Dynamic Rheology for the Prediction of Surgical Outcomes in Autologous Fat Grafting

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Background: Because of the abundance and biocompatibility of fat, lipotransfer has become an attractive method for treating soft-tissue deficits. However, it is limited by unpredictable graft survival and retention. Currently, little is known about the viscoelastic properties of fat after various injection methods. Here, the authors assess the effects of cannula diameter, length, and shape on the viscoelastic properties, structure, and retention of fat.

Methods: Human lipoaspirate was harvested using suction-assisted liposuction and prepared for grafting. A syringe pump was used to inject fat at a controlled flow rate through cannulas of varying gauges, lengths, and shapes. Processed samples were tested in triplicate on an oscillatory rheometer to measure their viscoelastic properties. Fat grafts from each group were placed into the scalps of immunocompromised mice. After 8 weeks, graft retention was measured using micro–computed tomography and grafts were explanted for histologic analysis.

Results: Lipoaspirate injected through narrower, longer, and bent cannulas exhibited more shear thinning with diminished quality. The storage modulus (G′) of fat processed with 18-gauge cannulas was significantly lower than when processed with 14-gauge or larger cannulas, which also corresponded with inferior in vivo histologic structure. Similarly, the longer cannula group had a significantly lower storage modulus than the shorter cannula, and was associated with decreased graft retention.

Conclusions: Discrete modifications in the methods used for fat placement can have a significant impact on immediate graft integrity, and ultimately on graft survival and quality. Respecting these biomechanical influences during the placement phase of lipotransfer may allow surgeons to optimize outcomes. (*Plast. Reconstr. Surg.* 140: 517, 2017.)

CLINICAL QUESTION/LEVEL OF EVIDENCE: Therapeutic, V.

Autologous fat grafting has demonstrated great utility for the reconstruction of soft-tissue deficits and contour abnormalities. However, fat graft survival and volume retention are notoriously variable and unpredictable, which limits the consistency of clinical results. Complicating the issue is the fact that the steps of fat grafting have yet to be fully standardized among surgeons, and the specific parameters leading to optimum outcomes remain largely unknown.

Although the Coleman technique is perhaps the most widely used fat grafting protocol, small injection cannulas (17-gauge, 18-gauge, or smaller) are often used to place small aliquots of fat.[1–3] However, literature on the effects of injection cannula size and geometry is limited. Erdim et al. found no differences in adipocyte viability between fat samples injected by means of 14-, 16-, and 20-gauge needles.[4] However, other studies have demonstrated a critical role for mechanical forces in fat graft outcomes: Tambasco et al. and Kirkham et al.

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found the use of larger aspiration cannulas facilitated increased adipocyte viability[5] and fat graft survival,[6] respectively. Such outcomes are thought to be attributable to the decreased shear stress associated with use of large-diameter cannulas.[5,6]

Our laboratory previously investigated the effects of shear stress during fat injection by comparing the use of a modified Coleman procedure with an automated, low-shear device (Advanced Adipose Tissue Injector; LifeCell Corp., Bridgewater, N.J.), concluding that the low shear conditions provided by the device resulted in significantly greater fat graft quality and retention.[7] To explain these findings, our group analyzed the rheologic properties of fat injected using the two techniques.[8] Rheology, the study of material flow and deformation, is particularly useful for assessing changes in the structure of adipose tissue caused by the injection process. As fat is a viscoelastic material, it demonstrates both elastic and viscous responses to deformation. Initially, adipose tissue behaves like a solid material, responding to applied mechanical force with elasticity: by deforming, then returning to its original shape. However, as more stress is applied over time, fat begins to respond like a liquid, becoming less viscous and changing shape (flowing).[8] This process is termed shear-thinning, meaning that material viscosity decreases as shear stress increases, and suggests that increasing force on adipose tissue causes it to break apart.[9] Indeed, adipocytes are easily broken down under force, and injection under low shear has demonstrated decreased lipolysis and less histologic injury (vacuoles, infiltration, fibrosis).[7] Thus, the use of a low-shear device may improve graft outcomes, as it preserves cell integrity and therefore the natural structure of adipose tissue.

As little is known about the effects of injection cannula geometry on the viscoelastic integrity of fat, the goal of this study was to first assess the rheologic properties of fat injected through cannulas of different gauges, lengths, and shapes. To do this, we used an oscillatory rheometer to apply a sinusoidal shear stress to lipoaspirate and determine the storage modulus (G') of each sample. A measure of elasticity, the storage modulus reflects the ability of adipose tissue to store mechanical energy and maintain its original, semisolid structure (instead of dissipating it and undergoing a conformational change, as liquids do). Higher storage modulus values thus indicate more "intact" fat. Second, we sought to determine whether our rheologic data correlated with the biological activity of processed fat, and thus assessed the in vivo volume retention and quality of fat grafts placed with each type of cannula.

MATERIALS AND METHODS

Lipoaspirate Sample Preparation

Fresh lipoaspirate from three healthy human female donors was obtained after informed consent in accordance with Stanford University Institutional Review Board approval (protocol no. 2188). All samples were obtained using suction-assisted lipectomy. Lipoaspirate was allowed to settle for 15 minutes for layers to separate by gravity sedimentation, and then blood/liquid and oil layers were removed by vacuum aspiration. The remaining fat layer was centrifuged at 1300 relative centrifugal force for 3 minutes at 4°C. Any remaining blood/liquid was removed again, and tubes were overturned on top of absorbent gauze for 10 minutes to remove remaining oil.

Fat was transferred carefully to 10-cc syringes, and then divided into groups for evaluation. For rheologic testing, fat was injected through five 8-cm cannulas of varying diameters (11-, 12-, 14-, 16-, and 18-gauge) and two 14-gauge cannulas of varying shapes (14-gauge, 25-cm straight; and 14-gauge, 25-cm bent). A syringe pump was used to control flow rate (0.1 ml/second) and volume delivered during injection (Fig. 1, *left*).

Dynamic Oscillatory Rheology Testing

Rheology testing was performed using an ARG2 rheometer (TA Instruments; New Castle, Del.) with 40-mm parallel plate geometry and a gap size of 1 mm, as described previously (Fig. 1, *right*).[8] Samples were allowed to reach room temperature before testing on a rheometer stage held at 37°C. Analysis was performed using rate sweeps from 0.1 to 100 seconds and an oscillatory stress of 0.2 Pa. Storage modulus was reported at 1 Hz to quantify the elastic-like stiffness of the fat, as described previously.[8]

Fat Grafting

Fat was transferred into 3-cc Luer-lock syringes, and 200 μl of fat was then injected at 1 ml/second using various cannulas into the subcutaneous plane of the scalps of adult Crl:NU-*Foxn1*^{nu}CD-1 nude mice (Charles River Laboratories International, Inc., Hollister, Calif.) ($n = 3$ per patient per group), in accordance with an approved Stanford University Administrative Panel on Laboratory Animal Care protocol. Cannulas used were as follows: 14-gauge, 8-cm; 18-gauge, 8-cm; 14-gauge,

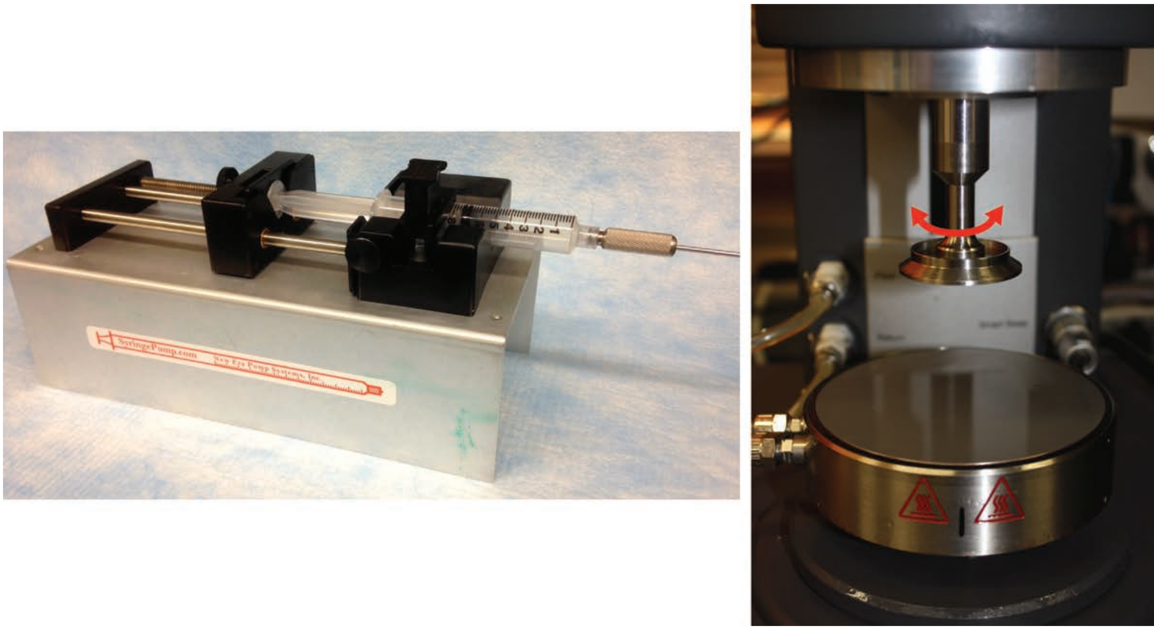

Fig. 1. (*Left*) A syringe pump was used to deliver precise volumes of fat at a controlled flow rate. (*Right*) A parallel plate rheometer was used to apply an oscillatory shear stress (*red arrow*) to lipoaspirate samples.

25-cm straight; or 14-gauge, 25-cm bent. The 14-gauge, 25-cm bent cannula is a 14-gauge, 25-cm cannula with a gradual 7-degree bend located over the last 10 cm along its length. These geometries were chosen based on clinical relevance, with the 14-gauge, 25-cm bent cannula being applicable for fat grafting in line with the body's natural curvatures.

Micro–Computed Tomographic Analysis

Fat graft volume was assessed at baseline and again after 8 weeks using micro–computed tomographic analysis, as described previously.[10] Briefly, mice were scanned in the ventral position using a Siemens micro–computed tomography scanner (Siemens, Munich, Germany). Three-dimensional volumes were reconstructed using Inveon Research Workplace software (Siemens) through cubic-spline interpolation, which allowed the in vivo measurement of volume at each time point. Volume retention was calculated as a percentage of the baseline volume.

Histologic Analysis

Fat grafts were explanted after 8 weeks for histologic analysis of graft structure using hematoxylin and eosin staining. Mice were killed and grafts were removed from the scalp, fixed immediately in 10% formalin, dehydrated, and embedded in paraffin for sectioning. Images were taken using a light microscope at the 10× objective (Leica DM5000 B; Leica Microsystems, Buffalo Grove, Ill.). Six sections from each group were chosen randomly for scoring by three independent, blinded investigators. Graft quality was determined using an established method, based on the assessment of overall integrity and the presence of fibrosis, inflammation, and cysts/vacuoles.[11,12]

Statistical Analysis

All data are presented as mean ± standard deviation. Statistical analysis was performed using a one-way analysis of variance followed by the Tukey multiple comparison test for direct comparisons between two groups. A value of $p \leq 0.05$ was considered statistically significant.

RESULTS

Cannula Geometry Affects Fat Intactness

Significant differences were observed in the storage modulus of fat injected using cannulas of different diameters, with larger cannula diameters producing larger values (Fig. 2, *above*). Specifically, the storage modulus of fat injected with 16-gauge and 18-gauge cannulas was significantly lower than that of fat injected with 14-gauge or larger cannulas ($p \leq 0.001$, $p \leq 0.0001$). There were no significant differences between storage modulus for the 11-, 12-, and 14-gauge groups. In addition, fat injected through the 16-gauge

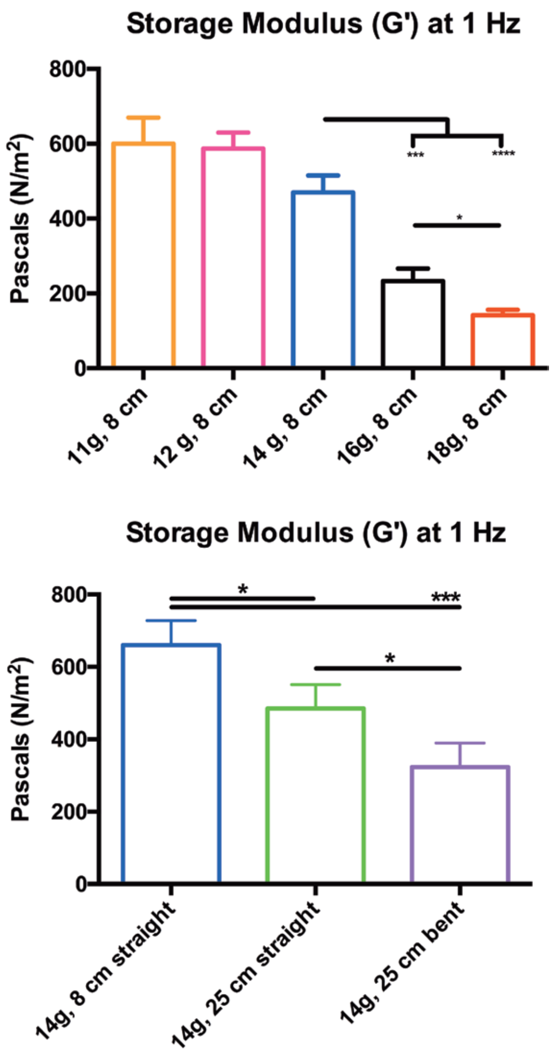

Fig. 2. (*Above*) Measurement of storage modulus of lipoaspirate injected through 8-cm fat grafting cannulas of various diameters showed significant differences between the 14-gauge cannula and the 16-gauge (***$p < 0.001$) and 18-gauge (****$p < 0.0001$) cannulas. (*Below*) Storage modulus of lipoaspirate injected through the 14-gauge, 8-cm cannula was significantly higher than that injected through the 14-gauge, 25-cm (*$p < 0.05$, ***$p < 0.001$) cannulas. The addition of a bend in the 14-gauge, 25-cm cannula significantly decreased (*$p < 0.05$) the storage modulus of injected fat.

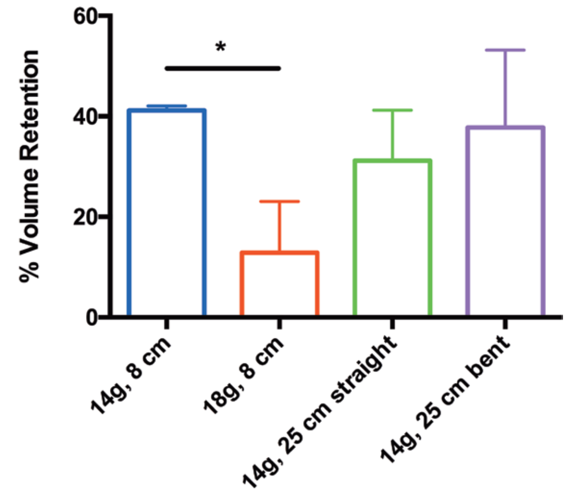

Fig. 3. Fat graft retention at 8 weeks after grafting were highest among grafts placed with the 14-gauge, 8-cm cannula, and significantly higher (*$p < 0.05$) than those placed with the 18-gauge, 8-cm cannula.

cannula had a higher storage modulus than that injected through the 18-gauge cannula ($p \leq 0.05$).

Among 14-gauge cannulas of varying geometries, fat injected using the shorter (8-cm), straight cannula had a significantly higher storage modulus than fat injected using either 25-cm straight ($p \leq 0.05$) or 25-cm bent ($p \leq 0.001$) cannulas (Fig. 2, *below*). Finally, the addition of a curvature to the 25-cm cannula geometry significantly decreased the intactness of the injected fat ($p \leq 0.05$).

Cannula Geometry Affects Fat Graft Volume Retention

Micro–computed tomographic analysis of fat graft volume retention showed that injection with the 14-gauge, 8-cm cannula produced the highest volume retentions (41.18 ± 0.8938 percent), followed by the 14-gauge, 25-cm bent (37.77 ± 15.43 percent); 14-gauge, 25-cm straight (31.19 ± 10.02 percent); and 18-gauge, 8-cm (12.88 ± 10.17 percent) cannulas. Likely because of the large standard deviations seen in all groups except the 14-gauge, 8-cm cannula, the only significant difference in volume retentions was seen between the 14-gauge, 8-cm and 18-gauge, 8-cm groups ($p < 0.05$) (Fig. 3).

Cannula Geometry Affects Histologic Properties of Fat Grafts

Histologic analysis of fat grafts explanted after 8 weeks showed an overall trend of the highest quality grafts being produced with use of the 14-gauge, 8-cm cannula, followed by the 14-gauge, 25-cm straight; 14-gauge, 25-cm bent; and 18-gauge, 8-cm cannulas (Fig. 4). Specifically, the 14-gauge, 8-cm cannula grafts had significantly more integrity (Fig. 4, *above*, *left*) and less inflammation than all other groups ($p < 0.05$, $p < 0.01$, $p \leq 0.001$, and $p \leq 0.0001$) (Fig. 4, *center*, *left*). This cannula also produced significantly less graft fibrosis than the 18-gauge, 8-cm and 14-gauge, 25-cm bent cannulas ($p < 0.05$, $p < 0.01$) (Fig. 4, *center*, *right*). Paralleling the fat graft volume retention rates, the 18-gauge, 8-cm cannula produced

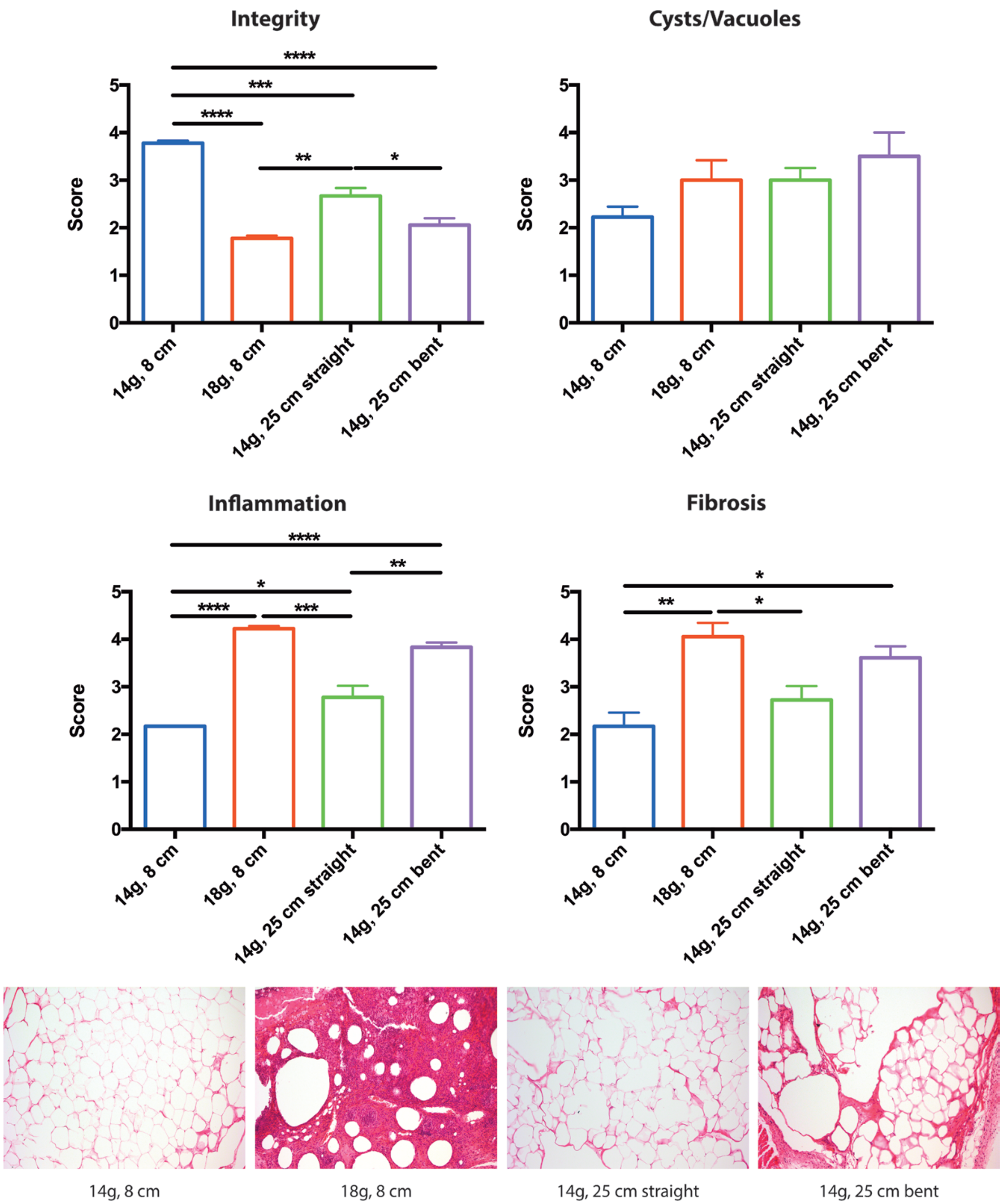

Fig. 4. (*Below*) Histologic differences were apparent between fat grafts injected through cannulas of various geometries (original magnification, × 10). Histologic scoring, wherein a higher numerical score represents increased levels of the factor being assessed, showed significant differences (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$) in the integrity (*above*, *left*), fibrosis (*center*, *right*), and inflammation (*center*, *left*), of fat grafts placed with cannulas of varying geometries. Differences in the presence of cysts/vacuoles (*above*, *right*) between groups did not reach statistical significance.

the worst quality grafts, with the lowest integrity and highest levels of inflammation and fibrosis.

DISCUSSION

Unlike standard "hard" materials such as ceramics and metals that display elastic properties, or standard liquid materials that display viscous properties, soft materials display both elastic and viscous characteristics. Adipose tissue is a viscoelastic material, meaning it does not behave as a purely elastic solid or a purely viscous fluid, but rather exhibits properties that lie between these two idealized states, over time shifting from a solid-like to a liquid-like response to applied stress. Storage modulus is a measure of the immediate response of a viscoelastic material to an applied stress, essentially quantifying the degree to which fat will respond like a solid and maintain its original structure.

We found that increased intactness, indicated by a larger storage modulus, correlated with improved fat graft volume retention and quality in vivo. Injection through the smallest gauge cannula (18-gauge) resulted in lipoaspirate with not only the lowest storage modulus , but the smallest percentage of original volume retained at 8 weeks. Furthermore, grafts made using the 18-gauge cannula demonstrated the largest amounts of inflammation and fibrosis. The second lowest storage modulus was found in lipoaspirate injected through the 14-gauge, 25-cm bent cannula. Interestingly, although the 14-gauge, 25-cm bent grafts demonstrated higher volume retention on micro–computed tomography, these were of worse quality than the 25-cm straight grafts. This is likely because of increased levels of inflammation and cyst/vacuole formation, which are not resolvable on computed tomography but can artificially inflate overall graft volume. Although the 14-gauge, 25-cm straight cannula produced the second highest storage modulus, fat injected through the 14-gauge, 8-cm cannula not only had the highest storage modulus overall, but ultimately produced the best graft outcomes, with the highest volume retention and graft quality.

These results can be explained using the principles of fluid mechanics. By normalizing the rate at which shear stress is applied to lipoaspirate during injection at flow rate Q, by the volume V of the cannula, we can calculate the instantaneous, volume-averaged shear rate, denoted as γ', for a cannula of given length L and radius R (Equation 1).

$$\gamma' = (8/3) \times (L/R) \times (Q/V)$$

Thus, because of the 47.5 percent reduction in cannula diameter, injecting lipoaspirate with an 18-gauge cannula results in a greater than 690-fold increase in the amount of shear compared with injection with a 14-gauge cannula. Meanwhile, total shear exerted on the lipoaspirate linearly increases with cannula length, leading to a greater than 310-fold increase in shear with use of the 25-cm cannula in this study. The addition of a bend in the cannula further increases the shear rate. In our study, lipoaspirate subjected to a low shear rate maintained a larger storage modulus and thus remained more intact. Conversely, lipoaspirate burdened by a high shear rate was associated with a decrease in storage modulus, reducing fat intactness and resulting in poor fat graft quality and overall retention rates. Thus, cannula size does appear to affect volume and viability, with longer and narrower cannulas performing worse than shorter and wider cannulas because of a mathematically predictable increase in shear.

In addition to cannula geometry, other injection parameters under investigation include pressure and flow.[13] Consistent with studies that have found increased injection pressure and flow to adversely affect fat graft retention,[14] the principles of fluid mechanics can again help to explain these effects in relation to shear. Equation 1 shows a linear relationship between shear and flow rate, that is, that a 2-fold increase in flow rate results in a 2-fold increase in shear exerted on the lipoaspirate. With regard to the effects of pressure, the pressure exerted during injection can be described as the pipe flow head loss, h_l, which is the pressure drop along a cannula as lipoaspirate flows through it and where the end pressure is atmosphere. The pressure is directly proportional to average flow velocity U of a fluid of viscosity μ and density ρ, through a cannula of given length L and diameter D (Equation 2).

$$h_l = 32 \times (L/D) \times (\mu U/\rho D)$$

Thus, as pressure is directly proportional to flow rate, it also is directly proportional to shear and affects shear exerted on the sample in a similar manner. Therefore, by contributing to increased shear, increasing either the pressure or flow rate at which lipoaspirate is injected can also harm graft viability and retention as observed previously.

Limitations of these calculations include the assumption that each cannula can be modeled as a discrete cylinder with constant radii.

In addition, the calculations were done assuming a Newtonian fluid undergoing laminar flow, whereas lipoaspirate behaves in a non-Newtonian manner and demonstrates shear thinning: its viscosity decreases as it is exposed to progressively more shear stress. Although further analysis would be needed to provide a more precise quantitative assessment of the biomechanical effects of various injection cannulas, the present study effectively demonstrates the deleterious effects of shear stress on the biological behavior of grafted lipoaspirate.

Our data suggest that, when possible, shorter, larger gauge cannulas should be used for injection. Furthermore, the improved accessibility of recipient sites facilitated by use of a bent cannula may or may not be worth the potential for decreased fat graft quality. Also, when use of cannulas that produce increased shear stress may be necessary, such as in superficial regions where smaller cannulas and lower volumes are preferred, it may be advisable to reduce the injection pressure or flow rate, as this variable directly influences the amount of shear to which fat is exposed during injection. Finally, although fat graft volume retention is notoriously variable and unpredictable, our data suggest the possibility that optimization of injection parameters may increase consistency of retention. The 14-gauge, 8-cm cannula provided not only the best outcomes, but also the most consistent results, suggesting that variability in graft outcomes may be exacerbated by injection conditions that affect the structure and viability of fat. Conversely, variability in outcomes may potentially be reduced by improved methods of grafting.

Finally, we would like to use the correlation we observed between our rheologic and in vivo data to emphasize the applicability of biomechanical testing in surgical planning, as a way to improve prediction of outcomes and reduce potential patient morbidity. The amount of literature on the rheologic properties of lipoaspirate is currently limited: aside from our previous work,[8] we have found only one other study that uses rheologic data in the setting of autologous fat grafting. Kochhar et al. used oscillatory rheology to compare the material properties of several artificial extracellular matrices with human lipoaspirate, to identify the best candidate material for soft-tissue replacement. They reported a storage modulus of lipoaspirate at 1 Hz to be 382.1 ± 66.8 after injection through a 20-gauge needle (unknown length).[15] As we have shown that the storage modulus of fat is affected by injection cannula type, it may be worth evaluating the performance of artificial soft-tissue fillers under a variety of injection conditions as well.

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