Comparison of Three Methods of Derivation of a Biologic Scaffold Composed of Adipose Tissue Extracellular Matrix

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Statement of Purpose: Surgical methods for the repair of adipose tissue are generally sufficient for tissue repair, however there are limitations regarding long-term survival and functionality of transplanted tissue. Synthetic and biologic materials have been utilized as constructs for soft tissue repair. However, these materials are often subject to resorption with a loss of mechanical integrity or to a foreign body response and an undesirable outcome. Biologic scaffold materials composed of extracellular matrix (ECM) have been harvested from a variety of tissues and organs and used successfully in a number of regenerative medicine approaches to tissue reconstruction. Adipose tissue represents an abundant source of ECM and may also represent an ideal scaffold material for growth, differentiation, and maintenance of cells harvested from adipose tissue. The present study characterized adipose ECM materials resulting from three methods of decellularization to determine the most effective method for derivation of an adipose ECM scaffold which was largely free of cellular content while retaining tissue-specific structural and functional components.

Methods: Porcine adipose tissue was frozen at -80°C and sliced into 3 mm sheets. Tissues were then treated with one of the decellularization methods described below. Method A included manual massaging of the tissue, treatment in 0.02% trypsin/0.05% ethylenediaminetetraacetic acid (EDTA), 3% Triton X-100, 4% deoxycholic acid, 0.1% peracetic acid, and 100% n-propanol. Method B included treatment in collagenase (3mg/g tissue), 0.02% trypsin/0.05% EDTA, 10 U/mL DNAse, and 10 U/mL lipase. Method C included treatment in collagenase (3mg/ tissue), 0.05% EDTA, 0.1% nonyl phenoxylpolyethoxylethanol, 4% sodium deoxycholate, 1% sodium dodecyl sulfate, and 0.9% NaCl in TRIS-HCl containing protease inhibitors. The resulting scaffold materials were evaluated for removal of cellular content (histologic staining, PicoGreen DNA Assay, agarose gel electrophoresis), the effects of each method upon scaffold ultrastructure (scanning electron microscopy; SEM), maintenance of ECM components and growth factors (ELISA and immunolabeling), and ability to support growth and differentiation of adipose derived stem cells (ADSCs) towards an adipogenic lineage (cell culture and labeling).

Results: ECM produced using Method A was a dry, white, fibrous material, while Methods B and C both produced white materials with wet, shiny surfaces. Oil red O staining showed little lipid in Method A scaffolds, however lipid was present throughout Method B and C scaffolds. No intact nuclei were observed in histologic sections of scaffolds resulting from any of the three methods. Method A scaffolds did not appear to contain DNA on agarose gel electrophoresis. Scaffolds produced using Methods B and C were shown to contain DNA of large bp length. No DNA was detected by PicoGreen assay for Method A and B scaffolds. Method C scaffolds contained 78.1 ng DNA/mg weight. SEM showed that Methods B and C resulted in an uneven globular appearance indicative of high lipid content. Method A resulted in a rough and uneven surface architecture indicative of collagenous ECM components. Lipid droplets were observed in Method A scaffolds, but were smaller in size and less in number than Method B and C scaffolds. Immunolabeling showed differences in the morphology and spatial distribution of the ECM components, and that differences were dependent upon decellularization protocol. Basic fibroblast growth factor was present in Method A, B, and C scaffolds at 2551.8 +/-148.1, 1840.5 +/-92.3, and 54.49+/-6.39 pg/g dry weight, respectively. Vascular endothelial growth factor was present in Method A and B scaffolds at 15.2 +/-13.0, and 27.6 +/-1.2 pg/g dry weight, respectively, but was not detected in Method C scaffolds. Glycosaminoglycans were present in Method A, B, and C scaffolds at 1109.0 +/-43.1 ug/g weight, 768.3 +/-52.2 ug/g weight, and 95.2 +/-4.3 ug/g weight, respectively. 95% of ADSCs seeded onto adipose ECM scaffolds were viable 24 hrs after seeding and viability was 99% at 72 hours post-seeding, regardless of preparation method. ADSCs seeded onto the surface of the three different types of porcine adipose ECM maintained an adipocyte phenotype as evidenced by positive labeling using Adipo-Red, suggesting that all three methods produced scaffolds which were able to support the growth and differentiation of ADSCs along an adipocyte lineage.

Conclusions: The results of the study showed that each decellularization method was associated with distinct structure and composition of the resulting material. Despite these differences, the ability to support the growth and adipogenic differentiation of ADSCs was unaffected, likely due to the adsorption of culture media to the surface of the materials. However, only Methods A and B achieved effective decellularization of the adipose tissue, and only Method A was shown to remove the majority of the lipid content of the adipose tissue. The presence of cellular remnants, including excess lipid, may affect the ability of an adipose ECM material to function as a template for constructive remodeling *in vivo*. This study shows the importance of the decellularization protocol and suggests that adipose ECM scaffolds derived using Method A as described herein may represent an effective substrate for use in tissue engineering and regenerative medicine approaches to soft tissue reconstruction.