Effects of Simulated Microgravity on Human Osteoblast Behavior: A Proteomics Study

Heather L. Nichols, Sarah Holton, Ning Zhang, Xuejun Wen
Clemson-MUSC Bioengineering Program, Department of Bioengineering, Clemson University
173 Ashley Ave., CRI#605B, Charleston, SC, 29425

Statement of Purpose: Major physiological changes occur during spaceflight, including cephalic fluid shift, bone and muscle loss, negative calcium balance, cardiovascular alterations and reduced immune response. Of these, reduction in bone mass, or osteopenia, is one of the changes with serious consequences, especially for prolonged space travel (astronauts can lose up to 2 percent of their bone mass per month in space). In order to develop highly effective countermeasures and prevent spaceflight induced diseases, there is a critical need to understand the mechanism of how microgravity causes these problems. In addition, the countermeasures to prevent bone loss on earth, such as physical exercise, do not help in space. Therefore, drugs that can block specific strain/stress-sensing pathways could be the ultimate "answer" for the control of osteopenia. Osteoblasts and osteocytes have been shown to be responsive to mechanical stimuli and previous studies have indicated that cell morphology, gene expression and protein expression are altered under microgravity conditions [1-3]. However, systematic investigation on microgravity induced protein expression is lacking, which is the key information to ultimately unfold the mechanism behind microgravity induced bone loss. To this end, the effects of simulated microgravity on protein expression of human osteoblast cells were studied using a proteomics approach.

Methods: Human osteoblasts were seeded into elastic 3-D interconnective porous scaffolds. Microgravity group was cultured in a rotating wall vessel bioreactor to simulate human bone tissue behavior under spaceflight free-fall conditions. Normal gravity 3D static culture, dynamic compression culture, dynamic tension culture, and dynamic compression-tension culture were used as comparison groups. Cell morphology, intracellular protein expression, and extracellular matrix production were examined.

3-D porous Scaffolds: Scaffolds were fabricated using a combined freeze-drying and particle leaching technique. Elastic polyurethane (SG80-A) was dissolved in dimethylacetamide (DMAC) at 60 °C. Grounded sodium chloride particles at 100 ~ 150 um in diameter were added to the polymer solution and mixed thoroughly. This was then poured into stainless steel molds, which were placed in a dry ice/ethanol bath for 5 minutes, then freeze-dry. After removal of the skin layer, scaffolds were cut into 10 x 10 x 5 mm rectangular slices, placed into DI water for one week to allow the salt to dissolve, and then coated with gelatin to enhance cell attachment.

Cell Culture: Human osteoblasts were seeded at a density of 2 x 10^6 cells/ml on scaffolds and cultured at static condition in a 37ºC incubator for 24 hours. Some scaffolds were then removed from static culture and placed in different types of bioreactors. Samples were fixed after 7 days in culture.

Proteomics: Samples were washed and lysed for proteomics analysis. For 2-D differential gel electrophoresis, different samples were pre-labeled with fluorescent dye, such as Cy2, Cy3, and Cy5. Samples were loaded on IEF strips and run on IEF, then run SDS-PAGE. For Liquid Chromatography Mass Spectroscopy (LC-MS), sample was injected into a linear ion trap mass spectrometer equipped with an electrospray ionization source to identify the protein in the cells at different culture conditions.

Immunostaining & SEM: Cells were stained for either bone sailoprotein (BSP), osteocalcin (OC), osteonectin (ON) or osteopontin (OP). Cell morphology was examined using SEM.

Results / Discussion: *Proteomics:* numerous differences in protein expression between osteoblasts under static and microgravity conditions were observed. A few of the proteins of interest that were down-regulated under microgravity conditions include Osterix (Osx), Cathepsin L, Catenin (alpha-like), and prostatic acid phosphatase (PAP). Some of the proteins up-regulated in osteoblasts under microgravity include ornithine decarboxylase (ODC) antizyme, Calbindin D28k, Calcineurin B-like protein (CBLP), mitogen activated protein kinase kinase kinase 1 (MAP3K1), transforming growth factor-ß inducible early gene 2 (TIEG2) and heat shock protein 70 (HSP-70). *Morphology*: After 7 days in culture, human osteoblasts under simulated microgravity to have elongated processes when compared with static cultures, which appeared more rounded with less cell processes. These results suggest that the anchorage-dependent osteoblasts developed the elongated processes in order to achieve a more secure attachment to the 3-D scaffolds when subjected to microgravity. *Extracellular matrix production*: Osteocalcin, a mature extracellular matrix in bone tissue, is down-regulated in microgravity group.

Conclusions: Proteomic approach is very to identify the differences in protein expression by cells under different environments. Microgravity bioreactor and dynamic compression/tension force bioreactors are very useful research tools that allowing long term investigation of human cells and tissue under microgravity. Microgravity affects many proteins expression of human osteoblast cells. Many of those proteins know to influence osteoblast function, such as differentiation, proliferation, and maturation. Studies are in progress to confirm the proteomics data with gene expression data using RT-PCR and gene microarray.

References:

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