Osteocytes are theorized to be the mechanosensors and transducers of mechanical forces in bone, yet the biological mechanism of this action remains elusive. Recent evidence suggests that SOST/Sclerostin is an important regulator of mechano-transduction. For in-vitro experiments, MLOY-4 osteocytic cells were seeded at a concentration of 250,000 cells onto 3D collagen scaffold (BD). Scaffolds (4 per condition) were either rotated in a vertical 50ml NASA/bioreactor vessel at a speed of 18 rpm (simulated microgravity), or cultured in a static 7-7.5 cm dish (static condition) for 7 days. For ex-vivo experiments, calvaria bones were harvested from 5-6 week old C57/B6 mice and sequentially digested with type I collagenase to remove periosteal osteoblasts. Calvaria halves (10 per condition) were then exposed to the same set of culture conditions described above.

Simulated unloading, as achieved in the NASA RWV, resulted in enlarged, round osteocytes, as assessed by H&E staining, that was reminiscent of prior reports of unloading causing loss of osteocyte morphology and dendritic network connectivity. Semiquantitative real-time qPCR and immunohistochemistry from both in-vitro and ex-vivo RWV experiments demonstrated a four-fold increase in mRNA expression of Mef2C in MLO-Y4 cells cultured in simulated microgravity (panel B). Immunolabeling for Sclerostin expression (panel A) showed an increase in Sclerostin expression (black staining) in MLO-Y4 cells cultured under simulated microgravity condition (rotating bioreactor, panel B). Immunohistofluorescence for Sclerostin (C) and MEF2C (D) showed an increase in Sclerostin expression (black staining) in MLO-Y4 grown under simulated microgravity condition (panel D).

Simulated Microgravity induces a 4-to-6 fold increase in SOST and mRNA expression comparable to response in hindlimb unloaded mice. Results are expressed as relative RNA and are normalized by RPL13. Data are expressed as mean ± SD of triplicates. *: student unpaired two-tailed t-test p<0.05

Future work is focused on validating results with additional osteocytic cell lines. Our lab is developing in preparation for a International Space Station (ISS) experiment.

CONCLUSIONS & FUTURE WORK

• Simulated Microgravity induces: Osteocyte morphologic changes similar to immobilization
  Increase in SOST/Sclerostin & Mef2C expression relative to rotating control

• Future work is focused on validating results with additional osteocytic cell lines. Our lab is developing in preparation for a International Space Station flight experiment

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SOST -/- mice are resistant to disuse-induced bone loss (Lin, et al., 2009)
Hindlimb unloading in mice upregulates SOST/Sclerostin expression in osteocytes
Antisclerostin antibody treatments in clinical development pipeline

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Figure 1: Proposed hypothesis for mechanical unloading regulation of SOST/Sclerostin

Figure 2: MLO-Y4 Osteocytic cell Line 5 mm x 3 mm Collagen Scaffold

Figure 3: Unloading causes characteristic osteocyte morphologic changes: Osteocytes from immobilized rat (A and C; Krempien, 1976) and MLO-Y4 cultured on 3D collagen scaffold under simulated microgravity (B and D; H&E staining). Loaded osteocytes are spindle-shaped with small nuclei; unloaded osteocytes have enlarged round nuclei and cytoplasm.

Figure 4: Simulated Microgravity increases SOST & Mef2C expression • 7 days: Real-time qPCR for SOST and Mef2C mRNA in MLO-Y4 cells (A1 and A2) and calvaria (A2) and B) placed in simulated microgravity. Simulated microgravity induced a 4-to-6 fold increase in SOST and mRNA expression comparable to response in hindlimb unloaded mice. Results are expressed as relative RNA and are normalized by RPL13. Data are expressed as mean ± SD of triplicates. *: student unpaired two-tail t-test p<0.05

Figure 5: Sclerostin protein expression up-regulated • 7 days: Sclerostin expression in MLO-Y4 cultured on 3D scaffold for 7 days under static (A), rotational control, (B), or simulated microgravity (C).