Pre-Clinical Assessment of an Adipose-Derived Allograft Scaffold

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INTRODUCTION

In recent decades, the usage of autologous fat grafting (AFG) has exponentially increased in the clinical setting, now being used for a plethora of applications in both aesthetic and reconstructive interventions [1]. The use of AFG has shown significant efficacy in many facets of surgery and over a significant range of injection volumes, beginning with small-volume fills translating to scar revision [2] or facial rejuvenations [3], to fills requiring much larger volumes such as breast or buttocks augmentations [4, 5]. Despite the clinical success of AFG it has not been completely free of challenges; there is consistent debate regarding the most optimal methods for fat harvesting and preparation with respect to centrifugation times and speeds, harvesting apparatuses and techniques, which are all thought to be directly correlated to the success of an autologous fat grafting procedure [6]. Additional challenges may also include inconsistent graft retention, donor site morbidities, insufficient harvest, or excessive harvesting times leading to complications and patient dissatisfaction [7]. Based cooperatively on the needs of the clinician, patient, and the availability of adipose tissue, an adipose-derived scaffold, Allograft Adipose Matrix (AAM), known commercially as Renuva®, has been developed, suitable for allograft transplantation; acting physiologically like autologous fat while also having an ease of application in a ready to use (off the shelf) platform resembling dermal tissue fillers. The processing of AAM is optimized to maintain the structural and endogenous components thought to be relevant toward promoting vascular angiogenesis, essential for leading to localized adipogenesis as the graft is incorporated and remodeled. This *in vitro* and *in vivo* evaluation of AAMdemonstrates a cell-friendly scaffold that allows for cell repopulation and differentiation while providing cushioning and volume retention.

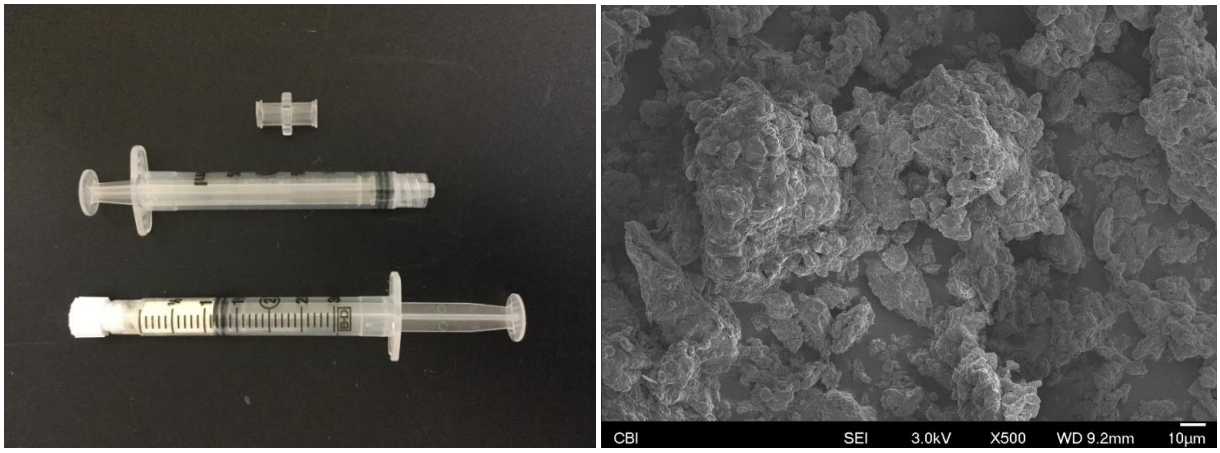

Figure 1: Allograft Adipose Matrix unit and rehydration kit (left) and scanning electron microscopy (SEM) of dehydrated Allograft Adipose Matrix (right)

METHODS

A method of processing allograft adipose tissue was developed to remove lipid and cells from the tissue, and disinfect and dehydrate the remaining adipose extracellular matrix Through this process the natural AAM components are preserved. The AAM may be stored at ambient conditions and at the time of use is rehydrated and delivered through an appropriate sized needle or cannula to the site. Adipose matrix membrane components were confirmed via immunofluorescent staining (HistoTox, Boulder, CO); adipokines and cytokines were quantified via multiplex (Molecular Resource Facility, Rutgers Medical School, Newark, NJ); residual lipid was quantified gravimetrically (NP Analytical Laboratories, St. Louis, MO) and residual dsDNA was quantified via fluorescent binding (Invitrogen, Carlsbad, CA). Cell-based *in vitro* characterization (cell attachment, proliferation and matrix deposition) was performed at Musculoskeletal Transplant Foundation (Edison, NJ) using human adipose-derived stem cells (University of Pittsburgh Medical Center, Pittsburgh, PA) where immunohistochemical staining for adiponectin was

performed at HistoTox (Boulder, CO) and Scanning Electron Microscopy at Rutgers University (Piscataway, NJ). *In vivo* studies in athymic mice with subcutaneous AAM injections were performed at Sinclair Research (Columbia, MO). Adipogenesis and revascularization of the graft were assessed histologically at various time points.

IN VITRO RESULTS

The processing of Allograft Adipose Matrix (AAM) is optimized to maintain the structural and endogenous materials thought to be relevant toward promoting vascular networks throughout the graft upon implantation, leading to localized adipogenesis as the graft is incorporated and remodeled. AAM processing allows for retention of key matrix proteins such as Collagen IV and Collagen VI (Figure 2) as well as adipogenic and angiogenic factors such as leptin, adiponectin, FGF-2 and VEGF (Table 1). The process by which AAM is produced successfully removes residual lipid, cellular fragments and dsDNA content.

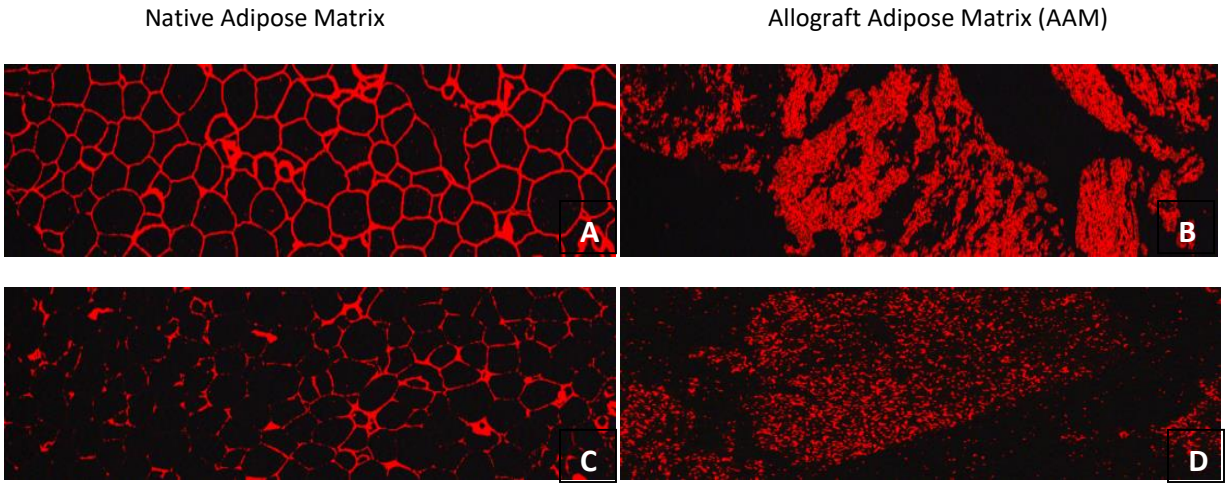

Figure 2: Key matrix proteins Collagen IV (A, B) and Collagen VI (C, D) are preserved through processing of AAM.

Table 1: Representative angiogenic and adipogenic growth factors and cytokines that are preserved through aseptic processing of AAM.

	Factor	Lipoaspirate	AAM
Key angiogenic factors	Angiopoietin-2	✓	✓
	EGF	✓	✓
	FGF-1	✓	✓
	FGF-2	✓	✓
	VEGF	✓	✓
Key adipogenic factors	Adiponectin	✓	✓
	IGF -1	✓	✓
	Leptin	✓	✓
	IL -6	✓	✓

Additionally, when human adipose-derived stem cells (hASC) are seeded on top of AAM and cultured in basal media, they readily attach to the scaffold and stretch as demonstrated by active cytoskeleton staining (Figure 3) and within 7 days in culture secrete adiponectin, an early marker of adipocyte differentiation [8], as evidenced by immunohistochemical staining of the cell-seeded scaffolds. Adiponectin was not present in the unseeded scaffolds (Figure 4). Basal media was chosen such that the culture media would not influence cell differentiation and the lineage would be influenced by the scaffold alone. This suggests that any adiponectin present in the scaffold is a result of the differentiation of hASCs into mature adipocytes, possibly due to the adipogenic factors and matrix proteins retained in the AAM scaffold. Cell proliferation and matrix deposition on the AAM scaffolds is also indicated via Scanning Electron Microscopy (SEM) of the unseeded and hASC-seeded scaffolds after 7 and 14 days (Figure 5).

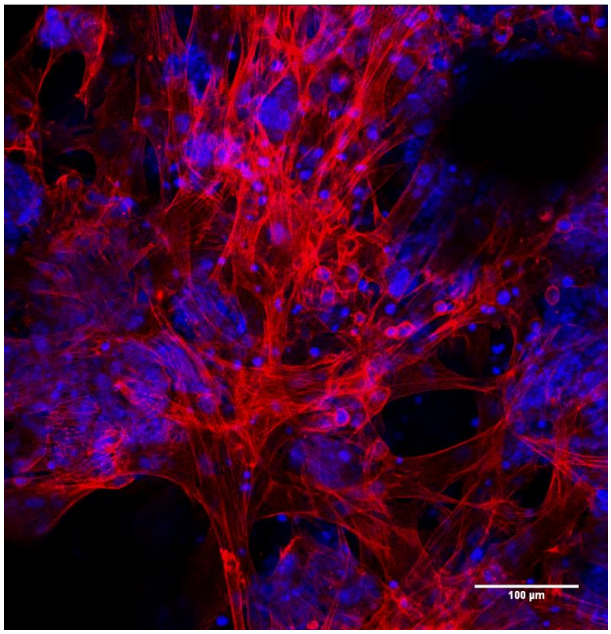

Figure 3: Actin cytoskeleton staining (red) of hASCs seeded on AAM after 3 days of culture in basal medium. Cells readily adhere to the matrix and stretch.

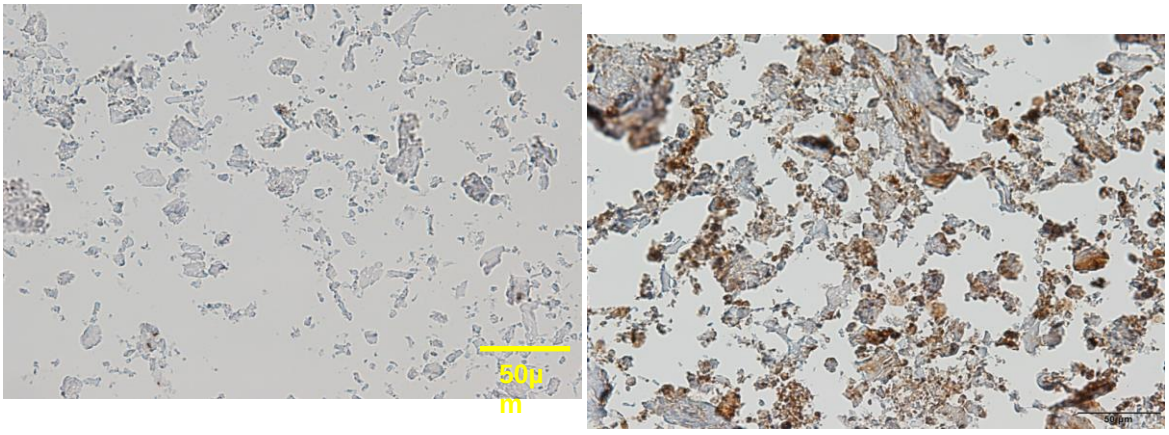

Figure 4: Image of unseeded AAM scaffold (left) and adiponectin deposition in an adipose-derived stem cell-seeded scaffold after a 7-day growth period in basal media (right) as evidenced with immunohistochemical staining

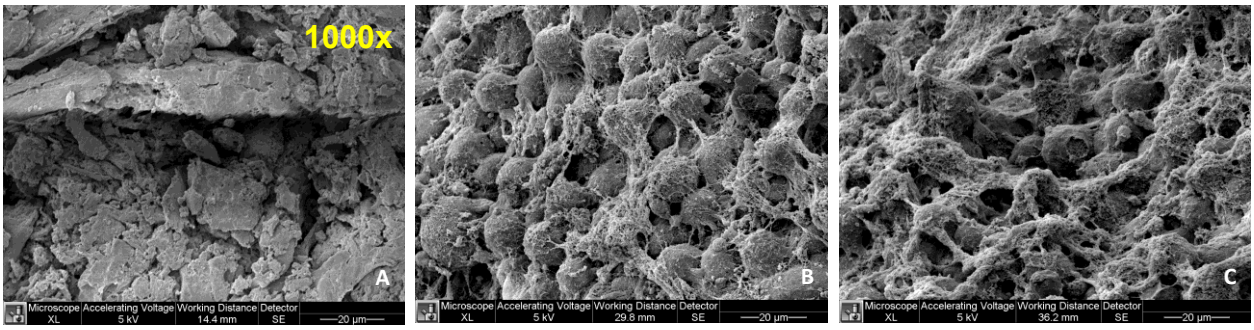

Figure 5: SEM pictures of unseeded (A) and hASC-seeded AAM after 7 days (B) and 14 days (C) of culture in basal medium.

IN VIVO RESULTS

To test the efficacy of the injectable scaffold *in vivo*, Allograft Adipose Matrix (AAM) was reconstituted in 0.9% saline and injected bilaterally in the dorsal flanks of athymic mice. The end-points for the study were volume retention and histological analysis and were evaluated at 3, 6 and 12 weeks post implantation. The explanted adipose material appeared well vascularized and of a soft, malleable consistency similar to the original adipose tissue in the mice (Figure 6).

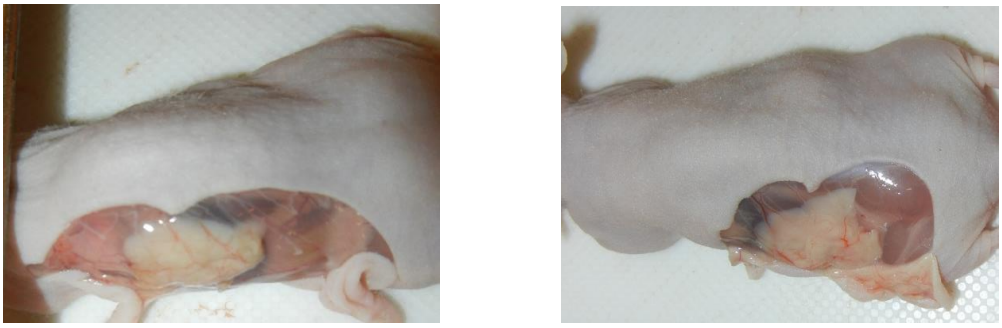

Figure 6: AAM explanted at 12 weeks demonstrates high vascularity and soft, pliable appearance

Hematoxylin and Eosin staining displays evidence of neovascularization and increasing presence of cells with adipocyte morphology in the implanted matrix over time; viable adipocyte presence was confirmed via immunofluorescent staining for perilipin-A, an expressed lipid-droplet protein of the mature adipocyte (Figure 7) [8].

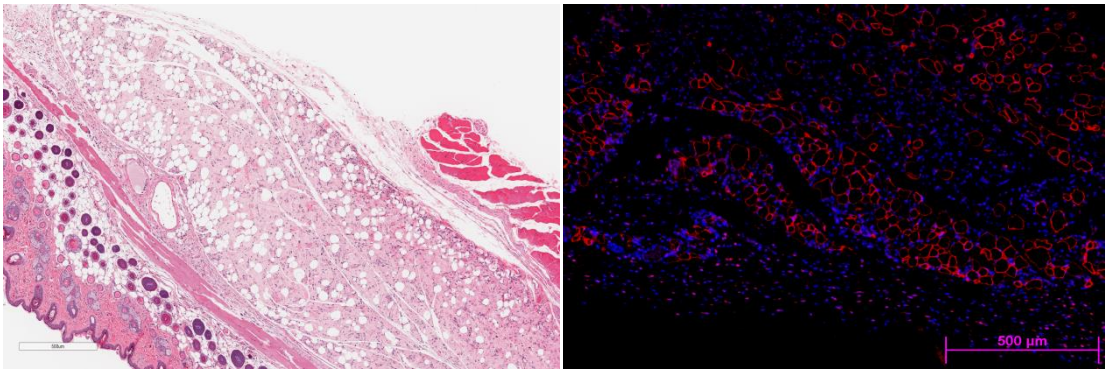

Figure 7 Twelve-week H&E (left) and perilipin-A (right) staining display viable host adipocyte presence within the implanted Allograft Adipose Matrix

CONCLUSION

With an increasing clinical demand, an off-the-shelf alternative to fat grafting in the form of an adipose-derived protein-rich allograft matrix may present an unmet clinical advantage, minimizing or eliminating fat harvest and donor site morbidity. AAM may also offer an option for patients without sufficient autologous fat. Thus far, *in vitro* and *in vivo* assessments have been performed investigating the biological aspects with respect to AAM. It has been shown that the AAM is not only remodeled by the host, but demonstrates clear signs of neovascularization, cell recruitment and adipogenic differentiation. The clinical translation of this off-the-shelf injectable scaffold's capability for long-term volume retention is undergoing further evaluation.

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