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Preliminary Report

Strategies for Stromal Cell, Platelet Dosing, and Micronized Fat Grafting After Facelifting: A Preliminary Retrospective 3D Vectra IRB Study

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Abstract

Background: Although new methodologies and techniques reported prolonged fat graft retention in facelift procedures with the utilization of stromal cells and platelets, no validated protocols have been accepted.

Objectives: The author carried out a quantitative volumetric and numerical approach to correlate stromal cell and platelet dosing and their ratios on similar volumes of micro-fat in the anterior midface.

Methods: Similar ratioed volumes of bladed micronized fat, dosed stromal cells, and platelets were delivered to 5 fat compartments in the anterior midface and in the palpebral-malar groove after lateral SMASectomy facelifts with or without neck lifts in 8 female patients and volumizing only in 1 male patient, who were followed by 3-dimensional (3D) imaging analysis for 12 months.

Results: At the final 12-month evaluation period, the average midface volume by 3D Vectra analysis was 61.6% of the initial micro-fat injected in the facelift patients and 64.5% in the volumized male patient. The observed average volumes reached their nadir of 31.9% (8 facelift patients) and 40.4% (male patient) at 5 to 7 months. The Investigator Global Assessment Improvement Scale 0 to 4 ratings at Month 12 observed scores of 4 (4 patients), 3.7 (1 patient), 3.3 (3 patients), and 3.0 (1 patient). There were no reported complications or prolonged side effects.

Conclusions: In this preliminary study, a combination of dosed stromal cell and platelet volumes and bladed micronized fat volumes resulted in 3D Vectra volumetric changes of progressive improvement from baseline values. Additional protocols with controls are needed to validate these findings.

Level of Evidence: 4 (Therapeutic)

Since the 1890s, plastic surgery adopted the utilization of autologous fat to restore, replace, or recreate functions and enhance the cosmetic appearance of soft-tissue defects from aging, disease, or damage.[1] Regardless of the implementation of evidence-based protocols, the utilization of autologous fat grafting has been disappointing because of unpredictable and variable outcomes with 20% to 80% resorptive volume losses.[2] Since the turn of the 21st century, however, a fundamental shift in fat grafting occurred, attributed largely to the isolation of adipose-derived stem cells and other regenerative cells by Zuk et al.[3]

Because current regulations prohibited the utilization of enzymatic processes to obtain adipose stromal vascular fraction (SVF), mechanical procedures became an alternative pathway to isolate total stromal cells in a safe, efficient, and approved manner. Copcu and Oztan recently introduced their novel method of cutting adipose tissue to release undamaged stromal and parenchymal cells from intercellular bonds.[4] These investigators defined this process as "adinization" or bladed micronized fat that utilizes sharp cutting bladed disks that downsize fat parcels to micro-fat and finally to total stromal cells (TOST). Because the utilization of either adipose stem cells, platelet factors (platelet-rich plasma, PRP), or a combination with adipose tissue has achieved mixed outcomes in fat grafting compared with those observed after traditional fat grafting, the utilization of proper dosing and bioformulations with these regenerative biologics

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may lead to improved understanding of cellular dynamics to obtain consistent and significant outcomes.[5-7]

Therefore, the objectives of this investigation were (1) to compare 3-dimensional (3D) imaging of progressive volumetric changes and retention of volumes in the anterior midface for a year when identical volumes of bladed micronized fat were mixed with identical volumes of stromal cells (TOST) and platelets (PRP) in a 5:1 v/v ratio and (2) to determine optimal cell numbers of TOST and PRP for safe and effective outcomes.

METHODS

Study Overview and Informed Consent

The single-center, prospective study was undertaken at the Huntington Specialty Center (Pasadena, CA) from November 2022 to May 2024 and complied with the Declaration of Helsinki, Subject's Bill of Rights, and local Ethics Committee at the Surgical Center. The study protocol and consent form were reviewed and retrospectively approved by the IRB of the Institute of Regenerative and Cellular Medicine (protocol number: GS-FFG-801; approval number: IRCM-2024-409, August 14, 2024). Each facelift patient was informed that the muscle adjustment procedure would be a lateral SMASectomy to reduce its effects on the added fat medially in the anterior midface. In addition, each patient was informed that the same total volume of micro-fat per malar fat pad and palpebral-malar groove compartment would be distributed according to site-specific needs to individual superficial and deep compartments to achieve clinical results. Patients were also informed that additional indicated areas of micronized fat grafting with stromal cells and platelets would be performed in other facial sites in a similar manner to that delivered in their anterior midface but would not be subjected to 3D analysis.

Selection Criteria

Primary inclusion criteria included candidates with ptosis and deflation of the malar fat pads and palpebral-malar grooves who never had facial or lower eyelid surgeries. Candidates were included 2 years after receiving temporary fillers and noninvasive/minimally invasive procedures to the anterior midface. Primary exclusion criteria included candidates with active systemic disease or local infections, pregnancy, breastfeeding, severe autoimmune diseases, bleeding disorders, and bony or alloplastic facial implants.

Preparation

Before surgery, medical histories and surgical clearances, blood panels, and electrocardiograms were evaluated in all patients. Patients at risk of herpetic infections received antiviral medication. Women of childbearing potential had a urine pregnancy test. BMI and baseline high-resolution digital facial photography in 3 standardized views were obtained for a year. To the greatest extent possible, imaging was performed in the same environment under standardized ambient lighting conditions by the same person.

Vectra H2 3D Volumetric Analysis Imaging

Each patient cleansed her/his face, removed jewelry, wore a hair band to expose the full facial features, and was directed to keep a neutral facial expression in a seated position throughout the capture process. Vectra imaging was confined to the anterior midface, composed of the malar fat pad and the adjacent palpebral-malar groove. The sensitivity of human error of the analysis was 0.2187 mL. Vectra analysis imaging (Canfield Scientific Inc., Parsippany, NJ) of each anterior midface was measured at baseline, 1 to 3, 5 to 7, and 10 to 12 months.

Preparation of Platelet-Rich Plasma and Platelet Dosage

The FDA-cleared automated device and collection containers (Pure PRP II 60 mL; Emcyte Corporation, Fort Meyers, FL) isolated a buffy coat containing high concentrations of platelets. A total of 54 mL of whole blood was withdrawn from an antecubital arm vein and mixing with 6 mL of citrate anticoagulant. After a double-spin centrifugation process, ~8.0 to 10.0 mL platelet-poor plasma was utilized to resuspend the buffy-coat pellet to a final volume of PRP. Aliquots of whole blood and PRP were counted for platelets using an automated device (Horiba ABX Micros 60 Analyzer, Irvine, CA).

Surgical Markings

Skin markings in the sitting position were traced transversely below the sideburn, along the preauricular crease, retro-tragal border, around the earlobe crease line, and in the postauricular sulcus for variable distances and to other surgical sites. The abdominal donor-site markings for fat harvesting were also drawn in the standing and sitting positions. As depicted in Figure 1, 3 deep and 2 superficial compartments in the malar fat pad, as well as the deep transverse palpebral-malar compartment, were outlined. Depending on topographic assessment and need, additional enhanced micro-fat grafting sites were outlined to the brows, temporal fossae, pyriform apertures, nasal tip, and mandibular borders.

Fat Harvesting

Lipo-harvesting from the anterior abdomen or back rolls utilized a wet tumescent technique with a solution containing 50 mL 0.5% lidocaine, 1 mg epinephrine, and 20 mL 8.4% sodium bicarbonate per liter of warm saline. Eight 20 mL syringes were typically filled with the utilization of an attached 7.5 cm long cannula with a blunt tip and 4 eccentric 1.8 mm holes. The lipoaspirate was transferred into 20 mL piston-lock syringes and centrifuged to obtain condensed fat.

Sizing Pathway for Micro-Fat

As depicted in Figure 2, usually 140 mL of condensed fat was obtained after centrifugation by discarding tumescent fluid, fat, and blood elements. Thereafter, 140 mL of condensed fat was downsized to micro-fat particles by passing condensed fat back and forth through ultra-sharp cutting blades in a 2400 µm disk (milli-fat) and then through a 1200 µm bladed disk to obtain about 120 mL of micro-fat. A 60 mL portion of micro-fat was set aside for eventual grafting.

Bladed Micronized Fat Pathway for Total Stromal Cell

The regenerative stromal cell product was processed through a micro-cutting mechanical device (Adinizer, Ultrasharp Blade System, South

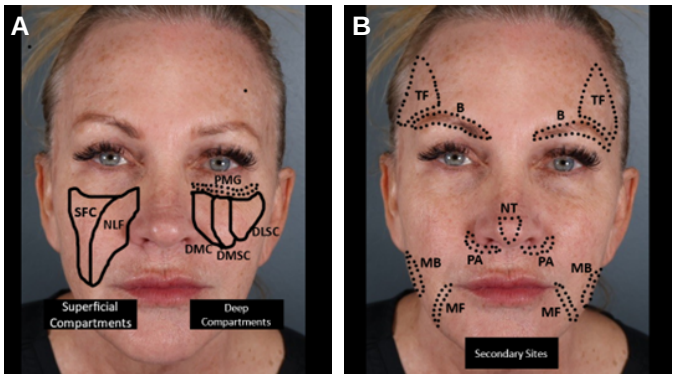

Figure 1. Treatment map for volume-depleted primary (3D Vectra) and secondary (non-3D Vectra) facial sites. (A) PMC, palpebral-malar compartment; SFC, superficial fat compartment; NLC, nasolabial compartment; DMC, deep medial compartment; DMSC, deep medial suborbicularis compartment; DLSC, deep lateral suborbicularis compartment. (B) TF, temporal fossa; B, brow; NT, nasal tip; PA, pyriform aperture; MF, marionette fold; MB, mandibular border.

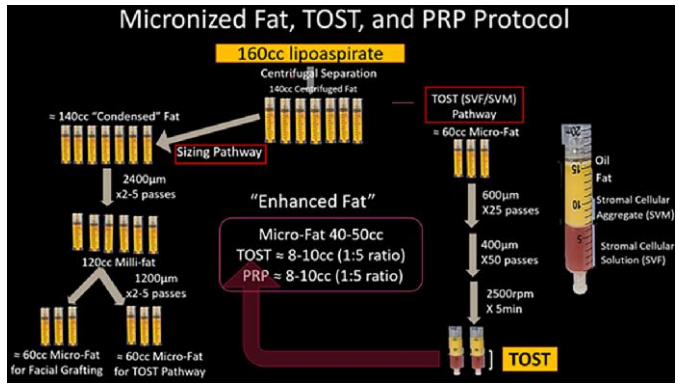

Figure 2. Sizing pathways for bladed micro-fat and total stromal cell (TOST) for enhanced fat. Left pathway from downsizing of centrifuged fat with 2400 to 1200 µm bladed disks for obtainment of micro-fat. Right pathway from downsizing of centrifuged micro-fat with 600-400 µm bladed disks for acquisition of total stromal cells (TOST) by combining stromal-cell solution (SCS) and stromal-cell aggregate (SCA). Enhanced micro-fat from the combination of micro-fat:TOST:PRP in a 5:1 ratio.

Table 1. Demographic Listing of Patients, Age, Sex, Ethnicity, BMI, and Surgical Procedures

Patient	Age/sex	Ethnicity	BMI (kg/m^2) (baseline)	Procedures
A	57 years/F	Caucasian	26.6	Face–neck lift/lower lid bleph./fat grafting/TOST/PRP
B	60 years/F	Caucasian	20.3	Face lift/lower lid Bleph./fat graft/TOST/PRP
C	63 years/F	Caucasian	21.8	Face–neck lift/fat grafting/TOST/PRP
D	66 years/F	Caucasian	27.6	Face lift/lower lid bleph./fat grafting/TOST/PRP
E	70 years/F	Caucasian	21.7	Face–neck lift/fat grafting/TOST/PRP
F	74 years/F	Caucasian	20.2	Face–neck lift/quad Bleph./fat grafting/TOST/PRP
G	76 years/F	Caucasian	21.6	Temporal/face–neck lift/fat grafting/TOST/PRP/buccal fat pad excisions
H	83 years/F	Caucasian	21.7	Face–neck lift/lower lid Bleph./Fat grafting/TOST/PRP
Average	68.6 years		22.6	
I	31 years/M	Caucasian	21.9	Fat grafting/TOST/PRP

Korea) that has a patented CE marking and ISO 13485–certified blade system that adhered to FDA policies of minimal manipulation.[8] No enzymes or additives were utilized, and the structure of the adipose tissue was unchanged. As shown in Figure 2, the remaining 60 mL of sized micro-fat parcels was passed in descending order through a bladed disk with a 600 µm diameter and then through a bladed disk with a 400 µm diameter to ensure proper sizing under minimal pressure. The final centrifuged product generated 2 visibly distinct layers. At the bottom of the syringe was the 6 to 8 mL stromal-cell solution (SCS), and above it resided the 2 to 4 mL stromal-cell aggregate (SCA) layer. The combined volumes of SCS and SCA represent the TOST and usually amounted to between 10.0 mL, contingent on patient variables and processing conditions.

Total Stromal-Cell Counting and Viability Analysis

The number and viability of nucleated cells in 3 separate aliquots of the stromal cell TOST solution were evaluated from the final products containing the combined SCS and SCA cells by utilizing the Luna-STEM Automated Fluorescence Cell Counter (Logos Biosystems, Seoul, South Korea).

Graft Injection Technique

After completion of the lateral SMAS-face or SMAS-face-neck lift procedures, secondary procedures such as temporal brow lifting, blepharoplasty, and buccal fat pad excisions were performed. The prepared micro-fat, PRP, and stromal-cell preparations were available in a cool water bath container for use within 3 to 4 h of processing.

As described in the author's previous publication, each malar fat pad received as close as possible a total volume of 10.0 mL micro-fat that was distributed into 2.0 mL aliquots into each of the 5 midface compartments through 1.3 mm single hole × 5 cm long blunt cannula entering a skin puncture in the mid-nasolabial crease line.[9]

Lastly, the same cannula was inserted through the puncture site at the lateral cheek, depositing 2.0 mL of micro-fat at the subperiosteal level across the transverse palpebral-malar groove inferior to the

Table 2. Injected Volumes of Micro-Fat With % Cell Viabilities

	Patient A 57 years (F)	Patient B 60 years (F)	Patient C 63 years (F)	Patient D 66 years (F)	Patient E 70 years (F)	Patient F 74 years (F)	Patient G 76 years (F)	Patient H 83 years (F)	Patient I[a] 31 years (M)
Temporal fossae, mL		8.0	6.0	6.0	4.0	4.0	8.0	6.0	
Brows, mL	4.0	4.0	2.0	6.0	4.0	4.0	4.0	6.0	
P-M grooves, mL	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Malar fat pads, mL	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	48.0
Pyriform apertures, mL	4.0	4.0	4.0	4.0	2.0	4.0	2.0	4.0	4.0
Nasal tip, mL						0.5			
Marionette folds, mL	4.0	4.0			2.0	2.0			
Mandible borders, mL	4.0		4.0		4.0	2.0	2.0		4.0
Total vol./both sides of face, mL	40.0	44.0	40.0	36.0	40.0	40.5	40.0	40.0	60.0
% Cell viability	92.0	95.1	90.5	93.9	91.0	92.4	89.3	94.8	93.5

[a]Familial facial lipoatrophy.

Table 3. Total Number of the Stem Cells (TOST) for Adinizer Micro-Fat Volume Retention in Palpebral-Malar Grooves and Malar Fat Pads

Patients	Micro-fat palpebral-malar grooves/malar fat pads, mL	TOST (1:5 ratio), mL	Concentration of TOST stem cells/mL	Total no. of stem cells
A	24.0	4.8	1.6×10^6/mL	7.9×10^6
B	24.0	4.8	2.0×10^6/mL	9.6×10^6
C	24.0	4.8	1.8×10^6/mL	8.7×10^6
D	24.0	4.8	1.6×10^6/mL	7.7×10^6
E	24.0	4.8	1.3×10^6/mL	6.0×10^6
F	24.0	4.8	1.2×10^6/mL	5.7×10^6
G	24.0	4.4	1.2×10^6/mL	6.0×10^6
H	24.0	4.8	1.7×10^6/mL	8.4×10^6
Average	24.0	4.8	1.6×10^6/mL	7.5×10^6
I	52.0	10.4	1.6×10^6/mL	17.1×10^6

orbital-malar ligaments and to the partially filled upper portions of the deep medial, medial suborbicularis, and lateral suborbicularis deep compartments.

Finally, volumes of TOST and PRP were added in ratioed portions to the 10.0 mL of micro-fat (malar fat pad) and 2.0 mL of micro-fat (palpebral-malar groove) to avoid a dilutional effect on the volume of micro-fat when added as an admixture. Secondary sites that received their indicated volumes of micro-fat are listed for each patient in Table 2. Each site similarly received up to 1:5 portions of TOST and PRP to micro-fat but their volumes and dosages are not listed. At these sites, only clinical evaluations and standardized photographs rated changes at 6- and 12-month intervals. In contrast, 3D Vectra imaging analysis of the anterior midface was performed at baseline, 3, 5 to 7, and 10 to 12 months.

Statistical Analysis

A sensitivity power analysis was performed to the predetermined sites using the normal distribution of the numerical variables utilized the Shapiro–Wilk normality test with SPSS statistics (SPSS version 17.0; SPSS, Chicago, IL) for small sample sizes (?50 samples). A 95% significance at the P ? .05 level was considered statistically significant.

RESULTS

Eight of 9 patients were females who underwent either a lateral SMASectomy facelifts (2), SMAS-face and neck lifts (5), and temporal

Table 4. Total Number of the Platelets (PRP) for Adinizer (TOST) Micro-Fat Retention in Palpebral-Malar Grooves and Malar Fat Pads

Patients	Micro-fat palpebral-malar grooves/malar fat pads, mL	PRP (1:5 ratio), mL	Concentration of platelet-rich plasma/μL	Total no. of platelets
A	24.0	4.8	1981×10^3/μL	9.5×10^9
B	24.0	4.8	1186×10^3/μL	5.6×10^9
C	24.0	4.8	1314×10^3/μL	6.4×10^9
D	24.0	4.8	1806×10^3/μL	8.6×10^9
E	24.0	4.8	1998×10^3/μL	9.6×10^9
F	24.0	4.8	1215×10^3/μL	5.9×10^9
G	24.0	4.8	2453×10^3/μL	11.8×10^9
H	24.0	4.8	1812×10^3/μL	8.6×10^9
Average	24.0	4.8	1721×10^3/μL	8.2×10^9
I	52.0	10.4	1751×10^3/μL	18.2×10^9

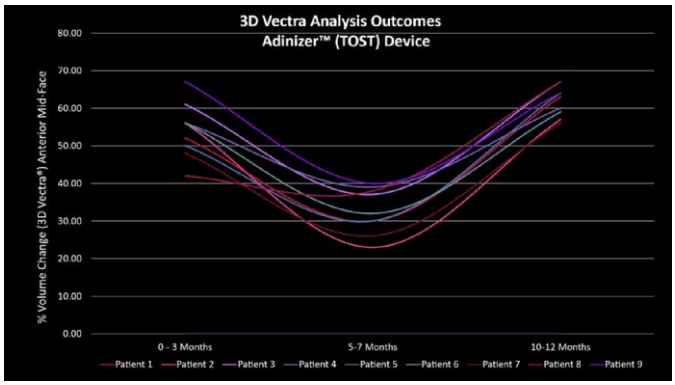

Figure 3. Percent volume change (3D Vectra) in bilateral palpebral-malar and malar fat pad zone in 9 patients from baseline, 3, 5-7, and 10-12 months.

SMAS-face and neck lift (1) in addition to enhanced micro-fat grafting with TOST and PRP as listed in Table 1. Average ages, BMI values, and additional surgical procedures are also listed. BMI values remained essentially the same at the year-end of the study. A 31-year-old male with familial facial lipoatrophy (BMI 21.9) underwent only facial enhanced micro-fat grafting with TOST and PRP, and his initial BMI of 21.9 remained unchanged throughout the study.

In Table 2, volumes of micro-fat with % cell viabilities on both sides in 8 facial sites, including the palpebral-malar grooves and malar fat pads, and the total grafted facial volumes for each patient are listed.

In Tables 3 and 4, total volumes (1:5 v/v ratio), concentrations, and quantities of stromal cells in TOST and platelets in PRP added to volumes of micro-fat in palpebral-malar grooves and malar fat pads are listed. Female patients received identical portions of micro-fat (24.0 mL), TOST (4.8 mL), and PRP (4.8 mL), whereas the male patient was injected with a larger volume of micro-fat (52 mL), TOST (10.4 mL), and PRP (10.4 mL). Further itemization of stromal cells and platelet numbers per side and together in patients' palpebral-malar grooves and malar fat pads are supplied in Supplemental Tables 1 and 2.

The sigmoidal patterns of volume changes are demonstrated in Figure 3, showing an elevated % volume at 3 months from baseline, a gradual decline between 5 and 7 months, and rebound from 10 to 12 months. The mean percentage volumetric change in each patient's face was calculated by dividing the sum of changes in the targeted anterior midface site by 2 and listed at 3 intervals over a year. Itemization for each patient's chronological volume changes is shown in Supplemental Table 3.

Three independent evaluators, who were not involved in any aspect of the study, utilized the Investigator's Global Assessment Improvement Scale (IGAIS) for each patient by assigning a "O" score to the baseline volumetric appearance of the palpebral-malar groove and malar fat pad units from preoperative photographs. Estimated levels of change at 12 months were scored from 0 = no change, 1 = slight change, 2 = improvement, 3 = much improvement, and 4 = significant improvement. In Table 5, evaluators estimated visual changes of 4 (4 patients), 3.7 (1 patient), 3.3 (3 patients), and 3.0 (1 patient) on Month 12 (Figures 4, 5).

None of the patients developed infections, fat cysts, irregularities, prolonged pain or dysesthesia, wound healing concerns, or other unwanted side effects. In 2 cases, minimal ecchymosis resolved spontaneously within 2 weeks. No patient requested any secondary procedure.

DISCUSSION

In this 12-month preliminary report, 1 surgeon assessed the retention of nearly equivalent volumes of micronized fat augmented with stromal vascular and matrix fractions and platelet-rich plasma to the palpebral-malar grooves and malar fat pads by 3D Vectra analysis. To the best of my knowledge, this report represents one of the first clinical studies to investigate the significance of dosing regenerative cells on micronized fat grafting. Of over 50 patients evaluated, 9 candidates with moderate-to-severe ptosis of the skin and face and other inclusion criteria qualified for study. In facelift patients, dissection extended 5 cm anterior to the preauricular incision line to about a centimeter from skin markings outlining the lateral borders of anterior midface, defined in this study, as the palpebral-malar groove and subjacent malar fat pad compartmental units. Although the surgical

Table 5. LipoCube Hybrid-SVF (SVF/SVM) IGAIS Scores at 12 Months

		A	B	C	D	E	F	G	H	I
Investigator 1	IGAIS score	3	4	3	4	3	4	3	4	3
Investigator 2	IGAIS score	4	4	3	4	3	4	3	4	4
Investigator 3	IGAIS score	4	4	4	3	3	4	3	4	4
	Average	3.7	4.0	3.3	3.7	3.0	4.0	3.0	4.0	4.0

0 = no change. 1 = slight improvement. 2 = improvement. 3 = much improvement. 4 = significant improvement.

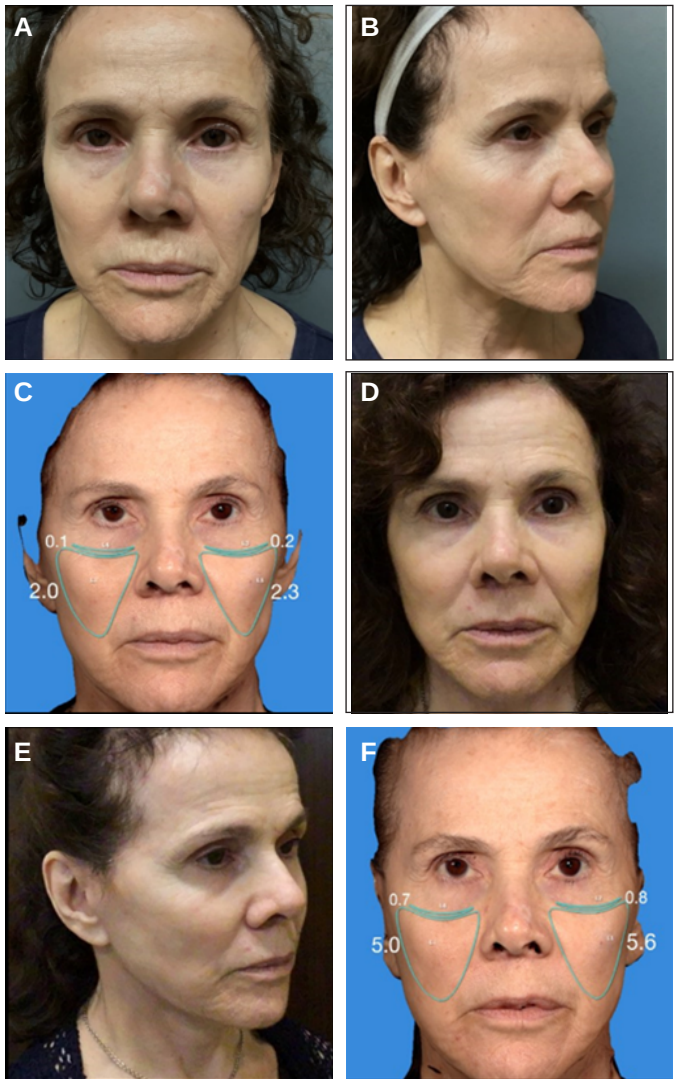

Figure 4. This 70-year-old patient "E" underwent a lateral face SMASectomy and neck lift augmented (TOST, PRP) fat grafting to palpebral-malar grooves and malar fat pads after skin closure. (A, B) Preoperative and (D, E) 12 months postoperative. 3D Vectra imaging at (C) Month 0 and (F) at Month 12 recorded a 64 ± 17.5% volume retention from baseline value. Three evaluators gave IGAIS scores of 3, 4, and 3 ("much improvement") from baseline to Month 12.

dissection and tension adjustments of the SMAS and skin units were separated from the anterior midface, their cumulative traction effects on the targeted grafted sites were not able to be completely eliminated. Thus, under less-than-ideal circumstances, the dynamic changes of volumetric reduction and recovery in the individual grafted areas were documented in the best way possible using Vectra H2 3D software for surface and volumetric assessments that might not be sensitive to specific fat compartments but to general volume changes over time. Although enhanced fat grafting was performed over multiple facial sites, the anterior midface was the primary focus for volumetric assessments. The initial utilization of equivalent volumes of 12 mL of micro-fat grafts on each side was chosen because of the (1) practicality of measuring progressive changes in larger volumes of fat within anatomically limited compartments, (2) comparison of matched starting volumes, and (3) previous report of statistically significant retention of 10 mL of grafted milli-fat to malar fat pads by Vector analysis.[7]

In the present study, a specialized mechanical device with bladed-equipped filtered disks obtained high number of viable micro-fat, total stromal cells and matrix, while avoiding excessive pressure emulsification.[4,8] The utilization of micro-fat-sized particles of 1 to 2 mm by bladed technique was preferred over milli-fat for grafting because of high % cell viability obtained in each patient, the improvement of survival chances of micron-sized particles upon vascular ingrowth at 2 to 3 days, and the promotion of stem cell up-regulation of phenotypic activity, differentiation, and proliferation of stem cells with mechanical separation methods.[10-12] Further downsizing of portions of micronized fat through 600 to 400 µm bladed filtered disks produced the final centrifuged total stromal cell end-product (TOST). The LunaSTEM device determined that the stromal cell % viability and cell concentrations in this study were similar to values reported in previous ultra-sharp bladed disk studies.[4,13]

Although there is evidence that fat should be injected soon after harvesting and processing, optimal times have yet to be determined that would achieve greater improvements in graft survival.[14] In this study, the % viability of the micro-fat and TOST cells remained high before injection 3 to 4 h later and might be partly because of the gentle method of mechanical processing and the effects of cooling. Also, the processing and usage of PRP within 3 to 4 h in this study followed current safe and effective guidelines that support the feasibility of platelet storage at room temperatures (20-24°C) up to 3 to 5 days with maintenance of their viability and hemostatic potential for transfusions.[15]

Presently, there exists no definitive consensus on an optimal volume/volume ratio of micro-fat to stromal cells or PRP nor a favorable dosage of cell numbers needed that might result in improved fat graft retention. An empirical approach was to utilize a 5:1 v/v ratio of micro-fat:TOST or micro-fat:PRP, because the usage of that ratio resulted in favorable outcomes in previous dose-dependent in vitro, translational, and clinical studies.[16-18] Furthermore, the rationale for separating

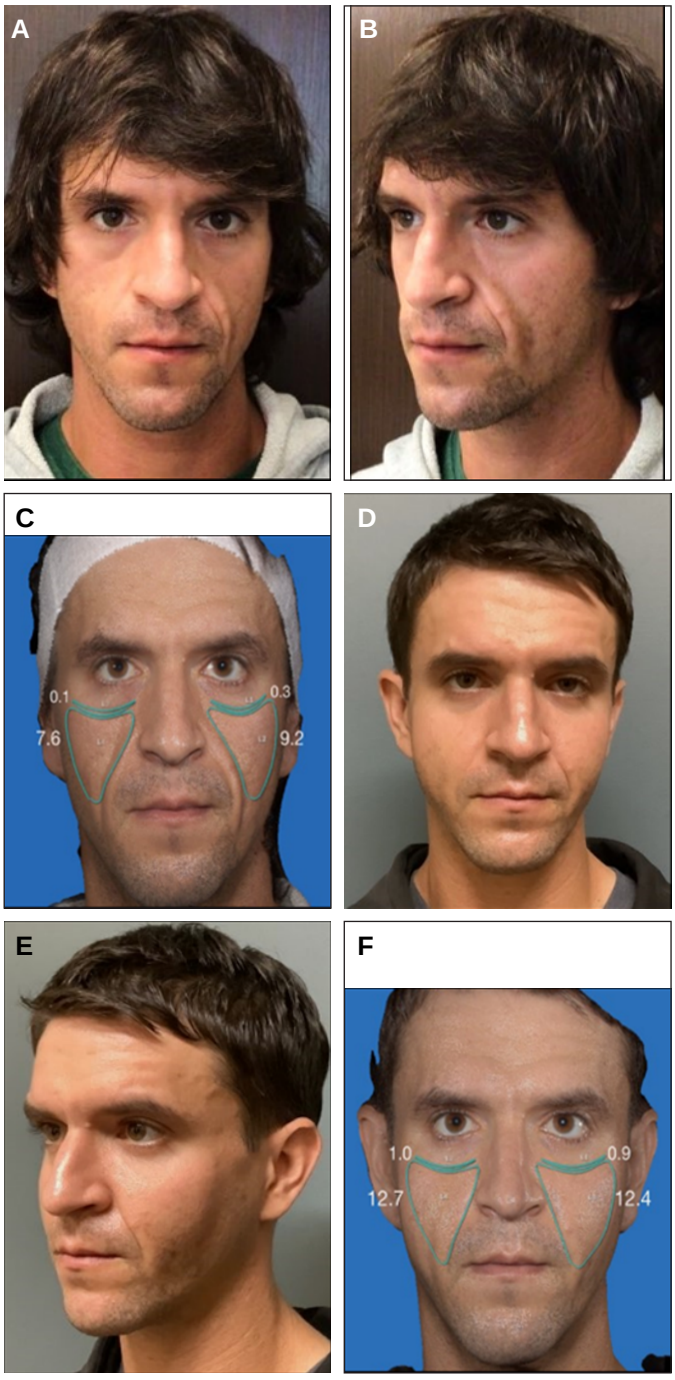

Figure 5. A 31-year-old patient "I" presented with familial facial fat atrophy and was treated with augmented (TOST, PRP) to palpebral-malar grooves and malar fat pads. (A, B) Preoperative and (D, E) at Month 12 postoperative. 3D Vectra imaging at (C) Month 0 and at (F) Month 12 observed 64.5 ± 11.3% graft retention from baseline measurements. Three evaluators awarded IGAIS scores of three 4s ("significant change") from baseline to Month 12.

micro-fat injections from TOST/PRP additions was to deliver first the requisite volume of fat to avoid a dilutionary effect that would occur when admixed with volumes of TOST/PRP before injection. That said, the delivery of TOST (and PRP) to micro-fat in precisely 1:5 blended volumetric proportions was probably imperfect to achieve in a clinical setting. Effort was made to disseminate as equally as possible TOST and PRP in a fan-shaped pattern by penetrations into the micro-fat grafts in the superficial and deep compartments of the malar fat pad and palpebral-malar groove.

In this study, 2 contending regenerative cell technologies, namely PRP-based and adipose mesenchymal stem cell–based therapies, were utilized to repair and restore volumes of grafted normal or damaged adipose fat cells.[19] Platelets are considered the frontline responses, because they release growth factors and exosomes that create appropriate conditions for mesenchymal cells to differentiate and regenerate for tissue repair and vascularization.[20] Stromal cells, on the other hand, consisted of immature progenitor cells and pericytes that provided potential stimulation, differentiation, and cellular replacement.[21] Although PRP therapies have been applied for many purposes for over 30 years, there was no consensus on standardization of preparation methods that employed qualitative or quantitative indicators for clinical results in fat grafting.[22] Marx's working definition of PRP consists of 1.0×10^9 platelets/mL in a 5 mL volume of plasma.[23] Guisti and his group demonstrated the need to deliver 1.5×10^9 platelets/mL to induce a significant angiogenic in vitro response in endothelial cell cultures.[24] To clarify the importance of dosage, Everts found that when platelet dosages were $<1.5 \times 10^9$ platelets in 14 studies, the results were not significantly different when compared with control groups.[25] The recent introduction of the DEPA classification for platelets proposed the following dosing definitions to predict clinical results: very high dosage (>5 billion platelets), high dose (3-5 billion platelets), medium dose (1-3 billion platelets), low dose (<1 billion platelets).[26] Based on the DEPA classification scale, the utilization of these dosages in this study would likely predict "high" chances to achieve positive results.

In contrast, a definitive and accepted specific adipose-derived SVF/SVM dosage classification has yet to be developed. In Copcu's study of 24 patients, the concentrations of total number of nuclear cells/mL (TOST) obtained was about 1.4×10^6 cells/mL by LunaSTEM Counter when utilizing his number 4 indication-based (gel/dense solution) protocol.[4] This concentration was close to values obtained in the current report.

Finally, sigmoidal trends in Vectra analysis among the 9 patients were tracked during the transitional phases toward graft stability. Although both parametric and nonparametric tests were run for small sample sizes (<50), the statistical findings were difficult to interpret based on a sample size, lack of control groups, addition of stromal and matrix cells and PRP, and concomitant SMAS facelifting. Nine individual trends exhibited matching timelines but differing volume percentages in their sigmoidal patterns of change. Of interest, in some studies, the authors have observed comparable sigmoidal patterns but with different timetables for troughing and peaking.[27,28] At this time, it is unclear whether these variances might be attributable to the utilization of dissimilar protocols, variable dosages of any added regenerative cells, multi-sizing of fat particles, different facial zones for 3D imaging, employing grafting alone or in conjunction with facelifting, and the patient's baseline and individual responses to these procedures. Although the pathophysiology and recovery events remain controversial, the initial grafting volume, gradual loss, subsequent remodeling, and recovery may be best understood by Yoshimura's graft replacement theory.[29] This dynamic course is postulated to involve overlapping and sequential events that began with increased volume from grafting, followed by hypoxic loss of mature adipose cells, partial cell survival from neovascularization, cell death at

the transplanted site and remodeling, and finally, the capability of stromal cells and pericytes to survive, differentiate, and replace dying cells.

Until results from controlled and larger sample-sized studies are completed, the results from this starter study remain speculative and cannot be solely attributed to the deliverance of a requisite number of TOST stem cells and platelets, although their released trophic factors are known to stimulate angiogenesis, stem cell proliferation and differentiation, and other anti-apoptotic properties. Although there is increasing evidence that the aging processes have been found to adversely affect paracrine functions from PRP and/or SVF in older patients, this observation was not appreciated in the 8 female patients whose ages ranged from 57 to 83 years of age.[30]

The study lacked control groups without surgery or surgery alone whose baseline data might clarify which factors after grafting with or without regenerative cells were important to account for volume changes. Moreover, the sample size was relatively small for such a complex study that requires not only larger subgroups but also a multi-center approach for standardization of procedures and importance of quantity (dosage) and quality of added cells and their regenerative proteomic factors. As a retrospective IRB study, a larger number of male patients and a wider spectrum of ages would be necessary to validate these preliminary findings. Longer patient follow-up by 3D Vectra analyses is ongoing to identify additional trends of recovery or demise that may occur over time.

CONCLUSIONS

Autologous fat grafting to the face continues to be an integral feature in facial aesthetics for volume restoration, symmetry, and skin rejuvenation despite historical and recent publications reporting unpredictable outcomes. New comprehensive methodologies and algorithms have emerged to obtain evidence-based data to clarify and validate longer retention outcomes but have not coalesced into generally accepted protocols. This clinical study was designed as a starter to obtain quantitative volumetric and numerical assessments to correlate the effects of stromal cell and platelet dosing and their ratios on similar volumes of micro-fat into concise anatomical compartments of the midface by Vectra 3D imaging. Although the reported values were too limited to provide definitive evidence that correlated high dosing of stromal cells or platelets to therapeutic outcomes, investigations are underway to verify these preliminary findings and determine dosing requirements that might be specific for different tissue types and treatment conditions.

Supplemental Material

This article contains supplemental material located online at https://doi.org/10.1093/asjof/ojaf066.

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