Osteogenic transcription regulated by exaggerated stretch loading via convergent wnt signaling

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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 osteoarthritic and bone 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 that 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 canaliculus (Figure 3A). Thus our experimental design will visualize 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 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.

Methods

MLO-Y4 osteocyte-like and MC3T3-E1 osteoblast-like cells were cultured in a custom designed biostimulator (Figure 5) and allowed to acclimate for 48 hours before 48 hours of stretch loading. Stretch loading was imparted by 2 Arduino controlled linear drive motors set to 0.1% tensile strain and 0.1Hz cyclic application. Measure of CX43 localization, cell number, metabolism, and phenotypic expression were taken at 10 minutes, 2, 12, 24 and 48 hours.

Figure 4. Experimental cell type morphology. MLO-Y4 represent critical dendritic cell processes inherent to functional osteocytes, while MC3T3-E1 pre- osteoblast cells present no such morphology.

Culture conditions were maintained at 5% CO2, 37°C and 90% humidity. Culture media was supplemented with 1% anti-anti, 10% FBS and changed every 48 hours such as to not interrupt the stimulation regime.

Results

Osteogenic Signaling Expression and Localization after Exaggerated Loading

Figure 5. Bistimulator CAD representation.

Figure 6. Fluorescence imaging demonstrating cell morphologies (actin – Green), connexin localization (CX43 primary antibody/secondary antibody D44 – Red) and counterstained with DAPI (nuclear –Blue). A) and insert are MLO-Y4 osteocytes after 48 hours of stretch culture, B) and insert are MC3T3-E1 osteoblast cells, and C) and insert are co-cultured MLO-Y4 and MC3T3-E1. A) illustrates typical MLO-Y4 morphology with extended cell processes along the direction of stretch (indicated by white arrow) and localized CX43 presence at the process peripheries and nuclear envelope. While in A) and C) CX43 is highly expressed at membrane interfaces in B) the extent of localization is non-specific in osteoblast cells. Osteoblast-Osteocyte co-cultured cells C) demonstrate both increased CX43 presence within the cell and membranous localization inherent to mechanistic communication both intercellular and intracellular.

Mechanotransduction signaling

Proliferation and Cellular Metabolic Activity with Exaggerated Loading

Cell number and metabolic activity of cultured MLO-Y4 cells demonstrate cellular viability. Stretch stimulation has a negative correlation with proliferation compared to unloaded controls. Cellular metabolism initially increases due to new stress applied to the cells however after acclimation metabolism reaches steady state after 24 hours.

Cellular Connectivity and Intercellular Regulation due to Exaggerated Mechanical Loading

The self organization of osteocyte cells is a critical metric of the cell population density. 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 canaliculus 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 8. Analysis of MLO-Y4 viability during stretch stimulation. *p <0.05

Cellular Connectivity and Intercellular Regulation due to Exaggerated Mechanical Loading

Table 1. Quantitative Assessment of MLO-Y4 Dendritic Process Length and Terminating Junctions per cell

Acknowledgements: This work is supported by NASA Space Biology Grant NNH14ZT001N-0062 to E. Blaber and NNH14ZT001N-003 to E. Almeida.