Injectable, Decellularized Adipose Extracellular Matrix as a Scaffold for Soft Tissue Engineering

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Statement of Purpose: Adipose tissue engineering strives to produce an alternative to artificial subcutaneous materials that simply fill voids, instead encouraging regeneration of damaged tissue or non-existing tissue. However, common synthetic polymers often see some degree of fibrous encapsulation in animal models, and natural biopolymers, such as collagen, are rapidly resorbed (Patrick C. Tiss Eng. 1999;5(2):139-151; von Heimburg D. Biomat. 2001;22(5):429-438). Clinical trials of hyalauronic acid scaffolds showed maintained shape and cell infiltration, but were encapsulated with fibrous tissue and had no mature adipocytes (Stillaert FB. Biomats. 2008;29(29):3953-3959). Several clinicians have pursued autologous alternatives by using free fat transfer to augment soft tissues, but these treatments suffer from necrosis and resorption, and are limited to small volumes (Meier J. Arch Facial Plast Surg. 2009;11(1):24-28). Thus, there remains a need for a material that will discourage fibrosis and resorption while mimicking natural adipose tissue.

Decellularization of tissues offers the possibility of producing a scaffold of extracellular matrix (ECM) components that closely mimics the physical and chemical cues seen by cells in the body. Several tissues have already been decellularized (Gilbert TW. Biomats. 2006;27(19):3675-3683); while they share many of the same ECM components, each tissue has its own specific combination of proteins and polysaccharides. Thus, it appears that the ideal naturally derived tissue engineering scaffold would incorporate ECM from that specific tissue. We therefore sought to develep a method to decellularize human lipoaspirate and produce an injectable scaffold from this tissue, thus offering a potentially autologous material for adipose tissue engineering.

Methods: Fresh human lipoaspirate was collected from 40-50 year old females undergoing elective liposuction treatments. Fresh-frozen lipoaspirate was stirred at room temperature in either 2.5 mM sodium deoxycholate in phosphate buffered saline (PBS) or 1% sodium dodecyl sulfate (SDS) in PBS. The detergent was replaced after 2 hours to remove cellular debris and red blood cells. This process was then repeated every 12 hr. After a total of 48 hr, 5000 units of porcine pancreatic lipase were added to digest any remaining triglycerides. Porcine colipase was added in an equimolar ratio to improve lipase activity. The lipase digest continued until the tissue was completely white, indicating full cell and lipid removal, generally 7-15 hours depending on the tissue sample. At the end of the digest, the tissue was rinsed and then stirred overnight in DI water to remove remaining detergents and enzymes. Decellularization and delipidization were confirmed with hematoxylin and eosin (H&E) and oil red O staining of OCT embedded frozen samples. The remaining ECM was lyophilized and then milled using a Wiley Mini Mill. The resulting powder was solubilized using pepsin from porcine gastric mucosa in 0.1 M HCl under agitation at room temperature, an adapted version of a protocol used for urinary bladder matrix (Freytes DO. Biomats. 2008;29(11):1630-1637). While the lipases will be removed from the material, fragments of pepsin will remain; pepsin can however be isolated from a patient, and thus the resulting material could be completely autologous. After 56 hours, the digest was neutralized on ice to pH 7.4 using NaOH, diluted with PBS, and analyzed with polyacrylamide gel electrophoresis (SDS-PAGE). To induce gelation, 100 µL injections at a concentration of 17 mg/mL were made subcutaneously into the back of female Sprague-Dawley rats with a 25 G needle. After 15 min, a solid gel was excised and analyzed with H&E staining.

Results: 1% SDS was unable to adequately remove lipids, regardless of the use of lipase, which prevented further processing of the tissue. Only 2.5 mM Na deoxycholate, when combined with lipase, could effectively decellularize and delipidize the tissue, as shown by the lack of nuclei with H&E (Fig 1A-C) and lipids with oil red O (not shown). SDS-PAGE confirmed the presence of collagen, as well as lower molecular weight proteins and peptides in the adipose matrix (Fig 1G). At a final concentration of 17 mg/mL, the solubilized matrix self-assembled into a gel within 15 minutes both *in vitro* and *in vivo* at 37 °C and pH 7.4 (Fig 1D-F). Excised *in vivo* gels formed a fibrous and porous network as indicated by an H&E stained section of the tissue (not shown).

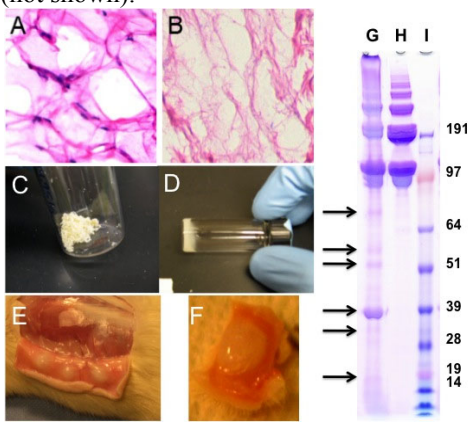

Figure 1. H&E of adipose tissue pre- (A) and post- (B) decellularization, lyophilized matrix (C), *in vitro* gelled matrix (D), *in vivo* gelled matrix (E) and (F), and SDS-PAGE of 17 mg/mL digested matrix (G), 6 mg/mL collagen (H), and standard (I) in kDa

Conclusions: The high density of lipids in adipose tissue imposes limitations to current decellularization techniques. We have demonstrated a new method for removing both cells and lipids from lipoaspirate, in a manner inspired by natural fat metabolism, to generate an injectable, thermoresponsive hydrogel. The relative ease of collection and injection of this adipose matrix hold promise for a potentially autologous, minimally-invasive option for *in situ* adipose tissue engineering.