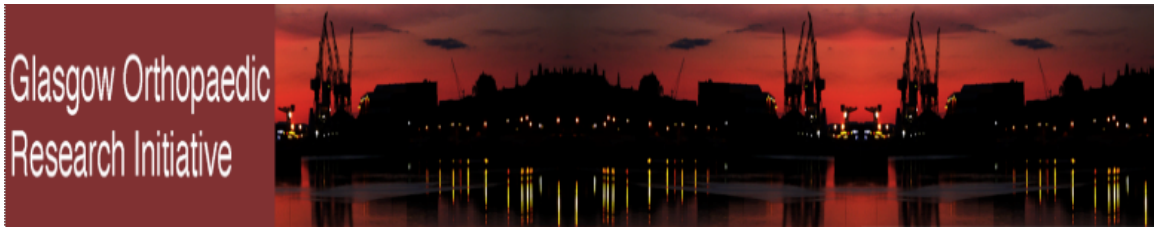

GLORI 26th March 2015

Bute Hall

University of Glasgow

Programme:
09.15 Coffee
Session 1 – chairs **Dr Monica P Tsimbouri** and **Mr Findlay Welsh**

09.30 Welcome from **Mr Dominic R Meek**

09.35 Sponsor talk from **Dr Duncan Sharp** from **Taragenyx**

09.40 Invited talk - **Prof Liz Tanner** FREng, School of Engineering, University of Glasgow
Mechanical testing to assess bone behaviour in a mouse model of RETT syndrome.

10.05 Invited talk - **Prof Maggie Cusack,** Head of School of Geographical & Earth Sciences
University of Glasgow
Oysters and Bone-an interesting connection.

10.30 **Dr Lesley Anne Turner**, Centre for Cell Engineering, University of Glasgow
Dr Anwer Saeed, School of Engineering, University of Glasgow
Development of nano-patterned substrates for the delivery of high quality stem cells.

10.40 Invited talk - **Dr Paul Rea**, Senior University Teacher and Licensed Teacher of Anatomy
Advances in Medical Visualisation for Education and Training.

11.00 Coffee

Session 2 – chairs **Dr Lesley Anne Turner** and **Mr Peter Young**

11:30 Sponsor Talk from **Mark Coldwell**, **Qiagen**

11.35 Invited talk - **Prof Nikolaj Gadegaard,** School of Engineering, University of Glasgow
From discovery to translation: harnessing nanotopography.

12.00 Keynote Speaker**- Prof Tim Board,** Consultant Hip and Knee Surgeon, Wrightington Hospital, Manchester and Salford Universities
Structural Bone Graft Tissue Engineering for Revision Hip Arthroplasty.

12.45 Invited talk -**Dr Chris West**, Registrar in plastic and reconstructive surgery, Centre for Regenerative Medicine, Edinburgh
Blood, sweat and ears - Identifying cells and scaffolds for cartilage engineering.

13.05 **Ms Suzanne Thomson,** Registrar in plastic surgery, Centre for Cell Engineering, University of Glasgow
A novel bioengineering approach to peripheral nerve surgery.

13:15 Lunch and posters

Session 3 – chairs **Dr Enateri Alakpa** and **Dr Aviral Vatsa**

14.05 Invited talk – **Mr Ashish Mahenda,** Consultant Orthopaedic Surgeon, Musculoskeletal oncology specialist, Glasgow Royal Infirmary
Primary Bone Tumours: Principles of Surgical Treatment.

14.30 Invited talk – **Prof Antony Chalmers,** Professor of Clinical Oncology, University of Glasgow
A novel 3-dimensional glioblastoma cell culture system reveals profound effects of the microenvironment on radiation sensitivity and DNA repair.

14.55 Invited talk- **Ms Lisa Lungaro** (PhD student in Prof D. Salter lab), University of Edinburgh
Development and utility of magnetic nanoparticles production by mammalian cells.

15.10 **Dr Monica P Tsimbouri,** Centre for Cell Engineering, University of Glasgow and **Paul Fairhurst,** Western Infirmary
Manipulation of cancer cells by nanotopography.

15:25 Coffee and discussion

4pm meeting ends

To register for the event please contact: glori2009a@gmail.com

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ORAL PRESENTATIONS

Coating and Implant Solutions for Orthopaedic and Dental Applications

Duncan Sharp[1,2], Lucie de Beauchamp[1], Andrew McNeill[1]
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Introduction
The aim of Taragenyx is to commercialise solutions to pressing clinical needs in the fields of orthopaedic and dental surgery. Three novel product streams are in development. The first involves the release of antibiotics from osteoconductive coatings on the synthetic polymer PEEK. The second enables the safe delivery of growth factors at a tenth of the normal therapeutic dose while retaining their osteoinductive and angiogenic effectiveness (data not shown). The third centres on 3D printing novel titanium/hydroxyapatite composites for the treatment of bone defects.

Experimental Method
The osteoconductivity of the coated PEEK was assessed using an ovine model. The release of gentamycin from the test implants was assessed using biochemical and bacterial growth inhibition assays. The cell compatibility of the gentamycin releasing coatings was examined by seeding human mesenchymal stem cells (hMSCs) onto the surface. The feasibility of manufacturing titanium/hydroxyapatite 3D structures using selective laser sintering was investigated.

Results
The *in vivo* pull out strength of PEEK was increased by a factor of 15. The release of gentamycin from PEEK was > 300 µg/cm^2 and was capable of inhibiting bacterial growth in a dose dependent manner. hMSCs grown on the surfaces were found to be morphologically normal. Titanium/hydroxyapatite 3D structures were also successfully manufactured.

Conclusions
With several "market-disrupting" technologies in development, Taragenyx seeks to commercialise innovative solutions to several long-standing problems in orthopaedics. We are actively seeking further clinical and academic collaborators to help us achieve our aims.

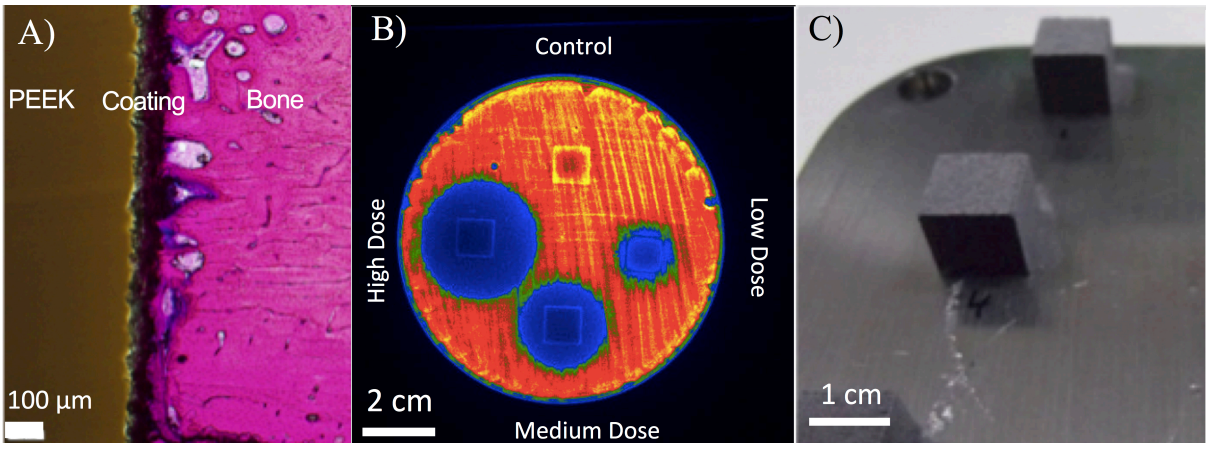

Figure 1: A) Osseointegration of coated PEEK, B) inhibition-zone assay using *E.Coli.*, C) 3D printed hydroxyapatite/titanium composite.

Biomechanical testing to assess bone behaviour in a mouse model of RETT syndrome

Bushra Kamal[1,2], Diego Constante[3], Stuart Cobb[1], Anthony Payne[2], Hanna Isaksson[4], **K.E. Tanner[3,4]**

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Introduction

Rett Syndrome (RTT) is an X-linked genetic disease considered a neuro-developmental disorder. However, the gene mutated in RTT, methyl-CpG binding protein 2 (Mecp2), is expressed throughout in peripheral tissues and in addition to neurological phenotypes, skeletal anomalies including reduced bone mass and fractures are common features. RTT is generally lethal in males, while girls survive but with profound disabilities. In order to explore whether Mecp2 protein-deficiency results in altered bone properties, we conducted biomechanical testing on both cortical and cancellous bone from mice in which Mecp2 is functionally silenced (Mecp2stop/y) and mice in which the RTT-like phenotype is rescued by Mecp2 gene reactivation (Mecp2stop/y/CreER).

Experimental Method

Mecp2stop/y male mice (n = 5; mean age = 14wk±0.7wks) and Mecp2stop/+ female mice (n≥3; mean age = 17±1 months) along with the age-matched Mecp2stop/y/CreER mice and wildtype controls were treated to reactivate the Mecp2 protein [Guy 2007]. The age difference between genders is due the differences in phenotype onset, severity and lethality in males. Tibial and femoral shafts were subjected to three point bending test to assess the cortical bone and the femoral necks to fracture test to measure the cancellous bone, using a Zwick/Roell Z2.0 testing machine. Microindentation testing was performed using a Wilson Wolpot Micro-Vickers 401MVA machine and at an applied load of 25gf. Small angle X-ray Scattering (SAXS) was used to measure mineral orientation.

Results

Both Mecp2stop/y male mice in which Mecp2 is silenced in all cells and female Mecp2stop/+ mice in which Mecp2 is silenced in ~50% of cells showed significant reductions in bone stiffness and hardness (Fig 1). Furthermore, unsilencing of Mecp2 in adult mice by cre-mediated stop cassette deletion resulted in a restoration of tibia stiffness, load properties, bending modulus and femur microhardness values to wild-type levels.

Conclusions

Previous studies [Jefferson, 2011; Shapiro, 2010; Zysman, 2006] have reported that reduced bone strength, bone mineral deficits, increase risk of fracture and bone related disorders are fairly common in RTT. We have shown the reversibility of bone defects identified in hemizygous male Stop/y and heterozygous female Stop/+ mouse model following Mecp2 gene reactivation. This significant improvement in bone properties after the Mecp2 gene reactivation points towards the potential application of intervention gene therapy for peripheral phenotypes in RTT patients.

References

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Oysters and Bone-an Interesting Connection.

Enateri Alakpa [1], Karl Burgess[2], Mathis Riehle[3], Matthew Dalby [3] **Maggie Cusack**[1]
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While the Mayan people are admired for their scientific knowledge, many of their social practices, such as shaping and ornamenting their teeth with semi-precious stones, are intriguing and mysterious. Even more intriguing is the fact that they used pieces of marine shells, made of calcium carbonate, as dental implants. We now know that this crossing of the Bone-Shell Divide [1] resulted in integrated implants indicating osteo-induction and osteo-integration [2]. The observation that nacre (mother of pearl) is osteo-inductive sparked research interest[3-4] and direct applications [5-6] in an attempt to identify which feature of nacre is responsible for this response. The co-occurrence of topography with mineral and organic components presents a challenge in identifying the causative component. Can the shell nano/microtopography alone determine cell fate?

Here we use the nacre and prisms of the pearl oyster *Pinctada maxima* shell as templates from which to isolate nacre and prism topography by forming bio-compatible polycaprolcatone (PCL) replicas of these two microstructures. Digital elevation models in scanning electron microscopy are used to quantify the fidelity of these replicas.

The response of mesenchymal stem cells (MSCs) to the nano/microtopography of nacre and prisms and control planar PCL was determined by quantitative PCR, histology and fluorescence and metabolomics. We demonstrate the suitability of this approach that enables the identification of topographic response to be isolated.

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Development of nano-patterned substrates for the delivery of high quality stem cells

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Lesley-anne.turner@glasgow.ac.uk

Previous research has demonstrated that engineered substrates with defined nanotopography can control stem cell behaviours[1,2]. The aim of this current research is to expand on these results in order to identify more 'hit' topographies that have the potential to direct stem cell differentiation. We have engineered a laboratory-relevant cell culture plate with a range of defined nanotopographies. Using this plate we are able to carry out high throughput analysis of mesenchymal stem cell (MSC) responses to a number of different topographic patterns using the In Cell Western technique.

Alongside this work we are using a metabolomics approach to characterise the metabolome of MSCs cultured on a SQ, or multipotency retaining, nanotopography. It is hoped that this approach will assist with the identification of biochemical mediators of multipotency. Understanding the biochemistry behind stem cell multipotency will further efforts to expand stem cells *in vitro,* bringing the goal of stem cell expansion to clinically relevant numbers closer.

References

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Advances in medical imaging techniques

Dr Paul M. Rea
Laboratory of Human Anatomy University of Glasgow

Advances in medical imaging techniques have rapidly evolved over recent years. This presentation will demonstrate how high end digital technologies are able to shape medical visualisation. Over the past seven years, there has been a long standing successful strategic partnership between the Laboratory of Human Anatomy, School of Life Sciences and the Digital Design Studio, The Glasgow School of Art. It has resulted in significant funding being provided by NHS Education for Scotland to create a highly accurate digital anatomical model of head and neck anatomy for dental education and training. This collaboration then resulted in the creation of an innovative taught MSc in Medical Visualisation and Human Anatomy to train students in the use of a variety of software packages to enable them to create interactive animations and educational and training products to benefit the wider medical and scientific communities, both from the learner and the trainer's perspective. Examples of medical visualisation applications will be shown, and expansion of the work into the cellular arena will be discussed, along with current digital technology use for current research.

From discovery to translation: harnessing nanotopography

Prof Nikolaj Gadegaard
School of Engineering, University of Glasgow

Structural bone graft tissue engineering for revision hip arthroplasty

Tim Board[1]

1 The Centre for Hip Surgery, Wrightington Hospital

1 Centre for Tissue Injury and Repair, University of Manchester

Presenting Author email: tim@timboard.co.uk

Revision hip arthroplasty is performed when a hip replacement fails. There are approximately 10,000 revision hip procedures performed each year in the UK (11th UK National Joint Register report) and this burden is set to rise significantly. A recent prediction of revision rates from the USA suggested that the rate of revision hip arthroplasty would increase by 300% by 2030. Current acetabular revision techniques using large metal implants do not reconstruct the skeleton biologically and set the patient on a path of increasingly complex reconstructions if the device fails. We prefer a biological approach to revision surgery using bone graft when possible. The results of acetabular bone grafting are good in simple contained defects but have been less than satisfactory in more complex cases that require the use of larger, solid bone grafts. This presentation will describe work that has been undertaken to understand the mechanisms of failure and the tissue engineering approaches used to improve the outcome of structural bone grafting.

Blood, Sweat and Ears.

Dr Chris West
University of Edinburgh

Plastic surgery is a discipline that is defined by the aim to restore form and function. This often requires complex reconstruction of damaged and diseased tissue with significant morbidity to patients. Tissue engineering offers the possibility of eliminating much of this morbidity be growing new tissues that can be transplanted into patients. Fundamental to any successful tissue engineering is identifying suitable cells and scaffolds.

Using immunohistochemistry to establish a unique surface marker profile, this work identifies microvascular pericytes as an in-vivo source of mesenchymal stem cells. Using Fluorescence Activated Cell Sorting, protocols were developed to prospectively purify these cells from human adipose tissue in numbers sufficient for clinical use without the need for ex-vivo expansion.

A high through-put polymer microarray platform was used to identify synthetic polymers that can be used as substrates for tissue engnineering. A library of over 2000 polymers was screened and 5 distinct polymers that support the attachment of cells and the stable proliferation over extended periods of culture were identified. In addition, the ability of specific polymers to support the subsequent differentiation into mesodermal lineages was evaluated.

Bioengineering approaches to improve nerve repair

Thomson SE[1], Tsimbouri PM[1], DeJardine T[2], Smith CA[1], Kingham PJ[3], Hart AM[1], Riehle MO[1].

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2. Institut Jacques Monod, CNRS, Université Paris Diderot, Paris
3. Department of Integrative Biology, Umeå universitet 901 87 Umeå SE

INTRODUCTION AND AIMS

Peripheral nerve injury is common (1/1000) and costly to the patient and society. Despite advances in microsurgical repair outcomes remain unsatisfactory. Commercially available nerve conduits fail to surpass the current gold standard of autologous nerve graft in the management of a gap defect. The neurobiology of the nerve repair must be unraveled. Work at the University of Glasgow Centre for Cell Engineering and collaborating laboratories has demonstrated that the directionality and rate of axonal regrowth following nerve injury can be enhanced in vitro by growing cells on micropatterned surfaces. Furthermore adipose derived stem cells have been differentiated towards a glial lineage to support nerve healing. Work is underway to further characterize these powerful strategies and incorporate them into the design of a novel bioactive nerve conduit.

MATERIAL AND METHODS

A review of the currently available nerve conduits is presented. Means of optimizing nerve conduit design will be discussed. 2 and 3-D topographically enhanced substrates were fabricated from polycaprolactone (PCL) and Polydimethylsiloxane (PDMS). The dorsal root ganglion organotypic explant model was used to study the effect of engineering the cellular microenvironment (including surface treatment, topography and co-culture with supportive cells).

RESULTS

Highly directed neurite outgrowth was demonstrated by immunohistochemistry in response to growth on topographically enhanced surfaces. The differential expression of the target genes studies over a timeline of 10 days is presented. Furthermore, imunohistochemistry was used to localize target proteins. Differentiated adipose derived stem cells adhered to, and proliferated on a PCL surface, whilst maintaining their markers of differentiation.

CONCLUSION(S)

These preliminary results further characterise a useful *in vitro* model and provide more detail on the complex interactions underlying nerve repair. It highlights some of the downstream effects of micropatterning and explores complimentary bioengineering approaches. A combinatorial approach, incorporating these strategies to bioengineer a novel nerve conduit is under investigation.

Primary Bone Tumours: Unanswered Questions

Mr Ashish Mahendra

Glasgow Royal Infirmary

The talk includes introduction to primary bone tumours, principles of diagnosis and management, therapeutic/diagnostic advances and future scope.

Radiation responses of 2D and 3D glioblastoma cells: a novel, 3D-specific radioprotective role for VEGF activation and/or EGFR inhibition through Non-homologous end-joining

Natividad Gomez-Roman, Katrina Stevenson, Lesley Gilmour, **Anthony J. Chalmers**
Translational Radiation Biology Group, Wolfson Wohl Cancer Research Centre, University of Glasgow
anthony.chalmers@glasgow.ac.uk

Introduction

Glioblastoma (GBM) is the most common primary brain tumour with dismal prognosis and novel targeted agents have failed to improve outcomes despite promising pre-clinical data. Tumours exhibit inherent resistance to radiation and chemotherapy which has been attributed to a subpopulation of cancer cells termed 'GBM stem-like cells' (GSC) characterised by multipotentiality and potent tumorigenic capacity [1]. The use of established cancer cell lines in simplified two-dimensional (2D) *in vitro* cultures might explain the observed discrepancy between pre-clinical and clinical responses to cytotoxic treatments.

Experimental Method

We developed a customised, 3D GSC culture system using a polystyrene scaffold (Alvetex®) that recapitulates key histological features of GBM including high cellularity and sparse extracellular matrix (ECM) and compared it to conventional 2D GSC cultures.

Results

2D and 3D cultures of three different primary GSC lines exhibited similar radiation sensitivities as measured by clonogenic survival. Previous studies have demonstrated radiopotentiating efficacy of the EGF receptor (EGFR) inhibitor erlotinib against GBM cell lines in 2D cultures [2]; however it failed in GBM clinical trials [3, 4]. Thus we evaluated the radiation modifying effects of erlotinib on 2D and 3D GSC cultures. Erlotinib enhanced radiosensitivity of 2D GSC cultures but had no effect on radiation responses of GSC in 3D culture or neurosphere formation assays, where cells grow in 3D conditions devoid of a scaffold or extrinsic ECM. We next examined VEGF inhibition, since anti-VEGF therapy in combination with standard radio-chemotherapy increases progression-free survival of GBM patients. VEGF deprivation was associated with significant radiosensitisation of 3D GSC cultures but had no effect on 2D GSC. Erlotinib treatment of VEGF-deprived 3D cultures increased the radiation resistance of 3D cells to the same extent as VEGF addition, indicating epistasis. EGFR has been shown to regulate repair of radiation-induced double-strand breaks by activating the non-homologous end-joining (NHEJ) repair protein DNA-PKcs [5]. A correlation between radiosensitivity, increased gH2AX foci and phospho-DNA-PK nuclear foci after radiation treatment was observed, In contrast, increased numbers of foci of the homologous recombination (HR) repair protein Rad51 were observed in radioresistant populations.

Conclusions

Our results show that EGFR inhibition and/or VEGF signalling induce a switch from ineffective NHEJ to more accurate HR repair leading to radiation protection. These 3D effects recapitulate data from clinical trials, strongly supporting the clinical relevance of this 3D model and its potential value in preclinical studies.

References

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Development and utility of magnetic nanoparticles production by mammalian cells

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Introduction

Nowadays there is an increasing interest in magnetosomes used as Superparamagnetic Iron Oxide Nanoparticles (SPIONs) for magnetic hyperthermia cancer therapy [1],[2]. Magnetosomes are magnetic organelles produced by Magnetotactic Bacteria (MTB), aquatic prokaryotes which can become aligned with the geomagnetic field. Studies of MTB genome show the importance of Mms6 and MmsF genes in magnetosome formation. The aim of this work is to create magnetosomes expressing mammalian cells, in order to obtain a new and non-invasive contrast agent that could be used for Magnetic Resonance Imaging (MRI) to precisely kill cancer cells by magnetic hyperthermia without affecting the neighbouring healthy cells.

Experimental Method

Osteosarcoma cells (MG63) were transfected with Mms6 and MmsF genes. Gene expression was assessed by RTPCR. Nanoparticles production was assessed by transmission electron microscopy (TEM). An alternating magnetic field was applied to fixed cells to investigate magnetic hyperthermia effects.

Conclusions

Experiments suggest that mammalian cells may be transfected with Mms6 and MmsF genes. Next *in vitro* experiments will test the ability to effectively kill the tumour cells in a co-culture of tumour and transfected cells, under the action of an alternating magnetic field.

References

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[2] Kievit et al., 2012.

Effects of Nanotopography on Cancer Cells

Stella Gkegka*[1], Deirdre M. Saunders*[1], T. Sjostrom[3], B. Su[3], P. Young[2], R.D.M. Meek[2], A. Machendra[4], M.J. Dalby[1] P. Fairhurst[5] and **P. M.Tsimbouri[1]**

[1]Centre for Cell Engineering, University of Glasgow, UK.[2]Southern General Hospital, Glasgow, UK.[3] School of Oral and Dental Science, University of Bristol, UK, [4]Glasgow Royal Infirmary, [5]Glasgow Western Infirmary.

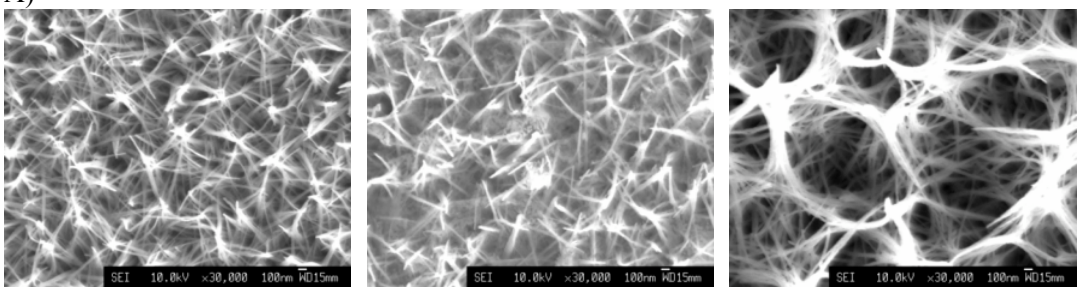
Introduction: Tumour cell migration is a major health focus that relies on soluble chemistry and invasive biology for its study. Nanotopographies have been shown to direct stem cell differentiation, suggesting that the environment and cell geometry can influence signal transduction pathways and change the fate of the cell. Here we propose the use of nanotopography as a non-invasive method of altering cancer cell migration, allowing mechanistic study and comparison to normal cells, with the possibility of discovering nanopatterns that could inhibit tumour growth and metastasis and allow enrichment of normal healthy cells by competition.

Aim: The purpose of this investigation is to identify topographies that would negatively influence cancer cell growth and determine the differences in the response of healthy cells, osteosarcoma cells, and cancerous epithelial derived cells lines with relation to the Mitogen-activated Protein Kinase (MAPK) pathway and apoptosis.

Methodology: Each cell type was grown on four surfaces with nanotopographical variation: flat (smooth polished) titanium and titanium nanowires created by treating the titanium discs with NaOH for 2h, 2.5h, and 3h (Fig.1A). Immunocytochemistry (Fig. 1B) was used to assess cell cytoskeleton, cell adhesion, proliferation, and apoptosis. MTT assay and BrdU were used to evaluate cell growth and proliferation respectively. qRT-PCR compared mean level gene expression for proapoptotic, antiapoptotic, and proliferative genes (BAX, BCL2, ERK2, cmyc, TNFa) on each of the surfaces.

Results/Discussion: The results showed possible effects of nanotopography on the proliferative and apoptotic abilities of cancer cell lines whereas healthy BM cells remained largely unaffected.

A)



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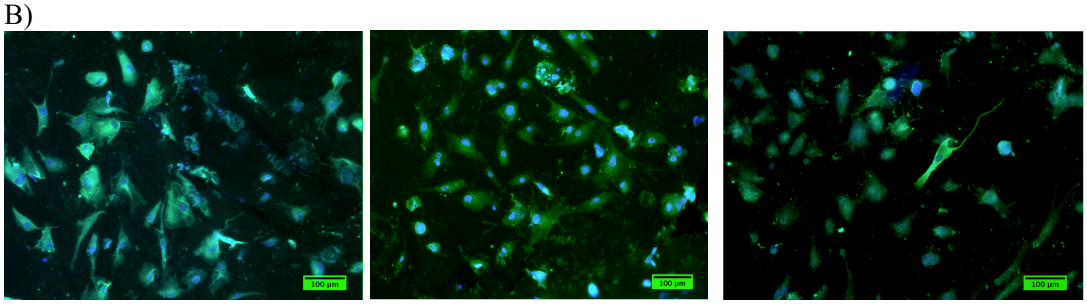

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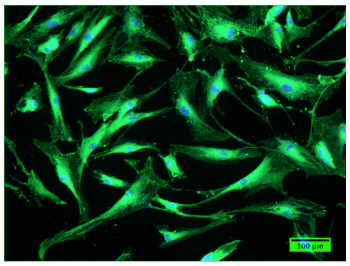

Flat control

Fig. 1 A) SEM images of titanium discs taken by TJS at Bristol University. B) BM cells cultured on the Ti surfaces with various adhesion and morphological changes.

*equal contribution to the work.

POSTERS

Cell Controllable Interfaces

Hilary J Anderson[1], Dr Jugal Sahoo[2]
Professor Matthew Dalby[1], Dr Catherine Berry[1], Professor Rein Uljin[2],
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Currently mesenchymal stem cell (MSC) culture is not efficient. We lack an appropriate enabling technology that allows the controlled maintenance and differentiation of MSC in the same platform. Using materials engineering, we aim to provide a dynamic culture medium that can not only maintain MSC markers but also promote differentiation in osteogenic lineage. By incorporating enzyme responsive peptide sequence we have the power to modify the material allowing controlled switching of MSC phenotype. The enzyme responsive sequence is hidden beneath a polyethylene glycol (PEG) blocking group, we have previously shown that PEG can maintain MSC marker stro1. When an enzyme digests this, the cell adhesive tri-peptide arginine-glycine-aspartic acid (RGD) is revealed; promoting increased intracellular tension and subsequent differentiation. What is novel about this material is that we can employ the enzymes that the cells themselves secrete (the matrix metalloproteinases) to modify the surface. It is therefore not user defined but cellularly controlled. The material is created using solid phase peptide synthesis (SPPS), a technique utilized in the past to produce peptides from a solid support that are then cleaved and purified. Here, we maintain the peptides on the solid support and by doing so we can biofunctionalise the properties of glass coverslips to promote both stem cell retention and differentiation.

Controlling the Enemy: Bacterial Biofilms for MSC differentiation

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[2] Centre for Cell Engineering, Institute of Biomedical and Life Sciences, University of Glasgow, Joseph Black Building, Glasgow G12 8QQ, UK

Introduction

As of 2007, there have been at least 50,000 hip replacements per annum in the UK alone, revision rates of about 6% after five years and 12% after ten years are to be expected and cause a huge economic burden on the NHS. Genetically modified Lactococcus lactis, a non-pathogenic bacteria expressing the FNIII7-10 fibronectin fragment as a protein membrane has been used to create a living biointerface between synthetic materials and mammalian cells. This FNIII7-10 fragment comprises the RGD and PHSRN sequences of fibronectin to bind a5b1 integrins resulting in cell adhesion. We have added the growth hormone bone morphogenetic protein-2 to MSCs grown on the biofilm and we have shown that this novel material can sustain MSC differentiation to an osteogenic lineage. This biointerface based on living bacteria can be further modified to express any desired biochemical signal, establishing a new paradigm in biomaterial surface functionalisation for biomedical applications.

Functional Nanoparticle Design: The importance of the Interplay between PEG density and Intracellular Glutathione for Efficient siRNA Release

Mark A. McCully,[1] Yulan Hernandez [2], Jesus M. de la Fuente [2] M.J.Dalby1, C.C.Berry[1]

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Introduction : Gene silencing by RNA interference holds great potential in nanomedicine, but needs successful delivery of the small interfering RNA (siRNA). This study used ***thiolated siRNA*** against C-Myc, conjugated ***gold nanoparticles*** (AuNPs), which rely on intracellular ***glutathione*** (GSH) for siRNA release. The NPs were also coated with biotin, to aid observation. The bone carcinoma cell line, MG63, were challenged with AuNPs with two differing PEG densities; 25 % and 40%. .

Methods : The AuNPs were assessed for (i) cytotoxicity via MTT assay; (ii) Cellular uptake via TEM and ICP-MS, and (iii) C-Myc knockdown via In-Cell Westerns (ICW) and BrdU analysis.

Results : Results indicated the highest knockdown was achieved with the lower 25% PEG density, equivalent to lipofectamine knockdown (figure 1). Noting the low levels of GSH levels in MG63 cells (figure 2), we believe the higher PEG densities block GSH access to the thiolated siRNA.

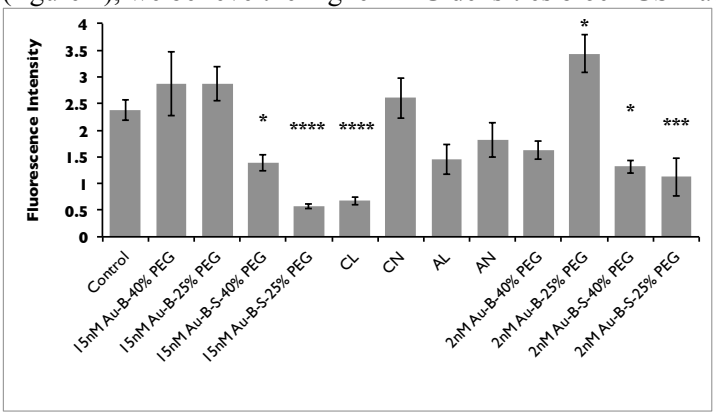

Figure 1. ICW of C-Myc protein levels in MG63s treated with AuNPs with either 40% or 25% PEG coverage at both 2nM and 15nM siRNA. MG63s were serum starved for 24hours before treatment to synchronise cell division. The treatments included plain NPs (PEG and biotin (B) only) and siRNA (S) loaded NPs. Control cells had no NPs. Additional controls included C-Myc siRNA with Lipofectamine (CL), Allstar nonsense siRNA with Lipofectamine (AL), and C-Myc or Allstar added directly to the media (CN & AN) (n=6; error bars denote standard error).

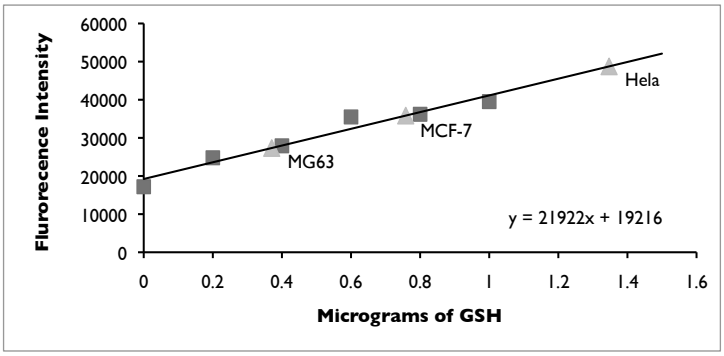

Figure 2. Colorimetric glutathione (GSH) assay indicating the varying intracellular GSH levels between cell lines.

Conclusion : The varying C-Myc knockdown observed appears related to the PEG density. As siRNA release is reliant on GSH interaction, it is surmised that the higher PEG densities cause steric hindrance and thereby reduce GHS accessibility to the siRNA and therefore reduce release and subsequent knockdown effect.

Blocking mir-31 with antisense RNA conjugated gold nanoparticles drives an increase in osteogenic markers

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Introduction: The therapeutic potential of miRNAs (short single stranded RNA molecules ~ 20 nucleotides) as a means of altering gene expression is huge, due to their increasingly realised role in cell regulation. However therapeutic progression suffers from delivery issues. A new role for ***Mir-31*** has recently been identified, linking mir-31 with osteogenic suppression and the osteogenic transcription factor ***Osterix*** (OSX). Here we report for the first time that a thiolated antagonist of mir-31 (antagomir-31; 50nM) can significantly increase OSX levels in the OSX suppressed cell line MG63 and suggestively in primary mesenchymal stem cells (MSCs), using ***gold nanoparticles*** (AuNPs) as a delivery vector.
Methods: The AuNPs were PEGylated and conjugated with either the 5' or 3' antagomir-31. Cells were assessed for (i) cytotoxicity via MTT assay; (ii) Cellular uptake via TEM and ICP-MS, and (iii) Osx Expression via fluidigm RNA analysis and In-Cell Westerns (ICW).
Results: Following 48 hour incubation with both 5' and 3' antagomir-31-AuNPs, OSX protein levels were increased in both MG63 cells (figure 1) and MSCs (figure 2).

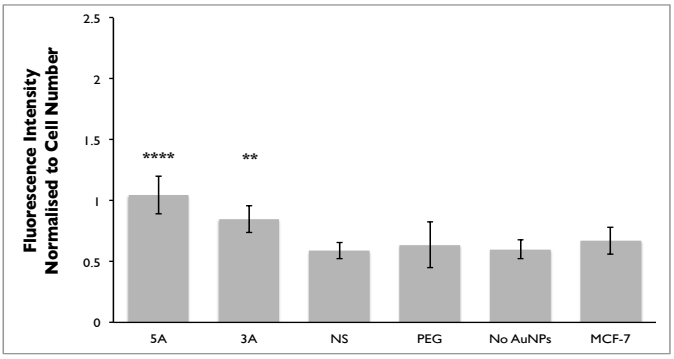

Figure 1. ICW protein levels of OSX in MG63 cells after 48 hours treatment with AuNPs (normalised to GAPDH). The treatments included 5A (complementary sequence to 5' mir-31); 3A (complementary sequence to 3' mir-31); NS (nonsense strand); plain NPs (PEG). The MCF-7 cells are a negative control that expresses low OSX levels (n=6; error bars denote standard error)

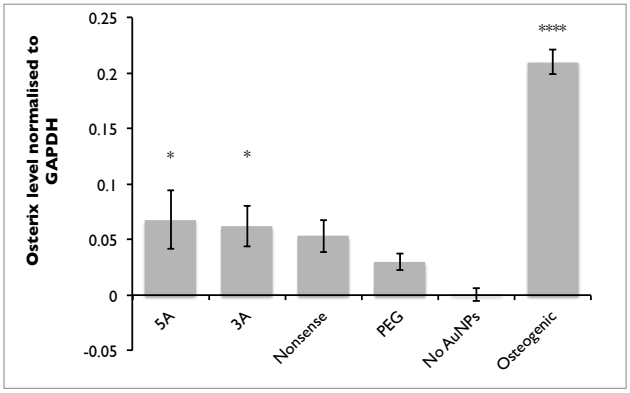

Figure 2. ICW protein levels of OSX in hMSC after 48 hours treatment with AuNPs (normalised to GAPDH). The treatments included are identical to figure 1 above. The osteogenic media serves as a positive control with high OSX levels (n=6; error bars denote standard error)

Discussion: This novel study demonstrates that antagomir-31, delivered via AuNPs, is capable of inhibiting the effect of Mir-31, allowing an increase in OSX protein expression. Such a delivery platform has excellent potential clinical application with regards to conditions such as osteoporosis and osteoinperfecta.

Investigating the Effect of Polymer Surface Mobility on Protein Organization and Cellular Response

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Introduction

Substrate properties, such as topography[(1)] or stiffness[(2)], are well known to affect stem cell differentiation. We have also found that polymer surface mobility, defined by their glass transition temperature (T_g), can also affect cell behaviour[(3)]. As cells interact with synthetic surfaces via extracellular matrix proteins (e.g. fibronectin)[(4)] we believe this polymer mobility translates to the interfacial mobility of the protein layer. In our current work we adsorbed fluorescently labelled fibronectin to glass surfaces, spun coated with polymers containing varying side chain lengths, $-COO(CH_2)_xH$. These polymers maintain similar chemical groups but differing surface mobility allowing for the analysis of how surface mobility can affect cell-mediated protein reorganisation. We have found that the physical properties of the fluorescent fibronectin are comparable to that of the unlabelled protein. Also, by plotting the intensity gradient change of bleach spot edges over time on each surface we found a non-monotonic dependence of gradient change on mobility. Furthermore, we have found that L929 cells showed an enhanced ability to reorganise the fibronectin on more mobile surfaces, with the most mobile surface still showing reorganisation even in the presence of high concentrations of the cytoskeletal inhibitor, blebbistatin. These results serve to further elucidate the underlying mechanisms of how surface mobility affects the protein matrix and the resulting cellular response.

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Nano-Kicking Stem Cells into Making Bone

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Introduction and Experimental: Mesenchymal stem cells (MSCs) have large regenerative potential to replace damaged cells from several tissues along the mesodermal lineage. The potency of these cells promises to change the long term prognosis for many degenerative conditions currently suffered by our aging population. Here, we present data demonstrating our ability to induce osteoblastogenesis in MSCs using high frequency (1000 Hz, 3000 Hz & 5000 Hz) piezo driven nano-displacements (30, 28 & 16 nm displacements respectively at each of these frequencies) in a vertical direction. We have used interferometry and the wavelength of light to measure these nanometre-level displacements.

Result: Osteoblastogenesis has been determined by the up-regulation of osteoblasic genes, accessed via quantitative real-time (qRT-) PCR. Further to this, we also provide corroborative quantitative results at a proteomic level and confirm the presence of *in vitro* bone nodule (calcium phosphate ($Ca_3(PO_4)_2$) deposition by these osteoblastic cells using Raman Spectroscopy as a very sensitive and specific technique [1].

Conclusions: This work investigates the effect of high frequency piezo displacements on the osteoblastic phenotype of MSCs. It shows that nano-topography and piezo can work in synergy to provide differentiation, but that even piezo stimulation alone is strongly osteoblastic. It is envisaged that using gels piezo could also prove its efficacy in 3D coming even closer to being biomimetic , and in the future potentially aiding in the recovery of musculoskeletal conditions.

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Growth by Stretch: An Interdisciplinary Approach to Improve Current Practice

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Tissue expansion is a technique used by plastic and restorative surgeons to cause the body to grow additional skin, bone or other tissues. For example, distraction osteogenesis has been widely applied in lower limb surgery (trauma / congenital), and congenital upper limb reconstruction (e.g. radial dysplasia). This complex and tightly regulated expansion process can thus far only be optimised by long-term animal or human experimentation.

Here the intent is to develop an in vitro model of tissue expansion that will allow to not only optimise the extension regime (µm/h, continuous/ intermittent), but also investigate using proteomic techniques which molecular pathways are involved in its regulation, and how drugs could be used to aid tissue expansion. Cells cultured onto sheets of polymer (PCL) can be stretched at very low, adjustable speeds, using a stepper motor and various 3D printed and lasercut designs. The system utilises plastic flow of the polymer, enabling the material to stay extended upon strain being released.

Plasma treating the material has shown an increase in cell (fibroblast) attachment with plasma treatment time; a decrease in water contact angle at higher plasma treatment times indicates increased cell attachment occurring due to the material becoming more hydrophilic. Tensile tests display the plastic behaviour of the polymer sheet when stretched. Further implications of stretch by the system on PCL can be seen using grids printed onto the PCL prior to it being stretched. Initial experimentation on fibroblasts aim to show that substrate stretching can be correlated to stretching of the cells cultured on it (immunofluorescence quantification).

Porous structure modulates microfibre stiffness and cell adhesion

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Introduction

There is a higher demand for bone replacement therapies due to an ageing population becoming more active that is outliving their implants [1]. The gold standard in osteo-implants is autografts [2] but this technique has various problems including that the procedure can be very painful and lead to donor site morbidity [3]. The overall aim of the project is to create alginate microfibers with controlled porous structures via microfluidics for driving differentiation of the mesenchymal stem cells (MSC).

Experimental Method

The alginate microfibers are created by using a microfluidic device with a flow focusing T-junction combined with a narrow focal aperture to allow the formation of microbubbles within the fibres. A 1.8% alginic acid solution is flowed through the device and nitrogen gas is used to make the microbubbles. The microfibers are cross-linked within a bath of 0.1 M strontium chloride ($SrCl_2$) solution.

Results

Analysis with microscopy and atomic force microscopy (AFM) is used to measure the size and stiffness of the fibres. We have shown that larger micro-bubbles decrease the overall stiffness of the fibre to around 30 mN/m. With the use of a population study using MG 63 cells, we have shown that the cells grow and proliferate on the micro-bubbled regions but not on non-bubbled regions.

Conclusions

In conclusion with the use of the microbubbles, a highly porous cell scaffold is created which allows customisability in regions of stiffness capable of allowing cells to better adhere and proliferate upon the surface, while also being biodegradable.

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Characterisation of tissue remodelling and inflammation in models of osteoarthritis

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ABSTRACT

Objectives. The role of proteinase activated receptor2 (PAR2) in regulating inflammation and tissue remodelling was evaluated in two experimental models of osteoarthritis (OA).

Methods. OA was induced by either destabilisation of the medial meniscus (DMM) or disruption of the anterior cruciate ligament (ACL) in PAR2 wild-type (WT) and PAR2 knock-out (KO) mice (1). Using histological scoring and computerized planimetry, the effect of PAR2 deficiency on cartilage damage, bone sclerosis, and ligament remodelling was assessed at 1 year post-DMM induction. Synovitis and immune cell characterization were evaluated at 4 weeks post-surgery. Flow cytometry was used to assess immune cell populations in spleens from both models.

Results. PAR2 KO mice showed a significant decrease in cartilage damage, bone sclerosis and ligament remodelling in one year DMM compared to WT ($n=5$ per group, $p<0.05$). Further characterization of the meniscotibial and anterior cruciate ligament in controls demonstrated the presence of PAR2, bone remodelling markers, hypertrophic-like chondrocyte cells and chondrocyte-like cells, with an absence of macrophages. At 4 week in both WT DMM and ACL, macrophages (F4/80) were observed in the ligaments. Immune cell populations remained unchanged between groups with no significant synovitis observed.

Conclusion. Disruption of PAR2 offers protection against cartilage degradation, bone sclerosis and ligament remodelling. There was no detectable inflammation in the OA models studied.

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Titanium alloy with nanotubular surface modification for orthopaedic applications

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Introduction Titanium and its alloys have been widely used in orthopaedics due to their good mechanical properties and biocompatibility. In order to accelerate healing and to improve the integration of the implant, various surface treatments can be performed. One of the possibilities is *in situ* preparation of nanotubes via anodic oxidation in the presence of fluoride ions. Nanostructured surfaces can mimic the extracellular matrix ability to trigger specific cell responses[1]. Our aim is to prepare and test nanoscale surface modification that can support osteodifferentiation of the bone mesenchymal stem cells migrating to the site of the damage when implanted to the body. This would then prevent undesirable fibrous layer formation on the interphase leading to better fixation of the implant.

Experimental Method The Ti-6Al-4V alloy with nanotubular surface modification (nanotubes of 50 nm and 100 nm in diameter) was tested using human mesenchymal stromal stem cells, human osteoprogenitors and human osteoblasts. Adhesion of MSCs and osteoprogenitors after three days was studied using fluorescent microscopy after immunostaining for vinculin and tubulin. Morphology of the cells and interaction with the nanotopography after three days was observed under scanning electron microscope. Metabolic activity of primary osteoblasts after one week was evaluated using MTT assay. After four weeks, markers of differentiation were determined using qPCR and morphology and cell numbers of MSCs were evaluated using immunofluorescent microscopy.

Results We observed that adhesion and morphology on the surfaces was dependent on the cell type (MSCs being more sensitive than osteoprogenitors). Vinculin and tubulin intensity was significantly lower on nanostructured surfaces compared to polished control in case of the first donor; however, in the second case with different donor, there was no significant difference. Although there was neither significant difference in cell numbers nor in metabolic activity on the tested materials compared to polished control, we could observe a trend that nanotubes of smaller diameter (50 nm) seemed to be more suitable than 100nm nanotubes. Using qRT-PCR, we were not able to detect any conclusive changes in expression of osteogenic markers. ALP expression was downregulated on both nanosurfaces, whereas OCN expression was upregulated on 100nm nanotubes. Other osteogenic markers (OPN, OSX, osteonectin) as well as other markers of adipo- and chondrodifferentiation (PPAR-gama and SOX9, respectively) remained unchanged. After four weeks of growth on the surfaces, MSCs on nanostructure had similar morphology to the polished control and there was no difference in cell numbers within the samples.

Conclusions Nanotubular morphology on titanium alloy did not impair growth of primary human osteoblasts, osteoprogenitors and hMSCs. However we didn't observe any strong effect of nanotubes on stem cell fate, which could be caused by interference of the fibrogenic character of the titanium material. Nanotubes of smaller diameter might have more pronounced effect.

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

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INTRODUCTION: Titanium(Ti) an orthopaedic applications material with 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 shown that 15nm high nanopillars are bioactive using human MSCs and could improve osseointegration. We have also developed an osteoblast/osteoclast co-culture system using nanopits on polycarbonate. Here, we have used these co-cultures as they give an accurate representation of the *in vivo* environment, allowing assessment of bone remodelling related to biomaterials.
AIM: Under co-culture conditions 15nm high Ti nanopillars, will induce osteoblastogenesis and reduce osteoclast activity, producing a method of enhancing secondary implant fixation.
METHODS: Osteoblast/osteoclast progenitors co-cultured on polished Ti (control) and 15nm Ti nanopillars fabricated by the block copolymer technique. 14d and 28d time points were selected for analysis of osseointegration in vivo. Quantification of osteoclasts and bone nodule formation performed using histochemical staining. Morphological changes were examined by SEM. qRT-PCR was used to quantify expression of osteoblast, osteoclast and inflammatory response related genes.
RESULTS: Using SEM, nanopillar Ti substrates were less inductive of osteoclastogenesis, with decreased maturity and activity with time, when compared to control (Fig.1a). Supportive TRAP staining showed macrophages/osteoclast projenitors present on control only (Fig.1b). Alizarin red staining indicated increased osteogenesis on the nanopillars (Fig.1c). qRT-PCR revealed a time-related decrease in osteoclastogenesis-related genes on the nanopillars with an associated increase in osteoclast inhibitors.

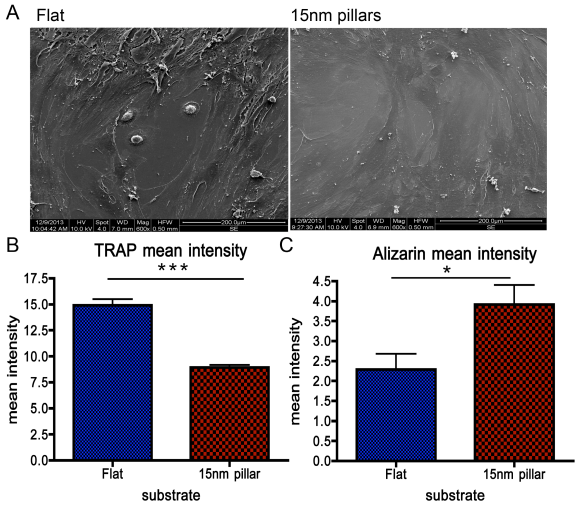

Fig.1 Showing a) SEM, b) TRAP and c) Alizarin staining results (n=3, T-test, *p<0.05, ***p<0.001).

DISCUSSION: Dramatic reduction in number of osteoclast progenitor cells and increased osteoblastogenesis on the nanopillar substrates versus control. Genomic data on osteoclast and osteoblast-related genes will be presented.

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HLA-B27 over-expression in rats alters central and peripheral monocyte populations.

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Background and objectives: HLA-B27 expression is associated with spondyloarthropathy (SpA) and transgenic rats expressing human HLA-B27 and b2-microglobulin (B27 rats) display systemic inflammation and bone loss, which resembles human SpA. We have previously demonstrated that these rats lack a dendritic cell population. However, the myeloid compartment has not been completely characterized. Here, we aimed to characterize monocyte and pre-osteoclast populations in the bone marrow and blood of B27 rats, and examined the potential of different monocyte/pre-osteoclast subsets to generate mature osteoclasts *in vitro*.

Materials and methods: 14-16 week old control (B7) and B27 rats were bled and plasma CCL2 levels were measured by ELISA. Monocytes subsets were analysed and quantified in both the bone marrow and blood of B27, B7 and non-transgenic animals, by evaluation of surface markers (CD172a, CD43, and CD11b) and intracellular uptake of fluorescent M-CSF and CCL2 by flow cytometry. Monocyte populations were FACS sorted and cultured in pro-osteoclastogenic medium for 7 days to evaluate osteoclastogenic potential. Cultures were stained with tartrate-resistant acid phosphatase (TRAP) and mature osteoclasts ($TRAP^+$ and $\geq$3 nuclei) were quantified by light microscopy.

Results: A previously unidentified $CD172a^+$ $CD43^{lo}$ $CD11b^-$ population of monocytes was observed in the bone marrow. These are *bona fide* monocytes, expressing CD115 and CCR2, as determined by uptake of fluorescent M-CSF and CCL2 respectively. Interestingly, this new monocyte population was significantly increased in B27 rats. Assessment of the osteoclastogenic potential of bone marrow monocyte subsets revealed that $CD172a^+$ $CD43^{lo}$ $CD11b^-$, but not $CD172a^+$ $CD43^{lo}$ $CD11b^+$ monocytes, can differentiate into mature osteoclasts. Furthermore, although $CD172a^+$ $CD43^{lo}$ $CD11b^-$ have osteoclastogenic potential, optimal osteoclastogenesis was observed only when all $CD43^{lo}$ ($CD172a^+$ $CD43^{lo}$ $CD11b^-$ & $CD172a^+$ $CD43^{lo}$ $CD11b^+$) were present. No differences in osteoclastogenesis were observed between B27 and controls rats. Finally, evaluation of circulating monocytes demonstrated that all blood monocytes express CD11b and that the $CD43^{lo}CD11b^+$ population was increased in B27 rats. This corresponded with an increase in CCL2 plasma levels in the B27 rats.

Conclusions: We have identified a previously unreported $CD11b^-$ monocyte population ($CD172a^+$ $CD43^{lo}CD11b^-CCR2^+CD115^+$, or Mo-2) in the bone marrow of rats, which can efficiently differentiate into mature osteoclasts. Interestingly, along with the numbers of total $CD43^{lo}$ monocytes, Mo-2 is significantly increased in the bone marrow of rats over-expressing HLA-B27. However, Mo-1 subset ($CD172a^+$ $CD43^{lo}CD11b^+CCR2^+CD115^+$) results increased in blood while decreased in the BM of B27 animals. The increase of circulating Mo-2 may correlate with the high plasma levels of CCL2 in B27 rats. These findings suggest that the HLA-B27-derived inflammation alters the monocyte compartment and in turn may enhance inflammation and bone loss.

Metabolic profiling and control of the Staphylococcal biofilm

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Mass-spectrometry based metabolomics has provided the tools required to study cell metabolism, becoming a fundamental technology used in the understating of microbial metabolism. The *Staphylococcus aureus* metabolic phenotype can change in response to environmental stimuli or growth state of the bacterium, whether planktonic or biofilm forming: the latter attributed to orthopaedic implant failure and antibiotic resistance.
Understanding the differences in metabolism between biofilms and planktonic cells provides crucial insight into the onset of persistent infection, drug modes of action and antibiotic resistance, while highlighting potential novel treatment and prevention strategies.
Here we use novel metabolome extraction procedures, employing a fast, reproducible bead beating method coupled with untargeted liquid-chromatography/orbitrap mass-spectrometry, to provide a snap-shot of the metabolic phenotype at different growth states of the bacterium.
We highlight significant changes in metabolism, specifically arginine and purine metabolism, which an orthopaedic clinically-derived strain of *Staphylococcus aureus* undergoes in its transition to a biofilm state. Results presented highlight the potential of metabolomics to provide new drug targets and areas of research to combat adaptive antimicrobial resistance mechanisms.