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NOVEL OSTEOCYTIC CELL LINES TO STUDY RANKL AND SOST FOR A INTERNATIONAL  
SPACE STATION (ISS) FLIGHT EXPERIMENT**Abstract**

**Purpose:** Although it is known bone responds to its mechanical environment, the mechanisms underlying this mechano-transduction are poorly understood. Osteocyte cells are the most abundant yet least understood bone cell type. To date, studies of osteocytes' response to microgravity have been limited by the lack of osteocyte cell lines that produce mechanical sensitive osteocytic genes, notably SOST and RANKL. To this end, we have generated osteocytic cell lines which fully recapitulate the hallmarks of osteocytes in vivo. These osteocytic cell lines were tested for their ability to maintain an osteocytic phenotype in bioreactors designed for use in microgravity and investigate the role of mechanical regulation in ground-based microgravity analogs in preparation of a future spaceflight mission. We demonstrate that simulated microgravity, as achieved by the NASA STLV bioreactors, increases RANKL and SOST in the osteocytic cell line, G454, that we have developed.

**Methodology:** Mice expressing the green-fluorescent protein (GFP) under the control of Dentin Matrix-Protein 1 (8KbDMP1-GFP) were mated with mice carrying a ubiquitously expressed SV40Ag (Immortomouse Charles River). Osteocytes were isolated from long bones of double transgenic offsprings by sequential collagenase digestions followed by FACS sorting for intrinsic GFP expression. As a carrier in the NASA STLV, we seeded 4 million cells/ml cells onto 200 um x 9 mm diameter collagen coated Alvetex three-dimensional scaffolds. Seeded osteocytic cell scaffolds were grown in the STLV bioreactors for 4 days and in the CO2 free cell culture environment of the spaceflight qualified eOSTEO (Calm Technologies, Ontario, Canada) bioreactors to validate cell culture conditions for an ISS flight experiment.

**Results:** Sorted cell displayed the dendritic morphology and expressed genes characteristic of an osteocytic population such as, SOST, DMP1, FGF23, RANKL, and had low levels of genes characteristic of osteoblasts, such as Fmod. Notably, after two weeks in culture, one cell line, G454 expressed level of SOST that was 72-fold higher than wild type osteoblasts. Furthermore, these cells increased SOST by 24demonstrating these cells can be grown in conditions necessary for an ISS experiment.

**Conclusion:** We have established novel DMP1/SOST enriched osteocytic cell lines from long bones that will be a tool to further investigate fundamental osteocytic biology in the microgravity environment of the ISS.