SPACE LIFE SCIENCES SYMPOSIUM (A1)
Interactive Presentations (IP)

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EFFECTS OF MODIFIED GRAVITY COUPLED WITH MECHANICAL STIMULATION ON MOLECULAR SIGNAL TRANSDUCTION AND TARGET GENE TRANSCRIPTION IN 3D OSTEON CELL NETWORK.

Abstract

The current attitude of NASA and other space faring agencies is that to combat the effects of microgravity experienced by astronauts in space, resistive exercises and nutritional supplements are adequate. However, evaluation of bone cell response to altered gravity and concurrent mechanical loading has yet to be conducted on a cellular/molecular signaling level.

In this investigation, MLO-Y4 osteocyte-like cells were cultured on a 3D collagen coated polystyrene mesh, placed in direct contact with MC3T3-E1 osteoblast-like monolayer and exposed to a fluid shear field at 4 dynes/cm2. This co-culture architecture is meaningful for altered gravity experimentation because it represents a functional osteon cell network, presenting interconnectivity of a 3D osteocyte network exposed to fluid shear and a lining osteoblast layer which experience minimal flow strain. The experimental culture was exposed simultaneously to the fluid shear regime and a constant 10G centrifugal force. Evaluation of cell morphology, connexin-43 intercellular communicative networking, osteogenic gene expression, and osteoblast mineralization at 10 min, 2, 12, and 48h post stimulation was conducted to evaluate modulation of molecular signaling and subsequent osteogenic phenotypic expression.

When comparing hypergravity stimulation to static controls, MLO-Y4 morphology changes drastically. Under hypergravity, cell processes elongate to an average of 8.4X the length of the static controls. Similarly MLO-Y4s exposed to fluid shear without hypergravity elongate to a lesser degree, an average 3.6X. Interestingly, the cell process elongation is not correlated with the expression of connexin-43, a gap junction protein hypothesized to play a significant role in bone cell signaling. Connexin-43 expression was up-regulated for all cells and culture conditions compared to static controls; however, localization of the protein at the cell membrane was only visualized in the MLO-Y4/MC3T3-E1 co-cultures exposed to fluid shear. This leads to the conclusion that connexin-43 signaling is dictated by dynamic mechanical loading and is not modulated by hypergravity. PCR evaluation of osteogenic markers (RANKL/OPG, RUNX) and specific mechanotransduction signaling molecules further corroborate that the co-cultured population's communicative networks regulate the translation of dynamic mechanical signals to molecular messaging and that constant gravitational loading only changes the degree of expression, not localization or activation of signaling cascades.

Our findings suggest that translating mechanical loading in the presence of altered gravity is dependent on the architecture of a highly interconnected osteocyte-osteoblast network. This study however utilized model cell lines that are immortalized down osteogenic lineages. Future work will investigate bone turnover mechanisms (osteoblastogenesis/osteoclastogenesis) and stem cell recruitment and activity.