Evaluation of Factors that Influence Adipogenic Cell Differentiation

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Statement of Purpose: The human breast is structurally and functionally a complex biological system. This dynamic organ is comprised of multiple tissue types and their associated secretory factors that play key roles in the developmental processes of the breast throughout the stages of female maturation (1). 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 (2). The complexity of the breast must therefore be considered when investigating strategies for engineering breast tissue; however, a complete understanding of the specific interactions that occur between the stromal adipose cells and epithelial cells of the breast remains limited. To successfully engineer large, stable volumes of adipose tissue for breast reconstruction, the factors that affect the differentiation and lipid-filling capacity of adipose cells must first be identified. This study was performed to identify the effect of fatty acid supplemented medium on adipogenic cellular behavior during various stages of cell culture *in vitro*.

Methods: D1 cells were seeded at 20% seeding density (7.6×10^4 cells/well) in 12-well culture plates and cultured in 2mL Dulbecco's Modified Eagle Medium (DMEM). Cells were supplemented with 4% volume/volume (v/v) linoleic acid-albumin (LA) (1mL linoleic acid/100ml DMEM) as follows:

Control samples:

- DMEM- basal medium only
- AC- adipogenic cocktail consisting of insulin, dexamethasone, and 3-isobutyl-1-methylxanthine

Experimental samples:

- LA Begin- linoleic acid added at initial seeding
- LA Confl- linoleic acid added at cell confluence
- LA Diff- linoleic acid added after cells differentiated with cocktail for 4 days

Cells were cultured with the specified conditions for 10 days. Samples were characterized using Oil Red O (ORO) staining, Total Triglyceride Assay (n=3), and Gas Chromatography (GC) to assess intracellular lipid presence, total triglyceride content, and lipid identity, respectively. All statistical analyses were performed using SAS 9.1. The least squares mean was used with a significance level of $\alpha = 0.05$.

Results: Measurement of the total triglyceride content (Figure 1) indicated that the addition of linoleic acid to the cell cultures at any culture stage resulted in higher lipid production than if cells were cultured without fatty acid in the medium. Additionally, the largest amount of lipid was found within the cells that received linoleic acid at the beginning of the culture period. This quantity was significantly larger than the amount of triglyceride measured in the other sample conditions. ORO staining (Figure 2) also showed that intracellular lipid was present within each of the samples; however, the lipid droplets within the LA Diff samples were clearly more rounded in shape and larger in size, characteristic of maturing adipocytes. Further characterization of these cells with GC analysis indicated that the lipid profile for cells receiving linoleic acid at any stage of culture was different than the lipid profile of cells not cultured in linoleic acid at all (results not shown here).

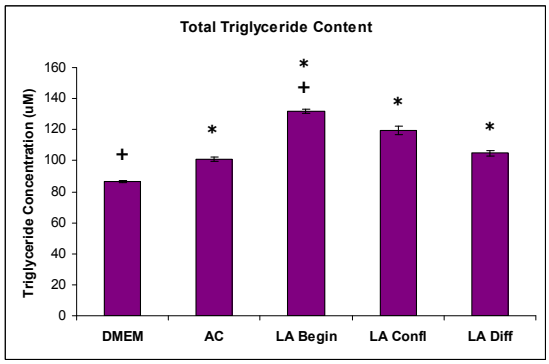

Figure 1: Total Triglyceride Measurement

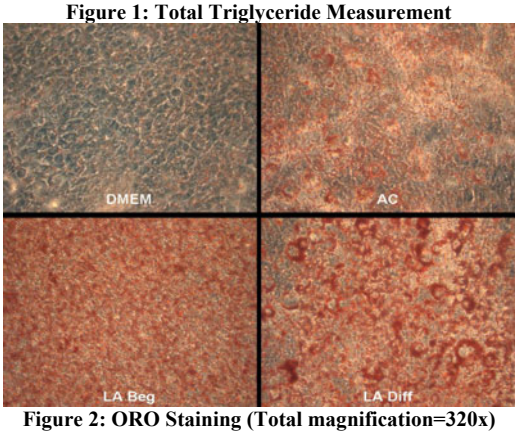

Figure 2: ORO Staining (Total magnification=320x)

Conclusions: It has been shown that the addition of fatty acids to adipose cell culture medium facilitates increased lipid production within D1 cells. While the quantity of lipid is increased with the addition of fatty acid alone, it appears that fully differentiated, mature fat cells are not formed with fatty acid media supplements alone; other stimuli may be necessary for complete differentiation to occur. For our long-term goal of breast reconstruction, it is feasible that the release of fatty acids incorporated within the microcarrier beads for our tissue engineered system will prove useful in maintaining the lipid present within implanted cells. This, in turn, would aid in maintaining the volume of adipose tissue formed after implantation *in vivo*.

References: (1) Lanigan F, et al. Cell Mol Life Sci. 2007;64:3159-84. (2) McGlohorn JB, et al. J Biomed Mater Res 2003; 66A:441-49.

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