Fatty Acid-Loaded Chitosan-Gelatin Beads for Adipose Tissue Engineering

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Statement of Purpose: The clinical success of adipose tissue engineering strategies has been limited due to complications that result in loss of tissue volume over time. Our research is directed toward the development of a minimally-invasive device for breast tissue reconstruction that uses biodegradable, injectable cell microcarrier beads and a hydrogel delivery medium to stimulate regeneration of host adipose cells and fill soft tissue voids in the breast (1,2). Within adipose tissue, adipocyte cells store lipids primarily in the form of triglyceride, which is comprised of three fatty acids attached to a glycerol molecule. Fatty acids are therefore often considered to be the building blocks of triglyceride, the stored fat within adipose tissue. We hypothesize that the inclusion of fatty acid supplements in our injectable composite system will enhance the success of our system for breast tissue reconstruction by stimulating lipid filling within the adipose cells to facilitate an increase in adipose tissue mass. The objective of this study was to evaluate the influence of fatty acid-loaded chitosan-gelatin beads on adipose cell differentiation.

Methods: Bead Fabrication: Sterile chitosan-gelatin beads, with and without fatty acid incorporated, were formed electrostatically. A 3% w/v chitosan-gelatin solution was prepared and the beads were formed after ejection into a 0.5N solution of sodium hydroxide (NaOH). Fatty acid-loaded beads were formed by mixing the chitosan-gelatin solution with linoleic acid-albumin (LA) (2:1 by volume) and ejecting the solution into NaOH. Beads were rinsed with sterile PBS and DMEM culture medium before use in cell cultures. The cumulative concentration of fatty acid released from the beads was measured using standards prepared from dilutions of the LA stock solution. **Cell Culturing:** Murine mesenchymal stem cells, D1 cells, were seeded onto plain and fatty acid-loaded beads (FA-Beads) in 24-well Ultra-Low Binding plates with $4.5x10^5$ cells and 0.6mL of beads per well. An adipogenic differentiation cocktail (AC) consisting of insulin, dexamethasome, and IBMX was added to the cells after 2 days of culture. Cells were evaluated at Day 10 and Day 17 to assess metabolic activity, intracellular lipid, and total triglyceride content.

Results: As shown in Figure 1, there was no difference observed in bead color or shape due to the addition of linoleic acid (A&B). The average size of the plain and fatty acid-loaded beads was 0.246 ± 0.013cm and 0.245 ± 0.022cm, respectively. The cumulative quantity of linoleic acid released from the volume of beads was approximately 85.83 ± 0.09 µg/mL during a 28 day incubation period. Oil Red O staining of intracellular lipid showed the presence of lipid on each bead type, however, a greater quantity of mature lipid droplets were observed in D1 cells cultured on fatty acid-loaded beads with the AC (Figure 1F).

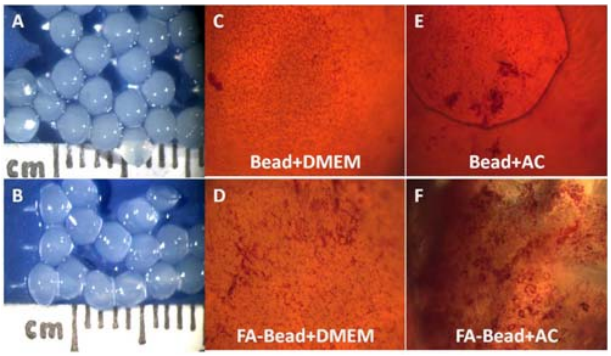

Figure 1: Representative images of chitosan-gelatin beads (A) and fatty acid-loaded chitosan-gelatin beads (B). Oil Red O staining of intracellular lipid showed larger, more mature lipid droplets when cells cultured on fatty acid loaded beads with adipogenic cocktail (F).

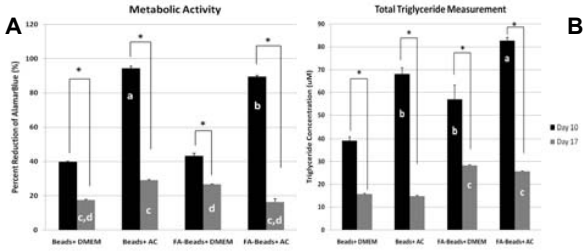

Figure 2: Measurement of metabolic activity and total triglyceride content. Asterisks (*) indicate statistically significant values. Letters a, b, c, and d denote values of statistical difference ($p<0.05$).

Results of the alamarBlue® (Figure 2A) showed significantly higher metabolic activity in cells at Day 10 than at Day 17 for each condition. Measurement of total triglyceride (Figure 2B) within the cells showed significantly higher amounts of triglyceride at Day 10 than at Day 17 for each condition. The cells grown on FA-Beads, receiving the adipogenic cocktail produced a significantly higher amount of triglyceride than all other samples at Day 10. At Day 17, the cells grown on beads with fatty acid had significantly higher amounts of triglyceride than those on plain beads.

Conclusions: Linoleic acid may be successfully incorporated into chitosan-gelatin beads and delivered to cells in culture. These results indicate that both chitosan-gelatin beads and fatty acid-loaded chitosan-gelatin beads are capable of supporting adipose cell growth and differentiation. Fatty acid-loaded beads could be useful for stimulating fatty acid uptake and lipid production in adipose cells of the injectable composite system.

References: (1) KJL Burg, *Tissue Engineering Composite*. 2006: United States Patent. (2) KJL Burg et al. *Trans 2000 World Biomat Congr.*

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