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MLO-Y4 OSTEOCYTE RESPONSE TO STEADY AND SETTLING REGIMES IN THE ROTARY CELL CULTURE SYSTEM

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 under fast (steady) and slow (settling) rotating regimes to determine optimal conditions for simulating mechanical unloading in these cells. 3D gel scaffolds composed of collagen I and calcium phosphate were used 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 modulated by altering the concentration of collagen and mineral phases to facilitate either steady or settling rotation, the latter requiring denser scaffolds. Osteocyte markers of mechanical stimulation were studied by quantitative real-time PCR and Western Blot to assess the response of MLO-Y4 osteocytes to the two rotation regimes. 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 studied as potential therapeutic targets to combat astronaut bone loss.