Mechanical Supplementation With the Stromal Vascular Fraction Yields Improved Volume Retention in Facial Lipotransfer: A 1-Year Comparative Study

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Abstract

Background: Unpredictable volume maintenance in the long term is a major limitation of autologous fat grafting.

Objectives: The authors compared results of autologous lipotransfer to the face with or without enrichment of fat with the stromal vascular fraction (SVF).

Methods: Thirty patients with asymmetric depletion of facial volume were included in a prospective study. Patients were randomly assigned to undergo a single session of autologous fat transfer with washed adipose tissue (control group) or with washed adipose tissue combined with the pellet of centrifuged lipoaspirate, which contained the SVF (enriched group). Patients were evaluated clinically and from photographs. A subset of 5 patients in each group underwent computed tomography (CT) preoperatively and 12-months postoperatively for quantitative assessment of graft retention. Washed and fractionated lipoaspirates were evaluated histochemically and with flow cytometry to determine relative abundances of viable cells.

Results: No major complications occurred. CT findings 12 months postoperatively indicated that patients who received SVF-enriched fat had significantly better volume retention (9.6% volume loss vs 24% in the control group; $P = 0.013$). Independent surgeons more frequently rated long-term aesthetic outcomes as "excellent" for patients in the enriched group (82.5% vs 47.6% for control group). Laboratory results indicated that each pellet contained approximately 16,000 intact adipose-derived stem cells.

Conclusions: Lipotransfer with SVF-enriched adipose tissue is safe and associated with improved volume retention, compared with transplantation of unenriched fat. The SVF can be dissociated from lipoaspirate by centrifugation to yield a large quantity of viable regenerative cells, without enzymatic digestion.

Level of Evidence: 2

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Autologous fat transplantation is an excellent option for treatment of tissue deficiencies. Fat is readily accessible in subcutaneous deposits and can be molded to reconstruct defects. Neuber[1] first described autologous fat transplantation in 1893; however, volume maintenance of fat grafts continues to be a major limitation of this technique. Results of fat transplantation are highly variable and operator-dependent. Poor graft retention is common, and the volume of large grafts has been found to decrease significantly over time.[2]

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The primary contributors to tissue loss in fat grafting appear to be insufficient vascularity and cell death; additional causes include mechanical disruption of cells, lipid-induced membrane damage, and apoptosis.[3,4] Adipose tissue is richer than bone marrow with regard to regenerative cells, such as stem cells. In 2001, adipose-derived stem cells (ADSCs) were identified by Zuk et al[5] as cells with the capacity to differentiate into several mesenchymal lineages. ADSCs can be isolated from the stromal vascular fraction (SVF) of adipose tissue by enzymatic processing and culturing. These cells adhere to and can be cultured on plastic, unlike other cell types in the SVF.[6,7] ADSCs express specific surface markers, including CD73, CD90, and CD105.[7] ADSCs also can be identified by the absence of CD45, CD34, CD14 or CD11b, CD79 or CD19, and HLA-DR.[7] Metabolic features of ADSCs—including angiogenic potential, antioxidative effects, immunotolerance, and paracrine and inflammatory modulation—have been demonstrated in the laboratory as well as in preclinical models and clinical settings.[7-10]

The multipotent stem cells contained in fat can be obtained by liposuction without loss of viability.[8-10] Investigators have applied procedures of fat harvest and cell isolation to develop tissue-engineered constructs and generate induced pluripotent stem cells from ADSCs for reconstructive and aesthetic purposes.[11-15] Transplantation of fat enriched with ADSCs—termed cell-assisted lipotransfer—has several potential advantages over conventional fat transfer. Aspirated fat has approximately half of the mesenchymal stem cells of excised whole fat.[16,17] The poor take and volume unpredictability associated with transferred lipoaspirate may be attributed, at least in part, to a relative deficiency of ADSCs.[9] Consequently, many authors have advocated cell-assisted lipotransfer for restoration or augmentation of tissue volume.[18-21]

The purpose of the current study was to compare the results of standard lipotransfer to those of a novel technique of cell-assisted lipotransfer in which fat was washed and enriched with the SVF. Our findings have potential implications for treating volumetric deficits of the face.

METHODS

Patients and Study Design

In this prospective, clinical, randomized comparative study, 30 patients were evaluated who underwent structural fat grafting for management of volumetric deficits of the face from January 2013 to July 2015. The study was approved by the Brazilian Investigation Ethical Committee Board (protocol no. 28063) and the Brazilian Clinical Trials Registry and was conducted in accordance with the Declaration of Helsinki.

Patients with facial deformities resulting from tumorectomy, Parry-Romberg syndrome, or trauma were included in the study. Patients with unstable clinical pathologies, including type 2 diabetes, uncontrolled blood pressure, cardiac arrhythmias, or coagulation abnormalities, were excluded. Also excluded were patients who were current smokers or had previously undergone volumetric restoration of the face, such as by lipotransfer or filler injection. Patients were randomly assigned to undergo either traditional lipotransfer with washed fat (control group) or cell-assisted lipotransfer with SVF-supplemented fat (enriched group). Randomization was conducted prospectively by order of enrollment with software available at http://www.randomization.com. Eligible patients were invited verbally to participate. Patients were clearly informed of benefits, risks, and operative complications, and all patients provided written informed consent.

The volume of fat to be transferred was determined by comparing facial volumes on the affected and unaffected sides of the face, with the goal of achieving symmetric facial volume. All fat grafting procedures were performed by the same surgeon (N.F.G.A.). All patients underwent preoperative evaluations, which included laboratory studies, blood tests, cardiac examination, and photography. Among patients with Parry-Romberg syndrome or trauma sequelae, 5 were selected randomly from each group to also receive X-ray computed tomography (CT) preoperatively and 12 months postoperatively.

Surgical and Laboratory Procedures

Patients received sedation and local or regional anesthesia at the hospital prior to surgery. Patients in both groups underwent the same procedures for fat harvest and lipotransfer. Patients in the enriched group received autologous fat that had been supplemented with the SVF.

Fat Harvesting

Chlorhexidine was applied to antiseptically prepare the skin of the lower abdomen, and a solution of normal saline, 0.5% lidocaine, and epinephrine (diluted 1:500,000) was injected through a 22-gauge spinal needle into the lower abdomen (donor site) at a ratio of 1 mL of solution per mL of tissue to be aspirated. Fat was collected with the superwet technique. Approximately 200 mL of fat was aspirated with a blunt cannula (diameter, 3 mm; length, 20 cm) (tip, 3B; Richter, São Paulo, Brazil) attached to a 10-mL Luer-lock syringe. Light negative pressure was created by slowly withdrawing the plunger by hand.

Conventional Fat Processing (Control Group)

The lipoaspirate, still contained within the Luer-lock syringe, was washed with saline to remove blood and cellular debris and then was left to separate by gravity. Three

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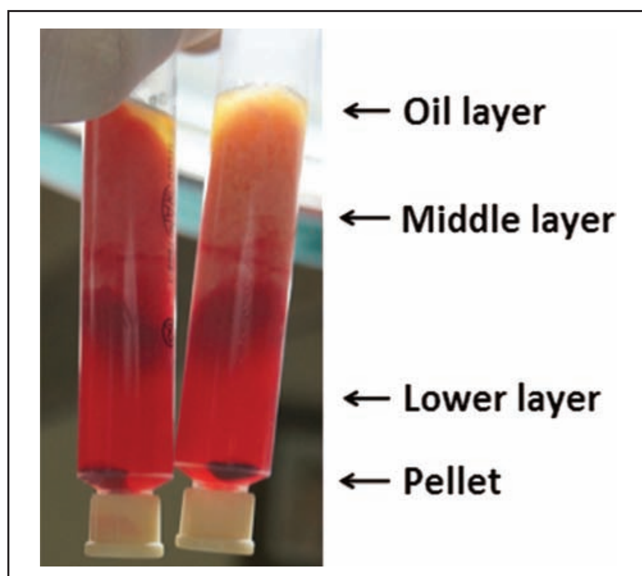

Figure 1. After centrifugation of the lipoaspirate, 4 fractions were observed: an upper layer composed of an oily liquid; a middle layer, consisting of firm yellow tissue with few blood spots; a lower layer, comprising blood, infiltrate fluid, and wash liquid; and a pellet, corresponding to the stromal vascular fraction (SVF). It was done only one section of centrifugation.

iterations of washing and gravity separation yielded a layer of saline and a supernatant layer predominantly composed of bright yellow adipocytes. Fat processing was conducted in a closed system with the piston retained in the harvesting syringe during washing.

Fat Processing and Supplementation With the SVF (Enriched Group)

For each patient, lipoaspirates that had been collected into 10-mL syringes were separated into 2 groups. Half of the lipoaspirates were processed by washing and gravity separation, as in the control group. The other half of were processed by centrifugation to enable isolation of the SVF. Specifically, syringes containing the lipoaspirates were capped and placed in a centrifuge (IEC Medilite Microcentrifuge, Thermoelectron Corporation, Byron Medical, Waltham, MA) set to 3000 rpm (1286*g*) for 3 minutes. After centrifugation, 4 layers were observed: (1) an abundant upper oily layer (more predominant than in the gravity-separated lipoaspirates); (2) a middle layer of firm, compacted yellow tissue (with fewer blood spots than in the gravity-separated fat); (3) a lower layer of blood and fluids from infiltration and washing; and (4) a bottom pellet that corresponded to the SVF. It was done only one section of centrifugation. This pellet comprised regenerative cells, growth factors, and cytokines and was separated mechanically, rather than by enzymatic processing (Figure 1).

Fat was enriched by adding the pellet from 1 centrifuged sample to a 10-mL syringe containing conventionally washed fat (ie, 1 pellet per 10-mL of washed lipoaspirate). The pellet was gently reconstituted in the washed fat by connecting a 3-mL Luer-lock syringe via a female Luer-lock adapter. Less than 24 hours after collection of adipose tissue, one 10-mL syringe containing washed fat and 1 pellet per patient were sent for laboratory analysis by a group of biotechnicians. The upper and lower layers of washed lipoaspirate were discarded, and the middle layer, which typically is applied for adipose tissue grafting, was retained for analysis.

Lipotransfer at the Recipient Site

Preoperative markings were made to indicate areas of volumetric deficit. Blockade of the supraorbital, infraorbital, and mental nerves was achieved with 1 to 2 mL of 2% lidocaine. Small scalp incisions were made with an 18-gauge needle along the temporal hair line and at the lateral base of the alar cartilage. Fat was injected into these target sites by means of a 3-mm blunt-tipped Coleman cannula connected to a 3-mL syringe. A multichannel retrograde technique was performed; the goal was to deposit fat homogeneously in multiple subcutaneous layers. Fat was implanted to correct the volumetric deficit and restore symmetry with the unaffected side of the face. Care was given to avoid overcorrection, which could bias subsequent assessments of graft take. The cannula access points were closed with 6.0 nylon sutures.

Postoperative Assessments of Facial Volume

Clinical analysis (ie, observation and palpation) and CT were performed 12 months after lipotransfer to evaluate graft take. The Ethical Committee Board specified that CT be restricted to patients with alterations of the bone. Therefore, 5 patients with bone manifestations owing to Parry-Romberg syndrome or trauma were selected randomly from the control and enriched groups to undergo CT.

All patients were photographed before and approximately 12 months after surgery for independent review by 5 experienced plastic surgeons (4 men, 1 woman; average age, 45 years; all > 10 years of practice). The surgeons were blinded to the groups, and no surgeon was an author of this article. The surgeons scored volumetric improvement of the treated region on a scale of 1 to 10 (excellent, 9-10; good, 7-8; satisfactory, 5-6; unsatisfactory, 1-4).

Histologic Analysis

Analysis of Adipocytes and ADSCs in Washed Lipoaspirates (Control Group)

Washed lipoaspirates were transferred to 50-mL tubes and weighed. After decantation, each sample comprised 3

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layers: an upper oily layer, a middle layer of fat tissue, and blood cells. The oily fraction was collected, the blood cells were discarded, and the layer of fat tissue was retained for subsequent analyses. Adipocytes in the oily fraction were counted in a Neubauer chamber under an inverted microscope (TS100, Nikon, Japan) and photographed (MicroPublisher 5.0 & 3.3 RTV, QImaging Corporation, Canada).

Retained adipose tissue was washed with phosphate-buffered saline (PBS) to remove any remaining blood cells and was digested by adding 1.76 mg of collagenase I per gram of tissue (Sigma-Aldrich, St. Louis, MO) for 30 minutes at 37°C with agitation. Alpha-MEM (minimal essential medium; LGC Biotechnology) supplemented with 10% fetal bovine serum (FBS; LGC Biotechnology, São Paulo, Brazil) was added in a 1:2 ratio to inhibit enzymatic activity. After centrifugation at 700*g* for 10 minutes, 3 layers were observed: a superior oily fraction containing adipocytes, (2) a middle fraction composed of nondigested extracellular matrix, and (3) a pellet of small cells (eg, endothelial cells, preadipocytes, and mesenchymal stem cells). The upper layer of adipocytes was counted and imaged.

Cells of the pellet were counted in a Neubauer chamber, and expression of surface and intracellular molecules was evaluated by flow cytometry. To capture mesenchymal cells, pellet cells were incubated with the following conjugated monoclonal antibodies for 30 minutes: anti-CD31-FITC (fluorescein isothiocyanate), anti-CD146-PE (phycoerythrin), anti-CD34-PE-Cy5, anti-CD45-FITC, anti-CD90-PE, anti-CD73-FITC, anti-CD13-PE, and anti-CD49d-PE-Cy5 (all, BD Biosciences, San Diego, CA). Cells then were washed and fixed with BD FACS Lysing Solution (BD Biosciences), and flow cytometric analyses were conducted with a FASCalibur system (Becton Dickinson, San Jose, CA). At least 70,000 total events were acquired, and data analysis was performed with CELLQuest software (Becton Dickinson).

Analysis of ADSCs in Pellets After Mechanical Dissociation (Enriched Group)

Two populations of cells were distinguished in the pellets after centrifugation: nonviable hematopoietic cells that expressed CD45, CD14, and CD16 and viable ADSCs that expressed CD105, CD90, CD73, CD146, and CD34 but not CD45. Cell populations were analyzed with a FACScanto device (BD Biosciences, Franklin Lakes, NJ) equipped with FACS Diva 4.0 software. Concurrently, cells also were stained with Tuerk's solution (Sigma-Aldrich) and were quantified on a hemocytometer under a microscope. The total cell number in the pellet was determined to obtain the relative and absolute numbers of stem cells.

ADSCs in vitro were induced to differentiate into osteogenic or adipogenic lineages, as described by Zuk et al.[5,6] For adipogenic differentiation, cells were cultured in 24-well plates containing 1 mL of Dulbecco's Modified Eagle Medium (HyClone Laboratories, Logan, UT) supplemented with 10% FBS, 1 mM of dexamethasone (Sigma-Aldrich), 0.5 mM of 3-isobutyl-1-methylxanthine (Sigma-Aldrich), 10 mM of human insulin (Humulin N; Eli Lilly & Co, Indianapolis, IN), and 0.2 mM of indomethacin (Sigma-Aldrich). The induction medium was changed twice per week. After a period of 14 days in culture, cells were fixed with 4% paraformaldehyde, washed with PBS, and stained with a solution of 0.5% Oil Red O (Sigma-Aldrich) to assess fat storage. For osteogenic differentiation, cells were cultured in 6-well plates containing 2 mL of DMEM-low glucose (LGC) supplemented with 10% FBS, 10 nM of dexamethasone (Sigma-Aldrich), 10 mM of β-glycerophosphate (Sigma-Aldrich), and 50 mM of l-ascorbic acid 2-phosphate (Sigma-Aldrich). The induction medium was changed twice per week. After a period of 14 days in culture, cells were fixed with 4% paraformaldehyde, washed with PBS, and stained with 1% Alizarin Red S (pH 4.2; Sigma-Aldrich)

Apoptosis and viability were determined by means of annexin V-FITC and propidium iodide tests (FITC Annexin V Apoptosis Detection Kit I, BD Pharmingen San Jose, CA, USA), according to the manufacturer's instructions.

Statistical Analysis

Unpaired *t* tests were performed to evaluate the significance of between-group differences. Parametric testing was applied because the variables had a Gaussian distribution. Results were expressed as mean ± standard deviation (SD). Statistical significance was defined as $P < 0.05$. Software program used to perform statistical analyses was Prisma Graph Pad., Version 7.0 (La Jolla, CA, USA).

RESULTS

A total of 30 patients (16 female, 14 male) who underwent liposuction and lipotransfer to the face were included in this study. They ranged in age from 20-55 years old in the control group (mean, 39.67 years; SEM, 2.681), and from 27-60 years old in the enriched group (mean, 42.40 years; SEM, 2.636). Body mass index (BMI) ranged from 24.6 to 30.9 kg/m^2 in Group A (control group) (mean, 27.89 kg/m^2; SEM, 0.539) and from 25.6 to 33.2 kg/m^2 in Group B (enriched group) (mean, 29.89 kg/m^2; SEM 0.659). Five patients from each group with Parry-Romberg Syndrome and trauma sequelae were evaluated with 3D tomography pre- and postoperatively.

Six men (40%) and 9 women (60%) were randomly assigned to undergo standard lipotransfer (control group).

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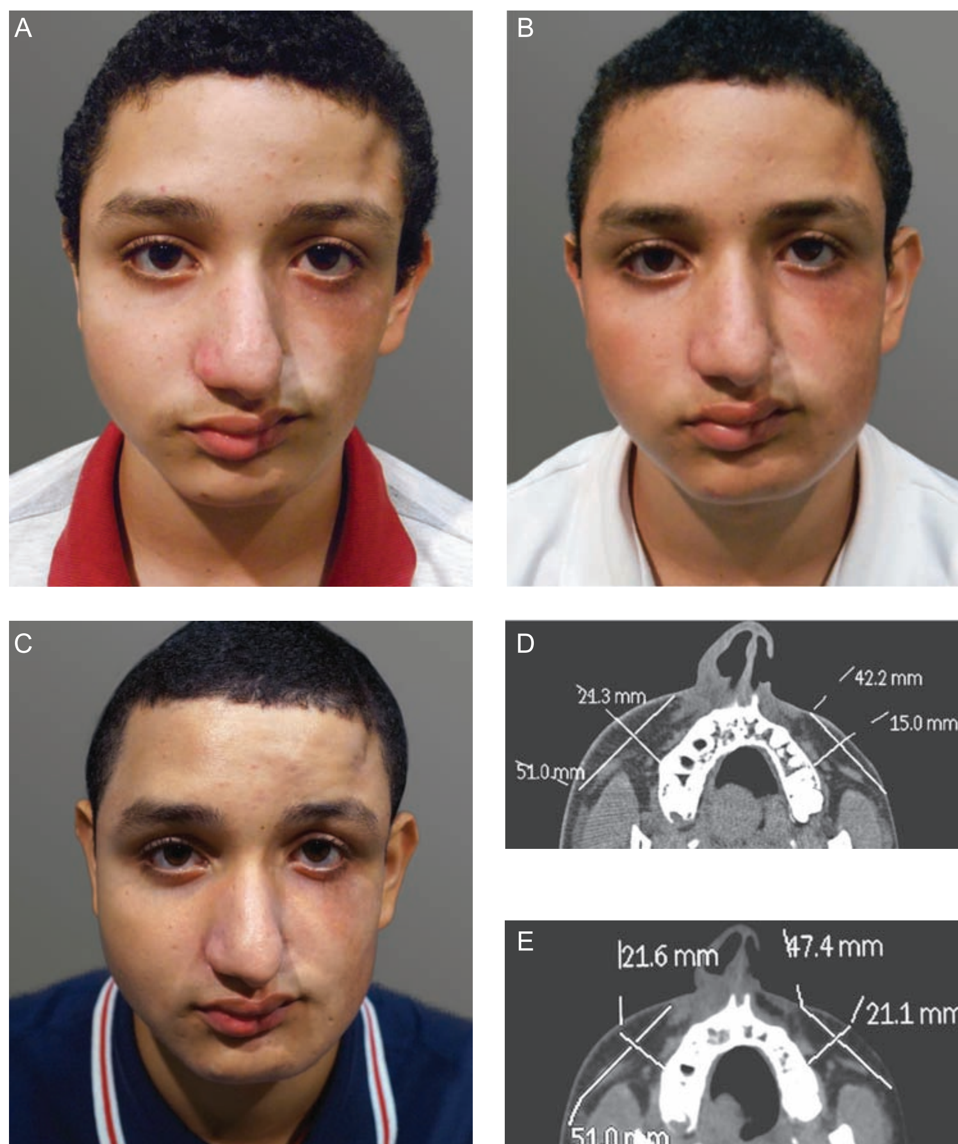

Figure 2. (A) This 18-year-old man with Parry-Romberg syndrome received lipotransfer to the face (70 mL) with unenriched autologous fat tissue (control group). (B) Six months and (C) 2 years postoperatively. The patient also underwent computed tomography (D) preoperatively and (E) 1 year postoperatively. CT results indicated a volume decrease of lipotransferred tissue to 60 mL (ie, a volume loss of 15%) 1 year after lipotransfer.

Included in this group were 2 patients with Parry-Romberg syndrome, 8 with trauma sequelae of the face, and 5 with volume loss after surgical procedures, including tumorectomy. The mean volume of fat transferred in this group of patients was 53 mL (SEM, 5.385 mL; range, 40-70 mL) (Figure 2). Eight men (53%) and 7 women (47%) were randomly assigned to undergo SVF-enriched lipotransfer (enriched group). This group included 3 patients with Parry-Romberg syndrome, 7 with trauma sequelae of the face, and 5 with volume loss after surgical procedures. The mean volume of SVF-enriched fat transferred in this patient group was 58 mL (SEM, 3.521 mL; range, 45-65 mL) (Figure 3). Although some patients had more severe volumetric deficiencies than did others, all patients received lipotransfer via a multichannel technique, and the average volumes of grafted fat in the groups were similar ($P = 0.46$) (Figure 4A).

The patients' mean ages were 39.67 years in the control group (SEM, 2.681 years; range, 39.20-55 years) and 42.40 years in the enriched group (SEM, 2.636 years; range 27-60 years). Mean body mass indices were 27.89 kg/m^2 in the control group (SEM, 0.539 kg/m^2; range, 24.6-30.9 kg/m^2) and 29.89 kg/m^2 in the enriched group (SEM, 0.659 kg/m^2; range, 25.6-33.2 kg/m^2). There is not significant difference among the groups considering age and BMI.

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Figure 3. (A, C) This 26-year-old woman with Parry-Romberg syndrome underwent transplantation (65 mL) with SVF-enriched autologous fat (enriched group). (B, D) Two years postoperatively. The patient also was submitted to CT (E) preoperatively and (F) 1 year postoperatively. This patient experienced a volume decrease of 6% at 1 year, based on CT findings. The side of the face that received enriched fat had similar tissue characteristics to the unaffected contralateral side, with no evidence of cysts, fibrosis, or microcalcifications.

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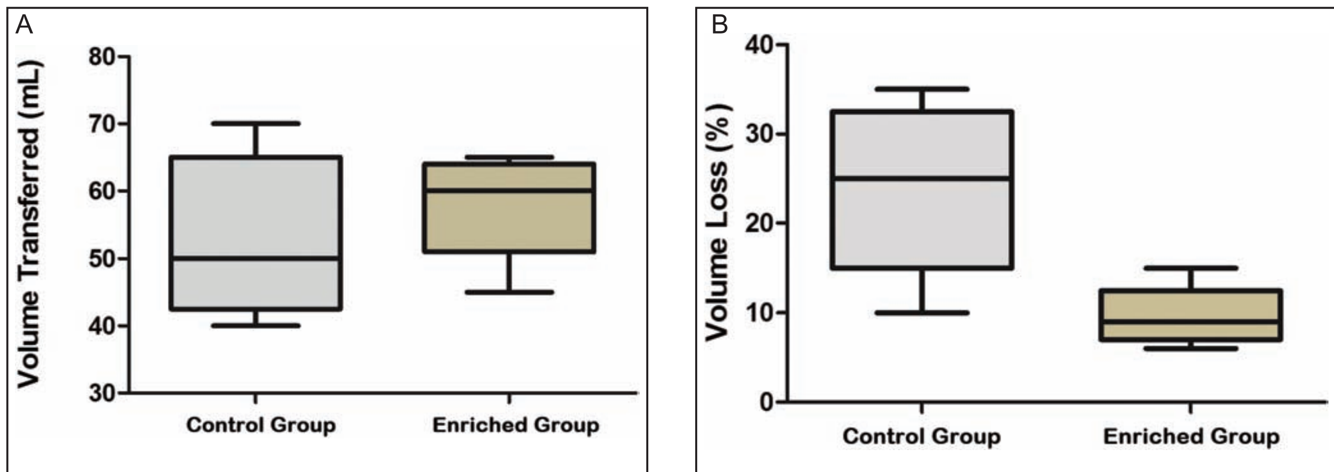

Figure 4. Volume of fat transferred and percentage volume retained in 10 patients (5 patients per group) with Parry-Romberg syndrome or trauma sequelae who underwent CT. Patients in the control group received conventionally processed fat tissue, and patients in the enriched group received fat supplemented with the SVF. (A) Mean fat volume transferred was 53 mL (SEM, 5.385 mL) in the control group and 58 mL (SEM, 3.521) in the enriched group ($P = 0.46$). (B) Percentage volume lost from grafts 1 year postoperatively. Patients in the enriched group had greater retention of the fat graft than did patients in the control group (mean volume loss in enriched group, 9.6%; SEM, 1.5%; vs in control group, 24%; SEM, 4.3%; $P = 0.002$).

Table 1. Blinded Photographic Evaluation by 5 Plastic Surgeons Preoperatively and 12 Months Postoperatively

	Surgeon Identifier	Excellent (%)	Good (%)	Satisfactory (%)	Unsatisfactory (%)
Control Group (N = 15)	1	46.70	52.4	0.9	0
	2	45.00	50	5	0
	3	45.6	42.3	12.1	0
	4	48.5	38.3	13.2	0
	5	52.0	36.60	11.4	0
	Mean	47.56	43.92	8.52	0
Enriched Group (N = 15)	1	78.90	19.3	1.8	0
	2	84.20	12.2	3.6	0
	3	87.3	10.4	3.3	0
	4	75.0	20.5	4.5	0
	5	82.30	12.30	5.4	0
	Mean	82.50	14.18	3.65	0

Results were graded on a scale of 1 to 10, as follows: excellent, 9-10; good, 7-8; satisfactory, 5-6; unsatisfactory, 1-4.

Clinical Evaluation

Patients were photographed preoperatively and an average of 1.5 years postoperatively (range, 1-2 years) for clinical evaluation by 5 plastic surgeons. Results of this blinded assessment are presented in Table 1. Long-term aesthetic results were scored as "excellent" more often for patients in the enriched group (mean, 82.5%) than for patients in the control group (mean, 47.6%).

CT Analysis

In the control group, 2 patients with Parry-Romberg syndrome and 3 patients with trauma sequelae underwent CT. In the enriched group, 3 patients with Parry-Romberg syndrome and 2 patients with trauma sequelae had this procedure. Preoperative and 12-month-postoperative CT findings for 2 representative patients are depicted in Figures 2D-E and 3 E-F. Five patients from Groups A and

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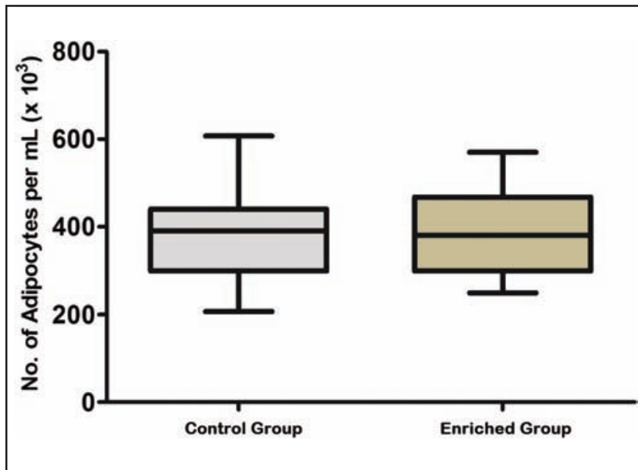

Figure 5. Histologic quantification of intact adipocytes in aspirated adipose tissue processed by washing in both groups (ie, prior to SVF supplementation in the enriched group). Mean cell count in the control group was 379,300 cells/mL (SEM, 25,590 cells/mL; range, 207,500-607,500 cells/mL). Mean cell count in the enriched group was 387,600 cells/mL (SEM, 28,120 cells/mL; range, 250,000-570,000 cells/mL) (P = 0.83).

B underwent 3D tomography scanning. Scans in the area treated with standard lipotransfer (Group A) and SVF-enriched lipotransfer (Group B) showed the same tissue characteristics as those of the healthy contralateral side. Specifically, cysts, fibrosis, and calcification were absent from the recipient sites of patients in both groups.

Twelve months postoperatively, results of CT demonstrated that 5 patients in the control group had significantly more volume loss at the recipient site than did 5 patients in the enriched group (mean volume loss, control group: 24%; SEM, 4.3%; mean volume loss, enriched group: 9.6%; SEM, 1.5%; P = 0.013) (Figure 4B).

In the control group, the 2 patients with Parry-Romberg syndrome who received CT had an average volume loss of 15%; the 3 patients with trauma sequelae had an average volume loss of 30% (Figures 2D-E, 3 E-F, and 4A-B). In the enriched group, the 3 patients with Parry-Romberg syndrome who underwent CT experienced a mean volume loss of 8%, and the 2 patients with trauma sequelae had a mean volume loss of 12%.

Skin quality was improved at sites that received SVF-enriched adipose tissue. This improvement was made on clinical examination, comparing with preoperative skin quality. No severe complications, such as infections, septic shock, cellulitis, fat embolism, or anemia, were recorded. Patients in both groups experienced only transitory events, such as ecchymosis and edema, which are common results of fat grafting in the early postoperative period.

Table 2. Percentages of Hematopoietic (CR45+) and Nonhematopoietic (CD45–) Cells Constituting Washed Lipoaspirates and Pellets

Cell Type	Washed Lipoaspirate	Pellet
Mean ± SD percentage of CD45+ cells[a]	2.5 ± 0.8	8.7 ± 1.3
Mean ± SD percentage of CD45– CD31+ cells[b]	4.7 ± 1.6	6.5 ± 2.0
Mean ± SD percentage of CD45– CD34+ cells[c]	4.2 ± 0.9	4.1 ± 1.9
Mean ± SD percentage of CD45– CD106+ CD90+ CD73+ CD105+ cells[c]	4.3 ± 1.2	4.6 ± 1.6

SD, standard deviation. [a]Hematopoietic cells. [b]Endothelial cells. [c]Mesenchymal cells.

Laboratory Analyses of ADSCs and Adipocytes

Hematopoietic (CD45+) and nonhematopoietic (CD45–) cells in the middle layer of conventionally processed lipoaspirates and in the pellet of lipoaspirates that were processed by centrifugation were compared quantitatively. In the middle layer, we observed many intact adipocytes with preserved surface morphology. The adipocytes intacts were determined by adipocyte counting and photography in a Neubauer Chamber using an inverted microscope (Nikon TS100). Viable intact adipocytes from uncentrifuged lipoaspirates (ie, lipoaspirates of the control group and half of the lipoaspirates of the enriched group) were quantified histologically. Mean cell density was 386,838 cells/mL (range, 210,000-600,000 cells/mL) (Figure 5).

In the pellet after centrifugation, high percentages of ADSCs (CD45– CD106+ CD90+ CD73+ CD 105+) and endothelial cells (CD45– CD31+) were found (Table 2) after centrifugation.

After laboratory analysis of the pellet, it was found that this layer was rich in ADSCs. Results of flow cytometry analysis of 25 pellets indicated that this fraction contained an average of 16,204 mesenchymal cells with the following expression pattern of surface markers: CD105+ CD106+ CD90+ CD73+ CD146+ CD14– CD45– CD34+ CD31+ (SD, 5516 cells; range, 3100-25,150 cells) (Figure 6).

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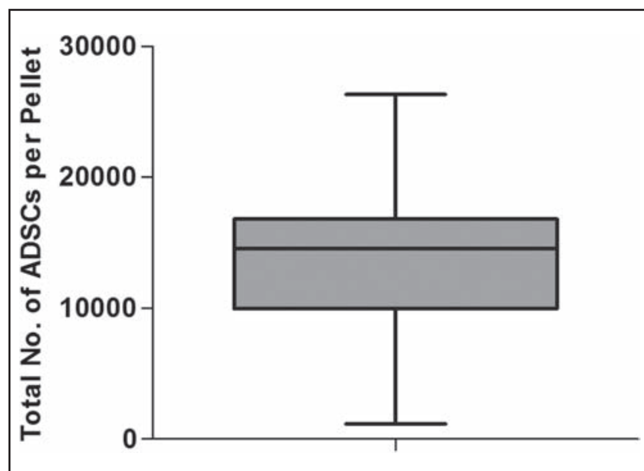

Figure 6. Quantitative representation of mesenchymal cells in 25 pellets, each obtained by centrifugation of 10-mL of lipoaspirate. Mesenchymal cells (CD105 + CD90 + CD73 + CD146 + CD14 − CD45 − CD34 +) were analyzed by flow cytometry, an average of 16,204 mesenchymal cells (SD, 5516 cells; range, 3100-25,150 cells). Cell subpopulations were not analyzed.

DISCUSSION

Applications of autologous fat transfer in plastic surgery have been changing ever since adipose tissue was found to be rich in adult stem cells (ie, ADSCs).[5-6] The process of lipoaspiration depletes the density of ADSCs. This relative deficiency may be responsible, at least in part, for poor retention of transplanted adipose tissue in the long term. Consequently, cell-assisted lipotransfer, in which fat tissue is supplemented with ADSCs prior to transplantation, may enable more enduring results.[18-21] Several metabolic properties of ADSCs, including angiogenic potential, antioxidative effects, immunotolerance, and paracrine and inflammatory modulation, have been found to influence grafted fat and its surrounding tissue.[7] Alternative lipofilling techniques have been described in recent years,[22,23] but no identical procedure has been adopted by all practitioners. Moreover, consensus is lacking regarding optimal fat-processing techniques for maximum viability and retention of the graft.

In this study, outcomes were compared for patients who underwent volumetric restoration of the face with washed lipoaspirate and patients who underwent the restoration with washed lipoaspirate that had been enriched with the SVF. We found that regenerative cells were concentrated in the pellet of centrifuged lipoaspirate. Other authors have shown that centrifugation preserves cells of the SVF and water-soluble growth factors.[23,24] Based on results of clinical analysis and CT in the current study, the control group experienced greater volume loss than the enriched group during approximately 1 year of follow-up. Although we found poorer graft retention in patients of control group, graft take was better in these patients than in similarly treated patients described in other studies (range of volume loss, 40-60%).[13-15] We attribute this difference, in part, to our technique of lipofilling in multiple channels—that is, Coleman's lipostructural technique.[13,25,26]

The SVF can be captured in the pellet of centrifuged fat and comprises circulating blood cells, macrophages, fibroblasts, pericytes, endothelial cells, and ADSCs.[23,24] The SVF of lipoaspirate provides a richer and more accessible source of ADSCs than obtainable from bone marrow.[26] Whereas 1 g of excised adipose tissue yields nearly 5000 ADSCs, the same mass of lipoaspirate contains approximately half of this quantity.[18] Therefore, we enriched washed lipoaspirate with pelleted SVF from centrifuged lipoaspirate to deliver a richer source of ADSCs to the recipient site. This cell-assisted strategy increased the number of ADSCs by approximately 1600 cells per mL of original lipoaspirate (Figure 6). Consistent with results of previous studies, our findings showed that SVF enrichment increases the efficacy of autologous fat grafting and yields grafts with a higher survival rate and improved volume maintenance, compared with unenriched adipose tissue.[18-20]

We isolated the SVF by centrifugation, which has been described as a low-yield form of ADSC enrichment, compared with enzymatic digestion.[27] However, we maintain that SVF enrichment by enzymatic processing is a less reproducible and more expensive procedure that can destroy growth factors and cytokines in the lipoaspirate. At time of fat harvest, cells are surrounded by a glycoprotein scaffolding of tissue factors. Matrix digestion, particularly with collagenase, eliminates this scaffolding.[28] Moreover, enzymatic processing with collagenase diminishes cell viability, as noted in laboratory protocols of primary cell cultures.[28] Mechanical separation of the SVF by centrifugation is reproducible and inexpensive and has been described as the preferred method for large-volume samples.[28-34]

The importance of preserving the stem cell niche has been recognized only recently. Maintenance of the milieu of cytokines and growth factors is crucial for intercellular interactions,[35] and survival of a fat graft is more dependent on these extracellular factors than on the presence of a large quantity of isolated ADSCs.[36]

Cells of the SVF exhibit biological properties of mesenchymal stem cells, such as self-renewal and multipotent differentiation. However, somatic stem cells have been demonstrated to exhibit limited functioning outside of the stem cell niche.[36,37] As opposed to enzymatic digestion or in vitro cell expansion, mechanical dissociation of the SVF by centrifugation yields an SVF pellet attached a scaffold of aspirated fat; this seems most likely to preserve the stem cell niche and support the interaction between fat tissue and supplemental regenerative cells.

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We attribute our findings of long-term volume retention to delivery of a comprehensive array of regenerative cells (mostly ADSCs), biochemical elements, growth factors, cytokines, signaling molecules, and extracellular matrix. In addition, we delivered fat to the recipient site by structural fat grafting. This technique has been shown by others to involve minimal manipulation and trauma and to maximize the likelihood of predictable and long-lasting aesthetic results.[25] During follow-up, we also noted that skin quality and tone were improved at recipient sites of SVF-enriched adipose tissue. This finding is consistent with the results of other investigators.[37,38]

Our study has several limitations. Our patient population was relatively small, and we included patients with a wide range of ages and a variety of diagnoses (eg, Parry-Romberg syndrome, trauma, and tumors). Patients in enriched group said that they noticed improvement in their skin quality; however, we did not perform a systematic assessment of patient satisfaction postoperatively. Another potential limitation is the limited number of patients who were evaluated by CT.due to ethical restrictions for pre- and postoperative 3D CT scans and absent of laboratory analysis of transplanted tissue by biopsy. In a subsequent study, we plan to analyze biopsy specimens from lipotransferred areas.

CONCLUSIONS

We evaluated a series of patients who underwent lipotransfer to correct asymmetric volume deficiencies of the face and demonstrated that fat grafts enriched with mechanically dissociated SVF yielded improved volume retention and aesthetic results compared with fat prepared conventionally. The methods we describe for SVF enrichment are relatively low cost, involve minimal manipulation, and avoid extensive preparation of specimens in a laboratory. Moreover, overcorrection was not needed, and no abnormal tissue characteristics were noted at the recipient site 12 months postoperatively. Results of 1 session of mechanically enriched fat grafting were safe, predictable, and enduring.

Disclosures

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