An Adipose Extracellular Matrix-Derived Biomaterial for Soft Tissue Reconstruction

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Abstract* – *An adipose extracellular matrix derived biomaterial, Acellular Adipose Tissue, can be used as an off-the-shelf alternative to autologous fat transfer for the treatment of soft tissue deformities and defects. This tissue engineering solution overcomes many challenges associated with autologous fat transfer and other common methods of soft tissue reconstruction. Subcutaneous Acellular Adipose Tissue implants display significant volume retention with minimal inflammatory response in both pre-clinical and clinical studies. The material triggers cell migration which supports development of new adipose and has also demonstrated the potential to modulate the immune response to create a more pro-regenerative microenvironment in the presence of trauma. These results indicate that Acellular Adipose Tissue could be a promising new therapeutic tool to treat soft tissue defects and promote wound healing.

I. Introduction

Adipose tissue is commonly used by surgeons for a variety of applications, including soft tissue reconstruction and wound healing [1]. Autologous fat transfer is the current gold standard treatment used to correct soft tissue defects and deformities caused by traumas, diseases, or congenital abnormalities [2]. However, this method has many limitations, including donor site morbidity, variability of outcomes, and inability to obtain sufficient volume to fill large deformities [3, 4]. To address these challenges, an off-the-shelf, injectable, human adipose derived extracellular matrix biomaterial was developed as an alternative to fat transfer.

Preclinical studies were performed in rat, mouse, and swine models to demonstrate the tissue regeneration potential and biocompatibility of the adipose extracellular matrix material, called Acellular Adipose Tissue (AAT). Mouse models of subcutaneous implantation and volumetric muscle loss (VML) injury were also used to assess the immune response triggered by AAT in both traumatic and non-traumatic environments. Recent studies characterizing the immune profiles of various ECM biomaterials suggest that the constituents of an ECM scaffold can alter the immune microenvironment of the tissue, a critical component in the wound healing process [5]. The ability of AAT to mimic native adipose tissue while potentially altering the immune microenvironment to promote healing indicates that AAT is a promising new material for the treatment of soft tissue defects.

In a Phase I clinical study, the material was implanted in eight healthy volunteers for 1 – 18 weeks prior to an elective surgery to remove redundant abdominal tissue (ie: panniculectomy). Following excision, the AAT implants and surrounding tissues were assessed histologically for inflammation and cellular migration. Additional outcomes included patient and physician satisfaction surveys and Panel Reactive Antibody (PRA) testing.

II. Methods

AAT is produced from cadaveric human adipose tissue using chemical and mechanical processing techniques which remove lipids and living cells while preserving the native architecture of the tissue. The tissue is then further processed into an injectable form preferred by patients and physicians.

For the preclinical animal studies, a murine VML wound was made by creating a critical size muscle injury in a mouse quadricep muscle. The area was then filled with 0.05 mL of material or saline solution as a control. For the murine subcutaneous model, animals received two 0.20 mL injections of material into the subcutaneous space at superior and inferior positions on the dorsal side of the animal.

In both preclinical and clinical studies, cell migration and inflammation were analyzed using hematoxylin and eosin staining of formalin-fixed, paraffin-embedded (FFPE) samples. Immune cell infiltration was examined by flow cytometry and reverse transcriptase polymerase chain reaction (RT-PCR) to quantify the presence of T cells, B cells, dendritic cells, macrophages, M1-polarized macrophages, and M2-polarized macrophages. In preclinical animal studies, adipogenesis was also examined using RT-PCR.

In the Phase I clinical study, eight patients were injected with AAT in redundant tissues previously scheduled for surgical removal in an elective surgical procedure. Implants were excised after 1, 2, 4, 6, or 18 weeks *in situ* and assessed by histopathological analysis, flow cytometry, and RT-PCR.

III. Results

Preclinical studies showed promising results for the use of AAT for soft tissue reconstruction in humans. Both VML and subcutaneous studies in mice showed good tissue integration with cell migration into the implant peaking at 1 or 3 weeks post-treatment respectively. Flow cytometric analysis of the AAT-treated quadricep muscles 1 week after injury showed significant infiltration of polarized macrophages and a moderate increase in $CD3^+$ T cells. VML wounds treated with both human- and pig-derived AAT attracted proportionately

more immature macrophages, monocytes, and other immune cells (F4/80^{lo}) to the wound area than those treated with saline (Fig. 1A). Animals treated with human AAT showed less infiltration of B cells than those treated with both saline and pig AAT.

Both human and pig AAT also promoted the migration of more M2-polarized, pro-regenerative macrophages (CD206) into the wound than saline treatment (Fig. 1B). RT-PCR analysis also showed that significantly more interleukin 4 (IL-4) was present in wounds treated with both pig and human AAT than those treated with saline. This indicates that AAT promotes the migration of immune cells which trigger the release of this key pro-regenerative cytokine. This increased expression of IL-4 is likely not due to an increased proportion of helper T cells in the wound, but may be due to the increased proportion of myeloid cells orchestrating upregulation of IL-4 secretion from T_H2 helper T cells, resulting in a more pro-regenerative microenvironment.

Because the clinical AAT is derived from human adipose tissue, transplantation into other species represents a significant challenge to obtain meaningful results that are not complicated by immunological differences. To address this, large animal testing was conducted in pigs using both same species (swine AAT) and xenogeneic (human AAT) implants. These experiments demonstrated that AAT is biocompatible, integrates with the host tissue, and forms new adipose tissue (data not shown).

Human Phase I clinical trial studies showed good volume retention and tissue integration up to 6 weeks post-injection. Histological analysis of the implants also showed significant

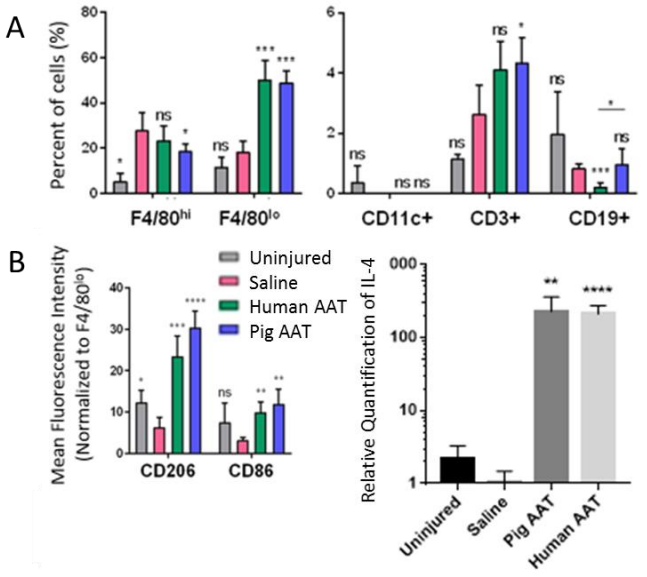

Figure 1. Immune cell characterization of AAT-treated muscle wounds after one week. (A) Composition of the immune cells in the injured quadriceps reported in percentage of total immune cells. (B) Macrophage polarization determined by flow cytometry and IL-4 secretion determined by RT-PCR. Statistics calculated using a unpaired t test, * $p < 0.05$, ** $p < 0.01$ *** $p < 0.001$. ****$p < 0.0001$.

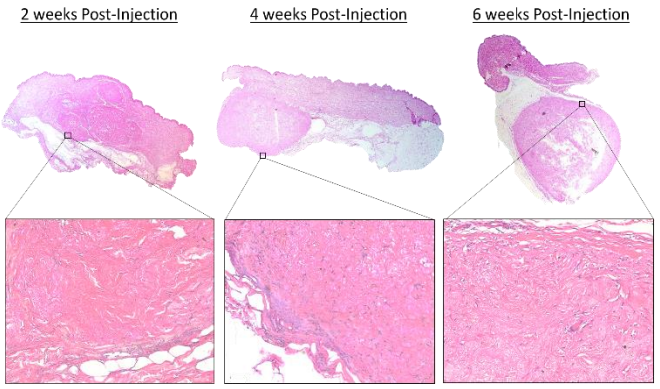

Figure 2. Hematoxylin and eosin stained FFPE sections of implant tissue with surrounding dermis and adipose tissue. Tissue sections show the entire injection site. Insets show the interface between implant and native tissue and moderate levels of cell infiltration.

cell migration into the implant from surrounding tissue (Fig. 2). PRA testing showed no increase in IgG HLA antibodies at 4 or 12 weeks post-injection for 7 of the 8 participants, with a clinically insignificant increase in one individual, indicating no adverse systemic reaction to the material.

IV. Conclusions

AAT demonstrated good volume retention, significant tissue integration, and minimal inflammation in preclinical mouse, rate, and swine studies. No serious adverse events were noted in Phase I clinical studies, and excised tissues showed minimal inflammation and moderate levels of cell infiltration in histological analysis. Recent animal studies have also evaluated the local immune microenvironment created by the AAT in both SQ and wound models. The ability of AAT to modulate immune response and induce a favorable pro-regenerative environment could potentially be harnessed to improve wound healing and reduce scarring after injury. This data indicates that AAT could be a good substitute for autologous fat transfer in the treatment of soft tissue defects.

V. Acknowledgments

This project was funded by the Armed Forces Institute of Regenerative Medicine (AFIRM).

VI. References

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