The Effect of Mammary Epithelial Cell Conditioned Media on Adipose Cell Differentiation in Two-Dimensional Culture

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Statement of Purpose: Adipose tissue engineering strategies have been widely investigated to provide methods useful for reconstruction of breast tissue. The relationship between mammary epithelial cells and adipose cells is one of great interest since fat and epithelial cells are two of the most common cell types found within mammary tissue of the breast. Our research is directed towards 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). Therefore, the adipose cell-epithelial cell relationship is of particular interest for our research, as adipose cells, will interact with epithelial cells once implanted into a patient's body. The objective of this study was to investigate the relationship between mammary epithelial cells and primary adipose cells to determine how adipogenic differentiation is influenced in two-dimensional culture.

Methods: Conditioned media was collected from confluent cultures of bovine and murine mammary epithelial cells, denoted as MACT-CM and NMuMG-CM, respectively; the medium was stored at 4°C until use. Immediately before use, the medium was warmed to 37°C and centrifuged at 1000 rpm for 5 minutes to collect residual cellular debris.

Primary murine and human adipose cells were isolated using an enzymatic digestion procedure and seeded in 12-well culture dishes at 20% seeding density (~$5.3x10^4$ cells per well). The cells in the well plates were cultured until confluence, at which time cells were cultured under the following specified conditions:

Controls:

- No additives (DMEM only)
- Adipogenic differentiation cocktail (AC)

Experimental:

- MACT-CM (one part DMEM + one part MACT-CM)
- MACT-CM +AC (one part AC + one part MACT-CM)
- NMuMC-CM (one part DMEM + one part NMuMG-CM)
- NMuMG-CM + AC (one part AC + one part NMuMG-CM)

At the completion of the study, cells were characterized using an Oil Red O stain and Adipocyte Differentiation assay (ZenBio) to evaluate intracellular lipid and total triglyceride content, 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: As shown in Figure 1, treatment of murine adipose cells with MACT-CM and MACT-CM+AC resulted in decreased triglyceride production when compared to the adipogenic control. When treated with NMuMG-CM, murine adipose cell differentiation was not inhibited. For cultures of human adipose cells, treatment with either MACT-CM or NMuMG-CM resulted in no significant effect on triglyceride accumulation, indicating no effect on adipogenic differentiation, as shown in Figure 2.

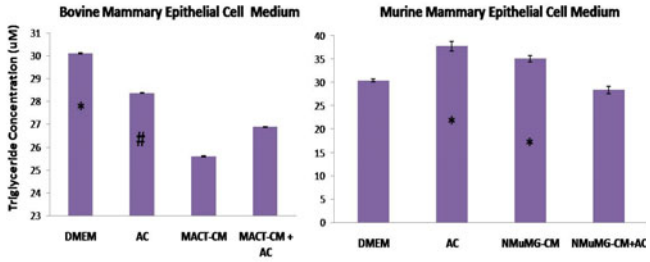

Figure 1: Triglyceride Measurement within Murine Adipose Cells

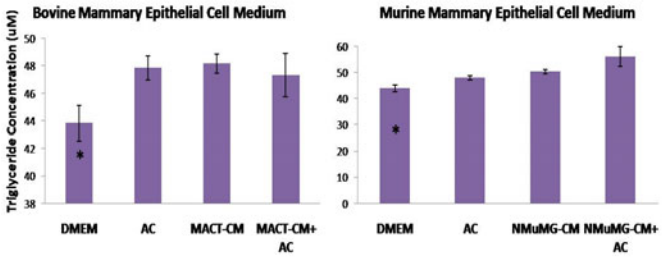

Figure 2: Triglyceride Measurement within Human Adipose Cells

Conclusions: The results of these studies, to date, suggest that the influence of mammary epithelial cell conditioned media on adipose cell differentiation is species dependent; i.e., adipose differentiation will either be positively or negatively influenced depending on the type of epithelial cell conditioned medium in which the fat cells are cultured. The culturing of primary adipose cells with mammary epithelial cell conditioned medium is therefore useful as an indicator for how cells used within our proposed breast reconstruction system will behave *in vivo*. Future work will include characterization of conditioned media components to identify specific factors expressed in the media that may play a role in adipogenesis. Additionally, the behavior of adipose cells cultured with conditioned media in three-dimensional cultures will be evaluated.

References: (1) McGlohorn JB, et al. J Biomed Mater Res 2003; 66A:441-49.

Acknowledgements: Funding was provided by Department of Defense (DoD) Breast Cancer Research Program Pre-Doctoral Fellowship (BC061439), DoD Era of Hope Scholar Award (BCO44778), and NSF Emerging Frontiers in Research and Innovation Grant (0736007).