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Developing a clinical grade human adipose decellularized biomaterial

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ABSTRACT

While tissue engineering investigators have appreciated adipose tissue as a repository of stromal/stem cells, they are only now beginning to see its value as a decellularized tissue resource. Independent academic investigators have successfully extracted lipid, genomic DNA and proteins from human fat to create a decellularized extracellular matrix enriched in collagen, glycoproteins, and proteoglycans. Pre-clinical studies have validated its compatibility with stromal/stem cells and its ability to support adipogenesis *in vitro* and *in vivo* in both small (murine) and large (porcine) subcutaneous implant models. Furthermore, Phase I safety clinical trials have injected decellularized human adipose tissue scaffolds in human volunteers without incident for periods of up to 127 days. This commentary takes an opinionated look at the under-appreciated but potential benefits of obesity as an increasingly available biomaterial resource.

The past as prologue

Over two decades ago, investigators recognized fat as a rich source of adipose-derived stromal/stem cells (ASC) (1). After identifying the many potential lineage differentiation pathways exhibited by ASC, De Ugarte, Hedrick and colleagues made the prescient prediction that fat would be a "raw material for tissue engineering" (1). As practicing plastic surgeons, they recognized the advantages of fat as an abundant and replenishable human tissue that was accessible through lipoaspiration, a relatively non-invasive out-patient surgical procedure (1). Furthermore, they appreciated that tissue engineering with ASC would require synthetic or biological scaffolds (1). Nevertheless, the complexity of removing lipid from the adipose tissue delayed its consideration as starting material for decellularization protocols. Despite this substantial challenge, Flynn extended the utility of fat by demonstrating that adipose tissue could itself be the source of a biological extracellular matrix (ECM). Using a combination of enzymatic, mechanical, and organic solvent extraction steps, Flynn isolated and characterized a decellularized adipose-tissue ECM (2). With these advances, investigators across multiple fields began to appreciate that adipose tissue would serve not only as a raw material for tissue engineering cells and growth factors but also as a unique scaffold.

Current attractions

Multiple pre-clinical studies have begun to explore the utility of decellularized adipose-derived materials in regenerative medicine. Recently, two independent academic groups have demonstrated the safety and utility of decellularized human adipose tissue scaffolds in both preclinical and clinical trials. The team at the University of Pittsburgh decellularized human cadaveric adipose tissue through sequential steps of lipid extraction with propanol, decellularization with sodium deoxycholate, and sterilization with peracetic acid, followed by lyophilization and milling (3,4). In their review of the literature, these authors noted that previous studies had demonstrated that these steps efficiently extracted genomic DNA and triglycerides which were likely to cause a foreign body reaction and immune rejection of a decellularized scaffold implant (5). Consistent with these observations from the literature, their preclinical examination demonstrated that the injected scaffold was tolerated in immunodeficient mice and served as the matrix for adipocyte differentiation *in vivo* (3). In clinical studies, patients with the scaffold implanted into the dorsum of the hand ($n = 14$) or into the pannus of patients scheduled for abdominoplasty ($n = 10$) safely tolerated the allogeneic biomaterial without evidence of rejection (3,4). Furthermore, the dorsal hand implants displayed evidence of volume retention over time and in both instances, histological analyses documented evidence of cellular infiltration and adipogenesis in a time dependent manner (3,4). Similarly, the team at Johns Hopkins University decellularized human abdominoplasty tissue by mechanical processing and rinsing followed by sterilization in peracetic acid to create a biomaterial scaffold (5). Using a global, unbiased proteomic mass spectrometry approach, they determined that the decellularized adipose tissue contained multiple collagens (I, II, IV, VI), proteoglycans (biglycan, decorin, lumican), and gly-

Abbreviations: ASC, Adipose stromal/stem cell; CD4, Cluster of differentiation 4; ECM, Extracellular matrix; M2, alternatively activated macrophage; MTF, Musculoskeletal Transplant Foundation.

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coproteins (cartilage intermediate layer protein, fibrillin, laminin), consistent with independent reports (5,6). When implanted subcutaneously into immunodeficient mice, the decellularized adipose tissue supported the formation of a fat depot. Indeed, the decellularized adipose tissue alone retained volume over time at a level comparable to that of intact fat grafting. Nevertheless, it was noteworthy that the addition of human ASC to the decellularized adipose tissue graft reduced the level of volume retention over time as compared to decellularized adipose tissue implants alone (6). Yorkshire pigs tolerated subcutaneous injections of the decellularized adipose tissue in volumes of 3 to 20 ml per site for periods of up to 4 weeks. Analyses of the infiltrating cell number between the edge and center of the implant indicated that there was minimal evidence of rejection (5). Further clinical studies were performed in patients scheduled to undergo abdominoplasty or panniculectomy ($n = 8$ subjects). Subcutaneous implantation of 2 to 4 ml of decellularized scaffolds for periods of 5 to 127 days were tolerated without severe adverse events. Flow cytometry analyses determined that the implants promoted infiltration of $CD4^+$ and $FoxP3^+$ T cells as well as activation of M2 macrophges (5). Together, these studies validate decellularized human adipose tissue as a novel biomaterial for clinical applications with early adoption likely for cosmetic and reconstructive plastic surgery, orthopedics, and dental/craniofacial repairs. Furthermore, global proteomics provides a reproducible and readily available technology to assess biomaterial product composition. This could be adapted as an industry-wide quality assurance/quality control methodology for product characterization and lot release.

Additional studies have manufactured decellularized adipose tissue in multiple formats suited for specific clinical applications. Flynn and colleagues have electro sprayed decellularized adipose tissue into liquid nitrogen followed by sieving-based size separation to create beads that can be self-assembled with luciferase-trackable ASC. Together, these can be cultured in molds to create constructs suitable for soft tissue reconstruction (7). This team has further advanced their processing methods enzymatically digesting the decellularized adipose tissue with α-amylase digestion prior to electrospray to create a microcarrier bead formulation. These have proved suitable for stromal cell adhesion and expansion in spinner flask bioreactors as well as subcutaneous implantation into immunocompetent mice (8). Likewise, decellularized adipose tissue can be processed as a hydrogel sheet, with or without the incorporation of ASC; this has been used to successfully repair critical sized femoral bone defects in an immunocompetent murine model (9). Finally, it has been possible to further modify the properties of decellularized human adipose tissue matrices by chemical cross-linking to polyethyleneglycol via thiol/acrylate addition (10). When implanted subcutaneously in immunocompetent mice, these chemically modified decellularized adipose ECM constructs supported host cell infiltration and adipogenesis (10). Thus, there is ample evidence that decellularized adipose tissue is compatible with multiple formats (beads, foam, hydrogel, sheet) applicable to a myriad of clinical situations and conditions requiring a range of viscoelastic and tensile properties.

Preview of future features

Research grade decellularized adipose scaffolds have been validated rigorously by multiple independent academic investigations in preclinical *in vitro* and *in vivo* models and are being explored in ongoing clinical investigations and trials. Indeed, MTF Biologics now distributes Renuva™ as a decellularized human adipose-derived product for soft tissue augmentation in the United States (https:www.myrenuva.com). At present, adipose tissue is routinely discarded as medical waste. Its use as a biomaterial offers the opportunity to deploy adipose tissue in a manner not unlike that of decellularized bone grafts, the most commonly transplanted human tissue other than blood. With hundreds of thousands of healthy individuals undergoing elective liposuction and abdominoplasty annually, it is feasible to consider the development of a pre-screening process to allow for the collection of clinical grade adipose tissue as a raw material. Adipose tissue received from donors meeting strict inclusion/exclusion criteria could be stored frozen for future decellularization processing with minimal complications. Unlike ASC isolation steps that must be performed within hours of tissue collection, ECM preparations from human adipose tissue can be delayed for weeks, months or longer if the starting tissue is kept appropriately cryopreserved during the interval. It is likely that clinical grade decellularized adipose scaffolds comparable to Renuva™ will soon gain full regulatory approval for specific clinical applications. As post-marketing data accumulates from such products, surgeons will gain confidence in the use of decellularized adipose tissue and adopt it as a routine tool for patient care. Of course, such post-marketing studies must focus on potential shortfalls in the product's performance, such as the ability of the implant to retain volume over time as this remains a confounding variable for autologous fat grafting procedures.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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