Facial Rejuvenation With Staged Injections of Cryopreserved Fat and Tissue Cocktail: Clinical Outcomes in the Past 10 Years

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Abstract

Background: Facial rejuvenation by autologous fat transfer is common in aesthetic plastic surgery. The main drawback is progressive resorption, requiring repeated harvesting and microfat grafting.

Objective: The authors present a method for cryopreservation of excess harvested fat and tissue to enable subsequent use of previously harvested excess material.

Methods: Fat grafts were harvested using a 50-mL syringe and a 3- or 4-mm cannula. A tissue “cocktail” composed of dermis, fascia, and fat was prepared from excised scar tissue, tissue from abdominoplasty, or tissue from reduction mammoplasty. Cocktail specimens were placed in sterile tubes, immersed in a liquid nitrogen tank (−196°C), and stored at −80°C. At 3- to 6-month intervals, repeated cryopreserved fat graft injections were performed. Patients were evaluated by comparing preoperative and postoperative photographs.

Results: Between 2000 and 2010, a total of 5199 cryopreserved fat or tissue injections were performed in 2439 consecutive patients (age range, 19-80 years). Nasolabial folds and lips were the most common injection sites. Clinical outcomes were satisfactory, and improved contour was achieved in most patients after repeated injections.

Conclusions: Cryopreservation of excess tissue for future injection is promising since repetitive injections are often required after resorption of microfat grafts. In our study, the survival of cryopreserved tissue cocktail or fat was comparable to that of fresh fat grafts and is therefore an effective adjuvant method for facial rejuvenation.

Level of Evidence: 4

Keywords
microfat graft, fat injection, fat transfer, tissue cocktail, autologous tissue transfer, cryopreservation, intradermal injection

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Aging is a progressive process, and the rate at which it occurs varies by person. Various internal and external factors contribute to facial aging, including genetics, reduction in bone density, hormonal changes, facial mimetics, sun damage, gravity, subcutaneous tissue atrophy, and smoking.¹⁻³ Facial rejuvenation can be achieved through major, invasive surgical procedures; through minor, less invasive surgical procedures; and through noninvasive skin management. Various injectable materials have been used for facial soft tissue contouring, including autogenous material (free fat, dermis), heterogeneous material (bovine collagen), natural materials (hyaluronic acid), and alloplastic material (silicone, methyl methacrylate spheres, polytetrafluoroethylene).⁴⁻²⁵ The advantage of autogenous tissue grafts over alloplastic and heterogeneous material is well known.²⁶⁻³¹ Volumetric reshaping of the face by autologous tissue injection is a popular and reliable method, and good long-term results have been achieved.¹⁸⁻³¹

Scan these codes with your smartphone to see the operative videos.

Since 1985, the senior author (O.E.) has gained extensive experience with fat injections. In 1989, he began using a tissue “cocktail”—a mixture of dermis, fat, and fascia—for facial autologous soft tissue contouring. The authors believe that this “cocktail” graft is superior to a fat graft alone in terms of graft retention or “take” and longevity. The main drawback of autologous fat transfer is its unpredictability; the vascularization and take of fat grafting may vary from 40% to 60% and even, rarely, 70%. Partial take of grafts, resorption of the original facial tissue, and transferred grafts in the recipient site make repeated injections necessary. Preservation of harvested fat for future use has been performed by the senior author and studied by many others. Several modifications and improvements have been made to enhance the effectiveness of this procedure and to make it more practical. In this article, we share outcomes of staged cryopreserved fat or tissue cocktail injections for facial rejuvenation over the past 10 years.

METHODS

Patient Selection and Evaluation

Patients were included in this series if they were 19 years or older and if they had received at least 1 fresh and 1 cryopreserved injection, after which they had been followed for at least 1 year after the last injection. Patients who had only 1 fresh injection were excluded. Marking of the recipient sites was performed preoperatively. Preoperative and postoperative photographs were taken of each patient to compare clinical results. All photographs were taken in the same studio and with the same equipment—a Nikon camera (Nikon Corp, Tokyo, Japan) with a 105-mm micro lens and 2 studio flash heads. The same film exposure, magnification, lighting, and angle were used for each photograph. During the first postoperative year, patients were seen every 3 months, and photographs were taken at every clinical visit, with annual follow-up and photographs taken thereafter (up to 10 years). At each visit, clinical assessment was made by the physician using medical records of the treatment conducted and by grading digital photographs. At least 1 year after the last injection session, 2 independent physicians at the clinic who were unfamiliar with the patients and the treatments assessed the clinical outcome by comparing the pre- and postoperative digital photographs of the patients. They rated the improvement in overall clinical appearance using the following scale: 0 = no improvement, 1 = minimal improvement, 2 = moderate improvement, and 3 = good improvement. In addition, patients were queried about their subjective satisfaction at follow-up visits; they were asked to rate their satisfaction on the same scale described above, and their responses were rerecorded in the medical records.

Harvesting and Preparation of Fat Graft

The senior author’s technique of fat graft harvesting has evolved. Fat grafts were collected using a 50-mL syringe and a 3- or 4-mm cannula (which has been part of the author’s technique since 1996). In this study, with the patient under general anesthesia and through a small incision, fat grafts were harvested from donor sites without injection of local anesthetic into the donor sites. After the syringe was filled with harvested fat grafts, the cannula was removed from the 50-mL syringe and grafts were put into the body of a 10-mL Luer-Lok syringe after removing the plunger and sealing the aperture. Then, the sealed Luer-Lok syringes filled with the harvested fat grafts were centrifuged at 3000 rpm for 3 minutes. The upper liquid lipid layer and the lower aqueous layer were discarded, and 1 g of first-generation cephalosporin was added for each 100 g of centrifuged fat tissue. The abdomen and flanks were the most common sites for fat tissue harvest. When these regions were insufficient for adequate harvesting, the trochanteric, buttock, or medial thigh regions were used.

Harvesting and Preparation of Tissue Cocktail

Mini-micro grafts of dermis-fascia-fat cocktail were prepared from excised scar tissue or tissue excised during abdominoplasty or reduction mammaplasty, without including any breast tissue. The tissue cocktail was cut into 0.5-mm pieces so they could pass through 16-gauge needles, and antibiotic was added before injection. Care was taken not to include epithelial tissue or hair follicles in the mixture.

Freezing and Thawing Protocol

After the required amount of fat or tissue cocktail was injected, remaining fat or tissue cocktail was cryopreserved. Specimens were put into 10-, 20-, or 50-mL sterile tubes, labeled, frozen at −196°C in a liquid nitrogen tank, and transferred to a UFS 601 medical refrigerator (Electrolux, Stockholm, Sweden) for storage at −80°C. To thaw, an estimated amount of cryopreserved graft specimens in sterile tubes was taken from the medical refrigerator 12 hours before use in the procedure, transferred to a regular refrigerator (−15°C), and, 1 hour before use, thawed slowly at room temperature.

Injection of Fat or Tissue Cocktail

Tissue cocktail injection was preferred and performed in every case where a simultaneous autologous tissue
Injection and breast reduction or abdominoplasty was performed and where excised tissues were available. The area of the face to be injected was marked while the patient was standing (Figure 1). A local anesthetic mixture of 20 mL 0.5% bupivacaine, 0.50 mg adrenaline, 30 mL physiologic serum, and 20 mg triamcinolone acetonide was injected into recipient sites to decrease postoperative edema and ecchymosis and to create vasoconstriction of vessels to prevent or diminish the risk for microembolism. For fat injection, an 18-, 20-, 22-, or 24-gauge disposable intravenous cannula was used. For tissue cocktail injection, a 16-gauge cannula was used to ensure passage of the semisolid graft particles through the disposable intravenous cannula. For patients who required additional facial contour correction, injections were repeated 1 to 3 times at 3-month intervals for 1 year. Touch-up injections were occasionally needed once a year for 2 to 3 years thereafter. Repeat injections were performed using cryopreserved tissue. Small amounts of fat or micro-mini tissue grafts were injected intradermally, subcutaneously, intramuscularly, and supraperiosteally, depending on the site of injection. This maneuver maximized the surface area of contact between newly transplanted tissue and the recipient bed.

Three videos demonstrating the author’s techniques are available at www.aestheticsurgeryjournal.com. You may also use any smartphone to scan the code on the first page of this article to be taken directly to the videos on www.YouTube.com.

### RESULTS

Between 2000 and 2010, a total of 5199 cryopreserved fat or tissue cocktail injections were performed in 2439 patients.
patients. Patient ages ranged from 19 to 80 years (mean, 44.66 years). Most patients (n = 2151; 88%) were female (Table 1).

A list of areas injected with the average amount of fat tissue or cocktail used is presented in Table 2. Repeated cryopreserved fat graft injections at 3 to 6 months or at 1-year intervals were performed in nearly half the patients. The average number of injection sessions was 6 for fat and 3 for tissue cocktail. Clinical improvement was obtained in most patients as assessed by 2 independent physicians, with minimal improvement in 0 patients (0%), moderate improvement in 220 patients (9%), and good improvement in 2219 patients (91%) (Table 3).

Clinical results for 6 patients are shown in Figures 2 to 7. An additional result is shown in Figure 9, which is available online at www.aestheticsurgeryjournal.com.

### Complications

Asymmetry was observed in 60 patients (2.46%) in the early postoperative period after injection but disappeared after edema resolved. No single infection introduced complications, probably due to the sterile conditions in which injections were performed and the addition of antibiotics to the graft material. No complications such as inclusion cysts or displacement of the injected grafts were observed.

### DISCUSSION

Traditional facial rejuvenation techniques lift facial skin and soft tissues in 1 or 2 dimensions. The senior author highlighted the importance of volumetric shaping of the face alone or combined with facelifting, and later, Ramirez and Little described a third dimension of facial aging—reduction of the skeletal framework and atrophy of the subcutaneous fat layer, which are not addressed by traditional facial rejuvenation procedures—as an advanced concept in facial rejuvenation. Various surgical procedures have been utilized to address the third dimension of facial aging, including use of alloplastic material and autogenous tissues. A wide range of facial alloplastic material has been used in solid and injectable forms. The ideal alloplastic material should produce no foreign body inflammatory response, should not support the growth of microorganisms, and should be sterilizable, nontoxic, nonallergenic, noncarcinogenic, and biologically compatible. Other criteria for ideal implant material include resistance to strain and deformation, ease of removal, ease of shaping into the desired form, and, in certain circumstances, radiolucency. Unfortunately, no alloplastic material fulfills the criteria for an ideal filler because of the complications associated with the material. Autologous tissue transfer—which includes fat, dermis, and fascia and is less invasive than skeletal framework procedures—addresses subcutaneous fat layer atrophy of facial aging. Use of autogenous tissue is safe, fulfills many of the criteria of an ideal filler material, and is associated with fewer complications than alloplastic material. Results of previous work with autologous fat transfer were disappointing because of significant volume loss due to central fat necrosis when fat was injected into a single layer in large amounts. However, recent refinements in and progress with fat transplantation support the injection of small amounts in different tissue layers. This technique maximizes the surface area of contact between newly

<table>
<thead>
<tr>
<th>Site of Injection</th>
<th>Range of Injected Amount, mL</th>
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<tbody>
<tr>
<td>Nasolabial sulcus</td>
<td>2-6</td>
</tr>
<tr>
<td>Marionette lines</td>
<td>0.5-1</td>
</tr>
<tr>
<td>White line of the vermilion</td>
<td>1-2</td>
</tr>
<tr>
<td>Philtral columns</td>
<td>0.5</td>
</tr>
<tr>
<td>Upper and lower lips</td>
<td>2-5</td>
</tr>
<tr>
<td>Zygomatic region</td>
<td>4-8</td>
</tr>
<tr>
<td>Infracrural region</td>
<td>2-6</td>
</tr>
<tr>
<td>Lower eyelids</td>
<td>2-4a</td>
</tr>
<tr>
<td>Upper eyelids fold</td>
<td>0.5a</td>
</tr>
<tr>
<td>Eyebrows</td>
<td>0.5-1</td>
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<tr>
<td>Glabella frowning lines (intradermal)</td>
<td></td>
</tr>
<tr>
<td>Forehead creases (intradermal)</td>
<td>1-2</td>
</tr>
<tr>
<td>Forehead augmentation (supraperiosteal)</td>
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<tr>
<td>Temporal region</td>
<td>2-4</td>
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*aOnly fat grafts injected.*

<table>
<thead>
<tr>
<th>Score</th>
<th>Clinical Improvement</th>
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<tbody>
<tr>
<td>0 (no improvement)</td>
<td>Patients, n</td>
</tr>
<tr>
<td>1 (minimal improvement)</td>
<td>0</td>
</tr>
<tr>
<td>2 (moderate improvement)</td>
<td>220</td>
</tr>
<tr>
<td>3 (good improvement)</td>
<td>2219</td>
</tr>
</tbody>
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Table 2. Areas Injected With Fat or Tissue “Cocktail” Grafts and Range of Injected Amount.

Table 3. Clinical Improvement in Patients Treated With Cryopreserved Fat or Tissue Cocktail Injections, as Rated by Independent Evaluators.
Figure 2. (A, C) This 40-year-old woman presented with a contour defect on her face, eyebrow ptosis, skin spots, and skin texture deficiency. (B, D) Six years after laser resurfacing, eyebrow suture suspension, and microfat grafting. Two cryopreserved fat grafting sessions occurred within a year of the original procedure. Marked improvement of facial contour and rejuvenated skin texture were achieved.
Figure 3. (A, C) This 21-year-old woman presented with severe facial contour deficiency. (B, D) Four years after microfat injection in the infraorbital area, cheek region, and mentum and 1 year after the last of 3 additional sessions of cryopreserved fat grafting. (E, F) One year after secondary treatment (5 years after initial treatment). Facial appearance after 4 and 5 years shows marked improvement.
transplanted tissue and the recipient bed for better revascularization and survival, with minimal resorption of the injected fat tissues.\textsuperscript{24,51,52}

Coleman demonstrated that autologous fat tissue is an ideal filler because it is biocompatible, versatile, stable, and can be long-lasting and natural appearing as it becomes integrated into the host tissue.\textsuperscript{51} As noted by Pu et al,\textsuperscript{43} fat grafts harvested and processed by the Coleman technique revealed more viable adipocytes and better cellular function than fat grafts harvested by the conventional liposuction technique. This finding was attributed to the application of low but consistent negative pressure during fat graft harvesting, preserving the integrity of adipocytes, which is a hallmark of the less traumatic Coleman technique.

Now, autologous fat transfer for facial rejuvenation and soft tissue augmentation is one of the most frequently performed procedures in plastic surgery. Although performance of the procedure itself has evolved, unpredictable resorption of the grafted fat often requires repeated harvesting of grafts. The preservation of fat grafts for future application has been of interest to aesthetic plastic surgeons. With cryopreservation of harvested fat grafts for future re-injection, repeated fat graft harvesting is unnecessary. Previous experimental studies in the preservation of harvested fat at $-16^{\circ}\text{C}$ and $-18^{\circ}\text{C}$ for 1 to 2 weeks showed variable results; injected fat survived in study and control groups,\textsuperscript{22} and a decrease was seen in viable adipocytes and fat cell necrosis in an animal model that received preserved fat.\textsuperscript{33} Dry freezing in liquid nitrogen at $-35^{\circ}\text{C}$ and $-195^{\circ}\text{C}$ revealed the ability to maintain the viability and histology of preserved fat grafts.\textsuperscript{34,35} Many studies have found that adding cryoprotective agents (dimethylsulfoxide, trehalose, or glycerol) achieves adequate protection of fat grafts during cryopreservation with different protocols of freezing and thawing.\textsuperscript{36-43,54} However, a survey conducted among 650 randomly selected members of the American Society for Aesthetic Plastic Surgery (ASAPS) revealed that only 6% of plastic surgeons freeze excess fat for later application.\textsuperscript{28}

In the present protocol of freezing and thawing, no cryoprotective agents were used. Gross appearance of

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure3.png}
\caption{(A, C) This 21-year-old woman presented with severe facial contour deficiency. (B, D) Four years after microfat injection in the infraorbital area, cheek region, and mentum and 1 year after the last of 3 additional sessions of cryopreserved fat grafting. (E, F) One year after secondary treatment (5 years after initial treatment). Facial appearance after 4 and 5 years shows marked improvement.}
\end{figure}
Figure 4. (A, C) This 30-year-old woman presented with nasal deformity, hollow eyes, cheek deficiency, mentum hypoplasia, eyebrow ptosis, damaged skin texture, and a tired appearance. (B, D) Thirteen years after rhinoplasty, microfat grafting to the face (including the infraorbital, cheek, and mentum areas), and suture suspension of the eyebrow. Ten cryopreserved fat grafting sessions occurred between the original procedure and 13 years postoperatively.
Figure 5. (A, C) This 40-year-old woman presented with hollow eyes, cheek deficiency, mentum hypoplasia, eyebrow ptosis, damaged skin texture, and a tired appearance. (B, D) Four years after microfat grafting to the face (including the infraorbital area, the cheek, and the labial, nasolabial, and mentum areas) and suture suspension of the eyebrow and cheek. Seven cryopreserved fat grafting sessions occurred between the original procedure and 4 years postoperatively. (E, F) Eight years after microfat grafting to the face (including the infraorbital area, the cheek, and the labial, nasolabial, and mentum areas) and suture suspension of the eyebrow and cheek. Eight cryopreserved fat grafting sessions occurred between the original procedure and 8 years postoperatively.
cryopreserved fat grafts maintained their quality even after a long period of cryopreservation (Figure 8). Unpublished results of histologic studies from the senior author’s laboratory did not show any difference in recipient site morphology between grafts and the recipient site in biopsies taken 6 months and 1 year postoperatively. Our technique of fat grafting is similar to Coleman’s, with some modifications. We use a 50-mL syringe instead of a 10-mL Luer-Lok syringe, but low negative pressure is still applied, as in the Coleman method. Using a 50-mL syringe makes fat harvesting less time-consuming, and a large amount of fat grafts can be harvested, as is necessary with buttock or calf augmentation. In addition, to protect the integrity of fat tissue, donor sites are not injected with local anesthesia.

Common sites for fat and tissue grafting in our study included the cheek, periorbital region, nasolabial folds, and lips. The periorbital region should be injected deeply with fat only since, in thin skin, semisolid tissue cocktail graft particles will be visible as palpable nodules. Overcorrection was avoided to ensure vascularization; if fat cells in the central part of some injected grafts do not contact the recipient bed, vascularization will not occur. Thus, staged injections with small amounts of autologous tissues were performed in each session to improve vascularization and facilitate better acceptance and survival of the grafts. Clinical assessments of the outcomes were made by physical examination of the injected areas and comparison of pre- and postinjection photographs, which is the assessment technique used by most practitioners of fat graft transfer.

Cryopreserved fat or tissue cocktails are advantageous because they are readily available, can be administered under local anesthesia and as an outpatient procedure, and are cost-effective. These benefits contrast with those of artificial substances such as hyaluronic acid, especially when injection takes place in a major part of the face. Anecdotally, we have found that use of this technique has increased the number of patients interested in microfat grafting, and it also has shifted interest from use of fillers to autologous tissues in such procedures in our clinic. The main drawback of our fat grafting procedure is that aging...
Figure 6. (A, C) This 38-year-old woman presented with contour deformity on the face, hollow eyes, damaged skin, and a tired appearance. (B, D) Eight years after brow and cheek suture suspension. Four cryopreserved fat grafting sessions occurred between the original procedure and 8 years postoperatively. Facial contour and skin texture are considerably improved.
is an evolving process; partial resorption of the injected grafts is inevitable and requires repeated injection. The survival rate of autogenous fat grafts is variable and is affected by many factors, including the technique used for fat graft harvesting and preparation, the vascularity of the injected areas, and the use of appropriate injections in different, multiple tunnels instead of a single layer. Reported rates of resorption vary from 25% to 90%. Dermis and fascia are tough and resilient, and they are readily vascularized, thus reducing the high resorption rates associated with autologous fat transfer when used in our cocktail. In the senior author’s experience, the average rate of fat graft survival after 6 months of injection has been approximately 60%. However, with injection of a tissue cocktail of dermis, fat, and fascia, the survival rate has increased to as high as 90%. This was supported by 2 experimental studies. Another minor drawback of our study concerns the possibility of more fullness, especially in the lips and cheeks, in patients who had fat injections to the face and experienced simultaneous weight gain. This was observed in some of our patients (Figures 6 and 9).

The debate regarding the best method of fat graft harvesting, preparation, and preservation, as well as modification of techniques, will continue until an optimum method is designed. In our experience comparing 2 fat tissue samples harvested 10 years apart from the same patient, the newly harvested fat tissue quality was markedly lower than that of the cryopreserved fat harvested and stored 10 years prior. This could be due to fat quality degradation after 10 years as a result of aging. This finding is consistent with the aging process, in which tissue collagen is diminished by quality and quantity. However, this finding needs to be supported by future histological studies. We stress that, in addition to traditional cryopreservation applications (such as for stem cells), fat tissue should also be cryopreserved for future microfat grafting.

CONCLUSIONS

Autologous fat or tissue cocktail grafting using our cryopreservation technique is an effective adjuvant method for facial rejuvenation, and it is our clinical experience that our outcomes are comparable with those resulting from fresh graft procedures. Injected tissue, with revascularization from the recipient site, can remain viable and survive similarly to the original tissue. Partial resorption remains a problem, requiring repetitive injections. Cryopreservation of harvested autologous tissue facilitates these subsequent treatments by creating a store of injectable tissue that is readily available for future use. Furthermore, acceptance by the patient’s body is much greater than with repeated fresh harvesting.
Figure 7. (A, C) This 53-year-old woman presented for facial rejuvenation. (B, D) Eight years after brow and cheek suture suspension and upper blepharoplasty. Eight cryopreserved fat grafting sessions occurred between the original procedure and 8 years postoperatively. Facial contour and skin texture are greatly improved, and the patient looks much younger.
REFERENCES

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Figure 8. The appearance of fat (A) 1 and (B) 7 years after cryopreservation. Samples maintain their quality after long periods of cryopreservation storage.