Human ECM Particles as an Injectable Bulking Agent for Adipose Replacement

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Statement of Purpose: While regeneration of adipose tissue may seem counterintuitive, there is a patient population of breast cancer and severe burn victims that would significantly benefit from therapies designed specifically for fatty tissue replacement. Adipose tissue engineering strives to produce an alternative to single-component subcutaneous injectables that simply fill voids, instead encouraging regeneration of damaged or non-existing tissue. Tissue Engineering by Self-Assembly (TESA) harnesses a cell's own ability to synthesize extracellular matrix (ECM) into natural scaffolding[1]. This approach has previously been used to produce sheets and threads of human ECM that can be assembled into completely biological repair devices such as valves, vessels, and ligaments. Current clinical fillers fail to recapitulate the biochemical complexity of native ECM. We therefore sought to develop a particulate form of TESA-produced ECM that will offer an injectable biomaterial to replace these lost ECM components.

Methods: Human fibroblasts were isolated from skin biopsies of consenting patients and used at either passage 6 or 7. Cells were cultured in DMEM supplemented with Ham's F12, FBS, glutamine, and sodium ascorbate for up to 8 weeks. After either 4 or 8 weeks of culture, sheets of cell-produced ECM were lifted from the culture vessel as an intact sheet, as previously described[2]. The sheets were subsequently rinsed with PBS, dried, and milled into a particulate form using a Wiley Mini-Mill. These particles could then be rehydrated in 0.9% saline for injection. Particle composition was assessed with mass spectroscopy and particle size distribution was examined using a Coulter Multisizer. For SEM imaging, particles were dehydrated and sputter coated with gold, then imaged with an Agilent 8500 SEM. To assess the DNA content of the particles, DNA was first extracted from the particles with a NucleoSpin kit and then quantified using a PicoGreen assay. Preliminary animal trials were then conducted to assess injectability and biocompatibility. Female Sprague Dawley rats received 0.5 cc subcutaneous dorsal injections of one of the following experimental groups: saline, particles from either younger or older ECM sheets, and Juvederm XC (Popular clinical filler; Hyaluronan-based; Allergan, Santa Barbara, CA). Injection sites were then excised at 1 and 5 days, formalin fixed, and embedded in paraffin. Tissue sections were then stained with both H&E and Masson's Trichrome.

Results: Particles produced from the ECM sheets (Fig 1A, 1B) ranged in size from ~50-250 μm. Rehydrated particles could pass through needles as small as 27G and could be concentrated up to 200 mg/mL in saline (Fig 1C). Mass spectroscopy revealed the particles were composed of a variety of ECM proteins and proteoglycans, including collagen, elastin, fibronectin, decorin, and lumican. DNA analysis indicated the particles produced from older ECM sheets contained only 0.98 ± 0.04 μg of DNA per mg of particles, which was 4 times less than the younger sheet particles at 3.96 ± 0.09 μg/mg. Furthermore, animal studies demonstrated the *in vivo* injectability of the particles, which did not initiate external irritation, erythema, or edema through 5 days *in vivo*. All particle and Juvederm injections were still palpable at Day 5 and could be visually identified upon excision. Particles produced from younger ECM sheets showed significant mononuclear cell infiltration (Fig 1D). However, particles from older ECM sheets (Fig 1E) caused a much milder response that was similar to Juvederm injections (Fig 1F) and slightly increased compared to saline injections.

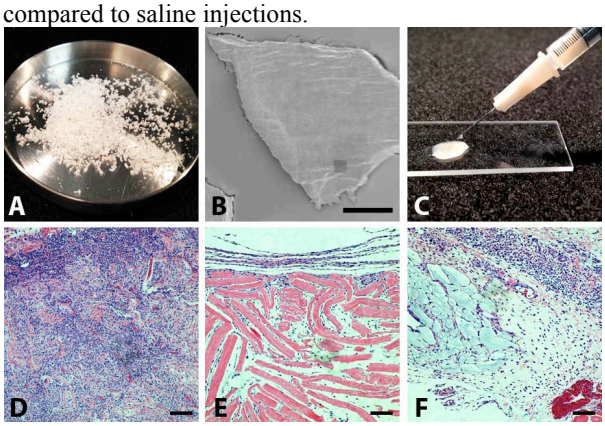

Figure 1. TESA-produced particles (A) were visualized with SEM (B) and could pass through a 27G needle (C). *In vivo*, younger sheet particles (D) produced a more significant immune response than older sheet particles (E) or Juvederm (F). Scale bars = 100 μm.

Conclusions: We have demonstrated a new method for producing a potentially autologous or allogeneic injectable dermal filler from human ECM. The TESA-produced particles from older ECM sheets could be injected minimally-invasively and initial results up to 5 days indicate a biocompatible response. This is especially significant considering that these human-produced particles were injected into an immuno-competent animal (xenograft), yet did not initiate a severe reaction. The increased immune response of the younger ECM sheet particles could possibly be attributed to the 4-fold increase in cellular content. Furthermore, the limited response to the older ECM sheet particles compared to younger ones suggest that there exists a threshold level of cellular material to trigger an inflammatory response, however, the actual component causing the response and its threshold level remain to be elucidated. Overall, these results demonstrate the development of novel injectable biomaterial for adipose replacement that is both biocompatible and comprised of many components that are characteristic of adipose ECM.

References: (1) L'Heureux N *et al*. Nat Med. 2006; 12(3):316-365. (2) L'Heureux N *et al*. FASEB. 1998; 12(1):47-56.