

Regenerative Cells For Facial Surgery: Biofilling and Biocontouring

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Abstract

Zuk et al in 2001 identified stem and regenerative cells within the stromal vascular fraction of fat. In preclinical studies, these cells appeared to stimulate angiogenesis and reduce inflammation, and soon thereafter, clinical use of stromal vascular fraction (SVF) evolved as researchers such as Rigotti, Coleman, Mojallal, our group, and others demonstrated that fat can be used for both therapeutic and aesthetic indications. The regenerative effects of fat and its contents on facial aesthetics have been shown at the histologic and cellular level. Regeneration of elastin and collagen fibers as well as improvement in capillary density and reduction of inflammation have been reported. We review our current approach to the use of regenerative cells and different types of fat grafts in facial surgery. The fat graft is classified, both from a regenerative point of view as well as a tissue product that can be modified into different tissue characteristics, depending on the area and condition treated. Clinical use of SVF enriched fat, millifat, microfat, and nanofat grafts as well as composite fat grafts are reviewed. Based on clinical experience and evidence to date, it appears that the regenerative effects seen with the use of SVF in aesthetic surgery are modest, but there appear to be definite histologic findings of regeneration. These improvements may not be clinically apparent to a patient when cell enriched fat grafts are compared to fat grafts alone. However, the subtle changes seen in histology may be cumulative over time. Three types of fat grafts are defined: millifat (parcel size 2.4<), microfat (1.2<), and nanofat (400-600 μm). Each are characterized by their injectability ratings and emulsification parcel size as well as amount of sSVF cells. Newer concepts of periosteal fat grafting, buccal fat pad grafting, pyriform aperture fat grafting, intraorbital fat grafting, and nanofat grafting are discussed. Composite fat grafts are presented as a new concept as is biofilling and biocontouring. The use of regenerative cells in facial surgery is evolving rapidly. Our understanding of the anatomic changes that occur with aging has become more precise and our ability to target histologic changes seen with aging has become more effective. Deep fat compartment grafting, superficial fat grafting, nanofat, and SVF are becoming important components of contemporary facial rejuvenation. The use of regenerative approaches in facial rejuvenation is a logical step in changing the paradigm from surgical treatment of aging to a more proactive prevention and maintenance approach that keeps up with changes in the tissues as they age.

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Zuk and coworkers¹ in 2001 identified stem cells and other precursor cells in fat and noted their potential for pluripotent development into differing tissues types such as myocardium, nerve, and bone to name a few. Soon thereafter, SVF cells, which are a heterogeneous population, consisting of stem and regenerative cells with growth factors, cytokines, and other cell signaling mechanisms were found to stimulate angiogenesis, prevent apoptosis, modulate immune responses, and could be isolated from fat for a variety of therapeutic uses.

The first application of adipocyte-derived stem and regenerative cells, also known as stromal vascular fraction

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cells, for facial aesthetics was performed by Cohen and Holmes in 2003 in 8 patients,² although Llull³ treated a patient with a hand and upper extremity deformity, and Howalt, a patient with a residual large cranial defect using SVF enriched fat and SVF with bone marrow and resorbable mesh, respectively a year earlier.⁴ In our early study, in addition to the effects of augmentation, regeneration of the skin's surface and thickness was noted.² In one patient, who returned 6 years later, regeneration of dermal soft tissue loss was still evident in the nasolabial folds and radial wrinkle lines of the lips.⁵ Coleman noted improvement in scars and overlying skin texture and pigmentation in patients undergoing conventional fat grafting using his technique² and a study by Mojallal in mice noted induction of epithelial and dermal changes by SVF interacting with the underlying host dermal tissues.⁶ A more recent study by Rigotti's group, revealed in biopsies in human subjects that fat grafts enriched with mechanically dissociated SVF cells demonstrated some degree of reversal of aging changes in elastin and collagen fibers with regeneration of tissue architecture 1 to 3 months after injection into the subcutaneous layer of the face.⁷

As early as 2000, others working in the field noted improvement in texture and pigmentation in photo-damaged skin with lower eyelid injection of stromal vascular fraction in fat delivered as primarily a cellular and stromal graft following enzymatic digestion, rinsing and centrifugation.⁸ "nanofat," a term popularized by Tonnard and Verperle was introduced to describe their method of preparation of an "adipocyte free product" composed mainly of free fatty acid, matrix, and stromal vascular fraction cells.⁹ First introduced by Ellenbogen¹⁰ and Guerrerosantos¹¹ large volume fat grafting during facelift was repopularized in the United States by Marten,¹² Cohen,¹³ and Rohrich.¹⁴

Observations by Lambros,¹⁵ contemporary, and practical descriptions of the fat compartments of the face by Rohrich and Pessa,¹⁴ and the craniofacial skeletal changes that occur secondary to aging as documented by Kahn¹⁶ and others¹⁷ compliment the advances in regenerative technologies and contribute to a comprehensive understanding of the aging process.

With the improvements in our ability to treat photo-aging and skin laxity as well as address volumetric changes with fillers and fat, progress in the treatment of surgical aging has rapidly progressed. Moreover, the etiology of skin laxity began to make more sense and with deep compartment fat grafting, more extensive deep plane facelift techniques have been largely eliminated in our practice. In essence, because of the groundwork in many areas, including a better understanding of the aging process and how it affects each of the layers and structures of the face along with improvements in biomaterials, surgical equipment for tissue, and cell

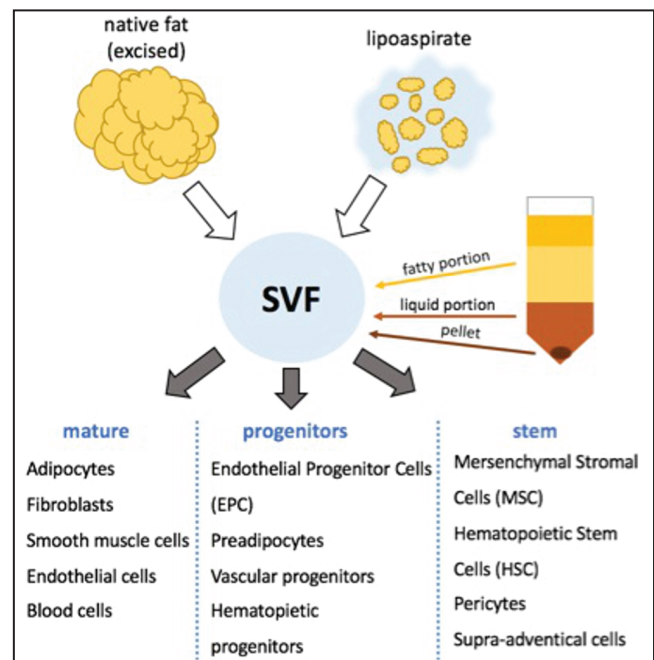


Figure 1. Stromal vascular fraction (SVF) is composed of stromal and vascular cells.

procurement and modification, the field of facial rejuvenation and facial contouring for both aesthetic and reconstructive purposes is poised to explode in the next five years.

We foresee a shift in how care is delivered from a more surgically oriented approach that is one of prevention and maintenance and gradual replacement of aging tissues with similar tissue. More kinds of repair therapies at the cellular level will be developed as we begin to understand how to alter the aging process in other organs. Herein, we describe the evolution of our strategies and techniques to treat facial aging, the role and clinical applications of autologous fat, fat-derived regenerative cells, and stromal vascular fraction in aesthetic facial surgery.

DEFINITIONS

Regenerative cells found in human fat are a mixed population of stromal and vascular cells found around blood vessels and between adipocytes in the matrix of fat. Most of these cells are covalently bound to small blood vessels in the matrices of fat which surround adipocytes. By definition, SVF is composed of stromal and vascular cells (Figure 1). A small proportion of the stromal cells are stem cells, which have been shown to be capable of differentiating into different structures (pluripotency). Adipocyte-derived stem cells, also known as mesenchymal stem cells, need to be expanded in culture in order to obtain large quantities.

Stromal vascular fraction cells participate in many functions throughout the human body. Adipose tissue is the only organ that undergoes considerable change in mass in the adult. Adipose tissue volume change is mediated by changes in adipocyte cell size. Blood vessels within adipose must expand (or be pruned) to support increased (or decreased) tissue volume. Vascular cells in adipose tissue are in a state of increased plasticity compared to those in other tissues. Stromal cells are such cells as preadipocytes, tissue macrophages, stem cells, and fibroblasts. Vascular cells consist of endothelial cells, vascular smooth muscle cells, and pericytes.

Stromal vascular fraction cells have been shown to have beneficial effects in wound remodeling, blood supply, differentiation, and wound immune modulation. Stromal vascular fraction cells home to injured sites and are associated with numerous trophic factors, growth factors, cytokines, cell signaling molecules, etc. and represent an orchestra of regenerative cells that can be found in human fat.

There are presently no Food and Drug Administration (FDA) approved devices that use an enzyme to digest lipoaspirate and obtain mesenchymal stem and regenerative cells for therapeutic application. A phase III FDA trial (The Star Study) treating Raynaud's in scleroderma based on Magalon's original study of 12 French patients¹⁸ is fully enrolled and may be the first approval if successful outcomes are reported.¹⁹

In addition, new devices that use mechanical dissociation are available to collect smaller amounts of SVF. These devices work in concert with the liposuction process to free SVF cells from fat. These cells should not be injected intravenously, but can be mixed back into the prepared fat

and/or used themselves in wounds, acne scarring, alopecia, and burns. The device we use is a vibratory device, (Millenium Medical Technologies, Inc., San Diego, CA) which causes mechanical dissociation of SVF (Video 1, available as Supplementary Material online at www.aesthetic-surgeryjournal.com). The infranatant is collected, washed, and centrifuged and a cell pellet of SVF is used for cell enrichment. The cell counts appear to be about one-third of what an enzyme might release as measured in the same patient. Lipogems is another means of obtaining mechanical SVF or SVF enriched fat. Lipogems works by ball bearings mechanically freeing SVF cells. This device, by anecdotal reports, releases less SVF than by vibration, but no head-to-head studies are available. We have no clinical experience with this device. Power assisted devices that reciprocate, such as Microaire, also release some SVF cells at the expense at slightly lower viability rates. The released SVF can be collected into a canister StromaCell.¹⁸

Nanofat technology based on Tonnard and Verpaele's original work⁹ has been developed by Tulip Medical, Inc. (San Diego, CA). Their device produces "nanofat," which is composed of smaller amounts of SVF plus some free fatty acid and other components of the matrix. A series of emulsifiers ranging from 2.4 mm to 400 to 600 μm are used to modify harvested fat into smaller parcels and nanofat. These products each have different injectability characteristics (Table 1) and are used for different applications, including deep fat compartment and periosteal grafting, superficial fat compartment grafting, and dermal and epithelial nanofat delivery. There are other products on the market that claim to produce nanofat, but each of these

Table 1. Different Types of Fat Grafts

Fat grafts	Harvest cannula	Process	Rx area	Cells/mL	Injectability rating
Macrofat (2.4 mm>)	2.4 mm or >	Tumescent delivery, preliposuction prep (ie, ultrasound, vibration, filtration, rinsing, decanting)	Breast, buttock, body	—	N/A
Millifat (2.4 mm<)	2.4 mm hole size	Washing, 2.4 mm emulsifier	Deep fat, lips, temporal, pyriform, mandible, chin, nasal bridge, orbit	—	18G-20G
Microfat (1.2 mm<)	1.2-2.4 mm hole size	1.2 mm emulsifier	Forehead, superficial fat in eyelids, brow, perioral, periorbital, nose, hands	—	23G-25G
Nanofat (400-600 μm <)	1.2-2.4 mm hole size	400-600 μm emulsifier	Superficial rhytids, intradermal	25-50K/mL	27G-30G
Composite Fat Graft [†]	1.2-2.4 mm hole size	Milli, micro, nano +/- SVF, CH, PLA	Superficial rhytids preperiosteal	—	18G
Mechanical SVF	Vibrationally dissociated SVF	Centrifuge, washing	Any	300-600K/mL (based on pt. variables)	
Enzymatic SVF (Celution)	Any	Centrifuge, washing, enzyme	Any	1 million/mL and >, based on pt. variables	
Decellularized Fat Graft	None	Good manufacturing practice (GMP) processing retains proteins (growth factors, cell signaling?)	Any	None	21 gauge needle or cannula

CH, calcium hydroxyapatite; GMP, good manufacturing practice; Micro, microfat; Milli, millifat; Nano, nanofat; PLA- polylactic acid; SVF, stromal vascular fraction.

products works by different mechanisms and there are no head-to-head comparisons that have been published in the medical literature. Presently, nanofat is characterized by the fat and tissue parcel size, which is 400 to 600 μm or less.

It is important to understand the tools for fat processing, stromal vascular fraction procurement, and nanofat creation as each produces a different tissue product. When 4 commercial SVF procurement systems using enzymatic digestion were evaluated by Aronowitz,²⁰ each had very different cell counts, numbers of stem cells, viable cells, and amounts of residual collagenase. Cell counts and profiles depend on many factors including the patient, the means of processing, and even the types of medications and conditions of the patient's health.²¹ The effect of dose of SVF on outcome is presently unknown and being evaluated in laboratory and clinical settings. It is important to understand the current regulatory climate in the United States. There are no devices that use an enzyme for SVF or stem cell separation that are approved by the FDA. In addition, there is little concern with washing, sizing, and decanting of fat.

Evidence that fat improves the appearance of soft tissue and scars has been observed by clinicians and has been shown by researchers in animal models.⁶ That said, it is more difficult for patients or physicians to discern differences between fat grafting and fat grafting with SVF cell enrichment in terms of outcomes unless one looks for histologic evidence. The use of SVF cells may give us incremental improvements in our tissues as we age such as: longer lasting results, better qualities of skin thickness and texture, and histologic reversal of aging seen in elastin and collagen, which have been demonstrated in humans by Rigotti's group in Italy.⁷ The article by Rigotti's team⁷ is perhaps the most important evidence to date that regeneration of aged tissue occurs after grafting with mechanically obtained SVF enriched fat. This article heralds a new approach to facial rejuvenation, where degenerating tissues are periodically replaced with like tissues in an effort to keep up with the aging process. By grafting biologically active scaffolds, cells and proteins with their associated growth factors, histologic regeneration of tissues can be accomplished. In many ways, this new paradigm is akin to upkeep of one's house with periodic replacement of carpet or new paint, etc. in an effort to keep up with the daily wear and tear rather than doing nothing until a total renovation is needed.

In this review, we present our current thoughts on fat grafting. Some of the concepts presented are new and untested, while others have been validated by prospective studies and close clinical observation.

TYPES OF FAT GRAFTING, STROMAL VASCULAR FRACTION ENRICHED FAT GRAFTING, AND CELL THERAPIES

Basic terminology is critical to the clinician in order to understand the meaning of stromal vascular fraction cells and to reclassify the traditional "fat" graft. Most surgeons still look at fat as simply a filler for augmentation. They are less aware of the regenerative properties in fat and are not well versed in how fat can be mechanically shaped into different types of tissue grafts, depending on how and where they are being used.

Types of Fat Grafts: Reclassifying the Fat Graft

Just as hyaluronic acid fillers have been characterized by their cohesivity, viscosity, and elasticity into different products for different uses such as Voluma for cheek volume vs Belotero for finer lines,²² fat can be modified by cleaning, compounding, emulsification, and filtering into specific shapes of the same tissue. Differences in parcel size are observed, which influences flow characteristics through specific sized cannula, and to some extent different compositions of cellular and extracellular components with different viabilities. It is important to remember that these cell counts and profiles are specific to the individual patient and quite a bit of variation may exist in similar age patients with similar ethnic backgrounds, making it more difficult to fully understand cause and effect. In addition, it is likely that these cells are in a dynamic state with possible feedback mechanisms, increasing in response to weight gain, in response to certain chemotherapeutic agents, and in response to injury and other endogenous and exogenous influences. Moreover, the complexities of the interactions among the multitude of growth factors, cytokines, and cells located within the stromal vascular fraction of fat are influenced by age and the specific diseases or disorders that calls them into action. Even the location and size of adipocyte may vary in individual patients. For instance, smaller fat parcels closer to hair follicles have different properties than fat parcels from the deeper layers beneath Scarpa's fascia.²³ Smaller adipocytes might also be associated with macrophages that have more anti-inflammatory properties than larger adipocytes.⁶

To simplify the understanding of these different types of fat tissues, we classified fat into 3 major types of fat grafts based on the size of the emulsifier utilized: millifat, microfat, and nanofat. These grafts are defined at present by their injectability ratings, their emulsified fat parcel size, and the degree of adipocytes that remain in the product (Table 1).

To surgically create these products, lipoharvest is carried out and the fat is cleaned. Lipoharvest may be performed under local anesthesia, intravenous sedation, or general anesthesia with tumescent anesthesia. Cannulae are introduced through a 14 gauge needle puncture. Tumescent solution is infused with a vibratory cannula or with an infiltration cannula and syringe. After 5 to 15 minutes, lipoharvest is carried out with a cannula whose hole size is no larger than 2.5 mm. We prefer to use the Carraway disposable cannulae by Tulip Medical, Inc. (San Diego, CA) or if we mechanically dissociate SVF, we use a vibratory harvest cannula with a hole size of 2.0 mm to 0.5 mm depending on the tissue sizes we are selecting for grafting. The infranatant produced is SVF cell rich from mechanical dissociation and can be centrifuged and rinsed to produce a cell pellet of SVF (Video 1).

Once the fat is removed, the fat is cleaned and the following three products are made using a series of emulsifiers and strainers (Tulip Medical, Inc., San Diego, CA):

1. **Millifat** is characterized as fat parcels of diameter 2.4 mm or less. Millifat is made by taking cleaned fat and passing it from one 20 mL syringe to another 20 mL syringe through a 2.4 mm emulsifier. Millifat is more structural in nature and injects easily through an 18 or 19 gauge cannula (injectability rating is an estimate of the ease of injection through a given size cannula indicating the size channel that the modified fat passes through). Nanofat can be placed with a 30 gauge needle direct intradermally, if necessary. Millifat is used for structural augmentation in the temporal regions, in the suborbicularis oculi fat (SOOF), the deep fat compartments of the cheek (medial and prezygomatic, the buccal fat pad, the pyriform region, on periosteal in the orbit, the mandible, the lateral eyebrow region, and the nasal bridge and columella as well as the chin and lips).
2. **Microfat** is characterized by fat parcel sizes of 1.2 mm or less in diameter and are made by passing the millifat through a second 1.2 mm emulsifier. Microfat is used for the forehead, eyebrow skin, deep on the periosteum of the tear troughs, perioral skin, and in the hands. Microfat is also used for some types of acne scars as well as a carrier for SVF and/or platelet rich plasma (PRP) for alopecia and in wounds. Microfat injects easily through a 19 or 20 gauge cannula.
3. **Nanofat** is characterized by parcels of tissue 400 to 600 μm . Nanofat is produced by taking the emulsified microfat and passing it through a final double strainer of 400 and 600 μm , which produces a more liquid product of matrix, stromal vascular cells, and free fatty acids that has been shown to improve fine rhytids in the perioral region, neck, and around the eyes. It can be easily injected through a 27 gauge needle and even

smaller. It is used for under the eyelids to improve pigmentation, in the fine lines of the neck, the fine rhytids around the mouth, and in the upper and lower lip and for wounds, incisions, scars, acne scars, and in cases of alopecia in combination with PRP and for preparing radiated breast tissue for implant reconstruction. My preference is not to inject with a needle around the eyes, but to use a blunt 27 gauge cannula. In addition, nanofat can be centrifuged to eliminate the free fatty acids and create a gel that can be applied in combination with a cr me that promotes dermal penetration and can be introduced by Mesotherapy techniques following laser resurfacing or facelift surgery. The nanofat can also be used prior to centrifugation as an injectable by needle, blunt cannula, or with microneedling techniques. In most patients undergoing facial aesthetic surgery, or full facial fat grafting, a combination of all three types of fat grafts are utilized. (Video 2, available as Supplementary Material online at www.aestheticsurgeryjournal.com).

STROMAL VASCULAR FRACTION ENRICHED FAT

Fat enrichment with SVF augments the fat graft with additional cells found in the matrix of fat to improve angiogenesis and reduce inflammation. Enriching fat with the SVF brings in more trophic factors to aid in the regeneration of tissues. After the landmark paper by Rigotti's group⁷ showing reversal of aging architecture in elastin and collagen in patients with the use of mechanically dissociated SVF combined with fat, many clinicians in the field are realizing the benefits of cell enrichment may be subtle, but are may be long lasting and cumulative. While histologic evidence exists, clinical efficacy of these SVF enriched fat grafts and nanofat is difficult to obtain. Patient satisfaction studies have not shown any differences in outcomes using either SVF enriched fat grafts or autogenous fat in conjunction with facelift surgery.²⁴ The histologic changes that are seen with mechanical SVF supplemented fat grafts are the best evidence we have in humans and point to regeneration of tissue architecture and improved blood supply when SVF is added.⁷ Many anecdotal reports are available which have demonstrated improved outcomes in refractory wounds and radiation injuries,²⁵ but again large controlled studies are still pending on the effects of these cells in patients with scleroderma and Reynaud's²⁶ and osteoarthritis.²⁷

There are a number of different devices on the market worldwide that are able to generate SVF from mechanical dissociation. The device we use is based on vibration and this movement dissociates the SVF from fat in situ. Fat is then

aspirated through specially designed cannulae with hole sizes of 2.4 mm to 1.2 mm into a device that permits the infranatant to be collected and processed with centrifugation and a rinsing regimen that generates a cell pellet of SVF, which is around one-third of the cell count of SVF produced by an enzyme from the same patient as the control (Video 1).

After tumescent solution is infiltrated, the cannula is activated and inserted through either a 14 gauge needle incision or an expanded 14 gauge needle incision into the adipose tissue without suction. After the cannula is permitted to vibrate in the adipose for 2 to 5 minutes/100 mL of tumescent solution, liposuction is commenced with a special cannula that selects the fat parcel size based on the hole size and shape. Fat is collected into one of either three reusable canisters, 250 mL, 1 L, and 3 L sizes. Because the fat has been agitated with vibration, more stem and regenerative cells from the stromal vascular fraction are mechanically dissociated. These are heavier and float to the bottom of the canister. This infranatant is collected after 15 minutes to permit settling of the SVF cells. The infranatant is removed, rinsed, and centrifuged to produce a serosanguinous suspension of SVF cells suspended in an aqueous Ringer's lactate solution. These mechanically dissociated SVF cells can be then added into any of the fat grafts.

Mechanical stromal vascular fraction (mSVF) has been obtained in our clinic using a vibrational device (Millenium Medical Technologies, Inc., Carlsbad, CA). Other devices are available such as Lipogems, but we have no experience with this device. In general, the mechanical dissociation technique with Aquicell, the device made by Millenium Medical Technologies, regenerative cell counts of around 300,000 cells/mL are obtained. This compares to 1 million cells/mL when an enzyme is used and SVF is processed with an enzyme using another device (Cytori, Inc.). We do not have comparative data on Lipogems, but nanofat with the Tulip device will generate around 25,000 to 50,000 SVF cells/mL and have been shown to be effective for perioral wrinkles and safer for periorbital injection closer to the surface. Devices requiring enzymatic digestion are not available on the US market outside of FDA studies. At present we use a mechanical dissociation technique for cell enrichment and/or nanofat, which may have certain advantages over SVF produced by enzymatic digestion of fat,^{28,29} including less cell surface changes.

Composite fat grafting is a term coined to represent a fat graft of any type (Milli, Micro, Nano with or without mSVF) combined with a commercially available filler (ie, calcium hydroxyapatite, poly-L-lactic acid, etc.) as a means of providing a scaffold (synthetic and biologic) in addition to cells (mSVF, nanofat) and is being used for bone contouring and/or deep fat compartment grafting (milli + mSVF + calcium hydroxyapatite) and for superficial fat grafting (micro or nano + mSVF + poly-L-lactic

acid [diluted to 1:25 mL]. In addition to synthetic scaffolds, decellurized fat can be used as a biologic scaffold with growth factor proteins that attract the patient's own SVF cells to repopulate the graft. Regenerative effects have been demonstrated.

CLINICAL APPLICATIONS: BIOFILLING AND BIOCONTOURING

Several events have advanced the field of regenerative medicine including simpler equipment that makes lipoharvest more of a "blood draw" procedure, eliminating the stigma and concern of liposuction. In addition to using smaller, disposable cannulae for harvest, a variety of cannulae of different internal diameters are used for injection of the fat graft. The fat is injected through small, 18 gauge needle incisions using disposable cannulae of different diameters, depending on where the fat graft is being placed. Tissue modification by emulsification permits fat to be customized for the clinical application. For example, nanofat can be directly injected intradermally into the rhytids of the lips.

As we have come to better understand the aging changes that occur in each tissue layer of the face, we are able to design better procedures and further customize care. The place of regenerative strategies involving fat and its contents are still evolving. The use of fat and its modifications and contents for both augmentation and regeneration have greatly improved our approaches to the surgical treatment of aging. A number of authors such as Tonnard and Verpaele, Cohen, Rigotti, Coleman, Rohrich and others^{2,7,9,13,14} are beginning to approach facial aging from a biologic as well as anatomic approach. In the next sections, we summarize our present use of regenerative tissues for aesthetic, reconstructive, and medical issues. The techniques we are using today are constantly changing. In the following sections, we have attempted to list areas where SVF and regenerative strategies have shown promise.

Regenerative facial surgery represents a potentially major advance and paradigm shift in how aging and contour deformities are treated. With the ability to reshape jaws, cheeks, and the chin with regenerative approaches, implants and certain osteotomies may be eliminated and even more precise outcomes can be accomplished by simple injection.

The other paradigm shift that is brought about by regenerative medical approaches is the treatment of facial aging itself. Fat grafting may be performed to actually keep up with the aging process and replace what is being lost over time. This ongoing approach may truly restore the blood supply to the skin and delay the aging process by basically helping the body do a better repair job.

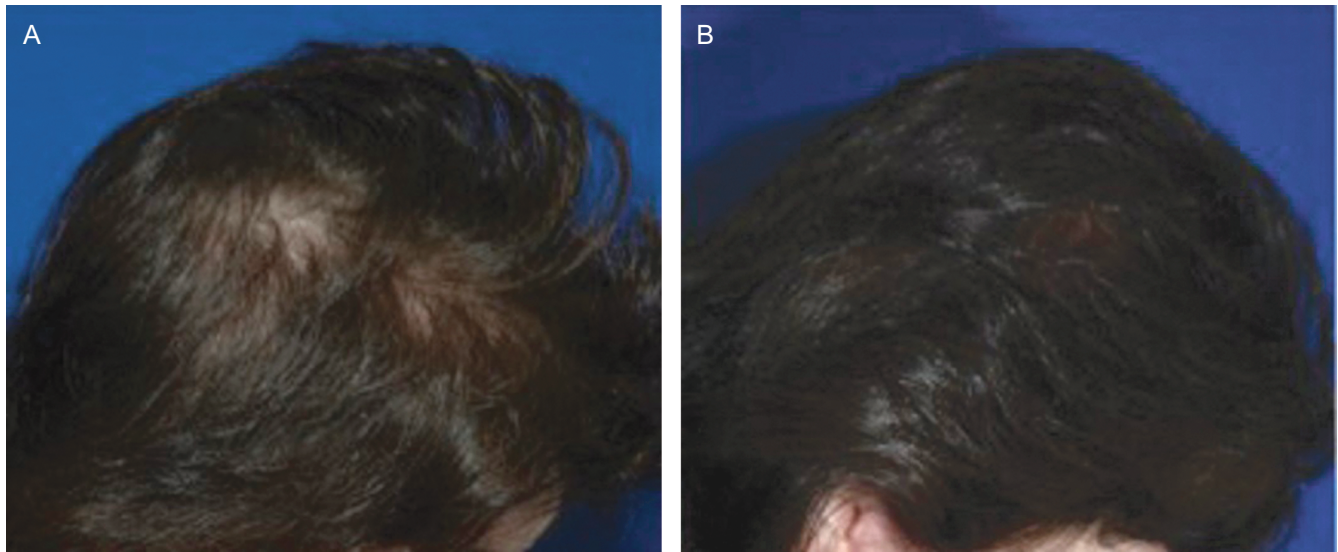


Figure 2. (A) Preoperative and (B) 9 month postoperative photographs of a 63-year-old woman with facial aging. She underwent facelift with high SMAS, submental platysmaplasty, periorbital, temporal, deep compartment, superficial compartment fat injection. In addition, microfat with enzymatically derived SVF was placed in her scalp to treat alopecia. A total of 26 mL of fat was used for the facial grafting and 15 mL of microfat supplemented with enzymatically procured stromal vascular fraction.

Alopecia

In males and females with a diagnosis of alopecia androgenetica, males with hair loss consistent with grades II, IIA, III, IIIA, III-Vertex, IV, IV-A, V-A, V-V based on Norwood-Hamilton scale and females with hair loss consistent with grades I to 7 based on the Savin scale might benefit from regenerative approaches.

The first use of adipocyte-derived stem and regenerative cells, also known as SVF, for alopecia were injected in combination with fat in 2010 for in a woman who demonstrated marked improvement in hair growth (Figure 2). Presently, no approach using enzymatically digested SVF is approved in the United States. In Europe, SVF can be used to treat alopecia in men and women. Mechanically dissociated SVF, nanofat, and PRP alone or in combination with fat grafts have been used to treat alopecia. In aesthetics, the lower priced, easier to deploy products, providing they achieve near equivalent results, will make the most sense. We have experience with eSVF + microfat and nanofat with mSVF delivered by mesotherapy. Patients with earlier stage hair loss are the best candidates for these strategies. Combination treatments are the standard and most patients are put on topical medications before and after treatment. Clinical trials in the United States³⁰ will document efficacy and help us better understand who are the best candidates for treatment.

In our clinic, during facial rejuvenation, if a man or woman has mild to moderate hair loss, nanofat with mSVF or SVF enriched fat obtained from mechanical dissociation, or a combination of both are used in conjunction with

microfat if the patient selects this approach. We also use enzymatically digested fat in property informed patients. We are presently exploring PRP products for use alone or in combination with nanofat and mSVF in alopecia.

The injection of nanofat or SVF enriched microfat is performed subcutaneously above the galea and in proximity to the hair bulb, which is the region where stem cells exist. An 18 to 19 gauge cannula is used for grafting. The fat is injected retrograde in a radial and crisscrossing fashion.

In our limited experience, we estimate about a 30% improvement in hair density and hair diameter with SVF enriched fat.

Forehead, Brows, and Temporal Regions

Our approach to the forehead depends on the physical findings of aging. We evaluate the severity of these aging changes in each tissue layer, from the skin to bone. The forehead is split vertically into 3 regions: the central forehead and the left and right temporal regions. The lower forehead blends into the brows and is evaluated for brow position and shape, glabellar projection, and the depth of the nasofrontal junction. If patients have moderate to severe photo-damage, then laser resurfacing is also recommended. If mild brow ptosis is associated with excessive upper eyelid skin lateral to the lateral canthus, fat grafting is performed to lift the lateral brow. If moderate to severe brow ptosis is present fat grafting is carried out first, followed by endoscopic or minimally invasive,

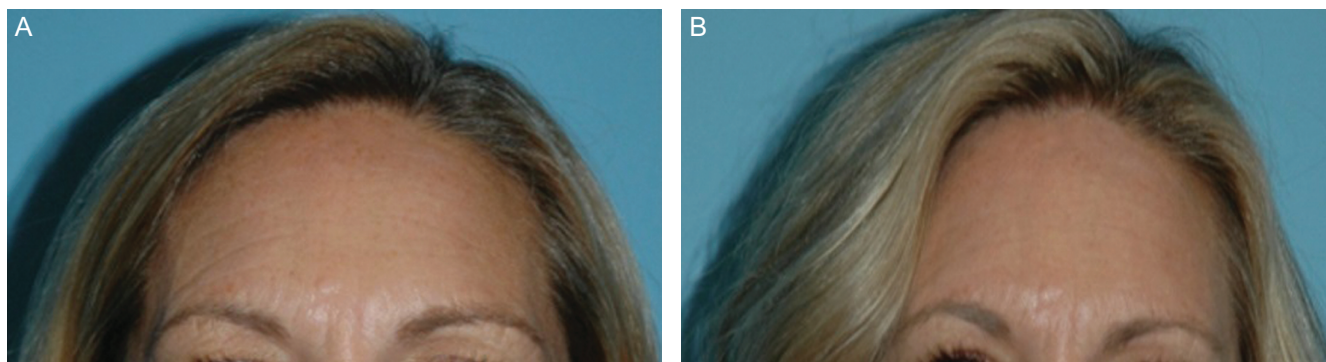


Figure 3. (A) Preoperative and (B) 6 month postoperative photographs of a 54-year-old woman post-fat grafting to the forehead. Microfat (10 mL) was used for superficial fat grafting to the forehead, whereas millifat (6 mL) was used for the temporal regions, 3 mL to each side, as well as 1.5 mL to each eyebrow. No laser was utilized to the forehead.

temporal browlift.³¹ When fat is grafted to the brow, millifat is used to provide a structural change in brow projection and is grafted retrograde in small threads or pulses, depositing the fat in the shape of a multidimensional wedge, so it blends into both the medial and superior brow. The fat graft is injected under the eyebrow and slightly biased inferiorly. Once the brow is lifted, microfat or nanofat can be used to replace subcutaneous fat loss throughout the central forehead, while millifat is placed in the temporal regions within the tissue above the temporoparietal plane (Figure 3). If an open temporal or modified endoscopic browlift is needed to reposition the lateral eyebrow, fat is injected first and then the browlift is performed. Additional microfat or nanofat grafting can be performed for refinement of the subcutaneous tissue of the upper eyelid. Nanofat can also be delivered by microneedling. The objective of fat grafting the forehead is to restore the subcutaneous and superficial fat compartments to improve angiogenesis and regenerate the overlying sun damaged epithelium and dermis. Nanofat is complimentary and can be injected directly into fine rhytids with a 27 gauge needle or delivered by mesotherapy devices, microneedling. We are working on combining a gel form of nanofat with a transport cream that permits increased delivery of nanofat to the deeper dermis. Structural losses in the prominence of the glabella and/or superior orbital rims as well as the nasal bridge can be addressed using millifat.

The brow itself is grafted using a lateral and medial approach through an 18 gauge needle incision. If more minor elevation of the lateral brow is needed, a small amount of millifat under the tail of the lateral brow will produce elevation and anterior projection of the lateral brow. The skin and subcutaneous tissue inferior to the eyebrow is addressed with microfat or nanofat placed in thin layers with threading techniques using a 19 or 21 gauge cannula. Indentations along the lateral orbital rim can be addressed with microfat or nanofat. Nanofat is used on the

periorbital rhytids and delivered with either microneedling or 27 gauge direct injections.

The temporal areas are best injected in the superficial plane above the superficial layer of the deep temporal fascia. This is the plane of the temporoparietal fascia and contains most of the veins. The objective is to reduce the appearance of these prominent veins and to restore volume to the temple. Typically, millifat is delivered in an 18 gauge cannula placed through an 18 gauge needle incision in the hair bearing sideburn or lateral temporal region. No attempt to overcorrect the volume is made.

The total amount of fat injected into the temporal region ranges from 2 mL to 6 mL per side. For the forehead, 10 to 15 mL of either microfat or nanofat is generally grafted. The eyebrows generally require 1 to 2.5 mL per side, depending on the amount used for blending the eyebrow superior and medial.

Periorbital Region

As we learn more about the aging orbit and its associated structures, our surgical approaches have become more refined (Figure 4). When performing blepharoplasty, fat injection is carried out first followed by blepharoplasty, which may become simpler once volume is restored. In patients with recession of the globe secondary to enlargement of bony orbital volume, our approach now includes intraorbital fat grafting. By placing small amounts of millifat to the inferior lateral orbit and superior orbit the senile enophthalmos can be improved and the globe will move forward and upward and take up additional soft tissues (Figures 4-5). Fat grafting to the orbit is almost always done prior to any incisions or excision of tissue. Once the soft tissue of the eyelids have been expanded by intraorbital fat, correction of the nasojugal region is carried out. In very thin skin patients, nanofat is used, but in most patients, microfat is used. Prior to doing the nasojugal fold, small microdroplets of millifat are deposited against the



Figure 4. (A, C, E) Preoperative and (B, D, F) 18 month photographs of a 60-year-old woman with facial aging. The patient underwent a facelift with high SMAS, submental platysmal plication, periorbital, temporal, deep compartment and superficial compartment fat grafting with millifat, microfat, and nanofat (total fat grafting was 22 mL). Note improvement in lower eyelid pigmentation and perioral texture. Also, note improvement in nasojugal fold and lid cheek blending. There is also evidence of reduced radial wrinkle lines and fuller lips (approximately 15% increase) at 18 months following perioral fat grafting.

orbital retaining ligament. From an inferior approach, the injector protects the lower lid with a finger while injecting microdroplets against the orbital retaining ligament using

the Celbrush. The gentle perforations are made with an 18 gauge blunt cannula. When the microfat or nanofat is placed retrograde in a herringbone fashion parallel along the periosteal surface of the nasojugal fold, the orbital retaining ligament is released with an incision. A pinch blepharoplasty may be all that is needed. The lateral canthal position is always evaluated to determine if a lateral canthopexy is necessary.

The treatment of choice for posttraumatic enophthalmos is orbital bone grafting or prosthetic implant to reconstruct the enlarged orbital volume, gently displacing the globe anterior and superior, filling out the eyelid, and correcting the pseudoptosis. With these principles in mind, we developed a technique for intraorbital fat injection during aesthetic blepharoplasty to correct pseudoenophthalmos of aging secondary to orbital expansion. By filling the intraorbital space with a fat injection just behind the equator of the globe, the globe moves anterior and superior, filling out some of the excess tissue (Figure 5). It is critical to not treat patients with exophthalmos and negative vector with placement of fat into the inferior orbit as this.

An 18 gauge needle incision just lateral to a perpendicular line dropped through the eyelid from the lateral aspect of the pupil corresponds to the lateral extent of the nasojugal fold or tear trough. We insert an 18 gauge blunt cannula with a side port injection hole and feel the superior edge of the inferior orbital rim. Once on the rim, one can feel the cannula and slowly “walk” the cannula along the bone and with one finger protecting the globe, small amounts of millifat can be placed after aspiration of the syringe. The fat is placed in the preperiosteal plane in the lateral inferior orbit and in the superior orbit. A second needle incision is made just lateral to the orbit at the lid cheek junction. Again, the 18 gauge cannula is guided first to the orbital rim and then walked inside the orbit where a small amount of fat is deposited. Similarly, 2 needle incisions are made in the upper eyelid and the preperiosteal or subperiosteal plane, in the upper lid, intraorbital fat is placed in the superior medial orbit.

This sets the stage for the next maneuver, which is microfat grafting of the skin inferior to the brow. Once this is done, the deep medial fat compartment is injected on the bony surface and with a finger along the inferior orbital rim, multiple, gentle, punctures are made into the arcuate line. Millifat is used for the deep compartment and SOOF injections. These deep fat compartment injections shift the malar eminence superiorly and help to blend the lid-cheek junction. Percutaneous release of the arcuate line is then performed and the tear trough is injected with microfat. Once the lateral canthal region and brow have been assessed and appear to be in good position with excellent lower eyelid tone, a decision is made about skin resection. Generally, the amount of skin removed is less after soft tissue volume restoration and can be accomplished with either a skin only blepharoplasty or pinch

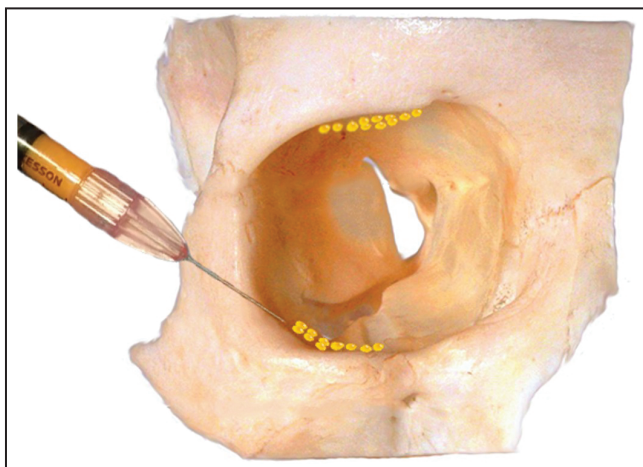


Figure 5. Intraorbital fat grafting. Larger parcels of fat (millifat, 2.4 mm parcel size) are grafted through an 18 gauge blunt cannula with side port injection to avoid intravascular injection. Both the larger cannula size, the blunt tip, the side port injection hole, and the fat graft parcel size make it virtually impossible to inject into a vessel in the orbit.

blepharoplasty. The last thing we do is inject nanofat with a 25 gauge cannula into the subdermal tissues around the eyelids. A 27 gauge needle for nanofat placement around the eyelid and orbit can be used, but we prefer the blunt 23 gauge blunt cannula. The cannula diameter should be larger than the orbital blood vessel diameter to avoid intravascular injection. This is also why we prefer an 18 gauge cannula for intraorbital fat grafting. The larger size of the cannula makes it easier to gently advance in a probing manner and is safer.

The specific steps of biofilling around the periorbital region in conjunction with upper and lower blepharoplasty are as follows:

1. Deep compartment fat grafting with millifat in 18 gauge cannula to the medial cheek fat and prezygomatic fat compartments through 18 gauge needle incision at base of nasolabial fold.
2. Deep fat grafting to the SOOF, inferior to superior through 18 gauge needle incision at base of nasolabial fold and superior to inferior placement through 18 gauge needle incision at the lateral superior malar region.
3. Millifat droplets and perforation or weakening of orbital retaining ligament.
4. Microfat to nasojugal fold from medial needle incision and 19 gauge cannula. Fat is grafted with the Celbrush permitting a microdroplet delivery which is done in a retrograde, herringbone pattern. This releases the retaining ligament.
5. Intraorbital fat grafting periosteal, behind the equator of the globe to inferior and lateral orbit and superior orbit with 18 gauge blunt side port disposable cannula and gentle probing motion.
6. Nanofat to subdermal eyelid tissue of the upper and lower lid with 21 gauge blunt cannula.
7. Skin excision, upper eyelid, rarely medial fat removal.
8. Skin excision, lower eyelid, +/- fat removal, +/- fat repositioning, +/- septal plication.
9. Canthal support suture if needed.
10. Orbitomalar suspension if needed.

Complications related to periorbital fat grafting are associated with the highest reoperation rates. In our experience with 190 periorbital fat injections, 3 patients required reoperation to remove excessive fat. Two of these patients had very thin tissues and the excess fat was not seen when they were supine. We now routinely sit patients up to see if there is any bulging in the lower lids from excessive fat. One other patient had fat injection to the SOOF which extravasated into the lower eyelids and had the appearance of lymphedema. Ultrasound demonstrated fat graft rather than edema and this patient underwent excision using a transconjunctival exposure.

Malar Midface

The malar midface (Figure 4) area is treated primarily by deep fat injection. The deep fat compartments of the midface and malar region are first accessed by 18 gauge needle incisions adjacent to the alar base attachment. Filling of these compartments, which are contained by ligamentous attachments, serves to restore tone to the ligaments and create angiogenesis in the soft tissues and bone surface. As described above, the deep medial fat compartment is injected on the bony surface with the operator's finger placed along the inferior orbital rim, protecting the septum and globe while multiple, gentle, probing, punctures are made into the orbital retaining ligament. Fat grafting of this compartment as well as the prezygomatic fat compartment and the SOOF, displaces the cheek soft tissue cephalad, which reduces the vertical height of the lower eyelid and begins to fill the tear trough. The punctate droplets placed along the inferior aspect of the ligament, weakens it and permits a 19 gauge cannula to be guided along the nasojugal fold. In a retrograde, herringbone style, the fold is injected with microfat, releasing the ligament and providing a nice transition into the cheek.

The other needle incision is made lateral to the malar prominence and the SOOF is grafted with millifat. Again, using this incision, the lateral aspect of the lower eyelid cheek junction can be filled with microfat.

When one grafts fat into the proper compartment, we can see a very rapid response in anterior projection of the soft tissue. In patients with prominent cheek bones and

substantial loss of buccal fat, the buccal fat pad can be injected directly with millifat using an intraoral approach. Stensen's duct is identified and avoided and the upper buccal sulcus is prepped with a betadine swab. An 18 gauge needle is used to make a puncture above the second molar in the unattached gingiva. An 18 gauge cannula is gently inserted into the buccal space with a probing motion and small amounts of fat graft (average, 2.5 mL) are placed.

Superficial fat injection into the cheek is generally avoided except in cases where there are acne scars or substantial sun damage. When the epithelial and dermal tissues are thinned and damaged, microfat or nanofat is used for treatment. A thin layer of fat is placed, not so much for augmentation, but as if the underlying soft tissue "carpeting" has been worn out and is now being replaced by the microfat graft. New subcutaneous tissue "carpeting" with fat is created by grafting a thin layer of carefully threaded microfat or nanofat. The microfat and nanofat can be enriched with SVF. Laser resurfacing can also be performed to treat the photo-damaged skin, recruit resident stem cells, and also works synergistically with the SVF cells to regenerate skin. Combining microfat and nanofat with a 1927-nm fractional laser may be used synergistically to treat photo-damage and superficial fat loss (Figure 6). The laser targets the epithelium and superficial dermal layers, while the nanofat addresses the deep dermis and epithelium and the microfat addresses the subcutaneous, superficial fat loss. The nanofat is used for the lower eyelids and the fine rhytids of the lips, while microfat grafts are used for superficial fat restoration in the skin of the lower lip and chin.

No patient suffered a complication from midfacial fat grafting. There were no instances of asymmetric absorption or visible fat grafts because of superficial fat grafting instead of deep compartment fat grafting for volume augmentation.

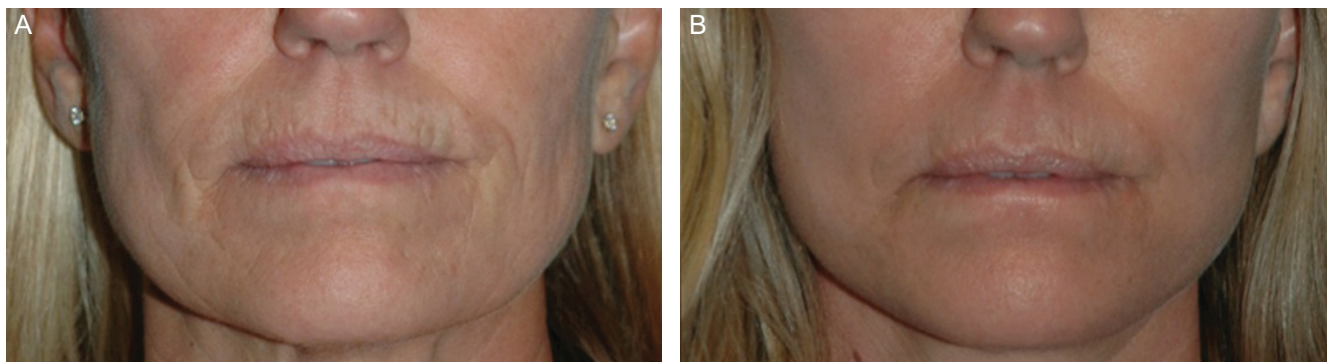


Figure 6. (A) Preoperative and (B) 6 month postoperative photographs of a 54-year-old woman postperioral fat injections. Approximately 2 mL of millifat was used in the upper lip and 2 mL of millifat in the lower lip. The chin and perioral skin was injected with microfat (4 mL) and the fine rhytids with intradermal nanofat (1 mL total) through a 27 gauge needle. A perioral fractional CO₂ laser was performed at the time of surgery as was mesotherapy, which delivered the nanofat into the dermis and epithelium.

Pyriform Aperture and Perinasal Regions

Resorption of the underlying bone of the pyriform aperture, prior upper teeth removal for orthodontic retraction, and genetically inherited perinasal hypoplasia may have pronounced effects on lip and nose posture. Treatment of the pyriform aperture is difficult to perform with implants because of their high infection rates. In addition, their propensity for bone erosion close to the apices of the teeth roots makes it difficult to augment the nostril sill and superior cutaneous upper lip. We prefer to use millifat for structural augmentation and are presently working on combinations of SVF enriched fat and calcium hydroxyapatite for long-term augmentation and modest regeneration of the bony surface. An 18 gauge cannula is used for fat grafting, which is performed through the same 18 gauge needle incision in the upper nasolabial fold through which the deep fat compartments are injected. A very effective change in the nasolabial fold depth is also noted with this injection and has caused us to change our approach with synthetic filler injection in this area.

Fat grafting of the pyriform region has been a very satisfying procedure with excellent correction of the nasolabial folds. Long-term outcomes and retention rates are presently not known. Pyriform aperture augmentation provides support to the upper lip, producing some degree of vertical shortening of the upper lip. In addition, the base of the nose is projected forward and upward, improving the appearance of nasal aging.

Lower Facial 1/3

The lower facial 1/3 represents a great treatment option for millifat grafting and potentially represents the best area for a composite biofiller material, decellurized fat in combination with SVF, and/or micronized demineralized and decellurized allogeneic bone. The use of fat and these

other composite or allogeneic grafts around the chin can be used to elongate the vertical height of the chin as well as blend the chin into the mandible, reduce the depth of the labiomental fold, augment the chin, and replace the use of mandibular implants for both chin projection and mandibular angle augmentation.

Millifat when indicated for buccal fat pad atrophy also fills out the marionette lines. Superficial fat grafting with microfat or nanofat through an 18 gauge needle incision lateral and superior to the marionette line is made and microfat or nanofat is injected as a single layer threading technique throughout the thinned areas of the perioral tissues.

In addition, the skin of the chin may be treated with mesotherapy using nanofat in an attempt to reduce pore size and resurface the photo-damaged skin.

In a recent abstract presented at the American Society of Aesthetic Plastic Surgery's annual meeting in 2017, progressive improvement in facial volume as estimated in the buccal region by 3-dimensional photometry was seen up to 18 to 24 months following deep compartment fat grafting and facelift.³²

Perioral and Lips

The perioral region is very amendable for improvement with millifat, microfat, and nanofat. The addition of SVF and PRP may also be beneficial. In addition, pyriform augmentation with millifat will have some effect on lifting the upper lip. Structural enlargement of the lip is performed using millifat placed through 18 gauge cannula through needle incisions in the lateral commissure on either side. The millifat is deposited retrograde along the vermilion cutaneous border with an 18 or 19 gauge disposable cannula. In addition to the vermilion cutaneous border of the upper lip, small droplets are placed at the inferior aspects of the philtral ridges. Also, small amounts can be placed in the tubercle and the 2 lateral pillows of the upper lip. Structural millifat is also placed along the vermilion cutaneous border of the lower lip and then deposited into the 2 pillows of the lower lip. Up to 2 mL per lip of millifat is used. Once millifat is grafted, the lip may be grafted with microfat or nanofat. In a very thin skin patient, nanofat is used as a single layer sheet graft in the subdermal tissue through a 21 gauge cannula and is inserted intradermal into the radial wrinkle lines of the lip. The effect of nanofat with fractional lasers and superficial sheet grafting with SVF microfat appears to be much longer term, even lasting for a couple of years and longer.

Once the lips are augmented, microfat is used to fill in around the marionette lines, which really are basins of lost subcutaneous fat. The upper lip skin thins out and often has many rhytids. We use SVF enriched microfat in these areas as a sheet graft that is placed in crisscrossing threads. Lastly, we use nanofat injected with a 27 gauge needle or

even smaller direct into the dermis of the rhytid as per Verpaele and Tonnards' SNIFF technique (Figure 6).³³

In the senior author's experience, when 4 mL of millifat is grafted in the upper lip (2 mL) and the lower lip (2 mL) about 10% to 15% of the lip augmentation by visual assessment appears to survive up to 1 year. There is presently no evidence in the literature to support this observation and we are working on evaluating intermediate-term outcomes of lip augmentation with fat.

Neck

The fine lines of the neck are amendable to nanofat injected into the dermis with a small 27 gauge or smaller needle. Scarring from the needle can occur, so one needs to be careful in patients who have a tendency toward hypertrophic scarring. We have had limited experience with this approach.

FACELIFT SURGERY AND FAT GRAFTING

Aesthetic surgery of the face requires a dynamic and highly personalized approach. It is ridiculous to think one size or approach fits all patients. Fat grafting has obviated the need for deep plane approaches in many circumstances as laxity in these tissues is generally corrected by deep compartment fat injection.

Large volume fat grafting has added the critical step of replacing lost soft tissue and bony volume to improve skin and soft tissue laxity. Observations by Lambros point to soft tissue volume loss as a major factor in soft tissue laxity.¹⁵ Guerrero Santos was one of the first to begin large volume fat injection during facelift surgery.¹¹ Cohen, Marten, Rohrich, and others more recently have popularized these new approaches.^{12,14,34} The important anatomic work of Pessa and Rohrich¹⁴ on the deep and superficial fat compartments of the face and the contributions of Kahn and others into the aging craniofacial skeleton, crystallized some of the technical aspects of these newer approaches to deep compartment regeneration. Cohen, Tonnard, and Verpaele began to present work on using SVF for skin dermal and epithelial regeneration.^{9,13} Tonnard and Verpaele presented their work with nanofat around the lips and eyes, while our group showed similar changes using enzymatically obtained SVF-enriched fat.^{13,33}

Some type of facelift is still the gold standard approach for significant skin laxity. Most patients undergoing more limited minifacelifts, as well as those needing more extensive skin removal and underlying submuscular aponeurotic system (SMAS) manipulation, benefit from fat grafting at the time of their procedures. Any of the fat grafting approaches we detailed in earlier parts of this manuscript are routinely performed during the facelift procedure,

depending on patient findings. The average amount of fat in all three forms (milli, micro, and nano) injected during aesthetic facelift is 25 mL (range, 13-70 mL).

In general, fat is harvested at the end of the facelift procedure because it is easier to determine where fat is needed after the skin has been lifted, with the exception of the eyelids. In the treatment of eyelid aging, fat grafting is performed first and then the eyelid surgery is tailored to the effects of the fat on the soft tissue excess. In the face, the fat grafting is done at the end of the procedure and before the eyelid surgery is carried out.

First, millifat is injected into the deep compartments of the cheek. If needed, fat can be used for chin augmentation, gonial angle and mandibular contour alterations, and buccal fat pad atrophy. In addition, the pyriform aperture and perinasal area are injected with millifat. It is rarely necessary to need fat in the nasolabial fold after the perinasal and pyriform deep compartments are filled. Next the temporal regions are addressed with millifat. Millifat is placed into the lateral brow tapering toward the medial eyebrow and glabella as well as blending into the forehead superiorly. Then, the forehead is injected with microfat or in thin skin patients, with nanofat. The nasal bridge is augmented with millifat or microfat. In patients who had prior rhinoplasty, if the skin has thinned, microfat or nanofat can be injected as a subcutaneous replacement, covering up small defects. If a browlift is planned, it is done along with the eyelids after periorbital, forehead, temporal, and brow fat grafting are completed.

Once the facelift is finished and fat has been injected as described above, the eyelids are addressed. Having already injected the deep medial compartments and SOOF, which shifts the fat superiorly toward the inferior orbital rim, the intraorbital fat injection is performed if indicated. This is an advanced procedure and should only be performed after seeing an expert do this operation or if one has a background in oculoplastic or craniofacial surgery. Using an 18 gauge, side port, disposable cannula (Tulip, Inc.), a needle incision is made at the lateral aspect of the tear trough, lateral to a perpendicular line dropped from the lateral iris. The cannula is inserted and the bone of the inferior orbital rim is felt by the cannula. Carefully, the cannula is "walked" over the inferior rim into the orbit, feeling itself along the bone. Once in the orbit, a finger or soft malleable is interposed to retract and protect the globe. The syringe is aspirated and small amounts of millifat are placed along the lateral inferior floor. A second incision is made at the lateral extent of the lower eyelid and cheek junction and the same cannula is guided along the rim and into the orbit for additional fat injection if needed or if this is the preferred approach. Once this is done, a small amount of fat is placed along the inferior orbital rim using microfat. The cannula is gently passed under the previously perforated orbital retaining ligament, permitting percutaneous release and filling.

Once the lower lid is completed, the upper lid is addressed by microfat injected into the skin below the eyebrow, while millifat in very small amounts is placed into the medial two-thirds of the superior orbit below the rim, gently "walking" the cannula along the preperiosteal, bony surface.

After the deeper fat injections have been done, nanofat is used for the skin and injected carefully with a smaller 25 gauge cannula into the subcutaneous space. Following fat injections, skin excision and canthal and cheek repositioning are performed as needed.

Outcome studies looking at patient satisfaction 18 to 24 months following facelift combined with fat grafting demonstrated high satisfaction rates that appeared to gradually improve over the initial 8 to 24 month period.

Progressive improvement in facial volume as estimated by 3D photometry demonstrated progressive improvements in facial volume at 18 to 24 months after facelift combined with fat grafting.³²

DISCUSSION

Limited data are available on the efficacy of using fat and/or its components for regeneration of skin, soft tissue, and bone in terms of clinical outcomes. Carefully performed histologic studies in humans undergoing facelift surgery have shown histologic evidence of reversal of some of the aging changes seen in elastin and collagen fibers as well as neoangiogenesis.⁷ Rigotti's group showed that mechanically dissociated SVF enriched fat demonstrated histologic evidence of regeneration in both elastin and collagen fiber architecture as well as angiogenesis. The angiogenesis was more pronounced with the addition of PRP, but the improvements in elastin and collagen were not seen to the same degree and these authors did not advocate the addition of PRP except in cases where there was more of a need for angiogenesis and perhaps less than a need for regeneration.⁷

Our group has presented data from an institutional review board clinical prospective study of the use of SVF enriched fat compared to traditional fat during aesthetic facelift surgery.²⁴ At 18 months, patients cannot tell a difference if they had SVF enriched fat or conventional fat grafting during their facelift. Both groups were very happy and in fact, satisfaction improved in both groups at 18 months relative to earlier reporting times. Patients, however, could not discern the reported histologic differences in outcomes, finding high satisfaction with both types of fat grafting in conjunction with facelift surgery. Efficacy of adipose tissue supplemented with stem and regenerative cells obtained from the SVF of fat has been demonstrated by Kolle,³⁵ Mailey,²¹ and Tanikawa.³⁶

Fat grafts have living cells and extracellular matrix that stimulate angiogenesis and reverse, to some extent,

histologic signs of aging in elastin and collagen fibers. Fat is really the prototype biofiller, demonstrating prolonged volume effects and subtle, but important regenerative effects. With off-the-shelf kits (Tulip Medical, Inc., Thibaud, Inc., France), it is simple to harvest fat through a needle incision with a small cannula. By performing a “fat draw” instead of liposuction, the fat can be removed, modified, and used for full facial and hand soft tissue rejuvenation in conjunction with lasers and/or surgery or simply as an alternative to fillers.

Presently, we recommend full facial fat grafting in patients presenting with the need for more than 2 to 3 different fillers or in patients requiring larger than 5 mL of total filler. When performed at younger ages as needed, fat and its tissue modifications can be used to replace the injured and lost tissue and regenerate elastin and collagen as well as blood supply. Instead of waiting for aging to progress to a point that surgery and/or more aggressive procedures are needed, this new paradigm involves gradually and serial replacement of the tissues as they age and become worn out.

The modification of fat into different tissue products with more even fat parcel sizes of different sizes makes sense from a surgical point of view. Much like fillers are used for different indications and in different sites, it seems that common sense would support the use of larger parcel sized fat (structural) in deep fat compartments and smaller sized fat parcels closer to the surface in the superficial fat compartments and intradermally. Whether regenerative effects are seen with nanofat on the epithelium occur and to what extent is unknown. Numerous clinicians and researchers have demonstrated that fat and its SVF cells exert beneficial effects in terms of increasing tissue thickness, improving the appearance of scars and favorably altering the skin's texture in aging skin, burned skin, and radiated skin. The benefit in this case is really more in the surgical approach. It seems to us that it would make more sense using smaller sized fat parcels that would present less of a tendency to clump in areas such as the eyelids and superficial tissues. By the same token, it seems sensible to use larger parcels in the deep compartments. These would be less dispersive and more structural. Both the Peer theory of fat cell survival³⁷ and Yoshimura's theory of fat cell replacement,³⁸ point toward using smaller diameter fat parcels for better revascularization.

Nanofat is a bit different, but again, can stand alone as a fat graft for superficial intradermal injections and other applications. One probably can assume there is a bit more of a regenerative effect in terms of angiogenesis and cell signaling because of a higher number of stromal vascular cells and matrix, respectively. One would probably expect smaller regenerative effects of nanofat than seen with enzymatic and mechanical SVF, both of which have shown

regeneration in elastin and collagen fibers and evidence of angiogenesis.

The effectiveness of millifat, microfat, and nanofat were neither evaluated nor quantified and this is a limitation of the study. Further evaluation of these approaches are needed. The absence of a control group and image methods to measure the absorption and retaining index of fat grafts are also weaknesses of the present study. These limitations are difficult for any study in that even magnetic resonance imaging evidence of fat survival is not accurate as dead and live fat may be difficult to assess.

Another limitation of this study was the limited laboratory analysis of these 3 different fat products. Cell counts and profiles of SVF will vary even from patient to patient and therefore, the only way to compare and contrast different products are to compare them in the same patient. In a limited sample size of 3 patients and summarized in [Table 1](#), are the total cell counts and cell viability in enzymatically digested fat (Celution, Cytori, not FDA approved), mechanical dissociated fat (Aquicell, Millenium Medical Technologies, Carlsbad, CA) and nanofat as produced by 400 and 600 μm strainers (Tulip nanofat). In this small sample, we found about 1 million SVF cells/mL for enzymatic SVF, 350,000/mL for mechanical SVF and 30,000/mL for nanofat. These are difficult numbers to come by, because the patient has to serve as their own control in comparing differences in preparations of the same tissue, but with different preparation techniques.

One of the concepts we discussed was the use of composite fat grafts. This is purely a theoretical approach combining available synthetic fillers that have some biologic effects with different types of fat grafts with or without SVF supplementation. It makes sense that polylactic acid fillers, which stimulate dermal thickness, may work in concert with nanofat, which supplies matrix growth factors along with SVF cells. Presently, we use this specific product in patients with severe perioral rhytids or extensive sun damage.

The other composite fat graft we have experience with is calcium hydroxyapatite. Theoretically, the calcium hydroxyapatite serves as a scaffold and is combined with one of the tissue products from fat, which supplies the cells. Whether or not this will stimulate small amounts of cortical bone formation either in response to its periosteal placement or because somehow the calcium hydroxyapatite combined with SVF cells draw osteoblasts and subsequently become calcified bone, we are not sure. We are doing early pilot studies and have had zero complications, but do not have any cephalometric or computerized tomographic proof of either prolonged skin changes or regeneration of small amounts of cortical bone that are pronounce because of their broad surface area. Researchers such as Yoshimura have shown that restylane against the periosteum stimulates small amounts of bone formation. Since erosion of the outer table of the skull and facial skeleton

occur with aging in a very gradual process, stimulation of even small amounts of cortical bone may be sufficient to keep up with gradual loss. At present the effects of composite fat grafts beyond their safety has not been established.

Another limitation of this study is that we are not able to provide data on intraorbital fat injection. In our limited experience in 6 patients, no complications occurred from intraorbital fat grafting. One patient was treated for posttraumatic enophthalmos and the others for aesthetic indications because of senile enophthalmos. This should be considered an advanced procedure unless one is comfortable with an intraorbital approach. Oculoplastic surgeons, craniofacial surgeons, and others with this type of background will be more comfortable with this approach. This is an idea in evolution and presented to stimulate others that might have other ideas on how to best address the orbital volume enlargement that occurs with aging of the craniofacial skeleton.

Another limitation of this report is the fact that it is an overview of a new approach and therefore should be considered as an "idea" that at a minimum makes sense in terms of fat modifications to improve safety. Unfortunately, at this time, the regenerative strategies of fat grafting can be mentioned, but other than numerous anecdotal reports and Rigotti's⁷ article demonstrating regenerative architectural changes in elastin and collagen fibers in aging skin in patients undergoing facelift surgery, there is still a paucity of convincing evidence. Mojallal's⁶ animal studies showed regeneration. In humans, regeneration of blood supply was shown by Magalon in scleroderma patients treated for their Reynaud's.¹⁸ The field is requires a bit of a paradigm shift -- small regenerative effects will be provided by a variety of biologics. These biologics will induce specific microscopic and cellular changes in aging tissues to keep up with the wear and tear. More studies are needed.

CONCLUSIONS

Regenerative cells in facial surgery are here to stay. Fat grafting is now being used for both filling and regeneration. Fat grafts are modified into different shapes for different anatomic indications. Millifat has larger parcel sizes (2.4 mm and <) and is used when structural fat grafts are performed for deep fat compartment filling, filling of the lips, temporal regions, brows, chin, nose, and pyriform. Microfat is used for superficial fat grafting, for instance, in the forehead, the hands, the perioral tissues (upper and lower lip and chin), and at times, the nose and deep in the tear trough. Lastly, nanofat is used for patients with thinner tissues in substitution for microfat; and is used more specifically for intradermal injection in the radial wrinkle lines of the lips, in acne scars, in the upper and lower eyelid in the subcutaneous fat superficial to the muscle, but

not below the muscles. Nanofat is also used as a mesotherapeutic agent delivered by microneedling into the dermis throughout the face, and also alone or in combination with PRP in the scalp at the level of the hair bulge.

Nanofat is used for scars, burns, and radiation injuries to the face and may be tried for neuropathies if the etiology is thought to be ischemia or inflammation. Stromal vascular fraction cells may be added to any of these types of fat grafts for enrichment. Presently, mechanical means of dissociating SVF from fat are utilized. The addition of these cells appears to regenerate elastin and collagen and stimulate angiogenesis, improving the thickness and texture of the skin. At the present time, no device is approved by the FDA for enzymatic digestion of fat to obtain SVF.

In addition to modifying the characteristics of fat to address anatomic needs and provide modest regenerative effects, it is critical to place the fat in the correct areas. It is dangerous to place millifat in superficial fat compartments as it may show as a loculated mass beneath the skin. It is also less effective to use nanofat for deep compartment fat replacement or bone regeneration as it is basically a liquid consistency. Fat should not be overcorrected to account for loss of fluid in the fat graft. During surgery or grafting, as some of the fluid disperses, one can add a bit more fat to improve aesthetics, but not to overcorrect. At this time, we prefer disposable injection cannulas and use a Cellbrush (Cytori, Inc.) for controlled microinjection.

Patient expectations need to be carefully managed. Fat being a natural material, just like our patient, is not going to last forever. That said, "permanent" survival of up to 75% of the fat injected is possible to see and gradual improvement of facial volume after fat grafting and facelift surgery may be a reflection of cell replacement over time as well as secondary to the large number of growth factors contained in the grafted in fat. Ongoing improvement for 6 months and even longer has been seen by the authors using the techniques presented above.

Facial aging is complex and the best outcomes are achieved when photo-damage, volume loss, and soft tissue laxity are treated simultaneously. Treatment targeted to each level of the facial soft tissue from skin to bone will become more sophisticated. Better biofillers that not only augment, but actually regenerate tissue will be coming in the future. Eventually, biocontouring through simple injections will permit the surgeon to perform in situ tissue engineering to construct chins and improve bony contours without the need for complex surgery.

The paradigm of surgical treatment of facial aging is changing from one of larger surgical procedures once facial aging is established to ongoing upkeep of the major anatomic, histologic, and cellular alterations that are gradually progressing. By improving volume at earlier ages with biomaterials such as our own fat, we are also stimulating angiogenesis, which in addition to sustaining the volume

augmentation, it is also regenerating tissues and perhaps, even preventing or decelerating aging at the cellular level. The next five to ten years will see a revolution in antiaging treatments. It is easy to see a time when the aging process is halted, not only by these newer strategies of upkeep and periodic replacement of degenerating tissues, but actually prevented by medication(s) that slow down or stop cellular aging.

Supplementary Material

This article contains supplementary material located online at www.aestheticsurgeryjournal.com.

Disclosures

Dr Cohen is a consultant, investigator, and minor shareholder of Cytori, Inc. (San Diego, CA); is a consultant for Tulip, Inc. (San Diego, CA), Millennium Medical Technologies, Inc. (Carlsbad, CA), Galderma (Lausanne, Switzerland), Allergan (Parsippany, NJ), and Merz (Raleigh, NC); and performs research with Thermigen (Southlake, TX) and Biologica (Carlsbad, CA). The other authors declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

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