MICROGRAVITY INDUCED PROTEOMICS CHANGES OF OSTEOCYTES IN A BIOMIMETIC 3D CELL CULTURE SYSTEM

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Astronauts exposed to microgravity environments during space flight experience significant and irreversible physiological changes.[1] The exact mechanism behind this phenomenon is yet to be comprehensively understood. Current theories predict that cellular mechanical unloading significantly affects the mechanosensitive cells in bone tissue causing significant loss of bone mineral density.[2] Here we have investigated protein level changes in osteocyte cells cultured in a biomimetic 3D cell culture hydrogel after exposure to simulated microgravity. Among different bone cell types, osteocytes were selected as they are the main mechanosensory cell type that orchestrate the bone remodelling process.3 We propose that the 3D cell culture system presented here provides more accurate protein expression data and enhanced endogenous biological relevance as the 3D system more accurately mimics the in vivo cellular microenvironment compared to 2D cell culture methods, particularly as the 3D system minimizes the fluid shear generated while simulating microgravity using a Random Positioning Machine (RPM). A polyisocyanopeptide-based hydrogel system was used to generate a biomimetic extracellular matrix (ECM) for the 3D cell culture experiments. This synthetic hydrogel is well-known for stress stiffening behaviours that are analogous with most naturally occurring ECM biopolymers.[4] Osteocytes cultured in 3D were exposed to RPM simulated microgravity out to 7 days, then examined for morphological changes using confocal microscopy and proteomics data using a liquid chromatography/mass spectrometry technique. Through the analysis of proteomics data, we observed separate distinct clustering of microgravity samples showing changes in the proteome due to exposure to microgravity. In addition, we observe a significant down regulation of proteins that affect the stability of the cell membrane and cytoskeleton, while also observing changes in metabolic processes related to purine containing compounds in the microgravity group compared to cells cultured in normal gravity.

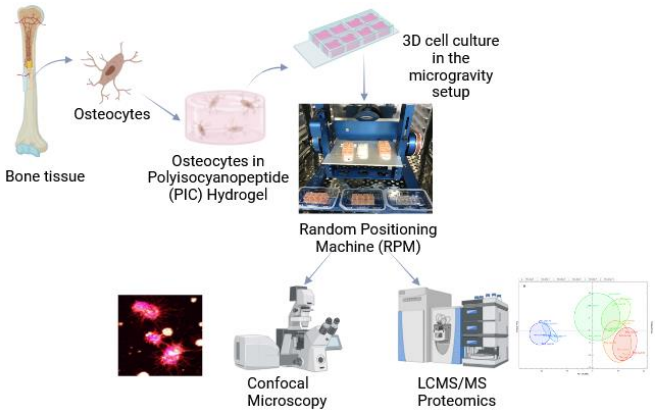

Figure 1: Schematic diagram of experimental procedure

References:
[1] White R. J. and Averner M. (2001) Nature 409, 1115-1118.
[2] Collet P. et al. (1997) Bone 20, 547-551.
[3] Choi J. U. A. et al. (2022) Frontiers in Cell and Developmental Biology 9.
[4] P. H. Kouwer P. H. et al. (2013) Nature 493, 651-655.