TRAM (Train and Retain Academic Musculoskeletal clinicians)
MB-PhD Project Summary

PhD project Title
Identifying bioactive metabolites for osteoporosis

PhD supervisors (please provide name, affiliation and email) *[At least two supervisors]*	
1. Matthew Dalby (Glasgow)	2. Dominic Meek (NHS, orthopaedics)
3. Karl Burgess (Edinburgh)	4. Monica P Tsimbouri (Glasgow)

Background

Osteoporosis is a prevalent degenerative condition that mainly effects women above 50 years old. Currently, osteoporosis effects >3 million people in the UK with 500k people pa being treated for fragility fractures. Fragility fractures, resulting from loss of bone density, occur notably in the wrist and hip result from low impact falls. Vertebrae collapse is another major issue of osteoporosis. Pain and immobility result in comorbidities and increased mortality.

We have developed a novel nanovibrational bioreactor that drives bone marrow mesenchymal stem cells (MSCs) to turn into bone forming osteoblasts. Osteoporosis is thought to result from an imbalance in the MSC population reducing their ability to form osteoblasts. We can thus test MSCs isolated from normal from patients without osteoporosis vs marrow from people suffering fragility fractures to see how the process of osteogenesis develops.

We have also developed a metabolomics pipeline where we can isolate metabolites from cultures of cells and use mass spectrometry to identify metabolites used up in the differentiation process. These metabolites then form 'activity metabolites' that help drive a desirable process/phenotype. We will use this approach to identify biologically active small molecules that can help drive osteogenesis in MSCs isolated from osteoporotic patients and avoid the side effects of present medications.

Aims

With the aim of better understanding how MSCs are linked to osteoporosis we will have the objectives of:

1. Use our nanovibrational bioreactor to understand bone-specific differentiation of normal vs osteoporotic MSC. We will look at 'amount' of differentiation and investigate change in activation of key pathways controlling the osteoblast phenotype (focal adhesion, extracellular signal-related kinase, SMAD, wnt).
2. Use metabolomics to survey metabolite changes in nanovibration stimulated normal vs osteoporotic derived MSC cultures. Using bioinformatics such as metaboanalyst and Ingenuity Pathway Analysis to build pathways.
3. Identify activity metabolites that can be used to enhance bone-related differentiation of MSCs derived from osteoporotic marrow.

Training and experience provided *[Include types of methodologies that will be employed]*

In this project we will offer training in stem cell isolation and culture, microscopy, qRT-PCR, protein expression analysis. We will also offer training in metabolomics and bioinformatic analysis and precise measurement for calibration of the nanovibrational bioreactors.

The Centre for the Cellular Microenvironment (CeMi), where the project will be hosted in Glasgow, is a partnership between the biological, engineering and biophysical sides of biomaterials and tissue engineering, it is a centre for excellence in fundamental and translational research. Not only do we regularly publish in some of the best journals in the world, but we are delivering impact. For example, we published on a polymer that presents fibronectin (FN) and growth factors (GFs) in a low-dose, physiological manner in Science Advances 2016. This went on to be used in a series of successful veterinary trials on 10 cats and dogs with non-union fractures. We now have funding for GMP manufacture of the polymer and full pre-clinical trials. In another example, we published on nanovibrational osteoinduction of MSCs in Nature Biomedical Engineering in 2017. We also secured funding for GMP manufacture and first-in-human trial (2023).

CeMi comprises 11 academics and 2 honorary clinical professors (orthopaedics and plastics) and 5 honorary clinical lecturers/senior lectures across UoG and the University of Strathclyde. At UoG, we are located in the flagship £100M Advanced Research Centre where we bring biology and engineering together in the same laboratory. We are currently undertaking major investment in equipment (including enhancing confocal microscopy, flow cytometry and cell sorting capabilities). We will collaborate with Dr Burgess, Scientific Director for Omics Technologies in Edinburgh to perform high-resolution mass spectroscopy for metabolomic analysis. We will have clinical direction and obtain tissue samples from Prof Meek, consultant orthopaedic surgeon at Queen Elizabeth II University Hospital in Glasgow.

We have long experience working with clinical scientists to MD and PhD level and will offer an excellent training environment.

Expected outcomes

This will identify potential new treatments for osteoporosis. For example, nanovibrations applied to the patient through similar technology as used in bone conducting headphones for runners (we vibrate at 1000 Hz and this is an audible frequency). Identification of activity metabolites can present a first step towards new small molecule therapies. It will also offer the clinical PhD student world class training in an environment used to training clinical scientists.

References

- Hodgkinson, T., et al. (2021). "The use of nanovibration to discover specific and potent bioactive metabolites that stimulate osteogenic differentiation in mesenchymal stem cells." Science Advances **7**(9).
- Tsimbouri, P. M., et al. (2017). "Stimulation of 3D osteogenesis by mesenchymal stem cells using a nanovibrational bioreactor." Nature Biomedical Engineering **1**: 758-770.
- Alakpa, E. V., et al. (2016). "Tunable Supramolecular Hydrogels for Selection of Lineage-Guiding Metabolites in Stem Cell Cultures." Chem **1**(2): 298-319.