Spring meeting April 8th- 9th 2019
University of Liverpool

Joint meeting between BSMB and MBI

"Stroma, Niche, and Repair

Abstracts

Invited Speakers

Name	Title	Time
Peter Friedl	3D tissue and cell jamming transitions in collective cancer progression	8/4/19 1.00-1.30
Michael Schmid	Regulation of liver fibrosis by macrophages	8/4/19 2.00-2.30
Erik Sahai	Generation and maintenance of the cancer-associated fibroblasts	8/4/19 4.00-4.30
Fiona Watt	Cellular heterogeneity in mammalian skin	8/4/19 5.00-5.30
Kenneth Yamada **(BSMB Medal Lecture)**	Extracellular Matrix Dynamics in Cell Migration, Invasion, and Tissue Morphogenesis	8/4/19 5.30-6.15
Catalina Vallejo	Neuroelectrode Functionalisation Through Heparan Sulphate Mimetics	9/4/19 9.00-9.20
Cian O'Leary	Engineering extracellular matrix analogues to model the epithelial Interface in 3D: prospects for developing novel in vitro models and tissue regenerative medical devices	9/4/19 9.30-9.50
Olga Piskareva	Reconstruction of Metastatic Tumour Microenvironment Using Collagen-Based Scaffolds	9/4/19 10.00-10.20
Colin Jahoda	Therapeutic tissue engineering Lessons from developmental biology	9/4/19 11.00-11.30
Michelle Peckham	Understanding the role of MEGF10 in skeletal muscle stem cells	9/4/19 12.00-12.30
Rama Khokha	Metalloprotease inhibitors regulate stem cell niches	9/4/19 2.00-2.30
Tim Johnson	Transglutaminases in tissue scarring and fibrosis	9/4/19 3.00-3.30

3D tissue and cell jamming transitions in collective cancer progression

(Monday 8th April, 1.00-1.30)

Authors

Peter Friedl[1,2]

Affiliations

[1]Department of Cell Biology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, The Netherlands;
[2]The University of Texas MD Anderson Cancer Center, Houston, Texas, USA

Abstract

Plasticity of cancer invasion and metastasis depends on the ability of cancer cells to switch between collective and single cell dissemination, under the control of cadherin mediated cell-cell junctions and the organization of the extracellular matrix. E-cadherin is considered a tumor suppressor, the downregulation of which causes single-cell scattering in 2D environments and enhances distant metastasis. In clinical samples, however, E-cadherin expressing and deficient tumors both invade collectively and metastasize equally, implicating additional mechanisms controlling cell-cell cooperation and dissemination, such as confinement caused by the 3D ECM. Using spatially defined organotypic culture, intravital microscopy in breast cancer in mice and in silico modeling, we here identify cell jamming by 3D tissue boundaries as dominant physical mechanism which supports collective invasion irrespective of the composition and stability of cell-cell junctions. Downregulation of E-cadherin and p120-catenin resulted in collective movement when tissue density was high and physically confining, whereas unjamming transitions followed by single-cell escape depended on locally high tissue space and porosity. These data reveal that steric hinderance by 3D tissue can substitute for cadherin-dependent cell-cell cooperation and dictates cell jamming and unjamming transitions in complex environments.

Regulation of liver fibrosis by macrophages

(Monday 8th April, 2.00-2.30)

Authors:
Michael Schmid

Affiliations:
Molecular and Clinical Cancer Medicine, University of Liverpool, UK

Introduction:

Pancreatic ductal adenocarcinoma (PDA) remains one of the deadliest cancer types worldwide, with a 5-year survival rate of only 6%. Thus, better therapies are urgently needed for this disease. PDA is a highly aggressive metastatic disease which mainly spreads to the liver. The colonisation of the metastatic lesions by disseminated PDA cells is accompanied by a progressive accumulation of immune cells, particularly macrophages, and the formation of an aberrant fibrotic reaction. Recent findings suggest that the formation of this inflammatory – fibrotic microenvironment in the liver plays a key role in PDA metastasis. However, whether and how metastasis associated macrophages affect the observed fibrotic reaction during PDA metastasis remains incompletely understood.

Materials and Methods

To study the role of macrophages in PDA metastasis associated fibrosis we are using a variety of models and cutting-edge technologies including pre-clinical mouse models, patient samples, and omic's based approaches.

Results

In my talk, I will discuss the role of progranulin expressing macrophages (PGeMs) in PDA metastasis. We have found that PDA liver metastasis critically depends on the recruitment of progranulin expressing macrophages (PGeMs) to the liver, resulting in the transactivation of resident hepatic stellate cells into myofibroblasts and the formation of an immunoprotective and growth promoting metastatic tumour microenvironment.

Discussion

I will discuss potential therapeutic approaches to block the formation of a growth promoting microenvironment in PDA liver metastasis.

Cellular heterogeneity in mammalian skin

(Monday April 8th, 5.00-5.30)

Authors
Fiona M. Watt

Affiliations
Centre for Stem Cells and Regenerative Medicine, King's College London

Introduction

Mammalian skin comprises two layers, the epidermis and dermis. Previous studies using mouse models and in vitro culture of human epidermis have shown that there is considerable cellular heterogeneity within the tissue.

Materials and Methods

We have used single cell transcriptomics to explore cellular heterogeneity in human adult skin.

Results

We show that there are at least four distinct fibroblast populations in adult human skin, not all of which are spatially segregated. We define markers permitting their isolation by flow cytometry and show that different fibroblast subpopulations retain distinct functionality following ex vivo expansion. We also find distinct cell populations within the epidermis.

Discussion

These studies are providing new insights into the cellular composition of the skin.

BSMB Medal Lecture

Extracellular Matrix Dynamics in Cell Migration, Invasion, and Tissue Morphogenesis

(Monday 8th April, 5.30-6.15)

Authors
Kenneth M. Yamada

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Abstract
Direct visualization of the dynamic interactions of cells with the extracellular matrix can provide novel insights into complex biological processes. Comparisons of cell migration and invasion using classical two-dimensional culture systems to analyses in the third dimension have confirmed some cell biological mechanisms, but they have also revealed multiple numerous differences in the mechanisms of cell adhesion, migration, and signalling. For example, myosin II often slows migration on 2D substrates associated with focal adhesions. But in 3D matrix environments, it can be required for effective migration, even though the cells display numerous, robust focal adhesions to 3D matrix fibrils. Modes of cell migration in 3D environments can switch between lamellipodial, lobopodial, and/or amoeboid modes of migration depending on the biophysical properties of extracellular matrices. Examples include “nuclear piston’ migration that can be switched off or on by local proteolysis or the induction of proteolytic invadopodia and proteolysis by malignant and normal cells, which can depend on the local matrix environment.

Particularly complex remodelling of both the extracellular matrix and tissues occurs during morphogenesis. ECM can support self-assembly and self-organization of embryonic tissues, but it must also be locally actively remodelled, e.g., for bud expansion and potentially for tissue-to-tissue communication. During branching morphogenesis, surprisingly focal remodelling of the basement membranes occurs during developmental to produce numerous tiny but well-defined perforations with an average area of 1-2 μm^2, resulting in a microscopically porous, flexible basement membrane meshwork. This mesh-like structure can permit tissue expansion. However, the perforations or holes in the basement membrane are usually accompanied by protrusion of a highly active bleb or protrusion extending up to 5 µm from the epithelium through the perforations towards the surrounding mesenchyme. Concurrently, however, the entire basement membrane is surprisingly undergoing translocation in a direction opposite to bud expansion. Underlying this slowly moving 2D basement membrane sheet translocation is highly dynamic individual cell 3D movements. Understanding how these concurrent processes and associated changes in basement membranes and cell signalling are coordinated remains an intriguing challenge. Further applications of live-cell imaging of cell-ECM dynamics are revealing other surprising findings.

Neuroelectrode Functionalisation Through Heparan Sulphate Mimetics

(Tuesday 9th April, 9.00-9.30)

Authors

Catalina Vallejo-Giraldo[1], Idir Ouidja Mohand[2], Minh Bao Huynh[2], Katarzyna Krukiewicz[1], Andrea Flannery[3], Anuradha R Pallipurath[4], Alexandre Trotier[1], Michelle Kilcoyne[3], Eilís Dowd[5], Dulce Papy-Garcia[2], Abhay Pandit[1], Manus Jonathan Paul Biggs[1]

Affiliations

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Introduction

Current strategies in the field of brain-machine interfaces focus on reducing impedance and the presentation of neurotropic moieties to enhance the integration with brain milieu and to help with electrode stability. The use of biochemical functionalisation through conducting polymers is being increasingly utilized to present biologically active dopants to promote or inhibit specific biological interaction with neural tissues. This study investigates electrode modification by entrapping a heparan sulphate mimetic (HM) in a conducting polymer to support the electrode stability and minimal glial scar formation *in vivo*.

Materials and Methods

PEDOT/HM coatings were polymerized galvanostatically on platinum/iridium microelectrodes. The cytocompatibility of the functionalized PEDOT coatings was evaluated in vitro with isolated rat ventral mesencephalic (VM) and quantitative immunostaining of relevant neural biomarkers (β tubulin for neural outgrowth and GFAP for astrocytes). The gliosis modulation and inflammatory response of the functionalized PEDOT coatings were studied by the secretion of pro-inflammatory cytokines and chemokine factors in vivo, as well as through the use of an in-house gliosis antibody microarray.

Results

Overall, the HM was successfully entrapped during electrodeposition and it preserved biofunctionality as assessed by the promotion of VM cell presence and neurite outgrowth on PEDOT/HM functionalized coatings for up to ten days in culture compared to pristine PEDOT coatings. A neurotrophic effect imparted by HM incorporated into the PEDOT polymer coatings is suggested. Further, the modulation of cytokine and chemokine activity in VM populations cultured on PEDOT/HM indicates the protective effect of the HM which may translate to an enhanced availability of neurotropic growth factors.

Engineering extracellular matrix analogues to model the respiratory epithelial interface in 3D: prospects for developing novel *in vitro* models and tissue regenerative medical devices

(Tuesday 9th April, 9.30-9.50)

Authors
Cian O'Leary [1, 2]

Affiliations
[1]School of Pharmacy, Royal College of Surgeons in Ireland (RCSI), Ireland.
[2]Tissue Engineering Research Group, RCSI, Ireland.

Introduction

Today, chronic respiratory disease is one of the leading causes of mortality globally, while major airway trauma has limited treatment options. The respiratory epithelium plays a central role in both issues, either as a significant contributor to aberrant signalling in disease, or as a crucial component of restoring damaged tissue. Engineered extracellular matrix analogues of respiratory tissue with organotypic cells, structure, and functionality can thus provide both novel *in vitro* platforms for disease modelling and drug discovery and also bioengineered constructs for regenerative medicine.

Materials and Methods

Using tissue engineering principles, a bilayered collagen-hyaluronate (CHyA-B) biomaterial scaffold was fabricated as an extracellular matrix analogue of the tracheobronchial region of the respiratory tract. Following scaffold characterisation, a 3D *in vitro* co-culture model of epithelial cells and lung fibroblasts was developed and analysed for markers of functional respiratory epithelium. For regenerative medicine applications, drug-loaded scaffolds and 3D-printed composites have been explored in the development of next generation tracheal implants.

Results

CHyA-B scaffolds were successfully developed and found to support mucociliary and barrier function in a 3D co-culture model. Drug-loaded scaffolds were found to further stimulate transcription and translation of mucociliary markers. 3D-printed composite scaffolds exhibited improved mechanical properties, facilitating the manufacture of tubular constructs with the necessary resistance to collapse in the respiratory tract.

Discussion

Engineered extracellular matrix analogues support, potentially enhance respiratory epithelial culture, and could have future roles as an innovative *in vitro* platform for applications in respiratory drug discovery or as an implant for regenerative applications.

Tissue-engineering concepts to reconstruct tumour microenvironment in neuroblastoma

(Tuesday 9th April, 10.00-10.20)

Authors

Piskareva, O.[1,2,3] Curtin, C.[3-5], Nolan, J.C.[1,2], Conlon, R.[1], Deneweth L.[1], Gallagher, C.[1], Cavanagh, BL[6], Stallings, R.L.[1,2], O'Brien, F.J.[3-5]

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Trinity Centre for Bioengineering, Trinity College Dublin, Dublin, Ireland
Cellular and Molecular Imaging Core, Royal College of Surgeons in Ireland, Dublin

Introduction

Neuroblastoma is a paediatric cancer of the sympathetic nervous system and the most common solid tumour of infancy contributing to 15% of paediatric oncology deaths. Current therapies are not effective in the long term treatment of almost 80% of patients with clinically aggressive disease. The accurate representation of the tumour architecture and patient diversity are two primary challenges in the identification of new agents for paediatric drug development particularly with the limited number of patients eligible for clinical trials.

Materials and Methods

Chemotherapeutic sensitive and resistant neuroblastoma cell lines Kelly/KellyCis83 were cultured in a 3D *in vitro* model on collagen-based scaffolds containing either glycosaminoglycan or nanohydroxyapatite and were compared to 2D cell culture and an orthotopic murine model. The model was characterised by cell viability and proliferation (DNA content), metabolism (Chromogranin A (CgA) secretion), scaffold colonisation (H&E) and fluorescent microscopy (DAPI). Response to cisplatin or miRNA-mediated treatment was assessed in 3D and compared to data obtained in 2D and an orthotopic neuroblastoma murine model.

Results

Both cell lines actively infiltrated the scaffolds and proliferated displaying > 100-fold increased resistance to cisplatin treatment when compared to 2D cultures, exhibiting chemosensitivity similar to orthotopic xenograft *in vivo* models. The efficacy of cellular uptake and gene knockdown by liposomes bearing miR-324-5p was similar in both 2D and 3D *in vitro* culturing models highlighting the proof-of-principle for the applicability of this model for validation of miRNA function.

Discussion

We successfully established and characterised a physiologically relevant, scaffold-based 3D neuroblastoma model, strongly supporting its potential value in the evaluation of

Therapeutic tissue engineering Lessons from developmental biology

(Tuesday 9th April, 11.11.30)

Author
Colin A.B. Jahoda

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Abstract
A key step underpinning embryonic hair follicle morphogenesis is the formation of an aggregation of mesenchymal cells in the skin dermis. This event is one of a series of epithelial-mesenchymal interactions leading to follicle development, with the progeny of the condensation cells eventually constituting the mature dermal papilla and dermal sheath structures. What makes the hair follicle unique is its cyclic regenerative activity in adult skin. This in turn imbues the hair follicle mesenchyme with distinctive properties. These include the capacity to regenerate after experimental amputation without scar tissue, and crucially the ability to induce new hair follicles, not only from skin epidermis, but also in association with other epithelia, including that of the cornea. Interestingly the same cells can also display mesenchymal stem cell-like properties if directed to do so in culture. Stemming from the induction work using animal models there has been keen interest in using this approach to create new hair follicles in human skin. However, a key difference between human papilla cells and those of the animal model is that the latter spontaneously aggregate in a manner that mimics the original embryonic condensation. In collaboration with others, our lab therefore used the simple strategy of forcing cultured human DP cells into 3D spheroids that partially restore the *in vivo* molecular signature of intact papillae as a means of eliciting human follicle induction. Subsequently we have developed a tissue engineered skin equivalent that uses this approach, and 3D printing, to create patterned follicles. We have also developed a double-layered spheroid model of skin that could be used for high throughput testing. This may also represent a useful means of introducing and maintaining cells in discrete skin locations for therapeutic purposes, and of creating protofollicle structures. One current interest is whether hair follicle dermal cells have epigenetic regulation that explains their multifunctional activities *in vivo* and *in vitro.*

Skeletal muscle satellite cell behaviour and the role of MEGF10

(Tuesday 9th April 12.00-12.30)

Authors

Ruth Hughes[1], Louise Richardson[1], Stuart Egginton[1], Colin Johnson[2] & Michelle Peckham[1]

Affiliations

[1]The Faculty of Biological Science and the [2]Faculty of Medicine and Health, University of Leeds, Leeds, LS2 9JT

Introduction

MEGF10 (multiple epidermal growth factor-like domains protein 10) is a membrane receptor protein involved in phagocytosis in macrophages and astrocytes. It contains an N-terminal EMI domain followed by 17 EGF domains, transmembrane and cytoplasmic domain (with NPXY and YXXL motifs). Mutations in MEGF10 cause early-onset myopathy, areflexia, respiratory distress and dysphagia (EMARDD) an autosomal recessive congenital myopathy, in which muscles are reduced in size. While loss of MEGF10 in disease may affect muscle satellite cell proliferation and differentiation, its precise role in muscle satellite cells is unclear.

Materials and Methods

We used a battery of techniques to explore the role of MEGF10, from in vitro culture of myoblasts, to a muscle hyperplasia model, as well as work on the expressed and purified protein.

Results

Cultured myoblasts express very low levels of MEGF10, as demonstrated by western blotting, immunofluorescence and RNAseq. Expression of GFP-tagged MEGF10 in cultured myoblasts reduces their motility and ability to fuse into multinucleated myotubes. In these cells, the majority of the eGFP-tagged protein is found in intracellular vesicles, and not at the membrane. In muscle fibres, MEGF10 is not expressed in PAX7+ quiescent satellite cells, but its expression is upregulated in satellite cells during muscle hyperplasia (in vivo) and in cultured muscle fibres in vitro, as satellite cells become activated and begin to express MyoD. The extracellular domain of MEGF10, expressed and purified from HEK cells, is highly post-translationally modified, supports myoblast attachment to non-adherent surfaces, and inhibits fusion. A shorter construct lacking the N-terminal EMI was less able to support attachment but did not affect fusion.

Discussion

Despite intensive effort, it is still not clear what the precise role of MEGF10 is in skeletal muscle satellite cells. However, it does appear to play a role in myoblast adhesion and high levels appear to block fusion. It may help to regulate when fusion occurs, with its loss

Skeletal and Cartilage Homeostasis Relies on Natural Metalloprotease Inhibitors

(Tuesday 9th April, 3.00-3.30)

Authors
Rama Khokha & Sanjay Saw,

Affiliations
Princess Margaret Cancer Centre, Toronto, Canada

Abstract
The four endogenous tissue inhibitors of metalloproteinases (TIMPs) regulate the pericellular proteolysis of a vast range of matrix and cell surface proteins, generating simultaneous effects on tissue architecture and cell signalling. We have recently summarized the hierarchy of the relationships between TIMPs, metalloproteinases, their plethora of substrates, as well as cellular processes that underlie cancer hallmarks[1]. Strong trends of specific TIMP alterations have been noted in multiple human cancers. Yet, the fundamental role of TIMPs in mammalian biology is still incompletely understood. To dissect the redundancy of TIMP function in tissue homeostasis and cancer biology, we have developed combinatorial knockouts of *Timp* genes and generated the TIMPless mice. Whole body quadruple knockout mice display multiple defects in the skeletal system, such as early growth plate closure, widespread chondrodysplasia and low bone mass[2]. Additionally, these mice have abnormal isometric scaling resulting in disproportionate bone segments. We have now uncovered novel molecular relationships between TIMPs and key signaling pathways in bone development, as well as between TIMPs and metalloproteases-sensitive proteoglycans in cartilage homeostasis.

References

1. Jackson et al, *TIMPs: versatile extracellular regulators in cancer.* Nature Reviews Cancer, 17: 38-53, 2017
2. Chen et al, *TIMP loss activates metalloproteinase-TNFα-DKK1 axis to compromise Wnt signaling and bone mass*. J Bone Miner Res, 34: 182-194, 2019.

Submitted Abstracts

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2	Prognostic Significance of Soluble and ECM associated VEGFA isoforms in High-Grade Serous Ovarian Cancer	***BL Agüero***
3	Patient-specific extracellular matrix-based 3-dimensional cultures are superior to 2-dimensional cultures conditions to model colorectal cancer and liver metastasis	***E D'Angelo***
4	In vivo SILAC labeling uncovers age and type dependent incorporation of extracellular matrix proteins in collagen rich tissues.	***Y Ariosa-Morejon***
5	Defining the Role and Mechanism of microRNAs in Osteoarthritis	***P Balaskas***
6	The effect of nerve growth factor on chondrocyte mechanosensing	***JJ Bara***
7	Preserved Neural Extracellular Matrix in Decellularised Human Femoral Nerve: Towards Developing a New Allograft for Peripheral Nerve Injury in the UK	***V Barrera***
8*	Biomimetic modelling of the lamina cribrosa region using tissue engineered scaffolds – a novel 3D model for glaucoma research.	***D Brennan***
9	Quasi-static loading inhibits endochondral ossification in ex vivo murine metatarsal culture	***S Caetano-Silva***
10	VPS33b is essential for collagen homeostasis	***J Chang***
11	Novel target discovery of miR140 targets in human articular chondrocytes	***N Chaudhry***
12	Simulating and modulating skin fibrosis in vitro: Multi-compartment collagen devices as multi drug delivery vehicles for fibrosis	***J Coentro***
13	Creating bespoke functionalised peptide gels to model the brain tumour microenvironment	***J Curd***
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16	Investigating glycosaminoglycan developmental diseases using fully defined 3D cell culture environments and human pluripotent stem cells	***K Dowding***
17	Interactions of silver nanoparticles with 2D and 3D human skin models	***R Deller[1]***
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19	Mechanoregulation of adipogenesis in orbital fibroblasts through Src family kinases	***V Eglitis***
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21*	Unravelling how bi-directional cell-ECM interactions direct stem cell fate in 3D	***E Gentleman***
22	The cartilage age-CpG cg23500537 represses target gene expression of PCDHB1 and shows changes with ageing in a tissue specific manner	***RC Fulea***
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30	Comparison of RNA Extraction Methods in Equine Synovial Fluid in the determination of the expression of Small Non-Coding RNAs	***YA Kharaz***
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49*	The regulation of DDR1 catalysis by its intracellular juxtamembrane region	***D Sammon***
50	The miRNA miR-21a-5p targets SMAD7, and its inhibition decreases lung fibrosis in the mouse.	***M. Scotto di Mase***
51*	Raman spectroscopy and second harmonic generation imaging reveal sexually dimorphic influence of osteoblast-derived VEGF on bone mineral and matrix composition.	***A Sharma***
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53	Laminin β1 with C-terminal Dendra2 fluorescent protein tag is inefficiently secreted from lung adenocarcinoma cells in culture	***L Shaw***
54	The articular cartilage proteome is dependent on zone, age and disease state	***A Smagul***
55	Development and validation of an inducible LaNt α31 overexpressing mouse model	***CJ Sugden***
56	Differential impact of matrix stiffness on adhesion, proliferation and differentiation potential of osteogenic and myogenic progenitor cells	***B Świerczek-Lasek***
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59*	LaNt α31 influences cell adhesion and migration through modulation of laminin organisation and hemidesmosome maturation	***LD Troughton***
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* indicates podium presentation

#1 Designing *in vitro* skin fibrosis model for testing the biological effects of anti-fibrotic molecules

Authors
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Introduction

Skin fibrosis is one of the common fibro-contractive diseases, characterized by the persistence of myofibroblasts, generated from the resident fibroblasts[1,2] in response to certain triggering molecules, especially TGF-β1[3]. In the fibrotic lesions, the myofibroblasts show characteristic *de novo* expression of □-Smooth Muscle Actin (□-SMA), in combination with interruption of the balance between collagen production and degradation[4,5]. Engineering 3D cultures that incorporate ECM and embedded cells provide interesting means to model more mature tissues and can be employed for testing different drugs *in vitro*. The anti-fibrotic molecules are designed for interfering with certain cellular or mechanical factors that control the formation of myofibroblasts. For instance, TD-139 is a recently developed clinical candidate with potent inhibitory effects on galectin-3, one of the main factors involved in the recruitment and activation of myofibroblasts[6].

Materials and Methods

A cell/hydrogel construct-based skin fibrosis model was fabricated: primary skin fibroblasts were seeded in collagen hydrogel with medium stiffness, and induced by TGF-β1 to change their phenotype into differentiated myofibroblasts, characterized by the expression of □-SMA, which was tested by Western blotting.

Results and Discussion

The different parameters for maintaining the viability of the distributed cells inside the hydrogels including the cell seeding density, method of loading, the period of culture in FBS-containing/free medium and the conditions for the fibrosis induction were assessed and optimized. Moreover, the conditions and parameters for the cell viability assays over the time course of the 3-D culture were pre-validated and qualified. These included the Alamar Blue assay, Picogreen DNA quantification assay, and the Live/Dead viability testing using ethidium homodimer-1 and calcein AM along with the protein extraction conditions for optimum quantity of isolated protein suitable for the Western blotting

For testing the applicability of the designed model for the *in vitro* testing of anti-fibrotic molecules, the cytotoxicity of seven different concentrations of TD-139 was tested, followed by an assessment of the efficiency of chosen three concentrations on eliminating the change in phenotype of fibroblasts to myofibroblasts based on the disappearance of □-SMA expression. This proves the future applicability of the designed model for evaluating the anti-fibrotic efficiency of new molecules.

Acknowledgements

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References

1. Luzina I.G., Atamas S.P. (2008) Fibrotic Skin Diseases. In: Gaspari A.A., Tyring S.K. (eds) Clinical and Basic Immunodermatology. Springer, London.
2. Rinkevich Y, Walmsley GG, Hu MS, et al.: Skin fibrosis. Identification and isolation of a dermal lineage with intrinsic fibrogenic potential. Science. 2015; 348(6232): aaa2151.
3. Bochaton-Piallat ML, Gabbiani G and Hinz B. The myofibroblast in wound healing and fibrosis: answered and unanswered questions. F1000Research 2016, 5(F1000 Faculty Rev):752.
4. Hinz B, Phan SH, Thannickal VJ, et al.: Recent developments in myofibroblasts biology: paradigms for connective tissue remodeling. Am J Pathol. 2012; 180(4): 1340–55.
5. Roberts AB, Sporn MB, Assoian RK, Smith J, Roche NS, Wakefield LM, Heine UI, Liotta LA, Falanga V, Kehrl JH, Fauci AS: Transforming growth factor type: rapid induction of fibrosis and angiogenesis *in vivo* and stimulation of collagen formation *in vitro*. Proc Natl Acad Sci USA 1986, 83:4167–4171.
6. Hirani N, Mackinnon A, Nicol L, Walker J, Ford P, Schambye H, Pederson A, Nilsson U, Leffler H, Thomas T, Francombe D, Simpson J , Gibbons M, Maher T. TD139, A Novel Inhaled Galectin-3 Inhibitor for the Treatment of Idiopathic Pulmonary Fibrosis (IPF). Results from the First in (IPF) Patients Study. American Journal of Respiratory and Critical Care Medicine 2017;195:A7560.

#2 Prognostic Significance of Soluble and ECM associated VEGFA isoforms in High-Grade Serous Ovarian Cancer

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Introduction

High Grade Serous ovarian cancer (HGSOC) is associated with high rates of mortality in women. Anti-VEGFA therapy (bevacizumab), in combination with chemotherapy, has shown promising results. However, not all patients benefit and side effects can be severe. VEGFA is expressed as a number of isoforms that vary in their affinity for the extracellular matrix (ECM). Preclinical data has suggested measurement of the short, soluble isoform $VEGFA_{121}$ could be a prognostic and predictive biomarker. Measurement of plasma VEGFA using an ELISA selective for $VEGFA_{121}$ failed to stratify patients for treatment, in part because plasma measurements do not reflect the quantity and variety of VEGFA isoforms present in the tumour. In light of these results, we hypothesised that high levels of $VEGFA_{121}$ expression, as measured within the tumour microenvironment may promote HGSOC disease progression and increased sensitivity to bevacizumab.

Materials and Methods

The Cancer Genome Atlas (TCGA) OV RNAseq expression data was used to identify HGSOC patients with increased expression of $VEGFA_{121}$ using isoform read counts and classification into clusters based on relative expression of $VEGFA_{121}$, $VEGFA_{165}$ and $VEGFA_{189}$ mRNA (Zscore of RSEM isoform percentage). Associations between isoform expression levels and survival were investigated using Kaplan-Meier analysis. Differential gene expression analysis was used to identify underlying mechanisms associated with VEGFA isoform expression and survival.

Results

A total of 221 patients with stage IIIC HGSOC were segregated based on VEGFA isoform expression levels. Patients with high levels of $VEGFA_{121}$ showed decreased overall survival compared to patients with high levels of ECM bound isoforms. Differential gene expression analysis showed high $VEGFA_{121}$ expression is linked to increased lymphocyte infiltration, metastasis and altered metabolism.

Discussion

Our data supports our hypothesis that high levels of the soluble isoforms of VEGFA in HGSOC tumours are associated with decreased survival, correlating with existing clinical and pre-clinical data. Differential gene expression analysis suggests women with HGSOC and increased $VEGFA_{121}$ expression have increased lymphocyte infiltration and gene expression associated with metastasis. We will now validate our findings in an independent clinical cohort and investigate response to anti-VEGFA therapy in an immune-competent murine ovarian cancer model expressing increased $VEGFA_{120}$.

#3 Patient-specific extracellular matrix-based 3-dimensional cultures are superior to 2-dimensional cultures conditions to model colorectal cancer and liver metastasis

Authors
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Introduction
Colorectal cancer (CRC) is the third most common cancer worldwide. The liver is the most common site of CRC metastasis and conventional culture models do not mimic the complex metastatic microenvironment. Here we developed a 3D model of CRC-liver metastasis (CRLM) using patient-specific decellularized extracellular matrices to investigate cell-matrix interaction and drug cytotoxicity.

Materials and Methods
Decellularization of patient-derived samples of healthy colon, CRC, healthy liver and CRLM was performed with a detergent-enzymatic process. Decellularized scaffolds were seeded with CRC cell line HT-29 transfected to express luciferase. Cell proliferation and migration were quantified with bioluminescence for up to 10 days of culture. Immunofluorescence analysis were performed for Ki67, Caspase3, E-cadherin and Vimentin.

Results
Decellularization preserved tissue-specific biological and ultrastructural properties of CRC and CRLM extracellular-matrix. The 3D model supported significantly higher cell proliferation and migration in seeded tumour-derived scaffolds compared to matched healthy tissues. Cells cultured in the 3D environment displayed significantly different gene expression profile in respect to 2D cultures, which together with the loss of E-cadherin and increased expression of Vimentin, suggested clear activation of epithelial-mesenchymal transition in CRLM seeded scaffolds. HT-29 cells grown in CRLM scaffolds were resistant to IC_{50} 5-Fluoruracil calculated in conventional 2D cultures.

Discussion
The 3D culture model developed with cell-seeded patient-derived decellularized ECM better recapitulates tissue-specific microenvironmental features compared to 2D culture conditions, and represents a relevant model for the study of CRLM.

#4* In vivo SILAC labeling uncovers age and type dependent incorporation of extracellular matrix proteins in collagen rich tissues.

(Podium Presentation: Tues 9th April, 11.50)

Authors
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Introduction

Major fibrillar collagens are regarded as highly stable proteins, while proteoglycans are known to maintain turnover throughout life. But, less is known about minor collagen turnover. Many studies have shown upregulation of fibrillar collagen mRNA levels in response to injury or disease, e.g. in Osteoarthritis. Other studies have suggested negligible or little incorporation into tissues after skeletal maturity, regardless of disease status. Here we use *in vivo* pulsed SILAC with quantitative proteomics to unravel the incorporation of new proteins into skin, cartilage and bone across the life course of the animal.

Materials and Methods

Four groups of four C57Bl6 male mice were fed with a stable isotope diet (SILAC) , $^{13}C_6$-Lysine (heavy) or control $^{12}C_6$-Lysine (light) for three weeks at different ages spanning the life course (skeletally 'immature', 'mature' and 'aged'). Two groups (A and B) of skeletally immature mice were fed with the heavy diet for 3 weeks, one of them was culled and the other was changed to the light diet for a further 3 weeks. Groups C (skeletally mature) and D (aged mice) were fed the heavy diet only for 3 weeks. At the end of every labeling scheme, plasma, knee articular cartilage, tibial trabecular bone and ventral skin were collected and analysed by HPLC-MS/MS.

Results

We determined protein turnover rates in the four tissues in skeletally immature animals. For each tissue a range of slow and fast turnover proteins were identified. Plasma proteins showed the highest turnover rates and cartilage the lowest. After skeletal maturity, both collagens and proteoglycans showed differential incorporation depending on animal age and protein. Fibrillar collagen synthesis was markedly reduced, especially in cartilage. The incorporation of $^{13}C_6$Lysine into proteins indicated new collagen synthesis for 14 different collagens in cartilage, 9 in bone and 7 in skin. New proteoglycan synthesis was detected for 17 proteoglycans in cartilage, 10 in bone and 7 in skin.

Discussion

Metabolic labelling combined with quantitative proteomics have proved to be a powerful approach to trace individual protein turnover in live animals. Our results agree with previous studies that showed negligible fibrillar collagen incorporation into aged articular cartilage, and also revealed higher incorporation in some non-fibrillar collagens. Using this approach, protein dynamics can be explored at the proteome scale during disease and potential tissue repair.

#5 Defining the Role and Mechanism of microRNAs in Osteoarthritis

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Introduction

Knee osteoarthritis (OA) is one of the most common age-related joint diseases in humans, characterized by articular cartilage degeneration and joint inflammation. Treatment is only symptomatic and most patients with late-stage OA undergo knee replacement surgery. MicroRNAs (miRNAs) are a class of small non-coding RNAs which function at the post-transcriptional level as important regulators of gene expression. There is increasing evidence of a role for miRNAs in cartilage ageing and OA. In this study we undertook a microarray analysis approach to determine alterations in the miRNA expression profile between young and old OA human knee cartilage and investigate how these changes can contribute to the pathogenesis of OA. This study could uncover novel molecular players and lead to potential therapeutic targets in OA.

Materials and Methods

Femoral articular cartilage was collected from the knee of young patients (n=9, 24±3.8 years) undergoing anterior cruciate ligament repair surgery and old OA patients (n=10, 63±7.3 years) undergoing total knee replacement, at Maastricht University Medical Centre under appropriate ethical approval. For the old OA group two samples were collected per patient; one cartilage sample from an area that was less affected, representing early-stage OA and one sample from an area that was heavily affected, representing late-stage OA. OA severity was confirmed histologically by two independent scorers using a modified Mankin's scoring system. For microarray analysis, total RNA was extracted and hybridized to Affymetrix GeneChip 125 miRNA 4.0 arrays. MiRNA log fold change (logFC) values were adjusted for multiple testing and significantly differentially expressed (DE) miRNAs were identified. Selected DE miRNAs were validated through quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) analysis in the same samples (dependent cohort) as well as in an independent cohort of cartilage samples (n=8-10 per group). Treatment of human articular chondrocytes with interleukin 1 beta (IL-1b) confirmed differential expression of selected miRNAs in an OA-like in-vitro model.

Results

Mankin's histological score showed a significant difference between early and late-stage OA samples (mean±SD; Early-OA: 2.6±1.3; Late-OA: 4.8±2.6). Microarray analysis revealed 484 DE miRNAs in young vs early-stage OA and 318 DE miRNAs in young vs late-stage OA. In contrast to histological findings, early and late-state OA groups showed a similar set of DE miRNAs when compared to young group, suggesting there is a similar miRNA expression profile in different stages of OA progression. MiRNAs with logFC > 4 and predicted significance in OA were selected for further validation. Among these, miRNA-361-5p, -379-5p, -107 and -143-3p were significantly decreased in OA cartilage compared to young cartilage. QRT-PCR analysis confirmed reduced expression of these miRNAs, both in dependent and independent cohorts of cartilage samples, validating microarray results. Finally, treatment of human articular chondrocytes with IL-1b decreased the expression of the selected miRNAs in the treated group compared to control, suggesting a potentially important role of these miRNAs in OA.

Discussion

Using microarray technology we identified several DE miRNAs, between young and old OA human cartilage of different disease stages. Early and late-stage OA showed a similar pattern of DE miRNAs indicating that dysregulation of important miRNAs in cartilage seems to be an early event during OA pathogenesis that precedes major histological changes. Differential expression of selected miRNAs was validated in an independent cohort of cartilage tissue samples as well as in an OA-like in-vitro model suggesting a potentially important role for these miRNAs in knee OA.

#6 The effect of nerve growth factor on chondrocyte mechanosensing

Authors
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Introduction

Nerve growth factor (NGF) and its high (trkA) and low affinity (p75) receptors are upregulated by chondrocytes in osteoarthritis; however, little is known regarding the function of NGF signaling in cartilage. In other cell types, NGF signaling is known to effect mechanosensing by modulation of ion channel activity. Our lab has previously identified the TRPV4, Piezo1, and Piezo2 ion channels as mechanosensors in cartilage. In this study we investigated the effect of NGF treatment on mechanosensitive ion channel activity in articular chondrocytes.

Materials and Methods

Articular chondrocytes and cartilage explants were harvested from the femoral condyles of 3-6 month old pigs. Chondrocytes were cultured in monolayer for 24hours with the addition of NGF protein. Piezo1 ion channel activity was quantitatively assessed by fluorometric calcium imaging following addition of the synthetic agonist/gating modifier Yoda1. In parallel cultures of chondrocytes and cartilage explants, expression changes in NGF family members, mechanosensitive ion channels, and matrix-modifying genes were assessed by qPCR.

Results

Calcium flux in articular chondrocytes was effectively induced by Yoda1 in a dose-dependent manner. Chelation and Ca2+ ATPase inhibitor experiments revealed this response was largely due to intracellular calcium flux. Pre-culture with NGF enhanced both the total and peak calcium flux induced by Yoda1. NGF treatment resulted in modest upregulation of mechanosensitive ion channels Piezo1 ($p<0.05$), Piezo2 and TRPV4 ($P<0.05$). In addition, MMP13 and PTGS2 were upregulated by cells cultured in the presence of NGF.

Discussion

Our findings suggest that NGF enhances mechanosensitivity in chondrocytes by modulation of ion channel activity. In vivo, this phenomenon may influence how chondrocytes respond to injurious strain, which may be either protective or pathological. In addition to being a pain target in the joint, NGF signalling may elicit diverse functions in maintaining joint health, following injury or in OA.

#7 Preserved Neural Extracellular Matrix in Decellularised Human Femoral Nerve: Towards Developing a New Allograft for Peripheral Nerve Injury in the UK

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Introduction
The development of a high-quality allograft for peripheral nerve repair following trauma is a high priority for NHS Blood and Transplant, as current options (autografts, allografts from US or synthetic conduits) present important limitations. The aim of the study was to develop a decellularised human nerve allograft, and to characterise it for effective removal of residual DNA and biomechanical strength whilst retaining its extracellular matrix.

Materials and Methods
Twelve femoral bundles were retrieved from 6 cadaveric donors within 48 hrs of death (age range: 20-66 years old; 1:1 male to female) and femoral nerves were dissected out. One nerve segment (min 6– max 25cm) from each donor was decellularised by a series of hypotonic, mild detergent, nuclease and hypertonic steps over a total of 5 days at 37-42°C in a closed system (CryoMACS). Histological analyses were performed on native versus decellularised nerve biopsies: Van Gieson, Sirius Red, DAPI, Fluoromyelin®, anti-pan Laminin, anti-fibronectin and anti-Collagen IV immunostaining. Absolute amount of residual double stranded DNA was measured by a PicoGreen assay on dried tissue; biomechanical testing was performed by using a uniaxial pull-to-break assay on a Lloyds universal tester (100N load cell). In addition, decellularised nerve segments (~2 cm, n=6) were cultured with primary rat Dorsal Root Ganglia (DRG) neurons to test the ability of the decellularised tissue to promote axon growth.

Results
Human femoral nerves were successfully decellularised, with minimal residual DNA (mean ± SD: 2.44 ng/mg ± 1.62; $p < 0.001$). Decellularised nerves retained native histioarchitecture and important basal lamina components, including collagen type IV and laminin. Biomechanical properties of the nerve were not significantly affected (mean load at failure ± SD: 9.32N ± 8.7 vs 12.2N ± 7 in native vs decellularised nerves; $p>0.05$). Neurons cultured on an acellular nerve scaffold remained viable, and progressive neurite extension was observed up to day 6.

Discussion
We obtained a decellularised human femoral nerve allograft with neural ECM components preserved, with clinical potential to promote axon regeneration in a transplanted patient. This study will ultimately lead on a clinical evaluation of decellularised human nerves in the UK to repair peripheral nerve injury.

#8* Biomimetic modelling of the lamina cribrosa region using tissue engineered scaffolds – a novel 3D model for glaucoma research.

(Podium Presentation: Tues 9th April, 9.20)

Authors

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Introduction

Fibrotic remodelling and loss of compliance (increased stiffness) are characteristic hallmarks of glaucomatous tissue change in the lamina cribrosa (LC) region of the optic nerve head. Mediating these glaucoma-related changes at the cellular level, are resident fibroblast-like LC cells. To accurately study the pro-fibrotic activity of LC cells, requires an appropriate cell culture model system to replicate the complex glaucoma tissue microenvironment. Thus, the purpose of this study was to create a novel, 3D *in-vitro* model of the stiffened glaucomatous LC utilizing bioengineered scaffolds.

Materials and Methods

A suspension of 84% collagen type I, 7% chondroitin-6-sulphate and 9% elastin in 0.05M acetic acid was freeze-dried to fabricate LC scaffolds (porosity 80-100μm). Scaffolds were chemically crosslinked to produce 'normal' and glaucomatous 'stiff' scaffold compliance reference values. Normal primary human LC cells were seeded onto scaffolds at a density of 3500cells/mm^2 and cultured for 5 days (n=2 biological replicates, n=11 scaffolds/group) which were further processed for microstructural assessment via micro-CT scanning and histological evaluation.

Results

The microporous 3D appearance of fabricated LC scaffolds was comparable in both 'normal' and 'stiff' groups. Micro-CT scanning provided a representative overview of the mesh-like structure of the bioengineered scaffolds. Histological analysis using eosin staining and scanning electron microscopy confirmed fabricated scaffolds had a porous ultrastructure throughout. The collagen type I composition, confirmed using picrosirius red staining with birefringence, was abundantly found throughout the scaffold structure. The elastin content was validated with immunofluorescence, and was localized to the scaffold beams using Verhoff-van Gieson stain. Analysis of primary human LC cell-seeded scaffolds showed similar successful infiltration and adherence of LC cells within both the 'normal' and 'stiff' scaffolds groups.

Discussion

Novel 3D biomimetic scaffolds were developed to have similar structure and composition to the native LC. Primary human LC cells have successfully been grown for the first time within these glaucomatous 'stiff' and 'normal' 3D scaffolds. This new *in-vitro* model of glaucoma has significant potential for deciphering the underlying fibrotic response of LC cells to stiffened glaucomatous conditions.

#9 Quasi-static loading inhibits endochondral ossification in *ex vivo* murine metatarsal culture

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Introduction
Osteoarthritis (OA), a chronic disease characterised by articular cartilage degradation, osteophyte formation and subchondral sclerosis, is the most common cause of pain and disability in adults. Its pathophysiology remains unresolved, however recent data indicate that the mammalian target of rapamycin (mTOR) and NF-κB pathways can regulate cartilage-to-bone transition recapitulated in OA. The aim of the current study is to determine whether regulators of mTOR and NF-κB signalling pathways interact with mechanical factors to control the endochondral ossification (EO) underpinning cartilage-to-bone transition.

Methods
E17 metatarsals from C57BL/6 mice were collected and cultured for 2 weeks. Treatment to modify mTOR and NF-κB signalling started at day 1 of culture with selective inhibitors and activators of either mTOR (rapamycin (100 nM), leucine (10 mM)) or NF-κB (betulinic acid (2.5 μM) and SC-514 (20 μM)). Metatarsi were also cultured in the presence of quasi-static mechanical loads by maintenance in hydrogel. Total, cartilaginous zone and mineralised tissue zone lengths were measured. Data were analysed using a linear mixed effect model.

Results
Metatarsi under control conditions showed ~2.5 fold expansion in their total lengths. Treatment with inhibitors/activators of mTOR and NF-κB did not alter length. The most significant modification was achieved when metatarsi were cultured in hydrogel, where total longitudinal growth was almost completely arrested; metatarsal length (and mineralised and cartilage tissue length) were not different between days 0 to 14 under these quasi-static mechanical loading conditions. Intriguingly, culture under such hydrogel-related, quasi-static loading conditions, revealed a growth-promoting influence for both activators and inhibitors of the mTOR or NF-κB pathways, which all tend to reverse the growth restricting effects of culture in the presence of hydrogel.

Discussion
These data reveal interaction between mechanical factors and the contribution of mTOR and NF-κB signalling pathways in the control of the EO. They suggest that the role for these pathways in OA development requires interpretation in the context of the modifications in mechanical loading conditions that underpin pathological bone-to-cartilage transition. These results show the potential to modify loading conditions in embryonic metatarsals that will allow the mechanical control of EO to be studied in isolation of systemic influences *ex vivo*.

#10 VPS33b is essential for collagen homeostasis

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Abstract
Understanding how ECM synthesis and turnover are balanced is key to developing new therapeutic strategies to treat ECM-driven maladies including fibrosis, musculoskeletal disease, and cancer. We recently showed that collagen-I synthesis is coordinated by the circadian clock, through the circadian-controlled expression of all nodes in the secretion pathway (1). However, how rhythmic synthesis is balanced by degradation was not known.
We found that vacuolar protein sorting (VPS) family member VPS33b is circadian clock rhythmic in tendons. VPS33b has been implicated in post-Golgi sorting of protein cargoes in the endocytic pathway. CRISPR-Cas9 knockout of VPS33b (VPSko) inhibited collagen fibril formation and dampened the circadian rhythm. Conversely, over-expression of VPS33b (VPSoe) led to increased collagen fibrils and enhanced circadian rhythm. Surprisingly, in VPSko cells, the loss of circadian rhythm and collagen fibril formation was not a result of ER-stress (2); indeed, ER-stress was alleviated in VPSko cells, potentially as a result of reduced transcription of both collagen and fibronectin. Treatment with Dyngo-4a, an endocytosis inhibitor, reduced collagen secretion as well as collagen and fibronectin transcript levels, indicating an endocytosis-dependent feedback mechanism, without impacting on the ER stress response. In VPSoe cells however, enhanced collagen fibril deposition was not accompanied by enhanced transcription, suggesting that VPS33b post-translationally controls collagen secretion. We are currently investigating whether VPS33b-induced increase in collagen deposition is due to increased secretion or turnover of pre-existing collagen, and how collagen turnover controls its own synthesis.
In conclusion, we propose that VPS33b is part of a matrix sensing mechanism that can couple and uncouple matrix recycling from degradation, via regulation of the endocytic pathway.
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1. *Pickard, A., Chang, J., Alachkar, N., Calverley, C., Garva, R., Arvan, P., Meng, Q. J., and Kadler, K. E. (2018). Protection of circadian rhythms by the protein folding chaperone, BiP. BioRxiv.* https://doi.org/10.1101/348078
2. Yeung, C-Y. C., Garva, R., Pickard, A., Chang, J., Holmes, D. F., Lu, Y., Mallikarjun, V., Swift, J., Adamson, A., Calverley, B., Meng, Q. J. and Kadler, K. E. (2018). Circadian clock regulation of the secretory pathway. BioRxiv. https://doi.org/10.1101/304014

#11 Novel target discovery of miR140 targets in human articular chondrocytes

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Introduction

Micro RNAs (miRNAs) are small, non-coding, negative regulators of genes. They play a major role in a wide variety of biological and pathological processes (1). Specifically, miR140, a chondrocyte-specific miRNA is required for normal cartilage development as determined with miR140 KO mice (2). Here we describe a CRISPR-Cas9 system to identify functional miR140 targets in isolated primary human articular OA chondrocytes. CRISPR-Cas9 editing offers a highly target specific and stable method for exploring functional miRNA targets without perturbing other miRNA levels.

Material & Methods

Guide RNAs (sgRNA) were designed using ATUM software (3) and transfected with purified Cas9 ribonucleoprotein (RNP) to primary human OA chondrocytes using Lipofectamine. To increase efficiency of editing, human chondrocytes were double-transfected and gDNA isolated to determine the gene editing efficiency using the T7 Endonuclease 1 (T7E1) assay. Isolated RNA was used for qPCR expresion analysis.
We screened a total of 48 genes by a TaqMan MicroFluidic card, which included validated miR140 targets (eg. *SEPT2, ATS5, FGF2*) as well as genes with defined roles in chondrocyte biology arrising from our group (eg. AGRN, retinoid acid pathway genes, primary cilium genes).

Results

A stable knockdown of miR140 expression (>90%) was achieved in 5 individual donors. Of the 48 genes three were significant after adjusting for multiple testing. These included *SEPT2*, *BMP2* and *RARG*. Of the top 9 targets (ranked according to their P value), 5 of these were also validated miR140 targets in a RNA-Seq validation of costal cartilage in 7 day old miR140-KO mice.

Discussion

We describe a method of double transfection using CRISPR-Cas9 to produce efficient and rapid genetically engineered primary human articular chondrocytes without prior need of clonal selection. We identified several miR140 targets of which *SEPT2, BMP2* and *FGF2* were known targets from the literature. *ATS5* was not a miR140 target in our experiment. A number of novel targets were identified including *AGRN*, a gene encoding for a heparan sulfate proteoglycan with chondrogenic potential as well as two members of the retinoic acid pathway, *RARG* and *CYP26B1* and finally two members of the primary cilium family, *IFT88* and *TTBK2*.
Taken together, our data does not support a direct role for miR140 in the control of matrix turnover but appears to suggest a role in control of anabolic tissue responses.

#12 Simulating and modulating skin fibrosis *in vitro*: Multi-compartment collagen devices as multi drug delivery vehicles for fibrosis

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Introduction
Fibrosis is a phenomenon characterised by the formation of excessive fibrous connective tissue, which can lead to the alteration of the skin's architecture and function, affecting millions of patients worldwide. In this study it was hypothesized that multi-compartment crosslinked collagen type I systems can modulate skin fibrosis *in vitro* through the controlled synergistic dual delivery of different anti-fibrotics.

Materials and Methods
Cell culture media was supplemented with 100 µg/ml Dextran Sulphate 500 kDa, 100µM L-Ascorbic acid 2-phosphate sesquimagnesium salt hydrate and 5 ng/ml TGF-β1 in order to recreate a fibrotic condition *in vitro*.
Multi-compartment collagen-based systems were made by mixing dialyzed type I collagen with 10x PBS, followed by neutralisation and crosslinking with 1 and 2.0 mM 4 arm-succinimidyl glutarate ester PEG, respectively, and incubated at 37ºC. The release of encapsulated drugs from the hydrogels was studied by fluorimetry and the effect of the delivered bioactive agents was assessed through imaging and quantification for fibrotic markers in a macromolecular crowding induced in vitro model.

Results
Macromolecular crowding and TGF-β1 supplementation resulted in a significant increase of collagen I deposition and α-SMA expression.
A pilot study using FITC-dextran proved that the inner compartment was capable of promoting a sustained release over a long period of time (7 days), fitting the intended therapeutic release profile.
Protein expression studies in an *in vitro* model of skin fibrosis showed a decrease of endogenous collagen type I and α-smooth muscle actin expression indicating amelioration of fibrosis.

Discussion
In summary, this indicates that this model is adequate to simulate a fibrotic condition *in vitro* and that the delivery system developed is suitable for the delivery of multiple bioactive agents, resulting in a controlled release *in vitro* and illustrating its potential in therapy.

#13 Creating bespoke functionalised peptide gels to model the brain tumour microenvironment

Authors
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Introduction
Current *in vitro* models used to study children's brain cancer typically fail to recapitulate the complexity of the brain tumour microenvironment. 2D monolayer cultures, or 3D models containing animal-derived products, unreliably represent human extracellular matrices (ECM), and raise ethical questions of using animal-derived products in research. Therefore, we aim to optimise a synthetic peptide gel model which can be selectively functionalised with various ECM components to mimic specific tumour microenvironments.

Materials and Methods
The self-assembling FEFEFKFK gelator octapeptide has been used to encapsulate a variety of cell types with excellent viability. Functionalisation of the gel can be achieved by entrapment of soluble ECM components during cell encapsulation or by covalent modification of the gelator peptide. Mouse embryonic stem (mES) cells have been used as a tool for detecting the biological impact of using specific glycosaminoglycans to functionalise the gels.

Results
mES cells grown in the peptide gels can be induced to differentiate in serum free culture. We have previously demonstrated that the glycosaminoglycan heparan sulphate (HS) is essential for mES cell differentiation to the neural lineage. Using mES cells deficient in HS synthesis we can probe the ability of exogenous HS, added in to the gel, to support differentiation. This provides a sensitive and relevant read-out for functionalisation with biologically active ECM components.

Discussion
Initial results demonstrate that cell types relevant to childhood brain tumours can be cultured in the peptide gels. Functionalisation of the gel was found to drive characteristic changes in the behaviour of at least one cell type. We have constructed a test environment to evaluate the biological effect of modifications to the hydrogel. Our aim is to develop a model that recapitulates the brain tumour microenvironment to improve upon current models, without the need for animal-derived products and their associated variability.

#14* Proresolving mediators counter inflammation in stromal cells from patients with Achilles tendon disease

(Podium Presentation: Tues 9th April, 11.40)

Authors

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Introduction

Achilles tendon disorders including tendinopathy and rupture cause pain and disability in athletes and non-athletic individuals. Growing evidence supports the contribution of inflammation to the onset and progression of these injuries, however the processes underpinning resolution of Achilles tendon inflammation are poorly understood.

Materials and Methods

We investigated the bioactive lipid mediator profiles of tendon-derived stromal cells isolated from patients with Achilles tendinopathy (AT) or rupture (AR) under baseline and IL-1b stimulated conditions using LC-MS-MS. RT-qPCR and immunocytochemistry were performed to determine if incubating these cells with two of the mediators produced by tendon-derived stromal cells, 15-epi-LipoxinA_4 (15-epi-LXA_4) or Maresin1 (MaR1), moderated their proinflammatory phenotype.

Results

AT cells showed concurrent increased levels of pro-inflammatory eicosanoids and proresolving mediators compared to AR cells. IL-1b treatment induced profound PGE_2 release in AR compared to AT cells. Incubation of IL-1b treated AT/AR tendon-derived stromal cells in 15epi-LXA_4 or MaR1 reduced pro-inflammatory eicosanoids and potentiated the release of proresolving mediators. 15-epi-LXA_4 or MaR1 also induced SPM biosynthetic enzymes ALOX12, and ALOX15 and upregulated the proresolving receptor ALX compared to vehicle treated cells. 15-epi-LXA_4 or MaR1 also moderated the pro-inflammatory phenotype of AT and AR cells, regulating Podoplanin, CD90, STAT-1, IL-6, IRF5, TLR4 and suppressed JNK1/2/3, Lyn, STAT-3 and STAT-6 phosphokinase signalling.

Discussion

Differences in the bioactive LM profiles between tendon stromal cells isolated from AT and AR patients likely reflect the temporal effects of disease stage. Proresolving mediators including 15epi-LXA_4 and MaR1 regulate the pro-inflammatory phenotype of patient derived Achilles tendon cells, dampening expression of fibroblast activation markers and regulating PGE_2, STAT-1, IL-6, IRF5, TLR4. These SPM also potentiated the further release of other proresolving mediators in IL-1b stimulated AT and AR cells. In summary, we identify proresolving mediators that are active in Achilles tendinopathy and rupture, and propose SPM including 15-epi-LXA_4 or MaR1 as potential therapeutics to resolve Achilles tendon inflammation.

#15 Transglutaminase-2 mediates stromal biomechanics in colorectal cancer.

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Introduction
Increased stiffness of the tumour microenvironment is linked to aggressive cancer cell behaviour through enhanced biomechanical signalling. The protein cross-linking enzyme transglutaminase-2 (TG2) is prominently expressed in the stromal tissue surrounding cancers, and in this work the contribution of TG2 to matrix remodelling and the biomechanical properties of the tumour microenvironment in colorectal cancer (CRC) were assessed.

Materials and Methods
Organotypic models were established using primary colorectal fibroblasts and CRC cells embedded in collagen. SiRNA was used to knockout TG2, and biomechanical analysis performed using unconfined compression analysis. Models were examined by H&E and sirius red staining, and TG2 detected by immunofluorescence. TG2 expression was assessed by western blotting and flow cytometry analysis, and clinical relevance validated by analysing TG2 expression and collagen structure in tissue sections from patients with CRC.

Results
TG2 inhibited cancer cell growth in the models, and biomechanical analysis demonstrated that CRC cells induced fibroblast-mediated matrix stiffening, which was blocked by silencing TG2. Biomechanical changes were associated with observed alterations to collagen fibre structure, notably fibre thickness. Structural changes were also observed in tissues from patients with CRC, with TG2 correlating positively with thicker collagen fibres and associating with poor outcome determined by disease recurrence post-surgery and overall survival. TG2 expression was lower in cancer-associated fibroblasts compared to patient-matched normal fibroblasts, CRC cells inhibited TG2 expression in fibroblasts, and regions of reduced TG2 expression appeared to promote invasive outgrowth of CRC cells.

Discussion
This work demonstrates that TG2 contributes to the stiffer microenvironment observed in CRC. Stiffness is linked to collagen fibre thickening presumably caused by TG2-mediated cross-linking, restricting CRC growth. Notably, these matrix changes are mediated by fibroblasts in response to the presence of CRC cells, and CRC cells appear to drive down-regulation of TG2 in fibroblasts which may be a mechanism to facilitate growth and invasion. These findings are consistent with a disease model in which matrix stiffening supports an early stromal defense to invading tumour, but drives eventual poor outcome through enhanced biomechanical signalling.

#16 Investigating glycosaminoglycan developmental diseases using fully defined 3D cell culture environments and human pluripotent stem cells

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Introduction
Mutations in genes for enzymes in the heparan sulphate (HS) synthetic pathway lead todevelopmental diseases, such as EXT1, EXT2 and Multiple Osteochondromas (MO), or NDST1 and Autosomal Recessive Intellectual Disability (ARID). These mutations result in abnormal glycosaminoglycans (GAGs) production, which impacts on GAG-regulated pathways. There is little genotype-phenotype correlation and disease progression is poorly understood. We have created human induced pluripotent stem cell (hiPSC)-based disease models by generating hiPSCs from patient tissue, and CRISPR-Cas9 gene editing of WT hiPSCs. As these disorders are associated with altered matrix deposition, we have used a fully-defined peptide gel to investigate abnormal matrix in disease-hiPSC differentiation.

Materials and Methods
MO patient tissue was reprogrammed to hiPSCs (MO-hiPSCs). CRISPR-Cas9 gene editing of WT hiPSCs was used to generate mutant EXT1, EXT2 and NDST1 cell lines. hiPSCs were differentiated in self-assembling peptide hydrogels under spontaneous (Essential 6 media) or neural induction (dual SMAD inhibition) conditions. Immunocytochemistry, flow cytometry, AMAC-labelling compositional analysis and radiolabelling will be used to characterise the GAG profiles of the cell lines.

Results
Immunostaining of MO-hiPSCs confirmed pluripotency and highlighted differences in HS deposition compared to WT controls. Genotyping confirmed successful knockouts of EXT1, EXT2 and NDST1. Flow cytometry and compositional analysis detected altered HS produced by the iPSCs. WT hiPSCs were successfully cultured and differentiated in the peptide gels without the need for serum, matrix addition or co-culture.

Discussion
Combining cellular disease models with a defined, GAG-free 3D system allows analysis of disease-specific GAG. Matrix components can be added to the system to test their impact on cell behaviour. We have developed a suite of models to study the mechanistic dysregulation of GAG synthethic pathways, providing insight into disease progression.

#17 Interactions of silver nanoparticles with 2D and 3D human skin models.

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Introduction

Silver nanoparticles provide the basis for the design and application of drug delivery vectors with tuneable surface chemistry. The interactions of silver nanoparticles with respect to their stability, cytotoxicity and uptake mechanisms following their administration is yet to be fully understood. Skin is an organ and target at the forefront of therapies to combat antimicrobial resistance and accelerate wound healing. Here we investigate the nanotoxicology of silver nanoparticles in 2D and 3D human skin models for use as drug delivery vectors.

Materials and Methods

A variety of standardised biophysical techniques and commercially available *in vitro* biochemical assays have been utilised in order to characterise the physical and chemical properties of polymer coated silver nanoparticles and subsequent impact on skin fibroblasts (WS1) and keratinocytes (HaCaT) *in vitro* in 2D. The development of a co-culture model focussed on the optimisation of culture conditions to ensure controllable and reproducible cell behaviour prior to the administration of tailored silver nanoparticles inferred from initial screening studies.

Results

We demonstrate the effect of size (10 nm - 100 nm), coating (+/- polymer) and concentration (up to 50 $\mu g.mL^{-1}$) of silver nanoparticles with respect to cytotoxicity (MTT), immunogenicity (ELISA; IL-6), cell motility (scratch assays) and cell proliferation (clonogenic) in human skin fibroblasts and keratinocytes cells in 2D. Furthermore we investigated the use of a 2D co-culture model and explore the use of a 3D commercially available multicellular skin equivalent (LabSkin).

Discussion

This allowed for the identification of factors that permit the rational design of silver nanoparticle based drug delivery vectors with chemical functionality and their subsequent interactions with skin cells.

#18 Maintenance of cartilage extracellular matrix in osteochondral allografts stored at physiological temperature

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Introduction

Approximately 160,000 hip and knee replacements are carried out in the UK every year. Many of these could be prevented with the transplant of viable osteochondral allografts. Living chondrocytes are essential in articular cartilage, as it is these cells alone that maintain the cartilage extracellularmatrix. Traditionally, storage of the allografts by tissue banks is performed at hypothermic temperature and is limited to a maximum of 28 days storage. The purpose of this study has been to develop a process by which living cartilage allografts harvested from knee joints can be stored at physiological temperature and maintain chondrocyte function beyond 28 days after retrieval. To validate this, assays were used to determine chondrocyte survival and matrix integrity.

Materials and Methods

Human femoral condyles were harvested from donors below the age of 45 with full consent. Each condyle was split into 2 hemicondyles. The tissue was then submersed in culture medium and incubated at physiological temperature for 12 weeks, with weekly media changes. At the beginning and end of the storage period cartilage biopsy samples were taken, with fresh biopsies cut and stained for the presence of living or dead cells, and fixed samples cut and stained for the presence of glycosaminoglycans in the matrix.

Results

Live/dead assay demonstrated that after 12 weeks of storage, chondrocyte viability was in excess of 90%. Safranin O histology showed that retention of glycosaminoglycan was also in excess of 90% coverage in sections.

Discussion

The storage regimen supports chondrocyte survival/function over a 12-week period. Viable cells are paramount to the success of a cartilage allograft with a minimum 70% [1] required to maintain the cartilage matrix, both during storage and once transplanted into a patient. Use of physiological temperature as opposed to hypothermic maintains the normal processes that occur in the cells, thereby increasing their shelf-life. We feel that extending the storage period will allow a more flexible approach to providing a service for surgeons and their patients.

References

[1]JL Cook, FM Pfieffer, *et al*. (2016) Importance of Donor Chondrocyte Viability for Osteochondral Allografts. AM J SPORTS MED. 44(5): 1260-1268.

#19 Mechanoregulation of adipogenesis in orbital fibroblasts through Src family kinases

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Introduction
Graves orbitopathy (GO) is an autoimmune disorder manifesting in 40-50% of patients with hyperthyroidism, which often leaves patients disfigured and/or with altered vision. The disease is characterised by an expansion of the soft tissue in the orbit at the back of the eye, through a unique combination of adipogenesis and fibrosis. We have shown previously that orbital fibroblasts spontaneously produce lipid droplets when cultured in soft 3D collagen matrices, with GO fibroblasts being more adipogenic and contractile than their healthy counterparts. Both phenotypes were sensitive to Src Family Kinase inhibitor PP2, and Src and Fyn have been previously proposed to facilitate fibrosis and regulate lipid droplet production, suggesting a possible role in GO.

Materials and Methods
Primary orbital fibroblasts from GO patients and control individuals, and conjunctival and dermal fibroblasts were obtained under ethical approval. The cells were placed within soft collagen gels (1.5 mg/ml type I collagen) to elicit spontaneous adipogenesis. Lipid vesicle formation was assessed using Oil-Red-O staining after 72h. To assess mechanosensitivity, cells were cultured on top of collagen gels, on PAA/bisAA coated coverslips (1KPa, 40KPa) or on silicone based substrates (CytoSoft Discovery Kit, Advanced Biomatrix). Src, Fyn, mTOR and AMPK expression and activity were assessed by Western Blot and/or immunofluorescence. Src and Fyn downregulation was performed using transfection with siRNA pools (Dharmacon).

Results
Baseline Src and Fyn expression in 2D showed opposite patterns in control and GO fibroblasts, with lower levels of Src and higher levels of Fyn in GO cells, correlating with their ability to form lipid vesicles in 3D. Similarly, Src and Fyn knockdowns had opposite effects on spontaneous adipogenesis: Src downregulation increased lipid vesicle formation while Fyn depletion decreased it. Lipid droplet formation was restricted to orbital fibroblasts, and linked to substrate stiffness, with GO fibroblasts more adipogenesis on high stiffness substrates. Src knock-down reduced mTOR levels, and Fyn knock-down increased AMPKα phosphorylation in 2D but surprisingly both Src, Fyn and mTOR levels were consistently downregulated on soft-substrate.

Discussion
Our data show that Fyn and Src both affect adipogenesis in orbital fibroblasts. Src may be limiting droplet development through mTOR, while FYN may block lipid droplet breakdown by blocking AMPK signalling. However, both kinases and downstream effectors are highly dependent on substrate mechanical stiffness, suggesting a link between lipid droplet formation in GO and altered mechano-sensitivity in orbital fibroblasts that may influence disease progression.

#20 CEMIP is induced by Proteinase-activated receptor (PAR)2 activation in human chondrocytes

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Introduction
PAR2 is a receptor for trypsin-like serine proteinases such as trypsin and matriptase. We have previously identified a role for matriptase in the pathogenesis of osteoarthritis (OA), acting through PAR2, to induce matrix metalloproteinase (MMP) expression and subsequent cartilage breakdown. PAR2 is involved in osteophyte formation in murine OA and PAR2 deficiency confers cartilage protection. Cell migration-inducing and hyaluronan-binding protein (CEMIP; also called KIAA1199) is a hyaluronic acid (HA) binding protein thought to have a role in HA depolymerisation and has been postulated to have a role in endochondral ossification with CEMIP deficient mice exhibiting delayed bone formation. We identify CEMIP as a PAR2-induced gene and investigate a role in osteogenesis and chondrogenesis.

Materials and Methods
PAR2 was overexpressed in SW1353 cells by lentiviral vector, and a gene expression microarray performed following canonical PAR2 activation by either matriptase or synthetic activator peptide SLIGKV-NH_2. SW1353 activation via PAR2 or interleukin (IL)-1 was assessed by RT-qPCR and Western blotting. Osteoarthritic cartilage in explant culture was stimulated with matriptase and protein in concentrated conditioned medium was assessed by Western blotting. Human mesenchymal stem cells (hMSCs) were subjected to either chondrogenesis or osteogenesis over 7 days, RNA was extracted, and RT-qPCR performed.

Results
Following canonical PAR2 activation in SW1353-PAR2 cells, *CEMIP* was the highest induced gene 24 hours post-stimulation. Induction was confirmed by RT-qPCR and protein expression was induced in both cell lysates and conditioned medium. CEMIP was also induced in wild-type SW1353 cells following IL-1 stimulation. CEMIP protein levels were also elevated in matriptase-treated osteoarthritic cartilage in explant culture. Following chondrogenesis and osteogenesis of hMSCs, *CEMIP* expression correlated with an osteogenic gene expression profile.

Discussion
CEMIP has previously been shown to have elevated expression in OA, and we show that CEMIP expression was induced by PAR2 activation and by IL1 stimulation, both pathways implicated in OA. The *CEMIP* expression profile in osteogenesis and chondrogenesis correlated with osteogenic genes, consistent with a previously described role in endochondral ossification. We aim to further elucidate signalling pathways involved in *CEMIP* expression and to assess a functional role for CEMIP in murine OA and in chondrogenesis and osteogenesis from hMSCs.

#21* Unravelling how bi-directional cell-ECM interactions direct stem cell fate in 3D

(Podium Presentation: Mon 8th April, 1.30)

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Introduction

Regenerative therapies that combine stem cells with materials offer tremendous clinical promise, but controlling differentiation and tissue formation remain a pressing challenge. In addition to soluble factors, physical characteristics of the extracellular milieu are known to direct lineage specification, however, how cell-ECM interactions influence this process in *in vivo*-like environments remains incompletely understood. Modifiable hydrogels can provide insight into this phenomenon, but in native tissues, cells do not passively receive signals from their ECM. Instead, they actively, and bi-directionally probe and modify their pericellular space to suit their needs. Nevertheless, the role these bi-directional interactions play in directing stem cell response within 3D hydrogels remains relatively unexplored.

Materials and Methods

Hydrogels were formed by cross-linking 8 mg/mL thiol-modified hyaluronic acid (HA) with varying concentrations of PEGDA. Human bone marrow-derived MSC (hMSC) were encapsulated within hydrogels. Secreted proteins were visualised by FUNCAT and identified by mass spectrometry (SILAC). hMSC-mediated changes in hydrogel Young's modulus were determined using AFM microindentation. hMSC differentiation was evaluated using gene expression (qPCR), histochemical and immunostaining techniques.

Results

hMSC within HA-based hydrogels secrete proteins (including fibronectin, collagens and periostin) pericellularly, but secreted protein density and distribution are dependent on hydrogel composition. Encapsulated hMSC modify the mechanical properties of their local environment by degradation (that softens stiffer hydrogels) or secretion of proteins pericellularly (that locally stiffen softer hydrogels). hMSC's interactions with their local self-modified environment have a role in regulating fate, with a secreted proteinaceous pericellular matrix associated with adipogenesis, and degradation with osteogenesis.

Discussion

Our observations suggest that hMSC encapsulated within HA-based hydrogels participate in a bi-directional interplay between the properties of their 3D milieu and their own secreted pericellular matrix, and that this combination of interactions drives fate specification.

#22 The cartilage age-CpG cg23500537 represses target gene expression of *PCDHB1* and shows changes with ageing in a tissue specific manner.

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Introduction

Osteoarthritis is the most common age-associated musculoskeletal disease of the joint. Our group has identified CpG sites in cartilage that show age correlation with methylation levels termed age-CpGs. Methylation of CpGs usually results in repression of genes. Analysis of age CpGs in non-musculoskeletal tissues suggest several cartilage age-CpGs may be tissue specific. The aim of this project was to investigate if methylation of one such CpG, cg23500537 also changes with age in other knee synovial joint tissues and to assess the impact the methylation of the age-CpGs has on target gene expression.

Materials and Methods

Using chromatin state predictors and chromatin interaction data from the ROADMAP and ENCODE projects data identified Cg23500537 as being located in a potential enhancer region with interaction data showing that it interacts with the gene *PCDHB1* promoter located 14 kb away. RNA and DNA were extracted from fat pad and synovium (n=26) of osteoarthritis patients. Gene expression levels of *PCDHB1* were evaluated using qRT-PCR and for the methylation level of cg23500537 through bisulphite pyrosequencing.
The impact of DNA methylation on gene expression and transcription activity was studied after exposure to the demethylating agent 5-aza-2′-deoxycytidine (5-Aza) in SW1353 human chondrosarco ma cells and luciferase reporter assays in the SW1353 and Tc28i2 rib chondrocyte cell lines.

Results +Discussion

The gene expression level of *PCDHB1* increased chondrosarcoma cell line SW1353 in response to the demethylating agent 5-Aza. This result suggests that *PCDHB1* is negatively influenced by methylation. This hypothesis was confirmed by luciferase assays where the enhancer activity of the age-CpG region was significantly reduced 4-fold ($p<0.0001$) by DNA methylation in SW1353 cells. This was not observed in TC28i2 cells. The methylation levels of the cartilage age-CpG cg23500537showed a significant correlation with age ($p<0.002$ $r^2= 0.34$) in the synovium but not in the fat pad tissues. However, DNA methylation levels only correlated with *PCDHB1* gene expression only in fat pad ($P<0.0001$ $r^2=0.55$) with no correlation observed between age and gene expression for either tissue In conclusion DNA methylation of the cg23500537 age-CpG can modulate the regulatory activity of this region and impact on *PCDHB1* gene expression in a cell and tissue specific manner.

#23 Macromolecular crowding and animal component free culture for stem cell phenotype maintenance

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Introduction

Xenogeneic- and serum-free reagents are gaining increasing interest for stem cell culture. Serum contains several molecules that are crucial for initial cell attachment and subsequent survival *in vitro*, creating a need for added attachment substrates in serum-free culture. Further, the requirement for exogenous attachment proteins also stems from the slow extracellular deposition (ECM) typically observed in dilute culture media. ECM deposition can be accelerated through macromolecular crowding (MMC), a biophysical phenomenon based on the volume excluded by macromolecules in an environment, which accelerates the conversion of procollagen to insoluble collagen *in vitro*. Therefore, it is hypothesised that MMC in serum-free stem cell culture can accelerate ECM deposition which will support multipotent phenotype maintenance during expansion.

Materials and Methods

Human bone marrow stem cells (BMSCs) were seeded at 25,000 cells/cm^2 in 24 well plates and were allowed to attach for 24 h. Subsequently, the medium was changed to medium with MMC (carrageenan or Ficoll) and 100 μM L-ascorbic acid phosphate (Sigma Aldrich). Medium without MMC was used as control. Media were changed every 3 days. SDS-PAGE, immunocytochemistry and FACS were conducted after 4, 7 and 10 days. Trilineage differentiation was performed at 21 days.

Results

Isolation with different serum-containing and animal component free media maintained the multipotent phenotype of BMSCs, evident by high expression of surface markers CD105, CD73, CD90 and CD44 and low expression of CD31, CD45 and CD146. Animal component free media maintained the spindle shaped morphology BMSCs up to passage 4. Cell viability and metabolic activity were not significantly altered by serum and animal component free conditions (p<0.05). Deposition of collagen type I was significantly enhanced in the presence of MMC in animal component free conditions (p<0.05).

Discussion

This indicates the suitability of BMSC-secreted ECM for phenotype maintenance during expansion.

#24* Intracellular trafficking of the invasion promoting cell surface proteinase MT1-MMP

(Podium Presentation: Mon 8th April, 4.30)

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Introduction

Membrane type-1 matrix metalloproteinase (MT1-MMP) is a type-I transmembrane proteinase that degrades the extracellular matrix (ECM) on the cell surface. MT1-MMP has been shown to promote the progression of different diseases including rheumatoid arthritis and cancer, by enhancing cellular invasion. MT1-MMP localisation to the leading edge is crucial for the proteinase to promote cellular invasion. However, the mechanism of this polarised localisation has not been clearly understood. We have found that MT1-MMP cell-surface exposure is achieved by intracellular trafficking of MT1-MMP containing vesicles along microtubules and identified four kinesin motor superfamily proteins (KIFs) involved in this process.

Materials and Methods

HT1080 human fibrosarcoma cells transfected with siRNA targeting KIFs were subjected to functional analysis of MT1-MMP, including gelatin film degradation assay, collagen film degradation assay, beads invasion assay. Cell surface localisation of MT1-MMP was analysed by confocal and total internal reflection fluorescence (TIRF) microscopies.

Results

Our data show that the knockdown (KD) of four KIFs affected MT1-MMP activity on the cell surface: silencing some kifs decreased MT1-MMP proteinase activity against gelatin and collagen film, while the KD of other KIF enhanced MT1-MMP activity; silencing these four Kifs decreased MT1-MMP-dependent migration in 3D collagen. Interestingly, the KD of these KIFs did not affect the overall level of MT1-MMP on the cell surface, while it significantly influenced MT1-MMP localisation at the substrate-attached sites of the cells.

Discussion

These data suggest that these four KIFs play key roles in regulating MT1-MMP cell-surface localisation. We hypothesise that each of the four KIFs has a different role in localising MT1-MMP to the substrate-attachment sites. Moreover, we have confirmed that these KIFs are involved in trafficking MT1-MMP-containing vesicles when cells are cultured in 3D collagen. Future experiments will aim to better understand the dynamics of MT1-MMP-containing vesicle transport in 2D and 3D culture conditions. Pursuing these goals will reveal novel mechanisms which govern MT1-MMP intracellular trafficking and cell-surface localisation, both crucial steps to fully understand cellular invasion within tissue matrices.

#25 Nuclear decoupling and chaperone regulation is part of a rapid protein-level cellular response to high-intensity mechanical loading

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Introduction

Mesenchymal stem cells (MSCs) have a well-characterised mechano-response, including mechano-sensitive commitment to lineage. Cells from mature tissues must also respond appropriately to the mechanics of their surroundings, with cells in stiff and mechanically stressed environments requiring more robust cellular structures. However, many musculoskeletal disorders and connective tissue pathologies begin at sites of aberrant mechanical loading, suggesting a link between high mechanical stress and musculoskeletal disease. Furthermore, many tissues that experience complex mechanical loads, including heart and muscle tissue, have been proposed to benefit from MSC-based therapies. Whether MSCs have the means to survive and function correctly within high-intensity mechanical strain environments remains unknown. We aimed to understand how molecular processes within MSCs are affected by mechanical stress, and how cells maintain tissue homeostasis in response to elevated mechanical loads.

Materials & Methods

Human MSCs (n≥3) adhered to type-I collagen-coated silicone membranes were dynamically strained (FlexCell system; low intensity (LI) 0 – 4% strain at 1 Hz and high intensity (HI) 2.6 – 6.2% strain at 5 Hz) for 1 hour, followed by a period of recovery. Cells were analysed using high-content imaging and quantitative –omics technologies (transcript and protein), with and without perturbations to SUN2 expression levels.

Results & Discussion

Nuclear and cytoplasmic spread areas increased with LI strain cycle. However, in cells subjected to HI strain cycle, nuclear area decreased, suggestive of chromatin condensation and decoupling between nucleus and cytoskeleton. We observed an increase in molecular chaperones, with heat-shock protein 70 translocating from nucleus to cytoplasm under HI strain cycle (the opposite to that reported during heat-shock). We report differential SUN -1 and -2 expression and post-translational modifications to SUN2 in cells exposed to HI strain cycle, suggesting a destabilisation of the LINC complex and a possible mechanism for HI strain-driven nuclear decoupling. The nuclear shrinking response, suggestive of chromatin condensation, was found to be TRVP4 (stretch-activated calcium channel) and SUN2 – dependent. We observed a SUN2-dependent decrease in phosphorylated γH2AX in response to strain, suggesting a strain-induced protective effect against DNA damage. Importantly, SUN2 overexpression prevented this protective effect, with strain then resulting in increased phosphorylated γH2AX, suggesting DNA damage.

#26 Laser capture microscopy – proteomics of the fibrogenic niche in Idiopathic Pulmonary Fibrosis

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Introduction:
The leading edge of Idiopathic Pulmonary Fibrosis (IPF) is enriched with hallmark lesions termed the fibroblastic focus, which can be envisioned as a fibrogenic niche. It contains activated fibroblasts that deposit excessive extracellular matrix (ECM) as it invades towards healthy tissue. Given that the ECM of IPF drives fibrosis progression, characterization of the IPF fibrogenic niche with be an important step forward. Herein, we will determine the ECM composition and organization of the fibroblastic focus (fibrogenic niche) and adjacent lung to inform mechanistic studies of fibrosis progression.

Materials and Methods:
A formalin-fixed paraffin-embedded IPF lung specimen (n = 1) was subjected to laser capture microscopy to microdissect the fibroblastic focus (site of active collagen synthesis), immediate adjacent normal lung tissue, and immediate adjacent mature scar tissue. These regions were subjected to a novel micro-mass spectrometry protocol to create proteomic profiles.

Results:
With our protocol, we detect over 1,600 proteins from each region of the IPF fibrogenic niche. We find that the fibroblastic focus, normal lung tissue, and mature scar tissue have distinct ECM profiles. The fibroblastic focus is enriched with collagen I, III, VI, fibronectin, tenascin C, and versican; Whereas normal tissue has an over-representation of collagen IV and laminin-β2. We also see that the mature scar tissue tracks more closely with the fibroblastic focus versus normal tissue. Finally, we replicate our data and show a strong correlation when comparing protein log2 fold-changes.

Discussion:
We developed a micro-proteomics technique to interrogate the composition of the IPF fibrogenic niche using laser capture microscopy. Our initial data yields promising reproducible results that are consistent with ECM constituents known to be deranged in IPF. These data will be used to create an ECM tissue atlas of the IPF fibrogenic niche which will serve as a platform to create data-driven hypotheses in understanding the molecular underpinnings of fibrosis progression and will lead to high-fidelity ECM systems to recapitulate IPF.

#27 Mechanical stimulation for tissue engineering: characterising load-induced changes by the 'collagen barcode'

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Introduction

Differentiated cells can be characterised by the composition of collagen isoforms (the 'collagen barcode') produce in response to specific mechanical loads. Collagen is currently known to exist as 28 different isoforms, each associated with specific functions: e.g. mechanical resilience, structure, proteoglycan-binding and cell regulation. The combinations and ratios of collagen types vary across all tissues, e.g. Type I, III, V, XI, XII, XIV in tendon, Type II, IV, V, VI, IX, XI in cartilage.

We have investigated the load-responsive changes in collagen isoform composition using 3D printing to generate bespoke bioreactor components which apply mechanical stimulus to cells seeded within 3D constructs. 3D printing has enabled us to customise our culture components around the existing bioreactor designs and replicate them at relatively low cost. The mechanical stimuli that have been applied include tensile loading and hydrostatic pressure.

Materials and Methods

Tensile (stretch) loading: Human mesenchymal stem cells (hMSCs) were seeded within 3D biomimetic hydrogel scaffolds (fibrin) around two loading posts within a 3D printed culture chamber and stretched using an EBERS-TC3 bioreactor. The constructs free swell for two weeks before tensile loading was applied as strain at 5%, 10% and 15% at 0.5Hz for 5 hours per day for three weeks.

Hydrostatic (compressive) loading: hMSCs were seeded within fibrin constructs. During the first two weeks, the constructs 'free swell' within a well-plate and then hydrostatic pressure was applied directly to them via filtered compressed air for three weeks. The hydrostatic pressure was applied as 280kPa at 1Hz for 1hour a day.

Results

Mechanical loading helps to regulate the physical properties of musculoskeletal tissue. In response to tensile loading and hydrostatic pressure, hMSCs alter the composition of the extracellular matrix they produce to create functional, load-bearing tissues. We are investigating how 'optimal' loading conditions may help generate engineered tissues with comparable ratios of collagen types as found in native tissue, and thus improved functionality and integration as implants.

#28 Effects of Fibrillin-1 mutation (Tight skin mouse) in joint health.

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Purpose:
Osteoarthritis development is a major chronic disorder affected >8million British people. Despite the high prevalence of OA, there are currently no therapies to slow disease progression. Fibrillin-1 is an extracellular matrix protein found in elastic fibres. One of its main roles is to control growth factor bioavailability, which play vital functions in joint homeostasis. Fibrillin-1 mutations, as those found in Tight Skin mice (TSK), can increase TGFß signalling, and leads to tight skin, myocardial hypertrophy, marfan-like skeletal phenotype (pectus excavatum, scoliosis, kyphosis, hip dysplasia, protrusio acetabuli, leg length inequality, patellar instability, early onset osteoarthritis; . and lung emphysema. The aim of this study is to assess OA development in TSK mice in spontaneous and in trauma-induced models.

Methods:
Immunohistochemistry for Fibrillin-1 was performed in mouse knee joints of spontaneous (Str/ort mice) and trauma-induced OA, as well as in human OA cartilage. For the ageing model, knees from 35wk-old TSK and littermate control male mice were collected, microCT scanned and used for histology. Preliminary analysis were also performed on intervertebral discs of male and female 35wk-old mice, using microCT and histology. The non-invasive loading model was used and the right knee of TSK and WT mice were loaded repetitively for 2 weeks to induce OA progression and joints assessed 6 weeks after the last loading episode. Histology was performed on these joints after decalcification (10% formic acid), serial sections cut across the whole joint at 6µm, and AC degradation severity assessed (OARSI grading system). Mechanical testing of the anterior cruciate ligament (ACL) of TSK Fbn-1 mice measured its viscoelastic region using an Instron dual column universal testing system (10N load cell).

Results:
Fibrillin-1 was found in the pericellular matrix of chondrocytes in both the growth plate (resting and hypertrophic cells) and in the uncalcified articular cartilage in healthy CBA mice. During the development of OA in all models assessed (Str/ort, trauma and human), Fibrillin-1 immunolabelling was significantly decreased around the lesions. Joint space mineralised tissue volume (including meniscus and ligament calcification) was increased in TSK mice with ageing and in response to mechanical loading. In addition, Tensile testing of the ACL of TSK Fbn-1 mice showed the expected viscoelastic behaviour and a decrease in stiffness noted in the stress-strain curve and the tangent modulus when compared to WT mice. Additionally ACLs of TSK Fbn-1 mice had a significant decrease in the maximum load to failure. In contrast, AC degradation was not significantly changed in any models of OA.

Conclusion:
This study showed that joint soft tissues are modified during ageing and post-traumatic OA when Fibrillin-1 fibrils are abnormal, but this did not affect AC degradation significantly. This suggest a possible role for Fibrillin-1 in maintaining ligament and meniscal function, but may not directly influence adult AC.

#29 Tissue sodium controls the release of heparin-binding growth factors, including hepatoma-derived growth factor (HDGF), from cartilage upon injury.

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Introduction
The extracellular matrix (ECM) of articular cartilage serves to protect chondrocytes from mechanical stress, the most important risk factor in the development of osteoarthritis (OA). It is also able to respond to mechanical stress to initiate repair. This response is mediated through the pericellular matrix (PCM), a distinct region immediately surrounding chondrocytes, rich in the heparan sulfate proteoglycan perlecan. Two PCM-bound growth factors have been characterised thus far; FGF2 and CCN2 (CTGF). By proteomic analysis of the PCM, two further growth factors have been identified; HDGF and CCN1 (CYR61). All four are known to be heparin-binding, and all four are released upon injury, suggesting they share a common mechanism of release. We hypothesise that upon injury sodium, sequestered on chondroitin sulfate, is mobilised, which is sufficient to displace heparan sulfate-bound growth factors to deliver an injury signal to chondrocytes.

Materials and Methods
Porcine articular cartilage was dissected from the metacarpophalangeal joint with a 4 mm biopsy punch, and rested in serum-free DMEM for 48 h. Tissue sodium concentration was manipulated either by adding additional sodium (NaCl) to tissue cultures, or by depleting chondroitin sulfate using IL-1 for 7 days. Dead cartilage was obtained by freeze-thawing. Cartilage was re-cut in medium. Medium conditioned by injured cartilage was analysed by Western blot.

Results
HDGF was released into culture medium upon explantation and re-cutting injury. Cutting in high sodium conditions led to increased release of HDGF and other heparin-binding proteins, in live and dead tissue. Depletion of chondroitin sulfate from the tissue decreased HDGF and other heparin-binding protein release after cartilage injury.

Discussion
These data are consistent with our hypothesis that mobilisation of sodium from chondroitin sulfate stores on aggrecan explains how heparan sulfate-bound growth factors are released upon tissue injury. Depletion of chondroitin sulfate in disease may affect bioavailability of such growth factors and reduce the capacity of cartilage for repair.

#30 Comparison of RNA Extraction Methods in Equine Synovial Fluid in the determination of the expression of Small Non-Coding RNAs

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Introduction
Synovial fluid (SF) is an integral articular component closely associated with other articular tissues in joints. It is a unique source of biochemical information which could be used as biomarkers aiding the early diagnosis of joint diseases including cruciate ligament disease and osteoarthritis (OA). Our previous studies have demonstrated alterations in the expression of small non-coding RNAs (sncRNAs) in several knee joint tissues with age and OA, which could be used as putative diagnostic biomarkers and eventual therapeutic targets. However, the optimal methods for RNA extraction from SF have not yet been determined. The aim of this study was to compare methods of RNA extraction from equine SF to determine subsequent sncRNAs expression.

Materials and Methods
Equine SF (200µl) was treated with hyaluronidase (1 µg/ml) and RNA was extracted using Qiagen exoRNeasy, miRNeasy advanced serum/plasma, miRCURY Exosome serum/plasma kit and standard TRizol-chloroform extraction. RNA concentrations were measured and a panel of sncRNAs including U6, snord77, snord33 and miR-188b were measured using RT-qPCR. Expression data were normalized to miR-191-5p. Differences between groups were determined using one-way analysis of variance test.

Results
TRIzol-chloroform RNA extraction in equine SF achieved greater RNA concentrations units when compared to the Qiagen exoRNeasy, miRNeasy advanced serum/plasma and miRCURY Exosome serum/plasma kit ($p<0.05$). Expression of sncRNAs U6, 33 and 77 were found to be significantly increased with Qiagen exoRNeasy kit in comparison to the other three extraction methods ($p<0.05$).

Discussion
This study has optimised a method to reliably process RNA extraction in equine SF for sncRNA RT-qPCR. The higher RNA concentration identified for the standard TRizol-chloroform method is primarily due to extraction of the total RNA in the SF, whilst with the Qiagen kits exoRNeasy and miRCURY exosome total RNA are extracted from the extracellular vesicles (EVs). As result, lower RNA yields were obtained with the Qiagen kits. The higher sncRNA expression identified with the Qiagen exoRNeasy kit indicate that several sncRNAs mainly originated from SF EVs and may therefore be a more appropriate method for differential expression of sncRNAs in EVs. However, further studies are required to allow for a more thorough conclusion.

#31 Extracellular LaNt α31 influences laminin deposition and cell-to-matrix adhesion

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Introduction

Laminin N-Terminus (LaNt) α31 shares structural motifs with the N-terminal domain of laminin α3b, and has been shown to impact migration speed and adhesion in limbal-derived epithelial cells as well as epidermal keratinocytes. However, whether these effects are cell- or matrix-mediated is not known. To address this question, we here investigated cell adhesion and migration of human corneal epithelial cells (hTCEpi) on LaNt α31 coated surfaces as well as the effect of exogenous LaNt α31 on laminin α3 deposition.

Methods

Cell adhesion to LaNt α31 was measured by fixing and staining hTCEpi cells one hour after seeding on recombinant LaNt α31 or laminin 111, then measuring absorbance. Adhesion strength was determined through measuring the dissociation rate of cells in response to trypsin exposure after seeding onto LaNt α31 or control coated wells for 6 or 24 hours. Immunofluorescence microscopy was used to visualise deposition of laminin α3 from cells seeded on LaNt α31- coated glass coverslips. Single cell migration rates were determined using time-lapse microscopy.

Results

LaNt α31 supported rapid attachment of cells to a similar level as laminin 111. Moreover, cells cultured on LaNt α31 coated dishes revealed an increase in adhesion strength at early and late time points suggesting a rapid maturation of cell-matrix adhesions. Consistent with this, laminin α3 organisation displayed distinct differences in cells cultured on LaNt α31 coated surfaces, with tight laminin clusters observed on LaNt α31 coverslips compared with more continuous arcs and rosettes in control conditions. However, hTCEpi cell migration speed did not differ for cells on LaNt α31 compared with controls.

Discussion

Together, these data suggest that LaNt α31 is able to mediate hTCEpi cell adhesion by supporting cell to matrix interactions and therefore could have possible implications in corneal epithelial cell maturation and differentiation.

#32* Self-assembled supramolecular tissue-like constructs for tendon enthesis repair

(Podium Presentation: Tues 9th April, 9.50)

Authors

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Introduction

The enthesis displays aligned collagen fibres of the tendon inserting into bone across fibrocartilage and mineralised fibrocartilage. Due to high stress concentrations arising at this, entheses are prone to overuse injuries. Consequently, due to a limited regeneration capacity of the tendon and cartilage layers, treatment remains challenging. Phenotypic drift of stem cells and insufficient production of extracellular matrix (ECM) are frequently observed in tissue-engineered constructs, posing major weaknesses of clinically relevant therapies. Macromolecular crowding (MMC) was shown to significantly increase ECM deposition and to promote chondrogenic differentiation of stem cells. Using a thermoresponsive electrospun scaffold, we aim to develop an ECM-rich, cell-based implant for tendon-enthesis regeneration.

Materials and Methods

The chondrogenic differentiation potential of human adipose-derived mesenchymal stem cells (hADSCs) will be investigated in pellet culture, collagen type I sponges, collagen type II sponges and electrospun thermoresponsive fibres using chondrogenic differentiation media. To assess tenogenic differentiation potential of hADSCs, cells will be seeded on collagen type I sponges and electrospun thermoresponsive fibres. Samples will be collected for histology and gene expression analysis.

Results

The use of MMC resulted in the formation of cells sheets with increased content of ECM. Both collagen type I and type II scaffolds showed high chondro-inductive potential with robust expression of chondrogenic marker genes after 21 days. Electrospun fibres supported the deposition of chondrogenic matrix of hADSC-sheets. Chondrogenic cell sheets showed increased expression of Sox9, Col2a1, Col1a1, Comp and Acan after 7 days when compared to pellet culture.

Discussion

MMC enabled the generation of ECM-rich cell sheets in reduced culture periods. Electrospun thermoresponsive fibres showed high chondro-inductivity and therefore pose a promising tool to generate scaffold-free multilayer constructs for tendon-enthesis repair within reduced culture periods.

#33 A *Col9a3* Exon 3 Skipping Mouse as a Novel Model for Multiple Epiphyseal Dysplasia

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Introduction

Multiple epiphyseal dysplasia (MED) is an autosomal chondrodysplasia characterized by earlyonset degenerative joint disease. Its genetic background is complex and heterogeneous and among the mutated genes identified in patients are those encoding for the pro-α chains of the Type IX collagen, COL9A2 and COL9A3 where the majority of mutations lead to the skipping of exon 3.

Materials and Methods

By CRISPR/Cas9 technology we generated two mice lines, one carrying a deletion of Col9a3 exon 3 (*Col9a3*$^{\Delta ex3/\Delta ex3}$), reproducing the splicing events reported in a MED patients group and a Col9a3-null mouse (*Col9a3*$^{-/-}$). On mutant mice, we performed skeletal X-ray phenotyping and growth plate analysis, including immunohistochemistry and BrdU labelling to monitor proliferation. Sequential protein extraction and Atomic Force Microscopy (AFM) were used to check cartilage stability. Bone density was assessed by Microcomputed Tomography (µCT).

Results

The phenotyping of CRISPR/Cas9 generated offspring through DNA and cartilage RNA analysis had led to the establishment of two transgenic mouse lines, one splicing as predicted (*Col9a3*$^{\Delta ex3/\Delta ex3}$) and a second lacking the Col9a3 transcript and Collagen type IX protein (*Col9a3*$^{-/-}$). Both lines are viable, however only *Col9a3*$^{-/-}$ mice displayed detectable phenotypic abnormalities: mild short stature and hip dysplasia, abnormal tibial epiphysis morphology and delayed ossification of femoral head in 18-week old mice. A reduced level of growth plate chondrocyte proliferation was detected in both mutant mice compared to WT, along with softening of the proliferative zone shown by AFM indentation measurements on new-born and 6-week old animals. Only in *Col9a3*$^{-/-}$ mice immunoblotting of sequentially extracted Matrilin-3 and COMP proteins revealed different cartilage extractability from controls.

Discussion

Analysis confirmed the production of a shorter transcript from cartilage of *Col9a3*$^{\Delta ex3/\Delta ex3}$mice. However, these mice still produced Collagen type IX protein and had no overt phenotype apart from reduced chondrocytes proliferation and softer proliferative cartilage. The *Col9a3*$^{-/-}$ mice had a mild skeletal phenotype and expressed no Col9a3 transcript or protein, resulting in overall more unstable cartilage.
Both mutant mice will represent an important tool to gain insights on Collagen IX role into the matrix. In particular, the *Col9a3*$^{\Delta ex3/\Delta ex3}$ line, by recapitulating human Col9-MED, can add to our understanding of MED disease mechanism.

#34 Differences in VEGFA isoform expression regulate modes of migration and their sensitivity to anti-VEGFA therapy.

Authors
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Introduction

Vascular endothelial growth factor A (VEGFA) is characterised by alternative mRNA splicing generating three principal isoforms, $VEGFA_{121}$, $VEGFA_{165}$ and $VEGFA_{189}$ in humans, with mouse one amino acid shorter (e.g. $VEGFA_{120}$). Our studies have shown sarcoma cells expressing $VEGFA_{120}$ have increased metastasis to the lungs of mice compared to cells expressing longer isoforms. Anti-VEGFA antibodies also selectively inhibited metastasis of $VEGFA_{120}$ cells. Surprisingly, anti-VEGFA therapy had no isoform selective effects on tumour growth, vascular biology or lung colonisation, indicating an alternative mechanism of action. $VEGFA_{120}$ tumours had greater levels of laminin compared to $VEGFA_{188}$ tumours that were rich in collagen-I. As we had previously found $VEGFA_{120}$ cells change morphology on laminin compared to collagen-I, we hypothesised their increased metastatic capacity is linked to an ability to adopt different modes of migration, and these are sensitive to anti-VEGFA.

Materials and Methods

Migration of $VEGFA_{120}$ and $VEGFA_{188}$ expressing cells (Fs120 and Fs188) cells was studied using live imaging of single cell migration. Chemotactic migration of serum-starved cells towards agarose containing serum was also studied. The anti-mouse VEGFA antibody B20-4.1.1, a preclinical mimetic of bevacizumab, and control IgG clone BE5 were kindly provided by Genentech Inc. Migration was studied on tissue culture surfaces coated with laminin, collagen-I, fibronectin or in a custom made chamber that enabled amoeboid/non-adherent migration to be measured.

Results

Fs120 cells showed significant differences in migration speed between laminin, collagen-I and fibronectin coated surfaces, favouring fibronectin and laminin over collagen-I. Fs188 cell migration was similar on all three ECM coated surfaces and faster than Fs120 migration. Non-adherent migration of Fs120 cells was significantly faster than that of Fs188 cells. B20-4.1.1 inhibited migration of Fs120 cells on laminin, fibronectin and on non-adherent surfaces but showed no inhibitory effect on migration of Fs188 cells.

Discussion

These results have identified a potential mechanism by which $VEGFA_{120}$ expression provides and advantage in sarcoma metastasis and why this is sensitive to anti-VEGFA therapy. We have recently found increased expression of $VEGFA_{121}$ in clinical sarcoma correlates with decreased survival suggesting further studies into mechanism could lead to improvements in response to anti-VEGFA therapy in these patients.

#35 Combinatorial effects of matrix stiffness and ECM proteins on cell behaviour and morphology

Authors
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Introduction

Stiffness of a matrix (resistance to deformation), is one of the many mechanical forces acting on cells and is increasingly appreciated as an important moderator of cell behaviour. It broadly regulates cell signalling, with major effects on growth, survival and motility. Particularly, the Hippo and YAP/TAZ signalling pathways have been identified as a major mechanical sensing signalling arms within the cell. Although, the effects of stiffness for different adherent cell types vary, generally, cell proliferation and differentiation increase with the stiffness of the matrix. This study explores the collaborative effect of matrix stiffness and various types of ECM proteins on cell behaviour and YAP/TAZ signalling.

Materials and Methods

Adherent cell line (hTERT RPE-1) was cultured in 96-well plates (Matrigen, SW96G-EC-HTS) with varying elastic modulus (0.2-50 kPa) polyacrylamide hydrogel coated wells. These, wells were further supplemented with ECM protein coatings: Collagen, Fibronectin and subtypes of Laminins. Cell growth and morphology were observed using transmitted light microscopy and immunofluorescence.

Results

While on soft matrix (0.2 kPa matrix) cells displayed reduced growth. This was further enhanced (2fold decrease) in presence of Laminin111. Consistently, Laminin111 increased the proportion of cytosolic YAP1 on soft matrix. Inversely, cells displayed increased proliferation and YAP1 nuclear translocation on stiff matrix (2 kPa) that was further enhanced in the presence of laminin511 (3-fold increase over no-coating control). Accordingly, cell proliferation phenotypes were shown to be sensitive to factors relating to both stiffness and ECM subtype.

Discussion

This study shows that combination of matrix stiffness and selected ECM proteins stimulate changes in cell morphology and rate of proliferation. In particular, we observed effects of YAP1 localisation that reflected a combination of stiffness and ECM rather than either being dominant. Many recent studies explore the effects of matrix stiffness on Hippo signalling or ECM protein influence on cell behaviour in terms of YAP1 localization in isolation. Here, we show the combination of the two parameters. We therefore propose, that there is a combinatorial effect of matrix stiffness and ECM proteins that drive cellular proliferation or arrest. Future studies will expand on these findings to explore these behaviours in various cell types, including stem cells. This study gives preliminary insight into factors affecting cell behaviour more reminiscent of the *in vivo* niche and will be implemented into an *in vitro* intestinal scaffold design going forward.

#36 The Natural Repair of Articular Cartilage in Humans: An Immunohistological Study

Authors
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Introduction:
Evidence suggests articular cartilage is capable of natural regeneration in some individuals, despite the oft-stated belief of its inability for self-repair. We have examined repair tissue formed following surgically induced cartilage defects in humans as part of an autologous cell implantation (ACI) procedure.

Materials and Methods:
Sixteen patients (12 males, 4 females, mean age 36±9years) underwent harvesting of macroscopically healthy cartilage from a low-load bearing part of the trochlea for ACI. The quality of repair was assessed macroscopically on MRIs taken at 14.7±3.7months and during arthroscopy at 15±3.5months post-harvest (using the Oswestry Arthroscopy Score (O-AS) and the International Cartilage Repair Society Arthroscopy Score (ICRS-AS), maximum scores of 10 and 12 respectively, where higher is better). Core biopsies of the repair tissue were assessed histologically for morphology (scored using the ICRSII and OsScore histology scores) and collagen types I, II, III and VI determined immunohistochemically and compared to healthy cartilage.

Results:
The mean O-AS and ICRS-AS of the repaired defects were 7.2±3.2SD and 10.1±3.5SD respectively with a mean defect area fill of 80%±23SD. The quality of the repair tissue formed was variable; hyaline cartilage was present in 50% of the biopsies and was associated with a significantly higher ICRS-AS (median 11 vs 7.5, $p=0.05$). The OsScore, but not the ICRSII score, correlated significantly with both the O-AS ($r=0.49$, $p=0.05$) and ICRS-AS ($r=0.52$, $p=0.04$). Collagen type I was detected in 12/14 biopsies, type II in 10/13 biopsies and types III and VI in 15/15 biopsies with variable staining patterns and intensities. Biopsies consisting of mostly hyaline cartilage had a similar staining pattern for collagen types III and VI to healthy cartilage.

Discussion:
These results clearly demonstrate the ability for articular cartilage to heal naturally following an injury, albeit with variable morphologies. The harvest defects may have an advantage in their ability to heal compared to condylar cartilage defects typically found in osteoarthritis, due to the lower loads at their location and macroscopically healthy cartilage having been removed. However, the mechanism by which this repair process occurs remains unknown. Understanding such mechanisms may lead to improved treatments for cartilage defects in the future.

#37 Evidence for alternative polyadenylation of the ADAMTS5 mRNA creating a heterogenous pool of transcripts that differ in their response to post-transcriptional cues.

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Introduction
Alternative polyadenylation affects many mRNAs, resulting in the generation of transcripts that encode the same protein but differ in their structural and regulatory properties. The influence of alternative polyadenylation on regulating genes related to osteoarthritis has not been well studied. This study examined the use of alternative polyadenylation sites in the ADAMTS5 mRNA and investigated the functional effects that this has on ADAMTS5 mRNA dynamics.

Materials and Methods
Potential alternative polyadenylation of the ADAMTS5 3' untranslated region (3'UTR) was performed by manually searching the sequence or using the "APADB" online resource. Primers specific to different regions of the 3'UTR were used in qRT-PCR assays, using absolute and relative quantification approaches, to determine levels of sequence present in primary human articular chondrocyte samples. RNA half-life was determined in cells subjected to actinomycin D treatment. Interaction of cellular proteins with ADAMTS5 RNA was determined in a range of cells using Electrophoretic Mobility Shift Assays (EMSAs).

Results
Sequence and ARED database analysis indicated that the ADAMTS5 mRNA's 6kb 3'UTR could be alternative polyadenylated. qPCR analysis identified strong evidence for a site 2.5kb 3' of the stop codon. The resultant long and short forms of the transcript appear to be present at an approximate 1:1 ratio in primary human articular chondrocytes. The shorter form of the transcript would be predicted to not include a considerable number of predicted protein and microRNA interaction sites and analysis of RNA half-life demonstrated that it was more stable than the longer version. EMSA analysis of an evolutionarily conserved region immediately 3' of our predicted polyadenylation site revealed multiple protein interactions, the patterns of which differed widely across a broad range of cell types. Following stimulation of chondrocytic cells with IL-1ß, the proportion of shorter form increased dramatically.

Discussion
ADAMTS5 has a crucial role in the progression of osteoarthritis and it exhibits many tiers of regulation, one of which is microRNA-mediated post-transcriptional control. This study indicates that, in chondrocytes, the ADAMTS5 mRNA pool is heterogenous with transcripts that will vary in their response to post-transcriptional regulatory inputs.

#38 A peptide gel-based method for fully-defined, adaptable studies of cell-matrix interactions in 3D culture.

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Introduction

Current in vitro disease models are limited by their poor similarity to human tissue, batch-to-batch variability and high complexity in composition and manufacture. Here, we present a "blank slate" culture environment that can be customized by incorporating matrix components specifically selected to match the target tissue, with mechanical properties controlled independently and simultaneously. Built from a self-assembling peptide gel, the engineered microenvironment contains no exogenous proteins or glycosaminoglycans: only those specifically added, or synthesized by encapsulated cells. This 3D culture platform therefore enables full control over biochemical and physical properties, allowing detailed study of the interplay between matrix composition and mechanics and cell behaviour.

Materials and Methods

We demonstrate excellent viability of multiple adhesive and suspension cell types in the peptide gels with and without matrix functionalisation. The system is shown to be compatible with commonly used analytical methods (in situ immunostaining, fluorescent/luminescent platereading, PCR) and flexible to incorporate direct and indirect co-culture. We used the welldocumented model of breast cancer progression as proof-of-concept of the model for studying cell-matrix interaction.

Results

Controlling the peptide gelator concentration allows peptide gel stiffness to be matched to normal breast (<1 kPa) or breast tumour (>1 kPa), with higher stiffness favouring the viability of breast cancer cells over normal breast cells. Modification of the peptide gels with matrix components relevant to human breast, such as collagen I and hyaluronan, or the introduction of relevant cells in co-culture (human mammary fibroblasts) altered cell behaviour.

Discussion

The choice and concentration of matrix additions control the size, shape and organisation of the breast epithelial cell structures formed in co-culture with fibroblasts. This system therefore provides a means of unravelling the individual influences of matrix, mechanical properties and cell-cell interactions in cancer and disease.

#39 FGF2 promotes regeneration of cartilage *in vivo* by promoting MSC chemokinesis

Authors
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Introduction

FGF2 is released from the pericellular matrix upon cartilage injury. FGF2 null mice exhibit accelerated degeneration of cartilage both spontaneously and after surgical induction of osteoarthritis (OA), which suggests a chondroprotective role. One possible mechanism could be that FGF2 released upon injury stimulates an intrinsic repair response. Healing of a critical cartilage defect in mice is dependent upon background strain and age. The DBA/1 mouse has good regenerative capability. It is assumed that repair is reliant on provoking tissue-resident progenitor or mesenchymal stem cells (MSCs) to proliferate, migrate, adhere to the injury site, and to undergo chondrogenesis. Here we test whether FGF2 is involved in intrinsic cartilage repair.

Materials and Methods

Full-thickness cartilage defects were created in the patellar groove of 10-week-old male DBA/1 wild-type (WT) or $FGF2^{-/-}$ mice that had been previously backcrossed on to the DBA/1 background. Mice were culled 8 weeks post-surgery and scored using a modified Pineda scoring system. 24 hours dynamic Juli-stage scratch assay was performed on $FGF2^{-/-}$ and wild-type MSCs treated with or without FGF2 and FGFR-inhibitor (SB402451). Explanted cartilage was used to measured adhesion of MSCs. Chemotaxis was measured using a transwell system. Human MSCs were cultured in a trans-well disc assay with standard chondrogenic medium (DMEM, TGFβ3 10 ng/µL, ITS 1X, sodium pyruvate 100 µg/ml, dexamethasone 100 nM, ascorbic acid 25 µg/ml, L-proline 40 µg/ml, L-glutamine 1X) in the presence of FGF2 or FGFR inhibitor. Discs were analysed histologically and by RT-PCR for expression of chondrogenic genes.

Results

$FGF2^{-/-}$ mice showed significantly lower repair scores compared with wild-type animals. MSC migration in the scratch assay was significantly reduced in $FGF2^{-/-}$ cells or wild-type MSCs treated with the FGFR inhibitor. FGF2 did not affect adhesion of MSCs to damaged cartilage nor did it act as a chemotactic agent. In addition, it suppressed in vitro chondrogenesis.

Discussion

FGF2 has a crucial role in cartilage repair *in vivo* most likely by enhancing the migratory potential of MSC.It does not promote adhesion of MSCs to damaged cartilage nor does it promote chemotaxis or chondrogenesis *in vitro.*

#40* Matrix adhesion site function in polarised invasive migration

(Podium Presentation: Tues 9th April, 2.30)

Authors

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Abstract

Cells migrating through 3D matrices form hybrid adhesion structures, which display hallmarks of both focal adhesions and invadopodia. We established novel methodology to analyse the composition of matrix adhesion complexes of invasive cancer cells in 3D matrices and compare them to conventional 2D matrix adhesion complexes using a BioID based technique.

Using this approach, we have identified a novel interaction module consisting of N-WASP/WIP -> ßPIX -> Myosin18A (M18A) that is enriched in invasive 3D adhesion sites. Loss of ßPIX /M18A abolished cancer cell invasion, but increased matrix degradation and pseudopod extension in collagen matrices. These seemingly contradictory results can be explained by our finding that ßPIX is part of a ßPIX-M18A-non-muscle Myosin 2A (NM2A) module that is required for adhesion site turnover and nuclear-force coupling (NFC). The nucleus can act as limiting factor in 3D cell migration. Migrating cells need to actively squeeze the nucleus through matrix pores. Knockdown of nuclear envelope actin binding proteins called Nesprins severely affect 3D cell migration. We have found a reciprocal regulation of Nesprin-2 and ßPIX/M18A function. We can show using a novel nuclear membrane FRET/FLIM force biosensor, that direct force coupling from 3D adhesion sites to the nuclear membrane through ßPIX/M18A is required for 3D cell migration. M18A has been shown to co-assemble with NMIIA to form mixed bipolar filaments. We propose a model where ßPIX and M18A promote the incorporation of NMIIA into myosin filaments at nascent adhesions and subsequent differential turnover of NM2 isoforms are responsible for their polarised distribution in invading cancer cells. The workload difference of NMIIA and NMIIA has the potential to generate contraction force gradients along actomyosin fibers in migrating cells. We propose that this mechanism establishes front-rear cell polarity during 3D cell migration.

#41 Untargeted extracellular chemical profiling by LC-QTOF-MS identifies novel markers of bone collagen degradation

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Introduction
Bone and its associated matrix is a highly regulated tissue, its maintenance balanced by the tightly coupled processes of bone formation and resorption. Bone resorption relies upon degradation of type I collagen, and dysregulation of this process occurs in a range of clinical disorders of increased bone resorption including osteoporosis and Paget's disease. Biochemical assays are important for assessing bone resorption *in vitro* and *in vivo*. Measurement of C-terminal telopeptide (CTX) is the most widely employed assay and CTX is considered the most specific resorption biomarker available. This study aimed to investigate novel biochemical markers of bone resorption through non-targeted chemical profiling of the extracellular media content from osteoclasts cultured on dentine.

Materials and Methods
Human osteoclast precursors were cultured on dentine wafers from hippo and walrus tusks in 96well plates in the presence of RANKL (66ng/ml) and MCSF (33ng/ml) (n=8). After 14 days culture medium was sampled and dentine wafers were fixed and stained to quantify resorption. Chemical profiling of media was performed by liquid-chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS; Agilent) in positive polarity electrospray ionisation, mass range 50-1700. Profiles of media samples were compared between the experimental condition (+RANKL/+dentine; as detailed above) and three control groups: osteoclast precursors cultured on plastic with RANKL (+RANKL/-dentine; n=6), osteoclast precursors cultured on dentine without RANKL (-RANKL/+dentine; n=6) and osteoclast precursors cultured on plastic without RANKL (-RANKL/-dentine; n=3).

Results
147 chemical entities were detected across all culture groups. 59 entities showed statistically significant differences in profiles ($p<0.05$, fold change >2) across the four culture groups. The clearest difference was in the profiles of media from +RANKL/+dentine compared to the other conditions. 28 unique entities were upregulated in +RANKL/+dentine media compared with all other groups (mass range=144-1541 Da). Based on high-resolution accurate mass, putative identifications for these markers of bone resorption were the collagen tripeptide glycylprolyl-hydroxyproline, and dipeptides arginyl-hydroxyproline and hydroxyprolyl-leucine. Subsequently glycylprolylhydroxyproline and hydroxyprolyl-leucine were detected in human and mouse serum and urine by LC-QTOF-MS.

Discussion
Untargeted extracellular chemical profiling of medium from osteoclasts cultured on dentine indicated novel and specific *in vitro* markers of type I collagen degradation. Further research aims to confirm the chemical structures of these biomarkers and to assess their potential as bone resorption markers *in vivo*.

#42 Tenascin-C: A Driver of Inflammatory Bowel Disease?

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Introduction
Tenascin-C (Tnc), founding member of the matricellular Tenascin family, is an ECM glycoprotein implicated in the pathogenesis of a variety of chronic inflammatory diseases. At sites of inflammation Tnc becomes highly upregulated where it acts to create a local pro-inflammatory 'niche' via stimulation of the TLR4 receptor on stromal and immune cells. Inflammatory Bowel Disease (IBD) is a chronic inflammatory disease of the large intestine with varied aetiology and presentation in patients. Tnc has been demonstrated to be increased in the inflamed mucosa and serum of IBD patients however the exact role Tnc may be playing in the disease has yet to be fully investigated.

Materials and Methods
Acute colitis was chemically induced in 8 week old 129sv mice by administration of 2-3% Dextran Sulphate Sodium (DSS) in their drinking water for 4 days. Mice were scored for gross and histological pathological changes while immunohistochemistry and RT-qPCR were used to study the dynamics of immune cell types and inflammatory mediators in the colon.

Results
Tnc was significantly upregulated at the protein level in the colons of DSS treated mice from day 6 to 8 in the colitis induction protocol. Additionally this was concurrent with the upregulation of other key pro-inflammatory factors such as IL-6, TNFα, and IL-1β. Immunohistochemistry revealed that Tnc is basally expressed in the colon, however, upon induction of colitis it is significantly upregulated in the mucosa appearing to co-localise with areas of ulceration. Additionally, co-staining with immune cell markers has shown that immune infiltration coincides with Tnc upregulation. Ongoing studies in Tnc KO mice have indicated a protective effect of Tnc's genetic ablation with percentage body weight loss significantly reduced in comparison to wild-type controls.

Discussion
Studies have previously demonstrated the role of Tnc in maintaining aberrant inflammatory responses, such as antigen-induced arthritis, with a protective affect observed upon Tnc ablation. This study further supports these findings in an IBD pre-clinical animal model and suggests Tnc may be playing a similar pro-inflammatory role. This suggests that therapies targeting Tnc may also benefit patients suffering from IBD. Further study of the exact role and best methods to target Tnc are warranted.

#43* Small non-coding RNA transcriptome signatures of chondrocyte ageing

(Podium Presentation: Tues 9th April, 2.50)

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Introduction

Osteoarthritis (OA) is linked to ageing but the mechanisms are incompletely understood. Small non-coding RNAs (SNCRNAs) are key regulators of gene expression (microRNAs), rRNA modification and protein translational capacity (snoRNAs). Short RNA fragments derived from parental RNAs, such as tRNA cleavage fragments, are novel regulatory RNAs that inhibit translation in response to stress. In this current study we profile SNCRNAs in normal young and old chondrocytes in order to elucidate age-specific changes.

Materials and Methods

RNA from freshly isolated chondrocytes derived from n=5 young (mean age ±SD; 4±1 years) and n=5 old (18±3.5 years) horses was subject to small RNA sequencing on the Illumina MiSEq platform generating 2x150 bp paired-end reads. Reads were aligned to the EqCab 3.0 genome. Additional analysis identified novel snoRNAs and tRNA fragments. Differential gene expression (DE) analysis was conducted using edgeR. Ingenuity Pathway Analysis was used to identify microRNA targets and pair data with our previous RNASeq study undertaken on young and old cartilage to determine the microRNA interactome in cartilage ageing.

Results

Chondrocytes were from normal joints; Kawcak gross scoring; young (1.25±0.5) and old (1.75±1). 50 DE SNCRNAs were identified in ageing; 16 microRNAs, 8 snoRNAs, 2 novel snoRNAs, and 24 tRNAs (FDR<0.05). tRNA fragments were from two distinct populations of approximately 50nts (tRNA halves) and 30nts (tRNA fragments) and these increased in ageing. DE microRNAs input into IPA were involved in cell death, movement, growth and proliferation pathways. After applying prioritisation towards likely miRNA-mRNA targets, a regulatory network of 16 miRNAs targeting 75 previously identified DE mRNAs was created. Subsequent pathway analysis identified genes within the pathways joint inflammation, activation and mineralisation of connective tissue and OA.

Discussion

One key characteristics of OA is an imbalance between protein anabolism and catabolism. Ageing leads to a loss of protein homeostasis. This data evidences specific microRNAs that may be potent regulators of gene expression at different levels during cartilage ageing. tRNA fragments increase in response to stress and reduce protein synthesis. We identified for the first time in tRNA fragments in cartilage ageing that may, to some extent, result in the loss of protein homeostasis evident in OA.

#44 Design and characterization of a three-layer collagen-based scaffold to modulate BMSC behaviour for enthesis regeneration

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Introduction
The enthesis is a specialised tissue interface between tendon and bone, essential for adequate force transmission. Following injuries and surgical repair, the enthesis is often not re-established and traditionally used tissue substitutes have lacked to reproduce the complexity of the native tissue. We hypothesised that a collagen-based three-layer scaffold that mimic the composition of the enthesis, in combination with bioactive molecules, will enhance the functional regeneration of the tissue.

Materials and Methods
A three-layer sponge composed of a tendon-like layer (collagen I), a cartilage-like layer (collagen II) and a bone-like layer (collagen I and hydroxyapatite) was fabricated. Bone-marrow stem cells (BMSCs) were seeded on the scaffolds and cultured in differentiation media (chondrogenic, tenogenic and osteogenic). Alizarin Red and Alcian Blue were performed to evaluate BMSC differentiation towards osteogenic and chondrogenic lineage. Tenogenic differentiation of BMSCs was evaluated through expression of collagen I and tenascin by immunofluorescence staining. Subsequently, the cartilage-like layer was functionalized with insulin growth factor 1 (IGF-1) and the analysis repeated.

Results
The scaffolds promoted osteogenic differentiation of BMSC selectively in the bone-like layer in scaffolds cultured in basal and osteogenic media. Alcian blue staining revealed the presence of proteoglycans selectively in the cartilage-like layer in scaffolds cultured in chondrogenic media but not in basal media. Increased expression of the tenogenic markers collagen I and tenascin was observed in the tendon-like layer of scaffolds cultured in tenogenic but not in basal media (**Fig.1**). The presence of IGF-1 increased osteogenic and chondrogenic differentiation of BMSCs.

Conclusion
The collagen composition of the non-functionalized 3-layer sponge was able to regulate BMSC differentiation in a localized manner within the scaffold. The functionalization with IGF-1 accelerated chondrogenic and osteogenic BMSC differentiation. Overall, functionalization of the 3-layer scaffolds holds promising potential in developing novel and more efficient strategies towards enthesis regeneration.

Acknowledgements
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#45* The role of WWP2 in cartilage.

(Podium Presentation: Tues 9th April, 11.30)

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Introduction

WW domain-containing protein 2 (WWP2) belongs to the Nedd4 family of ubiquitin ligases. WWP2 is involved in stem cell differentiation, cancer development and immune responses; it also plays a crucial role in craniofacial development. In cartilage, WWP2 expression is high and driven by the cartilage-specific transcription factor SOX9. Several isoforms of WWP2 have been reported with a C-terminal WWP2 (WWP2-C) isoform expressed only in cartilage, however its function remains unknown.

Materials and Methods

Chondrogenesis in the presence/absence of WWP2 was induced in human mesenchymal stem cells. A knockout mouse lacking the cartilage specific isoform of Wwp2-C was generated using CRISPR/Cas9 technology. RNA-seq from costal chondrocytes of 7 day old mice was performed to assess gene expression in Wwp2-C KO mice. The growth plate development in femur was assessed using Safranin O staining. The craniofacial development was assessed with X-rays and micro-CT. OA was induced surgically by destabilization of the medial meniscus.

Results

Depletion of WWP2 results in a drastic decrease of cartilage pellet formation during chondrogenesis, with a concomitant decrease in the expression of the cartilage-specific genes aggrecan and type II collagen. RNA-seq analysis showed significant up- or down-regulation of 120 genes, enriched in the involvement of regulation of ECM organisation, ossification, and skeletal system development. Deletion of the Wwp2-C results in delayed formation of the secondary ossification centers and craniofacial development. Preliminary data indicate that WWP2-C plays a role in OA development.

Discussion

These results show that WWP2 plays an important role in chondrogenesis. In addition, the WWP2 C-terminal isoform plays a role in the skeletal and craniofacial development in mice. Full-length WWP2 and microRNA140 (miR140) knockout mice have similar phenotypes in regard to craniofacial development. There is an ongoing controversy as to whether the WWP2 or miR140 is responsible for the craniofacial phenotype. Our data support the WWP2 involvement, however more research is needed to establish the distinct and overlapping functions of miR140 and its host gene WWP2.

#46 Targeting Retinoic Acid Metabolism as an Anti-inflammatory Treatment for Hand Osteoarthritis

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Introduction
Mechanical injury is a common risk factor at all sites of osteoarthritis (OA), including hands. Injury signals are transmitted through the cartilage matrix to activate inflammatory signalling pathways, which then induce a number of inflammatory genes including matrix degrading enzymes. Hand Osteoarthritis is the most prevalent OA. Polymorphic variants in *ALDH1A2* have been associated with severe hand OA in a GWAS. *ALDH1A2* encodes the key enzyme controlling the irreversible synthesis of all-trans retinoic acid (atRA). atRA is anti-inflammatory and has essential role in maintaining tissue homeostasis. Here we investigated the link between cartilage injury and cellular atRA, and the role of atRA as an anti-inflammatory target in hand OA.

Materials and Methods
We performed a SNP replication study using UK Biobank. Cartilage injury was conducted by explanting cartilage from porcine metacarpophalangeal joints and murine femoral heads. Microarray and quantitative PCR was carried out in injured cartilage at 0 and 4h. Talarozole, an atRA metabolism blocking agent (RAMBA), or atRA was added to isolated porcine chondrocytes together with IL1. To examine the role of atRA in injury induced inflammation, talarozole was injected into porcine joints prior to injury. RNA was extracted from the articular cartilage of 34 hand OA patients at the time of hand surgery, and was analysed for the expression of atRA dependent and inflammatory genes.

Results
The *ALDH1A2* SNP association with hand OA was confirmed in UK Biobank. Upon injury of porcine and murine cartilage, atRA-responsive genes were downregulated while inflammatory genes were strongly induced. Talarozole maintained endogenous atRA levels and suppressed disease-relevant inflammatory genes driven by injury or IL1. Hand OA cartilage from patients carrying the risk allelic variants of *ALDH1A2* showed reduced atRA-responsive and raised inflammatory genes.

Discussion
Our findings demonstrate a novel anti-inflammatory role for retinoic acid in injury- and cytokine-induced inflammation in cartilage. Targeting the metabolism of retinoic acid respresents a new pharmacological strategy for hand OA treatment.

#47 Biomarkers and mechanics of murine anterior cruciate ligament during osteoarthritis development

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Introduction
Osteoarthritis (OA) is the most common form of arthritis and the leading cause of disability among elderly. It is a multicomponent chronic disease and yet little is known about the role of ligaments in OA. Trauma of the ligament has been closely linked to OA making it a potential target for therapeutics. The goal of this study was to understand the biomarkers and the mechanical changes in the ligaments during disease progression in murine spontaneous and post-traumatic OA.

Materials and Methods
Two OA models were used: C57Bl/6 mice following non-invasive knee trauma, and CD1 outbred mice previously shown to develop spontaneous OA. Samples were imaged for µCT and stained with Toluidine Blue. Immunohistochemistry (IHC) markers included collagen type II, sox9 and asporin. Viscoelastic properties of the anterior cruciate ligament (ACL) were measured using an Instron (10N load cell).

Results
Toluidine Blue staining showed changes in the ligaments including increased extracellular matrix staining and cell hypertrophy. In the trauma model collagen type II deposition occurred in the mid-ligament region, sox9 expression in the hypertrophic regions, and asporin expression in the ligament and surrounding tissue. µCT of the joint space revealed an increase in mineralized tissue volume with increasing OA severity in the trauma model. Viscoelastic behaviour of the ACL at 90 degree of knee flexion showed viscoelastic behaviour and measured hysteresis, creep, strain rate dependence and maximum load of both OA models.

Discussion
Overall, ligament pathology was affected during OA progression, and pathology changes are potentially consistent with endochondral ossification. Marker expression shows changes in sox9 and collagen type II expression particularly in the post-traumatic model, which could affect mechanical properties. Further analysis of the CD1 mouse model and viscoelastic testing will reveal the similarities and differences with spontaneous OA. Next steps should aim at elucidating the role of asporin and the pathways altering the collagen matrix, and identifying potential therapeutic targets.

#48 Utilising a Novel Photoresponsive Hydrogel with Defined Surface Topography to Probe Primary and Immortalised Mesenchymal Stem Cell Morphology Response to Extracellular Stiffening

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Introduction
Mesenchymal stem cell (MSC) morphology responds to hydrogel stiffness and topography both in isolation and combination. However, in addition to static hydrogel stiffness, MSC morphology also responds to stiffness modulation. We previously reported the development of a photoresponsive hydrogel with photoswitchable stiffness and demonstrated its potential for studying the response of MSCs to extracellular stiffening. This work demonstrates that by modulating the fabrication of this novel photoresponsive hydrogel we were able to probe the morphological response of both primary and immortalised (Y201) MSCs to extracellular stiffening in the presence of a defined surface topography. Understanding the combined regulatory effects of multiple static and dynamic material stimuli will improve the regulation of MSCs for tissue engineering and regenerative medicine.

Materials and Methods
Fibronectin coated photoresponsive hydrogels were prepared with defined surface topography and softened via exposure to 30 mins UV light. Hydrogels were seeded with either primary bone-marrow derived MSCs or Y201s and cultured for 24 hours before being exposed to 60 minutes blue light exposure, to stimulate hydrogel stiffening, and cultured for a further 24 hours. Samples were fixed and stained with DAPI and phalloidin before being imaged and subjected to quantitative morphometric analysis using CellProfiler software. Hydrogel topography on both softened and subsequently stiffened samples was characterised via bright-field, fluorescence (following Fibronectin staining) and atomic force microscopy.

Results
Both primary MSCs and Y201s showed a significant and conserved morphological response to hydrogel stiffening. Cell and nuclear area increased by ~33 – 34% and ~20-22%, respectively, for both primary MSCs and Y201s. Hydrogel surface topography remained constant during stiffness modulation, with no significant change in surface area ratio or branch number, length, and angle.

Discussion
These data demonstrate that both primary MSCs and Y201s show a conserved morphological response to extracellular stiffening, even in the presence of a defined surface topography, in particular highlighting the conserved mechanosensitivity of the nucleus and further indicating a mechanosensitive link between the cell and nucleus, supporting the existing literature. We now aim to study the effect modifying surface topography has on this morphological response to stiffening, and utilise the Y201 line to perform time-resolved analysis via live-cell time-lapse fluorescence microscopy.

#49* The regulation of DDR1 catalysis by its intracellular juxtamembrane region.

(Podium Presentation: Mon 8th April, 1.40)

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Introduction

The discoidin domain receptor (DDR) DDR1 is a collagen-binding receptor tyrosine kinase (RTK). Aberrant DDR1 signalling is implicated in the progression and poor prognosis of several diseases, including many cancers. DDR1 is therefore an attractive target for pharmacological intervention. However, unlike many other RTKs, the processes governing DDR signalling are poorly characterised. Our study aimed to better understand these processes by investigating the functional role played by the intracellular juxtamembrane (JM) region of DDR1.

Materials and Methods

X-ray crystallography was performed on the intracellular domain of DDR1 with diffraction data collected at Diamond Light source. Immunoblotting of Baculovirus expressed DDR1 constructs was used to monitor receptor autophosphorylation over time, and the identity of phosphorylated tyrosine residues was confirmed by tandem mass-spectroscopy (MS/MS). Enzyme kinetics of each DDR1 phosphoform were determined using a luciferin/luciferase-based ADP-Glo™ assay.

Results

We solved a 2.5 Å resolution crystal structure of the DDR1 cytoplasmic domains. The structure revealed that the DDR1 JM region inserts into the kinase active site, disrupting transition between inactive and active catalytic states. This JM autoinhibition is shown to be relieved through a concerted two-step process beginning with the phosphorylation of tyrosine residues within the JM region. This initial catalytic event enables kinase activation loop autophosphorylation and facilitates the transition to an active conformation. Through isolating different phosphoforms of DDR1 we also show that JM phosphorylation alone is sufficient to drive a marked increase in substrate affinity (Km decreases 100 fold). Subsequent activation loop phosphorylation results in a five-fold increase in catalytic rate.

Discussion

These data identify the DDR1 JM region as a novel regulator of signalling, highlighting that phosphorylation of this region is the critical first step in DDR1 activation. This study will aid future structure-guided design of inhibitors that can specifically target DDR1 and which may have therapeutic applications.

#50 The miRNA miR-21a-5p targets SMAD7, and its inhibition decreases lung fibrosis in the mouse.

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Introduction
Idiopathic pulmonary fibrosis (IPF) is a debilitating disease of unknown aetiology. Life expectancy is around 5 years after diagnosis, and there is currently no treatment for the disease. Intra-tracheal administration of Bleomycin is the most used experimental model to recreate fibrosis in the mouse. microRNAs are small non-coding RNAs that operate an inhibition at transcript level by a binding on the 3'-UTR of mRNA coding region.
We found a significant 3-fold times increase in miR-21a-5p in the Bleomycin model. SMAD7 is one of the top predicted targets for this miR. The profibrotic cytokine TGFb induces SMAD signalling, resulting in increased matrix production. SMAD7 is a nuclear protein that inhibits the activation of the SMAD signalling cascade. The aim of the study was to investigate whether modifying miR-21a-5p expression results in changes in fibrosis in the Bleomycin mouse model.

Materials and Methods
Primary mouse lung fibroblasts (mLF) were treated for 48h with miR-mimic and antago-miR (resulting in upregulation and inhibiton of miR-21a-5p respectively). Gene expression was assessed using qPCRs.
Fibrosis was induced in mice *via* intra-tracheal instillation of Bleomycin. Levels of the microRNA miR-21a-5p were modified *via* intravenous injection of cholesterol-modified miR-mimic and antagomir, using a scrambled miR as control. Lung fibrosis was assessed by *in vivo* micro-tomography. Mice were sacrificed at day 18 of the experiment, and lung tissue harvested and analysed by qPCR and Sirius Red staining of histological sections.

Results
In the mLF the miR-Mimic reduced expression of SMAD7 of 20%, whereas treatment with the antago-miR resulted in a 35% increase. In the *in vivo* experiment, µCT showed decreased lung density in the antagomiR group compared to the control group, in contrast, treatment with the miR-mimic showed an increase and this was confirmed by histology. Treatment of the mice with the antagomir, lead to a 50% increase of SMAD7 expression, and a 60% decrease in Col1a1 expression.

Discussion
Our results show that miR-21a-5p inhibits SMAD7 mRNA levels. Furthermore , an antoginist of the miR reduced SMAD7 levels in vivo, and reduced fibrosis in the Bleomycin lung fibrosis model. Our results indicate that miR-21a-5p could be a target molecule for the treatment of lung fibrosis.**Title** The extracellular matrix critically influences tumor-fibroblast interactions in lung cancer

#51* Raman spectroscopy and second harmonic generation imaging reveal sexually dimorphic influence of osteoblast-derived VEGF on bone mineral and matrix composition.

(Podium Presentation: Tues 9th April, 2.40)

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Introduction

Vascular endothelial growth factor (VEGF) is crucial for coupling of angiogenesis with osteogenesis during development, growth and repair. Reductions in levels of VEGF has been linked to osteoporosis and increased risk of fracture[1]. Developmental differences exist between male and female skeletons, however the role of VEGF in this sexual dimorphism is poorly understood. Raman spectroscopy (RS) and second harmonic generation (SHG) imaging are sensitive, label-free techniques that facilitate characterisation of bone composition. In this study, these techniques were used to assess the influence of VEGF on collagen fibre organisation and hydroxyapatite arrangement in the matrix of male and female bone.

Materials and Methods

OB-derived VEGF was conditionally deleted (OBVEGFKO) in 16-week old male and female mice expressing floxed alleles of VEGF and Cre-recombinase under the osteocalcin promoter. Long bones were fixed, embedded in PMMA and sectioned at the tibiofibular junction. Spectral peak analysis highlighted VEGF effects on collagen (proline: 853cm^{-1}), B-type carbonate (1070cm^{-1}) and hydroxyapatite (960cm^{-1}) (n=75 spectra from 3 bone sections/gender/genotype). Fibre extraction analyses of SHG images enabled quantification of VEGF effects on collagen fibre width and length (n=9 regions of interest from 3 bone sections/gender/genotype).

Results

RS highlighted alterations in hydroxyapatite mineralisation in males and female OBVEGFKO mice versus wildtypes (WT) (-1.07; p=0.0294 and -1.27-fold; p<0.0001) in addition to modifications to crystallinity (-1.12; p=0.0311 and -1.02-fold; p=0.0165 respectively). Carbonation was reduced in female OBVEGFKO versus WT (-2.79-fold; p<0.0001) with the converse observed in males (+1.21-fold; p=0.002). Collagen species were 1.21-fold higher in males following OBVEGFKO (p=0.0001) versus WT however no significant differences were detected in females (OBVEGFKO vs WT mice; -1.06-fold; p=0.5830). Collagen fibre analyses revealed decreased fibre widths (-1.11; p=0.0945 and -1.09-fold; p=0.0189 respectively) and fibre length in OBVEGFKO males and females versus WT (-1.06; p=0.0395 and -1.08-fold; p=0.0462 respectively)

Discussion

Our data show OB-derived VEGF regulates mineral and matrix components in a sexually dimorphic fashion. Signatures obtained by RS and SHG imaging detailed alterations in hydroxyapatite levels and

crystallinity between genders, linking to modified collagen structure and organisation. Targeting the factors responsible for sex differences may provide a better means to treat age-related skeletal pathologies.

References

[1] Liu Y and Olsen BR (2014) Distinct VEGF Functions During Bone Development and Homeostasis. *Archivum Immunologiae et Therapiae Experimentalis* 62(5): 363-368.

#52 Utilising self-assembling peptide hydrogels for MSC mechanobiology research

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2: Biogelx Ltd, Glasgow, UK

Introduction

Understanding how bone marrow mesenchymal stem cells (BMMSCs) interpret and respond to mechanical stimuli is critical to understand the role of tissue mechanics on stem cell fate. Recent publications have highlighted the response of BMMSCs to the stiffness of the culture surface. On soft surfaces, BMMSCs have a small cell area, nuclear area and rounded morphology whereas on stiffer surfaces they have a large cell, nuclear area and spread morphology. BMMSC morphology is an early indicator of their phenotypic fate; rounded BMMSCs undergo adipogenesis and spread BMMSCs undergo osteogenesis. However, the synthetic polymer hydrogels used do not allow progression to more physiologically relevant 3D culture and rely on biochemical functionalisation for cell adhesion. To overcome these limitations with existing technologies, a self-assembling peptide (Fmoc-FF/S) hydrogel with tuneable stiffness was investigated to establish whether BMMSCs display the characteristic morphological response to stiffness in the absence of biochemical functionalisation (1,2).

Materials and Methods

Fmoc-FF/S hydrogels were created at set range of concentrations: 5-40mM. The storage and loss modulus of the hydrogels was analysed using oscillatory rheology. Gel layers were formed in Mattek dishes and seeded with primary human BMMSCs ($2.2x10^3/cm^2$). Viability was assessed after 24 hours using a fluorescent LIVE/DEAD assay. After 96 hours the BMMSCs were fixed, stain with Phalloidin 488 and Hoechst 33342 before fluorescent imaging. High throughput morphometric analysis was performed using CellProfiler to determine cell spread area, nuclear area and cell eccentricity.

Results

Oscillatory rheology confirmed storage moduli of the hydrogel between 0.5 and 30kPa. LIVE/DEAD staining confirmed equal viability and density of BMMSCs bound to all stiffness of the hydrogels. Morphometric analysis revealed an increase in cell area, nuclear area and eccentricity of BMMSCs cultured on increasing hydrogel stiffness despite the lack of biochemical functionalisation of the hydrogel.

Discussion

Together, these data highlight the biocompatibility of the peptide hydrogel for BMMSC culture and its suitability for future mechanobiology experiments with 3D culture.

#53 Laminin β1 with C-terminal Dendra2 fluorescent protein tag is inefficiently secreted from lung adenocarcinoma cells in culture.

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Introduction

Laminins are an essential structural component of the basement membrane and regulate a range of cell and tissue functions. However, laminin dynamics during basement membrane development, remodelling and turnover is difficult to observe in real-time at high resolution. Recent advancements in super resolution microscopy using photoactivatable or convertible fluorophores could allow these studies to be performed now. One such fluorophore is Dendra2, a photoconvertible monomeric protein. The non-reversible photoconversion from a green to red fluorophore could allow tracking of the movement of tagged laminins, as well as monitoring degradation and replacement rates simultaneously in two and three dimensional culture. A *C.elegans* ortholog of human laminin β1 tagged at the C-terminus with Dendra2 has previously been described. Here we aimed to determine whether this tag could be implemented into the human genome thereby achieving endogenous levels of expression of a laminin fusion protein.

Materials and Methods

CRISPR-Cas9 technology was used to edit the genome of A549 cells to express Dendra2 fused to the C-terminus of laminin β1. Single cell clones were isolated and expanded then screened by PCR, confocal and total internal reflection microscopy, and western blotting from whole cell extracts and conditioned media. Cell cycle analyses were performed using flow cytometry.

Results

A549 cells were successfully edited and western blotting of isolated clones revealed expression of LMβ1::Dendra2 fusion protein. However, secretion of the fusion protein could not be detected either by western blotting from conditioned media or by TIRF microscopy. Interestingly, edited cells displayed an 8% reduction in the proportion of cells in M phase of the cell cycle (LMβ1::Dendra2 18+0.4%, Wild-type 25+3.4%, $p<0.05$) indicating that the mutation had a cytotoxic side -effect on the division and growth of the cells.

Discussion

Overall, these data suggest that, unlike in *C elegans*, laminin β1 tagged at the C-terminus with Dendra2 is inefficiently secreted and is not a viable tool for dynamic basement membrane research

#53 The articular cartilage proteome is dependent on zone, age and disease state

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Introduction
Osteoarthritis (OA) is a major chronic age-related musculoskeletal disease, leading to pain and disability. Cartilage deterioration during OA is principally initiated from the tissue, thus stratifying the anatomical regions of articular cartilage and investigating them separately could add a new level of understanding of OA leading to therapeutic targets.

Materials and Methods
In this study we have divided donors into 3 groups: young (n=5, mean age=32), old (n=5, mean age = 71) and OA (n=5, mean age = 76). From each donor we collected superficial, middle and deep zones of knee articular cartilage using laser microdissection (LMD) technique, in total giving us 9 groups. Microdissected tissue was *in-situ* trypsin digested with the addition of RapiGest surfactant (Waters). Tryptic peptides from each samples were individually run using liquid chromatography-tandem mass spectrometry on an UltiMate 3000 Nano LC System coupled to a Q Exactive Quadrupole-Orbitrap instrument (Thermo Fisher). Progenesis™ QI for proteomics v4.0 was used for label-free quantification following protein identification using the Unihuman reviewed database in Mascot (Matrix Science), ANOVA values of $p<0.05$ and at least 2 unique peptides for protein identification were determined significant. Pathway analysis of differential abundant proteins was performed in IPA (Qiagen).

Results
Using LMD an average for superficial zone 5mm^2 of cartilage tissue was collected and for middle/deep zones 25mm^2 area was collected. In total 514 proteins were identified in this study. Label-free quantification revealed differentially abundant proteins in groups by age, OA and zones. Pair wise comparison of differentially abundant proteins in the superficial zone between young and old group demonstrated the 'activation of inflammatory response' ($p=7.47x10^{-6}$),' inflammation of joint' ($p=3.42x10^{-6}$) and 'apoptosis' ($p=6.96x10^{-5}$) pathways with ageing. Comparison of old and OA groups in this zone showed the 'activation of apoptosis' ($p=8.63x10^{-6}$) and 'cell death' ($p=9.28x10^{-9}$) in the OA group, whereas 'inflammatory response' ($p=1.35x10^{-4}$) was upregulated in the old group. Similarly to the superficial zone, the 'inflammation of joint' ($p=1.1x10^{-5}$) pathway was activated in the deep zone of old samples in comparison to young group. However, the deep zone OA group demonstrated the 'activation of inflammatory response' ($p=4.94x10^{-4}$) in comparison to the old group. Old group in contrast had 'activation in cell death' ($p=4.93x10^{-9}$) pathway.

Discussion:
In the current study we have demonstrated a difference in protein abundance dependent on zone within articular cartilage, and that during ageing and OA these proteins change. From the results of pathway analysis we suggest that the proteome in the superficial and deep zones in ageing are similar, as old group had activated state of inflammation pathway in these two zones. However, comparing old and OA groups there are major differences between superficial and deep zones. It seems that at the protein level in OA cartilage inflammation pathways were downregulated in the superficial zone but activated in the deep zone when compared to the old group.

#55 Development and validation of an inducible LaNt α31 overexpressing mouse model

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Introduction

LaNt α31 comprises a laminin N-terminal domain and short stretch of laminin-type epidermal growth factor like repeats. This structure is similar to the netrin family of proteins and suggests a role in signalling or influencing laminin network assembly. In vitro studies suggest a role in wound repair, cell migration and matrix deposition. Furthermore, IHC staining shows that LaNt α31 is redistributed during the wound healing process, and is upregulated in some cancers. In order to investigate the function that LaNt α31 plays during development, we have developed a tamoxifen-inducible, LaNt α31 overexpressing mouse model.

Materials and Methods

A UbC-LoxP-LaNt α31-T2A-tdTomato construct was generated and validated by transfecting the construct along with pCAG-Cre:GFP into HEK293A cells. Expression was confirmed by western immuno-blotting and fluorescence microscopy. Transgenic mouse lines were generated by pronuclear microinjection. To confirm transgene expression, fibroblasts were isolated from E13.5 embryos, and expression induced using adenoviral-delivery of Cre recombinase cDNA. Expressing mouse lines were mated with R26CreER mice. To confirm the transgene could be activated in vivo, pregnant mice were given tamoxifen at E13.5, and then cells were isolated from embryos at E19.5. PCR, western immuno-blotting, and fluorescence microscopy were used to confirm expression in isolated cells. Functional implications of LaNt α31 overexpression was investigated using scratch closure assays in vitro.

Results

Establishment of a UbC-LoxP- LaNt α31-T2A-tdTomato transgenic mouse line was confirmed via PCR, fluorescence microscopy, and immuno-blotting. Preliminary data suggests that primary dermal fibroblasts from mice expressing the transgene have a reduced capacity to close a scratch wound in vitro.

Discussion

This model allows us to overexpress LaNt α31 in any tissue, at any stage of development. This important model will allow us to investigate the role of LaNt α31 in normal and disease physiology, and to ask how this protein interacts with its environment to influence physiological processes.

#56 Differential impact of matrix stiffness on adhesion, proliferation and differentiation potential of osteogenic and myogenic progenitor cells

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Introduction
Satellite cells (SCs) and mesenchymal stem cells (MSCs) are progenitor cells with great potential for cellular therapies. *In vivo* these cells migrate to the sites of the tissue injury playing an active role in tissue repair and regeneration through secretion of chemokines and differentiation into "mature" cell types. Differentiation is initiated and directed by the cell sensing changes in local microenvironment including matrix elasticity. Previous studied have shown that MSCs express different cell lineage markers depending on the stiffness of the substrates on which cells grow. Here we evaluated the response of SCs and MSCs to variation in matrix stiffness ranging from 2.5 to 4 MPa.

Materials and Methods
$2x10^4$ cells/ml were seeded on fibronectin (FN) or matrigel pre-coated polydimethylsiloxane (PDMS) materials. Cell morphology and FN matrix were qualified by immunocytochemistry and confocal imaging while cell proliferation, the number of attached cells and osteogenic differentiation were quantified using CCK kit, Image J and Alizarin Red staining. Pappenheim staining was used to visualize SCs morphology and real-time PCR for expression of MyoD and myogenine.

Results
MSCs demonstrated a higher sensitivity to differences in the studied range of substratum stiffness than SCs. MSCs spread, produced FN and deposited calcium matrix in the greatest degree on PDMS with the lowest Young's modulus of 2.5 MPa while index of fusion and *MyoD* and *myogenine* levels of SCs were very similar on all PDMS materials. Proliferation rates of both cell types however were the lowest on PDMS with the lowest elastic modulus.

Discussion
Our results support the hypothesis that material stiffness influences *in vitro* proliferation and differentiation potential of progenitor cells in cell and substratum stiffness-dependent manner. PDMS material with elastic modulus of 2.5 MPa was optimal to induce osteogenic differentiation of MSCs while myogenic differentiation of SC cells was not affected significantly on the stiffness of PDMS materials in the studied ranges. This study highlights the potential of PDMS materials to be used as materials promoting regeneration of skeletal muscles and bone tissue.

#57 The extracellular matrix critically influences tumor-fibroblast interactions in lung cancer

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Introduction
Lung cancer is the leading cause of cancer-related deaths worldwide due to early metastatic spread and quickly acquired resistance to therapies. These processes can be critically influenced by cancer-associated fibroblasts and extracellular matrix (ECM). They were reported to enhance resistance to multiple drugs and increase tumorigenicity in a xenograft mouse model. However, an unbiased analysis of the dynamic changes in factors secreted upon tumor-stroma interactions was missing. In this study, we focused on the communication between cancer cells and fibroblasts in the context of varied ECM to gain insights into how these interactions drive tumor progression.

Materials and Methods
To better understand the influence of ECM on cell communication we characterized the lung cancer patient-derived ECM by mass spectrometry as well as developed an *in vitro* model for lung ECM deposition by lung fibroblasts cell line HFL1 and primary lung fibroblasts. Furthermore, we repopulated this produced and decellularized ECM with the lung adenocarcinoma cell line H1975 and HFL1 fibroblasts. Mass spectrometric proteome analysis combined with a newly developed cell type-specific proteome labelling method allowed us to distinguish the cell-of-origin of proteins in the supernatant.

Results
We identified multiple proteins differentially regulated between normal and cancer tissue-derived ECM suggesting tissue remodeling. Many of these differences could be recapitulated by producing ECM from fibroblasts with and without stimulation with Transforming Growth Factor β. Seeding cells on the ECM greatly influenced the levels of multiple proteins in the supernatants and cell lysates. Cocultures of cancer cells and fibroblasts altered the secretion of numerous factors including proteins related to tumor progression.

Discussion
These results suggest the existence of multiple feedback loops between cancer cells and fibroblasts. Such reciprocal interactions may further increase fibroblast differentiation and induce EMT in tumor cells that would facilitate tumor spread and lead to therapy resistance.

#58* A novel role for Syndecan-4 in Neovascular Eye Diseases

(Podium Presentation: Mon 8th April, 1.40)

Authors

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Introduction

Angiogenesis is a major process in the pathology of a number of eye diseases such as Wet Age Related Macular Degeneration and Diabetic Retinopathy. Considerable progress has been made in treating these conditions by targeting the pro-angiogenic cytokine VEGFA. Despite these successes there remain a number of issues, such as, patient none response to these therapies, highlighting the need for a better understanding of the underlying processes involved. In this work we identify a novel role for the proteoglycan syndecan-4 in angiogenesis and identify it as a downstream effector of VEGFA driven cellular responses.

Materials and Methods

We use a variety of methodologies both *in vivo* and *ex vivo.* We use important preclinical models of Wet AMD and diabetic retinopathy, and mice that are null for syndecan-4.

Results

We show that in preclinical models of neovascular eye disease syndecan-4 null animals show a substantially reduced angiogenic response and that this is linked to an impaired responses to VEGFA in endothelial cells from these animals. Analysis of the cellular localisation of syndecan-4 reveal a significant pool resides at endothelial cell junctions and that upon VEGFA stimulation syndecan-4 participates in trafficking VE-Cadherin away from these structures. Finally we show that targeting syndecan-4 in a model of Wet AMD leads to efficacious outcomes.

Discussion

This work identifies a novel role for Syndecan-4 in endothelial cells and may offer an alternative target to improve or replace existing therapeutic options for treating neovascular eye diseases.

#59* LaNt α31 influences cell adhesion and migration through modulation of laminin organisation and hemidesmosome maturation

(Podium Presentation: Mon 8th April, 4.50)

Authors

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Introduction

Laminin N-Terminus (LaNt) α31 is a relatively unstudied protein which shares structural motifs with the N-terminal domains of laminin α3b. LaNt α31 is upregulated during times of corneal wound repair, and modulating LaNt α31 expression influences migration speed and adhesion of epithelial keratinocytes, although the mechanism of these effects has not been determined. Based on its structural architecture, we predicted a role for LaNt α31 in matrix remodelling. To investigate this, we identified LaNt α31 expression levels in invasive epithelial-derived tumours where ECM-remodelling is evident, and upregulated its expression in cultured cells, investigating the effects on cell-matrix adhesive devices.

Materials and Methods

Formalin-fixed paraffin-embedded head and neck squamous cell carcinoma and breast adenocarcinoma sections were processed for immuno-histochemistry (IHC) using antibodies raised against LaNt α31. eGFP-tagged LaNt α31 over-expression was induced in tumour-derived or normal epithelial-derived cells, followed by migration and invasion assays. Indirect immuno-fluorescence (IF) microscopy and western immuno-blotting were used to assess changes in cell-matrix adhesive devices.

Results

LaNt α31 was upregulated in >80% of tumour cases analysed. Upregulation of LaNt α31 in tumour cells did not influence invasion into collagen I, however it did influence the way in which MDA-231 cells invaded into matrigel; instead of the characteristic multi-cellular streaming of control cells, LaNt α31 overexpressing cells invaded as individuals. In 2D culture, epithelial cells over-expressing LaNt α31 displayed increased cell spreading and reduced migration speed. Moreover, in live analyses of new matrix synthesis, LaNt α31 and LMβ3-mCherry co-distributed and were deposited together and immunoprecipitation confirmed complex formation. Immunofluorescence revealed changes to laminin α3β3γ2 organisation, where tight clusters were observed; with similar findings observed in the distribution of β4 integrin. Interestingly, these changes correlated with an increase in collagen XVII recruitment to the clustered β4 integrin and with an increase in laminin α3 processing; together pointing to early hemidesmosome maturation.

Discussion

Taken together, these data reveal that LaNt α31 can influence laminin organisation, LM-based adhesive devices, and invasion into a predominantly laminin-based ECM. These findings have implications for wound healing and tumour cell invasion/ metastases.

#60* Integrin αVβ6-EGFR crosstalk regulates bidirectional force transmission and controls breast cancer invasion

(Podium Presentation: Mon 8th April, 1.50)

Authors

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Abstract

The mechanical properties of the extracellular matrix within tumours control multiple cellular functions that drive cancer invasion and metastasis. However, the mechanisms controlling microenvironmental force sensation and transmission, and how these regulate transcriptional reprogramming and invasion, are unclear. Our aim was to understand how mechanical inputs are transmitted bidirectionally and translated into biochemical and transcriptional outputs to drive breast cancer progression. We reveal that adhesion receptor and growth factor receptor crosstalk regulates a bidirectional feedback mechanism co-ordinating extracellular matrix stiffening, force-dependent transcriptional regulation and invasion.

Integrin αVβ6 drives invasion in a range of carcinomas and is a potential therapeutic target. αVβ6 exhibits unique biophysical properties that promote force-generation and increase matrix rigidity. We employed an inter-disciplinary approach incorporating proteomics, biophysical techniques and multi-modal live-cell imaging to dissect the role of αVβ6-EGFR crosstalk on transmission of mechanical signals bidirectionally between the extracellular matrix and nucleus.

We show that αVβ6 expression correlates with poor prognosis in triple-negative breast cancer (TNBC) and drives invasion of TNBC cells. Moreover, our data show that a complex regulatory mechanism exists involving crosstalk between αVβ6 integrin and EGFR that impacts matrix stiffness, force transmission to the nucleus, transcriptional reprogramming and microenvironment rigidity. αVβ6 engagement triggers EGFR & MAPK signalling and αVβ6-EGFR crosstalk regulates mutual receptor trafficking mechanisms. Consequently, EGF stimulation suppresses αVβ6mediated force-application on the matrix and nuclear shuttling of force-dependent transcriptional coactivators YAP/TAZ. Finally, we show that crosstalk between αVβ6 & EGFR regulates TNBC invasion and therapeutic response *in vivo*.

We propose a model whereby αVβ6-EGFR crosstalk regulates matrix stiffening, but also the transmission of extracellular forces to the nucleus in order to co-ordinate transcriptional reprogramming and invasion. To exploit adhesion receptors and receptor tyrosine kinases therapeutically, it will be essential to understand the integration of their signalling functions and how crosstalk mechanisms influence invasion and the response of tumours to molecular therapeutics

#61 Lithium chloride triggers primary cilia elongation and inhibits hedgehog signalling in articular chondrocytes

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Introduction
In osteoarthritis (OA), the hedgehog signalling pathway is activated and promotes chondrocyte hypertrophy and matrix catabolism through the upregulation of ADAMTS-5 and MMP-13. Inhibiting this pathway reduces cartilage degradation in surgical models of OA. Hedgehog signalling requires the primary cilium, a microtubule-based organelle present on the majority of chondrocytes. The trafficking of hedgehog signalling proteins through the ciliary compartment is essential for pathway regulation. Recent studies indicate that lithium chloride (LiCl) is chondroprotective largely due to its antiinflammatory properties. LiCl modulates cilia structure in numerous cell types. We hypothesise that LiCl may also affect cilia-mediated signalling pathways in chondrocytes, like hedgehog, through the regulation of ciliary structure.

Materials and Methods
Articular chondrocytes were treated with 0-50mM LiCl for up to 24hrs. Immunocytochemistry and confocal imaging were used to measure primary cilia length and prevalence. Activation of the hedgehog signalling pathway in response to recombinant Indian hedgehog (r-Ihh) was quantified using real-time PCR for GLI1 and PTCH1.

Results
LiCl induced dose dependent primary cilia elongation such that mean cilia length was increased by 95% in response to 50mM LiCl. Cilia elongation was rapid, with the majority of growth occurring within the first hour resulting in an increased proportion of cilia with bulbous tips. Following r-Ihh treatment, the expression of GLI1 and PTCH1 was significantly increased by 5.22 and 4.23-fold respectively indicative of pathway activation. Co-treatment with LiCl inhibited this response in a dose dependent manner such that 50mM LiCl completely abolished pathway activation.

Discussion
These data show that LiCl stimulates rapid, dose dependent cilia elongation in primary articular chondrocytes and inhibits hedgehog signalling. Recent studies show that the modulation of ciliary structure can affect the organisation of proteins at the distal tip of the ciliary compartment resulting and disrupts ligand-mediated hedgehog signalling. We therefore propose that pathway inhibition may be linked to the effects of LiCl on cilia structure, future studies will investigate this by examining the effects of LiCl on the localisation of Kif7 and IFT81 at the ciliary tip. This study highlights the potential for targeting the ciliary structure as a novel therapeutic approach to modulate hedgehog signalling and matrix catabolism in OA.

#62 Biophysical and biological microenvironmental cues for tenogenic phenotype maintenance

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Introduction
In vitro expansion of tenocytes leads to phenotypic drift and loss of function. Different microenvironmental cues has been employed to maintain the phenotype of tenocytes. Herein, in this study we venture to assess the effect of macromolecular crowding and low oxygen tension.

Materials and Methods
Human tendons were kindly provided from University Hospital Galway, after obtaining appropriate licenses, ethical approvals and patient consent. Afterwards, tenocytes were extracted using the migration method. Experiments were conducted at passage 3. Optimization of MMC conditions was assessed using 50 to 500 μg/ml carrageenan (Sigma Aldrich, UK). For variable oxygen tension cultures, tenocytes were incubated in a Coy Lab (USA) hypoxia chamber. ECM synthesis and deposition were assessed using SDS-PAGE (BioRad, UK) and immunocytochemistry (ABCAM, UK) analysis. Protein analysis for Scleraxis (ABCAM, UK) was performed using western blot. Gene analysis was conducted using a gene array (Roche, Ireland). MINITAB (version 16; Minitab, Inc.) was used for statistical analysis.

Results
SDS-PAGE and immunocytochemistry analysis demonstrated that human tenocytes treated with the optimal MMC concentration at 2 % oxygen tension showed increased synthesis and deposition of collagen type I, which is the major component of tendon ECM. Moreover, immunocytochemistry for the tendon-specific ECM proteins collagen type III, V, VI and fibronectin illustrated enhanced deposition when cells were treated with MMC at 2 % oxygen tension. In addition, western blot analysis revealed increased expression of tendon-specific protein Scleraxis, while a detailed gene analysis illustrated upregulation of tendon-specific genes and downregulation of osteo-, chondro- and adipose genes again when cells cultured with MMC under hypoxic conditions.

Discussion
Collectively, results suggest that the synergistic effect of MMC and low oxygen tension can accelerate the formation of ECM-rich substitutes, which stimulate tenogenic phenotype maintenance.

#63 Insulin-like growth factor binding protein 6 (*Igfbp6*) as a gene expression marker of normal tendon phenotype in native tissue and cell culture systems

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Introduction
We have previously reported that insulin-like growth factor binding protein 6 (*Igfbp6*) expression is higher in normal tendon compared to cartilage and muscle tissue across species and could potentially serve as novel marker of differentiated tendon. Here we aimed to establish the alterations in Igfbp6 gene expression across different physiological states and *in vitro* systems.

Materials and Methods
Igfbp6 expression was determined by RT-qPCR in equine and human tendon samples. The impact of pathology was examined in torn human supraspinatus tendon (established pathology, n=13) and matched subscapularis tendon (early pathology, n=12) compared to normal hamstring tendon (n=8). The effect of development and ageing was evaluated in equine superficial digital flexor tendon (SDFT) in following age groups: 0-1m, 3-6m, 12-24m, 3-5y, 9-11y and 18-22y. Function-related differences were investigated by comparing SDFT (energy-storing tendon, n=5) and common digital extensor tendon (CDET, positional tendon, n=5) and two anatomically distinct regions within SDFT (compressional and tensional, n=5). Microarray profiles of rat tendon and cartilage cells isolated from tissue samples (primary) and subjected to culture in monolayer and three-dimensional systems were analysed for significant changes ($\log_2$ fold change $>\mp 0.5$, FDR <0.01, log-odds ratio of expression >0) in *Igfbp6* expression.

Results
Early pathology in human tendon was associated with decreased ($p<0.05$) *Igfbp6* expression comparing to healthy tendon. Expression of *Igfbp6* in compressional SDFT, displaying fibrocartilaginous morphology, was lower ($p<0.05$) than in tensional region. No difference was observed between SDFT and CDET. There was a trend for *Igfbp6* to increase between 0d and 12-24m of age in equine SDFT followed by stabilization at lower level (p=ns). Higher *Igfbp6* expression was demonstrated in tendon relative to cartilage cells in primary, monolayer and three-dimensional culture.

Discussion
Degenerative tendon and tendon with a transitional/fibrocartilage phenotype show clear changes in *Igfbp6* expression. Functional or age-related changes were not established. Moreover, it allows discrimination of tendon- and cartilage-derived cells *in vitro* independently of the culture condition. This data indicates that *Igfbbp6* is a robust tendon marker that may be used for the evaluation of tendon physiology and could facilitate guided differentiation of permissive cells towards functional tenocytes.

#64 VEGFA isoform switching in soft tissue sarcoma is associated with decreased survival

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Introduction

Soft-tissue sarcomas (STS) are a diverse group of mesenchymal malignancies that can occur almost anywhere in the body. The growth and dissemination of STS is dependent on angiogenesis. Vascular endothelial growth factor A (VEGFA) plays a central role in angiogenesis and is characterised by alternative mRNA splicing that generates three principal isoforms, $VEGFA_{121}$, $VEGFA_{165}$ and $VEGFA_{189}$. Our recent studies have shown VEGFA isoforms differentially promote metastasis and response to anti-VEGFA therapy in mouse pre-clinical models of STS. However, the role of differential expression of VEGFA isoforms in human STS is poorly understood.

Materials and Methods

VEGFA mRNA isoform expression in STS was investigated using data from The Cancer Genome Atlas SARComa (TCGA-SARC) RNAseq dataset. Patients were classified into clusters based on relative expression of $VEGFA_{121}$, $VEGFA_{165}$ and $VEGFA_{189}$ mRNA (Z-score of log2(normalised counts + 1)). Differential gene expression and gene set enrichment analysis (GESA) were used to identify significant differences in gene expression and enriched biological pathways between patient clusters. Differences in survival were calculated using the Kaplan-Meier estimation.

Results

Patients were classified into three clusters named VEGFA165_121, VEGFA165 and VEGFA165_189 after the most dominant isoforms expressed. Patients that clustered into the VEGFA165_121 isoform group with Myxofibrosarcoma (MYX) had particularly poor overall survival compared to patients in the rest of the isoform expression clusters (VEGFA165_121: 29.9 months, 95% CI 11.0 - 48.7, REST: 50.8 months, 95% CI 39.0 - 62.7, P=0.048). Gene set enrichment analysis results suggest that VEGFA165_121 MYX are highly proliferative and inflammatory with both PD-1 immune checkpoint (IC) signalling E2F target pathways enriched (FDR q-val ≤ 0.05). Interestingly, tumour mutational burden, hypothesised to be a biomarker for response to IC therapy, is also increased in the VEGFA165_121 MYX group (p-value ≤ 0.05). RNAseq analysis of our mouse model STS tumors expressing $VEGFA_{120}$ and $VEGFA_{188}$ also showed $VEGFA_{120}$ expression is associated with increased PD-1 and E2F signalling.

Discussion

In conclusion, we hypothesise the VEGFA165_121 MYX patient group may be responsive to IC inhibitors (PD1/PDl-1) and anti-VEGF/VEGFR combination therapy. Supporting data from independent clinical cohorts and pre-clinical models is now needed to validate our hypothesis.

#65 The role of the serine proteinase inhibitor SERPINA3 in chondrogenic differentiation

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Introduction
Chondrogenesis is a crucial process for the development of long bones, involving the condensation of mesenchymal stem cells (MSCs) in high density, promoting their differentiation into chondrocytes. Serpins are a superfamily of structurally similar serine proteinase inhibitors which also have roles unrelated to inhibition. Here, we investigate the role of serpins in MSC differentiation into chondrocytes.

Materials and Methods
Chondrogenesis was performed over 3, 7 or 14 days using standard pellet cultures in 15 mL Falcon tubes with defined chondrogenic medium. Gene silencing was conducted using small interfering (si)RNA transfection of MSCs, 48 hours prior to the induction of chondrogenesis. For RNA extraction, cartilage pellets were ground and isolated using Phenol/Chloroform followed by RNeasy column purification. RNA was reverse transcribed and subsequent gene expression was conducted by TaqMan real-time PCR. Cartilage was also fixed, embedded and sectioned prior to staining with Safranin-O to examine proteoglycan. Osteogenesis was performed using osteogenic differentiation medium in monolayer after siRNA transfection. Mineralisation was monitored by Alizarin Red staining while RNA extraction and gene expression analysis were carried out as described for chondrogenesis.

Results
SERPINA1 and *SERPINA3* were markedly induced during chondrogenesis. Silencing of the master chrondrogenic transcription factor, *SOX9*, abrogated the induction of both serpin genes. SiRNA targeting *SERPINA3*, but not *SERPINA1* reduced cartilage pellet size and Safranin-O staining. The induction of chrondrogenic genes *COL2A1*, *ACAN*, *MATN3* and *COMP* were all significantly reduced. A second siRNA with a different sequence was used to confirm the observed effect. For osteogenesis, silencing of *SERPINA3* increased matrix mineralisation and induced the osteogenic genes *RUNX2*, *BGLAP* and *ALPL*.

Discussion
Serpins are known to have roles outside of their inhibitory function, although little is known about their role in cartilage or chondrogenesis. These data highlight a role for SERPINA3 in an essential 'switch' mechanism, by directing MSCs down a chondrogenic lineage although the exact mechanism by which this occurs is still under investigation.

#66 Small GTPases Rap1b and Rab8a regulate integrin trafficking and activation of fibronectin binding integrins (α5β1 and αvβ3) to control angiogenic process

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Introduction
Integrin-mediated cell-matrix interactions contribute directly to tumour angiogenesis and metastatic dissemination. The fibronectin-binding integrin heterodimers, α5β1 and αvβ3, are up-regulated on angiogenic vessels and regulate angiogenesis. However, therapeutic agents targeting αvβ3 or α5β1 have elicited variable results both in the clinic and preclinical models. α5β1 and αvβ3 integrins exhibit distinct biomechanical and signalling properties and exert differential effects on cell migration. It is therefore necessary to determine the precise signalling mechanisms by which α5β1 and αvβ3 modulate cellular functions to control angiogenesis.

Materials and Methods
Adhesion complex enrichment coupled with quantitative proteomic analysis was used to identify changes to adhesion complex composition following treatment with inhibitory antibodies (L230 and Mab16) that differentially engage α5β1 and αVβ3 heterodimers. *In vitro* angiogenesis was investigated with tube-like structure formation and fibrin bead assay. Integrin and VEGFR2 recycling was measured with surface biotinylation/reduction method. Total and surface levels of integrins were assessed by WB and flow cytometry, respectively.

Results
Inhibition of α5β1 but not αVβ3 inhibits *in vitro* angiogenesis. Bioinformatic network and Gene ontology terms analysis of heterodimer-specific adhesions revealed Rab8a and Rap1b as key regulatory control points in αvβ3 engaged adhesion network. Depletion of either Rab8a or Rap1b significantly inhibited tube-like structure formation. KD of Rab8a resulted in increased surface levels of α5β1 but had no effect on surface levels of αvβ3 integrin. Recycling assay revealed that lack of Rab8a increased recycling of both FN binding integrins, however, only α5 internalisation rates were reduced leading to elevated levels of surface α5β1. Additionally, VEGF stimulation increased recycling of both α5β1 and αvβ3 but not VEGFR2. Rab8a KD increased unstimulated constitutive and VEGF-stimulated recycling of α5β1 and to lesser extent αvβ3. This negative regulation of integrin recycling is likely due to crosstalk with VEGFR2 signalling as VEGF treatment significantly reduced Rab8a activity. KD of Rab8a increased maximal integrin activation, whereas Rap1b KD didn't show similar effect.

Discussion
Recent studies show that the Rab8a activity is implicated in trafficking from trans Golgi to cell membrane, membrane dynamics and protrusion formation, all instrumental in cell migration. This study highlights novel role of Rab8a as a negative regulator of integrin recycling. We propose that during angiogenesis Rab8a controls surface availability of integrins, whereas Rap1b is responsible for integrin activation.

#67 Engineered recombinant collagens as substrates for cell adhesion

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Introduction

We have developed a new technology for the production in *E. coli* of recombinant proteins containing long collagen-like sequences. This technology is the result of our previous work on prophage and bacteriophage collagen-like proteins found in the genomes of several *E. coli* strains (Ghosh *et al*, 2012, *PLoS One* 7:e37872), which contain trimerization domains that help in the correct folding and increase thermal stability of engineered recombinant collagens. This technology provides a useful avenue for the manufacture of collagen-based polymers and has an intrinsic versatility for the design of collagen sequences targeting specific biomedical and biomaterial applications.

Materials and Methods

Recombinant collagen proteins were prepared by cloning and transformation of BL21 DE3 competent *E. coli* cells. Transformed cells were cultured on LB Agar plates supplemented with ampicillin or kanamycin according to the proteins. Pellet obtained from induced culture were processed for protein purification by means of nickelaffinity chromatography and size-exclusion chromatography. Purified proteins were characterized for their secondary structure and thermostability by circular dichroism spectroscopy. The different protein substrates activity was determined using Fibroblast spreading assays, testing different cell lines such as HT1080, HFF and others.

Results

We have demonstrated proof of principle that these engineered collagens are good substrates for cell adhesion and that improve cell proliferation *in vitro*. We show here cell adhesion data for different cell types on surfaces coated with recombinant collagens. These collagens are potentially attractive new biomaterials that could address some of the problems arising from using animal-derived collagens in tissue engineering applications.

#68 Can Raman Spectroscopy Detect Age-related Changes in Tendon Matrix?

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Introduction

Raman spectroscopy is able to provide a comprehensive profile of the chemistry of biomolecules within tissue in a non-destructive manner. The technique has generated much interest in the field of orthopaedics for detecting bone and cartilage pathology, however, few studies have investigated the utility of Raman spectroscopy in tendon tissue. Tendon pathologies are more prevalent in the older population and are likely related to the well-documented changes in matrix molecules with increasing age. This study aims to discover the Raman spectral features of different aged tendons and to create a putative link between spectral features and age-related matrix differences.

Materials and Methods

Human Achilles tendon samples from young (14-16 years, n=3) and old (65-81 years, n=3) donors and equine deep digital flexor tendons (young - 3-7 years, n=4; old - 21-24 years, n=4) were freeze-dried and crushed prior to Raman spectroscopy and biochemical analyses. Ten Raman spectra from each tendon sample were acquired from randomly selected locations and processed with a standardised spectral analysis procedure included scaling and baseline correction. Tendon composition was analysed by measuring collagen-linked fluorescence, non-enzymatic cross-links (pentosidine, glucosepane), enzyme-mediated crosslinks (hydroxylysyl-pyridinoline, lysyl-pyridinoline) in human tendon samples and collagen-linked fluorescence, total collagen content and sulphate glycosaminoglycans in equine samples.

Results

Raman spectral features differed between young and old tendons. Older tendons had smoother raw Raman spectra than the young tendons and consistently showed higher intensities at lower frequency peaks in baseline-corrected spectra. Biochemical analysis showed higher levels of collagen-linked fluorescence and non-enzymatic cross-links in old tendon tissue compared to young but no age-related differences in the enzyme-mediated crosslinks or collagen and glycosaminoglycans contents.

Discussion

Our data provide promising indications that Raman spectral features can distinguish between young and old tendons. These features may result from the increase in collagen glycation and accumulation of non-enzymatic crosslinks as measured in older tissue samples. Our next step is to investigate individual Raman peaks using principal component analysis and spectral deconvolution methods to better differentiate age-related spectral features and potentially link frequencies to specific molecular features.

#69 Macrophages stimulate a pro-fibrotic phenotype in orbital fibroblasts

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Introduction
Graves' orbitopathy (GO) is an autoimmune inflammatory disease, which leads to expansion and remodeling of the tissues at the back of the eye, creating significant visual impairment and often disfiguration. Orbital fibroblasts are key components of the pathology as they drive the tissue expansion and distortion through both adipogenic and fibrotic processes. Macrophages are thought to participate in the immunological stage of GO, but the detailed mechanisms are unknown. We previously showed that GO adipogenic and fibrotic phenotypes could be modelled in a 3D environment *in* vitro (collagen gels), using primary orbital human fibroblasts. Here, we used U937-derived macrophages to assess the effect of macrophages on fibroblast behaviour in 3D, including spontaneous adipogenesis, hyaluronic acid (HA) production, and contractility.

Materials and Methods
Human primary orbital fibroblasts from patient with Graves' orbitopathy or control donors were cultured in collagen gels in the presence of macrophages (derived from human monocyte line U937). Spontaneous adipogenesis was measured using Oil Red O staining. HA levels were assed using ELISA. Contractility was measured in a standard free-floating collagen gel contraction assay. Alpha-smooth muscle actin (αSMA) levels were evaluated using Western blot and quantitative microscopy. Down-regulation was achieved using a Dharmacon siRNA pool with Hiperfect reagent. Cells were imaged using a Nikon Eclipse Ti microscope.

Results
Macrophages did not significantly affect adipogenesis in orbital fibroblasts, but dramatically increased HA production and contractility, suggesting that they may promote primarily the fibrotic phenotype. While both TGF-β and PI3K inhibition reduced macrophage-induced HA production, macrophage-stimulated fibroblast contractility was only sensitive to PI3K inhibition. Interestingly, macrophages' stimulation of fibroblast contraction was found to be independent of αSMA expression but rather linked to an increase in cell protrusive activity.

Discussion
In conclusion, we show that macrophages stimulate HA production and contractile behaviour in orbital fibroblasts, suggesting they may play a key role in the fibrotic stage of GO. Interestingly, while signalling through PI3K appears to be one of the main drivers for the fibrotic phenotype, macrophages are able to trigger both HA production and contractility through non-canonical pathways, suggesting a potential for novel approaches to future treatment.

#70 Micromotion-induced neuroinflammation as a model of peri-electrode gliosis

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Introduction

Neuroprosthetic electrodes are routinely used in clinic to treat multiple diseases of the central nervous system including Parkinson's and Alzheimer's disease [1]. However, studies indicate that, in situ, mechanical traumas due to the insertion and micromotions of the electrode results in an adverse tissue response characterized by glial scar formation and electrode encapsulation [2]. In this study, we aim to develop an inflammatory model using fluid shear stress on neural cell populations to reproduce gliosis in vitro.

Materials and Methods

Using a parallel-plate flow chamber system, ventral mesencephalic mixed primary cells were exposed to different level of pressure-driven fluid flow allowing to apply a defined shear stress at either 0.1 or 0.5 Pa from 1 to 6 hours, to mimic micro-motions between the implant and the tissue. The cells were then kept in culture for 14 days before being assessed for gliosis hallmarks. The morphology and protein expression of neurons and glial cells were quantified by image analysis. qPCR and Western-blots were used to detect the expression level of neuroinflammatory proteins, along with the use of the DMMB assay and immunocytochemistry to quantify heparan and chondroitin sulphate changes.

Results

Data have shown that the applied shear flow leads to astrocyte reactivity and an overall pro-inflammatory environment. Micromotion stimulations have all significantly increased the GFAP protein expression, number and size of astrocyte cells, along with the up-regulation of glycosaminoglycan deposition and several neuronal pro-inflammatory marker expression.

Discussion

We developed an *in vitro* model using parallel flow shear stress that mimics damages at the interface of the neuro-electrode. This model will certainly be a precious tool for future researchers developing anti-inflammatory and anti-gliosis biomaterial approaches.

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Financial support was received from Science Foundation Ireland (SFI) and the European Regional Development Fund (Grant Number 13/RC/2073).

#71 Membrane tension orchestrates rear retraction in matrix directed cell migration

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Abstract

In development, wound healing and cancer metastasis vertebrate cells move through 3D interstitial matrix, responding to chemical and physical guidance cues. Protrusion at the cell front has been extensively studied, but the retraction phase of the migration cycle is not well understood. Here we show that fast moving cells guided by matrix cues establish positive feedback control of rear retraction by sensing membrane tension. We reveal a mechanism of rear retraction in 3D matrix and durotaxis controlled by caveolae, which form in response to low membrane tension at the cell rear. Caveolae activate RhoA signalling to control local F-actin organisation and contractility in this subcellular region and promote the translocation of the cell rear. A positive feedback loop between cytoskeletal signalling and membrane tension leads to rapid retraction to complete the migration cycle in fast moving cells, providing directional memory to drive persistent cell migration in complex matrices.

72 The role of Cryptochrome1/2 proteins in the regulation of TGF-β1 signalling pathway and their implication in tissue fibrosis

Authors

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Introduction

Chronic tissue scarring resulting from unresolved tissue repair contributes towards the leading causes of morbidity and mortality worldwide. One major research focus has been the analysis of the TGFβ-mediated pathways that control extracellular matrix synthesis (ECM). Recent studies demonstrate that genetic clock disruption in mouse models leads to increased susceptibility of tissues to fibrotic injury. Human and mammalian cells have an intrinsic biological clock, driven by a conserved set of core clock genes and proteins that form positive/negative feedback loops. Here, we investigated the role of the negative loop components of the clock, Cryptochrome 1/2 (CRY1/2) proteins, in the regulation of TGFβ target genes implicated in fibrosis.

Materials and Methods

The expression of TGFβ target genes was assessed in peripheral tissues from wild type (WT) mice over 24h using qPCR. Primary mouse embryonic fibroblasts (MEFs) from WT and Cry1/2 KO mice (a genetic model of arrhythmic clock) were used to test the expression and rhythmicity of matrix genes following clock-synchronisation. Matrix gene promoter activity was assayed using dual luciferase reporter. Pharmacological or genetic Cry1/2 rescue were used to assess matrix genes following TGFβ stimulation. The effects of siRNA-mediated knockdown of Cry1/2 was tested in human cell line using qPCR and real-time bioluminescence imaging.

Results

We show rhythmic expression of several TGFβ target genes implicated in fibrosis in the lung of WT mice. Elevated endogenous expression of target matrix genes was observed in Cry1/2 KO MEFs. TGFβ stimulation led to further induction of matrix genes in Cry1/2 KO MEFs, which was prevented using canonical TGFβ pathway inhibitors. Pharmacological stabilisation of CRY proteins reduced TGFβ-stimulated induction of matrix genes in WT MEFs. Consistently, *Cry1* siRNA-mediated knockdown in human cells exerted more profound effects on the TGFβ target genes compared to *Cry2.* Finally, we observed downregulation of Cry1/2 genes in the lungs of fibrotic mouse models.

Discussion

These results demonstrate that *Cry1/2* represses expression of target matrix genes in a TGFβ-dependent manner and that fibrotic conditions lead to reduced *Cry1/2* rhythms. Thus, *Cry1/2* may play an important role in the negative feedback regulation of TGFβ pathway during resolution of tissue repair.