Osteogenic transcription regulated by exaggerated stretch loading via convergent wnt signaling
Cassandra M. Juran1,2, Elizabeth A. Blaber1, Eduardo A.C. Almeida1
1Space Biosciences Division, NASA Ames Research Center, Moffett Field, CA
2Universities Space Research Association, Mountain View, CA

Cell and animal studies conducted onboard the International Space Station and formerly the Shuttle flights have provided data illuminating the deleterious biological response of bone to mechanical unloading (Figure 1). Loss of bone mass and inherent microarchitecture is a feature similar to osteoarthritis, the causal mechanism of which has been highly researched. In vivo down regulation of molecular intra- and inter-cellular signaling cascades has been demonstrated in osteoarthrosis and unloading studies. Specific to osteocytes the canonical wnt and Connexin43 induced CAMP signaling cascades have been shown as critical regulators. However the intercellular communicative cues and mechanotransductive cascades responsible for osteogenic transcription and stem cell recruitment are still largely unknown.

Bone is a dynamic tissue undergoing constant remodeling and repair from stem cell precursors in the bone marrow (Figure 2). Osteocytes are believed to be responsible for the controlled regulation of cell activity in living bone. Thus how mechanical stimulation modulates biochemical activity of the osteocyte is a definitive factor in the study of bone biology and homeostasis maintenance.

A significant feature of interest in mechanical regulation of bone biology is the mechanism of loading experienced by the cell modulates the cells reaction. Many of the previous osteocyte studies investigating response to loading have evaluated how the cells respond to fluid flow induced shear adjacent to cells in monolayer. This model however, is not representative of the critical osteocyte dendritic process activation within the canalculus with which we are concerned (Fig 3A). Thus our experimental design will utilize stretch loading, such that the cell process directly experience load to better represent the physiologic response of cells in vivo (Fig 3B).

In this investigation, MLO-Y4 osteocyte-like and MC3T3-E1 osteoblast-like cells (control cell) were culture under dynamic tensile conditions and evaluated for expression of CX43 and wnt-signaling proteins influential in driving cell-cell communication as well as stem cell recruitment and differentiation.

Hypothesis
We hypothesize stretch loading induces gap junction and wnt11 choreographed convergent wnt signaling which regulates a cascade of molecular events terminating in osteogenic gene transcription.

Results
Osteogenic Signaling Expression and Localization after Exaggerated Loading
Figure 3. Cellular response to mechanical stimulation dependent on the mechanism of cell deformation, in this study is strain applied through the cell attachments, in contrast to fluid flow studies which are regulated by membrane strains.

Results
Proliferation and Cellular Metabolic Activity with Exaggerated Loading
Figure 4. Analysis of MLO-Y4 viability during stretch stimulation. Ty < 0.05
Cellular Connectivity and Intercellular Regulation due to Exaggerated Mechanical Loading
Figure 8. Calcium –AM live cell membrane stain and MitoTracker automated dendritic process length measurement program and processing.

Table 1: Quantitative Assessment of MLO-Y4 Dendritic Process Length and Terminations

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Process Length</th>
<th>Terminations</th>
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<tbody>
<tr>
<td>Control</td>
<td>300 ± 5.2</td>
<td>0.5 ± 1.6</td>
</tr>
<tr>
<td>1.16 Hz</td>
<td>300 ± 5.2</td>
<td>0.5 ± 1.6</td>
</tr>
<tr>
<td>2.29 Hz</td>
<td>300 ± 5.2</td>
<td>0.5 ± 1.6</td>
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The self organization of osteocyte cells is a critical metric of the cell population number, MLO-Y4 cells cultured in unloaded conditions will form dense overlapping networks with short dendritic process lengths and little CX43 membrane localization. In vivo examination of osteocyte networks have been shown to be highly interconnected (Figure 3) and these connections are made between dendritic cell processes with lengths averaging 20-30µm. Within the osteon the cell processes are organized in the canalicus network, the microarchitecture of which amplifies mechanical stress sensed by the processes which in turn lengthens the process. This supposition is supported by our measurement of MLO-Y4 dendritic cell process lengthening under stretch loading stimulation. Additionally, the shared terminating junctions were quantified and demonstrated greater MLO-Y4 population interconnectivity when cells were exposed to stretch loading.

Conclusion
Figure 9. Hypothetical feedback regulation of osteogenic transcription in MLO-Y4 osteocyte cells due to mechanical loading. This theoretic feedback loop implies the critical importance of coupled wnt signaling control of CX43 intercellular communication channel.

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