Composite Hydrogel Scaffold for Increased Fat Retention After Surgical Graft Procedure

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Statement of Purpose: Soft tissue reconstruction for the repair congenital deformities or defects from tumor resections/trauma often requires an adequate replacement of adipose tissue. Autologous fat grafting using processed lipoaspirate is a minimally invasive option in reconstructive surgery. However, results are unpredictable due to post-graft resorption which can sometimes result in as little as 10% original fat volume retained. Several researchers have investigated traditional biomaterials but most show some degree of fibrous encapsulation or struggle to resist rapid resorption *in vivo*. In order to match the unique composition of the native adipose extracellular matrix (ECM), donated decellularized whole fat matrix was chosen the material of choice to develop a hydrogel scaffold. Dexamethasone, a well-known and researched glucocorticoid, was encapsulated in poly lactic-co-glycolic acid (PLGA) microspheres (MS) are added to the hydrogel to help increase adipogenesis. We hypothesize that the use of this biomaterial composite improve autologous fat grafting results, more specifically, retaining fat volume. In the current research, *in vitro* testing of adipose derived stems cells were cultured on top of the composite hydrogel to assess viability of cells.

Methods: Abdomen whole fat was donated from a non-diabetic female (age: 41, BMI: 26.3) undergoing a routine surgery at the University of Pittsburgh Medical Center. The decellularization process includes four main phases taking up to a week of rinses, delipidization, and disinfection of the adipose matrix. After processing, the matrix is snap frozen using liquid nitrogen and lyophilized via laboratory freeze dryer. A Mini Wiley Mill (Thomas Scientific, Swedesboro, NJ) breaks down the lyophilized matrix into a powder for pepsin digest and hydrogel formation. PLGA (50:50) (Sigma Aldrich, St. Louis, MO) was used as the base polymer to encapsulate dexamethasone in MS using a single and double emulsion mixing technique. An *in vitro* release study was conducted via ultraviolet-visible spectroscopy of microsphere samples in phosphate buffer saline (1X) at 37 °C. Adipose-derived stems cells (ASCs) were acquired using an isolation protocol on abdominal fat donated from a non-diabetic female (age: 38, BMI: 24.8) following a routine surgical procedure. *In vitro* study was conducted in a tissue culture well plate over a four day period. Live/dead assay cell viability assay (Life Technologies, Grand Island, NY) was used for assessing ASC survival on composite hydrogel scaffold. Immunohistochemistry confirmed decellularized adipose ECM content and effectiveness of decellularization process. Scanning electron microscopy (Jeol USA, Inc., Peabody, MA) was used to examine the structure of lyophilized matrix.

Results: Decellularization and delipidization process were confirmed by immunohistochemistry staining with hematoxylin & eosin staining and adipored reagent assay, respectively. SEM image in Figure 1 also supports the decellularization with the lack of cellular imagery while also giving a microscopic look at the matrix. Matrix structure is similar to results seen in the literature. Collagen IV protein content was confirmed after staining and results can be seen in Figure 2. In vitro release studies of MS confirmed dexamethasone encapsulation under both single and double emulsion methods. In vitro studies of ASCs with the composite hydrogel gave promising results with cell viability assessed at over 90% for each of the experimental groups (hydrogel alone, hydrogel w/ single emulsion MS, and hydrogel w/ double emulsion MS).

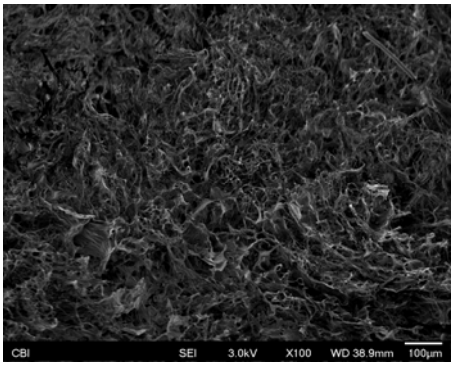

Figure 1. Scanning Electron Microscope Image of Decellularized Adipose ECM. Scale bar is 100 µm.

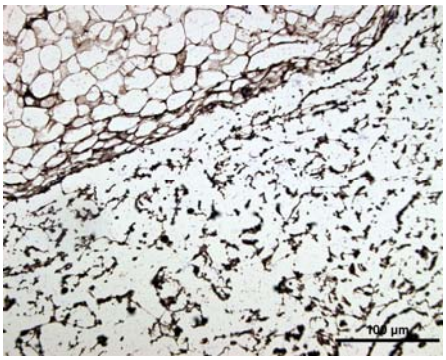

Figure 2. Brightfield Microscopy Image of Paraffin-Embedded Decellularized Matrix with Collagen IV stain.

Conclusions: From the results which have been gathered so far, it can be concluded that the development of a composite hydrogel was successfully achieved. In vitro studies of the composite hydrogel with ASCs demonstrated that a safe and biocompatible material was developed from donated adipose fat tissue and drug encapsulated microspheres. These results are the first steps in the process of creating an injectable scaffold for increasing fat retention in soft tissue reconstruction procedures. The next step will be to further analyze the composite hydrogel scaffold and complete an in vivo study with a longer time point and endpoint assays which assess cell differentiation, proliferation, and lipid accumulation.