Abstracts for Posters

P3 - Supplementing the Corneal Donor Pool using Human Decellularised Corneas

Samantha L Wilson, Laura E Sidney, Siobhán E Dunphy, Harminder S Dua & Andrew Hopkinson
Academic Ophthalmology, DCN, University of Nottingham, Nottingham, UK
Samantha.wilson@nottingham.ac.uk

INTRODUCTION: There is a clinical need for reliable & quality biomimetic corneas that are as effective, preferably superior to cadaveric donor tissue[1]. Decellularised matrices are advantageous compared to synthetic or semi-synthetic engineered tissues in that the complex native milieu is preserved to retain intrinsic biological cues including growth factors, cytokines & glycosaminoglycans (GAGs)[2]. However, there is currently no effective, standardised, decellularisation protocol suitable for human corneal tissue[1]. Therefore, the purpose of this work was to provide a systematic evaluation of existing decellularisation methods in terms of their applicability to human corneal tissue.

METHODS: Corneal eye-bank tissue unsuitable for transplantation was utilised. NaCl, SDS, Triton-X100 & mechanical agitation, followed by nuclease treatments were investigated. Removal of detectable cellular/immunoreactive material was evidenced by immunofluorescence & Picogreen® analysis. Macroscopic preservation of optical properties & light transmittance was evaluated. Retention of corneal architecture & GAGs was assessed *via* histological, immunofluorescence & quantitative analysis.

RESULTS:

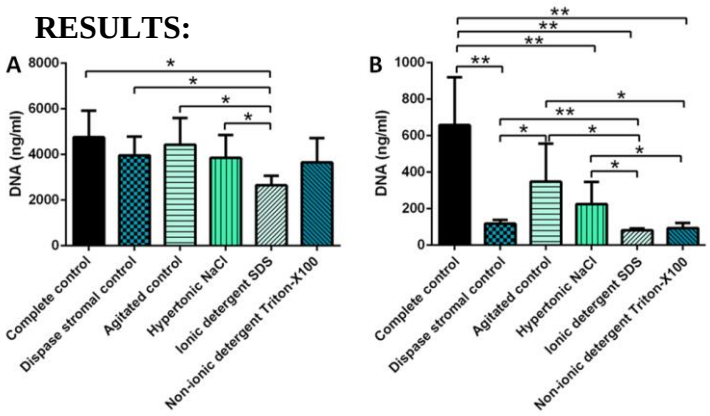

*Fig. 1: Picogreen® quantification of residual DNA following decellularisation treatments (**A**) & decellularisation with an additional nuclease treatment (**B**), n = 5, * $p \leq 0.05$, ** $p \leq 0.01$.*

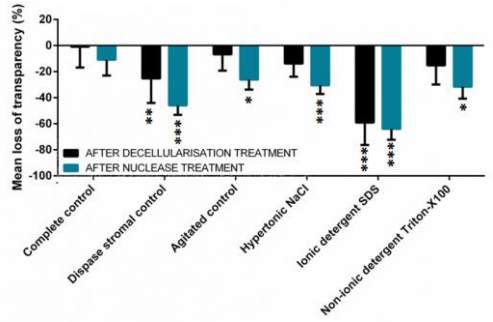

*Fig 2: Quantitative analysis of reduction of light transmittance following decellularisation & nuclease treatments, n = 5, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, when compared to corneas prior to any treatment.*

None of the employed decellularisation techniques successfully removed 100% of the residual cellular material (Fig. 1). The techniques which had the least residual DNA were the most structurally compromised, with the greatest loss of transparency (Fig. 2). GAG analysis demonstrated the stripping effects of the different decellularisation treatments.

DISCUSSION & CONCLUSIONS: The ability to utilise, reprocess & regenerate tissues deemed "unsuitable" for transplantation allows us to salvage valuable tissue. Reprocessing the tissue has the potential to have a considerable impact on addressing the problems associated with cadaveric donor shortage, which would have a significant economic benefit. Patients would directly benefit by having access to greater numbers of corneal grafts & health authorities would fulfill their responsibility for the delivery of effective corneal reconstruction to alleviate corneal blindness.

REFERENCES: [1]S.L. Wilson et al (2013) *J. Funct. Biomater.* **4**:114-161; [2]S.F. Badylak (2004) *Transpl. Immunol.* **12**:367-277.

ACKNOWLEDGMENTS: The authors would like to thank the EPSRC (EP/I01701/1) for funding this research.

P-6: Surface functionalization of electro-spun Poly(L)Lactic Acid scaffolds with heparin to induce angiogenesis

Giulia Gigliobianco[1*], Sabiniano Roman[1], Chuh K. Chong[1] and Sheila MacNeil[1]

[1]Kroto Research Institute, University of Sheffield, Sheffield, S3 7HQ, UK
dtp11gg@sheffield.ac.uk

INTRODUCTION:

Delays in new blood vessel formation after implantation of a tissue-engineered construct within the host is a major limiting factor for the long survival of the constructs [1]. Smarter biomaterials are needed to be able to induce angiogenesis post-implantation and keep the newly formed tissue alive. Hence, biomaterials can be designed to be specifically functionalised for this purpose. The aim of this study is to develop a versatile approach to modifying scaffolds to induce angiogenesis once implanted.

METHODS:

Electrospun PLA scaffolds were plasma polymerized with PolyAcrylic Acid (PAA) and coated with alternative layers of PolyEthyleneImine (PEI) and PAA or PEI and Heparin for a total of seven layers, in a layer-by-layer (LBL) coating approach. Coated scaffolds were then dipped in heparin solution, dried and immersed in Vascular Endothelial Growth Factor (VEGF) solution. Surface chemistry was verified by X-Ray Photon Electron Spectroscopy. An ELISA was used to quantify the amount of VEGF bound to the scaffolds. The Chick Chorionic Allantoic Membrane (CAM) assay was used to assess *in-vivo* the angiogenic potential of the scaffolds over a period of 7 days.

RESULTS:

XPS showed that plasma polymerization of the scaffolds with PAA was successful as the presence of S indicates Heparin is present. Heparin bound well to LBL-coated scaffolds, compared to non-functionalised scaffolds, and showed an increase in VEGF binding. The CAM assay showed that the functionalized scaffolds induced blood vessel formation compared to non-functionalised scaffolds.

Table 1. Atomic ratios of elements present on the surface of different scaffolds, normalized by the amount of carbon atoms present (C= carbon, N=Nitrogen, S=Sulphur, O=Oxygen).

	Atomic Ratios		
Type of Scaffold	**O/C**	**N/C**	**S/C**
Not-functionalized PLLA	0.375	0.011	0.000
1 layer functionalized PLLA	0.468	0.128	0.052
5 layers functionalized PLLA	0.542	0.155	0.076
7 layers functionalized PLLA	0.546	0.149	0.082

DISCUSSION & CONCLUSIONS:

The LBL functionalization we describe allows heparin to be bound to the surface of the scaffold which can in turn bind VEGF. The CAM assay demonstrated that this bound VEGF is functional inducing directed neovascularisation. We conclude that this protocol (which can be applied to a range of scaffolds) offers a new approach to tackling the problems of delayed angiogenesis for tissue engineering .

REFERENCES:

1. Lovett, M., et al., *Vascularization Strategies for Tissue Engineering.* Tissue Engineering Part B-Reviews, 2009. 15(3): p. 353-370.

ACKNOWLEDGMENTS:

X-ray photoelectron spectra were obtained at the National EPSRC XPS User's Service (NEXUS) at Newcastle University, an EPSRC Mid-Range Facility.

P-7: Indirect Three Dimensional Printing (3DP)of Apatite-wollastonite (A-W) Scaffolds

Naif Alharbi[1],-Kenneth Dalgarno[1], Oana Bretcanu[1]
[1]Newcastle University, UK
naif.al-harbi@newcastle.ac.uk

INTRODUCTION:

The 3D printing (3DP) technique based on powder-binder system is an excellent method for the fabrication of porous structure scaffolds. Sintered apatite-wollastonite (A-W) of suitable porosity has been known to show similar mechanical properties to natural bone during controlled manufacturing. This research has investigated the potential of using the indirect 3DP process to create 3D porous A-W scaffolds.

METHODS:

A-W as defined by Kokubo et al.[1] with weight ratio of 4.6MgO, 44.7CaO, 34SiO_2, 16.2P_2O_5, 0.5CaF_2 was used in this study. The approach taken to indirect 3DP was: (i) Blending of the A-W with maltodextrin (MD) powder. A range of formulations was used as shown in Table 1. (ii) Using a Z Corp Z310plus 3D printer to selectively print binder into sequentially deposited thin layers of the blended powders in order to build up a 3D structure. Two binder solution were used: 98% distilled water and 2% glycerol (DW); and zb®60 (from Z Corp). (iii) The 3D printed parts were then heat treated up to 1150ºC to burn off the MD and sinter the A-W to create a consolidated 3D structure.

Table 1 Powder blends

Blend	Composition (wt %)
PB1	70% 54-90µm A-W 30% 0-53 µm MD
PB2	85% 0-53 µm A-W 15% 0-53 µm MD
PB3	80% 0-53 µm A-W 20% 0-53 µm MD
PB4	70% 0-53 µm A-W 30% 0-53 µm MD
PB5	55% 54-90µm A-W 15% 0-53 µm A-W 30% 0-53 µm MD

RESULTS:

Table 2 presents a summary of the porosity, shrinkage and strengths measured on the various samples which were approx. 40 x 4 x 3 mm. 30% MD was required for sufficient strength to develop. Table 2 also shows that PBs 1, 4 and 5 develop the highest strengths with sintering. Figure 1 and 2 shows the macro and micro porosity structures of PB4 and PB5.

Table 2 Summary of Strength, Shrinkage and Porosity Results

Samples	Average Volume Shinkage in Sintering (%)	Average Total Porosity (%)	Average Open Porosity (%)	Average Bend Strength (MPa)
PB1/DW	34.38	51.76	33.01	8.95
PB1/zb®60	-	47.95	15.43	23.65
PB2/DW	47.6	53.67	39.48	11.56
PB2/zb®60	34.96	-	-	8.23
PB3/DW	50.32	52.24	39.08	8.09
PB3/zb®60	15.77	-	-	6.13
PB4/zb®60	48.56	35.27	11.62	35.64
PB5/zb®60	41.30	41.85	12.4	25.68

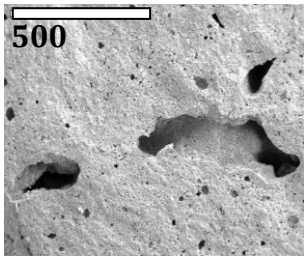

Fig. 1 SEM of the fractured surface of the sintered part of PB4

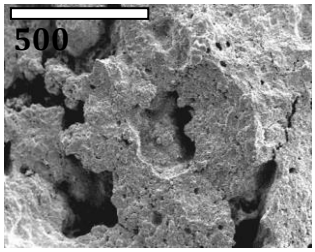

Fig. 2 SEM of the fractured surface for sintered part of PB5

DISCUSSION & CONCLUSION:

In all cases the increase in strengths is as a result of the increased consolidation during sintering, as indicated by the reduced porosity. Indirect 3D printing of A-W structures can be used to create strong, highly porous structures, but care must be taken to appropriately select binder and processing parameters.

REFERENCES:

1. T. Kokubo et al (1982), BICRKU 60: 260-268.

ACKNOWLEDGMENTS:

I would like to thank the interior ministry of Saudi Arabia for providing financial support to my study.

P-8: Optimising 405 nm HINS-light technology for patient safe decontamination during arthroplasty surgery

Praveen Ramakrishnan[1, 2], Michelle Maclean[2], Scott MacGregor[2], John Anderson[2], M. Helen Grant[1*]
[1] Department of Biomedical Engineering, University of Strathclyde, Glasgow, Scotland, UK
[2] The Robertson Trust Laboratory for Electronic Sterilisation Technologies, University of Strathclyde, Glasgow, Scotland, UK
p.ramakrishnan@strath.ac.uk

INTRODUCTION:

Infection rates following orthopaedic arthroplasty surgery are as high as 4%, while the infection rates are even higher after revision surgery [1]. The duration of routine arthroplasty surgeries is typically between 1 and 2 hours. 405nm High-Intensity Narrow-Spectrum Light (HINS-light) has bactericidal activity against Hospital Acquired Infection (HAI) related bacterial pathogens including MRSA [2] and hence may aid in reducing the incidence of infections that arise from environmental contamination during arthroplasty surgery.

METHODS:

Immortalised rat osteoblast (OST 5) cells were exposed to 405 nm light at an irradiance of 5mW/cm^2 in Dulbecco's Phosphate Buffered Saline (DPBS) at different dose rates (18, 27, 36 and 45J/cm^2) at 37˚C and 5% CO_2. Unexposed controls were treated in the same way. After 48 hours post treatment, cell viability (MTT assay), cell function (ALP assay)and cell proliferation rate (BrdU assay) were measured. Live/Dead cell staining was carried out using Acridine Orange/ Propidium Iodide (AO/PI) dyes after 48 hours post light treatment. Statistical analysis was performed using unpaired Student t-test and differences considered significant when $p<0.05$.

RESULTS:

After 48 hours post light treatment, no significant difference was observed between the unexposed and 405 nm treated samples for up to a dose rate of 36J/cm^2 in cell viability, function and proliferation rate (fig 1.a). More apoptotic and dead cells were observed for the 45J/cm^2 exposed samples compared to the 36J/cm^2 exposed samples (fig. 1.b).

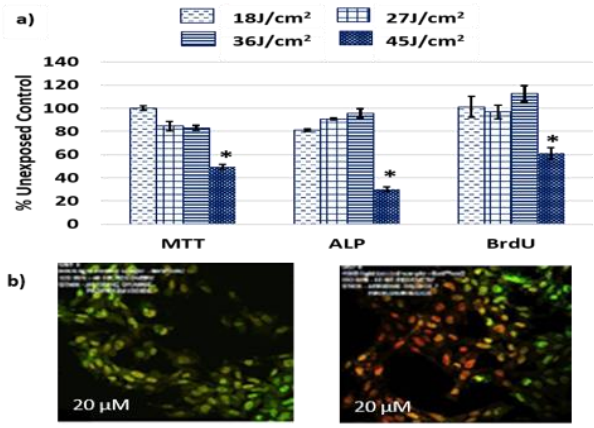

Fig 1. (a) Effect of 405 nm light treatment at 5mW/cm^2 on OST 5 cell response parameters after 48 hours incubation, (b) AO/PI staining. Left – 36J/cm^2 and Right – 45J/cm^2 of 405 nm light treatment after 48 hours incubation. AO (live - green, apoptotic- orange), PI (dead - red).

DISCUSSION & CONCLUSIONS:

From the quantitative and qualitative studies, it
is found that the cells were healthy for up to a dose rate of 36J/cm^2 (5mW/cm^2 for 2 hours) whilst cell death became evident with doses of 45J/cm^2.

These results suggest that exposure to a dose of 36J/cm^2 may be suitable for use for continuous decontamination during orthopaedic surgery whilst being safe for tissue exposure.

REFERENCES:

1. H. Hamilton, J. Jamieson (2008), *Canadian Journal of Surgery*. *51(2)*: 111-117.
2. M. Maclean et. al. (2009), *Applied and Environmental Microbiology 75(7)*,1932–1937.

ACKNOWLEDGMENTS:

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P-11: Analysis of osteoclastogenesis/osteoblastogenesis using human bone marrow derived co-cultures on nanotopographical titania surfaces.

P. M.Tsimbouri[1], R.K. Silverwood[3], P. Fairhurst[3], T. Sjostrom[2], B. Su[2], P. Young[3], R.D.M. Meek[3], M.J. Dalby[1]

[1]*Centre for Cell Engineering, University of Glasgow,*[2]*Southern General Hospital, Glasgow, UK.*[3] *School of Oral and Dental Science, University of Bristol, Bristol, UK.*
penelope.tsimbouri@glasgow.ac.uk

INTRODUCTION: Titanium (Ti) is currently used for orthopaedic applications due to its excellent load bearing properties. However, Ti is bioinert, and this can affect osseointegration and outcomes of implants.

Nanopatterning of implant surfaces could be the solution to this hurdle. We have previously shown that 15nm high nanopillars are bioactive [1,2] using human mesenchymal stem cells (MSC). We have also developed an osteoblast/osteoclast co-culture system using nanopits on polycarbonate[3]. Here we have used these osteoblast/osteoclast co-cultures as they are believed to give the most accurate representation of the *in vivo* environment, allowing assessment of bone remodeling related to biomaterials. Under co-culture conditions 15nm high nanopillars on titanium, will induce significantly increased levels of osteoblastogenesis and reduce osteoclast activity producing a method of enhancing secondary implant fixation.

METHODS: Co-culture of osteoblast and osteoclast progenitors on polished titanium and titanium patterned with 15nm nanopillars fabricated by the block copolymer technique was performed. Time points of 14 and 28 days were selected for analysis as a reasonable timescale for osteointegration *in vivo*. Histochemical staining was performed to identify and quantify osteoclasts and bone nodule formation. Scanning electron microscopy (SEM) was conducted to morphologically examine the effect on differentiation of untreated and nanopatterned titanium substrates on osteoprogenitors and osteoclasts. Real-Time, quantitative reverse-transcription polymerase chain reaction is currently being utilised to quantify expression of osteoblast, osteoclast and inflammatory response related genes.

RESULTS: On SEM nanopillar titanium substrates were shown to be less inductive of osteoclastogenesis, with decreased maturity and decreased activity with time visualised when compared to flat titanium substrates (Fig. 1a). This was supported by TRAP staining, which showed macrophages present on flat titanium substrates only (Fig.1b). Increased osteogenesis on the 15nm pillars was indicated by Alizarin red staining (Fig.1c). qPCR revealed a time related-decrease in osteo-clastogenesis related genes on the nanopillars with an associated increase in osteoclast inhibitors.

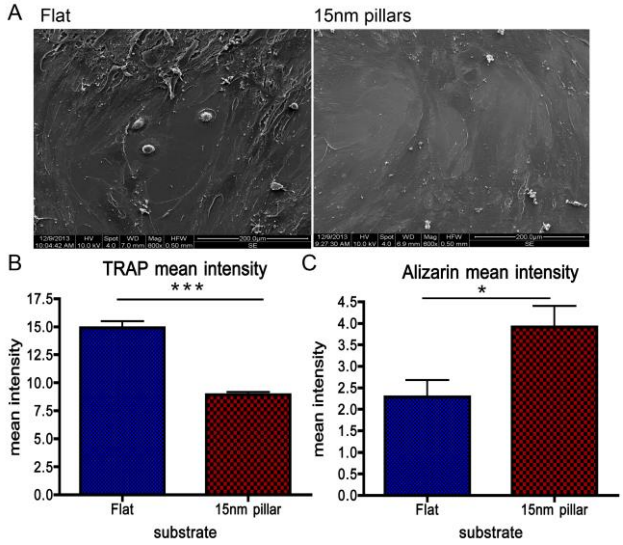

Fig.1 Showing a) SEM, b) TRAP and c) Alizarin staining results (n=3, T-test, *p<0.05, ***p<0.001).

DISCUSSION & CONCLUSIONS: Dramatic reduction in number of osteoclast progenitor cells on nanopillar substrates versus flat was noted. Alizarin red staining demonstrated osteoblast proliferation on both surfaces, with no difference in osteoblastogenesis. Genomic data on osteoclast and osteoblast-related genes will be presented.

REFERENCES:

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ACKNOWLEDGMENTS:

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P-14: Development of Mesenchymal Stem cell niche *in vitro* using magnetic nanoparticles

Emily E L Lewis[1], Mathew J Dalby[1] and Catherine C Berry[1]
[1]Centre for Cell Engineering, University of Glasgow, UK
e.lewis.1@research.gla.ac.uk

INTRODUCTION:

Stem cells are able to self renew and differentiate into other cell types.[1] In the body, stem cells reside within a special microenvironment, termed the 'niche'.[2] The niche protects the stem cells from over stimulation and apoptosis, whist maintaining quiescence and homeostasis.[2] Understanding the regulatory mechanisms within the niche allows the *in vitro* expansion of stem cells and desired differentiation for regenerating damaged tissue. This project aimed to mimick the bone marrow mesenchymal stem cell (MSCs) niche *in vitro.* The development of a 3D spheroid culture system 'niche' in collagen gels, demonstrated excellent cell viability, maintenance of STRO-1/nestin expression and quiescence compared to parallel MSCs cultured in monolayer.

METHODS:

Human MSCs (hMSCs) and magnetic nanoparticles (mNPs) were purchased from Promocell and Chemicell respectively. Magnets comprised of a NdFeB core (13 mm diameter, 350 mT).

MSC Labelling. The MSCs were labelled with mNPs (PEA coated, 200 nm diameter, Fe_3O_4) at 0.1 mg/mL mNPs (30 minutes + magnetic field). ***Spheroid Culture***. Labelled MSCs were suspended in media. A single magnet was placed on *the top of the culture well and the plate was incubated for up to 2 weeks.* ***Collagen gel Culture.*** *MSC spheroids were implanted into collagen gels for up to 2 weeks.* ***Analysis.*** *Monolayer, spheroid and collagen gel culture systems were assessed for viability (calcein/ethidium homodimer), cell architecture (actin), phenotype (STRO-1/nestin) and quiescence (BrdU) using fluorescence microscopy.*

RESULTS:

MSCs were successfully labelled with mNPs and were able to form a 3D spheroid culture system within hours. MSCs are adherent cells and previous studies have shown the formation of similar spheroids, which have very strong cell-cell interactions have provided a better representation of the natural niche environment.[3] The cultured MSCs remained strongly viable and quiescent over 2 weeks, with strong MSC marker expression, whilst the collagen gels provided a similar environment to bone marrow *in vivo*.

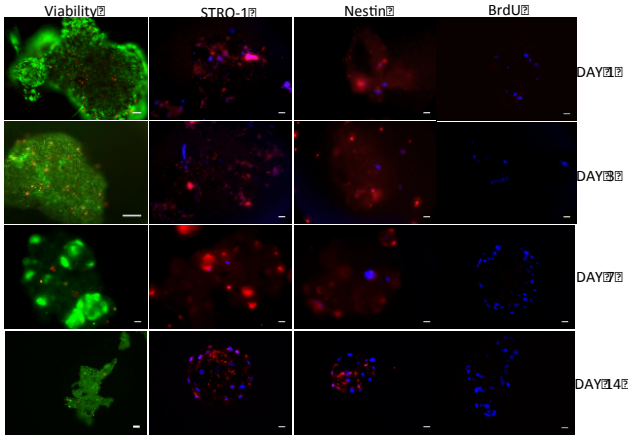

Fig. 1 Schematic diagram of a spheroid culture system. Fluorescence images of spheroid MSCs cultured for 14 days assessing cell viability (green: live cells, red: dead cells), phenotype (red: STRO-1/nestin, blue: nucleus) and quiescence (purple: proliferating cells, blue: quiescent cells). Scale bar: 20 μm.

DISCUSSION & CONCLUSIONS:

A simple, quick and highly efficient method has been developed to use mNP-labelled MSCs to form spheroid 3D niche cultures. The NPs allow for simple cell tracking and imaging *in vitro*, with a view to potential clinical use. The system was novel in mimicking the *in vivo* environment by creating a realistic 3D *in vitro* model. The spheroids remained viable and quiescent, whilst retaining their multipotency potential and exhibiting functional responses (results not shown here) over 2 weeks compared to conventional 2D cultures.

REFERENCES:

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P-15: Increasing Osterix Expression by Blocking Mir-31 with RNA Conjugated Gold Nanoparticles

M.McCully[1], Joao Conde[2], Pedro V. Baptista[2] M.J.Dalby[1], C.C.Berry[1]

[1] Centre for Cell Engineering, *College of Medical Veterinary and Life Sciences, University of Glasgow, Scotland*

[2] *Faculdade de Ciencias E Tecnologia, Universidade Nova De Lisboa, Portugal*

m.mccully.1@research.gla.ac.uk

INTRODUCTION: Controlled differentiation of stem cells into defined cell types is a major aim of tissue engineering. Mir-31a expression is involved in tumorigenesis, adipogenesis and skeletal muscle development, however a recent report has also indicated mir-31a's role in osteogenesis as a regulator of osterix. Osterix is a transcription factor and master regulator of bone differentiation. In this project we propose to increase osterix expression by blocking mir-31a, with antagonists to mir-31 (antagomirs) delivered to cells via gold nanoparticles (AuNPs). This hypothesis is tested initially with the pre-osteoblast like bone cancer cell line MG63 (known to have low osterix levels) and subsequently with primary human mesenchymal stem cells (hMSC) with a view towards influencing osteogenesis.

METHODS: Nanoparticles were synthesised (~12nm), protected with PEG and tagged with antagomirs. The AuNP toxicity was assessed by MTT; cellular uptake of AuNPs was visualized by TEM and quantified *via* ICP-MS. Osterix expression was assessed by qPCR (gene level) and in-cell westerns (protein level).

In-Cell Westerns. Briefly, cells ($1x10^4$ cells/ml) were seeded for 24 hours in DMEM/10% FBS (antibiotics & L-glutamine), and subsequently incubated in 50nM AuNPs. Following a further 48 hours, cells were fixed, permeabilised and blocked prior to incubation with an osterix primary antibody (Abcam, UK). Samples were washed and co-incubated with secondary antibodies (CellTag700 & donkey anti-rabbit IR800CW; Licor, UK). The plates were scanned by an Odyssey SA.

RESULTS: Osterix expression was significantly increased in MG63 cells incubated with the antagomirs, as determined by PCR, ICW and immunofluorescence. In addition, the antagomirs were found to produce an increase in osterix expression in MSCs, with particular enhancement via the 3' antagimir (figure 1).

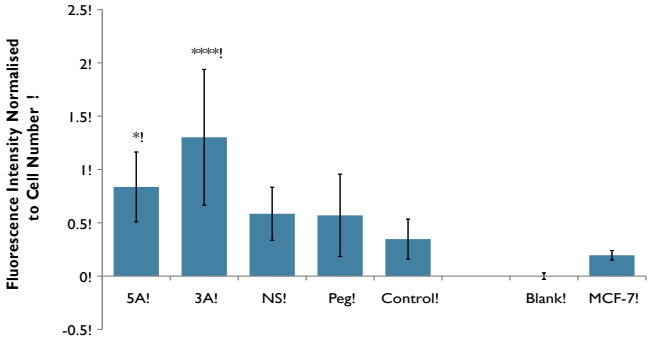

Figure 1. In-cell western showing osterix expression in MSCs. *Cells were treated for 48 hours. The treatments included four AuNP species (5A:antagonist to 5' end of mir-31, 3A:antagonist to 3'end of mir-31, NS:nonsense RNA, PEG:PEG only. Control:MG63 cells (low osterix), MCF-7:osterix negative cell line (error bar: St. dev, n=6).*

DISCUSSION & CONCLUSIONS: This study demonstrates the highly novel use of delivering small functional antagonists of miRNAs to cells *via* nanoparticles, and the potential capability of using such NPs to drive osteogenesis. Such studies highlight translational roles in clinical treatments including osteoporosis and osteogenesis imperfecta.

REFERENCES: 1. Baglìo et al (2013). Gene, 527 (1), 321–331

P-16*: In vitro* Modelling of Human Gastrointestinal Peritoneal Carcinomatosis: A Pre-Clinical Photodiagnostic & Photodynamic Therapy Treatment Tool

Kathleen E. Wright[1*], Thomas I. Maisey[1], Sarah L. Perry[1] and David G. Jayne[1]

[1]Academic Surgery Colorectal Research Group, Leeds Institute of Biomedical and Clinical Sciences, St James's University Hospital, University of Leeds, United Kingdom.

[*] k.e.wright@leeds.ac.uk

INTRODUCTION:

Gastrointestinal peritoneal carcinomatosis (PC) is a debilitating late stage disease that is characterized by multiple tumour lesions in the abdominal cavity. These tumours are associated with the peritoneal tissue, this is the thin tissue which covers organs & lines the abdominal cavity. Our aim is to develop an *in vitro* model system of PC for use in a feasibility study to use protoporphyrin IX (PpIX) fluorescence as a diagnostic aid, and photodynamic therapy (PDT) as a potential adjuvant focal treatment with surgical debulking.

METHODS:

Monolayer cultures and 3D hydrogels are being investigated for use in the construction of an *in vitro* PC-model. Human colon cancer cell lines were investigated, e.g. T84 & HT29. PC-models were constructed based on tumour to non-tumour cell ratios seeded at: 1 in 10, 1 in 50 & 1 in 100 and cultured for 3, 7 & 14 days. A range of 5-ALA concentrations (0-1mM) and incubation times (0-6h) were investigated for levels of PpIX fluorescence emitted using a spectrofluoreometer. Optimal doses were tested in our developing PC-model in order to estimate its future tumour diagnostic potential. In addition, a selection of antibodies was screened for tumour cell specificity in order to support our finding.

RESULTS:

1 in 10 ratio of tumour to non-tumour cells cultured for ~7 days showed promising results for use in constructing a PC-model. 6 h 5-ALA incubation gave the brightest PpIX fluorescence of the times tested, with HT29 cells showing the greatest fluorescence of the cell lines screened (Fig 1).

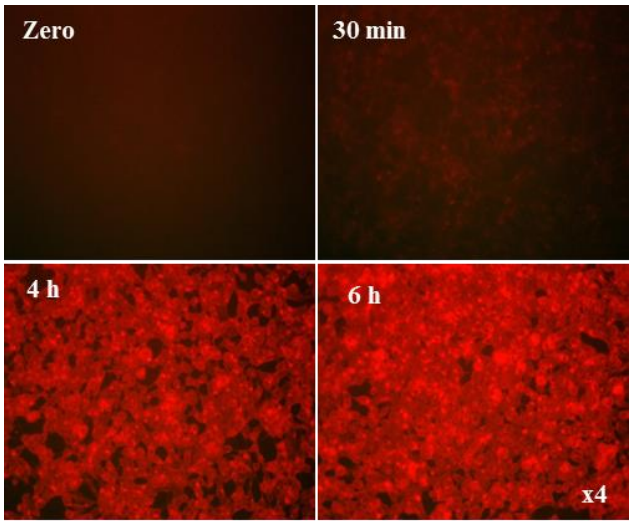

Fig. 1 Epi-fluorescence micrograph of PpIX fluorescence in HT29 cell line monolayers after incubation with 1 mM 5-ALA for 0, 30 min, 4h & 6 h.

DISCUSSION & CONCLUSIONS:

To be able to distinguish tumour from non-tumour cells & tissues, in a viable culture system would be a valuable research tool in the development of new diagnostic & therapeutic treatments such as PDT for treating local recurrence & metastatic diseases such as gastrointestinal-PC. We surmised, administering exogenously administered 5-ALA to a mixed-culture system show greater accumulation of intracellular localized PpIX fluorescence in tumour cell lines, such as HT29, to levels that are distinguishable from non-tumour cells.

ACKNOWLEDGMENTS:

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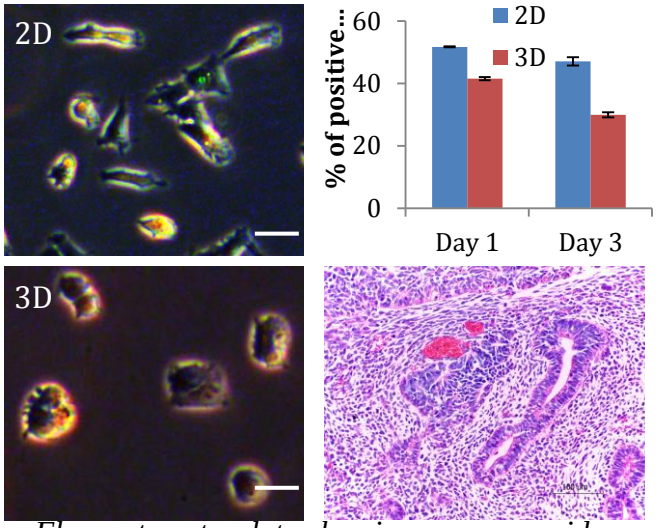
P-17: The Effect of Physical Microenvironment on the Shape of Pluripotent Stem Cells and Their Developmental Potential

C.H.C Sims[1], E.Knight[1], S.A. Przyborski[1,2]
[1]School of Biological and Biomedical Sciences, Durham University, U.K.
[2] Reinnervate Ltd, NETPark Incubator, TS21 3FD UK
Authors for correspondence: c.h.c.sims@durham.ac.uk and stefan.przyborski@durham.ac.uk

INTRODUCTION: Intercellular signalling between stem cells is vital for cell fate determination. Cells cultured on 2D (two dimensional) and 3D (three dimensional) substrates have different morphologies – cells grown in 3D possess a more *in vivo*-like and spherical structure rather than a flattened out shape as occurs in 2D monolayer cultures[1]. Differences in cell shape markedly affect gene and protein expression. This in turn changes the developmental potential of 2D flattened cells[1]. Cells cultured in 3D show enhanced differentiation compared with 2D monolayer counterparts[2]. It is proposed that changes to cytoskeletal structure and subsequently gene/protein expression profiles enabled by 3D culture enhance the pluripotent phenotype of stem cells *in vitro* and their ability to differentiate.

METHODS: Using Alvetex® Strata, a non-biodegradable commercially available porous polystyrene membrane, we established routine protocols for long term propagation of TERA2.cl.SP12 cells in 3D which maintain a stem cell phenotype[3]. Cells were continually maintained on Alvetex® Strata membranes and passaged every 4 days. Cell shape was assessed and flow cytometry performed on 2D and 3D propagated cells cultured and then differentiated as 2D monolayers in response to 1μM ATRA. Teratomas were produced in nude mice.

RESULTS: There was a noticeable difference in the shape of cells after 2D and 3D culture: 3D passaged cells maintained a spherical shape whilst 2D passaged cells rapidly flattened as normal. Compared to 2D monolayer culture, levels of SSEA-3 expression in 3D passaged cells rose as the passage number increased (p4<p6<p10) indicating enhancement of the stem cell phenotype. During differentiation, the stem cell marker SSEA-3 decreased more rapidly in 3D passaged cells than cells propagated in 2D. Teratomas produced from 3D propagated cells were significantly larger and more differentiated than tumours derived from 2D passaged cells.



Flow cytometry data showing a more rapid reduction in SSEA-3 during ATRA induced differentiation; Phase microscopy images of passage 10 2D and 3D cells put back into 2D culture. Scale bar: 50μm; Example teratoma generated from 3D propagated stem cells.

DISCUSSION & CONCLUSIONS:
We demonstrate the ability to propagate stem cells continually in 3D using a novel porous scaffold. We show that 3D passaged cells alter their shape, which in turn appears to affect the stem cell phenotype. Altering the shape of the cells can also influence their ability to differentiate *in vitro* and produce teratomas *in vivo*. Maintenance of 3D shape therefore has important implications on stem cell culture.

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P-19: Bioactive Scaffolds as Bone Templates for *in-vitro* Testing

Gifty Tetteh[1, 2], Ihtesham U. Rehman[1], and Gwendolen C. Reilly[1, 2]

[1] Kroto Research Institute, University of Sheffield, Sheffield, UK S3 7HQ
[2] INSIGNEO Institute for in silico Medicine, University of Sheffield, Sheffield, UK S1 3JD,
gifty.tetteh@sheffield.ac.uk

INTRODUCTION:

Bone tissue engineering (TE) aims at improving musculoskeletal health. Our aim is to develop a tissue engineered bone construct that could be used for studying *in-vitro* bone formation around prototype implant materials. In this study, we investigated polymer composite materials fabricated with electrospinning and particulate leaching (PL) as a support for tissue engineered bone matrix. The scaffold therefore needs to have good mechanical strength and resilience with a high porosity, low degradation rate and be osteoconductive.

METHODS:

Polyurethane (PU) and Polyurethane-Hydroxyapatite (PU-HA) composite solutions were electrospun to attain scaffolds with either aligned or random fibres, using 15%wt PU dissolved in 70/30 DMF/THF solvent for PU scaffolds. To create composite scaffolds, PU solutions were doped with either micro or nano-sized HA particles in a ratio of 3 PU: 1 HA. For particulate leached scaffolds, solutions were combined with NaCl particles (~250 µm). MLO-A5 and hES-MP cells were used for biocompatibility studies over a 28-day culture period. Mechanical testing of scaffolds; and assays of calcium and collagen deposition were also undertaken as part of this study.

RESULTS:

FTIR and Raman characterization confirmed the presence of HA in all composite scaffolds, whilst µ-CT confirmed good pore interconnectivity. Although all scaffolds supported proliferation of both cell types and the deposition of calcified matrix, random polyurethane scaffolds with nano-HA enabled the highest cell viability amongst electrospun composite scaffolds. Interestingly, within the PU-only group, aligned fibres allowed better cell support than random fibres.

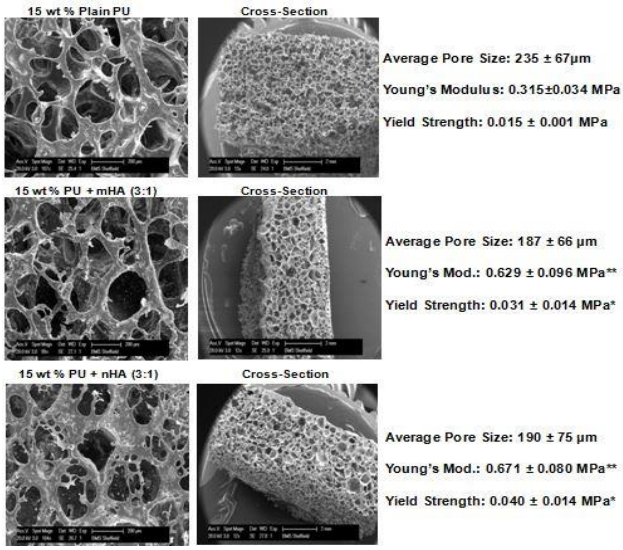

*Fig 1: SEM images of particulate leached scaffolds; left: top view (scale bar=200µm) and right: cross-sectional view (scale bar=2mm) Text: Mean ± standard deviation; (n=6) one way Anova **= p<0.001 and *= p<0.05*

DISCUSSION & CONCLUSIONS:

These particulate leached scaffolds composed of highly interconnected macro and micro porous network (>60% porosity) with pore sizes previously described as optimum for bone regeneration (>180µm)[1], and electrospun scaffolds support bone matrix formation and have good mechanical properties in relation to their good porosity. We therefore propose the use of these autoclavable scaffolds as a support for tissue engineered constructs, and for in-vitro studies of bone formation including testing of implant materials.

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[1] J. Klawitter, S. Hulbert, (1971) *JBMR* **5:** 161-229.

ACKNOWLEDGMENTS

The authors would like to thank the Ghana Education Trust Fund for providing financial support to the project.

P-20:Development of a three-dimensional model for ameloblastoma

T. Eriksson[1], S. Fedele[2], R. Day[3], V. Salih[4]

[1] Biomaterials and Tissue Engineering, [2] Oral Medicine and [3]Applied Biomedical Engineering, University College London, UK; [4] Plymouth University Peninsula School of Medicine & Dentistry, UK;
t.eriksson.11@ucl.ac.uk

INTRODUCTION:

Ameloblastoma is a benign, locally invasive neoplasm originating from epithelial cells of the tooth-forming apparatus. It is associated with extensive local bone destruction and high recurrence rate after surgical resection. Little is known of the molecular mechanisms regarding tissue invasion and bone resorption, recent studies suggest osteoclast activation by ameloblastoma cells as the main mechanism[1]. This study aims to develop *in vitro* organotypic models of the tumour to better detail the molecular mechanisms of tumour tissue invasion and destruction.

METHODS:

AM-1 cells[2] were cultured in flasks with keratinocyte serum-free media (KSFM) with 50ng/ml b-FGF. They were incorporated into collagen gels neutralised with NaOH, compressed[3] and incubated in KSFM for up to two weeks. Cells were then examined with confocal microscopy using phalloidin as a cytoplasmic stain and propidium iodide as a nuclear stain. In order to create cell spheres to accurately model the tumour, cells were placed on 6-well low-attachment plates (Corning) for 3 days in KSFM and imaged at regular intervals. As a control, HOS cells were cultured on low-attachment plates in DMEM.

RESULTS:

AM-1 cells form large cell aggregates within the 3D collagen scaffolds (fig.1), however no spheres are formed in 2D culture (fig.2).

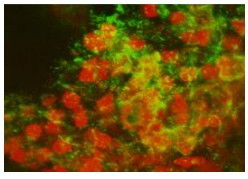

Figure 1: 40x confocal microscope image of AM-1 cells in compressed collagen. Nuclei – red, cytoplasm - green.

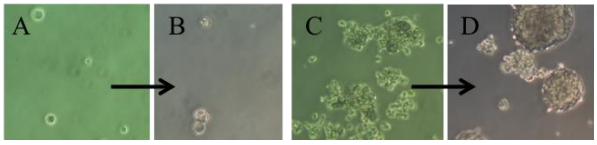

Figure 2: Low-attachment plate culture of AM-1 cells (A,B) and HOS cells (C,D) at 1 (A,C) and 3 days (B,D). HOS cells form large spheres.

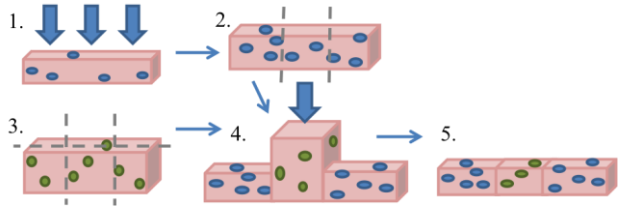

Figure 3. Creating co-culture model with AM-1 cells: 1. Compressed collagen gel with bone cells or fibroblasts, 2. Remove some of the gel, 3. Collagen gel with AM-1 cells, 4. Compress to combine gels, 5. Incubate and analyse.

DISCUSSION & CONCLUSIONS:

Despite being an invasive tumour cell line, AM-1 cells displayed attachment-dependence and did not form spheres. For further development of the 3D model, soft tissue scaffolds of compressed collagen and Matrigel (BD Biosciences) with gingival fibroblasts and bone models using Bio-Oss (Geistlich Biomaterials), compressed collagen and bone cells have been developed. AM-1 cells will be incorporated into these scaffolds as detailed in fig. 3.

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P-21: Optimisation of a Novel Neurite Outgrowth Model to Study the Role of Neurite Inhibition in Human Stem Cell Derived Neurons

K.E. Clarke[1], A. Whiting[2] and S.A. Przyborski[1,3]
[1]School of Biological and Biomedical Sciences, Durham University, South Road DH1 3LE, UK
[2]Department of Chemistry, Durham University, South Road DH1 3LE, UK
[3]Reinervate Ltd, NETPark Incubator, Thomas Wright Way, Sedgefield, Co. Durham, TS21 3FD, UK
Corresponding Authors: k.e.clarke2@durham.ac.uk and stefan.pryzborski@durham.ac.uk

INTRODUCTION: Spinal cord injury results in activation of molecules that suppress reinnervation of broken nerves and inhibit growing neurites. To fully understand the molecular mechanisms involved in this process, we are developing a novel *in vitro* cell-based model. This involves a combination of human stem cell-derived neurones and a highly efficient process of neuritogenesis within a 3D culture system. Initially, we are focused on the optimisation of neuritogenesis using novel synthetic retinoids to produce a robust model of neurite outgrowth.

All-*trans* retinoic acid (ATRA) has important roles *in vitro* during neural development.[1] It can be utilised *in vitro* to promote differentiation of pluripotent cells, [1] but is limited due to its ability to undergo photoisomerisation and degradation.[2] Synthetic analogues of ATRA such as EC23 and AH61 do not readily breakdown when exposed to light[3] therefore, are more stable for use *in vitro*. The plutipotent embryonal carcinoma cell line, TERA2.cl.SP12 differentiate into neural subtypes when treated with retinoids.[4] The purpose of this aspect of the study is to optimise the use of retinoids in neurite outgrowth from TERA2.cl.SP12 cell aggregates.

METHODS: TERA2.cl.SP12 cells were maintained as 2D monolayers with passaging every 3 days. Cells were seeded at a density of $1.5x10^6$ per sterile Petri dish and allowed to form cell aggregates in suspension for 24 hours prior to retinoid treatment. Aggregates were maintained in suspension for 21 days and were subsequently placed in a 48 well tissue culture plate coated in poly-D-lysine and laminin to promote neurite outgrowth in the presence of mitotic inhibitors. After 10 days, cells were fixed with 4% PFA and prepared for immunocytochemistry.

RESULTS: The stem cells differentiated into neurons and formed multiple TUJ-1 positive neurites that projected radially from the aggregate of neural perikarya. Quantification of the number and lengths of neurites was determined using Image J. Each retinoid tested induced the formation of neurites with synthetic retinoids (AH61 and EC23) inducing significantly more and longer neurites compared to ATRA. Neurite outgrowth appeared to be maximal at 1µM AH61 and 0.01µM EC23, whereas lower concentrations of ATRA resulted in less neurites and migration of cells from the aggregate, (see Figure).

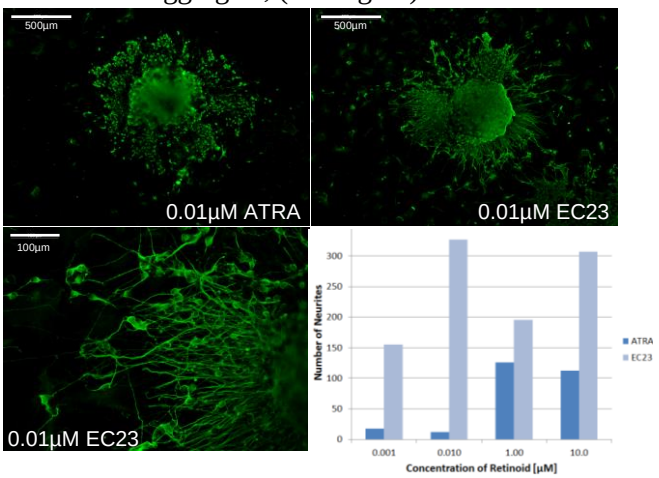

Fig. TUJ-1 expression for aggregates differentiated with 0.01µM ATRA and 0.01µM EC23 and a plot showing the relationship between neurite number and retinoid concentration for both ATRA and EC23.

DISCUSSION & CONCLUSIONS: The data demonstrate that synthetic retinoids can be used to efficiently induce neural differentiation from human stem cells, resulting in the formation of significant levels of neuritogenesis. Synthetic molecules EC23 and AH61 provide the added benefit of compound stability and greater reproducibility. This technology is being further developed to create a novel 3D cell culture system to investigate neurite inhibition in response to the glial scar that forms after spinal cord injury.

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P-22: Nutrient transport properties of tissue engineering membranes and scaffolds

Hazwani Suhaimi*, Shuai Wang and Diganta Bhusan Das
Department of Chemical Engineering, Loughborough University, UK
*Email: H.Suhaimi@lboro.ac.uk

INTRODUCTION:

The idea of growing artificial tissues in bioreactors such as hollow fibre membrane bioreactors (HFMBs) has started some time ago and preparation of biocompatible porous membranes and scaffolds has been attempted extensively. There have also been a number of studies on modelling glucose transport processes in HFMB. However, there is little information available that discusses specifically the glucose diffusivity across tissue engineering membranes or scaffolds and, importantly, its dependence on the properties of the materials (i.e., membrane and, scaffold)[1]. In this study, we construct a simple diffusion cell to determine scale dependent glucose transport processes for a number of porous membranes and scaffolds of different pore size and shapes, saturated with water and cell culture media (CCM). Porosity and tortuosity of the used materials are determined and consequently correlated to the glucose diffusion coefficient values.

METHODS:

Five different membranes and scaffolds were employed, which include cellulose nitrate membrane (CN), polyvinylidene fluoride membrane (PVDF), poly(L-lactide) scaffold (PLLA), poly(caprolactone) scaffold (PCL) and collagen scaffold (see Table 1).

Diffusion experiment:

The diffusion cell consisted of two half chambers with identical volumes, namely donor and receptor phase. The membrane/scaffold was fixed in between. The donor phase was filled with glucose solution while the receptor phase contained pure water or CCM. The whole apparatus was placed in a thermostated water bath at either 27 or 37 ± 1°C. Samples were taken from both the donor and receptor phase at intervals of 1 h until equilibrium was established. A UV spectrophotometer was used to monitor the change in glucose concentration over time for materials saturated with water while an YSI glucose analyser was used for experiments in CCM.

RESULTS:

Data revealed an increase in the diffusion coefficient at a larger pore size, indicating least resistance of glucose molecules diffusing through the pores. Data also showed a significant reduction of glucose diffusion coefficient through materials saturated with CCM at a given temperature. For instance, the glucose diffusion coefficient of PVDF membrane was reduced from 1.87 x 10^{-10} m^2/s to 7.68 x 10^{-11} m^2/s when the membrane was saturated in water and CCM at 37°C, respectively.

Table 1 Characteristics of the materials studied

Material	Thickness (µm)	Pore size (µm)
PVDF	125	0.1
CN	122.5	0.45
PLLA	50	12-18
PCL	50	20-30
Collagen	1500	80

DISCUSSION & CONCLUSIONS:

In this study, the significance of the increase of diffusion coefficient with increasing pore size of the materials was derived. It was also observed that glucose diffusion coefficients through membrane and scaffold pores saturated with CCM are significantly reduced at a given temperature which is contrary to what have been assumed in the previous studies on glucose transport processes in HFMB or similar bioreactors.

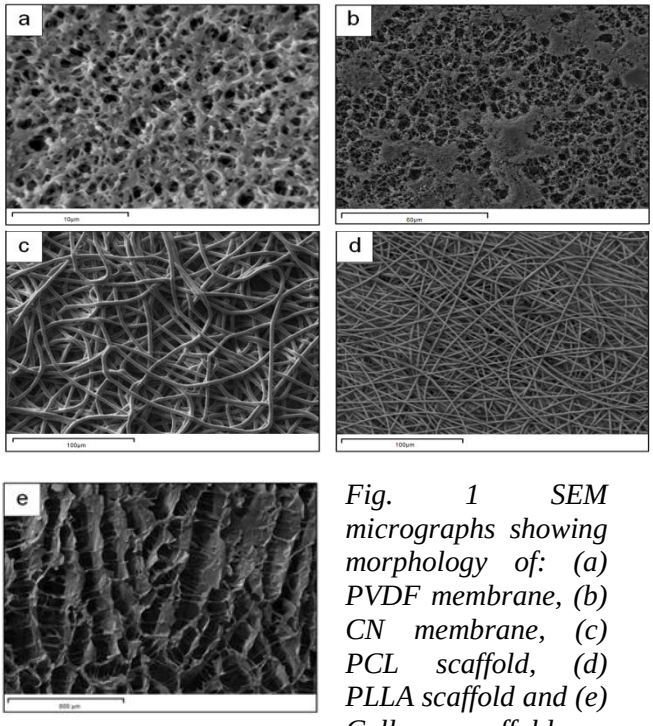

Fig. 1 SEM micrographs showing morphology of: (a) PVDF membrane, (b) CN membrane, (c) PCL scaffold, (d) PLLA scaffold and (e) Collagen scaffold

REFERENCES:

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ACKNOWLEDGMENTS:

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P-24: Development of Novel 3D Skin Models to Study Barrier Function

NDP Robinson[1], D Hill[3], P Lovat[3] and SA Przyborski[1,2],

[1]*School of Biological Sciences, Durham University, South Road, Durham, DH1 3LE*
[2]*Reinnervate Limited, NETPark Incubator, Thomas Wright Way, Sedgefield, TS21 3FD*
[3]*Dermatological Sciences, Institute of Cellular Medicine, Framlington Place, Newcastle, NE2 4HH*

n.d.p.robinson@durham.ac.uk and stefan.przyborski@durham.ac.uk

INTRODUCTION: The skin is the largest organ in the body and has evolved to perform many specialised functions including as a barrier to the environment and toxins, preventing water loss, acting in immune response and resisting mechanical stresses. Barrier function is of huge importance in maintaining homeostasis of the organism. Many disease states such as Netherton syndrome, icthyosis vulgaris and different types of xerosis are due to a loss of barrier function[1]. By studying how the skin is able to develop and maintain this barrier, will lead to insight into the pathways and controls of skin function. This knowledge will aid in the development of new treatments for such skin conditions.

This project aims to use novel three-dimensional (3D) skin models to investigate the roles of Vitamin A (retinol) in skin homeostasis. In particular, the development of barrier function in relation to tight junction (TJ) complexes. These 3D models will lead to a greater homology to *in vivo* samples over existing methods

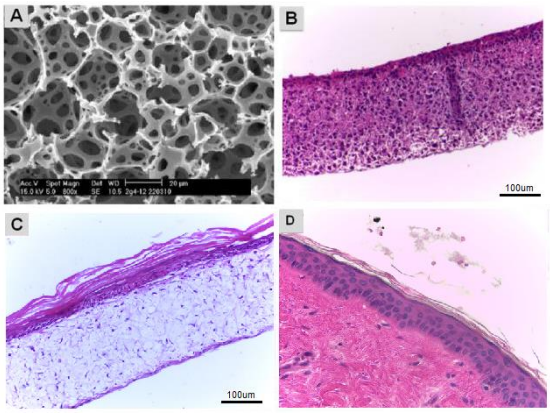

Fig 1A: SEM image of Alvetex®Scaffold and H&E images for B: HaCaT cells grown in Alvetex®, C: Primary human keratinocytes grown on top of Alvetex®Scaffold filled with human embryonic fibroblasts and D: human skin sample.

METHODS: Two 3D models of skin have been developed using Alvetex®Scaffolds. The first uses the established keratinocyte cell line (HaCaT) and the second utilises primary human keratinocytes isolated from tissue samples which are grown in co-culture with human embryonic fibroblasts. Growing the cells in 3D allows for the cells to develop and stratify which closely mimics structures that are seen *in vivo*. This allows more accurate assessment of the changes in barrier formation and TJ development by vitamin A. Results from these model systems are then collated back to data collected from *in vivo* samples from humans and mice, to further validate the models and findings collected from them.

RESULTS: Our data shows that when the HaCaT cells are grown within the scaffold they are able to grow in dense layers (Fig 1B) and have much greater contact with surrounding cells than is seen in traditional 2D culture. The cells also begin to differentiate at the top surface of the culture into the beginnings of a cornified layer, this mimics what is seen *in vivo*. The tightly packed nature of the cells also allows for the formation of more cell-cell junctional complexes (Fig 2), which are limited in standard 2D culture.

Co-culture of primary human keratinocytes on top of human embryonic fibroblasts grown in the scaffold (Fig 1C) shows even greater similarities to the structure of mammalian skin (Fig 1D). The cells show distinct stratification and cornification at the air-liquid interface.

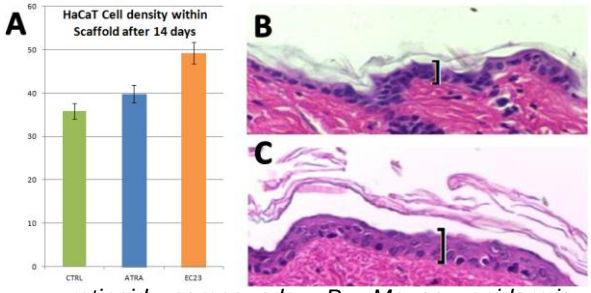

Fig 2: A; Graph detailing changes in cell density of HaCaT cells grown in Alvetex®Scaffold when treated with retinoid compounds. B; Mouse epidermis without treatment, C; mouse epidermis 10 days after topical retinoid treatment.

Treatment of keratinocytes with retinoid compounds, ATRA and ec23, causes notable changes to the cells behaviour and morphology in all model systems. The HaCaT model shows increases in cell density with application of retinoids (Fig 2A), which is correlated to an increase in epidermal thickness and more retention of the stratum corneum in mouse studies (Fig 2B/C). Treatment of the primary model with retinoid compounds is ongoing.

DISCUSSION & FUTURE WORK: Our models show great potential for studying cell morphology and protein expression during treatment with exogenous compounds. The models show close homology with *in vivo* skin samples. Ongoing work involving treatment with retinoid compounds will allow us to investigate the effects of these molecules on the structure and barrier properties of our skin models.

REFERENCES: 1 Ishida-Yamamoto et al (2011) Journal of Dermatology **38,** 645–654 .2 Simpson et al (2011) Nature Rev. Mol. Cell Biol. **12,** 565-580

ACKNOWLEDGEMENTS: This work was funded by a CASE studentship from BBSRC, with industrial support from Reinnervate Ltd.

P-25: Demethylation of a crucial NF-κB enhancer element orchestrates *iNOS* induction in osteoarthritis

María C. de Andrés[1], Atsushi Takahashi[1], Karl Alvarez[1], Richard O.C. Oreffo[1]
[1]Bone and Joint Research Group, University of Southampton, UK
mdag1d08@soton.ac.uk

INTRODUCTION:

Osteoarthritis (OA) is a complex disease of the joint, characterized by progressive degradation of the cartilage matrix by aggrecanases and collagenases. Nitric oxide (NO), the product of inducible nitric oxide synthase (*iNOS*), not only suppresses the synthesis of cartilage matrix, but also increases expression of proteases in OA. DNA methylation is an epigenetic mechanism implicated in the induction of *iNOS* in OA. We have examined the methylation profile of the NF-κB enhancer region at -5.8 kb of this gene, important in OA[1] to determine the role in *iNOS* induction of OA.

METHODS:

Methylation was determined in human articular chondrocytes and the chondrocytic cell line C28/I2. Specifically, percentage methylation was determined by pyrosequencing and gene expression by qPCR. Cell proliferation was determined using the MTT assay.

RESULTS:

In vitro de-methylation of the CpG sites localised at -5.8 kb showed decreased levels of DNA methylation in control chondrocytes (Fig1), which correlated with higher levels of *iNOS* expression. *In vitro* methylation of the NF-κB enhancer region at -5.8 kb showed high levels of apoptosis and G0/G1 arrest (Fig2).

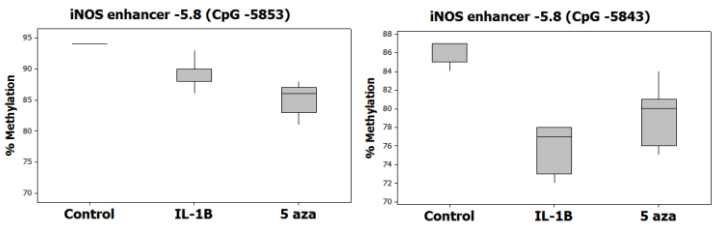

Fig. 1 Methylation of CpG sites in the -5.8 kb enhancer element of iNOS is affected by IL-1β and in vitro demethylation by 5-aza-dC.

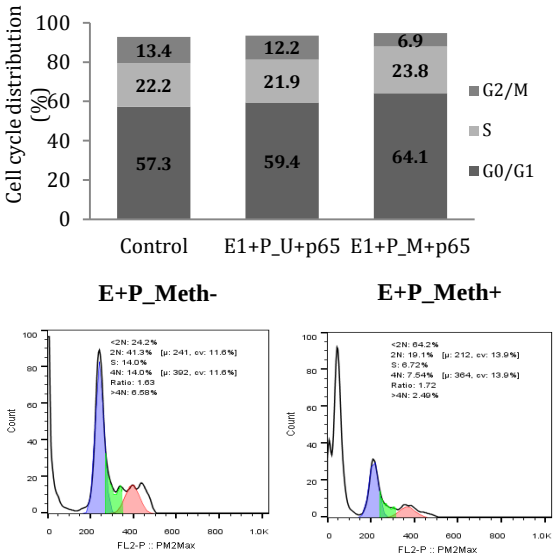

Fig. 2 Cell cycle analysis of C28/I2 chondrocytic cells transfected with the crucial NF-κB enhancer element before (Meth-) and after (Meth+) in vitro methylation treatment with a CpG methyltransferase. Results show the mean ± SD of three independent experiments.

DISCUSSION & CONCLUSIONS:

The NF-κB enhancer element localised at -5.8 kb upstream of the transcription start site is important for *iNOS* induction. Loss of methylation of this region correlated with lower levels of apoptosis, enhanced proliferation and increased cells at G2/M phase. The loss of methylation and consequent higher *iNOS* expression observed therefore, could be transmitted to daughter cells during cell division and, critically, contribute to OA pathology.

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ACKNOWLEDGMENTS:

The authors would like to thank the Leverhulme Trust for financial support for this project.

P-27: Effects of Hypoxia on Anabolic and Catabolic gene Expression and DNA Methylation in OA chondrocytes

Karl Alvarez[1], María C. de Andrés[1], Atsushi Takahashi[1], Richard O. C. Oreffo[1]
[1]Bone and Joint Research Group, University of Southampton, UK

INTRODUCTION:

Cartilage is an avascular tissue. Chondrocytes thrive in this environment of low oxygen tension and poor nutrient availability, leading to suggestions for hypoxia as a protective mechanism against the development of osteoarthritis (OA).[1]There is a growing body of evidence to support the role of epigenetic factors in the pathogenesis of OA.[2]However, few studies have investigated this effect within a hypoxic environment. Here we investigate the effects of hypoxia on gene expression and DNA methylation of anabolic and catabolic genes involved in the pathogenesis of OA.

METHODS:

Chondrocytes extracted from OA femoral heads were incubated in normoxia and hypoxia (21% and 5% oxygen concentrations respectively). Interleukin 1-beta (IL-1b) plus oncostatin M (OSM), 5-azadeoxycytidine (5-aza) or chondrogenic media alone (control) were added twice weekly to the incubated samples. After 5 weeks, levels of Collagen 9 A1 (COL9A1) and IL-1β gene expression were measured using SYBR Green-based qRT-PCR and were correlated with methylation status analysed by pyrosequencing methodology.

RESULTS:

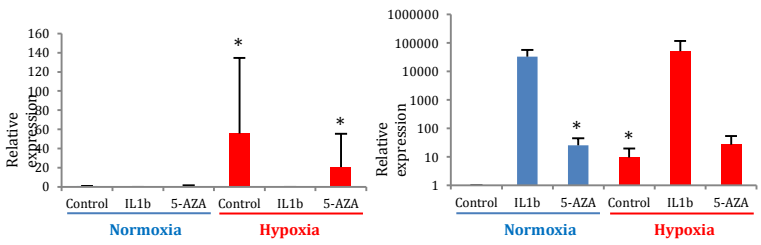

*Fig1. Relative expression of COL9A1 and IL-1β in OA chondrocytes analysed by qPCR (n = 5, *p =0.05).*

Hypoxia resulted in a significant increase in expression of IL-1β and COL9A1 (Fig. 1). This was inversely correlated to the DNA methylation status of these genes (Fig. 2).

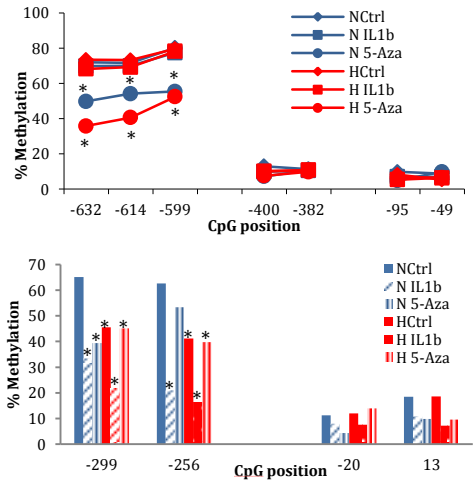

*Fig2. Percentage methylation of COL9A1 and IL-1β promoters in OA chondrocytes analysed using pyrosequencing (n = 5, *p =0.05).*

DISCUSSION & CONCLUSIONS:

These results demonstrate hypoxia upregulates anabolic and catabolic gene expression mirrored by a reduction in DNA methylation status in OA chondrocytes. These results further support the role of epigenetics in OA and, critically, highlight the complex relationship between the physiological environment of cartilaginous cells and the inflammatory processes in OA.

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[2]S Strobel et al (2010) *Arthritis Research and Therapy* **12:**R34.

ACKNOWLEDGMENTS:

The Authors would like to the Orthopaedic team at Southampton General Hospital for provision of samples, and the Leverhulme Trust for financial support for this project.

P-28: Surface modification of injectable microspheres for cell therapy applications

Abdulrahman Baki[1], Omar Qutachi[1], Toby Gould[2], Emily Overton[1], Kevin Shakesheff[1] and Cheryl Rahman[1]

[1]Drug Delivery & Tissue Engineering Department, School of Pharmacy, University of Nottingham, UK

[2]Centre for Children's Brain Tumour Research, School of Medicine, University of Nottingham, UK

Paxab12@nottingham.ac.uk

INTRODUCTION:

Injectable microspheres offer a minimally invasive approach to deliver cells for tissue repair. As substrate elasticity can direct stem cell fate[1], the aim of this study was to develop a method to alter the elasticity of PLGA microspheres with methacrylated gelatin (GelMA) using surface entrapment. Cross-linked GelMA supports cell growth[2] and has controllable elasticity[3]. Developing a technique to modify the surface of injectable microspheres with GelMA presents the opportunity to tune microsphere surface elasticity and thus influence cell fate for cell therapy applications.

METHODS:

GelMA was prepared by methacrylating porcine and fish gelatin. GelMA hydrogels were formed by UV crosslinking with photo-initiator (Ergacure 2959). Elasticity was assessed with a TA.HD+ texture analyzer. PLGA microspheres were surface modified with TFE/GelMA solution and immobilized GelMA was UV cross-linked. Microspheres were visualized using microscopy. Bicinchoninic acid (BCA) protein assay was used to quantify surface immobilized GelMA.

RESULTS:

Fit-C labelled GelMA was observed on the surface of microspheres using fluorescent microscopy (Fig.1A). Differences in surface morphology between non-modified (blank) and surface modified microspheres were visualized by SEM (Fig.1B). BCA assay results demonstrated the presence of protein (GelMA) on modified microspheres (Fig.2). Increased protein levels were observed with higher concentrations of (TFE) used in the surface entrapment process.

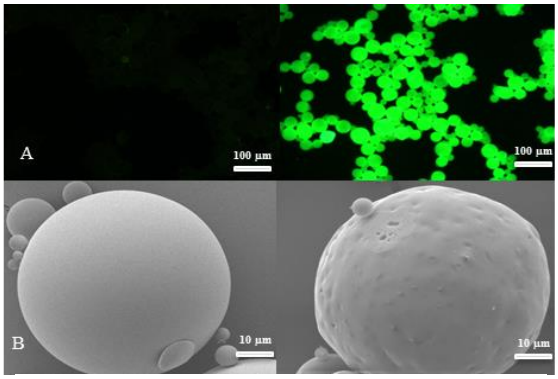

Figure 1 Images of blank PLGA microspheres (Left) and gelMA modified microspheres (Right) as shown in (A) fluorescent microscope, and (B) scanning electron microscope SEM.

DISCUSSION & CONCLUSIONS:

A promising approach for modifying the surface of PLGA microspheres with GelMA was developed. Future work will focus on altering GelMA elasticity to create a tuneable delivery system for cell therapy applications.

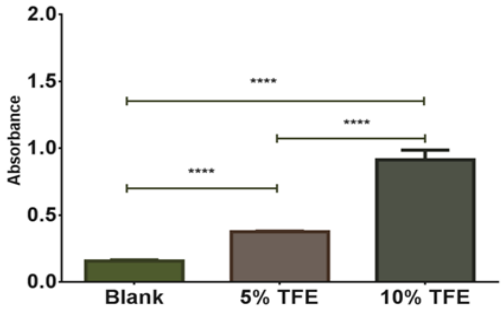

Figure 2 BCA assay results showing the change in gelMA concentration on modified PLGA microspheres.

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ACKNOWLEDGMENTS:

Authors would like to thank the UK RMP Hub for support in this research.

P-29: Dynamic Patterned Electrospun Fibres for 3D Cell Growth

Afnan Aladdad*, Lisa J. White, Cameron Alexander and Felicity R. A. J. Rose.
School of pharmacy, University of Nottingham, England
Paxama@nottingham.ac.uk

INTRODUCTION: Cell based therapies offer potentially revolutionary treatments for a number of diseases, but are dependent on the culture and supply of defined cell types in appropriate numbers. In turn, this supply requires culture environment that mimic the 3D structure *in vivo* and maintain the integrity of the cultured cells. The project was devised following the work published by Dey *et al.*, with the vision to take this work previously completed in 2D to a 3D matrix [1]. Here we demonstrate the use of co-electrospun poly (lactic-co-glycolic acid) (PLGA) and poly (ethylene terephthalate) (PET) with poly (poly (ethylene glycol) methacrylate) Poly (PEGMA) to produce three dimensional, non-woven and thermoresponsive fibres on which to culture red fluorescent protein expressing 3T3 fibroblast cells.

METHODS: Poly (PEGMA) was prepared by free radical polymerization [2], then mixed with 18.5% PLGA or 30% PET in six different concentrations (0%, 2%, 4%, 6%, 8%, 10% (*w/v*)) to form thermoresponsive scaffolds by a blend-electrospinning technique. Then, their thermoresponsive behaviors were characterized by ^{1}H-NMR, XPS and WCA measurements. 3T3 fibroblast cells were seeded on these fibres and cultured for five days. Subsequent cell viability tests (Almar Blue assay) were performed to measure the difference in cell populations while changing the culture temperature.

RESULTS: This study has demonstrated that blend-electrospinning is a promising way to create fibres with thermoresponsive surfaces, with poly (PEGMA) presence confirmed by XPS, ^{1}H-NMR and WCA measurements. These responsive scaffolds were able to support 3T3 fibroblast cells adhesion and proliferation at 37 °C. Also, it was possible to detach the cells from the scaffolds by decreasing the temperature to 15 °C, (Fig. 1), such that the poly (PEGMA) chains were strongly hydrated. Irrespective of the concentration of poly (PEGMA) used, all scaffolds exhibit thermoresponsive proprieties and cells were viable and proliferated in a similar manner to those cultured on control surfaces (PLGA or PET scaffolds) except with 10% concentration cell proliferation were less.

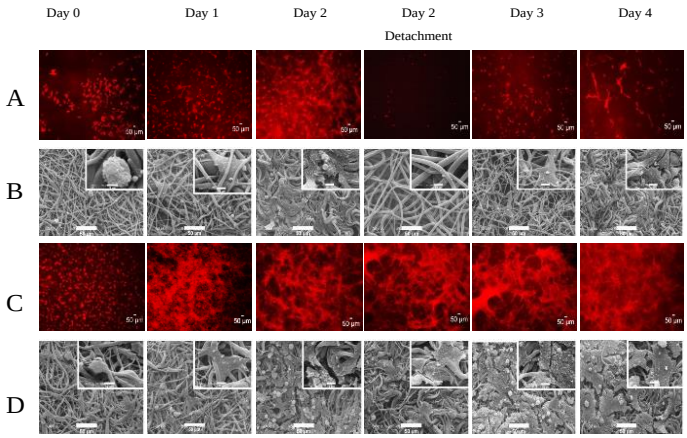

Fig. 1 Representative fluorescence microscopy and SEM images showing 3T3 cells attachment to (A-B) PLGA- 4% poly (PEGMA) and (C-D) PLGA scaffolds surfaces after 6 hrs at varying temperature–time regimes.

DISCUSSION & CONCLUSIONS: These thermoresponsive, non-woven, and bead-less co-electrospun fibres were found to be suitable for 3T3 fibroblast culture by supporting their attachment, proliferation and detachment. We are currently further investigating the use of this thermoresponsive system for prolonged cell culture and passaging experiments.

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ACKNOWLEDGMENTS: Funded by government of Saudi Arabia.

P-30: Directional Cues for Bone Regeneration in the Avian Growth Plate

Emma L. Smith[1], Carol A. Roberts[1] and Richard O.C. Oreffo[1]
[1]Bone & Joint Research Group, Centre for Human Development, Stem Cells and Regeneration, University of Southampton, U.K.
E.L.Smith@soton.ac.uk

INTRODUCTION:

Enhancement and application of our understanding of skeletal developmental biology is critical to developing new tissue engineering approaches to skeletal repair. We have previously developed a 3D *ex vivo* culture system of embryonic chick femora[1], used within this study to analyse critical directional osteogenic and chondrogenic cues present within the developing growth plate.

METHODS:

Central segmental defects were created in embryonic day 11 (E11) chick femurs. Regions of growth plate (resting and proliferative) were excised from separate E11 chick femurs, and implanted into the segmental defects. Orientation was either maintained the same, or reversed. In addition, to assess whether cues were matrix- or cell-based, resting and proliferative cells were isolated into matrix-dissociated cell pellets, and either placed into femoral defects or assessed *in vitro* for markers of bone (collagen I), cartilage (collagen II, collagen X, proteoglycan), and proliferation (PCNA). Femurs were cultured for 10 days in basal media, and assessed histologically for proteoglycan (Alcian blue), collagen (Sirius red) cell proliferation (PCNA) and the presence of skeletal stem cells (STRO-1).

RESULTS:

Isolated growth plate cells cultured *in vitro* demonstrated unique morphologies dependent on the regions from which they were isolated. Resting zone cells displayed a fibroblastic morphology, whereas cells from the proliferative zone displayed a small, rounded morphology with increased proteoglycan expression. PCNA and collagen II was expressed in both cell types, and no hypertrophy was detected (collagen X). Implanted whole growth plate regions initiated host tissue integration on only one side of the defect, depending on the orientation of the implanted growth plate region. Removal of growth plate cells from within their natural extracellular matrix disrupted this directional response with host tissue integration at both regions and a strong host periosteal induction (Fig. 1).

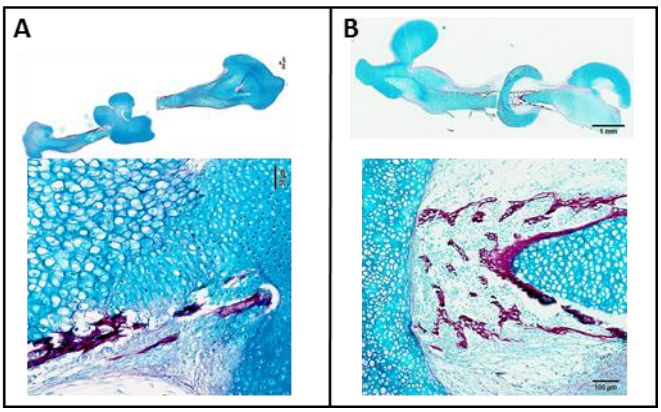

Fig. 1: Implanted growth plate regions integrate into host tissue dependent on orientation (A), which could be disrupted by removal of cells from their ECM (B).

DISCUSSION & CONCLUSIONS:

Implantation of whole growth plate regions into a chick femur defect model indicates the presence of directional cues within the growth plate that are critical for ordered directional bone growth *in vivo*. These studies provide new approaches to understanding construction of cartilage structures & chondrocyte organisation.

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ACKNOWLEDGMENTS:

This work was supported by the strategic longer and larger grant (LOLA) from the BBSRC, UK-grant number BB/G010579/1.

P-31: Do Chondrogenic ATDC5 Cells In Dense Collagen Make A Simple Cartilage?

X. Weng-Jiang[1,2], V. Salih[2], R. A. Brown[1]

[1] University College London, Institute of Orthopaedics and Musculoskeletal Science, CTRS, Division of Surgery and Interventional Science, Stanmore Campus, London. HA7 4LP. UK.

[2] University College London, Div. of Biomaterials and Tissue Engineering, Eastman Dental Institute, London, UK.

x.weng.12@ucl.ac.uk

INTRODUCTION:

Current *in vitro* models rely on flat monolayers of cells because they offer a quick, cost-effective and easy analysis. Nonetheless, in the body, cells are not attached flat, on one side to an ultra-stiff surface; they are embedded into a compliant, viscoelastic fibrous mesh of extracellular matrix (ECM) rich in 3D gradients of environmental cues. It is now widely understood that in vitro 3D tissue models are needed for more reliable, physiological test and screening systems. Our aim was to develop and characterise a living 3D model of cartilage using dense collagen (type I) and a chondrogenic cell line (ATDC5).

METHODS:

Different cellular concentrations of living 3D constructs were fabricated using the method known as Plastic Compression (PC). Constructs cultured for 1, 3 and 7 days were used to assess cell viability (Live/Dead staining) and aerobic metabolism (Alamar Blue). We used a novel optical instrument to quantify structural properties using in real time (CTS-2; Lein Diagnostics Ltd.). Matrix structure and composition was qualitatively assessed using basic H&E and Alcian Blue histology.

RESULTS:

Our findings showed that the PC did not alter viability-activity of ATDC5 cells (fig.1), though degradation/remodelling of the collagen network (data not shown) and deposition of cartilage specific matrix components by ATDC5 cells was identified (fig.2).

ACKNOWLEDGEMENTS:

I would like to acknowledge the invaluable work of Prof. Robert A. Brown and the assistance of Dr. Vehid Salih.

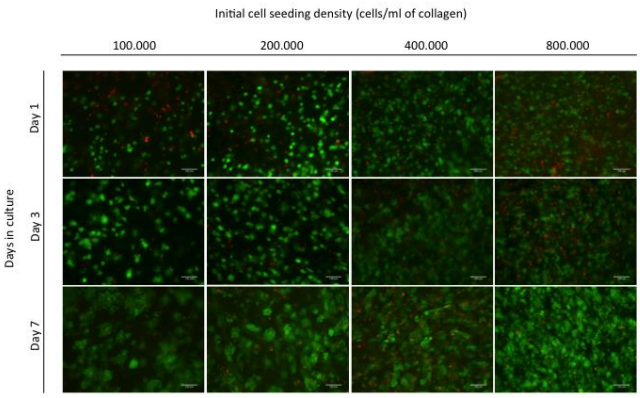

Fig1. Live/dead staining of different ATDC5 concentrations PC collagen gels at day 1, 3 and 7. Size bar = 100μm

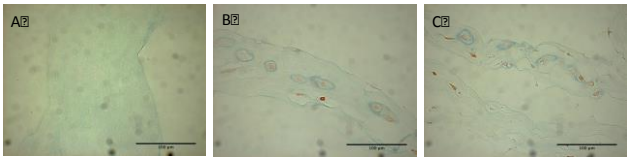

Fig. 2. Alcian Blue staining at day 14 of ATDC5 seeded PC collagen gels denoting accumulation of PG surrounding the cells. A) Control B) $2x10^4$ cells/ml collagen and C) $4x10^4$ cells/ml collagen. Size bar = 100μm.

Thickness measurements showed that constructs swelled in culture and that this effect was enhanced slightly by the presence of cells and their products, consistent with the idea that proteoglycans deposition of swell the mesh.

DISCUSSION & CONCLUSIONS:

We have showed that it was possible to culture ATDC5 cells in dense PC matrices with cartilage-specific matrix deposition with promise as a possible 3D model tissue of cartilage.

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P-32: Discrimination between High and Low Proliferative/Regenerative Dental Pulp Progenitor Cell Clones for Tissue Engineering by Raman Spectroscopy

Amr Alraies[1,2], Elisabetta Canetta[3] Rachel Waddington[1,2], Ryan Moseley[1,2] and Alastair Sloan[1,2]
[1]Tissue Engineering & Reparative Dentistry; Cardiff University, UK; [2]Cardiff Institute for Tissue Engineering and Repair, Cardiff University, UK, [3]School of Applied Sciences, St Mary's University College, London, UK

INTRODUCTION: Distinct dental pulp stem cell (DPSC) niches exist within dental pulp, with contrasting proliferative /regenerative potential[1]. Characterisation of DPSCs is a key consideration for tissue engineering, in terms of isolated clones undergoing sufficient *in vitro* expansion, whilst maintaining their regenerative potential. However, current methods are time consuming, which may be destructive for the such cells. Our recent work has confirmed clonal differences in proliferation and differentiation are partly due to contrasting telomere lengths. However, it is unknown if non-invasive techniques, such as Raman Spectroscopy, are also capable of distinguishing between DPSC clones and this initial study used Raman Spectroscopy to characterize the signatures of high and low proliferative/regenerative DPPC clones.

METHODS: Individual DPSC clones were isolated from the wisdom teeth of two patients, by fibronectin adhesion. Clones were previously identified as being high and low proliferative/regenerative by population doubling levels (PDs), telomere length and differentiation studies. Clonal suspensions of highly proliferative (A31 at 18PDs and 60PDs) and low proliferative (A11 at 8PDs, B11 at 7PDs) were fixed and analysed using an iHR550 Raman Spectrometer, equipped with a camera and linked to an Eclipse Ti-U Inverted Microscope. Single cell spectra were obtained for 20 cells for each clone over 600-1800cm^{-1}. Ten spectra were obtained for the nuclear and cytoplasmic regions from each cell and average spectra obtained. Spectra were analysed by Origin v7 and peaks assigned by comparison to references. Raman spectra were studied using principal component analysis to achieve clonal classification.

RESULTS: Raman spectra contained the typical peaks for DNA, proteins and RNA. Highly proliferative clone A31 (18PDs) possessed higher spectral band intensities that A31 (60PDs) or low proliferative clones, A11 (8PDs) and B11 (7PDs). This was particularly evident for DNA (729, 789, 1092, 1402cm^{-1}), RNA (1054cm^{-1}) and amino acids/protein peaks, such as phenylalanine, tyrosine and serine (958, 1167, 1245 cm^{-1}). PC3 and PC4 scatter plots demonstrated overlapping signatures between low proliferative clones, A11 and B11. In contrast, A31 at 18PDs and 60PDs did not overlap with each other, or with A11 and B11.

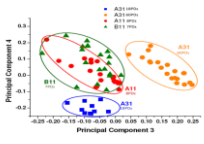

Fig.1 Mean Raman spectra obtained from three clones at early PDs and A31 at later PD, displayed in the fingerprint range 600 to 1800cm^{-1}.

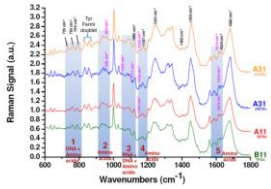

Fig 2 Principal component analysis.

DISCUSSION & CONCLUSIONS: This initial study demonstrates that Raman Spectroscopy may be capable of non-invasively discriminating between high and low proliferative DPSCs. As most spectral band differences identified between clone A31 and A11/B11 are implicated in the cell cycle, findings concur with our previous data that clone A31 is highly proliferative in nature. This study advocates the potential use of Raman Spectroscopy as a selective screening tool for the identification and isolation of highly proliferative/regenerative DPSCs from whole dental pulp for clinical use.

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ACKNOWLEDGEMENTS: Albawani Company for their financial support.

P-33: Introducing Complexity in Biomedical Devices throughout the Incorporation of Artificial Stem Cell Microenvironments

Ilida Ortega Asencio[1], Sheila MacNeil[2], Aileen Crawford[1], Paul Hatton[1], Frederik Claeyssens[2]

[1]The School of Clinical Dentistry and [2]Kroto Research Institute, The University of Sheffield, South Yorkshire, UK, i.ortega@sheffield.ac.uk

INTRODUCTION:

Stem cells are crucial for tissue regeneration and the ability of these unique cells to self-renew is thought to be at least partially due to their location within enclosed protective niches. Our aim is to design and manufacture biomaterial devices containing artificial niches for exploring the regeneration of soft tissues such as cornea and ultimately other tissues such as bone. In this study we have designed two types of microfabricated corneal outer rings (one biodegradable and the other non-biodegradable; fig.1D, 1G) and we have studied the effect of including microfeatures within the constructs.

METHODS:

PEGDA (polyethylene glycol diacrylate) non-degradable scaffolds were manufactured using microstereolithography[1]. PLGA (polylactide-co-glycolide) biodegradable scaffolds were manufactured using electrospinning[2]. The potential use of the microstructured rings as cell delivery devices was evaluated in a 3D rabbit cornea model using both limbal tissue explants and rabbit limbal epithelial cells.

RESULTS:

We specifically located cells in the artificial micropockets and we obtained promising results regarding epithelial cell transfer and re-epithalisation for both kinds of constructs (Fig.1). We also observed that the inclusion of microfeatures within the constructs affected epithelial cell migration and morphology. Moreover, a population of slow-cycling cells (positive for P63) was identified when tissue explants were cultured inside the artificial pockets and placed on 3D models; this was not observed when using cultured limbal epithelial cells suggesting that the enclosed niches together with the presence of native stromal cells within the explants play an important role in stem cell maintenance.

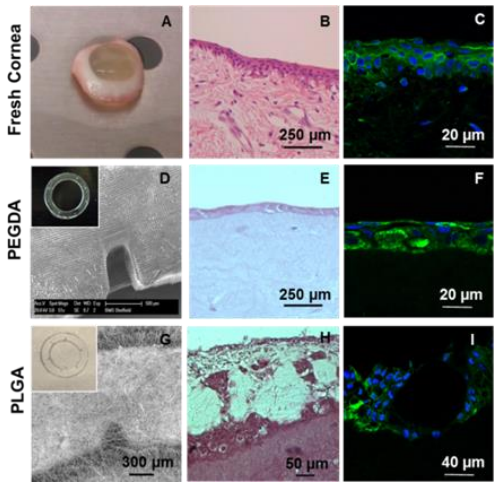

Fig 1. Rabbit cornea model (A); H &E and CK3 staining of fresh rabbit cornea (B, C); H &E and CK3 of tissue engineered cornea after 6 weeks organ culture with a RLE loaded PEGDA ring (E, F); H &E and CK3 of tissue engineered cornea after 4 weeks organ culture with a PLGA membrane with limbal explants (H, I); SEM images of PEGDA and PLGA microfeatured outer rings (D, G).

DISCUSSION & CONCLUSIONS:

This work provides techniques for producing artificial niches for studying stem cell behaviour using *in vitro* and *ex vivo* models. Using corneal ring models we demonstrated that the presence of microfeatures has a direct effect on parameters such as migration, cell morphology and stemness. Future work will explore the use of similar models for the regeneration of other tissues (e.g.bone).

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ACKNOWLEDGMENTS:

We thank the EPSRC Landscape Fellowship Scheme and the Wellcome Trust Foundation.

P-34: Silk-based Microparticles as an Osteogenic Scaffold for Tissue Engineering

Kim A. Luetchford[1], Julian B. Chaudhuri[2], Paul A. De Bank[1]
[1]Department of Pharmacy & Pharmacology, University of Bath, U.K.
[2]School of Engineering and Informatics, University of Bradford, U.K.
K.A.Luetchford@bath.ac.uk

INTRODUCTION:

Silk fibroin (SF) has long been considered a material with potential for tissue engineering as it is biocompatible, strong, light-weight and can be processed into a number of formats[1]. However, it is not highly cell-adhesive; hence it is frequently blended with other materials such as gelatin. SF has previously been investigated as a scaffold material for bone repair in the form of porous sponges and hydrogels. The work described here investigates microparticles produced from SF/gelatin (SF/G) as osteogenic cell scaffolds. The microparticles described will allow for the creation of larger tissue constructs by the process of moulding.

METHODS:

An aqueous solution of SF was extracted from the cocoons of *Bombyx mori.* Gelatin was porcine derived (Type A). Blends of SF and gelatin were processed into homogenous microcarriers by axisymmetric flow focussing[2]. Mesenchymal stem cells (MSCs), harvested from the bone marrow of juvenile Wistar rats, were maintained in MEM with 10% (v/v) fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin, at 37°C in a 5% CO_2 atmosphere. Osteogenic differentiation medium (ODM) was supplemented with 0.1 µM dexamethasone, 0.2 µM ascorbic acid 2-phosphate and 10 mM glycerol 2-phosphate. Cells seeded onto microparticles were cultured in either basal medium or ODM for a minimum of 21 days. Osteogenic differentiation was confirmed by positive alkaline phosphatase (AP) activity (BCIP/NBT assay).

RESULTS:

MSCs were successfully seeded onto all of the blended SF/G microparticles with high efficiencies. Seeding efficiencies of the blended microparticles were significantly higher than for SF alone. Cell seeding and viability was confirmed using LIVE/DEAD stain with confocal microscopy. A high proportion of cells remained viable after culture on the SF/G microparticles. MSCs showed evidence of differentiation in both medium types (Figure 1), suggesting the scaffold itself supports differentiation in non-supplemented medium.

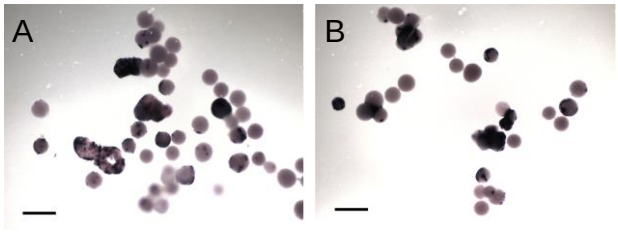

Fig. 1: Purple deposits show positive alkaline phosphatase activity in MSCs cultured on SF/G 25:75 microparticles in basal media (A) or ODM (B). Scale bar represents 500 µm.

DISCUSSION & CONCLUSIONS:

MSCs were successfully seeded onto novel microparticles composed of SF/G blends. The microparticle scaffolds supported osteogenic differentiation in both basal and supplemented media. The results described here suggest that the production of a macroscopic bone construct could be achieved using SF/G blended materials under appropriate growth conditions.

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ACKNOWLEDGMENTS:

Funding from the MRC is gratefully acknowledged.

P-35: GET: Glycosaminoglycan (GAG)-binding Enhanced Transduction of Functional Proteins

James E. Dixon[1*], Gavin Morris[1], Nina Lane[2], Chris Denning[3] and Kevin M. Shakesheff[1]

Wolfson Centre for Stem Cells, Tissue Engineering, and Modelling (STEM), Centre of Biomolecular Sciences, [1]School of Pharmacy, [3]School of Medicine; [2]Flow Cytometry Facility, School of Medicine; University of Nottingham, Nottingham, NG7 2RD, UK; University of Nottingham, Nottingham, NG7 2RD, UK.

james.dixon@nottingham.ac.uk

INTRODUCTION:

Protein transduction domains (PTDs) are powerful non-genetic tools that allow intracellular delivery of conjugated cargoes to modify cell behaviour. Their use in biomedicine has been hampered by inefficient delivery to nuclear and cytoplasmic targets.

METHODS:

mRFP1, Cre Recombinase, transcription factors, antibiotic resistance proteins and PTDs were cloned, fused and expressed in/purified from *E.coli* using GST affinity chromatography. Cells were as described[1]. Flow cytometry used a MoFlo^{TM} DP (DAKO) using a 488nm green laser. Cre assays used NIH3t3: LSL-eGFP cells created in-house.

RESULTS:

Here we overcame PTD deficiencies by developing a novel fusion protein that couples a membrane docking peptide to heparan sulfate glycosaminoglycans (GAGs) with a PTD. We showed this GET (GAG-binding enhanced transduction) system could deliver fluorescent reporters (mRFP1), functional enzymes (Cre, neomycin phosphotransferase) and transcription factors (NANOG, MYOD) at efficiencies of up to two-orders of magnitude higher than previously reported in cell types considered hard to transduce, such as mouse embryonic stem cells (mESCs), human ESCs (hESCs) and induced pluripotent stem cells (hiPSCs).

DISCUSSION & CONCLUSIONS:

This technology represents an efficient strategy for controlling cell behaviour and directing cell fate that has broad applicability for basic research, disease modelling and clinical application.

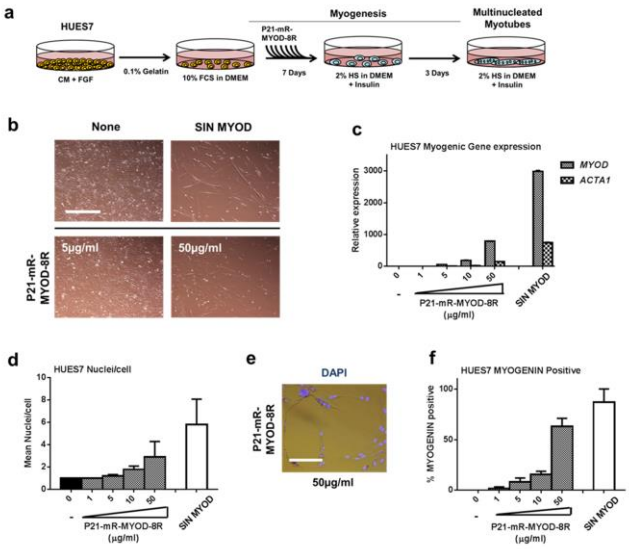

Figure 1. GET of MYOD promotes Myogenic differentiation of human embryonic stem cells. (a) *Scheme of testing the differentiation activity of transduced MYOD in HUES7 cells.* ***(b-f)*** *P21-mR-MYOD-8R drives myogenic differentiation of HUES7 cells to multinucleated Myotubes.* ***(b)*** *Light microscopy of HUES7 cells cultured under the myogenic regime. bar, 100µm.* ***(c)*** *Relative gene expression analyses of HUES7 cultures using quantitative PCR (QPCR). Error bars indicate s.e.* ***(d-f)*** *P21-mR-MYOD-8R differentiated cells are multinucleated and MYOGENIN positive. Error bars indicate s.d.*

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ACKNOWLEDGMENTS:

We would like to thank Dr. Andrew D. Johnson (University of Nottingham) and Dr. Catherine Merry (University of Manchester) for helpful discussions. We thank the European Research Council under the European Community's Seventh Framework Programme (FP7/2007-2013)/ERC grant agreement 227845 for funding. KMS acknowledges the support of the UK Regenerative Medicine Platform.

P-36: Nanoindentation of hydrogel composites determined by a flat punch tip

Jinju Chen[1,2*] and Samuel Wilson[1]
[1*]School of Mechanical & System Engineering, Newcastle University, UK
[2] Arthritis Research UK (ARUK) Tissue Engineering Centre, Newcastle University, UK
Jinju.chen@ncl.ac.uk

INTRODUCTION:

As a non-destructive approach, nanoindentation is effective to determine the materials properties of heterogeneous materials in tissue engineering with high-resolution[1]. This is important because cells sense local stiffness instead of bulk stiffness[2]. Nanoindentation with a flat punch tip is particularly good for indenting very soft biopolymer like hydrogels[3]. However, the relation between the nanoindentation elastic responses of the two-phase hydrogel composite and the stiffness ratio of these two individual hydrogels remains elusive. Therefore, we will use finite element (FE) simulations and mathematical model to explore such correlation.

METHODS:

An axisymmetric FE model is adopted to study the nanoindentation of the two-phase hydrogel via a flat punch with radius 0.5 μm and a fillet radius of 0.01 μm (see Fig.1). For phase 1, its elastic modulus is fixed at 10kPa and for phase 2 it is varied from 2 to 100kPa which covers a range of typical hydrogels for tissue engineering. Their viscoelastic behaviour is captured by Prony series model.

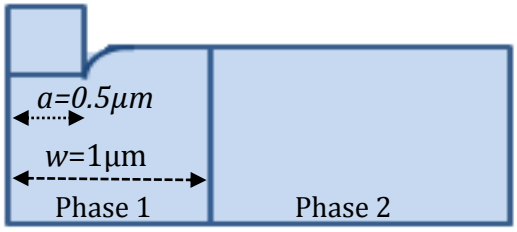

Fig. 1 Schematic of indentation of a two-phase hydrogel with a flat punch tip.

RESULTS:

Fig.2 displays some typical force-displacement curves during nanoindentation. Fig.3 shows the relation between the elastic responses of the two-phase hydrogel composite and the stiffness ratio of these two individual hydrogels based on the Clifford model[4].

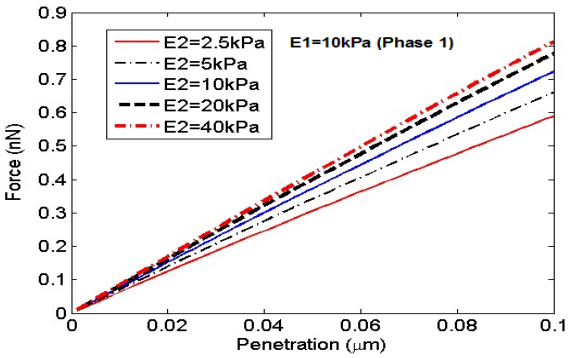

Fig. 2 Typical force-displacement curves.

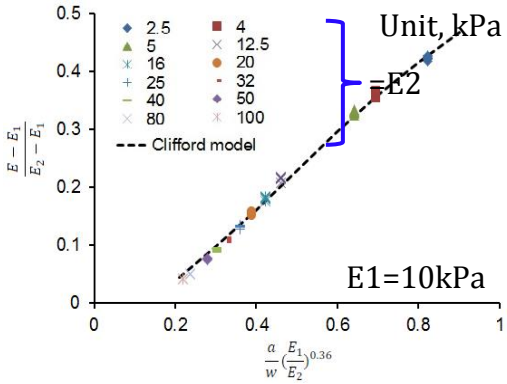

Fig.3 Relation between the elastic responses of the composite and the stiffness ratio of these two individual hydrogels.

DISCUSSION & CONCLUSIONS:

It has revealed the surrounding matrix has significant contribution to the measured modulus of the inclusion phase. The Clifford model originally developed for indenting elastic materials via a spherical tip can be modified to predict such an influence for indenting viscoelastic materials with a flat punch indenter.

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P-37: Nanomechanical responses of biopolymer composites determined by nanoindentation with a conical tip

Jinju Chen[1,2*] and Pengfei Duan[1,3]
[1*]School of Mechanical & System Engineering, Newcastle University, UK
[2] Arthritis Research UK (ARUK) Tissue Engineering Centre, Newcastle University, UK
[3] School of Chemical Engineering & Advanced Materials, Newcastle University, UK
Jinju.chen@ncl.ac.uk

INTRODUCTION:

Nanoindentation is particularly useful to determine the elastic moduli of individual phases in composite materials (e.g. biopolymer composites for tissue engineering) [1]. Indeed, the engineered tissues are also composite materials. When performing nanoindentation on these composite materials, it is essential to define the influence of surrounding second phase. In this study, we will use finite element analysis (FEA) and mathematical model to investigate such an influence.

METHODS:

An axisymmetric FE model is established to study the nanomechanical responses of the two-phase biopolymer composites indented by a conical tip (see Fig.1). The equilibrium elastic modulus for inclusion (Ei) is assumed to be 10MPa. The modulus of the matrix (Em) varies from 2 to 50MPa which covers a wide range of typical hydrogel biopolymers. Their viscoelastic behaviour is described by Prony series model[2].

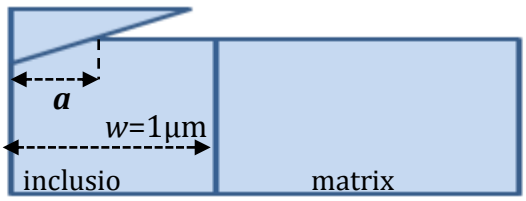

Fig. 1 Schematic of the indentation of two-phase biopolymer composites.

RESULTS:

Fig.2 displays some representative force-displacement curves and the corresponding spatial dependent elastic moduli when indenting this composite. Fig.3 shows the prediction about how the surrounding matrix contributes to the measured composite modulus based on the similar concept in Clifford model[3].

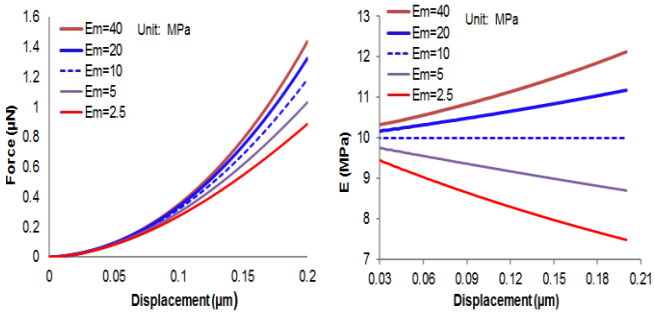

Fig. 2 The force-displacement curves and corresponding spatial dependent elastic moduli.

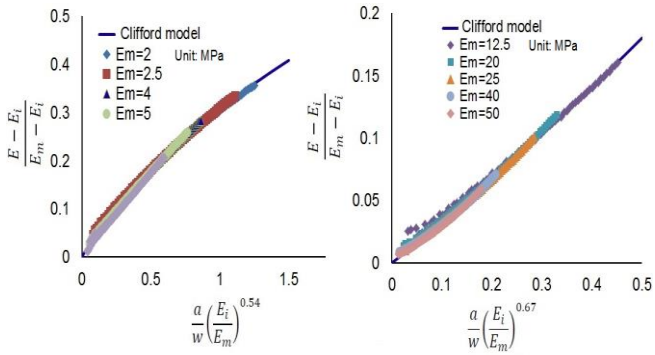

Fig.3 The contribution of the surrounding matrix to the measured composite modulus.

DISCUSSION & CONCLUSIONS:

It revealed that measured elastic modulus of the inclusion can be significantly affected by the surrounding matrix well before the indenter touches the matrix. The model presented here enables modelling such an influence, which will give guideline for modulus mapping when indenting various biopolymer composites.

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P-38: Nanomechanics of bioimplant infection related bacteria

Jinju Chen[1,2*] and James Hood[1]
[1*]School of Mechanical & System Engineering, Newcastle University, UK
Jinju.chen@ncl.ac.uk

INTRODUCTION:

Healthcare–associated infection is responsible for many biomedical implant failure. A recent study has shown that nanoprotrusions on silicon could kill various bacteria commonly presented at hospitals[1]. Such a nanostructure-bacteria interaction would result in the change of bacteria mechanics which can be assessed by nanoindentation[2]. In order to circumvent characterizing the localized mechanical properties of the cell, a relatively large penetration is preferred. However, in such case, the boundary conditions in the existing analytical model[3] violated due to limited dimension of the bacteria. The objective of this work is to develop a new model to tackle this.

METHODS:

An axisymmetric FE model is adopted to investigate the nanomechanical responses of the bacteria indented by a conical tip (semi-included angle=60°) with a trapezoidal loading function as shown in Fig.1. The elastic modulus of the bacteria is assumed to be 1kPa. Its viscoelastic behaviour is described by Prony series model. A similar mathematical framework presented by Chen et al [4] is adopted to account for a large deformation relative to bacteria size.

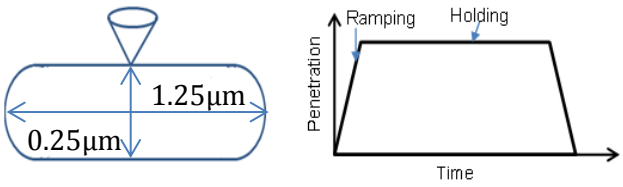

Fig. 1 Schematic of the indentation of rod bacteria and the trapezoidal loading function.

RESULTS:

Fig. 2 displays the comparisons of nanoindentation responses during ramping and holding period, determined by the simulation, the existing model and the new predictive model.

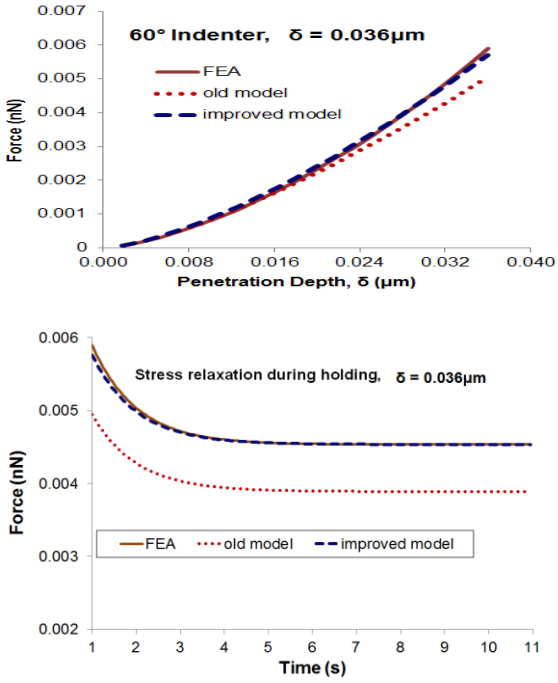

Fig2. Nanoindentation responses determined by the simulation and different models.

DISCUSSION & CONCLUSIONS:

A new model has been developed to determine the viscoelastic properties of a rod-shaped bacteria for a large deformation relative to bacteria size. Such an approach is also applicable to others cells in tissue engineering. Understanding how nanomechanics of cells are regulated by scaffold materials could guide the surface design of scaffold materials.

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ACKNOWLEDGMENTS:

EPSRC (EP/K039083/1) is acknowledged for providing financial support.

P-41: Non-Destructive *In Vitro* Tools for the Comparison of Degradable Biomaterials

Bardsley Katie[1], Wimpenny Ian[1], Bin Hu, Yang Ying[1] and El Haj J Alicia[1]
[1]ISTM, University of Keele, UK k.s.bardsley@keele.ac.uk

INTRODUCTION:

Degradable biomaterials are often required for regenerative medicine. These biomaterials are gradually resorbed and replaced by natural tissues. This rate of degradation often has an effect on cell proliferation and the production of extracellular matrix (ECM) proteins. Biomaterial degradation has been traditionally monitored using destructive techniques including weight loss and HPLC. This study, however looks at the production of fluorescent biomaterials whose degradation can be monitored online and compared to ECM production and cell proliferation to produce a turnover index which can be used as an online comparative tool for biomaterial selection.

METHODS:

Fluorescent biomaterials – PLGA 50/50 (Sigma Aldrich) and Chitosan (Sigma Aldrich) were solvent cast in chloroform and acetic acid respectively. PLGA was plasma coated with ammonia followed by treatment with TRITC, whereas chitosan was directly treated with TRITC. Alexa fluor-488 fibrinogen (Life Technologies) was combined with fibrinogen (Merck) and thrombin (Sigma Aldrich) in the presence of calcium to produce a fibrin gel

Cell Culture – MLO-A5 were seeded on to the biomaterials ($5x10^4$cell/cm^2) and cultured in osteogenic media for 7 days. Chitosan was degraded by addition of 0.2 mg/mL lipase to the media. Proliferation rates were assessed using Alamar Blue™ (Life Technologies) and ECM production was monitored using an Osteopontin ELISA (R&D Systems). Turnover indices (TI) were calculated as ratios between degradation rates and proliferation or ECM production.

RESULTS:

Fluorescently tagged biomaterials (*FIG 2*) were monitored with time and fluorescence was released into the supernatant at rates comparable to that observed using a weight loss technique.

MLO-A5 proliferation decreased on biomaterials with a higher degradation rate (*FIG 2(A)*), however the production and expression of osteopontin (*FIG 2(B)*) was increased.

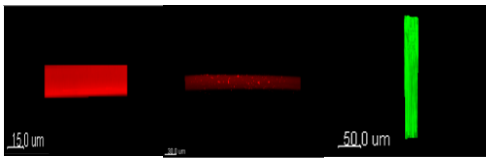
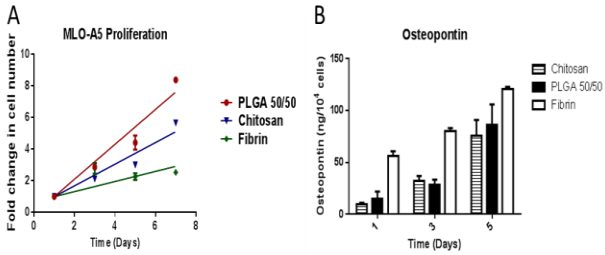


Fig. 1 Fluorescent Biomaterials (A) chitosan; (B)PLGA 50/50 and (C) fibrin.

A TI which can be used to compare the biomaterials can therefore be calculated. This



C

	Cell Proliferation Turnover Index	Osteopontin Turnover Index
Chitosan	0.028	15.54
PLGA 50/50	0.092	19.60
Fibrin	0.028	83.47

showed that fibrin which degraded quickest had the lowest proliferation rate and the highest production of osteopontin (*FIG 2(C)*).

Fig. 2 (A) MLO-A5 proliferation on fluorescent scaffold materials measured through Alamar Blue™ assays; (B) osteopontin production and (C) table of turnover indices.

DISCUSSION & CONCLUSIONS:

The use of online monitoring of fluorescent degradation is advantageous as it is non-destructive and allows for the online monitoring of the production of engineered tissues. The use of a TI value allows for the comparison of degrading biomaterials. Our aim is to generate an *in vitro* model and tools which allow the correct biomaterial to be selected for translation to clinic.

ACKNOWLEDGMENTS:

Authors would like to acknowledge Paul Roach for his help with plasma coating and Framework VII Programme BIODESIGN (Grant no: 262948) for providing financial support to this project.

P-42: Porous Chitosan Constructs Support Bone Marrow Stem Cells for Osteochondral Modelling

Alexander A. Popov[1*], George Roberts [2], David M. Grant[2], Colin A. Scotchford[2], and Virginie Sottile[1]

[1] Wolfson Centre for Stem Cells Tissue Engineering & Modelling, CBS, University of Nottingham, UK

[2] Bioengineering Group, Faculty of Engineering, University of Nottingham, UK

[*] mxzaap@nottingham.ac.uk

INTRODUCTION: Regenerative therapeutic solutions are required to address the increasing prevalence of bone and cartilage diseases within the population. Limitations of existing treatments, such as bone graft reconstructions or biomaterial implants, suggest that osteochondral tissue constructs with the ability to support differentiation of mesenchymal stem cells (MSCs) into both osteoblast and chondrocyte lineages are desirable[1]. The current project aims to overcome issues arising from the divergent differentiation requirements for each lineage by providing scaffolds with spatially resolved environments, each supportive of one of these cell lineages. The work reported here describes the porogen and cross-linker optimisation for the production of bi-layered chitosan scaffolds containing two distinct pore sizes. Scaffolds were characterised before cell seeding and 3D culture in a perfusion bioreactor.

METHODS: Porous scaffolds were produced using a freeze-gelation method from a 4% chitosan solution mixed with polycaprolactone (PCL) porogen and cross-linked using either 0.5% glutaraldehyde or 0.3% genipin solutions. Porogen microspheres were produced by an emulsion method. Porogen particles and scaffold porosity were evaluated using scanning electron microscopy (SEM) and micro-computed tomography (MicroCT). Cytocompatibility was assessed after seeding of human mesenchymal stem cells (hMSC) onto fibronectin-treated scaffolds and culture in a perfusion bioreactor for up to 3 weeks.

RESULTS: Dual porosity scaffolds were produced and analysed by MicroCT, confirming different porosity and pore size distribution for bottom, middle and top sections of scaffolds made with PCL microspheres (Table 1).

	Bottom	Middle	Top
% Porosity	74	73	73
Pore size (μm) ± SD	287 ± 10.5	232 ± 17.5	196 ± 5.5

Large (300-425 μm) pores were designed to promote osteogenic differentiation of hMSCs and smaller, (180-300 μm) pores to support chondrogenesis. SEM analysis of the scaffolds illustrated the graded pore size distribution (Fig.1), and the absence of delamination between layers.

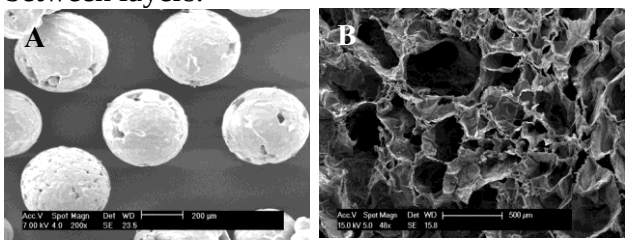


Figure 1 **SEM images of PCL porogen microspheres (A) and resulting bi-layered chitosan scaffolds (B).**

Scaffolds cross-linked with genipin displayed more uniform pore morphology than ones made with glutaraldehyde. Scaffolds were then seeded with hMSCs and cultured both on culture dishes and in a perfusion bioreactor for up to 21 days. Cell viability and differentiation were assessed at different time-points to measure osteochondral lineage maturation *in situ.*

DISCUSSION AND CONCLUSIONS:

A novel dual porosity chitosan scaffold was produced for osteochondral modelling. Results show that the PCL microspheres permitted good control over pore characteristics in the scaffolds. Subsequently, hMSC seeding methods were evaluated for optimal cell infiltration into the scaffold core to populate the construct. Scaffolds cross-linked with genipin allowed higher cell metabolic activity than glutaraldehyde, to a level comparable to tissue culture plastic. MSC differentiation analysed over 3weeks highlighted the influence of scaffold structure on cell compatibility in 3D.

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P-44: The effects of decellularisation on the mechanical properties of bone

Mohd Riduan Mohamad, Philip Riches, M. Helen Grant
Department of Biomedical Engineering, University of Strathclyde, G4 0NW, United Kingdom

INTRODUCTION: Bone defects as a result of trauma and physiological and pathological bone resorption represent a major challenge and have become a worldwide health issue[1]. Regenerative medicine strategies involving decellularised extracellular matrix scaffolds are developing very fast[2]. This project aims to demonstrate and establish decellularisation protocols to make donor bone scaffold, which could be used to repair bone defects in recipient patients and to determine whether the decellularisation process alters the mechanical properties of the bone scaffold.

METHODS: Bovine femur samples were decellularised by six cycles of overnight incubation at 37°C using two protocols: A – 10mM Tris, 1mM EDTA, 0.1% v/v Triton X-100; and B – method A plus 0.5% w/v trypsin. Decellularisation was confirmed by the absence of DNA staining with DAPI. Mechanical testing (Bose ElectroForce 3200) and porosity (mercury porosimetry) measurements were carried out before and after the decellularisation process for bone samples using protocol A and protocol B.

RESULTS:

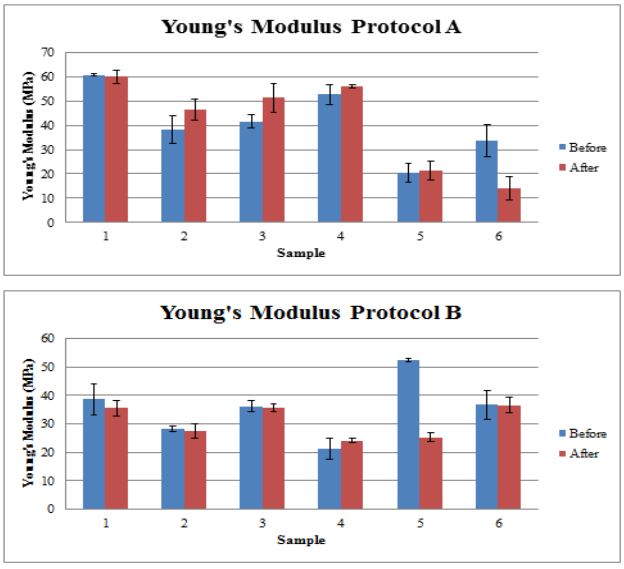

Figure 1: The comparisons of Young's modulus (E) in bone samples before and after six cycles of incubations with protocol A or B. Results are the mean ± SEM of n = 3. p>0.05 compared with the control (ANOVA for repeated measures).

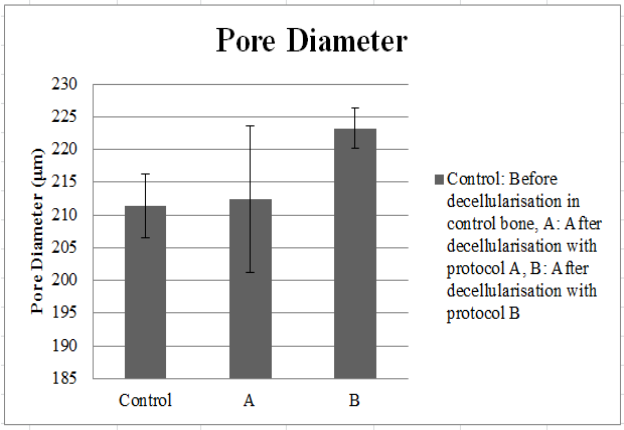

Figure 2: The pore size diameter of bone samples before and after decellularisation with protocol A or B. Results are the mean ± SEM of n = 3. p>0.05 compared with the control (ANOVA for repeated measures).

DISCUSSION AND CONCLUSIONS: The present study indicates that decellularisation process with protocols A or B did not affect the mechanical properties in terms of Young's Modulus (E, stiffness), and there was no significant difference in pore size diameters before and after decellularisation. The bone scaffold produced in all protocols had an average pore size of 211.349µm to 223.275µm. DAPI staining revealed that post-decellularisation by protocol B no measurable DNA was present on the bone samples. Protocol B using trypsin could therefore be used to make donor bone scaffold, which could be used to repair bone defects in recipient patients.

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P-45: Hydrostatic force bioreactor-a novel tool for mechanical conditioning of cells, tissues and tissue constructs

Y. Reinwald[1], Katie H.L. Leonard[2], James R. Henstock[1], Philippe Levesque[3], Alicia J. El Haj[1]*

[1]Institute of Science and Technology in Medicine, University of Keele, Thornburrow Drive, Stoke-on-Trent ST4 7QB, UK.
[2]Department of Computer Science, University of Oxford, Wolfson Building, Parks Road, Oxford OX1
[3]QD, UK. 3Instron TGT, 825 University Ave, Norwood MA 02062 USA.
y.reinwald@keele.ac.uk

INTRODUCTION: Cell fate and tissue development are affected by mechanical stimulation and chemical cues. Bioreactors provide growth environments to engineer tissues and to investigate the effect of mechanical forces on cells and cell-scaffold constructs. Hence, to define outcome, evaluation of the bioreactor environment during culture is critical. A novel hydrostatic force bioreactor imposing low levels of cyclical hydrostatic force at 0.005 Hz to 2 Hz frequency and 0-280 kPa on standard tissue culture multi well plates was developed in collaboration between Instron Tissue Growth Technologies, Ltd and Keele University. This study aims to investigate the growth environment within the bioreactor to define outcome for regenerative medicine and clinical applications.

METHODS: Physiological variables such as dissolved oxygen and carbon dioxide concentration in the medium and pH have been investigated experimentally. Physical forces (shear stress and pressure) in the bioreactor were determined by mathematical modeling and numerical simulation. The effect of hydrostatic pressure on differentiation and maturation of stem cell types was assessed using human bone marrow derived mesenchymal stem cells and chick femur fetal skeletal cells in monolayer and 3D hydrogel as well as organotypically cultured ex vivo chick foetal femurs and human embryonic stem cells.

RESULTS: The concentration of dissolved O_2 and CO_2 in the medium increased after mechanical stimulation, whereas the pH of the medium decreased. These changes were dependent on the applied hydrostatic pressure and were reversible when samples were transferred to a cell culture incubator. Further investigation is necessary to investigate the effect of these changes on potential cell fate and tissue development. Mathematical modeling and numerical simulation showed that the distribution and magnitude of physical forces such as shear stress and pressure depends on the shape and position of the cell-seeded hydrogels in the tissue culture well plates. Finally, cyclic hydrostatic force worked synergistically with chemical cues resulting in an increase in mineralised densities of cell-seeded hydrogels and chick foetal femurs leading to enhanced osteogenesis. In addition, hydrostatic force enhances the bi-refringent properties of 3D collagen hydrogels which suggested increased alignment of collagen fibrils.

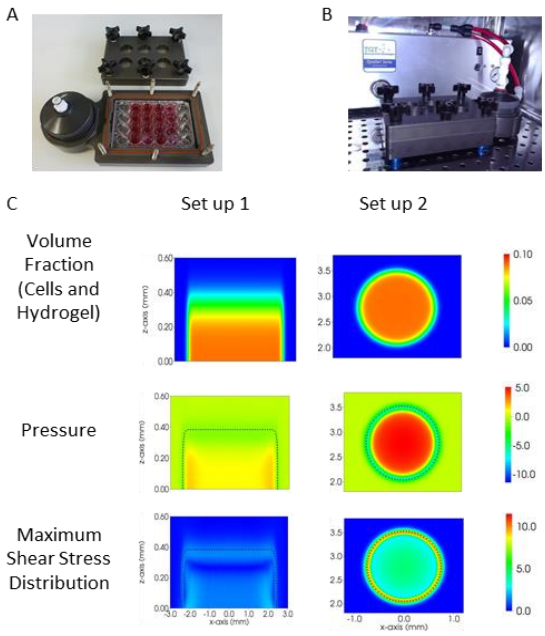

Figure 1: Hydrostatic force bioreactor. Images show the bioreactor chamber (A) and the bioreactor set up in the incubator (B). Distribution of volume fraction, pressure and shear stress are shown for two theoretical experimental set ups (C).

DISCUSSION & CONCLUSIONS: This novel bioreactor allows the application of hydrostatic force for gas-liquid interface culture at physiological relevant pressures and provides a growth environment for engineered tissues. The application of hydrostatic pressure resulted in changes of dissolved O_2, CO_2 and pH of the medium, which remained within the human physiological range. Moreover, this bioreactor is alignable to culture in standard culture environments and could be a suitable tool for pre-conditioning of cells and tissue for clinical tissue regeneration.

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ACKNOWLEDGMENTS: The authors would like to thank the EPSRC Centre for Innovative Manufacturing in Regenerative Medicine (ECP007/1010) and Instron TGT, Ltd for funding.

P-46: Promoting Vascularization in Bioactive Microenvironments

Vladimíra Moulisová, Marco Cantini and Manuel Salmeron-Sanchez
MiMe Research Group, Division of Biomedical Engineering, University of Glasgow, Scotland
vladimira.moulisova@glasgow.ac.uk

INTRODUCTION:

Forming functional vascular network remains a great challenge in tissue engineering[1]. One of the strategies how to achieve this is a targeted delivery of pro-vasculogenic growth factors. Use of ECM proteins able to bind various growth factors and to present them in order to modulate cell behaviour looks promising[2]. In addition, spontaneous fibrillogenesis of fibronectin (FN) on specific polymers was recently discovered[3] what can enhance GF sequestration, and contribute to stimulation of cell differentiation.

METHODS:

Sample preparation and testing: Polymers (poly (ethyl acrylate), PEA, and poly(methyl acrylate) PMA as a control) were spincoated on glass coverslips to obtain ~ 1 μm thin layer. Samples were incubated with FN to form a network, and coated with VEGF. Samples were tested for VEGF binding by ELISA and AFM.

Cell culture: HUVECs were used to test samples for stimulation of tubular network formation; cells were fixed and stained for actin and DNA.

RESULTS:

Binding of VEGF to fibronectin fibrils on PEA was characterized by ELISA, and visualized by AFM. There, VEGF molecules bind to a central part of a FN arm (Fig. 1).

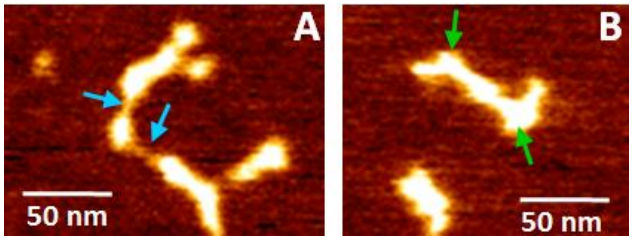

Fig. 1 AFM phase images of FN on PEA without (A) and with (B) VEGF bound. Blue arrows show unoccupied FN binding sites for growth factors, green arrows show VEGF bound to FN.

HUVECs cultured on samples for 6 days showed clear reorganization into network resembling structures (Fig. 2), while experiment with control polymer (PMA) did not show such cell behaviour.

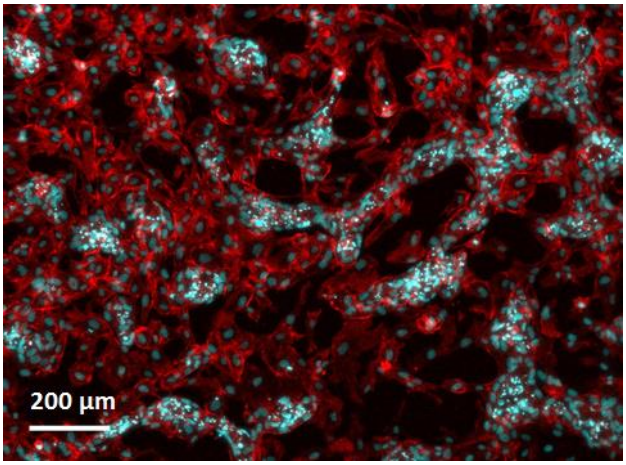

Fig. 2 HUVECs forming a network on PEA with FN fibrils binding VEGF covered with fibrin matrix. Actin is in red, nuclei are in cyan.

DISCUSSION&CONCLUSIONS:

A specific combination of a FN network formed on PEA and VEGF was shown to be able to provide conditions for triggering HUVECs formation of network structures, and it will be used for further testing of primitive vascular plexus formation *in vitro*. It represents a promising microenvironment to support so much needed circulatory system development in various tissues to allow their regeneration.

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ACKNOWLEDGMENTS:

The authors would like to thank the ERC through HealInSynergy (Grant No. 306990) for financial support.

P-47: Human Airway Smooth Muscle maintain *In Situ* cell Orientation and Phenotype when cultured on Aligned Electrospun Scaffolds

Gavin Morris[1], Jack Bridge[1], Alan Knox[2], Jonathon Aylott[3], Osama Eltboli[4], Christopher Brightling[4], Amir Ghaemmaghami[5], Felicity Rose[1]

[1]Division of Drug Delivery and Tissue Engineering, School of Pharmacy, University of Nottingham, UK.
[2]Division of Respiratory Medicine, School of Clinical Sciences, University of Nottingham, UK.
[3]Laboratory of Biophysics and Surface Analysis, School of Pharmacy, University of Nottingham, UK.
[4]NIHR Respiratory Biomedical Research Unit, University of Leicester, UK.
[5]Division of Immunology and Allergy, School of Molecular Medical Sciences, University of Nottingham, UK.
gavin.morris@nottingham.ac.uk

INTRODUCTION:

Human airway smooth muscle (HASM) contraction plays a central role regulating airway resistance in both healthy and asthmatic bronchioles. *In vitro* studies that investigate the intricate mechanisms regulating this contractile process are predominantly conducted on tissue culture plastic (TCP), a rigid, 2D geometry, unlike the 3D microenvironment smooth muscle cells are exposed to *in situ*[1]. It is increasingly apparent that cellular characteristics and responses are altered between cells cultured on 2D or 3D topographies[2]. Electrospinning is an attractive method to produce 3D topographies for cell culturing as the fibres produced have dimensions within the nanometre range; similar to cells' natural environment.

METHODS:

Polyethylene terephthalate (PET) was electrospun into uni-axially orientated nanofibres. The effect of this topography on HASM cell adhesion, alignment, morphology, and contractile protein characteristics was compared to both 2D-cultured and *in situ* smooth muscle.

RESULTS:

Fibre orientation provides contact guidance to cells enabling the formation of fully aligned sheets of HASM cells similar to *in situ* smooth muscle. Moreover, HASM cells cultured on the scaffold present an elongated cell phenotype with altered contractile protein levels and distribution.

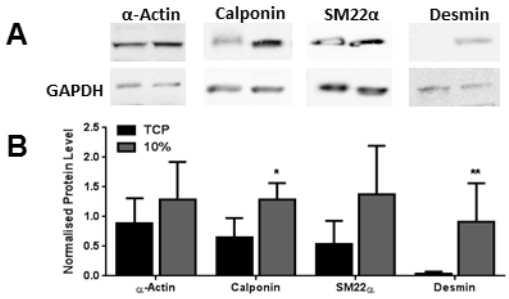

Fig. 1 ASM protein levels when cultured on 2D or 3D aligned topographies

DISCUSSION & CONCLUSIONS:

The platform presented provides a novel *in vitro* model that promotes airway smooth muscle cell development towards a more *in vivo*-like phenotype whilst providing topological cues to ensure good cell alignment.

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ACKNOWLEDGMENTS:

This work was funded by the National Centre for the Replacement, Refinement and Reduction of Animals in Research.

P-48: In Vitro Culture of Colonic Epithelial Stem Cells

Rehma Chandaria[1], Lisa J White[1], Mohammad Ilyas[2], Felicity R A J Rose[1]
[1]School of Pharmacy, University of Nottingham, UK
[2]School of Medicine, University of Nottingham, UK
Stxrc15@nottingham.ac.uk

INTRODUCTION:

Conditions such as inflammatory bowel disease which cause damaged intestinal epithelium are currently either treated with surgery to remove part of the intestine or with expensive medications which dampen the symptoms. Adult colonic stem cells could provide a new treatment option by forming tissue grafts that aid regeneration of damaged epithelium. Lgr5 is a marker of adult stem cells in various tissues. Lgr5+ 'organoids' have been cultured *in vitro* from mouse stem cells and transplanted into damaged intestine, where they have contributed to regeneration of epithelium[1,2]. Using this proof of concept, the first aim of this work is to culture stem cells from human colonic epithelium to form organoids containing Lgr5+ stem cells.

METHODS:

Normal colon tissue was obtained with local ethical approval from resections at the Queens Medical Centre, Nottingham. The isolation of colonic crypts was adapted from previously published work[3]. The tissue was sterilised in 0.04% sodium hypochlorite for 15 minutes. The tissue was then incubated in 3 mmol/L EDTA + 0.05 mmol/L dithiothreitol (DTT) for 90 minutes at room temperature. The EDTA/DTT solution was then removed, 15 mL PBS was added, and the tube was shaken manually for 20 seconds liberating the crypts from the submucosa. The PBS containing the crypts was transferred to another tube, centrifuged gently and counted. Crypts were cultured as described previously[4]. 100 crypts per 25 µL Matrigel per well were plated in a 48-well plate, overlaid with 250 µL culture medium containing nicotinamide, Wnt-3a, EGF, Noggin and R-Spondin.

RESULTS:

Individual crypts were isolated from normal colon, as shown in figure 1a. Within the first 24 hours in culture, the crypts became more rounded, forming cystic organoids. Proliferating cells have been found in budding organoids[4]. These budding structures were observed after 2 days in culture, as shown in figure 1b.

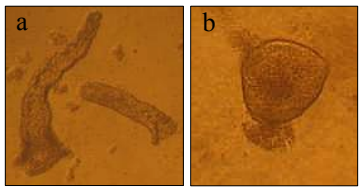

Figure 1: Crypts immediately after isolation (a) and a budding organoid 48 hours after plating (b)

DISCUSSION & CONCLUSIONS:

Organoids have been cultured from crypts isolated from normal human colon, as has been described previously[5]. Further work to confirm the expression of stem cell markers such as Lgr5 will be done. The more long term aim of this research is to grow organoids on tissue engineering scaffolds such as electrospun fibres to investigate the effect on differentiation. The scaffolds may also provide an efficient and convenient method of cell delivery.

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ACKNOWLEDGMENTS:

This research is funded by the BBSRC doctoral training partnership (DTP). We would like to thank the Nottingham Health Science Biobank and Kate Shepherd for colon samples.

P-49: Effect of cultured rat primary hepatocytes on the mechanical properties of collagen gel matrices

Peter E. Agbekoh, Catherine Henderson, Philip E. Riches and M. Helen Grant
Department of Biomedical Engineering, University of Strathclyde, Glasgow, UK
peter.agbekoh@strath.ac.uk

INTRODUCTION:

Collagen gels are widely used as matrices for in vitro cell culture and also for a variety of tissue engineering applications. The mechanical properties of matrices have been shown to be important parameters which greatly influence cell behaviour[1]. For successful support of long term cell growth and function, it is important for matrices to retain their mechanical integrity during the period of use. The presence of cells has been shown to alter matrix properties[2]. The aim of this study was to investigate the effect of seeding primary hepatocytes on the mechanical properties of collagen hydrogel matrices.

METHODS:

Adult rat primary hepatocytes were seeded at different densities on reconstituted type I collagen hydrogels (0.3% w/v) and maintained in culture for up to 7 days. Stiffness (aggregate modulus) and hydraulic permeability of the gels were evaluated at 48 h and 7 d using biphasic theory following confined compression testing[3].

RESULTS:

Stiffness and hydraulic permeability of the gels were altered when primary hepatocytes were maintained on them (Fig. 1). Presence of cells generally led to lowering of stiffness and an increase in hydraulic permeability.

DISCUSSION:

Primary hepatocytes influence the mechanical properties of collagen gels. The decrease in stiffness of collagen gels with presence of hepatocytes is most likely due to the cells degrading the matrix through the chemical action of degradation enzymes, and the physical exertion of mechanical forces on the gels by the cells. The increase in hydraulic permeability may also be due to the degradation of matrix which occurred with cell seeding.

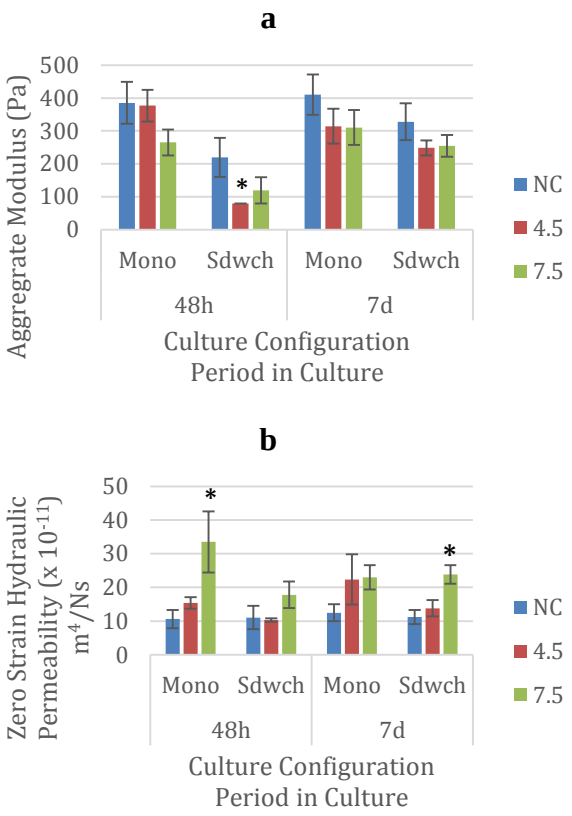

*Fig. 1: Effect of hepatocytes on stiffness (a) and hydraulic permeability (b) of collagen gels in monolayer (Mn) and sandwich (Sdwch) culture configurations. NC, no cells; 4.5, 4.5 x 10^5 cells/well; 7.5, 7.5 x 10^5 cells/well. Data are means ± SEM, n=3 independent experiments in triplicate, * represents significant difference from NC ($p < 0.05$) by ANOVA followed by Dunnett's multiple comparison test.*

CONCLUSION:

The change in mechanical properties observed in this study has implications for use of collagen gels as matrices for long term use.

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P-50: Preliminary Evaluation of Novel Bioceramics For Bone Restoration

E. Mancuso[1,2], M.A. Birch[2], M. Marshall[3], K.W. Dalgarno[1,2], O.A. Bretcanu[1, 2]
1. School of Mechanical and Systems Engineering, Newcastle University, Newcastle Upon Tyne, UK
2. Arthritis Research UK Tissue Engineering Centre, Institute of Cellular Medicine, Newcastle University, Newcastle Upon Tyne, UK
3. Glass Technology Services, Ltd, Sheffield, UK
e.mancuso@ncl.ac.uk

INTRODUCTION:

Since the development of the first Bioglass® in 1971, more than 40 different ceramic formulations have been used for bone reconstruction[1,2]. Nevertheless, regeneration of bone defects still remains a significant clinical challenge. The aim of this research work is the development of novel bioceramic materials with good bioactivity, biocompatibility and competent mechanical properties for bone tissue engineering applications.

METHODS:

Two novel silicate-based glasses, containing different oxide combinations including P_2O_5, B_2O_3, Na_2O, CaO, K_2O, MgO, ZnO, SrO, Ag_2O, V_2O_5, Cr_2O_3 have been prepared by GTS Ltd, Sheffield-UK, along with apatite-wollastonite (AW), a material previously adopted in FDA approved medical devices which has been used as control. The synthesised materials, labelled AW, NCL2 (45 mol% SiO_2) and NCL7 (50 mol% SiO_2), were ground and sieved to have particle sizes under 53 µm. Glass-ceramic pellets were then prepared with an automatic hydraulic press and sintered at temperatures determined from hot stage microscopy. The bioactivity was investigated by soaking the pellets in simulated body fluid. Subsequently in vitro tests were carried out on the sintered pellets and the modulus in compression was evaluated using a universal mechanical testing machine.

RESULTS:

Cell culture results showed a positive response in presence of both NCL2 and NCL7 sintered pellets (fig.1). According to the bioactivity definition[3], the novel materials possess a low level of in vitro bioactivity, although the amount of phosphorus increased from 0 to 4 weeks in immersion. The modulus in compression, measured on dense glass-ceramic pellets, (Table 1) was similar to that of AW.

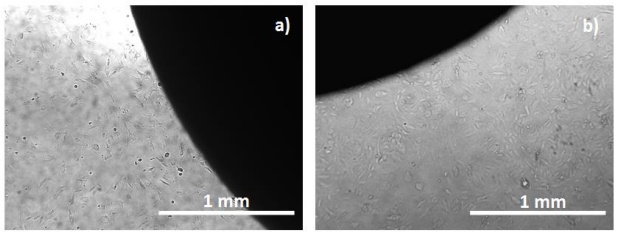

Fig. 1 Rat OB cells cultured in presence of a) NCL2 and b) NCL7 composition b) after 1 week

Table 1 Dry Compression Modulus

SAMPLE	YOUNG'S MODULUS (GPa)	STANDARD ERROR
NCL2	1.43	±0.03
NCL7	1.32	±0.01
AW	1.29	±0.03

DISCUSSION & CONCLUSIONS:

Two different novel SiO_2-based glasses with complex compositions have been obtained by a melting process. Morphological observations, image analysis, mechanical and in vitro tests showed that the new silicate glasses are good candidates for bone tissue engineering applications.

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ACKNOWLEDGMENTS:

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P-51: Oxygen Difference In Live Melanoma Muticellular Tumour Spheroids Using Novel Platinum Compound And Time-Resolved Imaging Microscopy

Ahtasham Raza[1], Helen Colley[2], Elizabeth Baggaley[3], Stanley Botchway[4], Sheila MacNeil[1], Julia A Weinstein[3] & John W. Haycock[1]

Corresponding Author: mdp10ar@sheffield.ac.uk

[1]Department of Materials Science & Engineering, [2] School of Clinical Dentistry, [3]Department of Chemistry, University of Sheffield, Sheffield, UK and [4]STFC Rutherford Appleton laboratory, Oxford, UK.

Introduction

More sensitive methods are continually in demand for the non-invasive detection of cancer cells, including melanoma. Quenching of phosphorescent compounds by oxygen is a novel approach for detecting hypoxia, which relies on the emission lifetime of compounds being sensitive to the oxygen concentration. Solid tumours display varied oxygen levels [1-3] so that there is a need to develop methodologies to determine these changes. Here we used novel non toxic phosphorescent platinum(II) complexes with long emission lifetimes in combination with Time Resolved Emission Imaging Microscopy (TREM) for hypoxic detection [4,5].

Materials and Methods

A human melanoma cell line (C8161) was used to create multicellular tumour spheroids (approximately 200-300µm diameter). Samples were incubated with a Pt(II) compound based on (Pt(dipyridobenzene)Cl) with an appended primary amine unit. Variation in emission lifetime was investigated using 2-photon excitation and time resolved emission microscopy, as well as in parallel studies using 'Hydroxyprobe' and histology of tumour spheroids for comparison.

Results

Spheroids were immersed in Pt(II), 100 µM solution for 12 hours, then washed with PBS, before imaging. Pt (II) lifetime distribution across spheroid ranges: 0.8-11.5 µs (Figure 1A). Time resolving lifetime clearly shows three distinct spheroid regions, outer proliferative rim (red, 0-3.0 µs), necrotic rim (green, 3.0-7.5 µs) and inner necrotic core (blue, 7.5-12.0 µs) (Figure 1B).

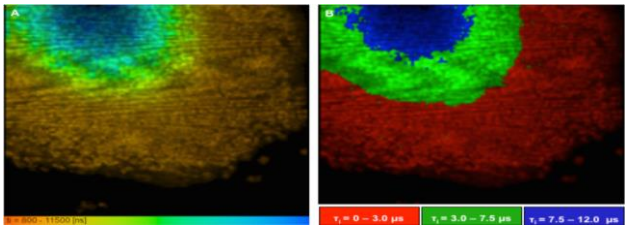

Figure 1. Lifetime distribution of Pt(II) in spheroid. (A) Continuous rainbow scale, range: 0.8-11.5 µs, (B) Discrete colours showing three distinct spheroid regions

Discussion and Conclusions

We propose the measurement of intra-tissue O_2 as a potential parameter for assessing cellular activity associated with tumour development. Depletion of O_2 is known to increase the emission lifetime of the Pt(II) labels described herein, and thus permits real-time information on the concentration of molecular oxygen in a tissue sample. This method therefore has the potential to detect changes in partial O_2 pressure, and related responses (e.g. necrosis) providing a novel parameter for real time non-invasive detection of tumorigenic processes e.g. melanoma.

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Acknowledgments

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P-53: 3D Micro-Printing of Nerve Guides for Peripheral Nerve Repair

Christopher J Pateman[1], Adam J Harding[2], Richard Plenderleith[3], Stephen Rimmer[3], Fiona M Boissonade[2] Frederik Claeyssens[1] & John W Haycock[1]

[1]Department of Materials Science and Engineering, University of Sheffield, UK, [2]School of Dentistry, University of Sheffield, UK and [3]Department of Chemistry, University of Sheffield, UK

INTRODUCTION

Nerve Guidance Conduits (NGCs) presently have a limited regenerative capacity, mainly due to the absence of physical guidance cues and materials, which do not ideally support the regeneration of injured neuronal and Schwann cell growth [1]. A need exists to fabricate more accurate NGC structures from more biocompatible materials to improve nerve regeneration following injury. The broad aims of this work are therefore to develop NGCs with improved bulk properties, physical design and surface chemistries to better support neuronal and Schwann cell growth, for surgical applications of nerve injury repair.

METHODS

Caprolactone and polyethylene glycol pre-polymers were microwave synthesised, methacrylate functionalised and characterised by THF-GPC, MALDI-TOF, MS and NMR. Pre-polymers were UV cured into 2D sheets and 3D structures via stereolithography [2]. The microSL set-up consisted of a 405 nm CW laser with a maximum output of 100 mW. Laser output was directed onto a digital micromirror device (DMD) used as a dynamic mask in the projection microSL set-up. NGCs were assessed *in vitro* via cell viability testing and immuno-fluorescence labelling of neuronal cells, rat-derived primary Schwann cells and dorsal root ganglia (DRG). Common fibular nerve regeneration was assessed *in vivo* via implantation in to a mouse Yellow Fluorescent Protein (YFP) short gap injury model.

RESULTS

NGCs of 5 mm length and 1.25 mm inner diameter were produced via microSL (Fig 1) with a minimum resolution of 50 µm from polyethylene glycol and caprolactone. *In vitro* testing using neuronal, Schwann and dorsal root ganglion culturing, cell viability testing and immuno-fluorescence labelling of ß-tubulin-III (for neuronal cells) and S100ß (for Schwann cells) demonstrating cellular adhesion and neurite outgrowth on these materials. *In vivo* implantation of NGCs in to a mouse YFP 3.0mm common fibular nerve injury model revealed axon regeneration equivalent to autograft control.

DISCUSSION & CONCLUSIONS

In summary, photocurable biodegradable polymers manufactured with accurate 3D structures by additive manufacturing techniques have considerable potential for a new generation of medical devices, including implantable NGCs. Devices have improved physical, biochemical and biological properties which are demonstrating to be as effective as 'gold standard' autograft methods for short gap repair.

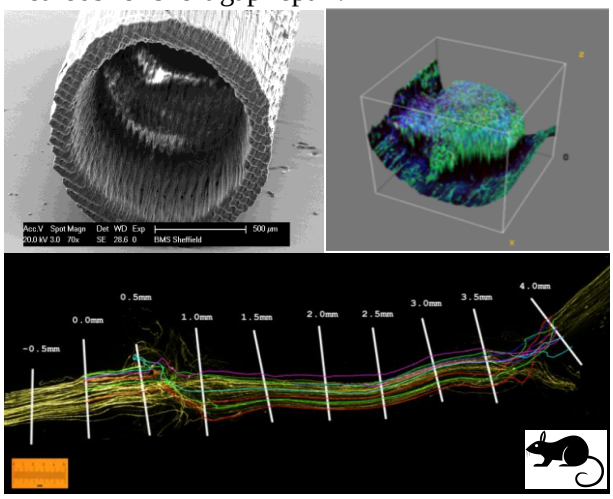

Figure 1: A nerve guidance conduit fabricated by microSL: (**top left**). 3D 2-photon microscopy of a DRG growing *in vitro* in an NGC channel showing neurite and Schwann cell outgrowth (**top right**). *In vivo* axon regeneration through a microSL fabricated NGC in a mouse YFP common fibular short gap injury model (**bottom**).

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ACKNOWLEDGEMENTS

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P-54: Assessing the Barrier Performance of an In Vitro Co-culture Model of the Intestine

Jamie Patient[1], Kate Harris[2], Bertil Arbahamsson[3], Christer Tannergren[3], Amir Ghaemaghammi[4], Lisa J White[1], Phil Williams[1], Clive Roberts[1] and Felicity R.A.J. Rose[1]

[1]School of Pharmacy, University of Nottingham, UK;
[2]AstraZeneca, Macclesfield, UK; [3]AstraZeneca, Mölndal, Sweden; [4]School of Molecular Medical Sciences, University of Nottingham.
paxjp@nottingham.ac.uk

INTRODUCTION:

In vitro models of human tissues are fundamental to academic and industrial research. Considering the caveats of *in vivo* animal studies (e.g. high cost, low throughput and poor human correlation) and the 3R's principle of refining, replacing or reducing animal use in research, it is imperative to develop more sophisticated *in vitro* models that better simulate the *in vivo* situation. This can be achieved by developing physiologically relevant scaffold technologies and culturing multiple cell types together to simulate the cellular microenvironment and cross talk experienced *in vivo*.

METHODS:

Nanofibre scaffolds were produced by electrospinning 10% poly (ethylene terephthalate) in a solution of dichloromethane and trifluoroacetic acid. Caco-2 cells (Epithelial) and CCD-18co (Myofibroblasts) were cultured on opposite sides of the Nanofibre scaffolds for 15-21 days. Throughout the culture period Trans-epithelial electrical resistance (TEER) measurements were used to assess barrier integrity. The apparent permeability (Papp) of two markers, Lucifer Yellow and Atenolol were used to assess the paracellular transport.

RESULTS:

Table 1. Apparent permeability values of Lucifer yellow and Atenolol

	Papp x10^{-6} (±SD)			
	Transwell		Nanofibre	
Substance	Caco-2	Caco-2/18co	Caco-2	Caco-2/18co
Lucifer Yellow	2.59 (±1.51)	2.69 (±1.75)	64.33 (±18.21)	56.63 (±14.21)
Atenolol	0.53 (±0.13)	1.63 (±0.11)	1.06 (± 0.27)	1.43 (± 0.94)

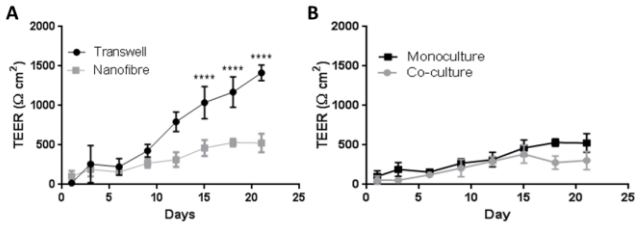

*Figure1. TEER measurments for Caco-2 monocultures and Caco-2/18co co-cultures on Nanofibre and Transwell scaffolds. **** p>0.0001*

Caco-2 cells cultured on nanofibre PET scaffolds show reduced barrier integrity with respect to TEER when compared to those cultured on Transwell™ supports (Figure 1). When Caco-2 cells were co-cultured with the 18co myofibroblast cells a reduction in TEER was observed which was significant for the Transwell™ system. Paracellular transport was assessed by Lucifer yellow and Atenolol transport (Table 1). Lucifer yellow transport increased around 20-fold for Caco-2 monocultures cultured on nanofibre scaffolds compared to the Transwell™ equivalent, this significant increase however was not observed for Atenolol.

DISCUSSION & CONCLUSIONS:

Initial results suggest that culturing Caco-2 cells on nanofibre scaffolds can produce a phenotype more akin to the small intestinal epithelium, the functionality of the model, however, is still to be fully assessed.

ACKNOWLEDGMENTS:

The authors would like to thank the EPSRC (EP/I01375X/1) and AstraZeneca for funding this work.

P-55: The Chorioallantoic Membrane (CAM) As A Capillary Bed For Human Bone Angiogenic Analysis: The Potential Of Laponite Clay Gel For Growth Factor Delivery Ex Vivo

Ines Moreno, Janos M. Kanczler, Nicholas R. Evans and Richard O. C. Oreffo

Bone and Joint Research Group, Centre for Human Development and Health, Faculty of Medicine, Southampton Univerity, England. i.moreno@soton.ac.uk

INTRODUCTION:

Bone disorders, such as osteoporosis, often lead to fractures which require bone substitute materials to promote bone healing. Lack of vascular invasion is often the cause of failure and is the focus of many current bone tissue engineering approaches[1]. Here we propose the use of a clay gel (laponite) as a vehicle for growth factor delivery into human bone, and the use the chorioallantoic membrane (CAM) as an ex vivo bioreactor to test our hypothesis.

METHODS:

To investigate this, 4mm diameter bone cores were obtained from fresh human femoral heads. Prior to incubation, bone fragments were perfused with (i) clay gel (laponite) and (ii) laponite with vascular endothelial growth factor ($rhVEGF_{165}$) or (iii) vehicle control. After 7 days' incubation either on the CAM or in organotypic culture, bone fragments were harvested and analyzed histologically (immunostaining for an endothelial marker, von Willerbrand factor (vWF) and standard bone tissue stainings).

RESULTS:

Results showed that the highest embryo survival rate was for the bone grafts containing both laponite and VEGF (90%), followed by the laponite group (75%). Histochemical analysis showed marked CAM cell migration and invasion in the laponite groups. Tight and close interaction between the human and chick tissue was observed. In the VEGF treated group vWF immunostaining showed increased blood vessel formation around the graft. Bone cores containing articular cartilage inhibited vascular CAM invasion.

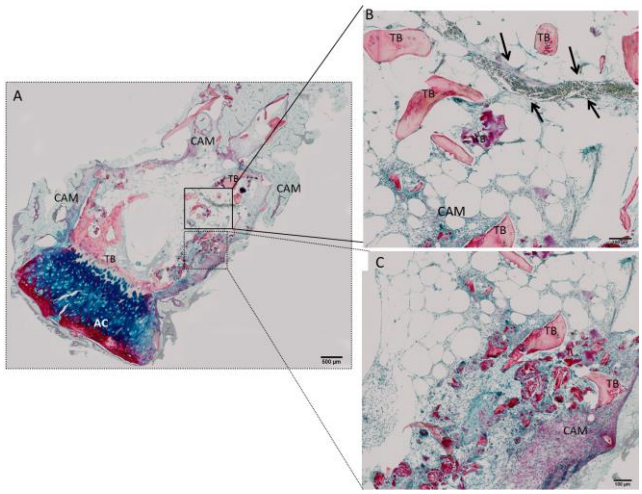

Figure 3: Histological section of bone graft with laponite and VEGF integrated and vascularized by the CAM within 7 days. (A-C) Alcian Blue (proteoglycans) and Sirius Red (collagen) staining. (A) Overview of bone graft surrounded by CAM. (B) Blood vessel (BV) invading marrow space. (C) CAM embedding human bone. Black arrows point to CAM blood vessels. TB (trabecular bone) AC (articular cartilage) CAM (chorioallantoic membrane).

DISCUSSION & CONCLUSIONS:

This data shows that CAM membrane was able to integrate human bone cores within 7 days and that its vasoproliferative response and invasion was increased in the presence of VEFG. Additional drugs in combination with biomaterials could be screened using the CAM model as a surrogate blood supply.

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ACKNOWLEDGMENTS:

The authors would like to thank the NC3Rs for providing financial support to this project.

P-56: Intracellular transduction of proteins in three dimensional gradients

Hoda M. Eltaher[1, 2], Jing Yang[1], James E. Dixon[1], Kevin M. Shakesheff[1]
[1] Wolfson Centre for Stem Cells, Tissue Engineering and Modelling (STEM), Division of Advanced Drug Delivery and Tissue Engineering, School of Pharmacy, University of Nottingham, NG7 2RD, UK.
[2] Department of Pharmaceutics, Faculty of Pharmacy, Alexandria University, 21521, Egypt.
paxhm3@nottingham.ac.uk

INTRODUCTION:

Methodologies to deliver cargo proteins directly into cells are useful tools to elicit changes in cell behaviour and direct stem cell differentiation and self-renewal. Cellular processes such as proliferation, angiogenesis and differentiation are guided and regulated by gradients of different physical and chemical cues[1]. The ability to control the 3D gradient of these signalling molecules within matrices is therefore important for inducing desirable differentiation of stem cells. We have developed a highly efficient system we term Glycosaminoglycan (GAG)-binding enhanced transduction or GET[2] and wanted to explore if GET protein delivery could be controlled spatiotemporally and ultimately allow us to direct cell responses.

METHODS:

Hydrogels (5x5x15 mm) with or without NIH3t3 mouse fibroblasts ($2x10^6$ cells/mL) were cast within a modified diffusion chamber[3] to create a 3 compartmental diffusion assembly of source-gel-sink. We used a GET- monomeric red fluorescent protein (mRFP) and compared this to non-transducing mRFP. Gradient profiles at different time points were defined as function of distance inside scaffold at 20 µm resolution by serially slicing the scaffolds perpendicular to the direction of protein diffusion using Leica CM1100 cryostat at -20 ºC. Protein content per slice was quantified fluorometrically using TECAN Infinite 200 PRO multimode reader supported with I-control ™ software.

RESULTS:

Our system demonstrated that gradient profiles at different time points reflected the transduction capabilities of the GET protein, with non-transducing mRFP diffusing to equilibrium throughout the hydrogel volume. Compared to the mRFP, cellular uptake of GET mRFP completely depleted the hydrogel of free diffusible protein and demonstrated that the cells themselves acted as sink that retained the GET-mRFP.

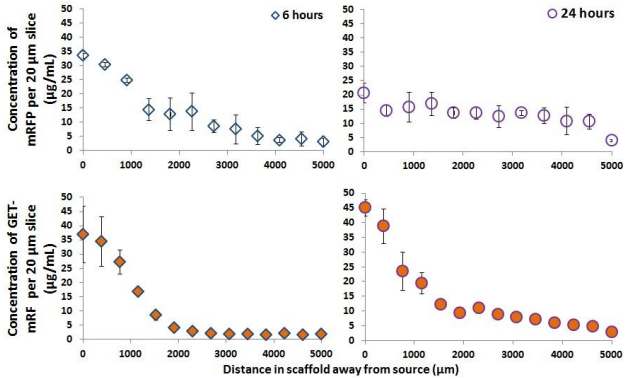

Fig. 1: Diffusion gradient profiles of mRFP vs GET-mRFP across cellular hydrogel scaffolds at 6 and 24 hours.

DISCUSSION & CONCLUSIONS:

With the ability to control the intracellular delivery of functional proteins spatiotemporally, we believe that GET technology will allow us to create gradients in hydrogels to direct cellular behaviour and ultimately control the differentiation of stem cells in 3D for regenerative medicine and tissue engineering applications.

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ACKNOWLEDGMENTS:

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P-58: Tissue engineered bone through the use of novel alginate/bone ECM hydrogels

[1] **David Gothard**, [1] Emma L Smith, [1] Janos M Kanczler, [1] Julia A Wells, [1] Carol A Roberts, [2] Lisa J White, [2] Omar Quatachi, [2] Mike S Sawkins, [2] Heather Peto, [2] Hassan Rashidi, [3] James Henstock, [3] Michael Rotherham, [4, 5, 6, 7] Luis Rojo, [4, 5, 6] Molly M Stevens, [3] Alicia El Haj, [2] Felicity RAJ Rose, [2] Kevin M Shakesheff and [1] Richard OC Oreffo

[1] Bone and Joint, Institute of Developmental Sciences, University of Southampton

[2] Wolfson Centre for Stem Cells, Tissue Engineering and Modelling, Centre for Biomolecular Sciences, University of Nottingham

[3] Guy Hilton Research Centre, Institute for Science and Technology in Medicine, Keele University

[4] Department of Materials, [5] Department of Bioengineering, [6] Institute for Biomedical Engineering, Imperial College London

[7] Institute of Polymer Science and Technology, CSIC and CIBER-BBN, Spain

Corresponding Author: D.Gothard@soton.ac.uk

Introduction

There is a growing socioeconomic need for new approaches to treating bone damage, especially within an increasingly aged population. Tissue engineering offers a new avenue to generate novel bone both *ex vivo* and *in vivo*. Here the authors have investigated the use of novel hydrogel constructs incorporating both inductive signaling, and an inducible cell source for the purpose of bone formation and regeneration.

Materials and Methods

Stro-1 enriched human BMSCs and growth factor loaded polymer microparticles [1] were combined with alginate/bone derived ECM hydrogel (60-40%) [2]. VEGF (fast release, 50 ηg/mL), TGF-β3 (fast, 15 ηg/mL), BMP-2 (slow, 100 ηg/mL), PTHrP (fast, 100 ηg/mL), and VitD3 (slow, 25 ηM) were each selected for temporal release of angiogenic, chondrogenic, and osteogenic signals. Controls included HSA loaded microparticles (carrier protein) and blank hydrogels. 5mm segments were subcutaneously implanted for 28 days within MF1 nude mice. Bone formation was assessed by both micro-CT and histological analysis. ANOVA and appropriate t-tests were used to assess statistical significance.

Results

Hydrogel constructs exhibited tissue invasion, mineralisation, and vascularisation independent of cell incorporation and loaded growth factor (Fig 1B). Dense bone tissue assessed by micro-CT (Fig 1A) correlated with mineralised bone assessed histologically (Fig 1C). Alginate/ECM hydrogel appeared highly osteoinductive.

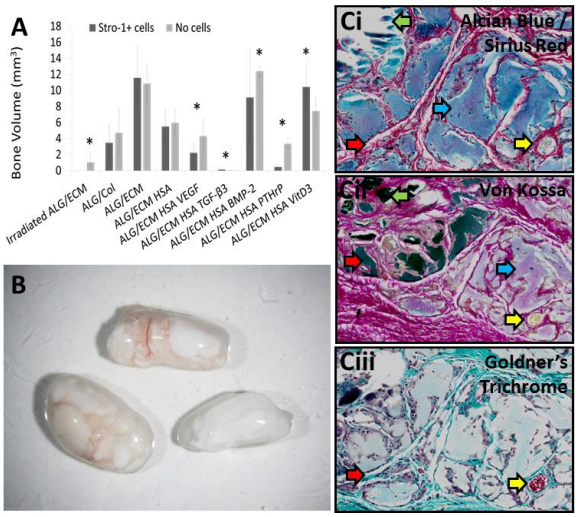

Fig 1: Subcutaneously implanted hydrogels (**B**) were assessed by micro-CT (**A**) and histological analysis (**C**). Green, red, blue, and yellow arrows depict mineralisation, tissue invasion, matrix deposition, and vascularisation respectively.

Discussion and Conclusions

Novel alginate/ECM hydrogel constructs offer an effective method for tissue engineering new ectopic bone *in vivo*. Future study would assess the capacity for bone defect repair *in vivo*.

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Acknowledgments

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P-60: Cell Micro-Patterning on PolyHEMA using 1,1'-Carbonyldiimidazole as a Surface Modification Intermediate

Emily R. Britchford[1], Kevin M. Shakesheff[1] Lee DK. Buttery[1], Stephanie Allen[2], Glen R. Kirkham[1]

[1]*Tissue Engineering Group, Centre for Biomolecular Sciences,* [2]*Laboratory of Biophysics and Surface Analysis (LBSA), School of Pharmacy, University of Nottingham, Nottingham, NG7 2RD, UK*

INTRODUCTION: Tissue function during development relies on correct cell organization (at micro and macro scales) within defined architectures. In this study we introduce a novel cell micro-patterning technique that utilizes aerosol deposition of CDI through a micro-stencil and subsequent protein immobilization to induce region selectivity, creating cell adhesive properties on a poor cell responsive surface. This methodology aims to produce a rapid and accurate cell micro-patterned area that can remain fixed, without loss or spreading of cells, over a macroscopic area for periods previously demonstrated[1].

METHODS: Micro-stencils[1] acted as a mask to selectively deposit CDI aerosols to polyHEMA coated surfaces. Fibronectin (Fn) or FBS was subsequently immobilised to the activated surface. Genetically labelled (mRFP) NIH3T3 cells[1] were seeded onto micro-patterned surfaces in defined media conditions (FBS containing or FBS free, calcium free DMEM). Cell proliferation was assessed every 24 hours for 5 days using MTS assay.

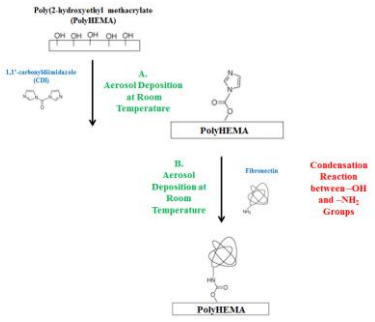

Fig 1. *Schematic diagram outlining reaction mechanism of CDI aerosol deposition and subsequent protein immobilisation.*

RESULTS: NIH-3T3 cells attached and proliferated to CDI modified regions and cell patterns remained faithful for several days ***(Fig 2 & 3).***

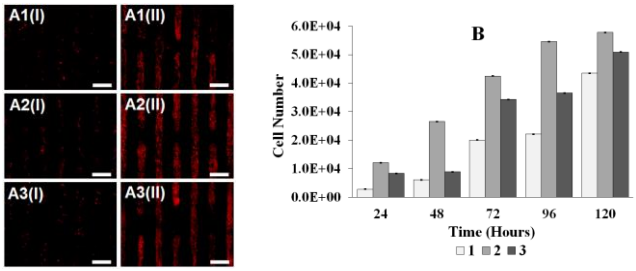

Fig 2. *Micro-patterning of cell lines. A. Fluorescent images and B. proliferation assay (MTS) of NIH-3T3 cultured with 1. 10% FBS immobilisation in FBS containing media, 2. Fn immobilisation in FBS containing media, and 3. Fn immobilisation in FBS free media. Images taken (I) 24 and (II) 96 hours post cell seeding. Scale bar = 20 µM.*

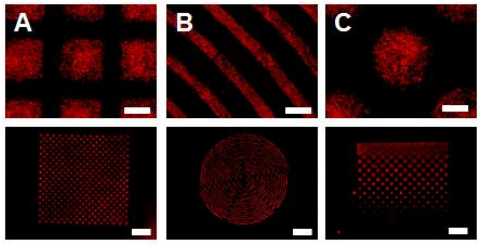

Fig 3. *Micro-patterning of more complex designs. Fluorescent images of NIH-3T3 cells cultured with Fn immobilisation in FBS containing media, showing A. Square, B. Spiral and C. Dot patterned structures. Images taken 96 hours post cell seeding. Scale bar = Top 20 µM and Bottom 2 mm.*

DISCUSSION & CONCLUSIONS: Changes to the surface chemistry of polyHEMA to a more adhesive cell substrate were successfully employed using aerosol deposition of CDI. Cells remained proliferative and responsive with limited spreading over a 120 hour period demonstrating high level precision and length scale micro-patterning for future tissue engineering applications and stem cell niche creation. This fabrication method represents a robust system that has micron resolution over a macro scale.

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ACKNOWLEDGMENTS: This work is funded by the EPSRC DTC for Regenerative Medicine.

P-62: Modification of Peptide Self-Assembling Hydrogels for Cell Culture Applications

Laura Szkolar[1,2], Aline F Miller[2,3], Julie E Gough[1] and Alberto Saiani[1,2*]
[1] School of Materials, The University of Manchester, UK
[2] Manchester Institute of Biotechnology, The University of Manchester, UK
[3] School of Chemical Engineering and Analytical Sciences, The University of Manchester, UK
Laura.Szkolar@Manchester.ac.uk

INTRODUCTION:

Self-assembling peptide hydrogels have been shown suitable for the culture of a range of cell types [1,2]. In our group, we have recently developed a family of β sheet forming peptides that have been successfully used for 3D cell culture applications [3,4]. Modifications to peptide sequence, in particular charge, has been discussed as a method to control the interaction of cells with peptide hydrogels. For this reason, a series of peptide hydrogels were produces which varied in charge density.

METHODS:

Cells were extracted from bovine cartilage [5] cultured in 5 % CO_2 atmosphere at 37 °C and maintained in DMEM supplemented with 10 % FBS, 1 % antibiotic and 50µg/mL ascorbic acid. Peptide solutions were prepared at 30 mg mL^{-1} and adjusted to pH 6.5 with 0.5M NaOH. Cells were suspended in media at $5x10^6$ cells per 300µL and cultured in 3D. Rheometry was performed on a TA AR-G2 with fixed strain and frequency of 1% and 1HZ. Gels were dissolved and cell counts performed using a haemocytometer.

RESULTS & DISCUSSION:

Hydrogels were produced by doping FEFKFEFK with charge. Subsequent hydrogels were rheologically tested (figure 1) and showed similar mechanical properties. This allows for any variation in cell number to be due to charge alone. Cells were cultured in 3D for 21 days (figure 2). It was seen that all gels were suitable for maintaining bovine chondrocytes over the 21 days, with an increase in cell numbers. In particular, an increase in cell numbers was seen on those hydrogels with 25-50% charge.

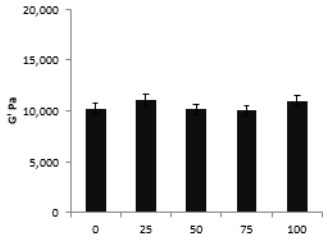

Fig. 1 Rheological data of peptide hydrogels produced using FEFKFEFK (0), peptide carrying 25% charge (25), 50% charge (50), 75% charge (75) and FEFKFEFKK (100).

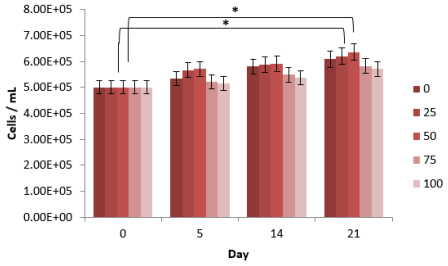

Fig. 2 Cells/mL of peptides FEFKFEFK (0), peptide carrying 25% charge (25), 50% charge (50), 75% charge (75) and FEFKFEFKK (100).

CONCLUSIONS:

We have shown that a range of stiffness equivalent hydrogels can be produced. The variation of charge has been shown to influence cell numbers, when cells are cultured in 3D.

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ACKNOWLEDGMENTS:

Peptisyntha (a member of the Solvay Group), Belgium **(**http://www.peptisyntha.com**)**

P-64: Towards the prevascularisation of tissue-engineered skin

L Dew[1], CK Chong[1], S MacNeil[1]

[1] *Kroto Research Institute, Department of Materials Science and Engineering, University of Sheffield, UK*

l.dew@sheffield.ac.uk

INTRODUCTION: Failure to achieve vascularisation is one of the major roadblocks in getting tissue-engineered (TE) substitutes translated from the laboratory to the clinic [1]. Knowledge of the major factors that drive vascular ingrowth is key to overcoming this hurdle. It is the aim of this project to create a model that induces neovascularisation within TE skin and in doing so allows the investigation of the effects of biochemical and mechanical factors on initial vascular ingrowth. We propose doing this through the use of a re-endothelialised biological vascular network pefused with a bioreactor to which TE skit will be added to explore formation of new vasculature into the TE skin. The current work focuses on the decellularisation of rat jejunum to establish the bioscaffolds with preserved vascular architecture and early stage recellularisation and angiogenic assay results.

METHODS: To produce the natural vascular nets jejunum was harvested from fresh rat cadavers. The main vessel of the mesentery was cannulated and flushed with heparin solution before treatment with 1% Triton-X 100 and 0.1% ammonium hydroxide. Distilled water was then circulated to remove residual detergent and the resultant matrix was sterilised using 0.1% peracetic acid. Residual peracetic acid was removed with sterile PBS. Blue dye was injected into the matrix to assess vascular patency. The presence of DNA, GAG, elastin and collagen before and after decellularisation were identified through histochemical staining and then quantified. The inherent angiogenic properties of the matrix were analysed using the chick chorioallantoic membrane (CAM) assay cultured for 8 days, with a poly-l-lactic acid (PLLA) negative control. To recellularise, a co-culture of dermal fibroblasts and human dermal microvascular endothelial cells (HDMECs) were injected through the mesenteric artery of the decellularised jejunum and subsequently perfused continuously with cell culture medium for 5 days at 37˚C. Samples were taken at days 1 and 5 for histological analysis and fluorescence microscopy.

RESULTS: A well-defined vascular tree with multiple branching was visible from blue dye injection. Quantitative analyses showed a 93% reduction in DNA following decellularisation. ECM components such as collagen were found to be retained whilst GAGs were diminished by around 40% and almost all elastin was removed by the process. The CAM assay showed good integration of the matrix with the host whilst indicating increased angiogenic properties of the decellularised matrix when compared to the PLLA control. Upon recellularisation, histochemical analysis showed that the cells attached to the matrix and occupied the blood vessels. Confocal microscopy showed a re-organisation of the HDMECs after 5 days in culture (Fig. 1), with the cells lining the vessel architecture.

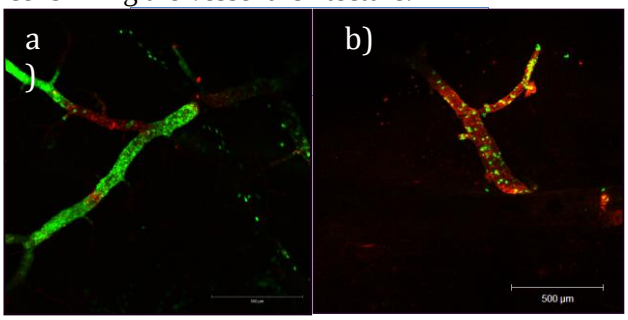

Fig. 1: Confocal microscopy showing the recellularisation of the decelluarised jejunum with dermal fibroblasts (green) and HDMECs (red) after a) 1 day and b) 5 days in culture

DISCUSSION & CONCLUSIONS: The decellularisation procedure was effective in preserving the architecture of the matrices whilst removing most of the cellular matter. The prp-angiogenic results from the CAM assay infer the preservation of growth factors whilst the recellularisation results illustrate that this matrix supports good cell attachment and reorganisation. We conclude that the re-endothelialised jejunum has the potential to act as a vascular bed from which neovascularization into TE skin can now be studied. .

REFERENCES: [1] Novosel, E.C., C. Kleinhans, and P.J. Kluger, *Vascularization is the key challenge in tissue engineering.* Advanced Drug Delivery Reviews, 2011. **63**(4-5).

ACKNOWLEDGEMENTS: This work is funded by the EPSRC.

P-65: Development of 3D Skin Models for *In Vitro* Testing

Joe Lemmens[1], Sheila MacNeil[1], Stefan Przyborski[2] and John W. Haycock[1]
[1]Department of Materials Science & Engineering, University of Sheffield, UK.
[2]School of Biological and Biomedical Science, Durham University, UK.

Introduction

For human 3D skin models to be physiologically relevant they must have a barrier function and appropriate epidermal architecture, with the correct proportion of proliferative through to differentiated cells. Importantly, fibroblasts help maintain proliferative epidermal cells and the formation of a dermal epidermal junction (DEJ), and also play a role in paracrine signaling. Critically, the inclusion of fibroblasts affects the response to potential irritants and the propagation of an acute inflammatory response. This is lacking in current 3D models. The aim of the present study was therefore to compare scaffold designs for supporting keratinocyte and fibroblast co-culture and organization in order to produce 3D skin models which can be used to study skin irritants.

Materials and Methods

Scaffolds: (i) electrospun poly-L-lactic acid (PLLA); (ii) PLLA / poly (3-hydroxybutyrate-co-3-hydroxyvalerate (PHBV) / PLLA trilayer[1] and (iii) Alvetex® (Reinnervate Ltd.) a highly porous polystyrene scaffold were investigated. Scaffolds were populated with $1x10^6$ human dermal fibroblasts (HDF) for 7-10 days. Collagen type-1 was coated over a subset of samples. Then $5x10^5$ of normal human keratinocytes (NHK) or HaCaT human immortalized keratinocytes were added. Cultures were submerged for 3 days, prior to air-liquid (A/L) interface culture for 10, 14, 17 or 21 days. Skin irritation using sodium dodecyl sulphate (SDS) was assessed by the '42 bis' protocol[2] by MTT viability and ELISAs for IL-6 and IL-8.

Results

The presence of fibroblasts with either HaCaT or NHK keratinocytes in Alvetex® produced a more developed morphology after 21 days at an A/L, than constructs without fibroblasts. NHKs developed a denser stratum corneum than HaCaT keratinocytes. HaCaTs cultured on electrospun scaffolds had a distinct close packed

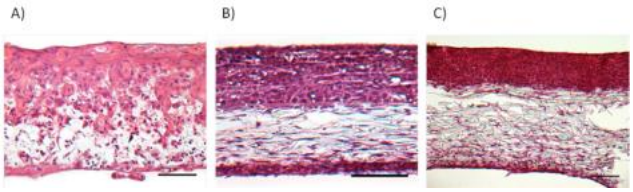

Figure 1. HDF and HaCaT keratinocytes cocultured for 21 days at an A/L on: A) Alvetex®, B) PHBV / PLLA electrospun trilayer and C) electrospun PLLA. Bar=100µm.

squamous appearance (Figure 1). When challenged with SDS All models responded to SDS at 10^{-4}M without any signs of toxicity. Using this non-toxic SDS concentration, it was found that SDS upregulated IL-6 and IL-8, as detected by ELISA, in the medium of all samples. NHKs generated greater levels than HaCats and challenge with SDS revealed NHKs to be more sensitive to irritation than HaCaT keratinocytes. HaCaTs developed a dense epidermal layer on electrospun PLLA, but had more organisation on the PHVB/PLLA trilayer scaffold

Discussion and Conclusions

NHKs produced a better-stratified epithelial formation than HaCaT keratinocytes and responded more to SDS challenge than the cell line. Work is on-going to evaluate which scaffold model is the most appropriate for production of 3D skin models for *in vitro* testing and to investigate the role of the fibroblast in the response of skin to irritants as determined by production of IL-1□, IL-6 and IL-8.

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Acknowledgments

EPSRC for funding and Reinnervate Ltd for samples.

Disclosures - The authors have no competing financial interests except Przyborski as a consultant for Reinnervate, who produce Alvetex technology.

P-66: Porous biodegradable microspheres that form highly porous scaffolds after injection

Omar Qutachi[1], Jolanda Vetsch[2], Daniel Gill[3], Helen Cox[3], Sandra Hofmann[2], Ralph Müller[2], Robin Quirk[3], Kevin Shakesheff[1], Cheryl Rahman[1]

[1]*School of Pharmacy, University of Nottingham, Nottingham, U.K*
[2] *Institute for Biomechanics, ETH Zurich, Zurich, Switzerland*
[3]*RegenTec Ltd, Biocity Nottingham, U.K*
omar.qutachi@nottingham.ac.uk

INTRODUCTION:

The use of injectable scaffolds for cell delivery can improve cell engraftment and survival [1]. Polymer microspheres can be used as such a scaffold for tissue repair [2]. For this application it is essential that scaffolds have the mechanical properties, porosity and pore diameter to support new tissue formation. This study demonstrates a method for fabricating porous PLGA microspheres that form scaffolds at body temperature, creating an injectable system capable of supporting cell growth *in vitro*.

METHODS:

Porous PLGA microspheres were produced by double emulsion using PBS as a porogen and treated with ethanolic sodium hydroxide. Scaffolds were fabricated by mixing microspheres with carrier solution and incubating at 37°C. Mechanical properties were assessed by TA.HD+ texture analyser**.** Porosity and pore diameter were analysed by micro-computed tomography. Viability of NIH-3T3 fibroblasts cultured on scaffolds was determined using PrestoBlue assay (Invitrogen).

RESULTS:

Porous PLGA microspheres were fabricated with an average size of 83µm. Treatment with ethanolic sodium hydroxide for 2 minutes increased surface porosity (Fig.1A) and enabled microspheres to fuse at 37°C into scaffolds (Fig1B). Average compressive strength of scaffolds after 24 hours at 37°C was 0.9 MPa. Scaffold porosity was 81.5% on average, with mean pore diameter of 54µm and maximum pore diameter of 306µm. NIH-3T3s attached and proliferated on the scaffolds *in vitro* (Fig1C).

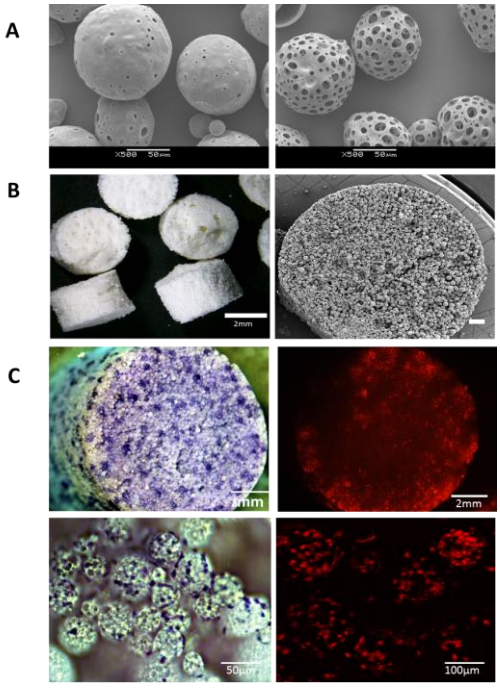

Fig. 1 (A) SEM of porous microspheres, non-treated (left) and EtOH-NaOH treated (right). (B) Scaffolds formed with treated microspheres. (C) Cells on scaffolds stained with toluidine blue (left), red fluorescent cells (right).

DISCUSSION & CONCLUSIONS:

This study demonstrates a method for fabricating porous PLGA microspheres that form solid porous scaffolds at body temperature, creating an injectable system capable of supporting cell attachment and proliferation.

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ACKNOWLEDGMENTS:

This work was funded by the European Community's FP7 project Biodesign and the European Research Council under the European Community's Seventh FP7 project.

P-67: Holographic Optical Tweezers as a Tool for the Study of Stem Cell Microenvironments

Glen R Kirkham[1], Emily Britchford[1], Thomas Upton[1], James Ware[1], Lee D Buttery[1], Stephanie Allen[1], Graham Gibson[2], Miles Padgett[2], Kevin Shakesheff[1]
[1]Centre for Biomolecular Sciences, University of Nottingham, Nottingham, UK
[2]School of Physics and Astronomy, University of Glasgow, UK
glen.kirkham@nottingham.ac.uk

INTRODUCTION:

The initial cellular organisation within early embryonic stem cell structures profoundly influences differentiation. Such fundamental cellular interactions also play pivotal roles within adult tissues. We have used holographic optical tweezers to precisely control and manipulate cellular position in three dimensions, providing a high precision tool for the study of stem cell microenvironments.

METHODS:

Holographic optical tweezers were constructed similar to a previous system[1] and various cell types were trapped and manipulated into 3D structures. Cultures were fixed into position using PEG[2] and agarose hydrogels or an avidin-biotin crosslinking method[3].

RESULTS:

Mouse embryonic stem cells (mES), mesenchymal stem cells (MSCs) and calvaria cells were positioned into precise 3D structures (Fig 1). MSCs were also seeded onto ECM fragments to replicate adult stem cell niche architectures (Fig 2 a/b). In addition mES were co-positioned with electrospun fibres (Fig 2c) and spatiotemporal control of chemical factors was demonstrated with positioned polymer microparticles releasing calcein-AM (Fig 3).

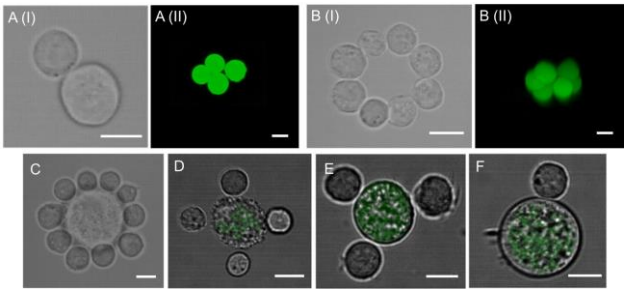

Fig. 1. mES (A-B), mES/calvaria (C-D), mES/MSCs (E-F) positioned into 3D structures. Scale = 14 µm.

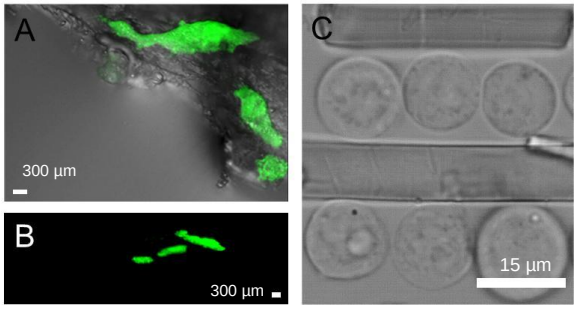

Fig 2. A/B - Single MSCs patterned onto ECM and cultured for 72hrs, C - mES and PLGA fibers.

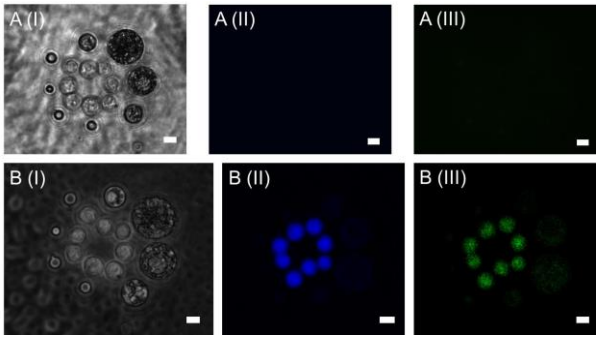

Fig 3. Patterned mES and microparticles releasing calcein-AM (blue and green) were cultured from 1 (A) to 10 days (B) in an agarose gel. Scale = 14 µm.

DISCUSSION & CONCLUSIONS:

We have developed a novel method to precisely position cells and polymer fibres forming stable constructs with varying configurations. Spatiotemporal control of chemical factors was also achieved with positioned polymer particles. This method has huge potential for the study of cellular microenvironments.

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ACKNOWLEDGMENTS:

ERC under the Seventh Framework Programme (FP7/2007-2013)/ERC grant agreement 227845.". Martin Ehrbar and Yannick Devaud.

P-69: In vitro Detection of PpIX Fluorescence in Human Colon Cancer Cells- Towards Better Pre-Surgical Photodiagnosis and Staging of Bowel Cancers

Kathleen E. Wright[1*], Thomas I. Maisey[1] and David G. Jayne[1]
[1]Academic Surgery Colorectal Research Group, Leeds Institute of Biomedical and Clinical Sciences, University of Leeds, United Kingdom.

[*] k.e.wright@leeds.ac.uk

INTRODUCTION:

Most bowel or colorectal cancer diagnosis and staging occur after the surgical removal of the suspicious tissue. The use of a real time fluorescent marker for bowl cancer tissue detection, in combination with laparoscopic or endoscopic techniques would take us a step closer towards better pre-surgical detection and staging (stratification) of these diseases in human patients. Our aim is to study the feasibility of utilizing protoporphyrin IX (PpIX) fluorescence in colorectal cancer cell lines, to distinguish them from non-tumour cells in *in vitro* model systems for diseases such as peritoneal carcinomatosis.

METHODS:

Monolayer cultures of human colon cancer cell lines (E.g. T84 & HT29) were investigated in comparison to non-tumour cells (E.g. HEK293 & 3T3). Incremental concentrations [0-1mM] of the pro-drug 5-aminolevulinic acid (5-ALA), and incubation times (0-6h) were investigated for levels of PpIX fluorescence detectable with a spectrofluorometer (Ex 405nm and Em 625nm) of 4 central points per well of 96 well black plates with transparent base (4 replica wells per plate). The % change above basal (no 5-ALA treated cells) was calculated using the formula below, and then plotted (Fig 1A).

% Change = [(Treated-Basal)/Basal] x 100

RESULTS:

PpIX fluorescence increased with increasing concentrations of 5-ALA and incubation times. HT29 tumour cells displayed the greatest detectable PpIX fluorescence in comparison to other cells tested in these experiments. 1 mM 5-ALA treated HT29 cells were approximately 1300 % brighter than non-treated cells, while T84 tumour cells are approximately 300 % brighter than non-treated cells after 6h incubation (Fig 1A). HEK293 cells under investigation showed lower levels of PpIX fluorescence than HT29 tumour cells (Fig 1B).

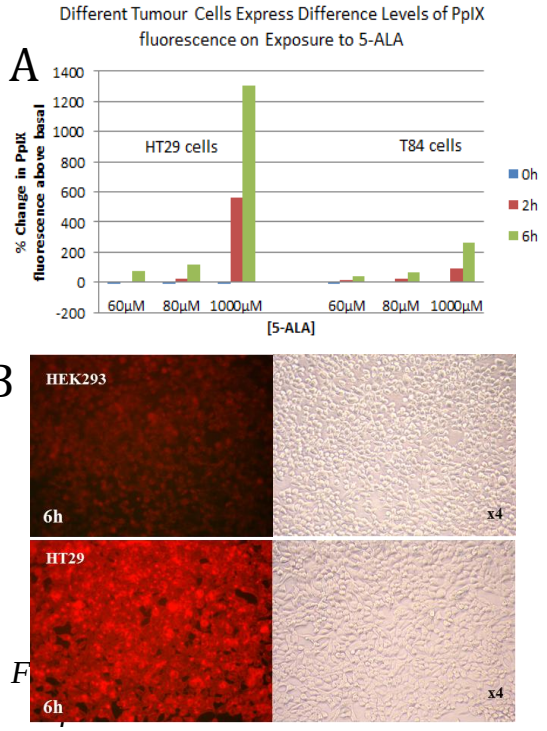

Fig 1: A) Different Tumour Cells Express Difference Levels of PpIX fluorescence on Exposure to 5-ALA. B) Fluorescence Microscopy Images of HEK293 and HT29 cells after 6h incubation with 1mM 5-ALA.

DISCUSSION & CONCLUSIONS:

These results indicate a possible difference in the generation and/or accumulation of PpIX by tumour cell lines investigated in this study. The level of PpIX fluorescence detected in bowel cancer cells/tissues may be a useful aid in classifying bowel cancer patients for treatment prior to surgery and/or chemotherapy.

ACKNOWLEDGMENTS:

The authors would like to thank NIHR and the CRUK Leeds Centre-Development Fund (Grant no: C37059/A16369) for providing financial support to this project.

P-70: Production of Growth Factors for Use in Regenerative Medicine Application

Noura Alom, Heather Peto and Kevin M. Shakesheff
Drug Delivery and tissue engineering division, Centre of Biomedical Science, University of Nottingham, UK
Paxna2@nottingham.ac.uk

INTRODUCTION:

Bone Morphogenetic Protein 2 (BMP2) is an osteoinductive growth factor which plays an important role in bone regeneration and repair. BMPs can be isolated directly from bones but the purification protocol is complicated and the yield is low (~1µg/kg) [1]. Another method to produce BMP2 involved constructing transgenic tobacco plants for eukaryotic expression system for BMP2. However, this approach required a long period of time for a growth and a very complicated purification protocol [2]. Commercially available BMP2 is produced in the prokaryotic hosts *Escherichia coli* (*E.Coli*). The BMP2 is produced as inclusion bodies but the solubilising and refolding procedures are complicated and costly [3].The high cost of BMP2 is prohibitive for tissue engineering research.

METHODS: This work aims to develop a low cost method to simply the production of BMP2 for tissue engineering, by producing it in its soluble form using a special tag dihydrolipoyl-dehydrogenase (Lip) which enhances its expression and solubilisation. A two-step purification was implemented to purify Lip-BMP2 based on the affinity and ion-exchange chromatography.

RESULTS: The expressed protein was in its soluble form in the supernatant, no further solubilising and refolding steps were needed The BMP2 was functionally active as demonstrated by the induction of alkaline phosphatase activity in C2C12 cells.

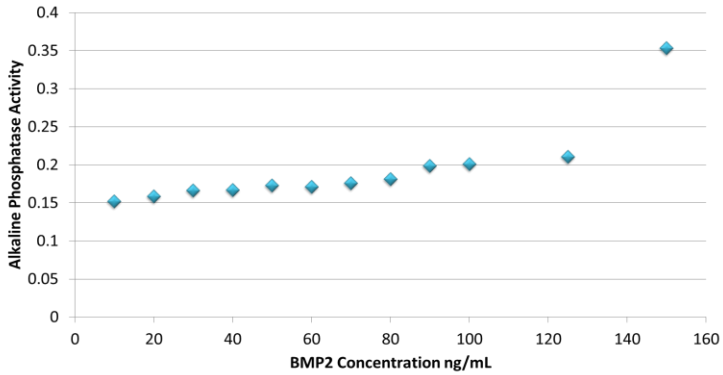

Fig. 1 : Biological activity of purified dimer BMP2 in C2C12.

DISCUSSION & CONCLUSIONS:

This work was carried out in order to develop an effective procedure to simply the production of active BMP2 without any refolding and solubilisation steps. The purified dimer BMP2 showed biological activity as demonstrated by the induction of alkaline phosphatase activity in C2C12. This procedure shown to be highly reproducible, it was able to produce active dimer BMP2 in short period of time with low cost and with no solubilisation and refolding steps.

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P-71: Serum-Free Medium Development for the Expansion of Bone Marrow Derived-Mesenchymal Stem Cells.

Steven Wilkinson[1*], Kenneth Dalgarno[2], Mark Birch[3] and Elaine Martin[1]
[1]Biopharmaceutical and Bioprocessing Technology Centre, Newcastle University, UK
[2]School of Mechanical and Systems Engineering, Newcastle University, UK
[3] Institute of Cellular Medicine, Newcastle University, UK
*s.a.wilkinson@newcastle.ac.uk

INTRODUCTION:

Adult human stem cells offer significant promise in regards to the repair and regeneration of damaged and degrading tissues and organs but due to poorly characterised and expensive expansion processes are rarely developed into mature commercial products. In an effort to help reduce the variability inherent to many adult stem cell-related culture methodologies and characterisation procedures, we have begun to develop a completely defined serum-free medium for use in the expansion of human bone marrow-derived mesenchymal stem cells (hBM-MSCs).

METHODS:

BM-MSCs were initially isolated from the trabecular bone of human femoral heads before being cultured using conventional serum-containing medium over a period of approximately five weeks. Utilising a series of fractional factorial experiments, the effects of a range of different medium supplements on total cell number and morphology were assessed.

RESULTS:

The results of this investigation appear to suggest that a number of the tested supplements may aid in the expansion of human bone marrow-derived mesenchymal stem cells during serum-free culture; including Fibroblast Growth Factor-2 (FGF-2) and Insulin-Selenium-Transferrin-Ethanolamine (ITSX).

DISCUSSION & CONCLUSIONS:

Whilst it is important to note that the total cell numbers generated under serum-free conditions were significantly lower than those seen when utilising serum supplemented medium, these results do support the idea that an optimal collection of growth factors could help sustain hBM-MSC growth within a defined environment. Future work will focus on encouraging serum-free cell adhesion and working towards hBM-MSC isolation using a completely defined medium formulation.

ACKNOWLEDGMENTS:

The authors would like to thank the Engineering and Physical Sciences Research Council (EPSRC) and Arthritis Research UK (ARUK) for providing financial support to this project.

P-72: Correlating Mesenchymal Stem Cell Delivery with Pain in an OA Rat Model.

Hareklea Markides [1], Devi Sagar [2], Oksana Kehoe [1], Robert H. Morris [3], Victoria Chapman [2], Alicia J. El Haj [1].

[1] Institute Science and Technology in Medicine Keele University, Stoke-on-Trent, UK,
[2] Arthritis Research UK Pain Centre, Nottingham University, Nottingham, UK and
[3] School of Science and Technology, Nottingham-Tent University, Nottingham, UK
h.markides@keele.ac.uk

INTRODUCTION:

Osteoarthritis (OA) is characterised by pain, inflammation and ultimately destruction of the synovial joint. Existing pharmacologic treatments are aimed at alleviating symptoms (pain and inflammation). These approaches do not target the disease process itself thus allowing the disease to progress in severity. Mesenchymal stem cells (MSCs) have been identified as suitable candidates in treating OA. They can be utilised as a therapy to address structural destruction and inflammatory aspects of the disease. To date, the relationship between MSCs delivery and pain has not been investigated. In this study, we have implemented a rat model of OA to investigate the effects of administering MSCs as a therapy on pain behaviour. Further to this, novel approaches to image and track implanted cell population's *in vivo* using superparamagnetic iron oxide nanoparticles (SPION) and MRI technologies were applied.

METHODS:

Murine Mesenchymal stem cells (mMSCs) were isolated, expanded and CM-DIL labelled prior to implantation. Meniscal transection model (MNX) of OA was surgically induced in adult Sprague Dawley rats. Upon OA induction, $1.5x10^6$ mMSCs (SPION-labelled or unlabeled) or serum free media were intra-articularly implanted. Pain behaviour (changes in weight-distribution and hindpaw mechanical withdrawal thresholds) was assessed up to 42 days post-surgery. MRI (Bruker 2.35T MRI scanner) of the knee joint was performed at the final time point and joint histopathogy was evaluated.

RESULTS:

Administration of MSCs significantly reduced weight bearing asymmetry, but not hindpaw withdrawal thresholds, indicating potential anti-nociceptive properties of MSCs. Good contrast was generated with SPION-labelled cell population located within the synovial cavity after 29 days by MRI and confirmed histologically.

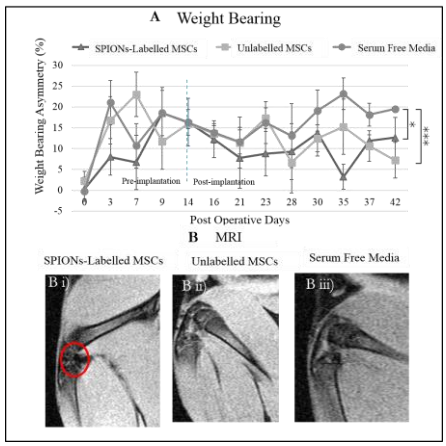

Figure 4. (A) Pain assessment; by weight-bearing asymmetry in response to the intra-articular implantation of SPION-labelled, unlabelled mMSCs and serum free media over 42 days. B) MRI tracking of implanted cell populations following intra-articular implantation of SPION-labelled, unlabelled mMSCs and serum free media. Red circle indicates location of SPION-labelled cell populations.

DISCUSSION & CONCLUSIONS:

This data highlights the potential anti-nociceptive properties of MSCs. The differential effects of the MSCs on weightbearing asymmetry versus hindpaw withdrawal thresholds suggest that this intervention may alter established peripherally-driven OA pain, but not established centrally-mediated OA pain. Whether reduced pain is linked to better repair or the anti-inflammatory actions of the MSCs will be further investigated. MRI tracking of cell populations proved to be a practical means of monitoring cell fate *in vivo*.

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Declaration: The authors declare no competing interests

P-73: The Role of Insulin –like growth factor (IGF) Binding Proteins in Osteogenic Differentiation of Human Dental Pulp Stromal Cells Derived from Healthy and Carious Teeth.

Hanaa Al-Kharobi, Reem El-Gendy, Deirdre Devine and James Beattie*
Department of Oral Biology/ Leeds Dental Institute, University of Leeds, UK
dnhea@leeds.ac.uk

INTRODUCTION:

The IGF axis plays an important role in osteogenic differentiation [1]. Although some of the molecular details associated with IGF ligand and receptor roles in osteogenesis are becoming apparent, much less information is available regarding the potential functions of IGF binding proteins (IGFBPs) in this process. Our research aims to investigate the role of the IGF axis in the osteogenic differentiation of human dental pulp stromal cells (HDPSCs) derived from healthy and carious teeth. Our translational aim is to investigate the potential use of IGF axis members for future therapeutic application in bone/dentine regeneration.

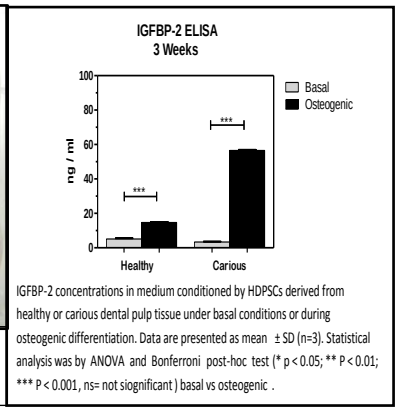
METHODS:

Freshly extracted third molars (3 healthy and 3 carious) were collected from adult age matched patients (20-40 years old). Pulp cells were isolated, and expanded until passage 4 then cultured in either basal medium (α-MEM, plus 20% FBS, 1% Penicillin Streptomycin and 1% L-Glutamine) or osteogenic conditions (basal medium + 10 nM dexamethasone and 50 µg/ml L-ascorbic acid) for 1 and 3 weeks. Osteoblastic differentiation was investigated in both cultures using alkaline phosphatase (ALP) and Alizarin red staining. qRT-PCR was used to confirm the expression of bone markers (*ALP*, OC, *RUNX2*) and to investigate the changes in gene expression of the IGF axis (10 genes). Concentrations of IGF binding proteins (IGFBPs) in conditioned media were determined by ELISA. All data were statistically analysed using ANOVA and Benferroni post-hoc test to report significant differences. Results were considered statistically significant at $p<0.05$.

RESULTS:

Healthy and carious HDPSCs showed positive staining for ALP indicating osteoblastic differentiation. However, carious HDPSCs showed higher proliferation rates and more intense ALP staining. Osteogenic differentiation was confirmed by osteogenic markers expression in HDPSCs isolated from both healthy and carious teeth; again, these markers showed higher expression in HDPSCs from carious teeth. The gene expression of specific IGF axis members was altered under osteogenic conditions in both healthy and carious HDPSCs, compared with basal cultures. There were reproducible changes in IGFBP-2 and -3 protein expression in all healthy and carious teeth.

ALP staining at 3Weeks



IGFBP-2 concentrations in medium conditioned by HDPSCs derived from healthy or carious dental pulp tissue under basal conditions or during osteogenic differentiation. Data are presented as mean ± SD (n=3). Statistical analysis was by ANOVA and Bonferroni post-hoc test (* $p < 0.05$; ** $P < 0.01$; *** $P < 0.001$, ns= not siognificant) basal vs osteogenic .

DISCUSSION &CONCLUSIONS:

We have clearly demonstrated reproducible and reciprocal changes in IGFBP-2 and -3 expression in HDPSCs during differentiation to an osteogenic phenotype. HDPSCs derived from carious teeth had greater potential to differentiate toward the osteogenic lineage compared to those derived from healthy teeth. These findings can have a future impact on clinical approaches for bone and dentin regeneration.

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1. Govoni, K.E.,(2012) *Insulin-like growth factor-I molecular pathways in osteoblasts: potential targets for pharmacological manipulation.* Curr Mol Pharmacol, 2012. **5**(2): p. 143-52.

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