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A METHOD FOR STUDYING MLO-Y4 OSTEOCYTE RESPONSE TO SIMULATED
MICROGRAVITY IN EMBEDDED 3D COLLAGEN DROPLET SCAFFOLDS**Abstract**

The limited number of opportunities for biological research in space has generated the need for ground-based microgravity simulators. The Rotary Cell Culture System (RCCS) has become one of the most widely used devices to simulate microgravity conditions in cultured cells. Nevertheless, the device is known to produce differing effects depending on the cell type, microcarriers used, speed of rotation, vessel dimensions and laboratory conditions. Osteocytes are the mechanosensors of bone, producing biological signals when mechanical forces are applied or removed from their environment. It is thought that these cells play an important role in astronaut osteoporosis, however the cellular mechanisms of the disease have yet to be elucidated. We sought to characterize the response of MLO-Y4 osteocytes in the device using 3D gel scaffolds composed of collagen I and hydroxyapatite nanoparticles to produce a bone-like environment for the cells. The gel was inoculated with a cell suspension and mixed to ensure homogenous cell distribution. The mixture was then formed into droplets to allow compatibility with the RCCS and to ensure good nutrient diffusion which would otherwise be hindered in a larger construct. The density of the scaffold was optimized such that the droplets were stably suspended in the rotating culture medium using a precise concentration of hydroxyapatite. The density of cells in the scaffold was also optimized to mimic the intricate network of the lacuno-canalicular system which osteocytes form in bone tissue. Osteocyte markers of mechanical stimulation were studied by quantitative real-time PCR to assess the response of MLO-Y4 osteocytes to the simulated microgravity environment. Our future work will utilize our optimized simulated microgravity environment in the RCCS to conduct a transcriptome analysis of MLO-Y4 osteocytes which will be compared to a static control regime and a mechanical loading regime. From these data, we will determine novel genes affected by mechanical unloading in osteocytes which can be further validated in true microgravity spaceflight. This work therefore marks an important first step in determining potential therapeutic targets to combat astronaut bone loss.