Simulated Microgravity Induces SOST/Sclerostin upregulation in osteocytes

Jordan Spatz1, Jean Sibonga2, Honglu Wu3, Kevin Barry4, Mary Bouxsein4, Paola Divieti Pajevic5

1 Harvard-MIT Division of Health Sciences and Technology (HST), Bioastronautics PhD Program, Boston, MA, 2 Universities Space Research Association, Houston, TX 3 NASA Johnson Spaceflight Center, Houston, TX, 4 Orthopedics, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston MA, 5 Endocrine Unit, Mass General Hospital, Harvard Medical School, Boston MA

ABSTRACT

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 mechanotransduction.

To investigate the molecular mechanisms of SOST/sclerostin regulation under in vitro and ex-vivo unloading we used the NASA Rotating Wall Vessel (RWV) Bioreactor. 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 18 rpm (unloaded), cultured in a static 50ml NASA/bioreactor vessel at 18 rpm (control for the sheared environment of vertical rotating vessel), or cultured in a static 7.7cm dish (static condition) for 7 days. For ex vivo experiments, calvaria bones were harvested from 3-to-5 day old mice and subjected to 6 collagenase digestions.

Simulated Microgravity Induces SOST/Sclerostin upregulation in osteocytes

Cells: MLOY-4 cells were grown to confluence, harvested and seeded onto 3D collagen scaffold (BD) at a concentration of 250,000 cells/scaffold.

Organ Culture: Calvaria were harvested from 3-to-5 day old mice and subjected to 6 collagenase digestions.

Simulated Microgravity & Rotational Control: Scaffolds (4 per condition) or calvaria were cultured were either rotated in a vertical 50ml NASA/bioreactor vessel at a speed of 18 rpm (simulated microgravity), cultured in a horizontal 50ml NASA/bioreactor vessel at a speed of 18 rpm (control for the increased sheared environment of rotating vessel), see Figure 2.

Statistical Controls: Calvaria or calvaria were cultured in a T-75 cm dish (static condition)

RESULTS

Simulated Microgravity:

MLOY-4 Osteocytic Cell Line 5 mm x 3 mm Collagen Scaffold

Simulated Microgravity

Static Control Simulated Microgravity

Figure 2: MLO-Y4 Osteocytic cells subjected to simulated microgravity: MLO-Y4 cells were grown on 3D collagen scaffold and subjected to static (A and C) or simulated microgravity (B and D) culture conditions. H&E staining (A and B) showed increase in cell proliferation in MLO-Y4 cultured under simulated microgravity condition (rotating bioreactor, panel B). Immunohistochemistry for SOST (C) and C (D) showed an increase in Sclerostin expression (black staining) in MLO-Y4 grown under simulated microgravity condition (panel D).

Figure 3: Unloading causes characteristic osteocyte morphologic changes: Osteocytes from immobilized rat (A and C, Krempien, 1976) and MLOY-4 cultured on 3D collagen scaffold under simulated microgravity (D and D, H&E and 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 MLOY-4 cells (A1) and calvaria (A2 and B) placed in simulated microgravity. Simulated microgravity induced a 4-to-6 fold increase in SOST and Mef2C 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:

SOST Expression

Mef2C Expression

Simulated Unloading Bioreactor

CONCLUSIONS & FUTURE WORK

- Simulated Microgravity induces:
  - Osteocyte morphologic changes similar to immobilization unloading
  - 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

Funding for this research was provided by: NIH UH2AR059665, NH R21 AR057222, NASA NNX05AE39G, NSBRI, NASA Life Sciences Division, & Northrop Grumman Aerospace Systems.