce a material capable of mimicking the characteristics of the smallest functional component of the lung, the alveoli. The main constituents of lung ECM are collagen and elastin, which play a key role in the biomechanical behavior of healthy lung tissue[1-2]. For this reason they are of particular interest in lung tissue engineering. The average Young's modulus of a single alveolar wall was calculated to be ~5kPa[3]. In this study collagen-elastin hydrogel constructs were examined for their mechanical properties. The incorporation of human lung fibroblasts was also observed.

METHODS: Hydrogel constructs were made using type I rat-tail collagen (BD Bioscience), soluble bovine elastin (Sigma Aldrich) and primary human lung fibroblasts using a previously described protocol[4]. Collagen only constructs and hybrid constructs with varying collagen to elastin ratios (4:1, 2:1 & 1:1) were produced. All gels contained a constant collagen concentration of 3.5mg/ml. The hydrogels with collagen only and with a collagen to elastin ratio of 1:1 were seeded with a cell density of either $2.5x10^3$ or $2.5x10^5$ cells per construct prior to gelation. The constructs were incubated for 8 days at 37ºC, 5% CO_2.
The hydrogels were tested for their mechanical and viscoelastic properties using a non-destructive spherical indentation technique previously described[4]. The thickness of the gels was measured using optical coherence tomography (OCT) system[5].

RESULTS: The addition of elastin increased the stiffness of the hydrogel construct (Figure 1). A correlation was found to exist between the elastin concentration and the elastic modulus for the constructs (Figure 2). Live/Dead imaging showed the addition of elastin had no adverse effect on cell viability.



Similar cell morphology was shown for all cellular constructs.

Figure 1: Central deformation of collagen constructs with different concentrations of elastin. Scale bar=2mm.

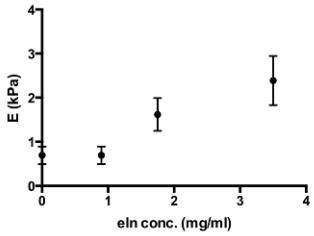


Figure 2: Mean Young's modulus ± standard error at different elastin concentrations.

DISCUSSION & CONCLUSIONS: The addition of elastin into a collagen hydrogel enhanced the mechanical properties of the construct. Increasing the elastin concentration resulted in a corresponding increase in Young's modulus. Cell-seeded constructs achieved a Young's modulus equivalent to the theoretical value for a single alveolar wall. Collagen-elastin hydrogels may have potential for use as a scaffold for lung tissue engineering applications.

REFERENCES: [1] H. Yuan et al. (1997) *J Appl Physiol.* 83:1420-31. [2] H. Yuan et al. (2000) *J Appl Physiol.* 89:3-14. [3] F.S.A. Cavalcante et al. (2005) *J Appl Physiol.* 98(2):672–9. [4] M. AHearne et al. (2005) *J R Soc Interface.* 2:455-63. [5] Y. Yang et al. (2006) Phys Med Biol. 51:1649-59.

ACKNOWLEDGMENTS.
The authors would like to thank the Engineering and Physical Sciences Research Council (EPSRC) Doctoral Training Centre for Regenerative Medicine (grant number EP/F500491/1) for funding this work.

P-80: Tissue engineering a physiologically and spatially relevant 3D *in vitro* model of colorectal cancer

Tarig Magdeldin[1, 2], Marilena Loizidou[1], Umber Cheema[2]
[1] UCL Cancer Nanotechnology Group, Division of Surgery and Interventional Science, Royal Free Campus, London, NW3 2QG, U.K.
[2] UCL Tissue Repair and Engineering Centre, Institute of Orthopaedics and Musculoskeletal Sciences, Division of Surgery and Interventional Science, Stanmore Campus HA7 4LP, U.K.
Tarig.magdeldin.11@ucl.ac.uk

INTRODUCTION: Collagen type I hydrogels are commonly used for cell culture as they provide a 3D environment in which to study cell behaviour. However, hydrogels do not accurately model tissue matrix density *in vivo*. Increasing collagen density alters scaffold properties such as matrix stiffness, a crucial parameter governing cell behaviour and can help recreate normal tissue barrier function *in vitro*. With this in mind, we have developed a spatially accurate 3D *in vitro* model of colorectal cancer known as a 'tumouroid' that recapitulates the dense architecture of tumours and the surrounding stroma.

METHODS: We created two ACMs (artificial cancer mass) of different matrix densities based on the removal of interstitial fluid in collagen type I hydrogels (plastic compression)[1]. These ACMs contained colorectal cancer cells (HT29 or HCT116), and were placed into a surrounding collagen hydrogel to construct tumouroids. Growth, morphology and invasion into the acellular compartment were assessed over 21 days. EGFR (epidermal growth factor receptor) expression was assessed by qRT-PCR and tumouroid response was investigated after treatment with the anti-EGFR monoclonal antibody cetuximab.

RESULTS: ACM matrix density was 2.63% ± 0.16% and 9.59% ± 0.64% for either partially compressed or fully compressed collagen hydrogels respectively (% w/v). Cells were cultured for 14 days and formed 3D cellular aggregates. EGFR expression levels revealed a 2-fold and 3-fold increase in 3D cultures for both HT29 and HCT116 cells in comparison to 2D monolayers respectively ($p<0.05$; $p<0.01$). Cetuximab efficacy was significantly lower in HT29 3D cultures in comparison to 2D monolayers whereas HCT116 cells in both 2D and 3D were non-responsive to treatment in agreement with their *KRAS* mutant status.

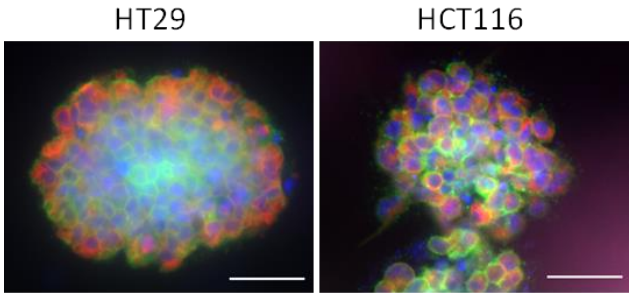


Fig 1: Immunofluorescent analysis of cytoskeletal proteins on colorectal cancer cultures. HT29 and HCT116 cells in 3D cultures were maintained for 14 days, fixed and stained for tubulin (red) and F-actin (green – phalloidin). Nuclei were stained with DAPI (blue). Scale bar – 50µm.

DISCUSSION & CONCLUSIONS: Cells behave similarly to the *in vivo* scenario, forming tumour-like cellular aggregates with visible cell-cell junctions *in vitro*. We established that cetuximab efficacy is significantly lower in 3D cultures in comparison to 2D monolayers, independent of EGFR expression levels. This signifies the increasingly important role of matrix density and cellular architecture on drug uptake and distribution.

REFERENCES:

[1] Brown, R.A. et al. 2005. *Adv. Funct. Mat.* 15: 1762-1770.

ACKNOWLEDGMENTS:

Umber Cheema is a David Philips BBSRC fellow and Tarig Magdeldin is funded by the UCL Impact studentship and TAP Biosystems.

P-81: Investigating Self-renewal of Mesenchymal Stem Cells on Nanotopography

Louisa CY Lee[1], Nikolaj Gadegaard[2], Stephen Yarwood[3],
Richard OC Oreffo[4], RM Dominic Meek[5], Matthew J Dalby[1]
[1]Centre for Cell Engineering, University of Glasgow, UK; [2]School of Engineering, University of Glasgow, UK; [3]Institute of Molecular, Cell, and Systems Biology, University of Glasgow, UK; [4]Bone and Joint Research Group, University of Southampton, UK; [5]Southern General Hospital, Glasgow, UK.
l.lee.1@research.gla.ac.uk

INTRODUCTION

Adult mesenchymal stem cells (MSCs) can be induced to undergo differentiation[1] or maintain self-renewal[2] with culture on nanotopography. Exploiting the latter property and retaining expression of stem cell markers would allow for expansion of autologous stem cells for use in future clinical treatments. A highly ordered nanopit arrangement (SQ) has previously been shown to promote self-renewal, and this work seeks to determine optimal conditions for MSC growth and to greater understand what mechanisms drive MSCs to undergo self-renewal on this surface.

EXPERIMENTAL METHODS

MSCs were isolated from human bone marrow aspirate using magnetic activated cell sorting and antibodies against surface markers (STRO-1/CD271). These were seeded onto polycarbonate SQ, an osteogenic near-square arrangement of nanopits (NSQ) and planar controls. Pit dimensions were 120nm diameter, 300nm centre-centre spacing, 100nm depth, with NSQ incorporating ±50nm disorder. MSC marker expression was assessed by fluorescence immunostaining and image analysis, with cell cycle protein levels detected using in-cell western methods. Coomassie blue staining, and scanning electron microscopy (SEM) was used to investigate growth patterns and cell morphology. Statistics: paired students t-tests with p values <0.05 taken as significant.

RESULTS AND DISCUSSION

Cell seeding density was important for effective use of our SQ nanotopography. Optimisation of cell number revealed that a degree of clonal growth (fig.1A) may be important for self-renewal and that some changes in cell cycle regulation were apparent in MSCs cultured on the different surfaces. On SQ, contact guidance of filopodia was observed (fig.1B) in comparison to more random filopodial distribution on flat and NSQ.

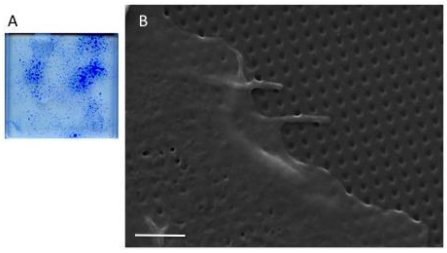


Figure 1. Optimisation of seeding density with subsequent morphology analyses (A) Coomassie stained MSCs on SQ at our optimal seeding density after 28 days culture showing colony growth (B) SEM imaging of MSCs on SQ showing contact guidance of filopodia. Scale bar: 1µm

Our observations suggest an association between adhesion-related events on SQ and intracellular biochemical changes.

CONCLUSION

We have identified an optimal seeding density for nanotopographical retention of stem cell marker expression and propose that contact guidance constraints together with changes in cell cycle regulation play a part in directing MSC self-renewal.

REFERENCES

1. Dalby *et al.* Nature Materials. 2007 6; 997-1003. 2. McMurray *et al.* Nature materials. 2011 10; 637-644.

ACKNOWLEDGMENTS

LCYL is funded by MVLS, University of Glasgow (UoG), MJD is funded by grants from BBSRC, EPSRC and MRC. We thank Julia Wells for STRO-1$^+$ MSCs and Peter Chung (UoG) for SEM imaging.

P-83: Bioprinting of vascularised liver tissues

Pritesh Mistry, Anne Canning, Kevin Shakesheff, Jing Yang[1](jing.yang@nottingham.ac.uk), [1]

1 Division of Drug Delivery and Tissue Engineering, School of Pharmacy, The University of Nottingham, UK

INTRODUCTION: Metabolically active tissues must reside within 200 μm of a capillary lumen to gain sufficient oxygen and nutrients for survival. Incorporation of a perfusing blood supply in tissue engineering design is vital for the scalability, survival and integration of almost all tissues with the exception of a few poorly vascularised tissues such as skin epidermis and cartilage. Delivery of angiogenic growth factors, genes and regenerative cell types as well as combination therapies have been employed to promote vascularisation. However, the integration of these engineered tissues with the host remains a monumental challenge. To meet this challenge, the establishment of functional vasculature in engineered tissue constructs is likely to be required before transplantation. To this end, a recent research highlight has been the use of a sacrificial lattice of carbohydrate fibres to fabricate vascular-like tubule networks.[1] Here, we demonstrate a novel method for engineering vascularised tissues using 3D printing. A construct with an embedded tubule network in a hydrogel matrix has been successfully printed at cell-compatible conditions. The tubule network was then perfused with endothelial cells to form vasculatures.

METHODS: A Fab@Home™ printer was used to print the tubule network and the hydrogels. To form tubule network, a thermo-responsive material and alginate were printed into a pre-designed structure. The printed network was then dissolved away at physiological conditions to form tubes which were later perfused with endothelial cells. In addition, human umbilical vein endothelial cells (HUVECs) were mixed in Matrigel, and printed into a network within alginate. Cells were labelled by live/dead fluorescent stains or labelled by a red cell tracker, and imaged using a fluorescent microscope.

RESULTS: HUVECs after 8-days culture were labelled with live/dead fluorescent stains (Figure 1). A tubule network facilitating the perfusion of HUVECs was printed within alginate (Figure 2).

CONCLUSIONS: The method reported here allows the fabrication of a vascular network at physiological conditions, which is an advance compared to methods in which materials are printed at elevated temperatures unsuitable for cells and biomolecules. The cell-compatible printing environment permits the patterning of the tubule networks which mimic blood vessels, biomolecules and multiple cell types in a single printing process. Future work will look to improve, measure and validate the architecture and function of the engineered vasculature.

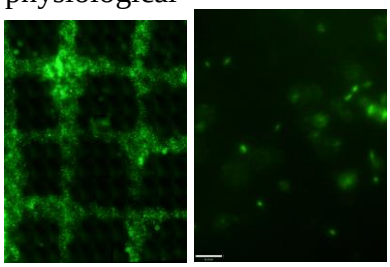


Figure 1 A stitched image of a printed network with HUVECs embedded in Matrigel (Left). An image of encapsulated HUVECs showing spreading of some cells (Right).

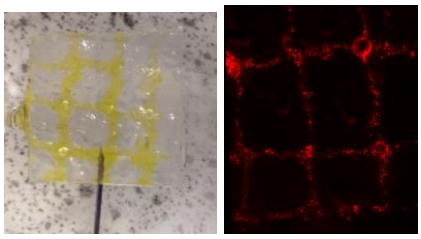


Figure 2 Images of tubule networks embedded in a hydrogel perfused with a protein solution (Left) and HUVECs (Right)

ACKNOWLEDGEMENTS: The authors would like to thank the Nottingham Research Fellowship scheme and the ERC Advanced Grant (227845) for supporting this work.

REFERENCES: [1] Miller, et al (2012) *Nat Mater* **11**:768-774.

P-84: Topographical Modification of Nanocomposite Polymer Vascular Graft Surfaces to Optimise Endothelialisation

Debra ST Chong[1] Nikolaj Gadegaard[2], George Hamilton[1], Alexander M Seifalian[1], Matthew J Dalby[3*]
[1]Division of Surgery and Interventional Science, UCL, UK
[2]Division of Biomedical Engineering, University of Glasgow, UK
[3*]Centre for Cell Engineering, University of Glasgow, UK
d.chong@ucl.ac.uk

INTRODUCTION:

The success of a vascular graft is well-known to depend on its ability to develop an endothelial layer within the luminal surface. However, current graft materials in clinical use do not support this and patency rates of these grafts low[1]. Surface topographical features are well-known to have an influence on cellular behaviour[2]. The aim of this study is to examine both the optimal plasma treatment and topographical feature size to enable endothelial cell attachment on a nanocomposite polymer for use within a vascular bypass graft setting.

METHODS:

Nanocomposite polymer, polyhedral oligomeric silsesquioxane and polycarbonate urea urethane (POSS-PCU) were embossed with both microgrooves (MG) of pitch 25µm and nanopits (NSQ), 120nm pits with centre-centre spacing of 300nm with ±50nm offset. These were then treated with different oxygen plasma treatments (40W, 60W and 80W for 60 seconds). The replication fidelity of the patterns was confirmed using AFM and SEM and water contact angle was used to measure the success of the plasma treatment. Human umbilical vein endothelial cells (HUVECs) were seeded onto POSS-PCU and Live/Dead staining were used to measure growth. Immunostaining was used to visualise the focal adhesions.

RESULTS:

AFM and SEM both confirmed the high replication fidelity of both the micro and nanofeatures on POSS-PCU. Water contact angle showed that hydrophilicity of the polymer can be fine-tuned by increasing the power of the O_2 plasma treatments ($p < 0.05$). HUVEC proliferation was seen to be optimal when seeded on a plasma treated surface with an average of 68° being achieved. There is also further preference of NSQ over MG features and planar surfaces after plasma treatment. Endothelial cell morphology and function was seen to be retained, especially on the plasma treated NSQ surfaces. There were also increased focal adhesions seen on these surfaces.

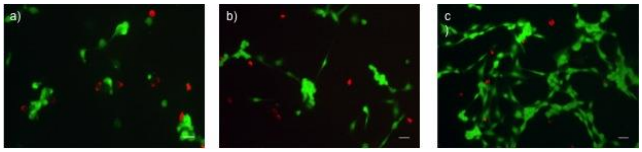


Fig 1. Live/Dead staining of HUVECs cells on a) planar b) micropattern and c) NSQ surfaces respectively*. Green indicates live cells and red dead cells (scale bar - 20*µm).

DISCUSSION & CONCLUSIONS:

This work shows the preference of HUVECs towards topographical features in the nanoscale over micro and planar surfaces, especially after plasma treatment. These are important observations as luminal surfaces of vascular grafts can be tailored-made using a combination of plasma treatment and topographical features to allow for self-endothelialisation potential.

REFERENCES:

1. DS Chong *et al* (2014), *EJVES* **In press**
2. MJ Dalby *et al* (2007), Nature Materials 6;997-1003

ACKNOWLEDGMENTS:

The authors would like to acknowledge the Wellcome Trust, Royal Free Charity and the European Society for Vascular Surgery for research grants.

P-85: A comparative genome wide transcriptional analysis of human bone marrow, synovial and periosteal mesenchymal stem cells

Shahida Shahana[1], Anke Roelofs[1] and Cosimo De Bari[1]
1Arthritis Research UK Tissue Engineering Centre, UK
Regenerative Medicine Group, Musculoskeletal Research Programme, Institute of Medical Sciences, University of Aberdeen, UK
sshahana@abdn.ac.uk

INTRODUCTION:

Osteoarthritis (OA) is a progressive degenerative disorder affecting several compartments of the joints, including articular cartilage. Current available treatments are mainly symptomatic. Recently, cell therapy with chondrocytes or mesenchymal stem cells to repair focal lesions of the joint surface has been intensely pursued[1-6]. However, there are only a few clinical trials with cell therapy in OA[4-6]. Although these studies demonstrated promising outcome in terms of patient well-being and cartilage repair, patient to patient variability to heal damaged cartilage hinders consistent outcome. To enhance consistency, cell therapy requires a better understanding of MSCs but little knowledge exists at the genomic level, limiting the scope of molecular studies and expression analyses of genes of interest. To overcome this limitation in this study, we hope to identify molecular signatures associated with tissue-specific MSCs.

METHODS:

We performed microarray of culture-expanded human MSCs from three different joint tissues (bone marrow=BM, human periosteum=HP, Human synovial membrane=HSM). In some cases, HSM and HP samples were obtained from the same donors. Twenty two microarrays were performed from 17 donors, using Affymetrix GeneChip Human Exon 1.0 ST arrays, to compare gene expression profiles of culture-expanded human mesenchymal stem cells (MSCs) between donors and across three different tissue sources.

Preliminary data analysis was done after the data was processed and statistics (ANOVA, posthoc, MTC $p<0.05$) performed. Differentially expressed genes were validated by q-RT-PCR.

RESULTS:

Analysis of the microarray datasets revealed striking differences in gene expression profiles between MSCs from the three tissue sources. In particular, MSCs from BM show a gene expression profile that is distinct from HSM and HP-MSCs (Fig.1). A total of 347 genes and 47 pathways [which include TGF Beta (10 matches), MAPK Signaling (9 matches), Endochondral Ossification (8 matches), EGF-EGFR Signalling Pathway (8 matches), Notch Signalling Pathway (4 matches), etc.] were differentially regulated. The q-RT-PCR study confirmed in multiple donors top most differentially regulated genes.

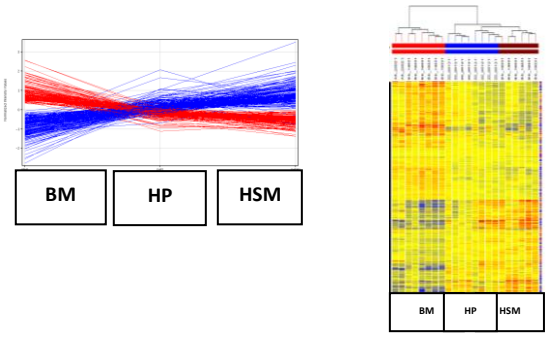


Fig.1a **Fig.1b**

Figure (1a) Profile plot *(*1b) Hierarchical clustering.

DISCUSSION & CONCLUSIONS:

We have demonstrated that HSM and BM-MSCs express distinct sets of genes. These could be a potential list of signature molecules for MSC identity in clinical applications and will provide opportunity to study them in joint diseases such as OA.

REFERENCES:

(1) Brittberg M et al., 1994 2) Brittberg M et al., 2008 3) Wakitani S et al., 2004 4) Orozco L et al. 2013 5) Sousa BR et al. 2014 6) Gopal et al., 2014.

ACKNOWLEDGMENTS: We are thankful to the ARUK Research Council for providing financial support to this project.

P-86: Elucidating Novel Roles for Dentine Matrix Components in Directing Stem Cell Biology

SJ Avery[1,2], AJ Sloan[1,2], RJ Waddington[1,2]
[1]Tissue Engineering and Reparative Dentistry, School of Dentistry, College of Biomedical and Life Sciences, [2]Cardiff Institute for Tissue Engineering and Repair, Cardiff University, UK

INTRODUCTION: Attenuated bone repair is traditionally resolved using autologous bone grafts, although with varying degrees of success. Previous studies have identified demineralised dentine matrix (DDM) to be highly bioactive, stimulating augmented bone repair in compromised situations[1,2]. The potency of DDM is attributable to a potent cocktail of growth factors acting synergistically to elicit optimum biological responses. Growth factors can also bind to matrix proteins, notably decorin and biglycan, which may aid biological activity by sequestering to the matrix, protecting from proteolysis and possibly modulating cell signalling[3,4]. Our current study aims to assess the responses of bone marrow stem cells (BMSCs) to DDM and identify factors within the matrix eliciting a bioactive role.

METHODS: Commercial BMSCs, representing a heterogeneous population, were selected further for α5β1 integrin immature cells by preferential adherence to fibronectin (FNA). FNA BMSCs were characterised for population doublings (PDs) and stem cell markers using RT-PCR and compared with unselected cells. DDM was extracted from mineralised human dentine with 10% EDTA. FNA BMSCs were cultured in DDM-conditioned media for 28 days to quantify differentiation towards osteoblast lineage via Von Kossa staining. Effect of DDM on cell expansion was assessed by MTT assay, apoptotic activity by luciferase activation of active caspase-3/7 and chemotactic properties using Boyden chambers. Growth factors and matrix proteins decorin and biglycan in DDM were assessed by Western Blotting.

RESULTS: FNA BMSCs express mesenchymal stem cell markers, including CD105, CD90 and CD73 and exhibit a greater sustained proliferative capacity compared to unselected BMSCs. Cells cultured in media supplemented with 10μg/mL DDM exhibited positive staining for mineral deposition compared to untreated (fig 1). DDM indicated a dose-dependent response in reduction of cellular expansion over 4 days and apoptotic activity after 48 hours. Cell migration is enhanced by DDM albeit attenuated with increasing concentrations. Western blot analysis identified the definitive presence of TGF-β1 in DDM.

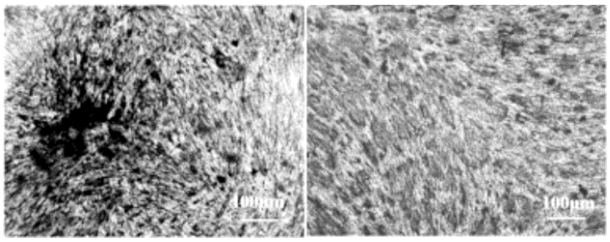


Fig 1: von Kossa stain of cells cultured in 10μg/mL DDM (left) and control (right) medium for 28 days

DISCUSSION & CONCLUSIONS: DDM stimulates BMSC differentiation towards an osteoblast phenotype and deposition of a mineralised matrix. In addition DDM appears to contain bioactive components that aid cell survival and migration. Although TGF-β1 has been identified within the DDM, it is hypothesized that a combination of components acting synergistically are responsible for the overall enhanced bioactivity of DDM.

REFERENCES:
[1]MF Gomes *et al* (2008). *Braz Oral Res* **22**(3): 275-280. [2]CR Reis-Filho, *et al* (2012). *Archives of Oral Biology* **57**: 469-476. [3]J Guiterrez *et al* (2006). *J Cell Physiol* **206**(1): 58-67. [4]AD Berendsen *et al* (2011). *Proc Natl Acad Sci USA* **108**(41): 17022-17027

ACKNOWLEDGMENTS:
We would like to acknowledge Rosetrees Trust for financial support of this project.

P-87: Arthroscopic delivery of bio-active materials: a preliminary study

Javier Munguía[1*] and Kenny Dalgarno[1]
[1]School of Mechanical & Systems Engineering. Newcastle University, UK
Javier.Munguia@ncl.ac.uk

INTRODUCTION: Current trends in tissue engineering for the restoration of chondral and ostheochondral lesions rely mostly on the in-vitro fabrication of scaffold structures, plugs and other constructs of various shapes and compositions which must be transplanted and fitted on the defect site with maximum accuracy and biocompatibility.
While a number of studies have demonstrated the capacity of 3D Printing, or Additive Manufacturing (AM) for creating biocompatible tissue repair constructs[1-3],few have considered the potential for in-situ delivery of bioactive materials. This work describes the principles of a novel arthroscope–based material delivery system for the in-situ fabrication of bioactive implants adopting some of the principles of 3D printing.

METHODS: A proof of concept system was built consisting on: a peristaltic pump system with Flow rate 0.001 to 3400 mL/min (Cole Palmer), silicone tubing (3mm internal diameter), and a single LED UV-light source. Three specimens were produced with different layer thicknesses (0.5mm, 0.75, 0.9mm). An off the shelf acrylate-based photo curable resin (wavelength 420 -480) has been used to measure the system's capacity for delivering layered materials in a controlled manner.

RESULTS: The system consistently delivered material volumes uniformly distributed across the different layers. High repeatability was achieved on cylindrical shape constructs with a regular cross section, however the UV curing mechanism exhibited its dependence on parameters such as light exposure time, distance from source and layer thickness as some uncured residues were trapped between layers for the 0.9mm thickness part.

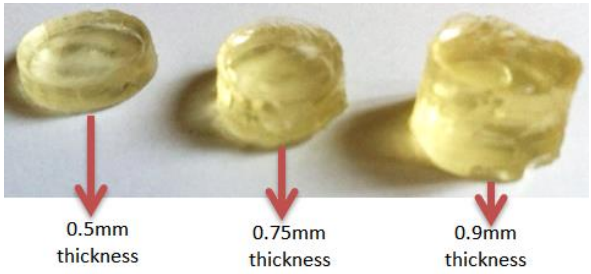


Fig. 1 Resulting UV-cured plugs with different layer thicknesses

DISCUSSION & CONCLUSIONS: The deposition mechanism showed its effectiveness delivering precise fluid material quantities over small timescales (10-12 mins) for the plugs to be made. The UV/light source cross linking activation must be tuned in order to reduce solidification time, thus accelerating the overall processing time-window. Future work includes making use of biopolymers designed specifically for the technique and further experimentation on thin extrusion profiles.

REFERENCES:

1. Cohen et. al. Biofabrication, 2010.
2. Sittinger et. al. Curr Opin Biotechnol, 2004..
3. Hockaday et. al., Biofabrication, 2012.

ACKNOWLEDGMENTS:

This work has been supported by the EPSRC Centre for Innovative Manufacturing in Medical Devices, MeDe Innovation
http://mede-innovation.ac.uk/

P-88: A Mathematical Framework for Nerve Regeneration in Implantable Conduits

O. R. Evans[1], J. B. Phillips[2], R. J. Shipley[3],
[1]CoMPLEX, University College London, UK
[2] Biomaterials & Tissue Engineering, UCL Eastman Dental Institute, University College London, UK
[3]Department of Mechanical Engineering, University College London, UK
owen.evans.13@ucl.ac.uk

INTRODUCTION:

Matching the performance of autografts with engineered scaffolds remains a challenge in peripheral nerve repair. A combination of 3D biomimetic architecture interspersed with 2D surfaces has been hypothesized to be an ideal environment for neurite regeneration[1]. However, the problem of how best to arrange material within a conduit is an open one[2]. Optimizing material parameters such as density, cross-sectional geometry and spatial distribution would require an extensive programme of experimental testing. By contrast, developing a modelling framework that is capable of testing key parameters may accelerate the design process, and reduce the dependency on animal testing.

METHODS:

A model was devised to simulate neuronal growth inside a cylindrical conduit. The trajectories of neurites are generated according to a 3D random walk process with a spatially dependent probability distribution. To mimic preferred neuronal growth on particular surfaces, a topographical bias is included. Parameters can be adjusted to mimic realistic dimensions and growth rates, as well as the rate of sprout formation at the proximal stump.

RESULTS:

The sensitivity of the hit ratio (proportion of neurites exceeding a prescribed longitudinal distance over a 2 week period) to changes in the porosity was investigated. Simulations were performed for a conduit featuring sheets of material. Fig. 1 shows the existence of an optimal porosity, representative of the competition between the total substrate available to aid growth and the obstruction of neurites at the entrance to the conduit.

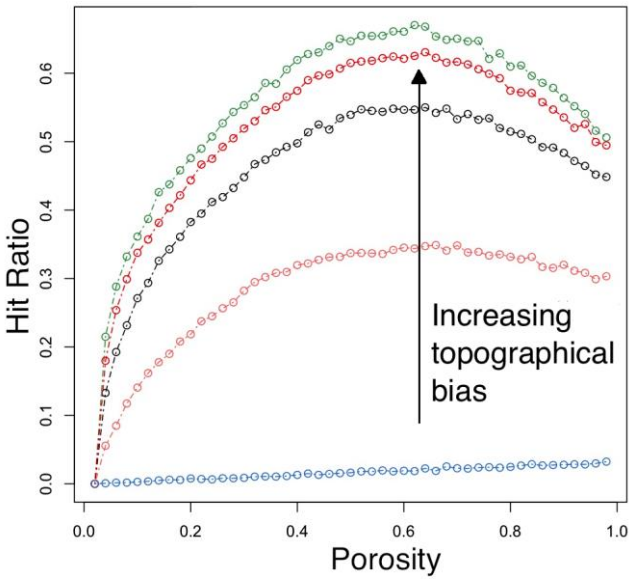


Fig. 1 Simulated hit ratios for different porosity values. Different curves correspond to varying topographical biases.

DISCUSSION & CONCLUSIONS:

We demonstrate the utility of a stochastic model for peripheral nerve regeneration and its ability to mimic experiments. The model presented predicts the existence of an optimal porosity value for one spatial arrangement of material, which could be adapted to investigate more complex designs.

More importantly, perhaps, is the promise indicated by this dual-approach, whereby wet & dry approaches can be built and updated simultaneously - experiments can verify and refine a model, and a model can help motivate experimental specifications and investigate novel biological hypotheses.

REFERENCES:

1. M. Bellamkonda (2006), *Biomaterials* **27**: 151-60.
2. M. Georgiou et al (2013), *Biomaterials* **34**: 7335-43.

P-90: Development of a stem cell therapy for repair of the degenerate IVD: Influence of pH on nucleus pulposus and mesenchymal stem cells

Hamish TJ Gilbert[1], Stephen M Richardson[1] and Judith A Hoyland[1]
[1]Centre for Tissue Injury & Repair, Institute of Inflammation & Repair, University of Manchester, UK
Hamish.Gilbert@manchester.ac.uk

INTRODUCTION:

Development of a mesenchymal stem cell (MSC) therapy for treatment of intervertebral disc (IVD) degeneration provides a challenge due to the unique microenvironment of the disc. Understanding how this hostile microenvironment may affect implanted MSCs is fundamental for the development of cell based tissue engineering/regeneration strategies for the treatment of IVD degeneration (a major cause of low back pain). During IVD degeneration nerve ingrowth has been shown to occur, which is thought to contribute to the pain associated with degeneration. Expression of pain-related neuropeptides is also increased in NP cells during disc degeneration. Low pH is one of the most detrimental microenvironmental factors, affects MSC biology[1,2], and has been linked to pain. Intradiscal pH has been shown to range from 7.1 in healthy discs to 6.2 in degenerate discs, with values as low as 5.7 recorded in severely degenerate discs[3]. To date, however, it is not known whether low pH affects expression of pain-related neuropeptides in MSCs or NP cells. The aim here was to assess the effect of a physiologically relevant low pH on cell viability, proliferation, intracellular pH and gene expression of pain-related and pH-regulating factors, in both human MSCs and IVD cells.

METHODS:

3 x human bone marrow MSC and 3 x human nucleus pulposus (NP) disc cell samples were expanded and then cultured in MEM or DMEM medium respectively, at a range of pH (pH 7.4, 7.1, 6.8, 6.5 and 6.2) for 1 and 7 days. Live/dead staining, Annexin V/PI staining, Pico Green assay, SNARF-5 staining and qRT-PCR were used to assess cell viability, apoptosis, proliferation, intracellular pH and gene expression of pH-regulating carbonic anhydrases IX/XII and pain-related factors (CGRP, Substance P and MCP-1), respectively.

RESULTS:

Intracellular pH decreased in line with external pH for both MSCs and NP cells. Both MSC and NP cell viability and proliferation were affected by changes in pH, with proliferation occurring at pH 7.4 and 7.1, a halt in proliferation at pH 6.8 and a reduction in viable cells (non-apoptosis related) at pH 6.5 and
6.2. The gene expression profile of CAIX&XII (data not shown), CGRP (Figure 1), Substance P and MCP-1 (data not shown) differed between MSCs and NP cells after 1 day of culture, with gene expression of pain-related proteins increased in MSCs but not NP cells; however MSCs altered their gene expression profile to mirror that of NP cells after 7 days of culture (Figure 1).

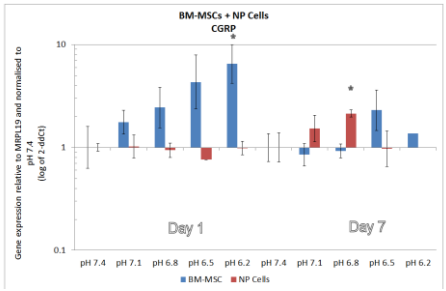


Fig. 1 Gene expression of pain-related factor calcitonin gene-related peptide (CGRP) in MSCs and NP cells cultured at different pH

DISCUSSION & CONCLUSIONS:

Acidic pH (similar to that found in degenerate IVDs) has a detrimental effect on MSC and NP cell viability and proliferation. This data suggests that MSCs may require "conditioning" prior to implantation into a degenerate disc. Low pH initially causes MSCs to increase their expression of pain-related factors, with expression levels decreasing similarly to NP cells after 7 days. This suggests that pre-conditioning of MSCs in a low pH may be necessary prior to implantation into a degenerate IVD in order to minimise a pain response.

REFERENCES:

1. K. Wuertz et al (2008), *Spine* **17**: 1843-9.
2. C. Liang et al (2012), *J Transl Med* **10**: 49
3. B. Diamant et al (1968) ,*Experientia* **12**: 1195-6

ACKNOWLEDGMENTS:

The authors would like to thank the Arthritis Research UK (Grant no: 19884) for providing financial support to this project.

P-93: Materials Processing for the Manufacture of Musculoskeletal Medical Devices at the Point of Need

Natacha Rodrigues[1], Naif Alharbi[1], Matthew Benning[1], Javier Mungia[1] and Kenneth Dalgarno[1]
[1]School of Mechanical and Systems Engineering, Newcastle University, Newcastle, UK
n.rodrigues2@ncl.ac.uk

INTRODUCTION:

Osteochondral defects result in severe pain and disability for millions of people worldwide and massive healthcare costs. There is a recognized and urgent need for developing novel treatments based in bone tissue engineering and three dimensional scaffolds design. The use of Additive Manufacturing (AM) has been growing in recent years due to its ability to directly print 3D porous scaffolds with pre-designed shape and patient customized, solvent-free, controlled pore size and interconnected porosity [1]. This paper reports the results of an initial scoping study on the development of new processes to support the in-clinic manufacture and configuration of hybrid bioactive devices for large defects which are load bearing, functionally gradient, and biologically enhanced.

METHODS:

As a starting point the aim was to develop a modular composite of a porous polylactic acid (PLA) block and wollastonite (A-W) cylinders. The overall aim is to have anatomical geometries derived from patient data. A Fused Filament Fabrication (FFF) 3D printer was selected to fabricate the PLA rectangular part with a 0º/90º laydown pattern and the AW cylinders were produced by a Z310 Plus 3D printer (Z-Corp, USA) using prepared AW and Maltodextrin powder. Afterwards the A-W green specimens were sintered at 1150ºC for 2 hours. Measurements were performed before and after sintering and all specimens were observed with a stereo microscope (Nikon SMZ1500).

RESULTS:

The specimens were successfully manufactured as presented in Fig.1.A-C and the modular composite was assembled (Fig.1.D). The PLA block presents an interconnected porous structure characterized by approximately 300 μm pore size (Fig.1.C) which was defined by the CAD model geometry and the laydown pattern. Moreover the walls are also porous and interconnected with the surrounding porous structure (Fig1.B), which is crucial for achieving an interconnected porosity throughout the hybrid device. Shrinkage of 15% and 18% for both diameter and length was observed after sintering of the AW cylinders.

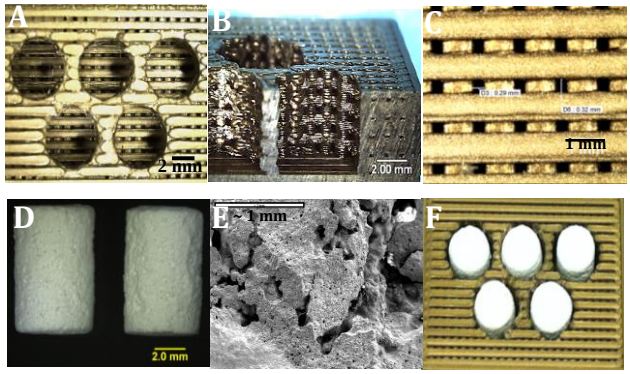


Fig.1. A.PLA block top view; B. cross sectional view; C. porous structure; D. AW cylinders after sintering; E. SEM of porous AW structure; and F. hybrid composite.

DISCUSSION & CONCLUSIONS:

The composite was successfully assembled and it is characterized by an interconnecting porous structure which is crucial for a good osteointegration. Additionally the pore size and geometry can be controlled during the design step.

REFERENCES:

1. Bose, S. et al. Materials Today, 2013. **16**(12): p. 496-504.

ACKNOWLEDGMENTS:

The authors would like to acknowledge the support of the EPSRC Centre for Innovative Manufacturing in Medical Devices MeDe Innovation; http://mede-innovation.ac.uk/).

P-94: Remotely Controlled Mechanotransduction *via* Magnetic Nanoparticles: Applications for Injectable Cell Therapies.

JR Henstock[1], M Rotherham[1], AJ El Haj[1]
[1] Institute for Science and Technology in Medicine, Keele University, UK.

INTRODUCTION: Bone requires dynamic mechanical stimulation to form and maintain functional tissue, yet mechanical stimuli are often lacking in many therapeutic approaches for bone regeneration. Magnetic nanoparticles provide a method for delivering these stimuli by directly targeting cell-surface mechanosensors and transducing forces from an external magnetic field, resulting in remotely controllable mechano-transduction. In this investigation, functionalised magnetic nanoparticles were attached to the mechanically-gated TREK-1 K^+ channels of human mesenchymal stem cells[1]. These cells were microinjected into an *ex vivo* chick foetal femur (e11) as a model for endochondral bone formation[2,3]. An oscillating 25mT magnetic field was used to induce mechanotransduction in the injected MSCs *via* the nanoparticles. Further analysis was performed *in vitro* in both monolayer and 3D hydrogel cultures[4].

METHODS: Human MSCs and TREK-1 antibody-conjugated magnetic nanoparticles were introduced into the cartilaginous epiphyses of an organotypically cultured *ex vivo* chick foetal femur using a glass capillary needle. 20nl of material was injected containing 10^3 cells per injection, whilst injecting unlabelled (nanoparticle-free) hMSCs was used as a control. The femurs were cultured organotypically for 14 days. An oscillating magnetic field was applied for 1 hour per day to remotely activate TREK-1 signalling.

RESULTS: Control (unlabelled) hMSC-injected femurs were shown to mineralise predominantly in the bone collar (diaphysis), whilst secondary mineralisation sites were observed in the epiphyses of the femurs injected with TREK1-labelled hMSCs (fig. 1). Significant mineralisation occurred in the region of the epiphysis surrounding the injection site as revealed by µCT and alizarin red staining for calcium.

DISCUSSION & CONCLUSIONS: In these experiments we demonstrated the effectiveness of targeting the TREK1 ion channel to remotely activate mechanotransduction and promote osteogenic effects at injection sites in an organotypically cultured chick foetal femur. Further research has shown that this method has the potential to act synergistically with other tissue engineering approaches and amplifies the effects of BMP2 delivered from microspheres, possibly by amplifying intracellular SMAD signalling[4].
Current work is ongoing to identify the differentiation and signalling mechanisms underlying the extensive mineralisation observed in the microinjection model.

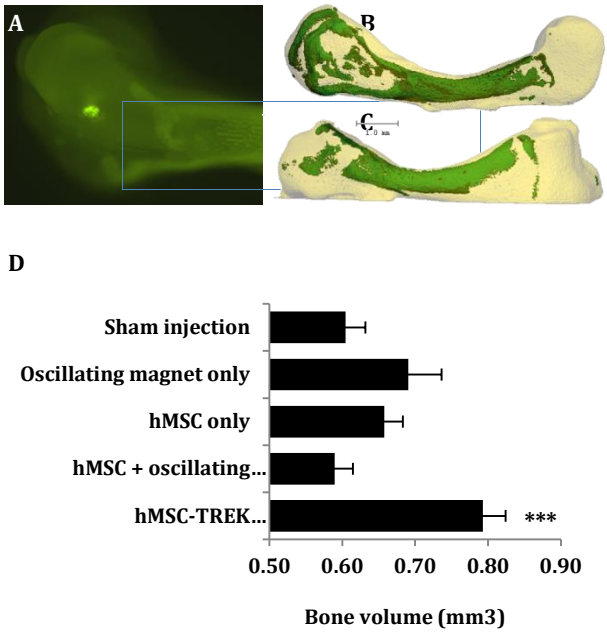


Fig. 1. The location of DiO-labelled hMSCs in the epiphyseal injection site within the chick foetal femur (a). After 14 days in vitro culture, the sites at which the TREK1-nanoparticle tagged cells were injected (b) were more mineralised than epiphyses injected with unlabelled hMSCs, (c) resulting in significant increases in bone formation in the TREK1-nanoparticle treated group compared to controls (d, n=9).

REFERENCES: [1]Kanczler JM, Sura HS, Magnay J, Green D, Oreffo RO, Dobson JP, El Haj AJ (2010). *Tissue Eng Part A* **16**: 3241-50.
[2]Smith EL, Kanczler JM, Roberts CA, Oreffo ROC (2012) *Tissue Eng. Part C* **18**: 984-994.
[3]Henstock JR, Rotherham M, Rose JB, El Haj AJ (2013) *Bone* **53***:* 468-77.
[4]Henstock JR, Rotherham M, Rashidi H, Shakesheff KM, El Haj AJ. (2014) *SCTM* (under review).

P-95: Scaffolds' characterisation for a multilayered construct simulating the tooth periodntium

Aliaa Khadre[1,3], Reem El-Gendy[1], Ourania-Menti Goudouri[2]

WaleedRefaat[3], Ahmed Ramadan[3], Aldo Boccaccini [2] , ElmostafaRaif[1]

[1]Department of Oral Biology /Leeds School of Dentistry, University of Leeds, UK

[2]Department of Materials Science and Engineering/ Institute of Biomaterials, University Erlangen-Nuremberg, Germany.

[3]Department of Orthodontics/ Faculty of Dentistry, Suez Canal University- Ismailia, Egypt.

denakh@leeds.ac.uk

INTRODUCTION:

Orthodontic forces are usually small forces used to move crowded or un-erupted teeth into place. The tooth periodntium is a complex tissue comprising a soft ligamentous tissue (periodontal ligament (PDL)) enclosed between 2 hard tissues (bone and cementum) [1, 2]. To understand the effect of orthodontic forces on capillaries and the balance between bone resorption and deposition ,in this study we have compared 4 different types of scaffolds as candidates to select: one scaffold representative of hard tissue components (bone and cementum)and one to represent the soft tissue component (PDL) of the periodontium to be used in a proposed multilayered construct simulating the tooth periodontium.

METHODS:

For the hard tissue component: PLGA 50/50 Poly (D,L-lactide-co-glycolide),and Sol-Gel sacffolds were compared. For the soft tissue component we have compared commercially available Geistlich Bio-Gide® and Flexcell® membrane. Each scaffold was seeded with ($2.5x10^5$) human periodontal ligament cells (HPDLCs) . The comparisons included assessing:

A. Cells' viability, adhesion, proliferation, and matrix formation (Confocal and SEM imaging) after 2 weeks of culture in basal media B. Mechanical properties were evaluated by measuring average compressive strength before and after cell seeding.

RESULTS: All types of scaffolds have displayed typical viable fibroblast like appearance in confocal images. SEM images indicated the presence of cells in sheets or layers, spreading and stretching within the scaffolds confirming the biocompatibility of theses scaffolds to HPDLCs and indicating various amounts of matrix formation by the cells within the scaffolds.

The average compressive strength of Sol-Gel scaffolds increased from 0.014±0.001 MPa in scaffolds without cells to 0.835 ± 0.3 MPa in cell seeded scaffolds after 2 weeks of culture under the same conditions. For the soft tissue component, the Bio-Gide® showed a higher initial compressive strength 0.020 ± 0.03MPa compared to collagen coated Flexcell® 0.0069±0.011Mpa.

DISCUSSION & CONCLUSIONS:

Since the four scaffolds tested in this study showed biocompatibility with the HPDLCs we considered the compressive strength as the deciding parameter for this study**.**

This study showed that matrix produced by HPDLSCs after 2 weeks has improved the Sol-Gel scaffold's mechanical properties. Hence, it was considered to be more suitable in representing hard tissue compared to PLGA.

Due to its higher compressive strength, Bio-Gide® was considered to be more suitable to represent the soft tissue component of our proposed multilayered construct.

Acknowledgement:

The authors would like to acknowledge the Egyptian cultural bureau for providing financial support to this project and the WELMEC, a centre of excellence in biomedical engineering (funded by Wellcome trust and EPSRC) for funding Reem El-Gendy.

REFERENCES:

1. Kawarizadeh, A., et al.(2005), Journal of dental research, 84(10): 902-906.
2. Krishnan, V. and Z. Davidovitch (2009), Journal of dental research,. 88(7): p. 597-608.

P-97: 3D printing of High Internal Phase Emulsions for Cell Culture Applications

Colin Sherborne [1] David W. Johnson[2], Matthew P. Didsbury[2], Chris Pateman[1], Neil Cameron[2] and Frederik Claeyssens[1]
[1]The Kroto Research Institute, University of Sheffield, UK
[2*]Department of Chemistry & Biophysical Sciences Institute, Durham University, UK
Mta06cs@sheffield.ac.uk

INTRODUCTION:

Stereo-lithographic production of 3D porous scaffolds using Poly-High Internal Phase Emulsions (Poly-HIPEs) have great potential for applications in tissue engineering as a support matrix. The tunability of the foam like morphology, and structural properties of a PolyHIPE scaffold are dependent on monomer choice, the addition of other components, and the processing conditions [1]. Typically the 3D printing capabilities of stereolithography has a trade-off between the resolution and subsequent fabrication time. We present a hybrid of structuring techniques to incorporate poly-HIPE's pre-processing control over micro porosity for macro structuring of highly porous materials [2]. Both the flexibility and porous nature of this fabrication approach makes it an excellent candidate for potential use for fabrication of scaffolds for *in vitro* cell culture

METHODS:

A water in oil emulsion was created using various blends of the water-immiscible monomers 2-Ethylhexy acrylate (EHA) and Isobornyl acrylate (IBOA) with a triacrylate crosslinking agent, photoinitiator and the surfactant Hypermer B246 stabilize the emulsion during the addition of water and agitation at 350 rpm. The viscous white liquid was cured into woodpile structures by selectively exposing the top surface to Ultraviolet light (355 nm) isolated from a ND:YAG microchip laser.

RESULTS:

SEM analysis of both poly-HIPE disks and 3D grid arrays produced via stereolithography were used to assess the porosity and surface features (Fig. 1). The tunability of the mechanical properties of PolyHIPE monomer blends were studied via nanoindentation and tensile testing. Cell culture studies have shown the porous discs to support proliferation of human fibroblast cells.

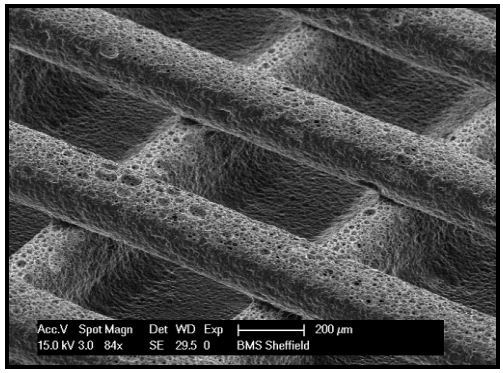


Fig. 1 SEM image of a PolyHIPE woodpile structure, pores can be seen on the top surface

DISCUSSION & CONCLUSIONS:

The Poly-HIPE's internal porosity is preserved during the stereolithography fabrication. This hybrid technique addresses the resolution trade-off between macro structuring with micro resolution, while maintaining user control over porosity parameters throughout the process. We illustrate the potential of this fabrication approach and that the scaffold supports the proliferation of human dermal fibroblasts. We foresee this material having a promising future with a wide range of tissue engineering applications.

REFERENCES:

[1] Bokhari M, Carnachan RJ, Przyborski SA, Cameron NR.. Journal of Materials Chemistry. 2007;17:4088-94.
[2] Johnson DW, Sherborne C, Didsbury MP, Pateman C, Cameron NR, Claeyssens F. Advanced Materials. 2013: 25, 23:3178-3181

ACKNOWLEDGMENTS:

We would like to thank EPSRC for funding the PhD studentship for CS.

P-98: Novel Hepatocyte-Like Cells and Biomaterial for Bioartificial Liver Design

Nelly Wung[1, 2], Zoë D Burke[1], Marianne J Ellis[2] and David Tosh[1]
[1]Department of Biology and Biochemistry, University of Bath, UK
[2]Department of Chemical Engineering, University of Bath, UK
N.Wung@bath.ac.uk

INTRODUCTION: Bioartificial liver (BAL) devices are a potential interim therapy to bridge patients with liver failure to transplantation and therefore reduce patient mortality from lack of donor livers by replicating liver function. The aim of this project is to develop a BAL device by using a novel hepatocyte-like cell (HLC) source (through the transdifferentiation (or conversion) of pancreatic cells to liver cells) and by developing a novel polymer for cell culture, PolymerX, to create a hollow fibre bioreactor (HFB). The rat pancreatic cell line AR42J-B13 readily displays normal liver cell functionality upon induction to HLCs [1, 2].

METHODS: AR42J-B13 cells were cultured on glass coverslips in 6 well-plates at a density of 30,000 cells per well. Cells were treated without or with 1 µM Dexamethasone (Dex) and 10 ng/ml Oncostatin-M (OSM) for 21 days and immunostained for pancreatic and liver markers to assess phenotypic changes. Characterisation of PolymerX was performed upon flat sheet membranes. Membranes were cast through phase inversion of 20% (w/w) PolymerX in NMP. Deionised water (DIH_2O) or 70% (v/v) industrial methylated spirits (IMS) was used as the nonsolvent. Membranes then underwent surface treatment for 1 min, 2 min or 5 min. Surface energy was analysed using the contact angle method by sessile drop. Statistical analysis of contact angle measurements was by a one-way ANOVA with Tukey's post-hoc tests using SPSS 21.

RESULTS: AR42J-B13 cells treated with Dex and OSM expressed glutamine synthetase (GS) and carbomoylphosphate synthetase I (CPS) (Fig. 1). Membranes undergoing surface treatment for 1 min showed significant reduction in the mean contact angle compared to the untreated control but thereafter showed no further significant change; this trend was seen for both nonsolvents (Fig. 2).

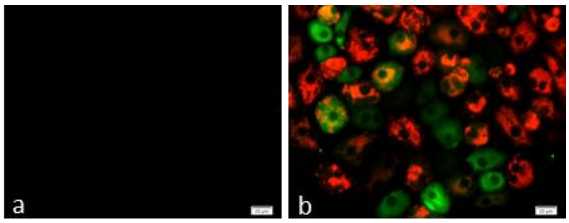


Fig. 1: Immunofluorescence of AR42J-B13 cells cultured on glass coverslips. Cells were treated without (a) or with (b) 1□M Dex and 10ng/ml OSM for 21 days and stained for GS (green) and CPS (red).

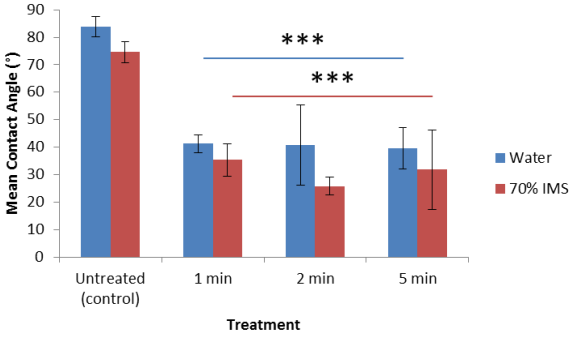


*Fig. 2: Surface treatment effect on PolymerX surface energy on 3 randomly selected membrane sections. Error bars = ± 1 SD. ***p<0.005.*

DISCUSSION & CONCLUSIONS: Surface treatment of PolymerX showed an increase in hydrophilicity compared to the control. The utility of AR42J-B13 cells to transdifferentiate to HLCs under Dex and OSM treatment has also been shown. Future work will assess the ability of the pancreatic cells to convert to HLCs on the novel polymer on both flat sheets and on the exterior of hollow fibre membranes.

REFERENCES: [1] Shen, C. N. *et al* (2000) *Nature Cell Biol* **2**: 879-887. [2] Burke, Z. D. *et al* (2006) *J Cell Physiol* **206**: 147-159.

ACKNOWLEDGMENTS: We would like to thank the BBSRC and the University of Bath for funding.

P-100: Developing an *in vitro* model to measure airway smooth muscle contraction

JC Bridge[1], GE Morris[1], J Aylott[1], MP Lewis[2], FRAJ Rose[1]

[1] *School of Pharmacy, University of Nottingham, England*

[2] *School of Sport, Exercise and Health Sciences, Loughborough University, England*

paxjb@nottingham.ac.uk

INTRODUCTION:

Airway smooth muscle (ASM) is the key effector cell in regulating airway contraction. Previous *in vitro* studies into ASM contraction have largely been limited to 2D single cell cultures and collagen gel contraction studies. Developments in tissue engineering have generated novel technologies for developing 3D models that better mimic the natural ECM. One method for producing such matrices is electrospinning, where non-woven mats of polymer fibres can be made by passing a polymer solution through a highly charged capillary. This is an attractive approach due to its simplicity, low cost and the wide range of both synthetic and natural polymers that are available to be electrospun.

The aim of this work was to culture contractile airway smooth muscle tissue using primary human ASM cells cultured on aligned electrospun scaffolds (fabricated from PET and gelatin) and in collagen gels. The contractile forces generated by the smooth muscle cells in response to contractile agonists can then be measured using a custom made culture force monitor (CFM).

METHODS:

Solutions of PET and gelatin were electrospun onto a rotating mandrel (separately) to produce aligned fibrous scaffolds. Electrospun gelatin was cross-linked using 1-ethyl-3-(dimethyl-aminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxyl succinimide (NHS) in an ethanol: water solution (9:1). Scaffolds were sterilised prior to seeding with smooth muscle cells. In addition, collagen gels were seeded with smooth muscle cells. Cells were cultured to confluency before cell-scaffold/gel constructs were placed on the CFM and stimulated chemically. The force of contraction was measured.

RESULTS:

The electrospinning process produced highly aligned non-woven fibres (50% of fibres within 5° of the mean angle).The fibre diameter of the produced scaffolds ranged from 250nm to 3.5µm dependant on concentration of polymer used. The PET and gelatin scaffolds differed gratly in their elastic properties with the PET scaffolds being much stiffer than gelatin. ASM cells were successfully cultured to confluency on aligned electrospun scaffolds forming a uniaxial population. Contraction of smooth muscle cells in collagen gels was measured on the CFM in response to contractile agonists.

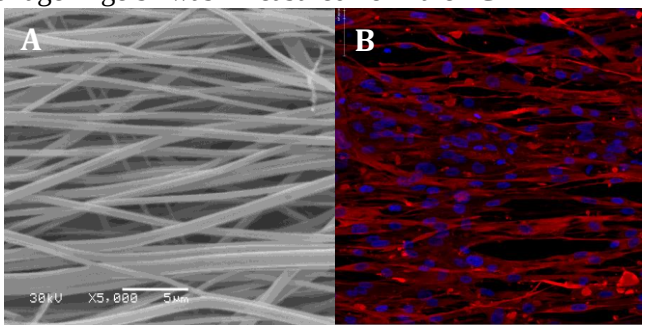


Figure 1: (A) SEM image of aligned electrospun PET and (B) an immunohistochemical image of ASM cells cultured on aligned PET stained for smooth muscle marker SM22α and DAPI.

DISCUSSION & CONCLUSIONS:

Electrospun scaffold properties can be tailored for purpose by changing the electrospinning parameters such as polymer type, concentration and flow rate. Additionally, the electrospinning method can produce highly aligned fibres regardless of the other parameters. ASM cells adhere to and proliferate on the electrospun scaffolds forming a highly aligned cell population. The contractile force generated by smooth muscle cells in response to a chemical agonist can be successfully measured using the CFM. This method of measuring cell contraction will be repeated using the electrospun scaffolds in place of collagen gels.

ACKNOWLEDGMENTS:

The authors would like to thank the EPSRC DTC in Regenerative medicine for funding this project.

P-101: Determination of drug permeability in Caco-2 monolayers under dynamic conditions using a novel magnetic force bioreactor technology

Antonella Lisella[1] and Alicia J. El Haj[2*]
[1,2]MICA Biosystems Ltd, Guy Hilton Research Centre, UK
[2*]Institute of Science & Technology in Medicine, Keele University, UK
a.lisella@keele.ac.uk

INTRODUCTION:

The Caco-2 cell line is the gold standard for the prediction of drug absorption and permeability *in vitro* by mimicking the small intestine[1]. However, this model does not closely resemble the physiology of the intestine as it lacks dynamic motion similar to peristalsis *in vivo*. In our research, we have developed a dynamic Caco-2 cell model which aligns with the standard assay using the MICA technology[2].
We have selected test compounds which characterise the properties across the biopharmaceutical classification system (BCS) classification[3]. In this way, we can assess whether a dynamic *in vitro* assay more closely resembles the adsorption seen *in vivo*.

METHODS:

The Caco-2 cells were labelled with bio-coated magnetic nanoparticles (MgNPs). With the use of our MFB technology (MICA BioSystems, UK), it is possible to interact with the MgNPs placed on the cell by applying an oscillating external magnetic field. The permeability experiments were performed with and without MFB (standard assay)[4]. The integrity of cell monolayer was assessed before and after the permeability studies by trans-epithelial resistance measurement (TEER), Lucifer Yellow passage in order to validate the experiments. Immunocytochemistry were used to assess the impact of MFB on the Caco-2 cell.

RESULTS:

MgNPs were successfully bio-coated, in order to apply MFB technology. A summary of all the permeability data obtained from our standard permeability assay (as internal control), and our developed dynamic permeability assay is provided in Table 1. The Figure 2 shows the cell monolayers before and after the application of MFB.

Table 1 Results from Caco 2 permeability assays.

Compound	BCS Class	% of P_{app} increase	P_{app} standard assay (nm/s)	P_{app} Dynamic assay (nm/s)
Ketoprofen	I	63%	27	44
Antipyrine	I	88%	34	64
Piroxicam	II	17%	24	28
Amoxicillin	III	20%	15	18

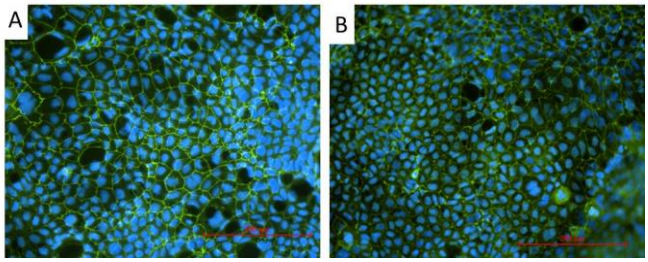


Fig.2 Immunocytochemistry of Caco-2: the cells were stained with an antibody against a tight junction protein, before (A) and after (B) the MFB performance.

DISCUSSION & CONCLUSIONS:

The use of our novel dynamic platform increased the permeability in all the standard drugs selected for the experiments respect to the control gold standard Caco-2 assay. The monolayer integrity was unaffected by the MFB technology or addition of magnetic particles.
The developed dynamic screening established an *in vitro* dynamic condition which more closely mimics the *in vivo* absorption rates.

REFERENCES:
1. P. Artursson et Al (2012), *Adv Drug Deliver Rev*, **64**, 280-289.
2. EL HAJ, A.J. and CARTMELL, S.H. (2010) *Proc. I.Mech E., Part H: J. Engineering in Medicine*, 224 (H12), 1523-1532.
3. A. Dahan et Al (2009), *AAPSJ*, **11**, 740-746.
4. I. Hubatsch et Al (2007), *Nat Protoc*, **2**, 2111-2119.

ACKNOWLEDGMENTS:

This work is funded by Marie Curie Intra-European Fellowship within the 7th European Community Framework: Functionalized Magnetic Nanoparticles and their Application in Chemistry and Biomedicine (MAGNETICFUN), project number 290248.

P-102: The Effects of Polymer Grade and Sterilisation on Electrospun Fibre Material Properties and Cell Response

Lucy A Bosworth[1], Anita K Ghag[2], Finella Tancred-Holmes[1] and Sarah H Cartmell[1*]
[1*]School of Materials, The University of Manchester, UK
[2]School of Chemical Engineering, University of Birmingham, UK
lucy.bosworth@manchester.ac.uk

INTRODUCTION:

Electrospinning biopolymers, such as poly(ε-caprolactone) (PCL), is a popular method for producing scaffolds that mimic the extracellular matrix of many tissues[1]. Yet, research intended for overall translation to the clinic and human use requires various regulations to be adhered to, which includes material purity and recognised sterilisation processes. This study investigated the effects of polymer grade (chemical or medical) and sterilisation (ethanol or gamma) on the material properties and cell response of electrospun scaffolds.

METHODS:

Grades of PCL investigated: medical (Purac) and chemical grade (Sigma). Three-dimensional (3D) fibrous scaffolds were prepared as described in Bosworth *et al.*,[1]. 3D scaffolds were sterilised in increasing concentrations of ethanol (50-100 %v/v) or gamma irradiated at 25 kGy (Synergy Health) prior to material characterisation, including tensile testing (Instron 1122, load cell 0.01 kN) and the morphology of seeded L929 fibroblasts (50,000 per cm^2) was assessed up to 48 hours by Scanning Electron Microscopy (SEM). Data was not Normally distributed. A Kruskal-Wallis test with Dunns post-tests was used for comparison of data sets for each PCL grade and Mann-Whitney test for comparison of as-spun (dry) scaffolds.

RESULTS:

The results demonstrated a clear difference in tensile strength and stiffness depending on PCL grade, with statistical significance when comparing the as-spun (or dry) scaffolds (Fig.1).

Fibroblasts attached to scaffolds irrespective of grade/sterilisation technique after 4 hours (Fig.2). By 48 hours, cells appeared flattened and spread-out on gamma irradiated scaffolds, whereas a rounder morphology was observed for cells seeded on ethanol sterilised fibres.

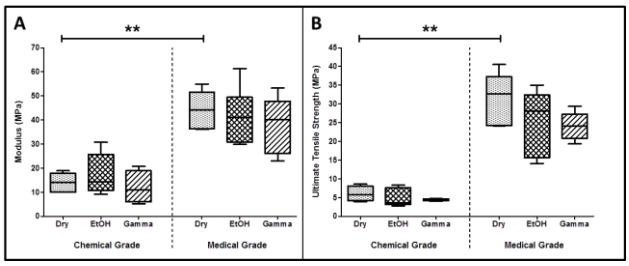


Fig. 1 – Tensile data comparing PCL grades and method of sterilisation for (A) Young's Modulus (MPa) and (B) Ultimate Tensile Strength (MPa). (n=5; p<0.01).

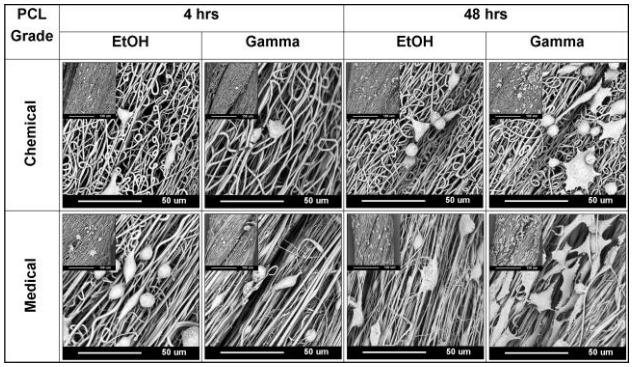


Fig.2 – SEM images demonstrating cell morphologies on PCL scaffolds. Scale bar = 50 µm (inset 100 µm).

DISCUSSION & CONCLUSIONS:

The data highlighted a significant difference in tensile properties depending on the material grade. Similarly, cell response appeared to be more favourable for cells cultured on gamma irradiated scaffolds. This study demonstrates the importance of incorporating the right materials and sterilisation processes early in the project timeline to aid translation to the clinic.

REFERENCES:

1. Bosworth *et al* (2013), *JMSMM* **24(6)**: 1605-1614.

ACKNOWLEDGMENTS:

This study was supported by the MRC-DPFS (Grant no: G1000788-98812).

P103: Controlled production of poly(3-hydroxybutyrate- co-3-hydroxyhexanoate) (PHBHHx) nanoparticles for use in tissue engineering

Richard Webb[1], Thomas Heathman[1], Cui Ling Dong[1], Xun Sun[2], Zhirong Zhang[2],Nick Forsyth[1], Alicia El Haj[1]

[1]ISTM, Guy Hilton Research Centre, Thornburrow Drive, Hartshill, Stoke-on-Trent, ST4 7QB

[2] Key Laboratory of Drug Targeting and Drug Delivery Systems, Ministry of Education, West China School of Pharmacy, Sichuan University, Chengdu 610041, PR China,

w.r.webb@keele.ac.uk

INTRODUCTION: Control of size and quality of nanoparticles during production is critical for their success in tissue engineering. This would allow reliable and reproducible manufacture of smaller nanoparticles for applications such as nanoparticle-mediated gene transfection. This holds much promise in regenerative medicine as non-viral gene vectors present a significantly reduced safety hazard compared to viral based gene vectors.

METHODS: A Design of experiment (DOE) method was utilised to determine the sensitivity of process variables and the repeatability of producing PHBHHx nanoparticles of a desired size. The DOE approach uses parallel multivariate designed experiments to investigate the action and interaction of variables to improve understanding, and therefore control of complex processes. We then encapsulated PDGF-ββ for controlled and sustained release to induce human mesenchymal stem cell expression of smooth muscle actin (SMA).

RESULTS: A size range of nanoparticles was produced by varying the number of sonication cycles whilst maintaining the same PHBHHx concentration at high and low sonication power. This process was first completed with blank nanoparticles to obtain a base curve for nanoparticle size production over two batches (to account for the associated common cause variability). This process produced a nanoparticle range of 90 - 215 nm with zeta potential (-35.2 ± 7.79) and PDI (0.168 ± 0.029) within accepted limits. This identified two curves of sonication power at 100 W and 500 W that could be used to produce nanoparticles of a controlled size. A controlled and sustained release of encapsulated PDGF-ββ over 80 hours which induced SMA transcription in hMSCs was observed.

DISCUSSION & CONCLUSIONS: In this study we created nanoparticles as small as 90 nm with a PDI of well below 0.3, which is far smaller than the theoretical minimum of 122.4 nm using PLGA-BSA.

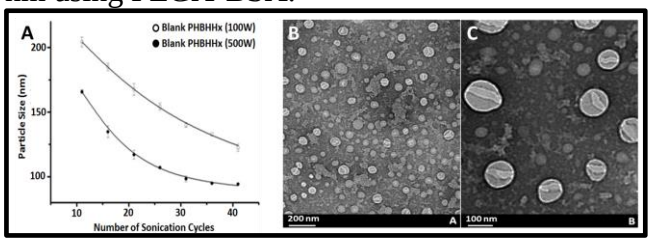


Fig. 1(A) Nanoparticle prediction Curve, (B&C) SEM of PHBHHx nanoparticles

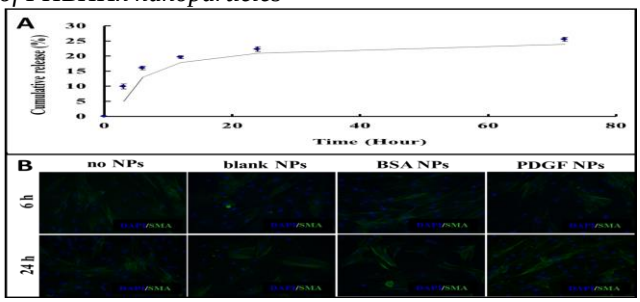


Fig. 2 Cumulative release curve for PDGF-ββ (A) and NP encapsulated PDGF-ββ induced differentiation on hMSC

This shows that the use of PHBHHx as a drug encapsulating polymer has greater potential compared to PLGA, producing smaller nanoparticles as required for intravenous injection and transport across the blood-brain barrier as well as opening up new avenues in the use of nanoparticles in gene transfection. The encapsulation and subsequent release of PDGF-ββ successfully induced SMA transcription in hMSCs. Our findings have added to the body of evidence that PHBHHx can be used not only in drug delivery, but also in sustained release of growth factors for controlled stem cell differentiation.

ACKNOWLEDGMENTS: Hyanji Scaffold Project: European Commission Framework 7 Program: SP3 People (PIRSES-GA-2008-230791)

P-104: In vitro degradation of novel resorbable polymers for maxillofacial applications

Sarah Upson[1], David Fulton[1], Matt German[2], Kenny Dalgarno[3,4]

[1]School of Chemistry, Newcastle University, UK
[2]Centre for Oral Health Research, Newcastle University, UK
[3]School of Mechanical and Systems Engineering, Newcastle University, UK
[4]Arthritis Research UK Tissue Engineering Centre, Institute of Cellular Medicine, Newcastle University, UK
Sarah.upson@ncl.ac.uk

INTRODUCTION:

Mandibular fractures are usually aligned using internal fixation plates which support the bones whilst they heal. Each year there are significant numbers of revisions required as a consequence of post-operative complications associated with current plate designs. The development of new biodegradeable fixation plates will reduce the need for these revisions, consequently reducing the economic burden on the health system, and improving quality of life for the patient.

We report use of an *in vitro* model to assess the initial degradation profile of a synthesised biodegradable polymeric material for use in maxillofacial bone fracture fixation.

METHODS:

Hydroxyethyl methacrylate terminated poly-lactic-*co*-glycolic acid (HEMA-terminated-PLGA) was synthesised *via* ring opening polymerisation of lactide and glycolide, (85 : 15 Mol %, 120 kDa). Commercially available PLGA (85 : 15, Sigma Aldrich) of molecular weights 50-74 kDa and 190-240 kDa were used as controls. Polymeric plates were prepared using compression moulding.

The plates (n = 5) were placed into vials containing phosphate buffered saline (PBS) (8 mL) in an incubator (37 ^{o}C) for 0, 1, 2, and 6 weeks, the PBS was changed weekly. On removal the plates were assessed for their mechanical properties (Instron 3-point bend test, 1mm/min).

RESULTS:

Table 1: Young's modulus of polymer plates (standard deviations can be seen in parenthesis).

	HEMA-term PLGA (120 kDa)	PLGA (50-75 kDa)	PLGA (190--240 kDa)
Time / weeks	Young's modulus / GPa	Young's modulus / GPa	Young's modulus / GPa
0	6.7 (2.0)	4.1 (0.3)	8.4 (2.8)
1	4.5 (0.3)	4.1 (0.8)	4.8 (0.4)
2	6.3 (0.5)	2.5 (1.1)	7.2 (0.3)
6	5.0 (0.2)	0.4 (1.5)	5.4 (0.1)

Analysis of the data reveals a difference between the Young's modulus of PLGA (50-75 kDa) and HEMA-terminated-PLGA after 2 and 6 weeks, indicating that HEMA-term PLGA is able to maintain its stiffness for longer. HEMA-term PLGA yields similar stiffness properties to PLGA (190-240 kDa).

DISCUSSION & CONCLUSIONS:

The results show that HEMA-terminated-PLGA maintains stiffness for 6 weeks in PBS, behaviour which is comparable with high molecular weight commercial PLGA, and which suggests that the HEMA-terminated-PLGA could be used within a biodegradable fixation plate.

ACKNOWLEDGMENTS:

The authors would like to acknowledge the support of the FP7 RESTORATION project (Award CP-TP 280575-2).

P-105: Cyclic Hydrostatic Pressure Enhances Bone Growth and Repair in an *ex vivo* Non-Union Defect Model

Joshua Price[1], James R Henstock[1], Alicia J El Haj[1]

[1]ISTM Guy Hilton Research Centre, Keele University, UK
j.price@keele.ac.uk

INTRODUCTION

Mechanical loading of bone *in vivo* results in the generation of dynamic hydrostatic forces as bone compression is transduced to fluid pressure in the osteocyte canalicular network. It has been previously shown that physiological hydrostatic loading regimes result in increased bone growth in *ex vivo* embryonic chick femurs[1]. The aim of this study was to investigate the effect of physiological mechanical loading on bone repair using a non-union defect in the embryonic chick femur model.

METHODS:

10^5 hMSCs were seeded into 0.5mg/ml collagen hydrogels. Defects were created along the mid-section of the (e11) chick foetal femur and the hydrogels inserted. A hydrostatic pressure regime of 0-280kPa at 1Hz, was applied to the femurs for one hour per day in a custom designed bioreactor for 14 days. The constructs were supported using a glass needle. Bone formation was assessed after 14 days by X-ray microtomography and quantified by histology.

RESULTS:

Total femur volume was significantly higher in hydrostatically stimulated samples (p=0.01). Hydrostatic stimulation significantly increased the bone volume in the implant (p=0.04) which was complemented by improved trabecular architecture and increased mineralized tissue bridging the defect site (fig. 1 b&d).

Table 1.µCT of femurs after 14 days culture

	Total Femur Volume (mm^3)	% Bone Volume (Defect)
Unstimulated	9.68	2.17
Stimulated	15.56	10.00

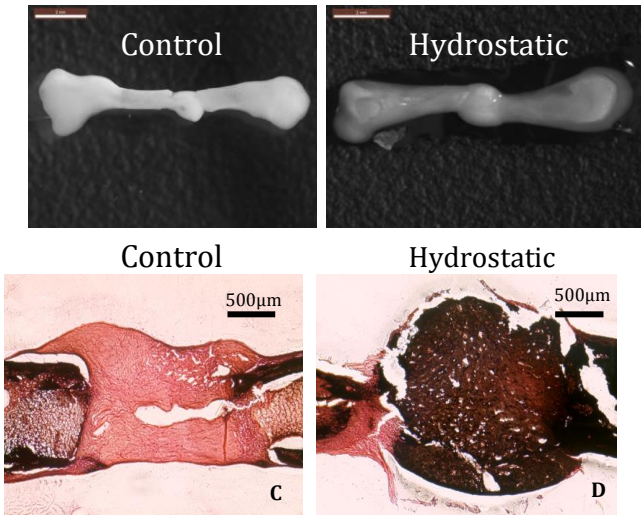


Fig. 2. (A&B) Whole mount imaging demonstrating enhanced cortical growth and improved integration of the construct in stimulated femurs. (C&D) Von Kossa staining for mineralisation shows osteogenesis in the implant in stimulated femurs.

DISCUSSION & CONCLUSIONS:

Hydrostatic stimulation enhanced femur growth and increased bone volume in collagen - hMSC implants in *ex vivo* chick femurs. Hydrostatic stimulation significantly increased mineralisation in hMSC collagen implants. The study also demonstrates a suitable model for future study of non-union repair *in vitro*.

REFERENCES:

[1] Henstock, JR, Rotherham, M, Rose, JB, & El Haj, AJ. (2013). Cyclic hydrostatic pressure stimulates enhanced bone development in the foetal chick femur in vitro. *Bone*, *53*, 468–477.

ACKNOWLEDGMENTS:

The authors would like to thank TGT/Instron and the EPSRC DTC in regenerative medicine.

P-107: Heterogeneity of Dental Pulp Progenitor Cells

Rachel Howard-Jones[1,2*], Marie Wiltshire[2], Alastair Sloan[1] and Rachel Errington[2]
[1]School of Dentistry, Tissue Engineering and Reparative Dentistry, Cardiff University, UK
[2*] School of Medicine, Institute Cancer and Genetics, Cardiff University, UK
HowardjonesRA1@Cardiff.ac.uk

INTRODUCTION:

Mesenchymal Progenitor Cells (MPCs) are the subject of intense scientific interest in biomedicine. We have derived and characterised populations of clonal human dental pulp progenitor cells (hDPPCs). Understanding how these cells behave as part of a community is fundamental to understanding processes of tissue repair and their potential for therapeutic application. This project proposes to study the heterogeneic behaviour of hDPPCs by labelling and tracking cells with fluorescent nanoparticles (Qtracker705®). These nanoparticles have a conserved signal therefore loss of signal is observed following cell division.[1]

METHODS:

Two clonal (A32 & B11) and one mixed hDPPC (M-hDPPC) population were cultured in α-MEM (10% Foetal bovine serum (FBS) and 100µM L-ascorbate). Cells were labelled with 4nM Qtracker® 705. After 24 hours, cells were sorted using FACS into two groups, high Qtracker®705 signal (Peak) and low signal (Lower). Cells were re-seeded at a density of $2x10^3$cells/cm^2 cultured for 72 hours and re-sorted using the same principle. RNA was extracted from the sorted cells. Biomarker analysis was carried out utilising RT-PCR for MPC markers, pluripotency markers, nerural crest and early neural markers.

RESULTS:

Clonal and mixed hDPPCs demonstrate differences in their initial Qtracker®705 loading and different signal loss over time. B11 loading and signal loss is similar to M-hDPPC. A32 cells maintain signal over 72 hours but lose signal after 144 hours. Differences exist in the expression of MPC, pluripotency and neural markers between the sorted cell populations based on their Qtracker®705 signal.

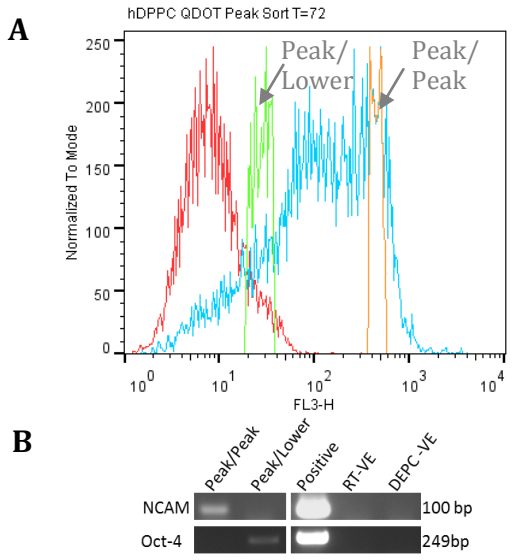


Fig. 1 ***A.*** *FACS analysis of QTracker705® labelled peak M –hDPPC 72 hours post-sort with re-sorted regions in orange and green.* ***B.*** *RT-PCR of NCAM and Oct-4*

DISCUSSION & CONCLUSIONS:

Major differences exist in cell behaviour between clonally isolated cell populations of the dental pulp and M-hDPPCs. Sorting cells in this way allows us to identify and characterise sub-populations of cells within a heterogeneous population based on simple labelling techniques. This is a novel technology utilising a biophotonic signal to identify different stem cell phenotypes from a heterogeneous population - a systems approach.

REFERENCES:

1. H. D. Summers et al, (2013) *ACS Nano. 7:6129-6137*

ACKNOWLEDGMENTS:

The authors would like to thank the EPSRC (Grant no: EP/F040954/1) for providing financial support to this project.

P-110: Ameliorating the effects of tissue engineered skeletal muscle atrophy with mechanical overload: relevance to osteoarthritis?

Hannah Stobbs[1], Darren Player[1], Adam Sharples[2] and Mark Lewis[1]
[1]School of Sport, Exercise and Health Sciences, Loughborough University, United Kingdom
[2]School of Sport and Exercise Sciences, Liverpool John Moores University, United Kingdom
h.c.stobbs@lboro.ac.uk

INTRODUCTION:

Osteoarthritis (OA) affects >80% of the population aged 55yrs and older[1]. Both OA and age lead to a significant loss in muscle force[2], mass (atrophy) and a decrease in the regenerative capacity of muscle following injury[3]. Therefore, it is of vital clinical relevance to study ways to ameliorate muscle atrophy in order to manage OA. A 3D *in vitro* model of replicative senescence has been established[4] to further understand the characteristics of atrophic muscle. This atrophic model can be manipulated *in vitro* to witness the effects of mechanical overload on its structure and genetic response.

METHODS:

MPD and CON C2C12 skeletal myoblasts were compared[6]. 4 x 10^6 cells/ml in 3ml type-1 rat tail collagen were plated in glass chambers between floatation bars and were cultured for 14 days [4 d in growth medium (20% FBS), 10 d in differentiation medium (2% HS)]. The constructs were placed on the t-CFM to undergo an acute intermittent stretch protocol or to remain un-stretched to serve as a control. The
RNA was extracted from each gel in preparation for PCR analysis of the following genes: MGF, IGF-I, IGF-IEa, Myogenin, MMP9, MMP2, Myostatin, MyoD, MuRF1, MAF-bx, IGFBP5, IGFBP2, IGF-II, IGF-IIR and IGF-IR.

RESULTS:

As previously witnessed[4], MPD C2C12 myoblasts have a reduced ability to fuse to form myotubes in monolayer, adhere and remodel the collagen matrix (Fig.1). Force data for the intermittent stretch protocol, and post-stretch PCR data will be presented at the conference.

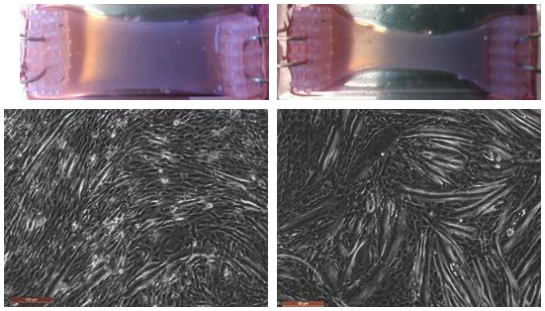


Fig.1 1. Macroscopic images of 3D collagen constructs (a) MPD (b) CON 2. Monolayer light micrographs of myotubes (a) MPD and (b) CON

DISCUSSION & CONCLUSIONS:

These early results show some differences between MPD and CON cells/myotubes in 2D and 3D. It is expected that there may be differences in the up regulation of a number of genes after mechanical overload between MPD and CON. This would show similarities between the exercise response in an atrophic phenotype *in vivo*, providing a controlled model to investigate these phenomena. This could then be translated to the context of osteoarthritis in an ageing population.

REFERENCES:

1. P.Bajaj et al (2001), *Pain* **93**: 107-14 2. W.Frontera et al (2008), *J Appl Physiol (1985)* **105**: 637-42 3. T.Pietrangelo et al (2009), *Exp Gerontol* **44**: 523-31 4. A.Sharples et al (2012), *Aging cell* 11: 986-95

ACKNOWLEDGMENTS:

This work is funded as part of the Arthritis Research UK Centre for Sport, Exercise and Osteoarthritis

P-111: Tissue engineering skeletal muscle to investigate glucose uptake.

Mark C Turner, Darren J Player, Neil RW Martin, and Mark P Lewis[1]
[1]School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough, UK
M.C.Turner@lboro.ac.uk

INTRODUCTION:

In vivo, skeletal muscle contains, multi-nucleated aligned myotubes surrounded by an extracellular matrix [1], and functionally, skeletal muscle is responsible for a large proportion of post-prandial glucose uptake [2]. The development of skeletal muscle tissue engineered constructs which recapitulate both morphological and physiological characteristics *in vitro* provides the potential to investigate metabolic diseases, such as skeletal muscle insulin resistance.

METHODS:

C2C12 skeletal muscle myoblast cells were seeded at $1x10^5$ cells in fibrin based constructs previously described [1]. Constructs were grown in growth medium supplemented with 250µg aminocaproic acid (AA) (DMEM, 20% FBS, 1% P/S). Upon confluence, media was changed to differentiation media (DMEM, 2% HS, 1% P/S, AA), for two days. Constructs remained in maintenance media for the duration of the experiment (DMEM, 7% FBS, 1% P/S, AA). Constructs were then analysed by qPCR for mRNA of GLUT1, GLUT4. Protein was obtained and separated by western blot to measure concentrations of protein kinase B (Akt) and GLUT4. Glucose uptake was quantified through scintillation counting using [H^3]-2-Deoxy-D-Glucose.

RESULTS:

Time duration in differentiation and maintenance media resulted in contraction of fibrin based constructs ($P < 0.0001$, ANOVA). At 14 days total in culture constructs had developed multi nucleated myotubes (*figure 1*) and express total Akt protein, a key mediator within the insulin signalling cascade (*figure 2*).

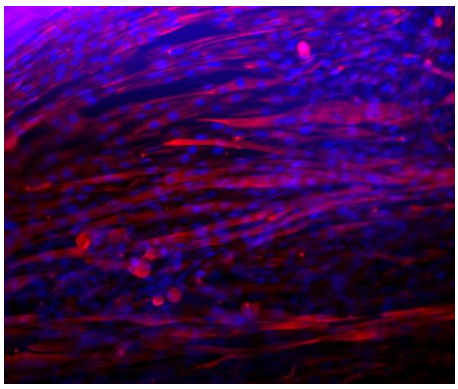


Figure 1: Immunofluorescence of C2C12 cells in fibrin gels at day 14 in culture. Myodesmin (Red) counterstained with DAPI (Blue). Scale Bar = 100µM (N = 1 construct).

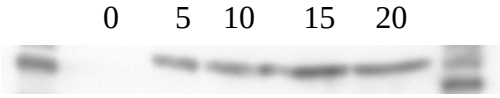


Figure 2: Immunoblot for total Akt with dose dependent protein concentrations (µg) from 14 day old fibrin gels.

DISCUSSION & CONCLUSIONS:

These findings present the characteristics of a skeletal muscle tissue engineered constructs which express fundamental genes and proteins required for insulin stimulated glucose uptake.

REFERENCES:

1. Khodabukus and Baar, (2012), *Tissue Engineering: Part C*, Vol. 18, 5.
2. DeFronzo RA, Gunnarsson R, Bjorkman O, Olsson M, Wahren J. (1985). *J Clin Invest*; 76(1):149-155.

P-112: Analysis of Positional and Rotational Variables for Cell Seeding on Hollow Fibres

Samuel Acott[1], Prof. Raimondo Acione[2], Dr. Marianne Ellis[1]
[1] Department of Chemical Engineering & Centre for Regenerative Medicine, University of Bath, UK
[2*]Bristol Heart Institute, Bristol Royal Infirmary, UK
S.M.Acott@bath.ac.uk

INTRODUCTION:

In recent years mesenchymal stem cells (MSCs) have shown increasing potential for use as a cell therapy for treatment of damaged heart tissue caused by myocardial infarction. MSCs can be extracted in limited quantities from adipose tissue surrounding the heart for use as an autograph to regenerate new cardiomyocites, however expanding the MSC population to a clinically applicable number remains elusive. To this end a dynamic method of seeding MG63 human osteosarcoma cells onto poly(lactic-co-glycolic) acid (PLGA) hollow fibres has been investigated.

METHODS:

PLGA hollow fibre membranes were fabricated using wet spinning phase inversion. A solution of 20% w/w PLGA in N-methyl-2-pyrrolidone (NMP) solvent was passed through a spinneret into deionised water, the fibres are left in the water, replaced twice a day, for three days to allow complete solvent removal[1].

Fibres of 1 mm external diameter were cut to lengths of 35 mm and placed within a cylindrical bioreactor housing of 0.1 ml. The fibre and internals of the bioreactor were sterilised with 70% ethanol for 12 hours at 6°C, soaked for 1 hour in Dulbecco's Modified Eagle Medium (DMEM), washed thoroughly with 1 ml DMEM and inoculated with 110,000 MG63 cells at a density of 100,000 cells/cm^2.

Bioreactors were attached to a Stuwart STR4 rotator drive at one of three different angles and spun at one of three rotational velocities for 6 hours at 37°C and 5% CO_2. Afterwards the residual media was collected, the bioreactor washed with PBS, and the hollow fibre trypsinised for 20 min and agitated to remove attached cells. Manual cell counts were also performed on the media and PBS wash.

RESULTS:

Cell attachment on bioreactors angled horizontally (17%), diagonally (20%) and vertically (16%) showed no statistically significant difference in their rates of cell attachment compared to each other (n=12) or static controls (23%) (n=59). This was also true of horizontally orientated bioreactors spun at 2 RPM (17%), 6 RPM (17%) and 8 RPM (11%) (n=12). Intermittent spinning at 6 RPM for 25 seconds and resting for 220 seconds also yielded no improved rates of cell attachment (12%) (n=8).

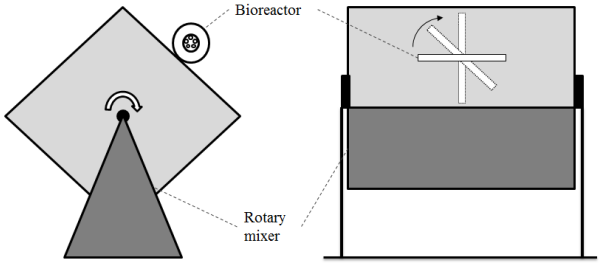


Fig. 1: A side and front facing view of a hollow fibre bioreactor attached to a Stuwart STR4 rotator drive.

DISCUSSION & CONCLUSIONS:

All forms of dynamic cell seeding showed no significant improvement over the same system seeded in static conditions. Large variations in each data set lead to a corresponding average error of ±33%. This indicates a high degree of variability in cell attachment on hollow fibres which needs to be mitigated if hollow fibre bioreactors are to comply with good manufacturing practices.

REFERENCES:

1. Ellis, M.J. & Chaudhuri, J.B. (2007). Poly(lactic-co-glycolic acid) Hollow Fibre Membranes for use as a Tissue Engineering Scaffold. *Biotech and Bioeng*. 96 (1), p177-187.

ACKNOWLEDGMENTS:

Funded by the University of Bath URS award.

P-115: Biological Characterisation of the Porcine Acetabular Labrum

Rachel L Pallan[1], Joanne L Tipper[1], John Fisher[1] and Sophie Williams[1]
[1]Institute of Medical and Biological Engineering, School of Mechanical Engineering, University of Leeds, UK
mn06rp@leeds.ac.uk

INTRODUCTION:

Femoroacetabular impingement (FAI) can impinge on the labrum, causing damage and has been recognised as an initiator of hip osteoarthritis[1]. Labral damage compromises the functions of the tissue (enhancing hip stability and protecting the articular surface); therefore it is advantageous to repair the labrum, in order to restore the hip to normal or near-normal mechanical and physiological function[2]. Few studies have characterised the biological properties of the labrum, such information could enhance current clinical repair strategies. The aim of this study was to characterise the acetabular labrum (in comparison to cartilage) both quantitatively and histologically.

METHODS:

Quantitative analysis of porcine acetabular labrum, acetabular cartilage and femoral cartilage (mean age 5 months and removed within 24 hours of slaughter) was carried out on load-bearing and non-load-bearing regions. Tissue water content was determined using lyophilisation. Glycosaminoglycan (GAG) and collagen concentrations were determined using quantitative assays[3]. Statistical analysis was carried out using a one-way ANOVA and student t-test (n=6). Histological analysis was used to qualitatively assess porcine acetabular labrum and cartilage. Samples were stained to identify the general architecture (haematoxylin and eosin [H&E]), collagen and elastin (Sirius Red and Miller's Elastin) and GAGs (Alcian blue)[3].

RESULTS:

The acetabular and femoral cartilage had significantly higher concentrations of water and GAGs, and a significantly lower concentration of collagen compared to the acetabular labrum (Table 1). There were no significant differences between the load-bearing and non-load-bearing regions of femoral and acetabular cartilage. Two key tissue types were identified within the labrum using H&E (Figure 1A). The majority of the tissue and the articulating surface were formed by a smoother more cartilaginous structure and the outer acetabulum was formed by a more fibrous structure. Sirius red staining under polarised light (Figure 1B) showed characteristic collagen orientation for the cartilage. The cartilaginous labrum region showed up dark under polarised light suggesting a radial alignment with the more fibrous region appearing random. Both tissues stained positively throughout for Sirius red and neither showed positive staining for Miller's elastin (Figure 1C). The labrum showed strong Alcian blue staining in the middle zone while the articulating surface and outer regions of the acetabulum showed reduced Alcian blue staining, highlighted by the presence of pink periodic acid-Schiff counterstaining (Figure 1D).

Table 1 Water content, GAG and collagen concentration of acetabular labrum and cartilage and femoral cartilage in load-bearing and non-load-bearing regions.

Sample	Water Content (%)	GAG Content (µg.mg-1)	Collagen Content (µg.mg-1)
ACL	75 (+1.35; -1.38)	186 ± 50	64 ± 11
ACN	75 (+2.01; -2.09)	197 ± 50	63 ± 23
FCL	76 (+1.48; -1.66)	176 ± 57	58 ± 15
FCN	75 (+2.37; -2.45)	176 ± 76	71 ± 7
ALL	70 (+2.69; -2.76)	67 ± 30	90 ± 21
ALN	67 (+3.23; -3.48)	29 ± 6	99 ± 16

AC – Acetabular cartilage, FC – femoral cartilage, AL – acetabular labrum, L-load-bearing, N- non-load-bearing

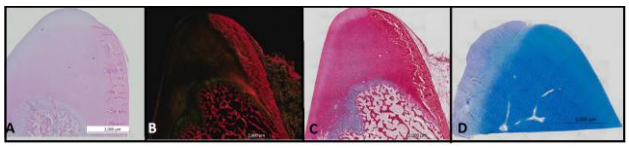


Figure 1 Histological images of the acetabulum taken at x10 magnification. A – H&E, B – Sirius red polarised, C – Sirius red bright field, D – Alcian blue

DISCUSSION & CONCLUSIONS:

This study has shown that the acetabular labrum demonstrates different material properties to the adjacent articular cartilage. The labrum has a higher collagen concentration to support its function of enhancing hip stability. The differences in fibre alignment may also support the tissues' functions; with articular cartilage requiring highly aligned surface fibres for articulation and the labrum requiring radial fibres for strength. GAGs enhance the tissues ability to support load and hence higher GAG concentrations were found in the load-bearing articular cartilage as opposed to the non-load bearing acetabular labrum. It is important to continue with further research to allow more specific labral treatments to be developed.

REFERENCES:
1. J. Clohisy et al (2010), CORR **468**: 555-564.
2. A. Grant et al (2012), J Child Orthop **6**: 357-372.
3. S-P Wilshaw et al (2006), Tissue Engineering **12**: 2117-2129

ACKNOWLEDGMENTS: This research was funded by the EPSRC DTC TERM.

P-116: A novel HDACi can improve osteogenic differentiation of human adipose derived stem cells

Liam Lawlor[1], Ronald Grigg[2] and Xuebin Yang[1*]
[1]Biomatierals & Tissue Engineering Group/School of Dentistry, University of Leeds, United Kingdom
[2]MIDAS Centre/School of Chemistry, University of Leeds, United Kingdom
mnll@leeds.uk

INTRODUCTION:
The ability to control stem cell differentiation into specific lineages is the key to functional tissue engineering. Histone deacetylase (HDAC) proteins play a key role in epigenetics and their inhibitor compounds (HDACis) are well researched cancer treatments. Novel HDAC3 selective HDACi, MI192, has potential in leukaemia, and rheumatoid arthritis treatments[1,2]. In recent years, researchers have investigated the effect of HDACis on stem cell epigenetics, with potential to control stem cell fate[3,4]. The aim of this study was to investigate the potential of using MI192 to control the osteogenesis of human adipose tissue derived stem cells (hADSCs), for bone tissue engineering.

METHODS:
The novel inhibitor MI192 was synthesised by a palladium catalyzed allene gas reaction and characterised using NMR spectroscopy. hADSCs were pretreated with different concentrations of MI192 for two days, prior to culture in osteogenic inductive medium for five days. HDACi effect on cell proliferation was assessed with MTT assays. The effect of HDACi pretreatment on osteogenic differentiation was assessed with alkaline phosphatase (ALP) staining.

RESULTS:
100 µM pretreatment of the HDACi MI192 caused ADSC death. Cell proliferation was halted at a lower concentration (30 µM). Pre-treatment of ADSCs with 30 µM HDACi MI192 for two days, followed by osteogenic media for five days, showed increased positive alkaline phosphatase staining, compared to the negative and positive controls (Figure 1).

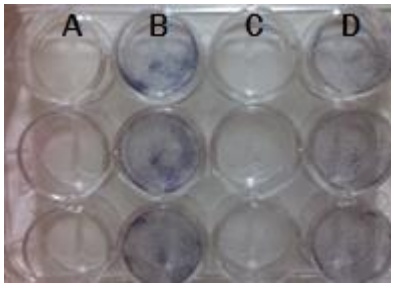


Figure 1 - ALP staining of ADSCs. ***A*** *- Basal media (no pretreatment),* ***B*** *&* ***C*** *- Cells pretreated with 30 µM (**B**) and 100 µM (**C**) MI192 for 2 days followed by 5 days in osteogenic media.* ***D*** *- Osteogenic media (no pretreatment).*

DISCUSSION &CONCLUSIONS:
The results from this study indicate that the high concentration of MI192 is cytotoxic to cells. However, low concentrations only inhibit cell proliferation. 30 µM pretreatment of MI192 promoted ADSCs differentiation along the osteogenic linage. Novel HDACi MI192 is known to be HDAC3 selective, which suggests the potential of using the selectively inhibiting HDAC3 for bone tissue engineering applications.

REFERENCES:
1. M. Boissinot et al (2012) *Leukemia Research* **36**: 1304-1310.
2. J. Gillespie et al (2012) *Arthritis and Rheumatism* **64**: 418-422.
3. J. Boer et al (2006) *Tissue Engineering* **12**: 2927–2937.
4. H. F. Duncan et al (2011) *Journal of dental research* **90**: 1377–1388.

ACKNOWLEDGMENTS:
The authors would like to thank the EPSRC funded DTC TERM for financial support.

P-117: Novel Polymer Topography to Influence Mesenchymal Stem Cell Behaviour

Walsh, Shane. B.[1], Dalgarno, K.W.[1,2] , McCaskie, A.W.[1,3] and Birch, M.A[1,3].

[1]Arthritis Research UK Tissue Engineering Centre, Institute of Cellular Medicine, Newcastle University.
[2]School of Mechanical and Systems Engineering, Newcastle University, UK
[3]Division of Trauma & Orthopaedic Surgery, Cambridge University Hospitals NHS, Cambridge, UK
Contact: S. B. Walsh. Tel: (+44) 191 223 8948; Email: s.walsh2@ncl.ac.uk

INTRODUCTION:

A cells response to the physical environment can regulate gene expression and differentiation[1]. Tailored architecture and topography of implants can regulate cell activity to augment the use of autologous progenitor cell populations to rebuild a diseased tissue more successfully. Adaptation of implant surfaces with appropriate temporal and spatial cues, possibly in combination with biomolecules and/or biomolecular motifs has the potential to influence multipotent cell activity influencing cell orientation, organisation and potentially cell differentiation leading to control of tissue formation and structure.

The aims of this project are to explore the influence of polymer surfaces on mesenchymal stem cell (MSC) biology and exploit different polymer chemistries to functionalize surfaces with biomolecules. These studies will assist scaffold design and fabrication, to aid regenerative applications.

METHODS:

Thin films of polymers were spread onto a flat substrate by spin coating at high speeds. Using a combination of immiscible polymers to generate a micro-scale landscape of opposing structures. Addition of water induces breath figure patterns, creating pores and crators to further accentuate the topographical pattern. Immiscible polystyrene (PS) and poly(methyl methacrylate) (PMMA) solutions were demixed at various ratio's (v/v) and spun at speeds > 8,000rpm under humid conditions[2] . Topographical surfaces were assessed using atomic force microscopy (AFM) and scanning electron microscopy. Primary adult human MSCs, isolated from bone marrow were characterized by flow cytometry and tri-lineage differentiation. Biological responses to surfaces were evaluated using immunofluorescece, histological staining and qPCR.

RESULTS:

After evaluation of a range of demixed ratios/ concentrations/ solvents a selection of surfaces [PS:PMMA 40:60/50:50/60:40] 3%w/v in toluene] were further characterized. These generated opposing raised PMMA and low-lying PS islands. Breath figure patterns generated crater-like features ranging from 0.5-1µm in height and depth (Fig. 1A). The raised *caldera* attracted increased cell interactions, where cells concentrate focal adhesion plaques (Fig1.B) to the raised features; altering their morphology (Fig.1 C) and gene expression (Fig.1D). Adaptation of demixed polymer ratios altered the spatial distribution of *Caldera*.

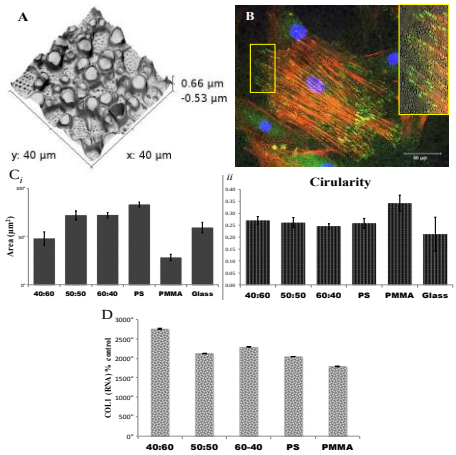


Figure 3. Demixed PS:PMMA [50:50] **A)** AFM 3D image **B)** Immunofluorescent staining of hMSC (P-2) 72hr. Focal adhesion plaques [*Vinculin*-FITC; green]; F-Actin cytoskeleton [*Rhodamine;* red]; Nucleus [Dapi; blue]. **C)** Quantification of cell morphology; ***i*)** Cell area and ***ii*)** Circularity. **D)** qPCR: COL1 expression (ΔΔCt) % control (glass) after 14days culturing on surfaces.

DISCUSSION & CONCLUSIONS:

Demixed polymer blends provide tuneable topographies for the stimulation of various cell responses through regulation of adherence dynamics. Utilizing the immiscible polymer chemistries we can restrictively alter the dispersion pattern of humidity induced pores and *Caldera,* to induce a biological response. Tailoring these surfaces to influence specific cell types has the potential to manipulate the tissue formation and hence tissue function.

REFERENCES:

1. C.M. Dekevsera *et al. polymer* 45, 2211-2219 (2004)
2. Park, M.S. and Kim, J.K. *Langmir* 20, 5347-5352 (2004)

ACKNOWLEDGMENTS:

Authors would like to acknowledge support from Arthritis Research UK (Award 19429).

P-119: Design and synthesis of hydrogels as tissue-engineering scaffolds

Abraham Gonzalez-Ruiz[1,3], Mario Flores-Reyes[1,3], Jaime Flores-Estrada[2], Miriam V. Flores-Merino[3*]

[1]Faculty of Medicine, Autonomous University of the State of Mexico (UAEM), Mexico

[2]Faculty of Chemistry, UAEM, Mexico

[3*]Research Center in Biomedical Science, UAEM, Mexico

mvfloresm@uaemex.mx

INTRODUCTION: Hydrogels are polymeric materials that are crosslinked in a 3D network. Their properties (*i.e.* biocompatibility) make them ideal as tissue engineering constructs. Network structure of hydrogel scaffolds is a key property for their performance in tissue engineering applications (*i.e.* migration of cells)[1,2]. Here, we studied the swelling behavior and the network structure of two poly(ethylene glycol) (PEG) hydrogel scaffolds synthesized by two different photoactivation methods.

METHODS: Poly(ethylene glycol) dimethacrylate (PEGDM) was obtained by a microwave synthesis of linear PEG (MW = 4000 $g.mol^{-1}$; Polioles, MX). UV light crosslinkable hydrogels were prepared by long wavelength UV light photoinitiated free radical polymerization of aqueous solutions of PEGDM and α, α′-azodiisobutyramidine dihydrochloride (Sigma-Aldrich, USA). Visible light crosslinkable hydrogels were synthesized mixing PEGDM, eosin Y, triethanolamine (initiator), and 1-vinyl-2 pyrrolidinone (Sigma-Aldrich, USA). Polymerization was carried out by exposing the solution to the visible light. Swelling behavior was studied by gravimetry of previously dried cylindrical samples of hydrogels in a phosphate buffer saline solution (PBS; pH=7.4) at 25±2º C. A kinetic study was carried out considering a second-order approach. Mesh size (ξ) and average molecular weight between cross-links (M_c) was calculated based on the weight swelling ratio experiments.

RESULTS: The value of the maximum weight swelling ratio of visible light activated hydrogels (8.87±0.62) was higher than the value of UV crosslinkable hydrogels (5.56±0.73; Figure 1). Also, the values of ξ and M_c showed to be higher for the hydrogels activated by visible light. The constant rate of swelling (k) according to the second order approach was 1.14 $g^{-1}h^{-1}$ (r^2=0.96) for the visible light activated hydrogels, meanwhile for the UV light crosslinked hydrogels was 10.16 $g^{-1}h^{-1}$ (r^2=0.97).

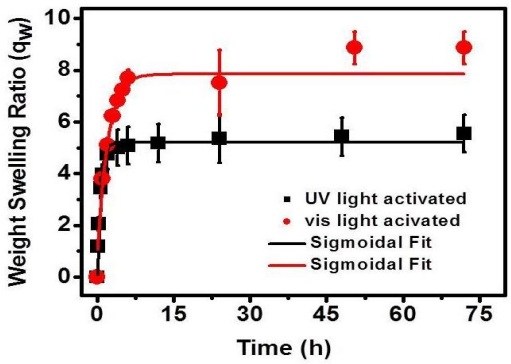


Figure 1 Weight swelling ratio (q_w) of UV (n=9) and visible light (n=3) crosslinkable hydrogels in PBS at 25±2º C (sigmoidal fit: r^2=0.99).

DISCUSSION & CONCLUSIONS: Two PEG hydrogels by different light activation methods were obtained and compared. Gels were obtained by UV light crosslinking and one molecule activation system, however we found a decrease in solvent uptake compared with visible activated gels. Longer photo-polymerization times showed a decrease in swelling and shorter time exposure to UV light did not polymerized.

REFERENCES:

1. A. Atala *et al* (2011), P*rinciples of Tissue Engineering* 2ed. Academic Press: Oxford.

2. M.L. de Vries-van Melle et al (2014), *ECM* **27**: 112-123.

ACKNOWLEDGMENTS: The authors would like to thank PROMEP (Grant no: 103.51/13/6535) and the UAEM for providing financial support to this project.

P-120: Development of Pre-Clinical Musculoskeletal Models to Investigate the Onset and Degeneration of Osteo-Arthritis

Darren J. Player[1], Neil R.W. Martin[1], Mark P. Lewis[1],
[1]Musculoskeletal Biology Research Group, School of Sport, Exercise and Health Sciences, Loughborough University, UK.
d.player@lboro.ac.uk

INTRODUCTION:

Osteoarthritis (OA) is a musculoskeletal disease characterised by the degeneration of hyaline cartilage. Recent reports suggest that the onset and degeneration of OA may reflect pathology across the whole joint, as an integrated 'organ'[1]. *In vitro*-based tissue engineered models allow for highly controlled investigations of potential factors involved in the onset and degeneration of OA, including biomechanics and inflammation.

METHODS:

The well characterised type-1 collagen-based skeletal muscle model[2], has been used to investigate the acute cyto-mechanical responses of both myoblasts and myotubes to unloading. We also have recently established a tissue engineered cartilage model, to investigate the response of chondrocytes to different modes of acute mechanical compression.

RESULTS:

Utilising an established model of tissue engineered skeletal muscle, we have been able to demonstrate that myoblasts and myotubes respond differently to acute mechanical unloading. The force change is reduced in myoblast cultures, whereas in myotubes there is a degree of compensatory reloading to maintain tissue tensional homeostasis.

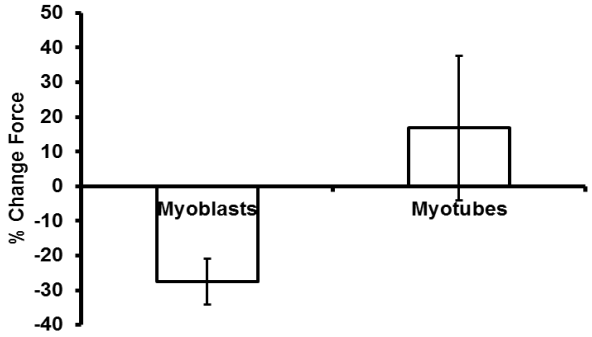


Figure 1. Response of myoblast and myotube cultures to -10% mechanical unloading.

Acute mechanical compression of tissue engineered cartilage, resulted in diverse chondrocyte morphology. Static loading contributed to the development of extensive actin networks, whereas dynamic loading reduced the number of actin positive processes compared to control.

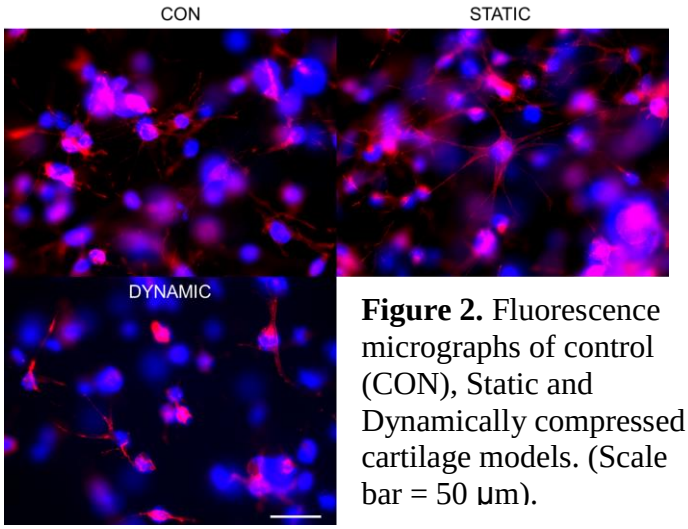


Figure 2. Fluorescence micrographs of control (CON), Static and Dynamically compressed cartilage models. (Scale bar = 50 μm).

DISCUSSION & CONCLUSIONS:

The experiments presented herein, demonstrate a programme of research to identify mechanisms that contribute to the onset and degeneration of OA. The mechanisms by which compensatory tension exists in myotube cultures are yet to be elucidated, however it is thought to be mediated by both active (actin-myosin) and passive cytoskeletal-extracellular matrix (desmin, titin, integrins etc.) components. The mode-dependent response evident in the cartilage mechanical compression model, will allow future experiments to investigate potential cartilage-muscle cross-talk mechanisms.

REFERENCES:

1. Loeser et al (2012), *Arthritis Rheum.* 64(6):1697-707. 2. Player et al (2014), Biotech. Letters. DOI 10.1007/s10529-014-1464-y.

ACKNOWLEDGMENTS: This work is funded as part of the Arthritis Research UK Centre for Sport, Exercise and Osteoarthritis.

P-121: Fabrication & Functionalisation of Nerve Guidance Conduits

James Clarke[1*], John W Haycock[1] and Frederik Claeyssens[1]
[1]Kroto Research Institute, Department of Materials Science & Engineering, The University of Sheffield, United Kingdom
mtp12jc@sheffield.ac.uk

INTRODUCTION:

Nerve Guidance Conduits (NGC) are increasingly used in surgical peripheral nerve repair. Autografts give superior results, but require additional surgery at a donor site with resultant morbidity.

Improving NGC performance is challenging as existing FDA approved materials are bio-inert. Microtopographical features ameliorate neuroregeneration on such materials[1], but surface functionalisation may provide greater enhancement[2]. As biological coatings face significant challenges in clinical acceptance, synthetic coatings are an attractive option.

The aims of this work are to investigate: i) whether the plasma polymer surface coating of NGCs and ii) the inclusion of organised micro geometries improves their effectiveness at stimulating nerve regeneration.

METHODS:

NGC were produced from photocurable polymers by microstereolithography (µSL). Plasma chambers were used to etch and coat substrates with either Acrylic Acid (AAc), Allylamine (AAm) or Maleic Anhydride (MA).

Surfaces were characterised by X-ray Photoelectron Spectroscopy (XPS) to determine element composition and confirm consistent coating. The hydrophilic/hydrophobic nature was determined by Water Contact Angle (WCA) analysis.

NG108-15 neuronal cells were cultured on surfaces in serum-free DMEM to induce differentiation. MTT assay was used to confirm cell viability. After 72 hours, cells were fixed and stained with Phalloidin-TRITC and DAPI. Cells were imaged by confocal microscopy and neurite growth analysed using ImageJ software.

RESULTS:

Guidance features with dimensions less than 100 µm were created on the inner surfaces of NGC (figure 1).

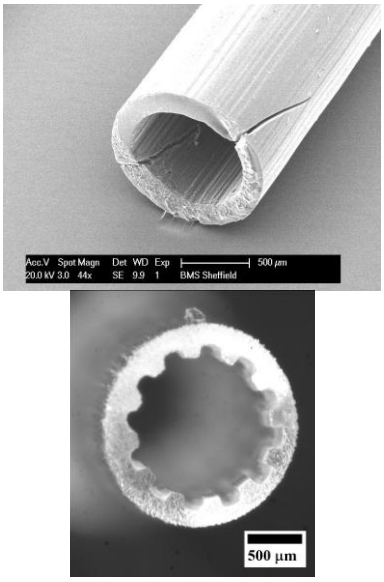


Fig. 1 NGC produced by µSPL

Plasma coating was achieved evenly on the inner surfaces of NGC <1 mm internal diameter with a length of 5 mm.
Preliminary results show increased metabolism and neurite growth on AAc coated surfaces. Cells adhered well to other coated surfaces but neurite growth was less extensive.

DISCUSSION & CONCLUSIONS:

We have produced NGC with user-defined topography and surface chemistry. Plasma treatment may be used to enhance neuronal cell response to bio-inert materials.

REFERENCES:

1. D. Hoffman-Kim et al. (2010), *Ann. Rev. Biomed. Eng*, **12**:203-231.

2. J. H. A. Bell & J. W. Haycock (2012), *Tiss. Eng. B* **18**:2 116-128.

ACKNOWLEDGMENTS:

The authors would like to thank the EPSRC for DTA funding and the National EPSRC XPS User's Service (NEXUS) at Newcastle University. Confocal microscopy was performed at the Kroto Research Institute Imaging Facility.

P-122: Preclinical Musculoskeletal Junction Testbed: Optimisation of a Reproducible Skeletal Muscle Construct

NM Wragg[1], DJ Player[1], Y Liu[2], MP Lewis[1]

[1]*School of Sport, Exercise and Health Sciences, Loughborough University*

[2]***Healthcare Engineering Group, School of Mechanical and Manufacturing Engineering, Loughborough University***

n.m.wragg@lboro.ac.uk

INTRODUCTION: Prior to new material or drug use in humans, a series of preclinical and clinical tests to determine compatibility with human subjects must be passed[1]. Tissue engineered constructs allow for high throughput testing in a tightly controlled environment whilst maintaining strong mimicry of *in vivo* architecture. However, this is often costly in terms of resources and cell numbers. Therefore, it was sought to optimise the manufacturing variables to limit use of resources whilst maintaining characteristics of the *in vivo* tissue. This work proposes that variation in cell density (2, 3 and 4 million cells/mL) and alterations in attachment forces governed by gel width and surface area of attachment will regulate the formation and maturation of tissue engineered skeletal muscle constructs.

METHODS: 3D type-1 rat-tail collagen (2.20mg/mL) neutralised constructs were seeded with C2C12 murine myoblast cells as previously described[3]. The constructs were tethered at either end by bespoke polythene mesh floatation bars to create longitudinal lines of isometric tension *(Fig 1)*. Constructs were placed in 20% FBS high glucose DMEM for 4 days and then cultured in 2% horse serum high glucose DMEM to induce differentiation. Preclinical work relies on high numbers of replicates; therefore, this study is conducted in a scaled down model (0.3mL-0.8mL) to reduce resource use whilst maintaining basic characteristics of the previous model[3] (3mL total volume)

RESULTS: Variation between 2, 3 and 4 million cells/mL of collagen and construct designs (volume and attachment area variation) had no significant effect on contraction time or width reduction (ANOVA P>0.05). Preliminary analysis indicates that the width of the construct has an effect on alignment and higher cell densities produce greater alignment. Physical success of the muscle construct with an extension past the anchor point is 30% greater than without [~100% (n=6) vs. ~70% (n=6)]. High cell densities displayed greater number of unattached cells. Immunohistochemical staining for the intermediate filament protein Desmin showed the capacity for alignment and differentiation of C2C12 myoblasts within the collagen system (Fig. 2).

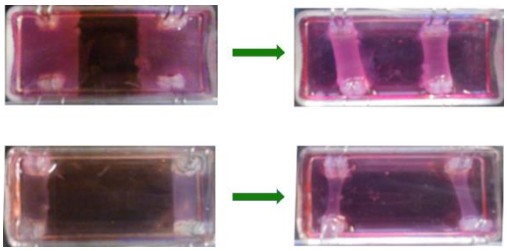


Fig. 1 Muscle construct variables: Observed effects of initial width past anchor point and cell density. (left) Day 0 (right) Day 14 (top), 4million cells/mL (0.8ml, ~15mm initial width) (bottom) 2million cells/mL (0.3mL ~5mm initial width)

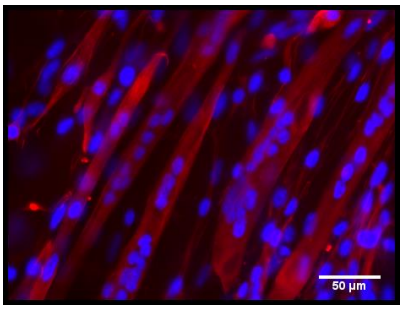


Fig. 2. Immunohistochemistry shows Desmin intermediate filament (red) and DAPI nuclear stain (blue) (40x magnification).

DISCUSSION & CONCLUSIONS:

The optimum extension lies between 0%-50% of the anchor point width based on alignment and physical success of the construct. This data replicates published work in a similar model[3] whilst expanding previous alignment data[4]. Further phenotypic and genotypic analysis is to be completed.

REFERENCES:

1. C. Chuang-Stein (2004), *PS*, *3*(3), 157–159.
2. ISO 10993-5, (2009).
3. A. P Sharples et al (2012), AC, 11(6), 986–95.
4. M. Eastwood et al (1998), *CMC 40*(1), 13–21

ACKNOWLEDGEMENTS: With thanks to the EPSRC Doctoral Training Centre in Regenerative Medicine as the funding body. This work was carried out in affiliation with ARUK.

P-123: Towards the smart prosthesis: Joining materials and biology

M.M. Pardo-Figuerez [1, 2], D.J. Player[1], N.R.W Martin[1], S.D.R. Christie [2], and M.P. Lewis[1]
[1] School of Sport, Health and Exercise Science, Loughborough University, Loughborough, LE11 3TU
[2]Department of Chemistry, School of Science Loughborough University, Loughborough, LE11 3TU
M.M.Pardo-Figueres@lboro.ac.uk

INTRODUCTION:

Prosthetics aimed at restoring natural human functions are becoming very popular forms of limb replacement. Newer prostheses are looking at the integration of different components (i.e. electrical components [(1)]), but real functionality of a robotic limb can be achieved only if the user can control it as part of their own body. This requisite could be accomplished by developing an interface between the natural and the artificial systems so the user can interact with the artificial device in a natural manner. [(2)]
We are focused on this interface between the artificial device and the remaining limb. By joining musculoskeletal biology and materials which can be chemically modified, we will find the optimum conditions compatible with the biological needs, whilst also optimising the prosthesis in terms of mechanical properties: structural integrity, flexibility etc.

METHODS:

Glass slides were chemically modified by attaching different polymers chains to the surface. The chemical modifications were performed attending to the different functionality of the end groups: PHEMA (-OH), PMMA ($-CH_3$), APTES ($-NH_2$) BIBB (-Br), PMETAC ($-N^+CH_3)_3Cl^-$), PKSPMA ($-SO_3^-K^+$), plastic (polystyrene treated) and glass coverslips (glass no coated). 50,000 cells per well of SHSY-5Y Neuroblastoma cells were seeded onto each surface. Cells were cultured in DMEM media, 10% FBS, and 1% P/S until a 50% of confluence was reached. Neurite length was measured at this stage as pre–RA (pre-retinoic acid); surfaces were subsequently cultured in similar media containing 10 µM retinoic acid (RA). Neurite length was measured at day 1, 3, and 5 in RA media (DM1, DM3, and DM5).

RESULTS:

Neurite length increased up to 73µm in SHSY-5Y cells cultured on the BIBB surface after 1 day in RA. Differences in the neurite length could be observed between coated and non coated glass.

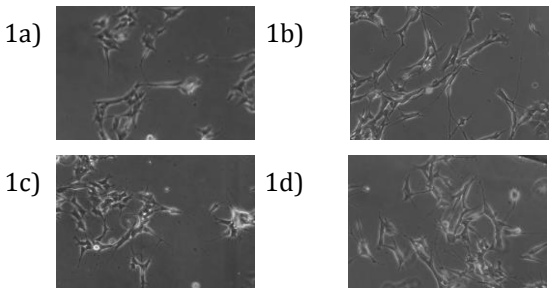


Figure 1. a)BIBB surface PRE-RA b)BIBB surface at DM1 c)Glass no coated PRE-RA d)Glass no coated at DM1 Scale bar = 50µm.

DISCUSSION & CONCLUSIONS:

The mechanisms of cell interaction with foreign surfaces are known to be influenced by various factors such as hydrophilic/hydrophobic balance in the surface [(3)]. Here we show that neurite length is at least partly dependent on the interaction of the cell and the surface to which it adheres, inducing their differentiation, and/or influencing their survival. Gene expression analysis will subsequently be undertaken to identify the specific cell phenotype (e.g. motoneuron, sensory neuron) induced by each chemical modification. Furthermore, different polymers (e.g. block copolymers, different monomers with different end groups) will be used to determine which specific conditions influence cell behaviour, helping us to obtain the requirements for cellular survival and differentiation in an adequate material.

REFERENCES:

1) T.Stieglitz et al. (2005) *IEEE Eng Med Biol Mag*. **24**, 58–65.
2) S. Micera. et al(2008) . *IEEE Trans Neural Syst Rehabil Eng.* **16**, 453–472
3) A. Kostadinova et al (2013) *Bulg. J. Agric. Sci.* **19**, 178–181

ACKNOWLEDGMENTS:

This work was supported by Loughborough University.

P-125: Effect of nano- and microscale roughness on cellular behaviour

Sotiria Toumpaniari [1,2], Mark Birch[1,3], Andrew McCaskie[1,3] and Kenny Dalgarno [1,2]
[1] Arthritis Research UK Tissue Engineering Centre, Institute of Cellular Medicine, Newcastle University, United Kingdom
[2] Mechanical and Systems Engineering, Newcastle University, United Kingdom
[3] Orthopaedic Research Unit, University of Cambridge, United Kingdom
s.toumpaniari@ncl.ac.uk

INTRODUCTION:

Apatite/Wollastonite (A/W) is a glass-ceramic that combines biocompatibility, bioactivity, osteoconductivity and bioresorbability[1] that can be used for bone scaffolds. The objectives of this study were to investigate how the difference of micro and nano- roughness affect the human mesenchymal stem cell behaviour.

METHODS:

A/W glass powder (GTS, Sheffield) was milled and then sieved to separate the particle fractions of <20μm, 20-53μm and 54-90μm. A/W powder was mixed with water resulting in a slurry and sintered at 1150ºC for 1 hour. Two different groups were fabricated with particle range 20-53μm (AW1) and combination of 54-90μm:<20μm (80:20) (AW2) to vary the surface roughness. Surface profile was determined using interferometry and morphology through SEM. Immortalised human MSCs were used to evaluate cell number and alkaline phosphatase (ALP) activity. Gene expression was quantified using real- time PCR. Statistical analysis was performed using a student's t-test.

RESULTS:

Profilometry revealed a significant difference in surface roughness between AW1 scaffolds (Ra=397nm) and AW2 (Ra=2.2μm).
Alkaline phosphatase activity showed lower levels on A/W scaffolds compared with control surfaces. Scaffolds with smoother surfaces have elevated alkaline phosphatase levels in both basal and osteogenic media in comparison to rougher surfaces (Figure 1).

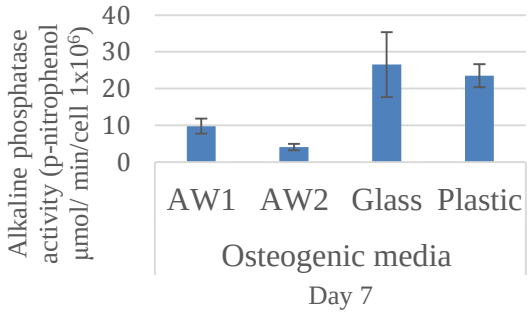


Fig 1: Normalised ALP activity of immortalised human MSCs cultured on various surfaces for 7 days.

DISCUSSION & CONCLUSIONS:

According to previous research, roughness stimulates osteogenesis[3]. However, the increase of ALP activity on the smoother surface can be explained by variations in cell number that could be due to primary cellular adhesion.

REFERENCES:

1. J. A. Dyson et al. (2007), Tissue Engineering 13:2891-2901
2. T. Kokubo et al. (1991), Biomaterials 12:155-163
3. J. Costa-Rodrigues et al. (2012), Acta Biomaterialia (8:1137-1145)

ACKNOWLEDGMENTS:

The authors would like to acknowledge the support of Arthritis Research UK (Award 19429), the EPSRC, and the FP7 RESTORATION project (Award CP-TP 280575-2).

P-127: POPULATION KINETICS OF MSC'S IN THE DEVELOPMENT OF BIO-ARTIFICIAL BONE SCAFFOLDS

Sylvia Müller[1,] Lyndsey Nicholson[1], Julian R. De Havilland[2*], Anne Dickinson[1], Xiao Nong-Wang[1]
[1] Institute of Cellular Medicine, Newcastle University, UK
[2*] Newcastle Cellular therapies facility, Newcastle University, UK
s.muller@ncl.ac.uk

INTRODUCTION:

Our group is assessing the potential for Mesenchymal Stem Cells (MSC's) to populate synthetic biomaterials for manufacturing scaffolds to support the growth and differentiation of MSC's into osteocytes for bone repair in osteoarthritis. We compared the use of non-haematopoietic medium with MSC basal medium across three cultures of bone marrow derived MSCs taken from the femoral heads of osteoarthritis patients. No difference was found in doubling potential, and growth rate using either growth media.

METHODS:

Selecting the use of MSC growth media to promote growth of undifferentiated, plastic adherent MSC's, we cultured MSCs through six passages, testing their tri-lineage potential to differentiate into osteocytes, chondrocytes and adipocytes at early, mid and late time points. We also assessed the surface phenotype by flow cytometry of undifferentiated MSCs against the ISCT standard classification guidelines (>95% expression of CD73, CD90 & CD105 with <2% CD14, CD19, CD34, CD45 and HLA-DR).

RESULTS:

Results showed the MSC's cultured could be differentiated into the three specific lineages and demonstrated conformance with the ISCT classification, except for CD34. Data showed no presence of CD34 in early cultures, but in two of three experiments, by day 50 in culture CD34 positivity was 3% and 8% - exceeding the classification criteria by this single parameter. By day 89 in culture, CD34 expression advanced to 15% and 46%. Those CD34+ cells remained >95% positive for CD73, CD90 & CD105.

DISCUSSION & CONCLUSIONS:

The data show that in our culture conditions, there is potential for MSC's to upregulate CD34 expression, whilst retaining all the other surface markers identifying them as MSC's. The retention of CD73 expression and the preferential culture medium used mitigates against the suggestion that CD34+ cells are haematopoietic cells, and instead suggests expansion of CD34+ MSC-like cells. The impact of this occurrence in manufacturing cells for regenerative medicine therapies is currently being assessed.

ACKNOWLEDGMENTS:

The authors would like to thank Arthritis Research UK for providing financial support to this project.

P-128: The use of polymersomes in stem-cell specific targeting for bone regeneration

Edoardo Scarpa[1], Joanne L. Bailey[2], Agnieszka A. Janeczek[1], Richard O.C. Oreffo[1] Tracey A. Newman[2] and Nicholas D. Evans[1]

[1] Bone and Joint Research Group, Institute of Developmental Sciences, Southampton General Hospital, Tremona Road, Southampton, SO16 6YD, UK. [2] Clinical Neurosciences, Building 85, Highfield Campus, University of Southampton, Southampton, SO17 1BJ, UK.

E.Scarpa@soton.ac.uk

INTRODUCTION:

Musculoskeletal disorders in the UK cost ~£5billion/year, and new therapies are urgently required[1]. Mesenchymal stem cells (MSCs) are fundamental in bone regeneration given their intrinsic capacity to differentiate into osteoblasts. Wnt signalling plays an essential role in the osteoblastic differentiation of MSCs[2], but can have stimulatory or inhibitory effects depending on the timing of delivery. Polymersomes (PMs) are nanosized carriers composed of amphiphilic block copolymers that can be loaded with hydrophilic and hydrophobic compounds, and can be engineered for specific targeting. Our hypothesis is that PMs can deliver Wnt agonists specifically to populations of MSCs to induce bone regeneration.

METHODS:

PMs were produced via nanoprecipitation using transactivator of transcription (TAT) conjugated polyethylene glycol 5.8K (PEG)-b-poly-ε-caprolactone 19k (PCL) block copolymers. PMs were loaded with 0.1 M sodium fluorescein; at this concentration the dye inside PMs is self-quenched, but fluoresces upon release. Loaded PMs were sized by dynamic light scattering or added to a murine fibroblast cell line (L929) for evaluation of cellular uptake and cytotoxicity using live imaging and FACS.

RESULTS:

PMs were produced with an average diameter of 85.90±33.24nm. FACS analysis demonstrated that after 3hrs of incubation, 92.53% of cells had internalised loaded PMs. Cytotoxicity, measured by propidium iodide (PI) staining demonstrated only 3.27% dead cells after 24 hrs.

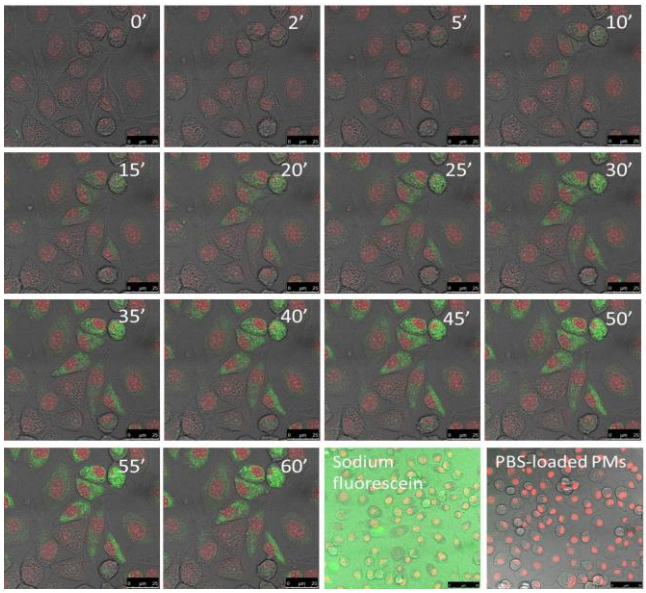


Fig. 1 Live imaging of L929 cells incubated with 1 mg/ml of sodium fluorescein-loaded TAT-PMs

DISCUSSION & CONCLUSIONS:

These results demonstrate that PEG-PCL PMs can be loaded with a hydrophilic cargo, are quickly internalised into cells and are able to release their payload without cytotoxic effects. These characteristics make polymersomes a promising candidate for targeted delivery in bone regeneration.

REFERENCES:

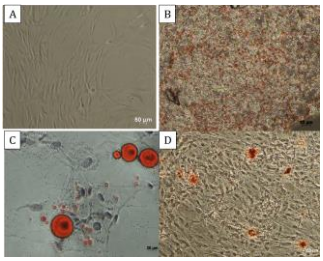
1. R. Dimitriou et al. *BMC med (*2011) **9,** 66.
2. Baron, R. and Kneissel, M. *Nat Med* (2013) **19,** 179-192.

ACKNOWLEDGMENTS:

Authors wish to thank the Institute for Life Sciences (Ifls) Southampton and the Wessex Medical Research charity for funding.

P-130: Limbal Mesenchymal Stem Cells; Alternative Stem Cells for Transplantation

Bakiah Shaharuddin[1,2] Charles Osei-Bempong[1] Sajjad Ahmad[3,4] Paul Rooney[5] Simi Ali[6]
Rachel Oldershaw[7] Annette Meeson[1]
[1]Institute of Genetic Medicine, Newcastle University, Newcastle Upon-Tyne, U.K
[2]Advanced Medical and Dental Institute, Universiti Sains Malaysia, Pulau Pinang, Malaysia
[3]St Paul's Eye Unit, Royal Liverpool University Hospital, Prescot Street, Liverpool, UK.
[4]Department of Eye and Vision Sciences, Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, UK.
[5]Tissue Development Laboratory, NHS Blood and Transplant, Liverpool, UK.
[6]Institute of Cellular Medicine, Newcastle University, Newcastle Upon-Tyne, UK.
[7]Musculoskeletal Biology I, Institute of Ageing and Chronic Disease, University of Liverpool, Leahurst Campus, Chester High Road, Neston, Cheshire, CH64 7TE
b.shaharuddin@ncl.ac.uk



INTRODUCTION:
Ex vivo expanded limbal stem cell (LSC) transplantation using amniotic membrane as a carrier system has been used as a cellular-based therapy to reconstruct the ocular surface. Here we look at a population of cells isolated from the limbus region that have the characteristics of mesenchymal stem cells (MSC).

METHODS:
Limbal epithelial cells were isolated from cadaveric corneo-scleral rims and the cells resuspended in a MSC growth promotion medium. Immunophenotyping for expression of cell surface antigens known to be expressed by human MSCs (CD44, CD90, CD105, CD106, CD146 and CD166), hematopoietic lineage markers CD19 and CD45 and expression of MHC Class I and Class II were performed. The ability for these cells to undergo tri-lineage differentiation (chondrogenic, adipogenic and osteogenic) was determined and lineage differentiation confirmed with histological staining and mRNA expression. Chemotaxis transwell assays were performed to study cell migration. Cells were also plated on to cryopreserved amniotic membrane to determine if they would adhere, and proliferate.

RESULTS:
Limbal MSC were adherent, rapidly proliferated on plastics and were positive for antibodies specific to human MSC, and negative for markers of lineage committed haematopoietic cells. They stained positive for mineralisation (Alizarin Red), cartilaginous deposition (Safranin O) and oil droplets (Oil Red O) on histology and expressed markers of differentiation to the three lineages at mRNA level. Limbal Mesenchymal stem cells (LMSC) showed high expression of CXCR4. Transwell migration analysis demonstrated LMSC migrated at higher rates than human corneal epithelial cells and breast cancer cells MDAMB231 ($P<0.05$). LMSC were also able to grow and proliferate on amniotic membrane.

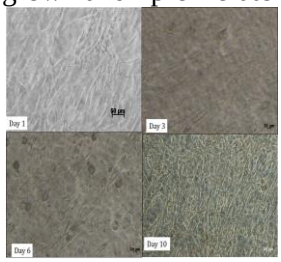


***Figure 1**. Histology of LMSCs. A) undifferentiated B) Cartilaginous deposition stained by Safranin O for chondogenic differentiation C) Osteogenic differentiated cells stained with Oil Red O. D) Osteogenic differentiated cells show evidence of mineralisation by Alizarin Red staining.*
***Figure 2**. Growth and proliferation of LMSC on cryopreserved amniotic membrane over ten days*

DISCUSSION & CONCLUSIONS:
Limbal mesenchymal stem cells can be successfully isolated from cadaveric corneo-scleral rings and grown initially in low oxygen (5%) in mesenchymal growth promotion media. These cells showed classic expression of human MSC markers. Using our tissue culture protocols, limbal MSC show tri-lineage differentiation as confirmed by histology and mRNA expression. Amniotic membrane (AM) as a niche for limbal stem cell transplantation is a widely acceptable practice. LMSC adhere to the AM and proliferated rapidly on this tissue substrate. The potential of these cells to contribute to improved outcomes in tissue transplantation bears further investigation.

REFERENCES:
1. Pellegrini, G et al (1997),*The Lancet*, **349**(9057), pp. 990-993. 2. Sun, T.T et al (2010), *Nature*, **463**(7284), pp. E10-E11.3. Knuth, C et al (2013), *Stem Cell Reviews and Reports*, **9**(5), pp. 599-608.

P-132: Low Density Culturing Maintains hMSCs in Their Highly Proliferative State and Improves Their Osteogenic Differentiation Potential

Richard Balint[1] and Sarah H. Cartmell[1*]
[1]School of Materials, The University of Manchester, UK
sarah.cartmell@manchester.ac.uk

INTRODUCTION:

Human Mesenchymal Stem Cells (hMSCs) enjoy widespread use in tissue engineering and regenerative medicine. Hundreds of clinical trials are performed and numerous articles are published each year linked to this very important adult stem cell type. However, reliable expansion of hMSCs, without compromising their stem cell capabilities, remains a challenging task. In this study the effect of expanding hMSCs using the traditional method (from 40% to 70% confluence – Protocol A) was compared to a low cell density technique (from 10% to 50% confluence – Protocol B) on proliferation, and osteogenic and adipogenic differentiation potential.

METHODS:

Commercial hMSCs were expanded to passage 4 using either the Protocol A (40% to 70%) or Protocol B (10% to 50%) method and were used for experiments at passage 5. To assess the proliferation rate cells were seeded at 1575 and 3150 cell/cm^2. Cell numbers (n=6) were measured each day up to day 4 using the PicoGreen assay. To assess differentiation potential cells were seeded at 3150 cell/cm^2 and cultured for 14 days in growth, osteogenic and adipogenic medium. Samples at day 14 were assayed for cell numbers (PicoGreen); gene expression of osteogenic (alkaline phosphatase, collagen type I, osterix, osteopontin, osteocalcin) and adipogenic markers (adiponectin, leptin) (qRT-PCR); alkaline phosphatase activity; and lipid formation.

RESULTS

Protocol B cells produced greater cell numbers and showed a significantly higher (+10-15%) proliferation rate. At day 14 Protocol B samples contained 23% more cells in growth, 12% more in cells in osteogenic and 78% more cells in adipogenic medium compared to Protocol A. Differentiation marker gene expression was comparable between the two protocols. Alkaline phosphatase activity was three times higher, while lipid levels were significantly lower when normalised to cell numbers, with Protocol B.

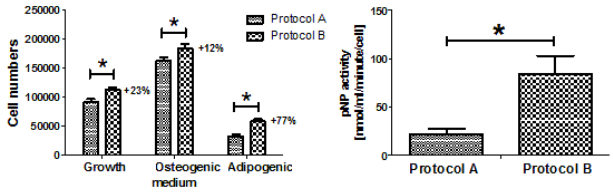


Fig. 1 – Cell numbers in growth, osteogenic and adipogenic medium (lelft) and alkaline phosphatase activity in osteogenic medium (right) at day 14

DISCUSSION & CONCLUSIONS:

Our results show that low density culturing improves the proliferation rate of hMSCs, not just during expansion but in differentiation experiments as well. This can potentially have a great impact on both research and clinical applications. The two culturing methods were comparable at the gene expression level, however at the secretional level Protocol B cells showed better osteogenic, while Protocol A cells showed better adipogenic differentiation. This study highlights the importance of identifying the optimal culture conditions for stem cells.

ACKNOWLEDGMENTS:

The authors would like to thank Orthopaedic Research UK and the EPSRC DTA award for funding this work.

P-133: Corneal stromal cells: A potential cell source for ocular surface regeneration

Laura Sidney, Matthew Branch, Owen McIntosh, Harminder Dua, Andrew Hopkinson
Academic Ophthalmology, Division of Clinical Neuroscience, University of Nottingham, UK
laura.sidney@nottingham.ac.uk

INTRODUCTION:

Keratocytes of the corneal stroma are quiescent, dendritic cells, which maintain the extracellular matrix. Upon injury, or during *in vitro* culture in serum, keratocytes differentiate into a fibroblastic repair phenotype[1]. It is believed that there is a subpopulation of keratocytes that act as multipotent progenitor cells[2]. These cells repopulate the stroma after damage and may play a role in the regeneration of other layers of the cornea, such as the epithelium.

METHODS:

Primary human corneal stromal cells (hCSC) were extracted from corneal rims. The optimum culture media was determined from a selection available, and the effect on cell phenotype, of passaging and expanding the cells was assessed. *In vitro* differentiation of hCSC into mesenchymal lineages was investigated, alongside the potential for differentiation back into a stromal keratocyte phenotype and transdifferentiation into corneal epithelial cells.

RESULTS:

The phenotype of hCSC is strongly affected by extraction and passage (figure 1). Culture media used also has an effect on cell phenotype. Under the correct conditions, hCSC show potential as a multipotent stem cell, expressing the indicative markers and differentiating *in vitro* down osteogenic, chondrogenic and adipogenic lineages.

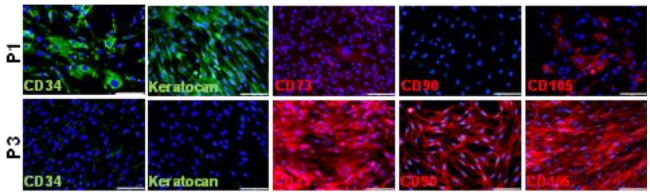


Figure 1: Passaging of hCSC affects cell phenotype. At passage 1 (P1) cells express high levels of CD34 and keratocan, but low levels of CD73, CD90 and CD105. This is the reverse at passage 3 (P3).

CD34$^+$ cells can be isolated from hCSC at low passage. This population demonstrates a higher stem cell potential than the unsorted cells. Under the correct conditions, CD34$^+$ hCSC show evidence of differentiation to corneal epithelial cells, showing a rounded morphology and high expression of key markers such as cytokeratin 3 (KRT3), transcription factor HES1, and desmoglein 3 (DSG3) (figure 2).

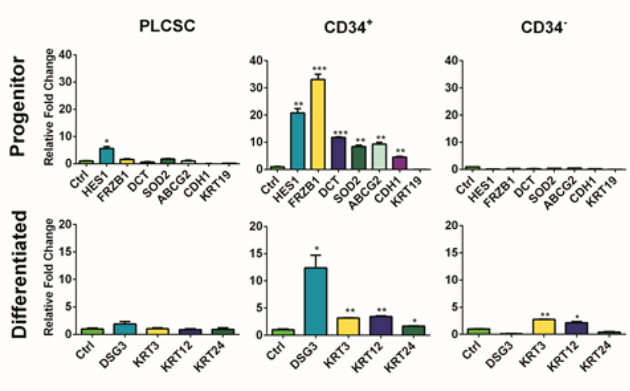


Figure 2: Differentiation of CD34$^+$hCSC to corneal epithelial cells. RT-qPCR showing significant upregulation of corneal epithelial genes in CD34$^+$ cells.

DISCUSSION & CONCLUSIONS:

Corneal stromal cells demonstrate a multipotent stem cell potential, independent of the role they are traditionally associated with in the cornea. *In vitro*, this potential may depend on the culture environment and on isolation of certain subpopulations. In future, these cells show potential in the regeneration of the ocular surface in cases of disease or trauma.

REFERENCES:

1. Poole et al. (1993), *J Cell Sci*, **106**: 685–91.
2. Pinnamaneni and Funderburgh. (2012), *Stem Cells*, **30**: 1059-63.

ACKNOWLEDGEMENTS:

This work was funded by the Royal College of Surgeons Edinburgh and Fight for Sight.

The TCES gratefully acknowledges the generous support from the following sponsors:

EPSRC Centre for Innovative Manufacturing in Regenerative Medicine

The EPSRC Centre for Innovative Manufacturing in Regenerative Medicine is one of the few centres in the world providing academic and industrial translational research in regulated regenerative medicine manufacturing. The EPSRC Centre:

- Creates next generation platforms for manufacturing regenerative medicines
- Tests and implements ideas in clinical and industrial settings
- Informs business models, policy and public debate

The EPSRC Centre is a collaboration between Loughborough, Nottingham and Keele universities and is developing a UK regenerative medicine manufacturing community through its national engagement strategy. Recent activities have include raising careers awareness among regenerative medicine early career researchers and leading a new schools programme engaging learners of ages 5-19 with the variety of science and engineering research underpinning the field of regenerative medicine.
www.epsrc-regen-med.org
www.heartblog.net

BOSE
Better products through research®

Bose Corporation is a leading supplier of materials testing and durability simulation instruments to research institutions, universities, medical device companies, and engineering companies worldwide. Bose® ElectroForce® test instruments help customers design better products and get them to market faster. Proprietary linear motors developed by Bose are at the heart of ElectroForce instruments, and they provide exceptional performance and energy-efficiency without compromising either.

Bose test instruments provide exceptional dynamic performance and precision for a variety of testing applications, including characterization of engineered materials, soft tissues and biomaterials; biomechanical testing and tissue growth solutions; fatigue testing of components used in industrial and consumer applications; and durability simulation of medical devices, including stents, endovascular grafts, and orthopaedic implants.

Panasonic

Panasonic Biomedical Sales Europe BV are specialists in the sales and support of temperature controlled biomedical laboratory products for sample preservation and storage, cell and tissue culture and in-lab sterilization. Our latest generation of products are designed to provide high performance, energy efficiency and sample security.

don whitley scientific

Don Whitley Scientific Limited is a leading international supplier of innovative equipment and services to the microbiology and tissue culture industries. We develop, manufacture, market and service instrumentation and associated products for the public and private sector - primarily for use in hospitals, public health laboratories, food testing organisations, pharmaceutical companies and research institutions. Brands include: Whitley Workstation, Hypoxystation, RABIT (Rapid Automated Bacterial Impedance Technique), and WASP (Whitley Automated Spiral Plater). We also provide extensive contract research microbiology from our UK-based GLP-compliant laboratories. We offer maintenance and repair contracts for a variety of laboratory equipment, not just the products we sell and distribute. As official distributors for AES, Rodwell, Copan, Ebers and Synoptics, our engineers are trained by the actual manufacturers – training that is not available to everyone. We are also accredited to BS EN ISO/IEC 17025:2005 to carry out calibration and validation services for heat sterilisation equipment, temperature controlled processes and temperature indicators.

www.dwscientific.co.uk
Tel: 01274 595728
Email: sales@dwscientific.co.uk

EBERS Medical Technology SL Profile

EBERS Medical Technology SL develops, manufactures and commercialises devices for research in cell culture, with special emphasis in the field of **bioreactors and culture chambers for tissue engineering**. Such bioreactors try to reproduce in an in-vitro culture environment, some of the conditions which cells experience when growing in-vivo by using two different approaches: the application of controlled flow rates or the direct deformation of the scaffolds in which cells are seeded.

The TEB series bioreactors allows a wide variety of **flow profiles on the cultures**. Used in combination with any of the available EBERS culture chambers, it creates both perfusion and perifusion conditions to culture cells on porous **bone-type scaffolds** as well as on **cylindrical vessel-type scaffolds**.

The TC-3 bioreactor allows applying**direct compression on the scaffolds** to simulate in the culture the deformation occurring in the in-vivo tissues. Tension and compression can be alternatively applied to simulate the growing conditions in ligament, tendon or bone scaffolds. Moreover, our experienced engineering offers a consultancy service for the adaptation of bioreactors and culture chambers to the particular demands of each researcher.

http://ebersmedical.com/
Tel: (0034) 876 013 826

Bioquell are the world leaders in the use of hydrogen peroxide vapour (HPV) technology in the bio-decontamination of rooms, safe transfers of materials and the manufacturing and filling of biopharmaceuticals under aseptic conditions. With a comprehensive range of instruments, consumables, services and validation, Bioquell assures the highest levels of performance whilst maximising uptime for facilities it serves. Whether you are performing sterility tests, manufacturing products or filling vials and syringes; an aseptic environment can make all the difference. From specific isolators for manufacturing and filling, to transfers of materials in and out of cleanrooms, Bioquell HPV bio-decontamination technology eliminates doubt.

Cambridge Bioscience is a leading distributor of life science products with a passion for bringing new and exciting technologies to researchers. Working with over 50 specialist suppliers around the world, we offer an innovative, extensive and diverse range of high quality products, services and instruments supporting research in the areas of cancer, cardiovascular research, cell culture, cell analysis, endocrinology, epigenetics, inflammation, immunity, metabolism, molecular biology, neuroscience, regenerative medicine and much more.

Cambridge Bioscience - A Great Choice For Life Science Research

Cambridge Bioscience, Munro House, Trafalgar Way, Bar Hill, Cambridge, CB23 8SQ

Tel: +44 (0)1223 316 855; Fax: +44 (0)1954 781 323
Email: support@bioscience.co.uk
Website: www.bioscience.co.uk

World Precision Instruments represent CellScale in Europe. CellScale is an industry leader in biomaterial and mechanobiology test systems.

Their mechanical test systems are specifically designed for characterizing the material properties of biomaterials. With integrated features such as image capture and analysis, media chambers, and a range of gripping mechanisms, their biaxial and micro-scale compression test systems are capable of generating high quality test data from day one.

Their mechanobiology technologies help advance research by providing insights into the response of

cells to mechanical stimulation. CellScale has systems optimized for high-throughput, real-time imaging, and 3D scaffold use. In addition to their standard products, CellScale can also develop a custom solution for your application.

Our products are used by researchers around the globe to study everything from tissue strength and stiffness to cellular response.

Our new name reflects the strategic direction for the company and positions all of our brands under one complete portfolio. Our brands:

R&D Systems entered the field of biotechnology early, becoming the first company to commercially market the multifunctional cytokine TGF-beta 1. R&D Systems has since established itself as a developer and manufacturer of high-quality proteins and the world leader in immunoassays as well as a producer of antibodies, arrays, stem cell products, cell selection and detection products among other cell biology tools

Tocris is the leading supplier of biologically active molecules for life science research. Used in experiments to understand biological processes and diseases and as part of the initial drug-discovery process, Tocris life science reagents include receptor ligands, ion channel modulators, enzyme inhibitors, caged compounds, fluorescent probes, and screening libraries.

Boston Biochem is the leading developer of ubiquitin-related research tools that facilitate basic research and drug discovery efforts. Ubiquitin has been the focus of an emerging field of research, and ubiquitination has been found in a wide variety of cellular processes and implicated in the genesis of several diseases and genetic disorders.

Together, we are Bio-Techne. Please come and find out more by visiting our stand 39

Orla Protein Technologies Ltd designs, engineers and manufactures proteins, peptides, antibodies and antigens for immobilisation on surfaces. Orla's elegant technologies preserve protein structure, function and bioactivity and underpin our products for cell biology, stem cell research, assay development and bioanalytical surfaces. Radically simplify and streamline your workflow with Orla's innovative products and services. Optimise tissue culture conditions in one quick and easy experiment with ready-to-use OrlaExplorer plates pre-coated with animal-free proteins known to enhance cell attachment, differentiation and behaviour. Reduce your use of expensive growth factors and cytokines by using Orla's surface immobilised, animal-free growth factor and cytokine plates.

JRI Orthopaedics Ltd is a UK manufacturer of innovative and quality orthopaedic solutions for healthcare providers and patients worldwide. The company was established in 1970, by Mr Ronald Furlong FRCS a consultant orthopaedic surgeon at St Thomas' Hospital, London, and in 1977 manufacturing facilities were set up in Sheffield to produce total hip replacement prosthesis to Mr Furlong's design.

In 1985, Mr Furlong introduced the world's first hydroxyapatite coated hip stem and in 1993, JRI received the Queens Award for Technological Achievement for this innovation. Today, the Furlong HAC total hip system has achieved clinical success globally and continues to be one of JRI's flagship products. The company has gone to develop other innovative hip replacements prostheses and instrumentation and in 2012 introduced the VIAOS Shoulder arthroplasty system. JRI also has an expanding portfolio of co-development projects, which include regenerative medicine products. JRI has an expanding portfolio of governement grants from the UK and the EU for co-development of new technologies.

JRI is a medium sized British Company, wholly-owned by a charity, Orthopaedic Research UK, to which it provides significant funds to dedicate to the advancement of orthopaedic research and clinician education. The new state-of-the-art manufacturing facility in Sheffield includes

modern computerised Vacuum Plasma Spraying, CNC machinery and measuring technology. Research, product development, customer services, marketing, warehousing and distribution are also based at the same site.

PeproTech is the trusted source for the development and manufacture of high quality cytokine products for the life-science and cell therapy markets.

With over 2,000 products, PeproTech has developed and refined innovative protocols to ensure quality, reliability and consistency.

- PeproTech offers customers a comprehensive line of products for culturing and maintaining several specific cell types
- Most of the Cell Culture Media products are animal component free, complete, chemically-defined compositions
- Products support the growth of specific cell types in a manner that is comparable, if not superior, to growth seen with serum containing media formulations

European Headquarters, PeproTech House, 29 Margravine Road, London W6 8LL
Email: info@peprotech.co.uk

Collagen Solutions Plc manufactures medical grade collagen components for use in regenerative medicine, medical devices and in-vitro diagnostics. Our current products range from minimally processed to highly purified collagen formulations.

Our capabilities include the provision of soluble and powdered collagen formulations and also the development and contract manufacture of tissue scaffolds and medical devices. Collagen Solutions products are produced from BSE-free bovine Type I collagen supplied from a source that has been providing materials for use in FDA and EMEA-approved medical devices for more than 20 years. The material has BSE-free status, is from a Geographical BSE Risk I country (GBR I) and has full sourcing and traceability upon receipt into our own facilities, including EDQM certification. Collagen Solutions manufacturing facility also holds its own ISO 13485 and ISO 22442 certifications.

In addition to the provision of our own collagen products we work with clients to develop their products and processes, providing contract manufacturing and distribution services for a range of markets and territories.

At Collagen Solutions we believe we have a unique offering which goes beyond raw material supply and contract manufacture. Through our expertise, we can add value and assist customers exploring other opportunities for their products and technologies.

CELLEUROPE

Marie Curie Multi Partner Initial Training Network: Developing a future generation of entrepreneurial researchers and investigating biomarkers and cellular therapies for improving haematopoietic stem cell transplantation outcome

The goal of the research is to gain insight into immunobiology of haematopoetic stem cell transplantation (HSCT) including graft versus leukaemia (GvL) and graft versus host (GvHD) effects, in order to improve current therapies and develop and test novel ones via clinical trials and/or animal model experiments. The research is therefore necessarily multidisciplinary including clinical medicine, immunology, genomics, proteomics, molecular biology and pathology.

Twelve researchers, nine of whom are Early Stage Researchers in the first four years of their research career, will be trained with the knowledge to understand the immunobiology of HSCT and develop skills to assess and identify biomarkers and novel targets for therapy for graft versus leukaemia (GvL) and graft versus host (GvHD).

Involvement of the private sector aims to enhance the intersectoral employability of the researchers and training in current Good Manufacturing Practice (GMP) for both clinical and non-clinical scientists.

Programme: FP7: MARIE CURIE ACTION: INITIAL TRAINING NETWORKS (ITN)

MULTI PARTNER, PART OF THE 'PEOPLE' WORK PROGRAMME. *Call Reference FP7-PEOPLE-2012-ITN*

Collaborative Project – Consortium of 10 partners

Project Coordinator: Anne Dickinson, Newcastle University, UK
Principal Investigator: Professor Anne Dickinson, Institute of Cellular Medicine, Newcastle University
Website: http://celleurope.eu

List of Delegates

First Name	Last Name	Insitution
Dave	Adams	Liverpool University
Peter	Agbekoh	University of Strathclyde
Sajjad	Ahmad	Liverpool University
Afran Mohammed	Aladdad	University of Nottingham
Naif	Alharbi	Newcastle University
Hanaa	Alkharobi	University of Leeds
Noura	Alom	University of Nottingham
Amr	Alraies	Cardiff University
Moyassar	Al-Shaibani	Newcastle University
Mahetab	Amer	University of Nottingham
Peter	Andrews	University of Sheffield
Angela	Armiento	University of Aberdeen
Ilida Ortega	Asencio	University of Sheffield
Sadaf	Atarod	Newcastle University
Steven	Avery	Cardiff University
Yanny Marliana	Baba Ismail	Keele University
Abdulrahman	Baki	University of Nottingham
Richard	Balint	University of Manchester
Ioannis	Bantounas	University of Manchester
Ivana	Barbaric	University of Sheffield
Katie	Bardsley	University of Keele
Amanda	Barnes	University of York
Mike	Barrow	Liverpool University
Gillian	Bell	Newcastle University
Richard	Bennet	Panasonic
Matthew	Benning	Newcastle University
M Riduan	Bin Mohamad	University of Strathclyde
Steven	Blagden	WPI
Lucy	Bosworth	University of Manchester
Mark	Bradley	University of Edinburgh
Jessica	Bratt	Keele University
Gemma	Bray	University of Nottingham
Oana	Bretcanu	Newcastle University
Jack	Bridge	University of Nottingham
Emily	Britchford	University of Nottingham
Gary	Brookes	ReNeuron
Robert	Brown	UCL
Anthony	Bullock	University of Sheffield
Darren	Burke	Bose
Graham	Busby	Collbio
Lee	Buttery	University of Nottingham
Kate	Cameron	University of Edinburgh
Andy	Carr	Oxford University

Sarah	Cartmell	University of Manchester
Gabriel	Cavalli	Surrey University
Rehma	Chandaria	University of Nottingham
Amit	Chandra	University of Loughborough
Julian	Chaudhuri	University of Bradford
Jinju (Vicky)	Chen	Newcastle University
Aixin	Cheng	University of Manchester
Alisha	Chhatawal	Durham University
Sanchita	Chowdhury	University of Nottingham
Stuart	Cain	University of Manchester
James	Clarke	The University of Sheffield
Kirsty	Clarke	Durham University
Philippa	Clarkson	University of Leeds
Joanne	Comenge	Liverpool University
Sharon	Crouch	University of Nottingham
Zhangfeng	Cui	University of Oxford
Sophie	Dale-Black	Loughborough University
Kenny	Dalgarno	Newcastle University
Katie	Daniels	TSB
Davide	Danovi	King's College London
Neil	Darroch	R&D Systems
Jonathan	Dawson	University of Southampton
Adam	Day	UCL
Richard	Day	UCL
Robert	Deans	Athersys Inc
Fabio	d'Agostino	Newcastle University
Maria del Carmen	de Andres Gonzalez	University of Southampton
Cosimo	de Bari	University of Aberdeen
Lindsey	Dew	University of Sheffield
Anne	Dickinson	Newcastle University
James	Dixon	University of Nottingham
Phil	Driver	University of Cambridge
Pengfei	Duan	Newcastle University
James Duan	Dugan	University of Sheffield
Reem	El-Gendy	Leeds school of dentistry
Alicia	El-Haj	Keele University
Hoda M	Eltaher	University of Nottingham
Tuula	Eriksson	University College London
Nicholas	Evans	University of Southampton
Owen	Evans	University College London
David	Farrar	Smith & Nephew
Francisco	Figueiredo	Newcastle University
Miriam Veronica	Flores Merino	Autonomous University of the State of Mexico
Stuart	Forbes	University of Edinburgh
Nicholas	Forsyth	Keele University

James	Fox	University of York
Robin	Franklin	University of Cambridge
Peter	Freeman	University of Edinburgh
Paul	Genever	York University
Giulia	Gigliobianco	University of Sheffield
Hamish	Gilbert	University of Manchester
Adam	Glen	University of Sheffield
David	Gothard	University of Southampton
Julie	Gough	University of Manchester
Helen	Grant	University of Strathclyde
John	Gray	CM Technologies
Liam	Grover	University of Birmingham
Shukry	Habib	King's College London
Omar	Hamid	University of Nottingham
Ross	Hawkins	NIBSC
David	Hay	University of Edinburgh
John	Haycock	Sheffield University
Thomas	Heathman	Loughborough University
Frances	Henson	University of Cambridge
Chris	Hewitt	DTC
Zoe	Hewitt	University of Sheffield
Araida	Hidalgo	Manchester Metropolitan University
Sam	Hollings	University of Leeds
Christine	Horejs	Imperial College London
Rachel	Howard-Jones	Cardiff University
Claire	Hutchinson	Liverpool University
Johan	Hyllner	CT Catapult
Imran	Iqbal	Imperial College London
Eleanore	Irvine	Biogelx
Andrei	Ivanenko	Bose
Colin	Jahoda	Durham University
Agnieszka	Janeczek	University of Southampton
Marcus	Johns	University of Bath
Julia	Jones	UCL
Tammy	Kalber	Liverpool University
Faysal	Karagoly	University of Bath
Helen	Kelt	Newcastle University
Caitlin	Kerr	Jellagen Pty
Aliaa	Khadre	University of Leeds
Ilyas	Khan	Swansea University
Cay	Kielty	University of Manchester
Susan	Kimber	University of Manchester
Paul	Kingham	Umeå University- Sweden
Jessica	Kirk	University of Leeds
Glen	Kirkham	University of Nottingham
Charlotte	Knight	University of York
Mallappa	Kolar	University of Umeå

Hatice	Kose	Keele University
Deepak	Kumar	University of Manchester
Majlinda	Lako	Newcastle University
Liam	Lawlor	University of Leeds
Helen	Lee	University of Leeds
Louisa	Lee	University of Glasgow
Christine	Le Maitre	Sheffield Hallam University
Joseph	Lemmens	Sheffield University
Tommaso	Leonardi	University of Cambridge
Sophie	Leow-Dyke	NHS BT
Raphael	Levy	Liverpool University
Mark	Lewis	Loughborough University
Emily	Lewis	University of Glasgow
Antonella	Lisella	Keele university
Chaozong	Liu	UCL
Lauren	Lowe	Peprotech
Wei-Yu	Lu	University of Edinburgh
Simon	MacKenzie	Bioascent
Thomas	Maisey	University of Leeds
Elena	Mancuso	Newcastle University
Hareklea	Markides	Keele University
Julia	Marshall	The University of York
Andrew	McCaskie	University of Cambridge
D	McFerran	Peprotech
Jane	McLaren	University of Nottingham
Emma	McMorrow	University of Southampton
Nick	Medcalf	University of Loughborough
Cathy	Merry	University of Manchester
Aline	Miller	University of Manchester
Stuart	Moore	Don Whitley Scientific Ltd
Ines	Moreno	University of Southampton
Gavin	Morris	University of Nottingham
Vladimira	Moulisova	University of Glasgow
Vivek	Mudera	UCL
Syliva	Muller	Newcastle University
Javier	Munguia	Newcastle University
Trish	Murray	Liverpool University
Celia	Murry-Dunning	UCL
Michael	Nation	Kidney Research UK
Lindsay	Nicholson	Newcastle University
Laura	Oakes	University of Leeds
Fergal	O'Brien	Royal College Surgeons Ireland
Rachel	Oldershaw	University of Liverpool
Richard	Oreffo	University of Southampton
Caitriona	O'Rourke	Open University
Gizem	Osman	University of Nottingham
Rachel	Pallan	University of Leeds

David	Pan	UK Regenerative Medicine Platform
Maria	Pardo-Figueres	Loughborough University
Kevin	Park	Liverpool University
Simon	Partridge	Newcastle University
Imran	Patel	Imperial College London
Stuart	Paterson	Bioquell
Jamie	Patient	University of Nottingham
Jennifer	Paxton	University of Edinburgh
Gabriel	Pemberton	University of Glasgow
Sian	Philips	Orla Proteins
James	Phillips	UCL
Benjamin	Pierce	Imperial College London
Darren	Player	Loughborough University
Graeme	Pollock	Cambridge Bioscience
Polina	Prokopovich	cardiff university
Stefan	Przyborski	Durham University
Marsela	Qesari	Alcyomics Ltd
Omar	Qutachi	University of Nottingham
Cheryl	Rahman	University of Nottingham
Praveen	Ramakrishnan	University of Strathclyde
Parisa	Ranjzad	Liverpool University
Hassan	Rashidi	University of Nottingham
Mike	Raxworthy	Neotherix
Ahtasham	Raza	Sheffield University
Yvonne	Reinwald	Keele University
Monica	Reis	ICM- Newcastle University
Stephen	Richardson	University of Manchster
Marcelo	Rivolta	Univesity of Sheffield
Natacha	Rodrigues	Newcastle University
Sally	Roberts	Keele University
Deborah	Robinson	Don Whitley Scientific Ltd
Neil	Robinson	Durham University
Abraham Gonzalez	Ruiz	Col Universidad
Aram	Saeed	University of East Anglia
Manuel	Salmeron-Sanchez	University of Glasgow
Chris	Sammon	Sheffield Hallam University
Prasad	Sawadkar	UCL
Edoardo	Scarpa	University of Southampton
William	Scott	Newcastle upon Tyne Healthcare Trust
Shahida	Shahana	University of Aberdeen
Kevin	Shakesheff	University of Nottingham
Jack	Sharkey	Liverpool University
Ruchi	Sharma	University of Cambridge
Colin	Sherborne	University of Sheffield
Laura	Sidney	University of Nottingham
Cole	Sims	Durham University
Alastair	Sloan	Cardiff University

Chris	Smith	University of Manchester
David	Smith	Loughborough University
Emma	Smith	University of Southampton
Jenny	Smith	University of Leeds
Glyn	Stacey	NIBSC
Philip	Starkey	Liverpool University
Molly	Stevens	Imperial College London
Hannah	Stobbs	Loughborough University
Hazwani	Suhaimi	Loughborough University
Mike	Sullivan	TSB
Julia	Sung	NIBSC
Laura	Szkolar	The University of Manchester
Gifty	Tetteh	University of Sheffield
Dominique	Thomas	University College London
Rob	Thomas	University of Loughborough
Oliver	Thompson	University of Sheffield
Joanne	Tipper	University of Leeds
Sotiria	Toumpaniari	Newcastle University
Mark	Turner	Loughborough University
Lesley-Anne	Turner	University of Glasgow
Sarah	Upson	Newcastle University
William	van Grunsven	University of Southampton
Lisa	Valenti-Randell	Star Lab
Loriana	Vitillo	University of Cambridge
Rachel	Waddington	Cardiff University
Shane	Walsh	Newcastle University
Jamie	Ware	University of Nottingham
Lorraine	Waugh	Newcastle Surgical Training Centre
Malcolm	Wilkinson	Kirkstall Ltd
Steven	Wilkinson	Newcastle University
Anna	Williams	University of Edinburgh
Dave	Williams	University of Loughborough
Bettina	Wilm	Liverpool University
Stacy-Paul	Wilshaw	University of Leeds
Lisa	White	University of Nottingham
Sam	Whitworth	University of Leeds
xiao-nong	Wnag	Newcastle University
Josephine	Wong	University College London
Andy	Wood	University of Sheffield
Adrian	Woolf	Liverpool University
Nicholas	Wragg	Loughborough University
Kathleen	Wright	University of Leeds
Nelly	Wung	University of Bath
Jing	Yang	Keele University
Leyla	Zilic	Sheffield University