Low pH of Trauma Environment Elicits Cellular Changes on Bone Biomaterial – Development of an *In Vitro* Model

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Statement of Purpose: Traumatic events causing open fractures of the tibia incur high re-operation rates and the use of bone grafts in up to 40% of procedures [1]. Autografts, the gold standard for bone grafts, are often not viable options or do not provide sufficient material. Many surgeons have turned to allografts, but these exhibit inconsistencies in osteogenic potential due to donor variability and sterilization. Because of these limitations, there has been a marked increase in the development of synthetic bone grafts. With over 200 synthetic bone substitutes available to surgeons, it can be difficult for surgeons to determine effective materials for a particular clinical scenario. Accordingly, our goal is to develop a simple *in vitro* model to test particular trauma environmental parameters. The model will simulate specific factors of the *in vivo* fracture environment and allow investigation of the biomaterial interaction and effectiveness in promoting bone growth. Multiple factors contribute to the fracture environment; the fracture environment is characterized by an initial decrease in pH as the healing cascade begins to take place. It has been established that fatty acids, found in the bone marrow, will influence bone cell function [2,3]. Hence, we investigated the effects of a lower pH on mesenchymal stem cell secretion of fatty acids in a 2D and a 3D environment.

Methods: Commercially available chronOS granules were heat sterilized at 200°C for 2 hours and placed into wells of a 24-well plate, 150mg of chronOS per well. D1 mesenchymal stem cells (ATCC) were seeded onto the chronOS granules and a control 24-well plate in 1 mL of Dulbecco's Modified Eagle's Medium (DMEM, Atlanta Biologicals) at 30,000 cells/cm^2 and 10,000 cells/cm^2, respectively. Cells were allowed to adhere overnight at incubator conditions, 37°C and 5% CO_2. To alter the pH, DMEM was supplemented with 15mM HEPES buffer and placed in incubator conditions to equilibrate. After equilibration, a specified amount of 3N HCl was added to each treatment to prepare DMEM aliquots with pH range of 6.6-7.6; the pH values were verified using a modified colorimetric model [4]. The DMEM was removed from the 24-well plates and replaced with treatment medium. After 3 days of culture, the treated DMEM was removed and analyzed for fatty acid and cell metabolite content.

Results: The level of cell metabolites suggests higher cellular activity on the chronOS granules than on the plastic surface at all pH levels. Additionally, the level of cell metabolites decreases with decreasing pH in both the granule and the control surface environments. The fatty acid levels suggest different behavior. As the pH reaches physiological conditions, the amount of fatty acids extracted from the control medium decreases. The amount of fatty acids extracted from the chronOS granule environment indicates no general increase or decrease due to pH.

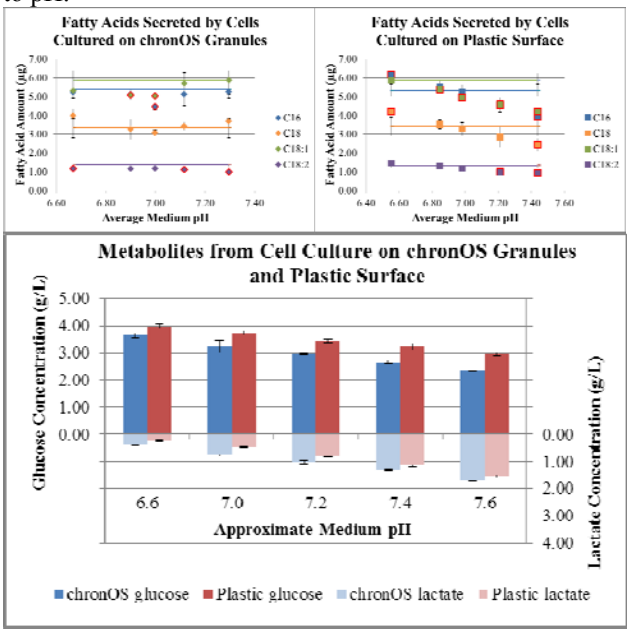

Figure 1. *Top.* Straight lines indicate fresh DMEM levels of respective fatty acids. Red outline indicates t-test significance of treatments in comparison to fresh DMEM level of respective fatty acid ($p<0.05$). C16-Palmitic acid; C18-Stearic acid; C18:1-Oleic acid; C18:2-Linoleic acid. *Bottom.* Metabolism of D1 cells on respective surface, chronOS or plastic. Cells on chronOS biomaterial consume more glucose and produce more lactate than those on the plastic surface, at all pH levels ($p<0.05$). Additionally, all metabolites differ significantly from physiological medium pH levels ($p<0.05$).

Conclusions: Preliminary results indicate that, while decreasing pH levels alter cell metabolites similarly on both chronOS granules and plastic, the chronOS granules alter fatty acid consumption suggesting the granules influence cell function. This work demonstrates the importance of understanding cellular changes on biomaterials in environments relevant to the implant site. Future work includes differentiation studies investigating cell-biomaterial interactions at pH levels simulating the levels found *in vivo*.

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References:

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