HEALIKICK Translation Session / Glasgow Orthopaedic Research Initiative

Thursday 9th May 2024

Wolfson Centre (WC203) | Department of Biomedical Engineering |University of Strathclyde | 106 Rottenrow East | Glasgow | G4 0NW

09:00 Registration/coffee

PROGRAMME

Session 1

(Chaired by Dr Monica Tsimbouri)

09:25 Welcome
Dr Monica Tsimbouri and Dr Miguel Pineda

09:30 Cardiac function with elevated blood cobalt levels
Mr Mark Jenkinson, NHS GGC

10:00 Orthopaedic Infection and need for Regenerative Strategies
Mr Dave Shields, NHS GGC

10:15 Metastatic Bone Disease: Stem Cell Perspective
Mr Peter Young, NHS GGC

10:30 HEALIKICK overview
Prof Manuel Salmeron-Sanchez, University of Glasgow

10:45 Tea & coffee

11:00 HEALIKICK Translation Session

13:00 Lunch & posters

Session 2

(Chaired by Dr Miguel Pineda)

14:00 The Implications of the Human Cell Atlas for Musculoskeletal Diseases
Professor Christopher Buckley, University of Oxford

14:45 Finite Element Simulation of Osteogenic Vibration Transmission via External Fixation Devices
Dr Peter Childs, University of Strathclyde

15:00 Biofilm Growth over Medical Devices in Response to Targeted Antimicrobial Treatment
Parna Mandal, University of Glasgow

15:10 Modelling Human Immune Responses to Functionalised Biomaterials
Jessica Roberts, University of Glasgow

15:20 Bioengineering 3D Leukemic Niches To Develop Stem Cell Therapies
Angelos Tsigkos, University of Glasgow

15:30 Extracellular Vesicle Bioactivity and Clinical Utility is Determined by Mesenchymal Stromal Cell Clonal Subtype
Dr Savvas Ioannou, University of York

15:40 Towards new ways of understanding bone formation and osteocyte function
Prof Natalie Sims, St Vincent's Institute of Medical Research, The University of Melbourne

16:00 Closing remarks

Poster Presentations

1. **Julia Isakova**
   Advancing RA Diagnosis: Linking Cell Glycome Changes to Disease Phenotypes via Raman Spectroscopy
2. **Dr Rosalia Cuahtecontzi Delint**
   Nanovibration Driven Chondrogenesis
3. **Zarina Issabekova**
   Tuneable Microgels for Guiding Cellular Response in Tissue Repair
4. **Emily Maxwell**
   Advanced Viscoelastic 3D Bioprinted Alginate/DNA Scaffolds for Stem Cell Engineering
5. **Dr Piaopiao Pan**
   Undenatured Collagen Protects Against Experimental Arthritis by Rewiring Gut-Joint Immune Networks
6. **Sarah Eccles**
   Antibiotic Elution Profile and Antimicrobial Efficacy of a Polymer-Rifampicin Coating for Prevention of Orthopaedic Implant Infections
7. **Dr Hussain Jaffery**
   An Axis of Wnt and Proinflammatory Signals Underlies Mechanically Driven Osteogenesis
8. **Yusuf Ayten**
   Bioengineering Surfaces to Maintain Mesenchymal Stromal Cell Self-Renewal In Vitro
9. **Alexandra Ligeti**
   Validation of a IMU wearable sensor for treadmill walking and stair ascent/descent
10. **Dr Jonathan Williams**
    Metaphyseal Trabecular Bone Separation is Bimodal

ORAL PRESENTATIONS

Cardiac function with elevated blood cobalt levels

Mark Jenkinson

NHS GCC

Aims

Elevated blood cobalt secondary to metal-on-metal (MoM) hip arthroplasties is a suggested risk factor for developing cardiovascular complications including cardiomyopathy. Clinical studies assessing patients with MoM hips using left ventricular ejection fraction (LVEF) have found conflicting evidence of cobalt-induced cardiomyopathy. Global longitudinal strain (GLS) is an echocardiography measurement known to be more sensitive than LVEF at diagnosing early cardiomyopathies.

Methods

Sixteen patients with documented blood cobalt ion levels above 13µg/l (13ppb, 221nmol/l) were identified from a regional arthroplasty database. They were matched with eight patients awaiting hip arthroplasty. All patients underwent echocardiography, including GLS, looking for signs of cardiomyopathy. Rats were exposed to cobalt treatment.

Results

Patients with MoM hip arthroplasties had a mean blood cobalt level of 29µg/l (495nmol/l) compared to 0.01µg/l (0.2nmol/l) in the control group. GLS was significantly reduced in 7 patients with MoM hip arthroplasties compared to 6 without (-15.5% v -18%, (MoM v control) p= 0.025). Pearson correlation demonstrated that GLS is significantly correlated with blood cobalt level (r= 0.8521, p=0.0002). However, there was no difference or correlation in other echocardiography measurements including LVEF (64.3% v 63.7% (MoM v control) p=0.845). Rats exposed to chronic (28-day) cobalt treatment (1mg/kg) developed ventricular fibrosis and cardiac contractility was impaired in rats exposed to chronic cobalt treatment.

Conclusions

Our studies support the hypothesis that patients with elevated blood cobalt above 13µg/l in the presence of a MoM hip implant may have impaired cardiac function compared to a control group of patients awaiting hip arthroplasty. It is the first study to use the more sensitive parameter, GLS, to assess for any cardiac contractile dysfunction in patients with a MoM hip implant and a normal LVEF.

Orthopaedic Infection and need for Regenerative Strategies

Dave Shields[1],[2]

[1]Consultant Trauma and Orthopaedic Surgeon, Queen Elizabeth University Hospital Glasgow
[2]Honorary Clinical Associate Professor, University of Glasgow

Musculoskeletal infection is a devastating complication following trauma or extremity surgery, with rates between 2% & 30% reported. Many factors are associated with an increased risk of infection, however, despite contemporary techniques and improved anti-microbial strategies infection remains a plague to the orthopaedic surgeon. In particular, the presence of infection (typically bacterial but occasionally fungal) in proximity to a surgical implant ('periprosthetic infection'), usually renders the implant unsalvageable. Aggressive surgical and antimicrobial management results in a deteriorated function, loss of native tissue, large resource investment and high risk of reinfection. Multiple strategies are in place to mitigate for orthopaedic infection, primarily focussing on prevention, however limited solutions exist for the regeneration of tissue loss.

Metastatic Bone Disease: Stem Cell Perspective

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Improvements in cancer treatments have improved patient survival, however, certain cancers have a predilection for affecting the bone, causing pain, immobility and fracture. We will discuss the improvements in cancer survival and the challenges facing treatment of metastatic bone disease, with a view to research targets and opportunities in the future.

The implications of the Human Cell Atlas for Musculoskeletal Diseases

Christopher Buckley

Kennedy Professor of Translational Rheumatology, Director of Clinical Research
Kennedy Institute of Rheumatology; University of Oxford

Career summary: I obtained a degree in Biochemistry from the University of Oxford (1985) with subsequent undergraduate training in Medicine (MBBS) at the Royal Free Hospital, London (1990). My postgraduate medical training was in General Medicine and Rheumatology at the Hammersmith Hospital, London (Mark Walport, Dorian Haskard), and John Radcliffe Hospital, Oxford. I obtained a DPhil arising from a Wellcome Trust Clinical Training Fellowship with John Bell and David Simmons at the Institute Molecular Medicine, Oxford in 1996.
Funded by a Wellcome Trust Clinician Scientist Fellowship, I joined the Department of Rheumatology in Birmingham later that year. In 2001 I was awarded an MRC Senior Clinical Fellowship and in 2002 became Arthritis Research UK Professor of Rheumatology. In 2012 I was appointed Director of the Birmingham NIHR Clinical Research Facility.
In May 2017 I took up a new joint academic post between the Universities of Birmingham and Oxford at the Kennedy Institute of Rheumatology Oxford and Director of NIHR Infrastructure in Birmingham for Birmingham Health Partners to Direct the Arthritis Therapy Acceleration Programme(A-TAP). In September 2021 I moved to the Kennedy Institute of Rheumatology Oxford as Director of Clinical Research

Lecture: While we know in exquisite detail the anatomical structures that make up human organs and all the genes in the human genome, rather embarrassingly we do not know how many different cell types make up a human. Establishing the cellular basis for disease is a major challenge for drug discovery in the 21st century. Unlike haematological diseases where the gene (Hb), cell (red blood cell) and clinical features (anaemia) map well onto each other, the cellular basis for most inflammatory diseases remains enigmatic. The Human Cell Atlas (HCA) has a key role to play in solving this problem [1].
The HCA is an international consortium, established to construct a map of the different cell types involved in forming all human organs. Its aim is to provide physicians with a “medical periodic table” analogous to the “chemical periodic table”. Clinicians, scientists, and those involved in designing new drugs now have a golden opportunity to contribute to and benefit from the insights that the cartographers of the HCA are delivering [2]. This approach has real potential to design better drugs by providing access to a high- definition maps of human tissue as opposed to our current low-level histological maps used to define and classify human pathology especially in musculoskeletal diseases [3].

Reading

1. https://www.humancellatlas.org/
2. *Cross-tissue, single-cell stromal atlas identifies shared pathological fibroblast phenotypes in four chronic inflammatory diseases*. Korsunsky I, Wei K, Pohin M, Kim EY, Barone F, Major T, Taylor E, Ravindran R, Kemble S, Watts GFM, Jonsson AH, Jeong Y, Athar H, Windell D, Kang JB, Friedrich M, Turner J, Nayar S, Fisher BA, Raza K, Marshall JL, Croft AP, Tamura T, Sholl LM, Vivero M, Rosas IO, Bowman SJ, Coles M, Frei AP, Lassen K, Filer A, Powrie F, Buckley CD, Brenner MB, Raychaudhuri S. *Med* . 2022 May 26:S2666-6340(22)00184-2 oi: 10.1016/j.medj.2022.05.002. PMID: 35649411
3. A roadmap for delivering a human musculoskeletal cell atlas. Baldwin M, Buckley CD, Guilak F, Hulley P, Cribbs AP, Snelling S.Nat Rev Rheumatol. 2023 Nov;19(11):738-752. doi: 10.1038/s41584-023-01031-2. Epub 2023 Oct 5.PMID: 37798481

Finite Element Simulation of Osteogenic Vibration Transmission via External Fixation Devices

Aaminah Shafi,[1] Peter G. Childs, [1] Jonathan Williams, [1] Matthew J. Dalby[2] , Stuart Reid[1]

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Fractures of the long bones, are common, with an incidence in England of roughly 45 per 10,000 population per year [1]. Non-union fractures in particular pose a challenge to clinicians due to the requirement of additional measures, including further surgery. The use of external fixation is one such measure which can be relevant for long bones such as the tibia, femur or humerus. However, fixation must balance mechanical support with delivery of natural levels of strain, which can drive bone growth and remodelling. Frost's Mechanostat theory, indicates strains in excess of 2000 microstrain (με) are required to drive this process [2]. At the cell level, mechanical stimulation of osteogenic cells can drive osteogenic differentiation and mineralisation. We have previously shown this to be the case with nano-amplitude (circa 30 nm) vibration when delivered in the 1 kHz range [3].

This study aims to establish the feasibility of delivering an osteogenic nanovibration stimulus into the fracture region of a long bone, seeking to stimulate osteogenesis of endogenous cells. Specifically, vibration could be conducted into the tissue through the pins of an existing external fixator (e.g. Ilizarov style). Using finite element modelling (FEM), modal and harmonic analysis of a simulated fixator and leg bone were performed using ANSYS Workbench (Fig 1). The model was used to investigate the levels of deformation and strain which could be delivered via the fixation device, looking in particular at the bone callus region. The impact of fixation pin diameter and vibration actuator placement were also explored for vibration transmission.

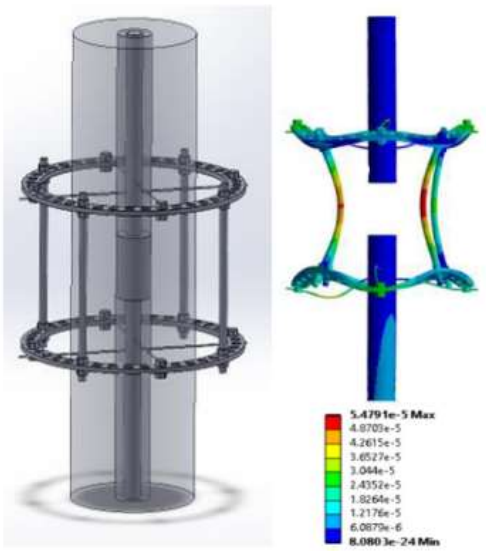

Figure 1: FEM of Ilizarov fixator attached to a fractured long bone along with deformation output stemming from applied vibration.

It was found that it is feasible to delivery osteogenic levels of strain into the callus region under certain conditions. Specific bone pin diameters, e.g. 1.5 mm diameter, could deliver 2260 με to the callus region when vibration was applied to the wire-fixator connection bolts. Higher levels of strain may be possible depending on resonance characteristics of the system. Further research is required in order to establish the impact of biological and fixator variability (e.g. using CT scan data), but this study provides a positive first step to explore a potential new therapeutic approach to fracture repair.

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Biofilm Growth over Medical Devices in Response to Targeted Antimicrobial Treatment

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Bacteria can be free-floating or adhering to a surface, producing biofilm communities. Biofilm formation is a multi-stage dynamical process with initial attachment of the proliferative bacteria, production of an extra polymeric substance (EPS) that helps to stabilise the biofilm, maturation where we see interchange between different bacterial phenotypes such as proliferative, persister and finally due to various reasons we get dead bacteria which for some time can act as a nutrient source, and possibly, detachment. The majority of natural settings contain biofilms made up of different species, whereas single-species biofilms are much less prevalent and typically only occur in specialised infection sites, including the surfaces of medical implants. Implant infection is a serious clinical problem, with treatment usually involving systemic delivery of antibiotics. However, due to the ability of bacteria within biofilms to survive antibiotic dosages that would ordinarily kill free-swimming proliferative bacteria, biofilm infections are extremely difficult to eradicate. Antibiotic resistance and tolerance confound the problem, often associated with nutrient insufficiency, hypoxia in the deeper layers of biofilm and antibiotic concentration at levels above the Minimum Inhibitory Concentration (MIC) [1]. An alternative approach is to delivery antibiotics locally in a sustained manner. In this study, we present a mathematical model of biofilm growth subject to antibiotic delivery, with the aim of understanding how the biofilm growth and composition depends on the drug dose and release rate.

We have formulated a 1D biofilm growth model in which we introduce controlled antibiotic release directly from the implant. Infection can take hold if the antibiotic release is insufficient to stop bacterial growth, but excessive drug release could hinder the healing of healthy tissue around the implant. This is an example of a delicate balance that can be investigated and optimised by mathematical modelling. A more effective biofilm prevention technique might be achieved by modelling the growth of the biofilm while concurrently optimising the antibiotic dose and release rate. The model consists of different bacterial phenotypes, self-produced extra cellular polymeric substance (EPS), nutrient concentration, water volume fraction in the biofilm pores, growth of the biofilm and a porous implant filled with antibiotic [2]. We gained a better knowledge of drug delivery for the prevention of infection through the simulation of the two moving boundaries in the model, which were the drug level inside the implant's pores and the thickness of the biofilm, respectively. Additionally, we have run simulations to see how certain model factors, such as nutrient content, affect the development of various bacterial phenotypes. The composition and time-course of biofilm formation are impacted by various antibiotic-release techniques from the polymer-free nanoporous implant [3]. This model takes into account both natural and antibiotic-induced mortality of living organisms. As would be predicted, the density of proliferating bacteria reduces with increasing antibiotic dose and increases as one moves away from the implant, where the antibiotic is being supplied from. Since the proliferative bacteria undergo a phenotypic change into the persister bacteria in order to survive the antibiotic dosage, persister bacteria, one of the primary causes of antibiotic resilience, rises with increasing antibiotic dose.

As the thickness of the biofilm and the number of proliferating bacteria cells decrease with increasing antibiotic dosage, our model shows that carefully regulating the antibiotic release could aid in preventing biofilm growth for implant-associated infection. The model is able to depict experimentally observed resilience to antibiotic shown by persister cells. Our next step would be to identify the best antibiotic administration arrangement so that the infection and persister cells are eliminated, which will prevent any subsequent infections on the implant.

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Modelling Human Immune Responses to Functionalised Biomaterials

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Introduction: Bone healing remains a significant challenge for reconstructive orthoplastic surgeons, reflecting bone's slow healing time, the morbidity of currently available treatment options and the lack of available donor sites for larger defects1. Major advances have shown functionalised biomaterials capable of promoting osteoblast differentiation from mesenchymal stem cells (MSCs) to promote bone regeneration2,3. The clinical feasibility of using these MSC biomaterials for bone reconstruction depends on a thorough understanding of the interplay between potentially allogenic donor MSC biomaterials and the recipient human adaptive immune system. Humanised in vitro models are increasingly used in the pre-clinical trial phase of biomaterial testing. We aimed to establish a novel, in vitro, humanised model of T cell activation to test responses to MSC biomaterials and allow for immunomodulation experimentation.

Materials & Methods: The biomaterial comprises a poly(ethyl acrylate) coating, functionalised with fibronectin with BMP-2, with an MSC layer which is differentiated over 4 weeks to form osteoblasts. This has shown efficacy in murine models and a canine veterinary trial, achieving bone healing in critical defects2,3. Peripheral blood mononuclear cells were isolated from healthy human volunteers and T cells were 'primed' with aCD3/aCD28 activator. Subsequently, they were rested before co-culture with biomaterials. T cell activation was investigated using flow cytometry. Experiments were carried out in technical replicates and for multiple biological donors, including both male and female sex.

Results & Discussion: Positive and negative controls demonstrated model efficacy with appropriate T cell responses to no further stimulus or restimulation with aCD3/aCD28 activator. Utilising the model to test human T cell responses to the biomaterial showed significant CD25 activation responses by 5 days coculture with the undifferentiated MSC biomaterial ($p=0.0365$). This was seen by 3 days with the differentiated osteoblast condition ($p=0.0036$), leading to greater surface activation (CD25, ICOS, PD1). Preliminary immunomodulation approaches using material-driven methods involved changing the biomaterial protein from fibronectin to laminin. Repeat modelling demonstrated significant T cell responses to both biomaterials but with no significant difference driven by change in glycoprotein.

Conclusions: We have established a novel, in vitro humanised model of T cell activation that can be used to test different cellularised biomaterials and immunomodulation. Future work will characterise the T cell cytokine milieu around these biomaterials and model the impact of the proinflammatory microenvironment on response. Ultimately, we aim to use the modelling to demonstrate material-driven immunomodulation at the material-immune cell interface on the path to clinical translation.

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Bioengineering 3D Leukemic Niches To Develop Stem Cell Therapies

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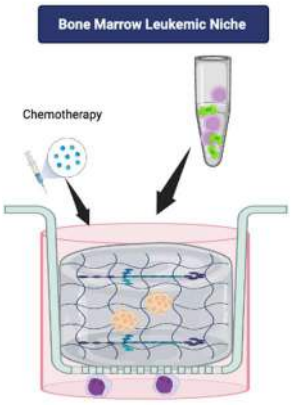
Introduction: Leukemia is a group of hematopoietic malignancies that can be mainly classified to myeloid or lymphocytic according to the cell from which the disease was originated while acute or chronic depending on the stage of the disease (1). Acute myeloid leukemia is the most lethal and most prevalent among all the leukemic types while patients that are relapsed or refractory for standard chemotherapy can only benefit from hematopoietic stem cells transplantation (HSCT). However, even then, up to 55% of the patients will experience disease relapse while after this their prognosis is poor (2, 3). HSCs are the regulators of hematopoiesis nevertheless, mutations can lead to their differentiation arrest, malignant expansion, and leukemogenesis (4). All the currently available *in vivo* models fail to recapitulate the tumor microenvironment comprehensively (5); therefore, the development of humanized models might confer a greater potential.

Materials & Methods: In this project we culture bone marrow hMSCs in aggrewell plates as spheroids, followed by their encapsulation in soft laminin-biofunctionalized polyethylglycol gels. THP1 (leukemic cells) are added outside of the insert which contains the gel, allowing the remodeling of the niche via their secretory profile. The leukemic niche is treated with daunorubicin chemotherapy to investigate its effects on both the THP1s and spheroids. Assessment of viability of the spheroids was performed via live/dead staining while the viability of leukemic cells was assessed via Alamar blue, and MTS. ELISA was used to characterize the secretory profile of the spheroids after the addition of the THP1s. Immunofluorescence was used to investigate the protein expression as well as morphology of spheroids.

Results: Preliminary data have shown that the addition of THP1s does not affect the viability of the spheroids. However, the THP1s induce the disaggregation and deformation of spheroids. The spheroid activation results in disaggregation, which allows single cells to escape as well as more elongated spheroids which tend to secrete more ECM proteins such as fibronectin and collagen. Furthermore, the addition of THP1s, upregulates the secretion of IL-6, the most prominent marker of chemoresistance in the leukemic niche. The addition of chemotherapy does not affect the viability of the spheroids due to their reduced proliferation and perhaps because of their quiescent phenotype. Nevertheless, it dramatically affects the viability of the THP1s. The presence of spheroids contributes to the development of chemoresistance in the niche, potentially via the secretion of IL-6. Finally, it has been observed that the addition of leukemic cells educates the MSC spheroids towards an adipogenic phenotype as well as to the upregulation of stemness associated markers such as the nestin. Moreover, a



Schematic representation of the leukemic model.

downregulation on the tumour suppressor p53 can be observed potentially because of the spheroid activation.

Discussion: Overall, we believe that the development of humanized models can mimic more precisely the tumour microenvironment at different stages of the disease. Moreover, the development of a model that accurately recapitulates leukemogenesis as well as leukemic progression can facilitate the reliable screening of novel anticancer therapies. More importantly, we propose that the current model has the potential for development of personalized models that could be used for the testing of targeted therapies. Future experiments will focus on the addition of HSCs in the leukemic niche followed by assessment of their phenotype.

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Extracellular Vesicle Bioactivity and Clinical Utility is Determined by Mesenchymal Stromal Cell Clonal Subtype

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Introduction

Mesenchymal stromal cells (MSCs) have been used in numerous clinical trials but very few reach phase 3. Progress has been hampered largely by the use of non-clonal, heterogeneous and uncharacterised MSC cultures and lack of mechanistic understanding. There is limited evidence of MSC engraftment in vivo and disease resolution may be the direct result of the paracrine effects of the MSC secretome, rather than the cells per se. Extracellular vesicles (EVs) are key components of the MSC secretome and there is growing interest in the use of EVs as cell-free therapies. EVs may be able to offer a more cost-effective route to clinic compared to their parent cells, with improved safety profiles and amenable transport, storage and administration options. However, similar to MSCs, heterogeneity within any therapeutic EV pool will hamper clinical development. Here we used immortalised clonal MSC lines, termed Y201 and Y202, to examine how the source MSC phenotype influences EV character and function. Y201 and Y202 were isolated from the same donor and identify as “MSCs” by surface protein expression and independent transcriptomic profiling. Y201 are model mesenchymal stem cells with strong trilineage differentiation and immune-suppressive properties, whereas Y202 MSCs have weak differentiation capacity and appear to function primarily as immune-regulatory cells.

Materials and Methods

EVs were isolated by ultracentrifugation and characterised by nano-sizing, ultrastructural morphometric analysis (diameter, perimeter, area, roundness), western blotting, mass spectrometry (proteome and lipidome) and miRNA screening. Bioactivity was determined by phosphorylation of ERK1/2, proliferation (live imaging and single cell tracking; cell counts, confluency, dry mass and doubling times), suppression of activated T cells, T cell polarisation and in vivo models of peritoneal inflammation and adjuvant-induced arthritis (AIA).

Results and Discussion

EVs isolated from Y201 and Y202 MSCs had similar peak average sizes, morphological characteristics and lipidomic profiles, however, Y201 EVs were more abundant in ALIX, flotillin-1, CD63 and CD81 versus Y202 MSC EVs, with an enhanced miRNA content (10 versus 2 significantly elevated, $q<0.05$) and EVome (68 versus 2 significantly increased proteins, $p<0.01$). Computational analysis of the Y201 EVome identified significant enrichment in matrix-associated proteins that were predicted to contribute to an elaborate EV corona particularly

abundant in the RGD-containing proteins fibronectin and MFG-E8, which was confirmed by western blotting.

Both Y201 and Y202 EV subsets significantly reduced proliferative index scores of activated T cells and Y202 EVs also reduced number of T cell proliferative cycles. Y201 EVs, but not Y202 EVs, caused a significant increase in the development of anti-inflammatory Th2 cells from naïve T cells as indicated by intracellular IL4 staining. We demonstrated that Y201 EVs, but not Y202 EVs, significantly increased the proliferation of articular chondrocytes in a dose-dependent manner, and that the proliferative effect of Y201 EVs was mediated at least in part via an RGD (integrin)-FAK-ERK1/2 axis. In models of inflammatory peritonitis, Y201 EVs, but not Y202 EVs, suppressed the accumulation of peritoneal exudate cells including a reduction in macrophage and neutrophil recruitment to the peritoneal cavity. Finally, in a disease-relevant AIA model we demonstrated that intra-articular injection of EVs derived from Y201 MSCs induced a significant decrease in all measures of disease activity compared to vehicle controls, including joint swelling, synovial infiltrate, joint exudate, synovial hyperplasia and overall arthritis index. Y202 EVs significantly reduced joint swelling compared to controls but otherwise did not affect any other disease score measures.

Conclusions

EVs released by closely related MSC subtypes within the same heterogeneous population differ significantly in terms of cargo abundance, bioactivity, and pre-clinical in vivo efficacy. Analysis of defined EV subsets will aid mechanistic understanding and prioritisation for EV therapeutics.

Towards new ways of understanding bone formation and osteocyte function

Natalie A Sims

St. Vincent's Institute of Medical Research, and Department of Medicine at St. Vincent's Hospital, The University of Melbourne, Australia

Bone formation occurs throughout life, and contributes to the establishment of peak bone mass, the maintenance of the skeleton during bone remodelling, and the repair processes required for fracture healing and implant integration. However, the region-specific mechanisms that control bone composition, including the way collagen and mineral are deposited during each of these processes, are poorly defined. These are the questions that my laboratory focuses on, using genetically altered mouse models, cell culture studies, and archived forensic bone specimens.

In this talk, I will introduce my laboratory's work by providing a brief overview of some of our current major directions of research. This will include our approaches to: (1) understand the difference in control of bone formation between periosteal (muscle-facing) and endosteal (marrow-facing) surfaces, (2) understand the process of cortical bone consolidation through the study of genetically-mutated mice, (3) investigate how osteocytes modify the composition of their surrounding bone matrix, and (4) determine whether older men and women produce bone with an inherent defect in its composition and strength through the use of archived samples in the Melbourne Femur Research Collection. I hope that this overview will provide food for thought, a tool for conversation while I am in Glasgow, and perhaps result in initiation of new collaborations.

POSTER PRESENTATIONS

Advancing RA diagnosis: Linking cell glycome changes to disease phenotypes via Raman spectroscopy.

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Introduction:

Rheumatoid arthritis (RA) is a degenerative autoimmune disease, affecting approximately 80 million people worldwide. The main target is the joint, inducing pain and stiffness, but disease present highly heterogenous pathophysiology, extraarticular symptoms and response to drugs. Around 5-20% of patients are deemed difficult to treat, due to unresponsiveness to medication and progression of symptoms. These differing disease phenotypes are likely to be the cause for different degree of response to treatment.

Healthy synovial fibroblasts are responsible for maintaining ECM of synovial fluid in the joint. However, in RA, synovial fibroblasts become activated and undergo epigenetic changes to perpetuate local inflammation by releasing inflammatory mediators and recruiting immune cells to the joint. The molecular pathways controlling fibroblast activation are not fully understood. Previous work in the lab showed that reduction of sialic acid (SA) upon TNF exposure is sufficient to transform healthy fibroblasts into activated cells. This may be related to the biological role of surface SA-containing glycoconjugates in cell-cell interaction and signalling.

This study will observe the changes in synovial fibroblast glycome, focusing on levels of SA, and link it to varying RA phenotypes. For this, Raman spectroscopy will be applied to investigate whether RAMAN signatures of activated synovial fibroblasts correlate with cell activity and inflammatory stages. As Raman is fast, label-free and non-destructive to samples, it could provide a novel tool for differentiating between different RA phenotypes, based on the amount of SA present on cells.

Materials and Methods:

Human fibroblasts from synovial and non-synovial tissues were used as a model to investigate the presence of sialic acid; immunofluorescence (IF) and in-cell western (ICW) assay were performed. Biotinylated *Sambucus Nigra* lectin was selected as a primary detection reagent due to its high affinity to sialic acid. It was added to the samples alongside DAPI and Streptavidin Alexa Fluor 555 for IF, and CellTag 520 and IRDye 800CW Streptavidin stains for ICW assay. For IF, all cells were permeabilised with 0.05% PBS Triton.

Furthermore, literature was reviewed for Raman applications in sialic acid detection and inflammatory conditions. To provide initial proof of concept, the proteins fetuin and asialofetuin (latter without sialic acid) were analysed with Horiba Raman spectrometer, using 532nm laser wavelength.

Results and Discussion:

Our results confirmed the presence of sialic acid on fibroblasts, exhibiting fluorescence on cell membrane and Golgi apparatus, in line with the presence of sialic acid on the outside and inside of the cells. Future work will include experiments with lectins showing complementary affinities,

in resting and in vitro activated fibroblasts. Additionally, fibroblasts from more cell lines will be tested in aforementioned conditions.

Raman spectroscopy shows potential to improve diagnostic process of RA, as it offers initial signs of differentiating between complex molecules. The obtained spectra of the compounds were very similar with a difference of an intense peak observed around 1000cm^{-1} in fetuin, which could be indicative of chemical bonds present in sialic acid. Further testing by analysing pure sialic acid would be able to confirm this.

Nanovibration Driven Chondrogenesis

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Cartilage is avascular and so suffers poor healing and unattended cartilage damage results in osteoarthritis (OA), the most prevalent chronic pathology of joints. OA costs the UK £13B pa including indirect costs such as days lost from work; which makes novel strategies for OA a research priority.(1) Current therapies such as autologous chondrocyte implantation (ACI) or matrix-assisted ACI (MACI) have been a focus on cartilage regeneration for >25 years. Success is limited as cultured chondrocytes from human mesenchymal stromal cells (hMSCs) require Transformong growth factor beta (TGFb) to regain phenotype and this results in deep cartilage (collagen X rich) rather than hyaline cartilage (collagen II rich) phenotype in culture, therefore approaches to substitute TGBb in the clinic are needed. The Centre for Cell Microenvironemnt (CeMi) have previously used nanovbration as a mechanotransduction cue to differentiate hMSCs into bone cells. Here, we want to direct the differentiation of Stro1+ enriched hMSCs into chondrocytes that produce matrix rich in collagen II and low in collagen X, i.e. a hyaline phenotype. The process consists in seeded the hMSCs as a dense micro mass on the well plate to mimic 3D conditions (**Figure 1**). After 21 days, the level of chondrogenesis was measured at the gene level using qPCR and at protein level using western blot and immunofluorescence to look for chondrogenic markers SRY-box transcriptor factor (SOX9), collagen II, collagen X, and aggrecan. Additionally, histology staining such as safranin O has been performed on the cultures. The obtained results so far have shown the possibility of using nanovibration to direct chondrogenesis at 21 days in a donor and age dependent manner. More studies are being carried out to optimise the system.

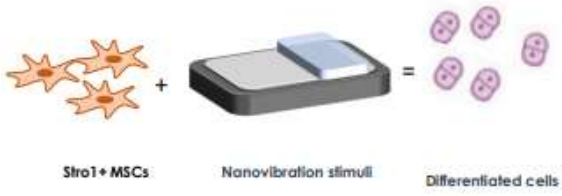

Figure 1. Schematic representation of the method used to induce chondrogenesis using nanovibration as a mechanotransduction cue.

Acknowledgements: The EPSRC for funding this project.

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Tuneable Microgels for Guiding Cellular Response in Tissue Repair

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Introduction: Controlled delivery of therapeutic proteins, such as growth factors, is a highly promising treatment strategy in wound healing and deteriorative conditions, such as non-union bone defects [1]. The primary obstacle in successful protein therapy lies in the lack of injectable carriers that are both biocompatible and capable of delivering proteins directly to the affected area with optimal bioactivity and controlled release rates. Hydrogels, highly hydrated 3D cross-linked polymer networks, have been studied as potential protein carriers that can mimic the properties of the extracellular matrix in native tissues. The encapsulation of bioactive molecules within the hydrogels would allow for the targeted and sustained delivery to the site of defect or injury, providing enough time for the tissue to heal and stimulating the tissue growth and cell differentiation. Injectable microgels with highly controlled biomechanical properties and protein release mechanisms can be developed, allowing a minimally invasive administration in the site of injury [2]. Microgels can be produced by using microfluidics approaches, that allow the high-throughput production of size-controlled, spherical and monodisperse microparticles [2]. Natural collagen- and synthetic poly(ethylene) glycol (PEG)-based scaffolds are promising biomaterial candidates for the microgel production for the applications such as bone and cartilage tissue repair [3]. The aim of this project is the development of controllable and robust natural and synthetic microgel platforms for the sustained protein delivery and modulation of cellular response by designing collagen-based (COL) and maleimide-functionalised (PEG-MAL) microgels, via droplet-based flow-focussing microfluidics, with tuneable mechanical properties and degradation rates.

Materials and Methods: Microfluidic devices with flow-focussing geometry and 200 µm nozzle size were produced by casting PDMS onto a silicon-based wafer. COL microgels are produced by crosslinking collagen with 8arm-PEG via NHS-ester chemistry in a 2:1 ratio using fluorinated oil as a continuous phase. PEG-MAL microgels are synthesised by crosslinking PEG-MAL macromers with PEG-dithiol via a Michael-type addition chemistry in a 1:1 ratio, at a buffer pH of 5.6, using mineral oil as a continuous phase. The polymer and crosslinker solutions are pumped inside the microfluidic device using syringe pumps through designated inlet channels and collected from a serpentine-like outlet channel. The droplets are then washed from the oil suspension and analysed for the size distribution and mechanical properties via nanoindentation. Protein encapsulation is carried out by incorporating the labelled protein in the crosslinking phase, and the release is evaluated by analysing the supernatant in a plate reader. Different strategies are investigated with the aim of increasing the sustained release of the encapsulated protein. Cell viability on the microgels is assessed on the mesenchymal stem cells (MSC) using a LIVE/DEAD assay, and cell adhesion is evaluated via imaging.

Results and Discussion: COL and PEG-MAL microgels were successfully synthesised inside a microfluidic device with a diameter of 200±20 µm and 100±20 µm, respectively, and FITC-BSA was successfully encapsulated inside the microgels and bulk hydrogels. The protein release was

evaluated at different time points for 5 days for the COL system. The protein encapsulation in microgels entrapped in a bulk hydrogel provided a granular network that improved the sustained release compared to the direct encapsulation in the bulk hydrogel. MSC viability and proliferation on the COL bulk 2.5D system was performed in preparation for the in vitro studies involving the COL microgel system, followed by the assessment of viability on microgels. Conclusions: We are developing a tuneable microgel platform for the encapsulation of proteins and guiding cellular response, involving collagen- and PEG-based scaffolds, via microfluidics, where the protein release and MSC behaviour can be precisely controlled by fine-tuning the microgel composition and size. Fig 1. FITC-BSA protein encapsulation and release in the COL microgel system.

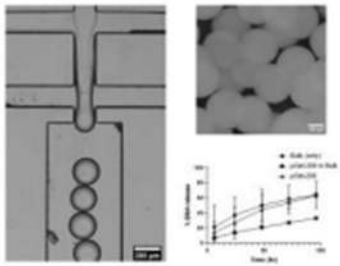

Fig 1. FITC-BSA protein encapsulation and release in the COL microgel system.

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Advanced Viscoelastic 3D Bioprinted Alginate/DNA Scaffolds for Stem Cell Engineering

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Introduction:

Extrusion 3D bioprinting is a method of scaffold fabrication that allows for predesigned scaffold structure with the combined ability to include multiple ink and cell types in one construct. This technology offers patient specific tissue models and enables the production of complex architectures, therefore enhancing biomimicry of tissue specific structure and function. The bioinks required for 3D bioprinting using extrusion-based methods must have certain properties such as having shear thinning behaviour, a temperature dependent viscosity and ability to form a gel after printing. Alginate is a natural polysaccharide that can be used in bioprinting and subsequently ionically crosslinked to form a gel, however pure alginate inks are not able to be printed due to their low viscosity at printing temperatures and their inability to maintain shape fidelity post printing. In this work we hypothesise a method of thickening unprintable alginate using DNA with consequential removal of this thickener post printing and crosslinking using DNase, leaving a pure self-supported tissue scaffold. By utilising the different molecular weights of alginate and DNA, a family of printable bioinks with different viscoelasticity's will be obtained to ultimately create bioinks that can support and drive stem cell differentiation, closely mimicking the mechanical properties of native tissue.

Materials and Methods:

DNA was isolated from *E. Coli* Nissle following the mini prep protocol. The extracted DNA was then reconstituted in illusion buffer to then be used to the stock solution, and was stored at 4° C. The alginate DNA gels were fabricated by gently mixing different ratios of alginate and DNA before inserting into a syringe.

Rheological sweeps were used to assess the viscosity of the gel solutions with increasing shear rates up to 300m/s repeated for differing temperatures (37°C - 25°C).

Results and Discussion:

Alginate DNA bioinks were shown to have a shear thinning behaviour and a temperature dependent viscosity, offering initial signs of suitable use as bioinks in extrusion 3D bioprinting. The precipitation of DNA when mixed with the alginate results in an inhomogeneous gel solution, so the inclusion of restriction enzymes to reach the length of the DNA molecule will be investigated.

Undenatured Collagen Protects against Experimental Arthritis by Rewiring Gut-Joint Immune Networks

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Keywords: UC-II, glycosylation, oral tolerance, experimental arthritis, gut immunology.

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disorder affecting synovial joints. Collagen-derived products have been used to induce oral tolerance and undenatured Type II collagen from Lonza (UC-II®), which preserves the physiological structure of collagen fibres, has shown an enhanced ability to induce tolerance, although its protective mechanism are not yet fully understood. Most research on its therapeutic potential in RA has focused on the modulation of immune and tolerogenic responses in the gut, while less attention has been given to stromal cells within the local microenvironment. However, our recent investigations of the gut-joint axis hypothesis suggest that the pathophysiology of RA is dependent on changes in the mucosal tissue, such as barrier integrity and microbiome diversity.

We thus, hypothesized that the protective effects of UC-II are associated not only with the induction of oral tolerance, but also with the restoration of the gut tissue integrity. Here, we used the mouse Collagen-Induced Arthritis (CIA) model as a surrogate for RA and investigated UC-II-mediated protective mechanisms. Mice were divided into naïve, CIA, and CIA+UC-II groups and incidence of pathology and associated clinical scores were monitored throughout, whilst gut and joint pathology were evaluated at the end of the study period.

Our results confirmed the ability of UC-II to ameliorate inflammation and even reduce the incidence of joint disease. Thus, pro-inflammatory cytokines, such as IL-17 and IL-22, were found to be down-regulated in the joints by UC-II and such treated mice displayed distinct cytokine and cellular networks, involving Th17 cytokines, immune system and stromal cells, relative to their CIA controls. Moreover, whilst mice undergoing CIA showed severe gut damage, this was not seen in the UC-II group which also exhibited a rewired gut glycosylation profile and gut microbiome composition.

Collectively, these results increase our understanding of mechanisms underlying oral tolerance induction by UC-II, paving the way for novel therapeutic interventions targeting glycosylation/microbiota-related pathways in RA.

Antibiotic elution profile and antimicrobial efficacy of a polymer-rifampicin coating for prevention of orthopaedic implant infections

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Introduction: Periprosthetic implant infection develops weeks to months after orthopaedic surgery. Infections caused by bacterial biofilms are difficult to treat, with revision surgery often necessary. This delays recovery and prolongs hospital stays, meaning new solutions for prevention are critical. Antibiotic-eluting biodegradable polymeric coatings are a potential solution, but they require optimisation. This study characterised the long-term *in vitro* release profiles and antimicrobial efficacy of rifampicin and poly(lactic-co-glycolic acid) (PLGA) coatings.

Methods: Three ratios of PLGA:rifampicin (50:50, 60:40, and 75:25) were dip-coated onto stainless steel samples and submerged in PBS (37°C) for 16 weeks. Elution was measured by UV-vis spectrophotometry. A subset of 50:50 coated samples were removed from PBS at selected elution time points and incubated in *Staphylococcus aureus* suspension at 37°C for 24h. Adhered bacteria were removed by sonication and enumerated by standard microbiological methods.

Results: All coatings demonstrated distinct elution behaviour ($P<0.05$). The 50:50 and 60:40 coatings displayed a 'burst' release within the first 24h, while the 75:25 had very little release before 28 days, after which a slow release was observed. Release from the 50:50 coating was >99% complete by 7 days while 60:40 and 75:25 continued to release up to at least 16 weeks. Preliminary analysis suggests that the release is dominated by diffusion, although other mechanisms may also have an influence. Bacterial inhibition >99% was maintained up to 12 weeks by the 50:50 coating, demonstrating bacterial attachment is significantly inhibited ($P<0.05$) by surface rifampicin even when elution has mostly completed.

Conclusions: The developed coatings provide a means of tuning drug release kinetics, which with further optimisation, may provide protection against infection that can be tailored to particular clinical scenarios. This can be informed by in silico modelling work which is now underway, opening up future opportunities for better prevention of infections in orthopaedic implants.

An Axis of Wnt and Proinflammatory Signals Underlies Mechanically Driven Osteogenesis

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Introduction | Musculoskeletal conditions, affecting approximately 1.71 billion individuals worldwide, stand as the predominant cause of disability globally. Mesenchymal stem cells (MSCs), naturally found in diverse bodily reservoirs, serve as multipotent progenitors for osteoblasts, the cells responsible for bone formation. The process of osteogenic differentiation, leading to the formation of bone, is influenced by a myriad of factors, including the response of cells and the entire organism to biomechanical forces. In our research, we have innovated a nanovibrational bioreactor, a cutting-edge biomechanical approach for directing MSCs towards bone formation. This technology has demonstrated significant efficacy in promoting targeted osteogenesis with high precision in preclinical models. However, the detailed molecular mechanisms underlying this process are yet to be fully elucidated. Our current research explores the impact of nanovibrational stimulation on the osteogenic Wnt signaling pathway, a critical component in bone development.

Materials & Methods | Adipose-derived human MSCs were cultured on our novel bioreactor, delivering nanovibrational stimulation of 30 nm vertical displacement, at 1000 Hz in basal medium ("nano-kicked"; NK) compared to controls stimulated with the osteogenic metabolites (OGM) L-ascorbic acid, b-glycerophosphate and dexamethasone, or controls kept in basal conditions with no stimulus (Ctrl), for 1 to 21 days. Parsing of various pathways involved incorporating Wnt inhibitors, including LGK974, XAV939, AMBMP hydrochloride, CHIR99021, alongside Rock inhibitor Y-27632 and BCL3 mimetic peptide BDP2. Relative gene transcription and protein expression studies were carried out to determine cell molecular responses.

Results | (**A)** An axis of proinflammatory, non-canonical Wnt pathway and osteogenic genes all have higher transcriptional expression in nanovibrated stem cells, compared to metabolite-induced cells. (**B**) Treating nanovibrated cells with Wnt pathway inhibitors XAV939 (targeting canonical-Wnt via Tankyrase) and LGK974 (targeting pan-Wnt secretion via Porcupine) reveals divergent gene transcription signatures – generally, LGK974 reduces expression of genes in the axis, while XAV939 increases axis gene expression. (**C**) In the presence of nanovibration, a BCL3 mimetic peptide (inhibitor of NF-kB signalling, associated with non-canonical Wnt) inverted the transcriptional signatures produced by XAV939 and LGK974 inhibition. (**D**) Further studies inhibiting the Wnt pathway with AMBMP and CHIR99021 recapitulated earlier observations that the non-canonical Wnt pathway is integral to forming the axis of osteogenic differentiation. (**E**) Transcriptional observations throughout were validated with protein expression and use of Rock inhibitor Y-27632 demonstrated functional reversal of nanovibrational signal. (**F**) In complement to hMSCs, BCL3 knockout murine monocytes abrogated the increase in axis genes observed in wild-type cells during nanovibration-driven osteoclastogenesis. While nanovibration itself did not increase osteoclast number or size, XAV939 treatment of BCL3 knockout cells had a dramatic increase in osteoclastogenesis – exemplary of the axis of proinflammatory, non-canonical Wnt in bone cell differentiation.

Discussion & Conclusions | Broadly, a cell signalling axis comprised of non-canonical Wnt signalling, proinflammatory cytokine signalling and NF-kB regulation determines stem cell

osteogenic and osteoclastogenic differentiation in response to nanovibrational biomechanical force.

Bioengineering Surfaces to Maintain Mesenchymal Stromal Cell Self-renewal *In Vitro*

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Introduction

Mesenchymal stromal cells (MSCs) have been identified as a promising candidate for use in cell therapies due to their unique properties such as multipotency (tissue regeneration) and immune-modulation (reducing transplant rejection)[1]. MSCs have the ability to differentiate into various cell types such as osteoblasts, adipocytes, chondrocytes, and fibroblasts [1,2]. However, in order to use them for cellular therapy, many millions of cells from a single donor are required for allogeneic approaches [3]. Further, maintaining their immunomodulatory and multipotent phenotype is of critical importance during cell expansion. Unfortunately, while growing these cells in cell culture, a common challenge is encountered. MSCs easily differentiate into other cell types (phenotypical drift) or growth exhaustion *in vitro* [3]. This is because the cell culture differs significantly from the natural environment of the MSCs, their niche. It is widely known that MSCs are greatly influenced by their environment [4]. In the bone marrow (BM) niche, MSCs have intermediate levels of intracellular tension, while osteoblasts have higher tension levels and adipocytes have lower tension levels [4]. In light of this understanding, we hypothesise that the growth of MSCs can be supported *in vitro* by modifying the cell culture environment, and in turn, obtain a higher yield of high-quality MSCs. Here, we use laminin, an extracellular matrix protein that has been shown to promote cell adhesion while lowering intracellular tension, organised by polyethylacrylate (PEA) [5,6,7]. PEA is a polymer that has been shown to organise extracellular matrix proteins in a more biomimetic manner [8,9].

Materials and methods

To optimise cell culture conditions, the culture surfaces are coated with PEA. PEA was applied by plasma polymerisation of the monomer, ethylacrylate, onto polystyrene culture plates. Stro-1 selected MSCs were cultured on the surfaces for a certain time depending on the technique. Following culture, qPCR transcript analysis was used to quantify senescence-associated phenotypes (SASP) like p16, p21, p53, IL-6, and TNF-a, and In-Cell Western protein analysis was used to quantify caspase3 and caspase6. Further, immunofluorescent microscopy was used to study nuclear lamina components, especially Lamin A/C.

Results and Conclusion

According to the results obtained from the different techniques used, MSCs exhibited better growth on PEA-laminin compared to on tissue culture plastic. They expressed less age-related markers and exhibited lower levels of lamin A/C, a marker of MSC phenotype. The data provides evidence that better conditions, that draw on the natural cell interactions with their extracellular matrix, can be used to produce better cells for cell therapies. The mechanism behind the process remains to be investigated.

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Validation of a IMU wearable sensor for treadmill walking and stair ascent/descent

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Introduction:
100,000 total knee arthroplasty (TKA) procedures take place in the United Kingdom annually [1], and 94% of these procedures occur in individuals 50 years and older [2]. The need for home-based rehabilitation is high, however, compliance is low [2]. MotionSense™ (Stryker, US) is a wearable technology that remotely supports post-operative TKA rehabilitation by continuously monitoring knee motion remotely. This allows for personalised patient rehabilitation. However, validation of such devices against a gold standard measure across a broad range of activities of daily living is important for confident interpretation of resulting clinical data. The aim of this study therefore was to validate the accuracy of MotionSense™ against Vicon, a clinical motion capture standard.

Methods:
Thirty-four healthy, able-bodied adults attended a laboratory session (Younger: n=20, age 24 ± 4 years, mean ± SD; Older: n=14, age 71 ± 5 years). Movement was tracked using Vicon motion analysis (100Hz) and MotionSense™ (~50Hz) wearable sensor. Plug-In-Gait lower body model was applied to determine knee flexion angles, while the sensor exported data in real time to a mobile device on which a proprietary algorithm determined knee angle. To time synchronise the technologies, MotionSense™ data were up-sampled to 100Hz, and cross-correlated. After a 1-minute acclimatisation period, 10 gait cycles were manually determined using heel strikes identified from foot marker trajectories via a bespoke graphical user interface. As the zero point for knee flexion depends both on marker and IMU placement, a bias was applied to the MotionSense™ data to account for any differences in calibration. The root mean square error (RMSE) between the technologies was determined. T-tests compared the older and the younger populations and significance was taken at the 5% level.

Results:
For both age groups and for all activities the RMSE remained below 3° (Table 1, Figure 1A). No difference between older and younger participants was evidenced, despite older volunteers walking significantly slower than the younger volunteers (0.94 ± 0.12 ms-1 vs 1.17 ± 0.07 ms-1 , $p < 0.001$). The combined RMSE for all adults was 2.4° for walking, 2.7° for the stair ascent, and 2.59° for the stair descent.

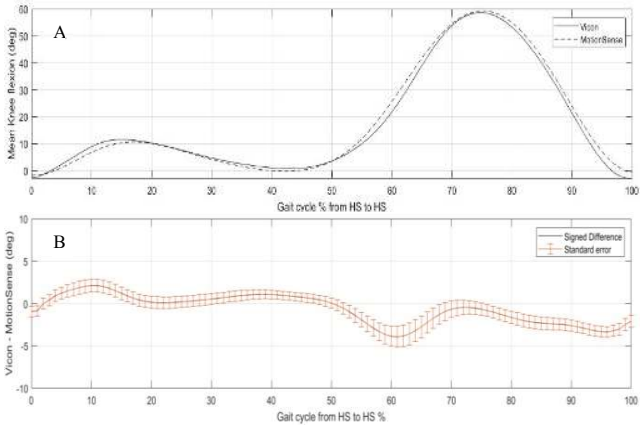

Figure 1. A) Comparison between the averaged Vicon and MotionSenseTM

Discussion:

The signed error increased during the swing phase of gait (Figure 1B). This may be due to high frequency transients. RMSE values indicate the MotionSense™ platform performs better in comparison to comparable systems [3,4]. MotionSense™ does not require any form of calibration from the user, and the algorithm out-performed one method which involved functional self-calibration movements [3].

Table 1. RMSE between Vicon and MotionSense™ for treadmill walking, stair ascent/descent for younger and older populations

	Younger	Older	Pooled
	RMSE (°)		
Walking	2.41 (0.85)	2.39 (0.68)	2.40 (0.77)
Stair Ascent	2.77 (0.83)	2.60 (0.96)	2.70 (0.88)
Stair Descent	2.41 (0.77)	2.83 (0.99)	2.59 (0.88)

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Metaphyseal Trabecular bone separation is Bimodal

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Micro-computed tomography is the gold standard methodology for skeletal phenotyping of small animal models of bone disease. Specifically, it can quantify the 3-dimensional morphology of trabecular bone. Metaphyseal trabecular bone, within load-bearing long bones, is the most common site of assessment. The 2010 guidelines paper for the assessment of bone microstructure recommends that a minimal set of four parameters should be used to describe trabecular bone morphometry[1]. These are bone volume fraction (BV/TV) and trabecular number (Tb.N), thickness (Tb.Th) and separation (Tb.Sp), calculated using the maximal sphere fitting method[2].

Here we showcase, on two separate species (mouse and rat) and two different osteoporosis models (spinal cord injury-induced and ovariectomy-induced) at two distinct anatomical locations (proximal tibia and distal femur), that metaphyseal Tb.Sp is bimodal (Figure A-C). We propose that Tb.Sp should be reported as two distinct values, preliminarily named Tb.Sp1 and Tb.Sp2, and that further detailed information on the type of osteoporosis can be gleaned from quantification of the standard deviation, skew and kurtosis of the distributions (Figure C). Furthermore, the easily implementable methodological tweak to existing methods will be provided. We propose that this bimodality is primarily the consequence of two bone remodelling events: i) thickening and/or thinning of trabeculae and ii) marrow cavity expansion. This methodological update should enable a more sensitive distinction of skeletal phenotypes.

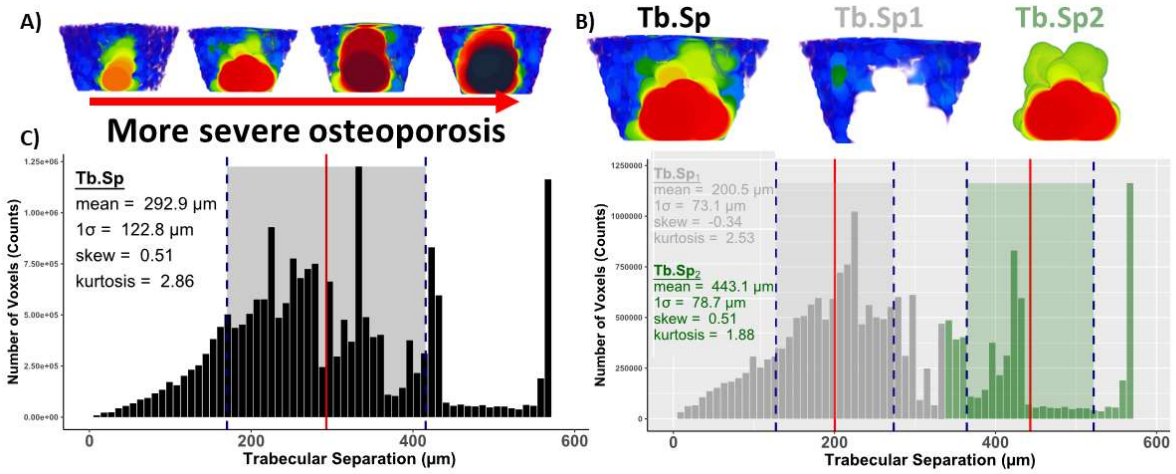

Figure 1. Trabecular bone is bimodal. A) Trabecular bone separation colour thickness maps, illustrating progression of marrow cavity expansion, compared to trabecular thinning, as a mechanism for trabecular separation increase, in spinal cord injury models of osteoporosis with increasing severity. B) Comparison of intra-trabecular separation (Tb.Sp1), compared to marrow cavity separation (Tb.Sp2). C) Trabecular separation histograms, highlighting the fact that average metaphyseal trabecular separation takes the average of a non-normally distributed histogram.

References

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