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# 3D DROPLET SCAFFOLDING FOR OSTEOCYTE MECHANICAL UNLOADING IN A ROTATING WALL VESSEL

**Abstract**

Astronauts face a serious challenge in space: how do they prevent their bones from becoming weak and fragile due to microgravity? Recently, it has been theorized that osteocytes play a central role in astronaut bone loss and other disuse-related bone pathologies. However, research on this cell type has been greatly limited as they are deeply embedded in the dense, calcified matrix of bone tissue. Osteocytes are therefore difficult to purify and impossible to see under light microscopy. Our research addressed this limitation by developing 3D type I collagen-based gel droplets and culturing the osteocyte cell line MLO-Y4 within it to produce an environment more closely resembling osteocyte organization in vivo. The droplets were compatible with traditional rotating wall vessels such as the Rotary Cell Culture System (Synthecon, Inc., USA) which allowed us to observe and analyze the response of MLO-Y4 cells to simulated microgravity by mechanical unloading. Droplets were fabricated by combining an MLO-Y4 cell suspension with a diluted and neutralized solution of type I rat tail collagen (ibidi GmbH, Germany) and pipetting 2.5$\mu$L onto a hydrophobic surface. In our preliminary work, we have successfully imaged MLO-Y4 osteocytes in these droplets by confocal microscopy and scanning electron microscopy. This enabled visualization of changes to both the intracellular and intercellular 3D architecture of cells in simulated microgravity which will be compared to static 1g and mechanically loaded controls. Furthermore, we have shown that mRNA and protein can be quickly and easily extracted from cells encapsulated in gel droplets for downstream analysis of gene and protein expression. Our method greatly improves current models used to study osteocyte mechanical unloading as we incorporate three-dimensionality which allows MLO-Y4 osteocytes to extend their dendrites, the membrane branches where most mechanosensing occurs, in all directions. This improves sensitivity to mechanical forces and the physiological relevance of the model. Upon further validation of our method, we intend to utilize next generation mRNA sequencing to identify novel genes affected in simulated microgravity. These genes will be studied to elucidate the mechanisms by which osteocytes are responsible for astronaut bone loss. Once these mechanisms are established, therapeutic intervention can be explored to mitigate bone loss in astronauts and patients afflicted with osteoporosis. This research will have a profound impact on astronaut health and their ability to undertake long-term space missions.