Role of Fractionated Fat in Blending the Lid-Cheek Junction

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Background: Fat grafting has been used extensively in plastic surgery in the past two decades. Here, the authors report the retrospective comparison of patients who underwent fractionated fat injection to blend the lid-cheek junction with those who had regular fat injection.

Methods: After obtaining institutional review board approval, a retrospective review of patients who underwent lower blepharoplasty with fractionated fat injection for blending the lid-cheek junction from January of 2014 through October of 2015 was performed. The results were compared to those of lower blepharoplasty patients who did not have fractionated fat injected before January of 2014. Twelve prospectively selected patients underwent histopathologic and gene expression comparisons.

Results: A comparison of complications between the two groups revealed no significant differences. Furthermore, there was no significant difference between the two groups for sequelae of fractionated fat injection and regular fat injection. The gene expression analysis of the fractionated and regular fat did not show any difference between undifferentiated and differentiated cells. In addition, Oil Red O staining of the fractionated and regular fat after differentiation showed that cells from both fat groups differentiated equally well.

Conclusions: Fractionated fat injection appears to be a safe addition in blending the lid-cheek junction in the five-step lower blepharoplasty. There is no fat nodule formation with injection of fractionated fat injection compared with injection of regular fat performed superficially in the tear trough area. Contrary to what has previously been shown, the presence of viable cells in fractionated fat was noted. (Plast. Reconstr. Surg. 142: 56, 2018.)

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any difference in the number of viable stem cells derived from the stromal vascular fraction from macrofat, microfat, and nanofat. In 2001, there was an important discovery made about the presence of adipose-derived stem cells in liposuction samples. Since then, the medical community has been interested in showing the presence of multipotent mesenchymal progenitor cells in liposuction samples. Mashiko et al. recently demonstrated squeeze and emulsification techniques of fat and showed the presence of adipose-derived stromal cells and endothelial cells after selective removal of adipocytes by mechanical micronization. They showed an increased adipose-derived stromal cell-to-adipocyte ratio by condensation. Both studies stressed the importance of more clinical studies using nanofat or micronized fat graft to see the effect of injected adipose-derived stem cells.

Microfat or nanofat grafting allows smooth and equal distribution of nanofat within the deep dermal or superficial layers of skin. The volume-enhancing effect of nanofat would be limited because of the absence of any adipocytes; however, it may improve the skin quality because of the presence of stem cells. There are isolated case reports of fat grafting showing beneficial effect on aged tissue, diabetic ulcers, irradiated tissue, and scar contractures. However, there has been no clinical study with a large number of patients demonstrating tissue revitalization/regeneration with nanofat or micronized fat. To demonstrate the clinical effect of fractionated fat, we report a retrospective review of the use of fractionated fat in blending the lid-cheek junction in lower blepharoplasty and compared it with patients who did not undergo fractionated fat injection. The senior author (R.J.R.) uses the term “fractionated fat,” or “FractoFat,” because it is basically a fractionalization of fat by emulsification.

PATIENTS AND METHODS

After obtaining approval from the University of Texas Southwestern School of Medicine Institutional Review Board, we conducted a retrospective review of patients who underwent fractionated fat injection for blending the lid-cheek junction from January of 2014 through October of 2015 performed by a single surgeon (R.J.R.) and compared it with patients who did not have fractionated fat injections before January of 2014. All charts were reviewed for demographic data, including age at the time of surgery, medical comorbidities, concurrent procedures performed, history of face lift, and duration of follow-up. Histopathologic and gene expression comparison was performed between fractionated fat and regular fat in 12 patients prospectively.

Fat Harvest and Preparation

The harvesting process is accomplished with manual low-pressure lipospiration of the inner thigh using a blunt 3-mm cannula with multiple small holes. Of note, the inner thigh and abdomen have been shown to contain the highest concentration of stromal vascular cells; their fat is morphologically similar to facial fat because of the relative small cell size. Our group prefers harvesting from the inner thigh because of less postoperative pain. No epinephrine or local anesthetic is used in the fat harvesting process to ensure and maximize adipose cell viability. In addition, the lipoaspirate should fill approximately half of a 10-cc syringe and be placed in a centrifuge for no longer than 1 minute (2250 rpm) at low pressure to remove cellular debris. The supernatant and infranatant are removed and the isolated middle fat is transferred to a 1-cc syringe and injected without delay, minimizing air exposure.

Fat Injection Technique

We prefer to transfer autologous fat at the beginning of the procedure; this previously described protocol ensures meticulous injections, accurate tailoring of the superficial musculoaponeurotic system over the augmented fat compartments, and minimal fat environmental contamination. A 16-gauge needle is introduced adjacent to the alar base and a blunt-tip 2-mm Micrins cannula (Eriem Surgical, Inc., Lake Forest, Ill.) is used for injection in the deep nasolabial and deep malar compartments and the superficial compartments. Each injection is performed with a 2-cc cannula for precise, low-pressure distribution in an antegrade and retrograde injection manner, with constant movement of the cannula tip area. One to 3 cc is injected into the compartments, followed by a gentle massage. The superficial compartments are addressed in a similar manner.

Fractionated Fat Injection Technique

The lipoaspirate after centrifugation is treated differently to obtain fractionated fat. Fractionated fat is prepared by mechanical emulsification of fat by pushing fat between two 10-cc syringes connected by a Luer-Lok (Becton, Dickinson & Co., Franklin Lakes, N.J.) connector with two small 2-mm holes (Transfer Emulsifier; Tulip Medical,
San Diego, Calif.). (See Video, Supplemental Digital Content 1, which demonstrates fractionated fat preparation and injection technique, available in the “Related Videos” section of the full-text article on PRSJournal.com or, for Ovid users, available at http://links.lww.com/PRS/C798.) Fifty passes are made between two 10-cc syringes to emulsify the fat. This leads to fragmentation of adipose tissue structure, which therefore has been termed fractionated fat (fractofat). After mechanical emulsification of fat, the fractionated fat is grafted using a fine 2-mm blunt Micrins cannula into the specific cheek–orbital rim junction to disrupt the orbital malar ligament in the suborbicularis plane (1 to 2 cc) and then deep to the orbicularis muscle in the lower eyelid as well (1 to 2 cc) (see Video, Supplemental Digital Content 1, http://links.lww.com/PRS/C798). This effect is to optimally disrupt the orbital malar ligament and lid-cheek junction and blend this area into one aesthetic unit.

CD34 Stem Cell Isolation

Samples from six patients were used for CD34 stem cell isolation. Lipoaspirates from regular fat and fractionated fat were diluted with equal volumes of sterile phosphate-buffered saline containing 1% penicillin/streptomycin and 0.1% collagenase D (50-720-3640; Fisher Scientific, Hampton, N.H.). The mixture was incubated in a shaking water bath at 37°C for 45 minutes. Then, 1 ml of fetal bovine serum was added to the lipoaspirates to stop enzyme activity and samples were centrifuged at 800 rpm for 5 minutes. The lipid phase and the adipose phase were aspirated and the stromal vascular fraction pellet was resuspended in phosphate-buffered saline containing 2% fetal bovine serum for cell counting.

Adipose-derived stem cells were isolated by magnetic separation using the anti-CD34 Human micro-bead kit from Miltenyi Biotec (130-046-702; Miltenyi Biotec, Bergisch Gladbach, Germany). Stromal vascular fraction cells were resuspended in 300 μl of buffer (0.5% bovine serum albumin in autoMACS Rinsing Solution) (150-091-222; Miltenyi Biotec). One hundred microliters of FcR blocking reagent was added for up to 10⁶ total cells. One hundred microliters of CD34 micro-beads was added (for up to a total of 10⁶ cells) to the cell suspension and incubated at 4°C for 30 minutes. Cell suspensions were washed by adding 5 ml of rinsing buffer and centrifuged at 300 g for 10 minutes. Supernatant was aspirated and pellets were resuspended in 500 μl of rinsing buffer. Antibody-bound CD34+ cells were then purified by magnetic separation using magnetic-activated cell sorting columns as described by the manufacturer’s instructions (Miltenyi Biotec).

CD34 Cell Culture and Differentiation

Purified CD34+ cells were resuspended in culture medium consisting of Dulbecco’s Modified Eagle Medium/F12, 1% penicillin/streptomycin, 0.1% gentamicin, GlutaMAX (10565042; Life Technologies, Carlsbad, Calif.), and 10% fetal bovine serum. CD34+ cells were seeded onto four wells of a 24-well collagen-coated plate. On reaching confluence, cells were serum starved overnight and then switched to serum-free induction medium containing 1% ITS premix (41400-045; Life Technologies), 500 μM isobutyl methylxanthine (I5879; Sigma, St. Louis, Mo.), 0.1 μM cortisol (G-106-1ML; Sigma), 1 μM dexamethasone (D4902-100MG; Sigma), 0.2 nM triiodothyronine (709719-1MG; Sigma), and 1 μM rosiglitazone (71740; Cayman Chemical, Ann Arbor, Mich.). After 4 days in induction medium, cells were maintained in serum-free medium containing 0.1 μM cortisol, 1% ITS premix, and 0.2 nM triiodothyronine until harvest.

Adipocyte Viability

Samples from three patients were used for adipocyte viability. Lipoaspirates from regular fat and fractionated fat were centrifuged at 600 g for 5 minutes. The oil layer was discarded and 1 ml of the adipocyte layer was transferred to a clean Eppendorf tube. One milliliter of phosphate-buffered saline containing 2 μl of calcein (1 μg/ml) (50850334; Fisher Scientific) and 2 μl
of propidium iodide (P4864-10ML; Sigma) was added to the adipocyte layer and incubated at room temperature for 10 minutes. Cells were then washed twice with phosphate-buffered saline and moved to a six-well plate for imaging by fluorescence and bright-field microscopy.

Gene Expression Analysis

Samples from four patients were used for gene expression analysis. Total RNA from cultured cells was extracted using the TRIzol reagent (Invitrogen, Carlsbad, Calif.) and purified with the RNeasy Mini Kit (Qiagen, Hilden, Germany). cDNA was synthesized with a High Capacity cDNA Reverse Transcription Kit (4374966; Life Technologies). Relative expression of mRNAs was determined by quantitative polymerase chain reaction using the SYBR Green PCR system (Applied Biosystems, Foster City, Calif.) and values were normalized to levels of RPS18 using the ΔΔCt method.

Oil Red O Staining

Differentiated cells were fixed in 10% formalin for 10 minutes at room temperature. After fixation, the cells were washed with deionized water twice and incubated in 60% isopropanol for 5 minutes. The cells were then completely air-dried at room temperature before Oil Red O working solution (2 g l:1 Oil Red O in 60% isopropanol) (0625; Sigma) was added. After incubation at room temperature for 10 minutes, the Oil Red O solution was removed and the cells were washed with deionized water four times before images were acquired for analysis.

Statistical Analysis

All data were entered into a spreadsheet and analyzed using SPSS Version 18 (SPSS, Inc., Chicago, Ill.) statistical analysis software. Categorical variables are expressed as frequencies and percentages. Continuous variables are expressed as means, medians, ranges, and standard deviations as appropriate.

RESULTS

Table 1 shows that there is no difference between the two groups in terms of sex, age, and body mass index. There were more patients with secondary face lift in the fractionated fat group than in the regular fat group. Mean follow-up was longer in the regular fat group (12 months; range, 0 to 36 months) in comparison with the fractionated fat group (6.57 months; range, 0 to 21 months).

Table 1. Demographics and Comorbidities

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Fractionated Fat Injection (%)</th>
<th>Regular Fat Injection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>121 (92.4)</td>
<td>92 (92)</td>
</tr>
<tr>
<td>Male</td>
<td>10 (7.5)</td>
<td>8 (8)</td>
</tr>
<tr>
<td>Age, yr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>59.42</td>
<td>60</td>
</tr>
<tr>
<td>Range</td>
<td>39–77</td>
<td>44–89</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>23.64</td>
<td>23.28</td>
</tr>
<tr>
<td>Range</td>
<td>17–34</td>
<td>17.7–36.7</td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>30 (22.9)</td>
<td>22 (22)</td>
</tr>
<tr>
<td>Cardiac disease</td>
<td>3 (2.3)</td>
<td>5 (5)</td>
</tr>
<tr>
<td>Pulmonary disease</td>
<td>5 (3.8)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>2 (1.5)</td>
<td>0</td>
</tr>
<tr>
<td>Prior face lift</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>6.57</td>
<td>12</td>
</tr>
<tr>
<td>Range</td>
<td>0–21</td>
<td>0–36</td>
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Table 2 compares complications between the two groups. There was no significant difference between the two groups in terms of major complications such as hematoma, ectropion, cellulitis, and chemosis ($p = 0.25$).

Table 3 compares sequelae of fractionated and regular fat injection. More patients had bruising and swelling (>2 weeks) in the fractionated fat group compared with the regular fat group; however, there was no significant difference ($p = 0.20$ and $p = 0.25$, respectively).

We determined the viability of floating adipocytes from regular and fractionated fat liposapirates by calcein/propidium iodide cell staining. Fluorescent imaging of stained cells indicated that most adipocytes within the regular fat preparations were viable (i.e., calcein-stained), with little evidence of dead cells (propidium iodide–stained) present (Fig. 1, above, and second row).
Within fractionated fat lipoaspirates, viable adipocytes were obtained; however, they were present to a much smaller degree than in the regular fat preparations. Instead, far more dead cells were present in the fractionated fat samples (Fig. 1, third row, and below). Thus, the fractionation procedure significantly reduces the number of viable adipocytes within lipoaspirates.

We evaluated the adipogenic capacity of adipose-derived stem cells within regular and fractionated fat lipoaspirates through in vitro differentiation assays. We isolated adipose-derived...
stem cells from the stromal compartment of collagenase-digested lipoaspirates using antibodies raised against the stem cell marker CD34. CD34+ cells from both regular and fractionated fat lipoaspirates appeared fibroblastic and proliferated in culture; no obvious differences in morphology or growth rates were observed (data not shown). We induced adipocyte differentiation of confluent cultures using a standard proadipogenic differentiation protocol (see earlier under Patients and Methods). Under our conditions, lipid accumulation appeared as early as 2 days after the removal of induction medium, and large clusters of differentiated adipocytes were easily apparent by 2 weeks after induction. CD34+ cells from both regular and fractionated fat differentiated similarly, with high efficiency. This was evident by the overall accumulation of lipid in differentiated cultures and the classic round morphology of the differentiated lipid-laden adipocytes (Fig. 2). Gene expression profiling by quantitative polymerase chain reaction revealed the induction of adipocyte-selective genes (ADIPOQ, PPARγ, and FABP4) in all differentiated cultures (Fig. 3, above and below, left). The expression of the adipose progenitor marker DLK1 (PREF1) was diminished as cells differentiated (Fig. 3, below, right). Collectively, these data indicate that the adipogenic capacity of adipose-derived stem cells was not adversely impacted by the adipose fractionation procedure.

**DISCUSSION**

Blending of the lid-cheek junction remains an elusive goal in modern blepharoplasty. The addition of nanofat, and now fractionated fat, to enhance the lid-cheek junction is another adjunct for accomplishing this goal.

Since the description of the Coleman technique for fat grafting, the majority of plastic surgeons have used cannulas to deliver fat at the desired target site. Based on mesenchymal and fibrous tissue, it has been difficult to deliver a small amount of lipoaspirate at the desired thickness and in appropriate density in the thin lower eyelid area. Usually, there is some resistance to smooth delivery during fat grafting, leading to formation of clumps. The early experience with macrofat grafting in the lower eyelid areas was not good, with numerous complications, including fat clumping and irregular fat take at the tear trough area, that resulted in unsatisfactory results, and the technique was abandoned. With the formation of clumps, it is difficult to get neovascularization to the center of the fat deposited, leading to necrosis and an undesirable outcome during aesthetic procedures. Therefore, there is a need to find a better way to deliver adipocytes or stem cells in a smooth manner where they are deposited in a thin uniform manner to allow the maximum number of adipose cells get a proper blood supply for survival. To achieve that goal, microfat grafting was initially attempted, and nanofat grafting was reported by Tonnard et al.

It must be emphasized that this is completely different from the current harvesting, preparation, and use of fractionated fat. However, when histopathologic analysis of nanofat was performed, it was noted that there were no viable adipocytes in nanofat compared with microfat and macrofat. Therefore, the role of nanofat as a volume enhancer is questionable. Tonnard et al. showed that there is an equal amount of multipotent stem cells capable of evolving into adipocytes in macrofat, microfat, and nanofat. Tonnard et al. had concluded that further studies with a larger patient population are needed to obtain conclusive evidence on the safety and efficacy of nanofat. Our study shows the use of fractionated fat in 131 patients for blending the lid-cheek junction in lower blepharoplasty.

Contrary to what has been previously shown, our study showed the presence of viable fat cells even in fractionated fat, which is significant. Recently, Mashiko et al. demonstrated similar findings. The presence of viable fat cells is noted in both studies. Our study is the second study in literature to show the presence of viable fat cells in fractionated fat. Oisinga et al. and Banyard et al. had also shown the presence of adipose-derived stem cells in the stromal vascular fraction after mechanical processing as described by Tonnard et al. These studies show isolation of a greater number of CD34+ progenitor cells with mechanical processing only, without the need for any enzymatic or outside facility processing. On further evaluation of adipogenic capacity of adipose-derived CD34+ stem cells (adipose-derived stem cells), we found that regular and fractionated fat can equally differentiate into mature cells. This shows the potential benefit of injecting fractionated fat within the lower lid. Tonnard et al. have discussed use of nanofat to improve skin quality but limited filling capacity. Our study supports those findings in helping to blend the lower eyelid–cheek junction outcomes.

Previously, studies have shown the benefit of fat grafting in aged tissue, diabetic ulcers, radiotherapy ulcers, and scars. However,
**Fig. 2.** Adipogenic capacity of adipose-derived stem cells from lipoaspirates. Phase-contrast images of differentiated adipocyte cultures after Oil Red O staining of lipid. (Above, original magnification × 4; below, original magnification × 10.) Images are representative of differentiated cultures of CD34⁺ stem cells obtained from four patients.

**Fig. 3.** Relative mRNA levels of adipocyte (above and below, left) or precursor-selective (below, right) genes in undifferentiated or differentiated cultures.
none of those studies have shown a clear mechanism of action of fat grafting. The question remains of whether it is adipocytes, stem cells, or increased collagen and elastin synthesis/remodeling. Mashiko et al. recently showed the tissue regenerative and revitalizing ability of fat grafting because of the presence of adipose-derived stem cells. In the follow-up discussion to the findings of Tonnard et al. and Mashiko et al., similar conclusions were drawn by Stuzin and Tonnard and Verpaele, respectively. Our study gives a better understanding of the mechanism, suggesting that adipocyte differentiation is one of the important components needed to achieve a potential regenerative effect in aging skin. Kuno and Yoshimura have shown that a critical role is played by adipose-derived stem cells for tissue revitalization/fertilization.

However, it is still unclear whether the presence of viable fat cells in fractionated fat benefits the regenerative process. CD34+ cells in fractionated fat and regular fat appeared fibroblastic and proliferated in culture. This may be the reason for increased collagen and elastin synthesis/remodeling too. In our study, the differentiation of cells was easier in the fractionated fat sample group. Further studies will determine the beneficial clinical effect of easier differentiation with fractionated fat.

There was slightly more bruising and swelling seen in the fractionated fat group compared with the regular fat group (p = 0.20 and p = 0.25, respectively). This could be because of the presence of more reactionary factors in fractionated fat compared with regular fat. No nodules or fat necrosis was noted in the fractionated fat group. Our patients obtained a desirable aesthetic outcome at the time of follow-up. (See Figure, Supplemental Digital Content 2, which shows photographs of patient 1 before and after injection of fractionated fat. Patient 1 had 1-year follow-up, http://links.lww.com/PRS/C799. See Figure, Supplemental Digital Content 3, which shows photographs of patient 2 before and after injection of fractionated fat. Patient 2 had 9-month follow-up, http://links.lww.com/PRS/C800.) Our mean follow-up was 6.5 months (range, 0 to 21 months). One of the limitations of this study is shorter follow-up. Another limitation of our study is the retrospective nature of clinical data collection.

Another important point of discussion is the distinct difference between nanofat, micronized fat, and fractionated fat in many ways (Table 4). In our study, fat was harvested from the medial thigh without infiltration of Klein solution and with low-pressure manual suction. Mashiko et al. aspirated fat from the thigh after infiltration with tumescent solution (1 liter of normal saline plus 1:1,000,000 adrenaline). In contrast, Tonnard et al. prepared nanofat from fat harvested from the lower abdomen after infiltrating modified Klein solution and with a high-negative-pressure device. Both studies used a multiport 3-mm cannula with sharp side holes 1 mm in diameter. Mashiko et al. used a multiport 3-mm cannula with sharp side holes 2 mm in diameter. In our study, fat was centrifuged before fractionation, as in the study by Mashiko et al. Tonnard et al. did not perform any centrifugation. To prepare fractionated fat, fat is passed 50 times between two syringes through the Tulip connector with two 1-mm holes. Mashiko et al. passed the fat 30 times between two syringes using a similar Tulip connector with three holes instead of two holes in our study. For nanofat, fat is passed 30 times between two syringes through the female-to-female Luer-Lok connector with 2-mm holes. Tonnard et al. suggested that it is difficult to pass fat through very small holes, but we did not have any difficulty doing it in our study. Similar to Tonnard et al. and contrary to Mashiko et al., we did not perform a second centrifugation after the preparation of fractionated fat. The way fat was harvested may be the important reason...
why we still had viable cells in fractionated fat even though it underwent more aggressive processing than nanofat. Mashiko et al. also showed the presence of few viable cells in the emulsified micronized fat.\textsuperscript{13} Overall, the end product in all three studies was similar.

Multiple authors have stressed the importance of blending the lid-cheek junction to achieve desirable youthful facial features.\textsuperscript{31–35} Nanofat grafting has been shown to give good results in fine rhytides and some facial scars.\textsuperscript{6} Our study is the first to show a benefit of even further fractionation of fat than nanofat. Fractionated fat is a better terminology to describe the emulsification of fat achieved by mechanical processing of the adipocytes and because of the presence of few viable cells. Similar to the observations by Tonnard et al., fractionated fat cannot be used to add volume to deflated cheek or other areas. Fractionated fat can be used in different layers of skin to improve skin quality because of stem cell activity. We noted some early bruising and swelling before seeing final changes in skin quality at 4 to 6 weeks. We also agree with the conclusion by Tonnard et al. that mechanical processing to obtain adipose-derived stem cells is more cost-effective than using enzymes, animal products, and outside facilities.

Fractionated fat is easy to use with smaller cannulas and delivers a large number of condensed adipose-derived stem cells using a very small volume of product. Also, it can be used without any restrictions or regulations because it is an autologous product from the patient. Stuzin et al. described the potential use of the regenerative ability of fractionated fat for treating superficial dermal wrinkles and reversing skin aging changes.\textsuperscript{28} We concur with their findings and encourage further studies for using fractionated fat, not only to blend the lid-cheek junction but also to improve superficial dermal wrinkles and other aging changes. Like chemical peeling, it can help regenerate epidermal and dermal matrix.

**CONCLUSIONS**

Fractionated fat injection appears to be a safe addition in blending the lid-cheek junction in the five-step lower blepharoplasty. There is no fat nodule formation with injection of fractionated fat injection in comparison with injection of regular fat performed superficially in the tear trough area. Contrary to what has previously been shown, the presence of viable cells in fractionated fat was noted.

**REFERENCES**


