Injectable Adipose ECM Hydrogels Stimulate Neovascularization and Subsequent Adipose Regeneration

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Statement of Purpose:
Adipose tissue engineering strives to design therapies designed specifically for fatty tissue replacement and regeneration for treating patients recovering from 3rd degree burns, HIV lipodystrophy, or even breast cancer 3lumpectomy procedures. Current materials available in the clinic for filling adipose deficits are either rapidly resorbed or walled off with fibrous tissue, and none stimulate new fat formation. Decellularization offers the ability to produce a tissue-specific, extracellular matrix (ECM)-based material that potentially provides a template for natural regeneration. We have previously developed a method for decellularizing human lipoaspirate tissue and processing it into a liquid formulation that self-assembles into a hydrogel *in vivo*.[1] Our objective in the current study was to investigate this adipose ECM hydrogel as a novel therapy for adipose tissue engineering and compare its *in vivo* performance against a clinical standard.

Methods:
Liposuctioned adipose tissue was obtained from human donors following an approved IRB protocol. Adipose-derived adult stem cells (ASCs) were first harvested from the tissue.[2] The remaining adipose ECM was then decellularized and reduced to a liquid formulation following our previously published protocol.[1] Female athymic mice were used for this study, which was approved by the UCSD animal use committee. Each mouse was anesthetized and received 250 µL subcutaneous, dorsal injections from one of the following groups (n=4 per group): 1) adipose ECM hydrogel, 2) adipose ECM hydrogel + ASCs, 3) adipose ECM hydrogel crosslinked with transglutaminase (TG), 4) adipose ECM hydrogel + TG + ASCs, or 5) Juvederm. The ECM hydrogel was delivered at 12 mg/mL, ASCs at 1.5 x 10^5/mL, and TG at 0.1 U/mL. TG (Sigma-Aldrich) is a clinically-relevant enzyme that crosslinks free amines to gamma-carboxamide groups, and Juvederm (Allergan) is a crosslinked hyaluronic acid gel and clinical standard for treating adipose voids. After 1, 2, and 4 weeks, injection regions were biopsied, sectioned, and stained with H&E and Oil Red O, then analyzed by an experienced histopathologist. Sections were also fixed and stained with fluorescent antibodies against CD31, smooth muscle actin (SMA), and pan-macrophage. Statistical significance was determined using a two-way ANOVA with a Tukey *post hoc* test.

Results:
All injections were well tolerated by the animals, showing no signs of redness or irritation. The adipose ECM hydrogel alone showed accumulation of neutrophils at the material boundary with increasing infiltration over time, but negligible evidence of capsule formation (Fig 1A). Significant neovascularization was present at 1 week, but decreased over time with minimal evidence of mature adipocyte development. Addition of ASCs to the ECM dramatically improved vascularization and exhibited adipogenesis in half of the samples at 1 month (Fig 1B). Interestingly, adding TG to the adipose ECM produced a similar mild immune response but improved infiltration and vascularization at all time points and showed significant adipogenesis in all samples at 1 month (Fig 1D). Adding both TG and ASCs to the ECM produced a similar response (Fig 1E, F). All of these results are in contrast to the Juvederm injection sites, which displayed no cellular infiltration, neovascularization, or adipogenesis (Fig 1C). A thin fibrous capsule surrounded the Juvederm, which was also associated with edema.

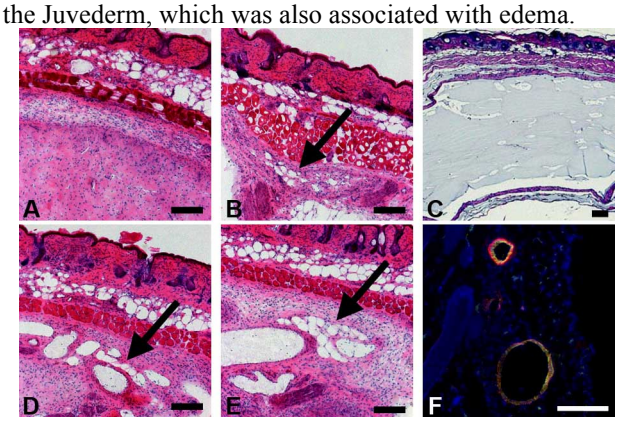

Fig. 1. H&E images of adipose ECM hydrogel (A), ECM+ASCs (B), Juvederm (C), ECM + TG (D), and ECM + TG + ASCs (E); and neovascularization in ECM + TG group (F, red=SMA, green=CD31) at 1 month. Arrows indicate new adipose formation. Scale = 100 µm.

Conclusions:
These results highlight that the adipose ECM hydrogel is a biocompatible material capable of stimulating natural adipose regeneration, whether delivered with or without exogenous ASCs. Importantly, it demonstrates that induction and sustainment of neovascularization is critical to support the development of this new adipose tissue, which in this case was provided by ASCs or the addition of a biocompatible crosslinker. This work provides proof of concept for a new class of materials that, unlike clinical standards, stimulate natural regeneration for the treatment of a variety of adipose deficits.

References
1. Young, DA *et al*. 2011. *Acta Biomaterialia*.
2. Bernacki, S *et al*. 2008. *Methods Cell Bio.*

Acknowledgments
Funding provided in part by NIH Director's New Innovator Grant and the NSF GRFP.