A Comparison of the Rheologic Properties of an Adipose-Derived Extracellular Matrix Biomaterial, Lipoaspirate, Calcium Hydroxylapatite, and Cross-linked Hyaluronic Acid

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IMPORTANCE Acquired soft-tissue injury with resultant volume loss may cause significant deformity in size, shape, and body or facial contour. Current autologous fat transfer techniques have several limitations, including availability, donor site morbidity, and unpredictable rates of resorption. We present an extracellular matrix (ECM) biomaterial derived from human adipose tissue as an off-the-shelf alternative for soft-tissue volume restoration and compare clinically relevant rheologic properties.

OBJECTIVES To determine the rheologic properties of adipose-derived ECM and to compare it with lipoaspirate, calcium hydroxylapatite, and cross-linked hyaluronic acid.

DESIGN, SETTING, AND PARTICIPANTS Adipose-derived ECM (n = 4), lipoaspirate acquired from aesthetic liposuction (n = 4), calcium hydroxylapatite (n = 4), and cross-linked hyaluronic acid (n = 4) were obtained to determine the viscoelastic properties.

INTERVENTIONS Dynamic frequency oscillation measurements were conducted using a rheometer (ARES-G2; TA Instruments). All injections were performed using a 20-gauge needle, and all measurements were performed using serrated 25-mm parallel-plate geometry with a 1.0-mm gap at 37°C.

MAIN OUTCOMES AND MEASURES Oscillation measurements for storage modulus, a measure of the elastic properties, and complex viscosity were obtained over an angular frequency range of 0.01 to 100 rad/s.

RESULTS At 1 Hz, adipose-derived ECM had a mean (SD) storage modulus of 713.2 (42.9) Pa and a mean (SD) complex viscosity of 115.8 (6.9) Pa/s. Lipoaspirate had a mean (SD) storage modulus of 382.1 (66.8) Pa and a mean (SD) complex viscosity of 61.5 (10.7) Pa/s. Calcium hydroxylapatite had a mean (SD) storage modulus of 1122.1 (67.1) Pa and a mean (SD) complex viscosity of 207.2 (11.6) Pa/s. Cross-linked hyaluronic acid had a mean (SD) storage modulus of 63.9 (3.0) Pa and a mean (SD) complex viscosity of 10.9 (0.5) Pa/s.

CONCLUSIONS AND RELEVANCE Of the 4 materials tested, calcium hydroxylapatite has the highest mean storage modulus and mean complex viscosity, and hyaluronic acid has the lowest. The viscoelastic properties of adipose-derived ECM are most similar to those of lipoaspirate, suggesting that it may be an ideal candidate for soft-tissue reconstruction.

LEVEL OF EVIDENCE NA.
A soft-tissue defect is a large volume loss within the subcutaneous fat layer of the skin that often results in scarring or adherence of dermis to underlying muscle, with subsequent alteration in the tissue contour. The primary cause of these defects is traumatic injury, such as burns, tumor resection, and congenital defects. These defects can lead to significant anxiety and perception of disfigurement. Therefore, the goal of reconstruction is to restore the contour to its natural state.

Some options for repair of soft-tissue defects include surgical reconstruction such as autologous tissue transfers (free or pedicled), implants, and fillers. Revisions are common to improve tissue contour and can be performed with collagen, calcium hydroxylapatite (CaHA), or cross-linked hyaluronic acid (HA), which are approved for reconstructive and aesthetic use. Unfortunately, these options are mostly temporary. The availability of adipose tissue in the human body and the ease of obtaining it through liposuction make it an attractive option for transplantation as lipoaspirate. However, the use of this method has not been consistently successful in patients. When autologous fat is transplanted from one location to the defect site, significant resorption is common over time and results in 40% to 60% of graft volume loss.

Several factors contribute to the high rate of resorption of lipoaspirate. One significant contributor is thought to be the lack of sufficient revascularization of the tissue following transplantation to a new location. It is believed that the fat grafts do not acquire sufficient vascularity, and poor centralized graft blood flow is inadequate for long-term survival. Insufficient tissue vascularization limits the supply of oxygen and nutrients to the tissue, prohibiting long-term tissue viability.

In addition to the biological properties of filling agents, the biomechanical or rheologic properties (viscosity and elasticity) are critical for behavior after implantation. Two important rheologic properties that must be considered for soft-tissue gels are storage modulus and complex viscosity. These factors are clinically relevant because they can predict how a gel behaves during and after injection and result in varying clinical outcomes. During injection, storage modulus of the gel depicts its stiffness or ability to resist elastic deformation, and complex viscosity relates to how well it resists shearing forces from the needle and physiological forces after administration. Shearing forces can alter a filler’s flow during injection by affecting its viscosity and elasticity. After injection, storage modulus and complex viscosity are important because they affect how well the gel will retain its physical structure and withstand facial movements and skin tension forces.

In an ideal solid, external deformation forces result in elastic strain, which recovers completely once the stress is removed. Elastic modulus is a measure of the stiffness of the material and is defined as the ratio of stress to strain because the sample stress is proportional to the strain. An ideal viscous fluid is unable to store energy elastically and is irreversibly deformed when subjected to external forces. The applied stress is proportional to the rate of strain deformation, and this ratio is defined as the viscosity of a fluid.

However, materials such as lipoaspirate and dermal fillers can exhibit viscous and elastic behavior, whereby some of the deformation force is stored elastically in the structure, while some is irreversibly lost through flow. By performing oscillatory dynamic tests using a rheometer, the elastic and viscous properties of a material can be determined. The viscoelastic properties are characterized by the following 2 dynamic moduli obtained from the oscillatory tests: storage modulus, which is a measure of the sample’s elastic behavior, and complex viscosity, which is the overall resistance to deformation (including elastic and viscous contributions) over the angular frequency.

A typical configuration of a rheometer used for oscillatory rheology consists of a motor that induces oscillatory strain deformation to a sample and a transducer that measures the resultant stress response or torque. The sample is loaded onto a test geometry such as a cone-plate or parallel-plate system (Figure 1). In this study, the parallel-plate geometry was chosen because of the heterogeneous composition and variable particle size of lipoaspirate. A serrated surface was selected to prevent slippage during the oscillatory tests.

Regenerative medicine approaches to soft-tissue reconstruction provide a potential alternative in which biomaterials facilitate tissue regeneration in the defect area, avoiding the need for tissue transplantation from a donor site. Our group has previously described the development of a novel adipose-derived extracellular matrix (ECM) biomaterial that is nonimmunogenic and serves as a 3-dimensional scaffold for tissue regeneration. Histologic analysis of the adipose-derived ECM 2 weeks after implantation into rats showed minimal inflammatory reaction and good tissue integration. By 12 weeks, the adipose tissue development continued in the implant, and areas of collagen remodeling were also observed by histologic analysis. To better understand the biomechanical properties and to better predict applications for this new biomaterial, we investigated storage modulus and complex viscosity of adipose-derived ECM and compared it with lipoaspirate, CaHA, and HA.
Methods

Study Approval and Informed Consent
The use of subcutaneous adipose tissue from patients undergoing liposuction procedures was approved by the Johns Hopkins Medicine Institutional Review Board. Written consent was obtained.

Commercial Filling Agents
A cross-linked HA soft-tissue filler (juvérderm Ultra Plus; Allergan) and a soft-tissue filler consisting of CaHA microspheres in a carrier gel containing carboxymethyl cellulose (Radiesse; BioForm Medical, Inc) were obtained. Three replicates each were studied.

Lipoaspirate
Subcutaneous adipose tissue was obtained from the lower abdomen of patients undergoing liposuction procedures with approval from the Johns Hopkins Medicine Institutional Review Board. This tissue was then subjected to mechanical processing and rinsing using the technique by Coleman.16

Adipose-Derived ECM
The method for processing adipose-derived ECM from human subcutaneous adipose tissue has been previously described.15 Briefly, subcutaneous adipose tissue was mechanically processed and then underwent extensive rinsing, followed by incubation with 3% peracetic acid for 3 hours to remove cells. Samples were then brought to physiologic pH using phosphate-buffered saline and were incubated overnight with 1% Triton X-100 in 2mM edetic acid to remove residual lipids. Samples were then milled to reduce particle size, and each sample was stored in a 2-mL sterile syringe and kept at 4°C.

Rheologic Evaluation
Rheologic measurements were performed to assess the flow and deformation of each material (adipose-derived ECM, lipoaspirate, CaHA, and HA) and to quantify elastic modulus and complex viscosity. All measurements were performed using a rheometer (ARES-G2; TA Instruments). A serrated 25-mm parallel-plate geometry with a 1.0-mm gap at 37°C was used (Figure 1).

Deformation oscillation measurements were obtained over a frequency range of 0.01 to 100 rad/s. Samples (1 mL) for each material were injected using a 20-gauge needle, and 4 separate samples were evaluated for each material. We chose 1 Hz as the frequency for comparison of the mean storage modulus and complex viscosity of each material because it was considered physiologically relevant for stresses that are common to the skin.

Statistical analysis to compare storage modulus and complex viscosity was performed using the paired t test. P < .05 was considered statistically significant. All statistical analyses were performed using a spreadsheet (Excel; Microsoft Corporation).

Results
Adipose-derived ECM had a mean (SD) storage modulus of 713.2 (42.9) Pa and a mean (SD) complex viscosity of 115.8 (6.9) Pa/s at a frequency of 1 Hz (Table). Lipoaspirate had a mean (SD) storage modulus of 382.1 (66.8) Pa and a mean (SD) complex viscosity of 61.5 (10.7) Pa/s. Calcium hydroxylapatite had a mean (SD) storage modulus of 1122.1 (67.1) Pa and a mean (SD) complex viscosity of 207.2 (11.6) Pa/s. Cross-linked HA had a mean (SD) storage modulus of 63.9 (3.0) Pa and a mean (SD) complex viscosity of 10.9 (0.5) Pa/s (Figure 2 and Figure 3).

Discussion
A common method for repair of soft-tissue defects is autologous fat transfer, but the morbidity of harvesting and the variability of retention make it difficult to consistently predict outcome.1,2–12 Furthermore, the need for revision is high. Other options for repair of soft-tissue defects include using commercial fillers, but most are temporary, with few permanent options. Through the use of regenerative medicine, our group has developed a biomaterial using adipose-derived ECM, which we believe has the following advantages: it is derived from a read-
In the present study, we evaluated the rheologic properties of adipose-derived ECM and found that it has a significantly higher storage modulus and complex viscosity than liposaprist. This is true across all frequencies tested between 0.01 and 100 Hz for storage modulus and complex viscosity (data not shown). This is clinically relevant because it indicates that adipose-derived ECM is more resistant to deformation and less likely to undergo shearing forces than liposaprist. The low viscosity and storage modulus, in addition to the poor vascular supply, likely contribute to the variability in retention rate found in autologous fat grafting.

Of the 4 materials tested, CaHA had the highest mean storage modulus and mean complex viscosity, and HA had the lowest, which is consistent with previous findings. Relative to all materials studied, the rheologic properties of adipose-derived ECM are most similar to those of liposaprist but avoid disadvantages associated with fat grafting such as requirement of a donor site, additional liposuction procedures, high metabolic need of the transplanted fat, and subsequent necrosis and calcification. Therefore, adipose-derived ECM may be well suited to clinical applications similar to those of liposaprist such as treatment for scars, burn contracture, or irradiation-induced fibrosis. Additional work in human models is needed to formally assess the utility of using adipose-derived ECM for volumization or lifting of facial zones that have high levels of muscle activity (and high skin tension forces) such as the nasolabial folds, midface, and lower face.

**Conclusions**

When deciding which method of reconstruction to use for defects of the face or body, choosing a graft material that is most similar to the defective region is of utmost importance. Emphasis is placed on color, size, texture, and functional match. Likewise, when using fillers for correction of soft-tissue defects, optimum contour and function are best achieved by returning volume in the form of what has been lost, primarily the subcutaneous fat layer. The biological and rheologic properties of adipose-derived ECM make it an ideal soft-tissue replacement for repair of contour defects.

**REFERENCES**