The Safety and Efficacy of Cell-Assisted Fat Grafting to Traditional Fat Grafting in the Anterior Mid-Face: A 3D Imaging Comparison Study

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Introduction

Current age-appropriate surgical rejuvenation of the face requires a site-specific analytical evaluation and harmonious treatment of the dimensional and differential aging changes to skin, muscle, fat and bone. Attention has focused within the last decade on the fate of adipose tissue as a consequence of landmark stereo-lithographic[1], radiologic[2] and anatomic[3] investigations. These studies demonstrated that the subcutaneous fat of the anterior mid-face existed in superficial and deep fat compartments, distinctly partitioned by septal boundaries and ligaments, and was subject to independent volume loss more than to descent with aging. The observed gradual occurrence of selective volume depletion of compartmental facial fat led to a fundamental paradigm shift from solely surgical "lifting and relocating" soft tissues to one of volume supplementation itself or in combination with lifting techniques for improved long-term results[4].

The use of autologous fat endures as a commonly accepted transplant material for correction of a wide variety of soft-tissue defects because of its biocompatibility, versatility, availability, similarity to like-tissue replacement, and point-of-care delivery in reconstructive and aesthetic surgery. The fate of using this ideal living tissue as a graft by traditional techniques, however, has been associated with unpredictable and inconsistent outcomes estimated between 25-90%, leading to variable degrees of necrosis, absorption, cyst formation, and fibrosis, necessitating over-correction and secondary procedures[5]. Despite cumulative information from years of numerous basic, preclinical translational and clinical pilot investigations, no consensus has been reached for an accepted evidence-base method to achieve superior and reliable clinical results after traditional fat grafting[6-7].

Within the past decade, two separate autologous cell approaches have emerged in the clinical setting as promising therapies to improve fat grafting. Of the two, the combination of adipose tissue either with adipose stem cells (ASCs) from stromal vascular fraction (SVF) or with isolated adipose-derived stem cells (ADSCs) isolated after SVF adherent-plating and culture expansion, has been the most controversial strategy[8-9]. In the last five years, surgeons reported both encouraging[10-12] and equivocal[13-14] outcomes with the SVF cell-assisted lipo-transfer (CAL) grafting method pioneered by Lull[15] and other clinicians[16-18].

The second less contentious clinical strategy is represented by the mixing of adipose tissue with platelet-rich plasma (PRP), which are concentrations of autologous platelets in small volumes of plasma[19]. Clinically, the use of PRPs may be considered a safer and more prudent pathway in cellular-adjunctive therapy, rather than the use of recombinant growth factors or even SVFs or ADSCs, to deal with the complexities of tissue repair and regeneration on the basis of its current safety profile and containment of multiple endogenous growth factors and adhesion molecules[20].

This clinical investigation was initiated to evaluate the safety and efficacy of stromal vascular fraction (SVF-assistance), platelet-rich plasma (PRP-assistance) and combined SVF + PRP-assistance in the maintenance of fat volume to the anterior mid-face on the basis of previously published case reports on successful facial fat grafting with the use of SVF-[21-22] and/or PRP-[23-24] assisted methods.

MATERIALS AND METHODS

Study overview: The single-center, prospective, case-controlled study investigated the safety and efficacy of combining a Baker-designed limited lateral SMASectomy (resection from Parotid Tail to over lateral aspect of malar eminence) or SMAS-plication face-lift[25] with simultaneous anterior mid-face fat grafting by 1) conventional Coleman's technique[26] or by 2) Yoshimura's CAL supplementation technique[10] mixed with 1) PRP, 2) SVF or 3) a combination of both cells at the author's AAAA-SF surgical center under local anesthesia from August 2010 to August 2014.

Patient Selection: Of over 1500 patients evaluated from August 2010 to August 2014, only 236 were qualified, provided informed consents and were categorized into four Study Groups. Primary inclusion criteria were candidates who never had facial surgery and presented with mild to severe ptosis of facial skin and fibro-muscular layers to the face and volume loss to the anterior mid-face. If candidates were indicated at the initial consultation for a lower lid blepharoplasty by fat excisional technique without anterior volumetric transposition, or a neck lift by complete skin undermining, medial platysmaplasty and lateral SMASectomy or plication, they were included in the study as these maneuvers would not have any significant impacts on topographical aging changes within the anterior mid-face. Secondary inclusion criteria were candidates who were more than two years from a previous facial surgery, including non-invasive lifting procedures, treatments with ablative and non-ablative skin procedures, temporary fillers and volumizers, or neurotoxins to the facial area. Primary exclusion criteria included candidates who presented with active systemic or local infections, acne or significant facial scarring, pregnancy, autoimmune diseases, uncontrolled diabetes mellitus and hypertension, altered wound healing, hemorrhagic disorders and hemostatic dysfunctions. Secondary exclusion criteria included candidates, who had undergone previous facial augmentation with boney and alloplastic implants, or had received permanent injectable fillers or volumizers. Patients evaluated and scheduled for endo-facelift, endo-laser facelift, extended-stacked SMAS/ sub-periosteal facelift), volumization only (fat/poly-L-lactic acid, dense HA material or facial implant), lower lid blepharoplasties with anterior transposition of fat, and non-surgical lifting and tightening techniques were excluded from participation in this study.

Informed Consent: All candidates were required to have at least three separate consultation sessions with the author, surgical charge-nurse and office manager until the candidate acknowledged that he/she was first fully informed and understood the offered procedures and, secondly, that the contemplated procedure would attempt to meet their clinical expectations and safety requirements. On the voluntary principle each candidate decided whether fat grafting would or would not be combined with their face-lift procedure. If a candidate decided on a fat grafting procedure, he/she was informed on the specific procedural aspects of each fat grafting procedures, the unique features of SVF and PRP cellular therapies, current FDA-status of devices and procedures related to SVF/PRP procurement and usage, safety and efficacy profiles, expectations and limitations of surgery, cost analyses, and the author's current or past disclosures as an investigator or consultant for any device employed in this study. Each candidate voluntarily selected one of four different techniques, which formed the basis for assignment into Group 1) conventional fat grafting, Group 2) PRP-assisted fat grafting, Group 3) SVF-assisted fat grafting, or Group 4) SVF + PRP-assisted fat grafting.

Each candidate was counseled that there would be only one fat grafting session without any additional fat grafting sessions during the one year study. In addition each candidate was informed that the same volume of fat per mid-face would be delivered but would be distributed according to the site-specific needs of depleted mid-face compartments for a customized result. If a subject desired to receive significantly more fat than allowed in this study, they were treated but were not included in the data

evaluation reported in this study. Each facelift candidate was informed that their muscle adjustment procedure would be a limited lateral SMASectomy or SMAS-plication in order to minimize its effects on the addition of fat grafting to their anterior midface. If a subject received an extended lateral SMAS procedure, they were treated but not included in the study. Follow-up was conducted every one to two weeks up to the postoperative 6th week and then every three months up to at least a year or longer to record any complications. Corrective procedures or management of any serious complications (bleeding, infections, nerve injuries, tissue loss) from surgery would be managed under the office guideline policies. The study protocol complied with the ethical guidelines of the 1975 Declaration of Helsinki with each candidate receiving a copy of the comprehensive written informed consent document.

Fat Harvesting and Processing: Fat was harvested from the hip rolls and/or anterior abdomen with the Coleman fat-grafting structural technique under local tumescent anesthesia with a solution containing 50ml 0.5% lidocaine, 1mg epinephrine, and 20ml of 8.4% sodium bicarbonate per liter of warm saline. Approximately 1.0-1.5ml of solution was infiltrated for every ml of fat to be harvested. After 20-30 min of vasoconstriction by epinephrine, syringe liposuction with a 3-4mm blunt tip Mercedes cannula collected the lipoaspirate in 60ml Toomey syringes prefilled with 20ml saline with the plungers withdrawn about 20ml to reduce as much as possible cellular injury from high negative vacuum pressures during aspiration. The filled syringes were capped and vertically positioned for at least thirty minutes in a standing-rack for initial gravitational separation of layers of oil, fat, and lipoaspirate fluid and debris. After decanting the fluid and oil portions, the collected fat was transferred to smaller syringes with a Luer-Lok plug attached to the distal end of the syringe to prevent spillage during centrifugation. The syringes were centrifuged for three minutes at a calculated G-force of less than 1200 g at a rotational speed between 3000-3200 rpms (Compact II Centrifuge, Becton Dickinson Co., Sparks, MD). After removal of oil and fluid layers, fat-filled syringes were recapped at the distal end with replacement of the plunger, placed in plastic bags, which were submerged in an ice water bath. The cooled fat was injected into predetermined site-specific compartments within 2-4 hours of preparation alone or combined with SVF preparations. In this small volume study, the grafting of low aqueous fat was critical to optimize graft-to-recipient site volume ratios within individual fat compartments and deliverance of a substantive real tissue volume rather than a sham fluid volume which would be quickly absorbed in the post-injection period. The donor sites underwent full traditional liposuction and contouring with a powered-assisted suction device (MicroAire® Surgical Instruments, Charlottesville, Virginia, USA) that utilized flared Mercedes 3.0mm cannulas.

Preparation and Injection of Platelet-Rich Plasma: For automated PRP isolation, an FDA-cleared device (SmartPreP2® System, Harvest Technologies Corp., Plymouth, MA, USA) was used to separate and concentrate a buffy-coat containing high yields of platelets. Fifty-four ml of whole blood was withdrawn from an antecubital arm vein mixing with six ml of ACD (adenosine-citrate-dextrose) within a 60ml syringe. Through floating-shelf space technology, one ml of platelet-poor plasma (PPP) was mixed with the buffy-coat pellet to a final approximate volume of 4mls of PRP. The capped PRP-filled syringe was placed in a plastic bag, which was submerged in an ice bath water bath ready for transdermal depot injections within 2-4 hours after preparation.

PRP Cell Counting Analyses: Since the final resuspension volume of the buffy coat by platelet-poor plasma (PPP) with the Harvest System could be varied, a smaller final volume of PPP was chosen to yield higher concentrations of platelets per microliter than would be obtained with larger recommended PPP volumes of 7-10mls. In this study, four ml of PRP was the selected final volume from each patient in order to distribute 2ml into each mid-face for comparison purposes. Aliquots from ACD-treated whole

blood and the 4ml PRP Harvest concentrate from five randomly selected patients within Group 2 and five randomly selected patients in Group 4 were transported overnight to Harvest Technologies for analyses of baseline platelet count from whole blood and baseline platelet cell Harvest concentration. The Coulter ACT DIFF 2 Series Analyzer™ (Beckman Coulter, Inc., Miami, Florida) sized and counted to derive a multiplication factor of number of platelets within the concentrated PRP samples. From this database, the baseline platelet concentration in 4ml of Harvest processed PRP (x 10^6 cells/µL) was calculated by multiplying the baseline mean platelet count (x 10^6cells/µL) by a factor of 10. Since the final prepared PRP volume was 4mls, the total number of platelets/ml could be derived by multiplying the baseline platelet concentration (10^6 cells/µL) by 4 to obtain the number of cells/ml (10^9 cells/ml) injected per site. Although the concentration of platelets are variable in healthy patients, the sampled counts in these ten patients represented 1) the range of values that may or may not provide an impact on their fat graft survival, and 2) provided a multiplication factor that was used in the non-sampled patients to obtain an estimated total cell concentration.

Preparation and Injection of SVF-Enhanced Autologous Fat Graft: For automated SFV isolation, a portion of the refined processed adipose tissue was loaded into the centrifuge Tissue Collection Container (maximum volume 360ml) of the Celution®900/MB System (Cytori Therapeutics, Inc., San Diego, CA, USA). After collagenase digestion, additional washes and centrifugation cycles, the SVF cell pellet was suspended in 4-5ml of solution. The SVF-filled syringe was capped with a Luer-Lok plug, placed in a plastic bag, which was immersed in the ice water bath until ready for use by uniform mixing with processed fat within 2-4 hours after preparation. The overall SVF extraction process was controlled through automatic sensors and processing algorithms for standard management of cells within 160 minutes.

SVF Cell Counting and Viability Analyses: In this study, the number as well as the viability of nucleated/nonnucleated cells in an aliquot of SVF were determined by an automated cell counter (Luna-STEM™, *logos* biosystems, Seoul, Korea) from five randomly selected patients undergoing SVF-assisted fat grafting in Groups 3 and from five randomly selected patients in Group 4. Although individual count can vary in healthy patients, the sampled counts from the ten patients would yield 1) their range of differences that may or may not provide a significant influence on graft survival, and 2) provide an estimation of total cell concentration that can be anticipated in the non-sampled patients with use of the Celution®system.

SURGICAL PROTOCOL

Preoperative Preparation: Before surgery, medical histories and surgical clearances, blood and chemistry panels, electrocardiograms, Body mass index (BMI), baseline high resolution digital facial photography and Vectra XT 3D Volumetric Analysis Imaging (Canfield Imaging, Fairfield, NJ, USA) were obtained. Oral antibiotics and pain medication were prescribed for ten days after surgery. Patients, who were at risk of viral infection, received a course of prophylactic antiviral medication up to six days after the procedure. Women of childbearing potential had a urine pregnancy test performed immediately before the procedure.

Surgical Markings: Since volume loss in the deep medial cheek fat compartment dominated differential facial deflation by promoting the formation of the tear-trough/nasojugal V-shaped deformity, markings first outlined its shape (Figure 1) by following the inferior border of the tear-trough sulcus and nasojugal groove and then curving superiorly outside of the nasolabial crease line and the nasomaxillary border, to the most medial part of the orbital rim, as defined by Rohrich and colleagues[27-28]. The outer border of

the medial sub-orbicularis fat compartment, located between the medial deep cheek fat and lateral sub-orbicularis fat compartments, was secondly outlined superiorly along the orbital rim to the lateral canthus and downward to the lower edge of the zygoma. Thirdly, the lateral sub-orbicularis fat compartment was marked from the lateral canthus to the lateral orbital thickening over the apex of the zygoma down to the inferior border of the zygoma. Of the superficial fat compartments, the nasolabial fold was most easily demarcated by visual inspection, while the medial fat compartment was outlined adjacent to the nasolabial fold spanning across the anterior midface to the middle cheek septum which corresponded to the vertical location of the zygomatic and parotido-masseteric ligaments. All fat within the deep and superficial fat compartments were located caudal to the orbital rim and the orbicularis retaining ligaments and lateral orbital thickening. Although the described locations and depths of these five compartments in the anterior mid-face were subject to variations, an attempt was made to create a practical treatment map based on the topographical volume loss in each of the compartments.

Graft Injection Technique:

After completion of the Baker limited SMAS-face lift under local anesthesia, about 3ml of a buffered lidocaine and epinephrine-containing solution in tuberculin syringes was distributed into the anterior mid-face targeting the five fat compartments through an entry point at the oral commissure. For patients in Groups 1 and 2, twenty mls of centrifuged fat were loaded into 1ml syringes for intended use in both mid-faces. Each mid-face received as close as possible a total volume of 10ml of centrifuged fat that was distributed into five mid-face compartments (deep medial cheek fat, medial sub-orbicularis fat, lateral sub-orbicularis fat, superficial nasolabial fat, superficial medial fat). Although each anterior mid-face could accept more than 10ml, the final volume was limited to nearly 10ml by protocol design with no attempt for overcorrection for comparison of data. For patients in Groups 3 and 4, twenty ml of centrifuged fat were thoroughly mixed with the entire 5ml volume of prepared SFV and loaded in 1ml syringes for both mid-faces of patients. Each mid-face of patients in Groups 3 and 4 received as close as possible a maximum volume of 12.5ml of mixed SVF/fat that was distributed in the same five mid-face compartments of patients in Groups 1 and 2. In all patients, an 18-gauge needle punctured the skin at the oral commissure for insertion of 1.3mm blunt cannula attached to a 1ml syringe filled with fat or enhanced fat combined with SVF cells. A fingertip was always positioned along the orbital rim to prevent cannula penetration into the subfascial space under the septal portion of the orbicularis muscle. The cannula was advanced first into the deep medial cheek fat compartment until the tip was palpated within the tear-trough deformity. Using retrograde injection technique, droplets were deposited in the channel of the retreating cannula at a slow flow rate. Without withdrawing the cannula, multiple injection passes placed aliquots in a fan-shape pattern from deeper to superficial planes within each compartment under bi-manual control. The same filling technique was repeated in the medial suborbicularis, and then the lateral suborbicularis deep compartments. As each deep compartment was filled in turn, the degree of correction to the tear-trough/nasojugal depression and volumization of atrophied compartments was assessed in order to alter subsequent fillings as changes occurred. Lastly, filling of the superficial nasolabial and medial compartments completed the grafting of the anterior mid-face compartments. As the need arose, a needle puncture to the lateral cheek was done for insertion of the same cannula for deposition of fat transversely below the orbital rim to treat any deficiencies inferior to the origin of the orbital malar ligaments at the orbital rim that was not adequately fill from the commissure. For patients in Groups 2 and 4, approximately 0.4ml aliquots of PRP were injected transdermally into each of the previously filled deep and superficial compartments. Transdermal depot injections of a small volume of PRP after fat grafting to the mid-face was intentional to maximize the volume of fat delivered into the selected recipient sites with respect to graft-to-capacity ratios. The injected sites were gently massaged and covered with a cold facial pack. An antibiotic ointment covered

the puncture sites. No external dressings were applied in the post-operative period. Patients were permitted to shower daily to the facial areas the day after surgery. Patient were asked to refrain from returning to normal activities for at least three to six weeks.

Clinical and 3D Vectra Evaluation and Follow up

All patients were seen on postop days 1 and 10 and at the 3rd week, and every three months thereafter for a year for clinical evaluation of graft persistence. Patients underwent standardized photography in a controlled light setting and in standardized positions at baseline, 3, 6, 9 and 12 months. The same medical photographer acquired and registered landmarks on the baseline 3D "mask" Vectra 3D Analysis Imaging (Canfield Imaging Systems, Fairfield, NJ) on each patient which was standardized to cover the diameter of 75mm selection of a sphere on the mid-face occupied by the five compartments at each evaluation session. The volumes were determined in milliliters at the latest follow up, from which volume changes were calculated as percent change from the baseline volume in each mid-face. Since the volume changes registered by the mirror software were nearly identical on each side at every assessment period, the per cent changes from both sides were averaged for statistical analysis at each time point.

***Statistical* Analysis**

The data was subjected to 1-way analysis of variance tests to test for significance between groups and for homogeneity of variances, respectively. Lastly, the data were submitted for Anova testing analyzing multiple comparisons within the groups of mean differences, standard error, and 95% significance at the $p < 0.01$ level.

RESULTS

Two hundred and thirty-six patients underwent a limited lateral SMASectomy or rarely SMAS-plication face lift procedure with autologous fat grafting alone or combinations with enhanced cells. As listed in Table 1, females (96.2%) of Caucasian ethnicity (81.8%) composed the majority of patients. The average ages and BMIs of patients in each group were nearly alike, but the distribution range of these parameter within each group exhibited variable spreads. As tabulated in Table 2, the total mean volume of processed fat and its distribution into each of the three deep compartments and the two superficial compartments in each side of the anterior mid-face of patients in Groups 1-4 were kept as close to 10ml for comparison of data. The average total volume of PRP, injected in depot aliquots of approximately 0.4ml/compartments/mid-face, was kept as equal as possible between patients in Group 2 and 4. The average total volume of SVF, combined with the processed fat, was nearly the same between patients in Group 3 and 4. The average volume of fat injected into each compartment was distributed according to need for optimal correction of the site-specific atrophy and resultant depressions. For example, patients, who presented with deeper tear-trough/nasojugal depressions received larger volume replacements up to 2.5ml in the deep medial cheek and adjacent medial suborbicularis compartments until full-correction was achieved. Other patients, who desired more lateral cheek projection, were injected with a larger volume to the lateral suborbicularis compartment. Since patients in Group III and IV received an additional volume of SVF mixed with their fat for each mid-face, their total injected average volumes were greater than the average volumes injected in Groups I and II, but all groups received close to 10ml of fat. The addition of PRPs by depot injections into the already fat-filled compartments in patients within Groups 2 and 4 did not affect the amount of fat delivered per site.

In Table 3, a range of volumes of fat in Group 3 and 4 were added to the Celution™device that, in turn, required the addition of differing amounts of digestive reagents. Despite these variances, the final derived SVF volumes, average nucleated cell counts, and percent viability of cells were nearly the same inspite of the variable range of values. Moreover, the ratio of SVF volume to fat volume was identical for both groups. The delivered volume of fat and SFV, however, varied slightly amongst the compartments due to the estimation of clinical fat wasting and judgment of volume replacement.

In Table 4, the average baseline levels of platelets in whole blood, average baseline Harvest platelet concentrations in 4mls of PRP), final average PRP volumes, calculated average number of platelets/ml in the concentrate, and PRP:Fat ratios (1:5;1:5) were closely alike for use in patients within Group 3 and Group 4 with the observed variable range of values. The delivered volume of PRP and injected cell numbers varied little from compartment to compartment.

In Table 5, the average percent change in mean volume retention, as profiled by Vectra Analysis in the Group 1 patients (fat), demonstrated a marked decline from baseline value at three months and progressively became less at a year. In Table 6, the average percent change in mean volume retention in the Group 2 patients (fat/PRP) also exhibited a marked reduction from baseline values at three months but gradually exhibited a positive recovery at a year ($p < 0.01$ from control group at one year). In Table 7, the average percent change in mean volume retention, as profiled in the Group 3 patients (fat/SVF), closely resembled the decline at three months in the PRP-treated patients in Group 2 with a similar positive recovery at a year ($p < 0.01$ from control group at one year). In Table 8, the average percent change in mean volume retention, as shown in the Group 4 patients (fat/SVF/PRP), displayed the same substantial reduction at three months but, in contrast to the recovery slopes in Groups 2 and 3, exhibited an irregular and lower final volume retention at one year ($p < 0.01$ from control group at one year). All four groups demonstrated a reduction of volume at the third month, but only the cell-assisted groups recovered slowly to graft stability from the sixth to twelve month period. At twelve months, there was no statistically significant difference in the mean percent changes by Vectra Analysis in Groups 2, 3, and 4. There was no clear explanation for the lower percent mean volume retention at 9 months compared to a higher percent mean volume retention at 6 month in Group 4, as no obvious deviations from protocol were found.

In the post-operative evaluation sessions beyond 6 weeks, none of the patients demonstrated chronic edema, calcifications, nodularities, dysethesias, muscle weakness or paralysis. All patients experienced transient swelling to the face and induration lasting between 2-3 weeks until full recovery. There were no recorded incidences of tissue loss, hematomas or infections. Less than 2% of patients commented on their mild volumetric asymmetries from grafting and declined further surgical or non-surgical corrective intervention. Over 90-95% of patients were satisfied with their results (Figures 2, 3, and 4) and did not request additional fat grafting or with other FDA-cleared materials at the end of the study period. At least 50% of patients in each treatment group have been followed for over 2 years since inception. About 50% of these long-term patients maintained their 1 year volumes for an additional year follow up, but about 10 -25%of them exhibited a 10-15% volume reduction beyond a year.

DISCUSSION

This twelve month clinical study by one surgeon assessed the survivability and retention of nearly similar volumes of fat alone or in combination with regenerative cells to defined compartments of the anterior mid-face in a relatively large cohort of patients in his private practice. The author attempted to control variables by standardizing protocol design for comparison of data in order to obtain evidence-based

data in a clinical setting over a one year period. By 3D Vector Analysis (Tables 5-8), PRP, SVF, or PRP/SVF cell supplementation demonstrated statistically significant percent mean graft retention over its baseline values and also at the twelve month evaluation period in comparison to the assessed volumes in the control ($P < 0.01$). The use of either PRP or SVF alone resulted in almost equal outcomes of graft enhancement. Of interest, the use of combining cell populations provided no synergistic or additive advantages over single cellular therapy. All transplanted tissue with or without supplemented cells exhibited mean percent volume reductions at an early stage up to three months. The presence of enhanced cells did not significantly reduce or prevent the extent of volume decline. The mechanism(s) postulated for initial reduction of volume have included resorption of sham fluid and non-viable cells. The final destiny of the retained fat graft is believed to be determined by the immediate availability of diffused nutrients and, secondly, by rapid neo-vascularization to reduce further apoptotic death, accelerate recovery, and facilitate potential cellular differentiation and proliferation[29-30].

Experimental studies[31] have demonstrated that activated platelets release a number of potent growth factors whose effects are believed to be responsible for angiogenesis[32], stem cell proliferation and differentiation[33-34], and anti-apoptotic properties[35]. Furthermore, Giusti's *in vitro* dose-dependent study[36] indicated the optimal concentration of activated platelets in PRP to stimulate angiogenesis occurred between 1.5 - 3.0 x 10^6 cell/μL, which coincided with the values obtained in patients within Group 2 and Group 4. In the clinical setting, however, evidence-base data for PRP efficacy has been difficult to obtain because earlier pilot studies have been anecdotal, conducted without randomized controls, or were inadequately powered[37]. After using a search filter for all PRP publications up to July 2011, Sommeling and colleagues[38] in 2013 extrapolated a total of only nineteen relevant clinically randomized or case-controlled studies on topics related to plastic surgery. Positive outcome studies with PRP-usage included eight of nine studies with improved wound healing rates, five studies with enhancement of bone graft regeneration, but only one of five publications with increased survival rate of facial fat grafting[39]. In another recent Pubmed-review from inception to October 2012, Jin and colleagues[40] assessed positive outcomes after PRP-assisted fat grafting in only one (breast reconstruction) of two clinical case-controlled trials and four of five controlled animal investigations. Thus far, a critical and comprehensive reading of the PRP-clinical literature leads to the conclusion that designed randomized controlled trials are needed to demonstrate unambiguous clinical outcomes, notwithstanding the promising results observed with *in vitro*[41] and translational animal model investigations[38].

Explanations for inconsistent reported long-term clinical results with SVF preparations, as observed with PRP outcomes, remain unclear particularly in view of the many positive findings from studies that demonstrated adipose stem cells' capabilities to secrete an array of proangiogenic and anti-apoptotic factors[42-43], differentiate and proliferate into adipocytes and vascular cells[44-45], and promote angiogenesis[46-48] that are believed to bring about increased fat survival. The SVF cell-supplemented graft volumes in Group 3 exhibited a gradual increase in percent mean volume recovery between three and twelve months. The nucleated cell counts in the Celution®-derived SVFs from five sampled patients within Group 3, averaged 2.0 x 10^5ASCs/ml prior to fat combination in an SVF:Fat ratio of 1:4. The sampled nucleated cell count values would appear to favor a sustained graft survival in these patients, as similar values of 3 x 10^5ASCs/ml vs 1.5 -3.0 x 10^6ASCs/ml from human donor fat were found to be optimal for graft survival in Kakudo's murine study[49] that also utilized the Celution™ system. Although patients in Group 3 achieved positive outcomes, the data was difficult to evaluate because of the small enrollment number. Factors that deterred patient selection for this method were unclear, but might be related to the relatively higher costs for cell procurement and its uncertain regulatory status.

The findings in PRP/SVF-assisted Group 4 were of great interest because its volume recovery pattern was similar to those observed in the PRP-assisted and SVF-assisted groups between 3 months to a year. One would have anticipated in this group the most consistent and greatest mean volume recovery because of the combined quantitative and qualitative synergistic effects of cytokines released by both cell populations. Currently, an explanation for this observation remains unclear as cellular optimization was

achieved with a PRP concentration around 2.3 x 10^6/μL (PRP:Fat ratio 1:5) and nucleated cell concentration about 2.3 x 10^5ASCs/ml (SVF:Fat ratio 1:4). It can be postulated that an anti-adipogenic signaling and transcriptional mechanisms may be in play to regulate excess adipocyte stimulation and replacement. Previous studies[36,49]have suggested the presence of such exquisitely balanced negative feed-back mechanisms by cytokine and bioactive molecules to control adipocyte proliferation and activity.

The scientific-clinical community has acknowledged that the cost, safety and procurement of SVF by any devices and laboratories, engaged in the isolation, proliferation or differentiation of ASCs prior to introduction into a patient in the United States, require the oversight of the Federal Drug Administration (FDA) with regards to more-than-minimal manipulation under Good Manufacturing Practices (GMPs).[50] Observations that ACSs have the immunosuppressive capacity to promote tumor growth or transformation in experimental models[51-53] emphasize the need for caution, even in the observed absence of any oncologic documentation to date in the clinical setting.[54] Currently, there exist no FDA-approved ASC isolation devices, procedures, or clinics in the United States for use in medical conditions. Thus far, the procurement and use of PRP "as is" from a 510K-cleared FDA device are classified in the "less than minimally manipulated" category. Unlike ASCs, there are no regulations by the FDA on the use of PRPs based on the degree of its manipulation and risk of adverse-related events to date. However, subtle changes in the federal drug code (21 CFR 1271.1) in 2004, when applied to activated PRP with calcium and/or thrombin, might classify this altered autologous tissue in the "more than minimally manipulated" category and therefore under the regulation of the FDA.[55]

In summary, the variable individual rates of absorption and maintenance recorded at each assessment interval in each of the cell-enhanced groups underscored the limitations of this study, as evidenced by our incomplete understanding of the influences conferred by the quantity and quality of ASCs and platelets, synergism or antagonism of released cytokine growth factors, characteristics of the recipient bed, and even subtle technical deviations from a "standardized" delivery method amongst patients. Moreover, the implementation of contemporary traditional fat grafting techniques in this study to capitalize on the unique characteristics of the cellular components of donor-site tissues, the perceived advantages of different methods of harvesting, processing, technical aspects of lipo-injection, and preparation of the recipient sites themselves to achieve more reliable long-term outcomes was disappointing. In addition the Vectra 3D technique represented an indirect technology with limitations to improper registration introducing errors in measuring volume changes of shifting facial tissues during simultaneous fat grafting in comparison to finding with MRI studies. The use of magnetic resonance imaging in future studies may provide a more objective, but expensive and time-consuming assessment of volume retention. An additional larger and longer prospective half-face IRB-study that includes comparative fat graft survival with regenerative cells at different concentrations and fat-ratios in different regions of the face may provide further evidence-based knowledge.

CONCLUSIONS

Autologous fat grafting continues as a useful adjunct in facial aesthetic surgery despite unpredictable retention rates and unintentional uncommon sequelae that range from fat nodules, asymmetrical maintenance volumes, and hypertrophic and hyperplastic changes with weight gain. Although the mechanisms responsible for fate of fat grafting are unclear, numerous methodologies and algorithms have been suggested to enhance its survival that have yet to coalesce into a universally accepted treatment regimen. In recent years, research has established that stem cells and platelets are capable of repairing and regenerating injured and normal tissue. Although this study demonstrated prolonged maintenance of fat grafting to site-specific compartments in the anterior mid-face with enhanced cells over control, further procedural refinements, standardization of technique, controlled randomized studies with regulatory approval, and a sophisticated understanding of the biology of fat cell regulation will be required to provide evidence-based support in this exciting new field. Current Investigation into defining optimal enhanced cell to fat ratios and determining the fate of graft survival within the individual injected compartments is under study. On the basis of the findings in this clinical study, the author would recommend the injection of non-activated PRPs to processed fat in-situ rather than SVF-CAL techniques in accordance to the FDA's position of minimally-manipulated procedures.

References:

1. Pessa JE. An algorithm of facial aging: verification of Lambros's theory by three-dimensional stereolithography, with reference to the pathogenesis of midfacial aging, scleral show and the lateral suborbital trough deformity. Plast Reconstr Surg. 2000;106(2):479-488.
2. Camp M, Filip ZA, Wong W, et al. A novel three-dimensional analysis of periobital facial aging. Plast Reconstr Surg. 2009;124(4S):41-42.
3. Rohrich RJ, Arbique GM, Wong C, et al. The anatomy of sub-orbicularis oculi fat: implications for periorbital rejuvenation. Plast Reconstr Surg. 2009;124:946-951.
4. Rohrich RJ, Ghavami A, Constantine FC, et al. Lift-and-Fill: Integrating the fat compartments. Plast Reconstr Surg. 2014;133:756e-767e.
5. Kaufman MR, Miller TA, Huang C, et al. Autologous fat transfer for facial contouring: Is there science behind the art? Plast Reconstr Surg. 2007;119:2287-2297.
6. Kaufman MR, Bradley JP, Dickinson B, et al. Autologous fat transfer national consensus survey: trends in techniques for harvest, preparation, and application, and perception of short-and long-term results. Plast Reconstr Surg. 2007;119:323-331.
7. Gir P, Brown SA, Oni G, et al. Fat grafting: evidence-based review on autologous fat harvesting, processing, reinjection, and storage. Plast Reconstr Surg. 2012;130:249-258.
8. Zuk PA, Zhu M, Mizuno H, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng. 2001;7:211-228.
9. Daher SR, Johnstone BH, Phinney DG, et al. Adipose stromal/stem cells: basic and translational advances: the IFATS collection. Stem Cells. 2008;26:2664-2665.
10. Yoshimura K, Sato K, Aoi N, et al. Cell-assisted lipotransfer for facial lipoatrophy: Efficacy of clinical use of adipose-derived stem cells. Dermatol Surg. 2008;34:1178-1185.
11. Chang Q, Li J, Dong, et al. Quantitative volumetric analysis of progressive hemifacial atrophy corrected using stromal vascular fraction- Supplemented autologous fat grafts. Dermatol Surg. 2013;39:1465-1473.
12. Li J, Gao J, Cha P, et al. Supplementing fat grafts with adipose stromal cells for cosmetic facial contouring. Dermatol Surg. 2013;39:449-456.
13. Peltoniemi HH, Salmi A, Miettinen S, et al. Stem cell enrichment does not warrant a higher graft survival in lipofilling of the breast: A prospective comparative study. J Plast Reconstr Aesth Surg. 2013;66:1494-1503.

14. Sterodemas A, de Faria J, Nicaretta B, et al. Autologous fat transplantation versus adipose-derived stem cell-enriched lipografts: A study. Aesthet Surg J. 2011;31(6):682-693.
15. Llull, R. Cited as Personal Communication (2005) in manuscript by Moseley TA, Zhu M, Hedrick MH. Adipose-derived stem and progenitor cells as fillers in plastic and reconstructive surgery. Plast Reconstr Surg. 118 (Suppl.):121S, 2006.
16. Cohen SR, Mailey B. Adipocyte-derived stem and regenerative cells in facial rejuvenation. Clin Plastic Surg. 2012;39:453-464.
17. Tiryaki T, Findikli, Tiryaki D. Staged stem cell-enriched tissue (SET) injections for soft tissue augmentation in hostile recipient areas: A preliminary report. Aesth Plast Surg. 2011;35:965-971.
18. Yoshimura K, Sato K, Aoi N, et al. Cell-assisted lipotransfer (CAL) for cosmetic breast augmentation-supportive use of adipose-derived stem/stromal cells. Aesth Plast Surg. 2008;32(1):48-55.
19. Marx RE. Platelet-rich plasma: evidence to support its use. J Oral Maxillofac Surg. 2004;62:489-496.
20. Sanchez AR, Sheridan PJ, Kupp LI. Is platelet-rich plasma the perfect enhancement factor? A current review. Int J Oral Maxillofac Implants. 2003;18:93-103.
21. Sterodimas A, de Faria J, Nicaretta B, et al. Tissue engineering with adipose-derived stem cells (ADSCs): Current and future applications. J Plast Reconstr Aesth Surg. 2010;63:1886-1892.
22. Choi J, Minn KW, Chang H. The efficacy and safety of platelet-rich plasma and adipose-derived stem cells: An update. Arch Plast Surg. 2012;39:585-592.
23. Sadati KS, Corrado AC, Alexander RW. Platelet-rich plasma (PRP) utilized to promote greater graft volume retention in autologous fat grafting. Am J Cosmet Surg. 2006;23:203-211.
24. Cervilli V, Gentile P, Scioli MG, et al. Application of platelet-rich plasma in plastic surgery: Clinical and *in vitro* evaluation. Tissue Eng Part C Methods. 2009;15:625-634.
25. Baker DC. Minimal incision rhytidectomy (Short scar face lift) with lateral SMASectomy: Evolution and application. Aesth Surg J. 2001;21(1):14-26.
26. Coleman SR. Facial augmentation with structural fat grafting. Clin Plast Surg. 2006;33:567-577.
27. Rohrich RJ, Pessa JE. The fat compartments of the face: Anatomy and clinical implications for cosmetic surgery. Plast Reconstr Surg. 2007;119:2219-2227.
28. Rohrich RJ, Pessa JE, Ristow B. The youthful cheek and the deep medial fat compartment. Plast Reconstr Surg. 2008;121(6):2107-2122.
29. Billings E Jr, May JW Jr. Historical review and present status of free fat graft autotransplantation in plastic and reconstructive surgery. Plast Reconstr Surg. 1990;85:378-386.
30. Ogawa R. The importance of adipose-derived stem cells and vascularized tissue regeneration in the field of tissue transplantation. Curr Stem Cell Res Ther. 2006.;1:13-20.
31. Rendu F, Brohard-Bohn B. The platelet release reaction: granules' constituents, secretion and function. Platelets. 2001;12:261-273.
32. Bir SC, Esaki J, Marui A, et al. Angiogenic properties of sustained release platelet-rich plasma: characterization *in-vitro* and in the ischemic hind limb of the mouse. J Vasc Surg. 2009;50:870-879.e2
33. Kakudo N, Minakata T, Mitsui T, et al. Proliferation-promoting effect of platelet-rich plasma on human adipose-derived stem cells and human dermal fibroblasts. Plast Reconstr Surg. 2008;122(5):1352-1360.
34. Cervelli V, Scioli MG, Gentile P, et al. Platelet-rich plasma greatly potentiates insulin-induced adipogenic differentiation of human adipose-derived stem cells through a serine/threonine kinase Akt-dependent mechanism and promotes clinical fat graft maintenance. Stem Cells Transl Med. 2012;1(3):206-220.
35. Fukaya Y, Kuroda M, Aoyagi Y, et al. Platelet-rich plasma inhibits the apoptosis of highly adipogenic homogeneous preadipocytes in an *in vitro* culture system. Exptl Mol Med. 2012;44(5);330-339.

36. Giusti I. Rughetti A, D'Ascenzo, et al. Identification of an optimal concentration of platelet gel for promoting angiogenesis in human endothelial cells. Transfusion. 2009;49:771-778.
37. Freshwater MF. The use of platelet-rich plasma in plastic surgery remains unproven. [Invited Commentary] J Plast Reconstr Aesthet Surg. 2013;66(3):311.
38. Sommeling CE, Heyneman A, Hoeksema H, et al. The use of platelet-rich plasma in plastic surgery: A systematic review. J Plast Reconstr Aesthet Surg. 2013;66:301-312.
39. Cevilli V, Gentile P, Scioli MG, et al. Application of platelet-rich plasma in plastic surgery: Clinical and *in vitro* evaluation. Tissue Eng Part C Methods. 200915:625-619.
40. Jin R, Zjang :. Zhang Y-G. Does platelet-rich plasma enhance the survival of grafted fat? An update review. Int J Clin Exp Med. 2013;6(4):252-258.
41. Kakudo N, Minakata T, Mitsui T, et al. Proliferation-promoting effect of platelet-rich plasma on human adipose-derived stem cells and human dermal fibroblasts. Plast Reconstr Surg. 2008;122(5):1352-1360.
42. Rehman J, Traktuev D, Li J, et al. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. Circulation. 2004;109:1292-1298.
43. Kilroy GE, Foster SJ, Wu X, et al. Cytokine profile of human adipose –derived stem cells: expression of angiogenic, hematopoietic, and pro-inflammatory factors. J Cell Physiol. 2007;212:702-709.
44. Tholpady SS, Aojanepong C, Llull R, et al. The cellular plasticity of human adipocytes. Ann Plast Surg. 2005;54:651656.
45. Thangarajah H, Vial IN, Chang E, et al. IFATS series: adipose stromal cells adopt a proangiogenic phenotype under the influence of hypoxia. Stem Cells. 2009;27:266-274.
46. Traktuev DO, Prater DN, Merfeld-Clauss S, et al. Robust functional vascular network formation *in vivo* by cooperation of adipose progenitor and endothelial cells. Circ Res. 2009;104:1410-1420.
47. Merfeld-Clauss S, Gollahalli N, March KL, et al. Adipose tissue progenitor cells directly interact with endothelial cells to induce vascular network formation. Tissue Eng Part A. 2010;16:2953-2966.
48. Song SY, Chung HM, Sung JH. The pivotal role of VEGF in adipose-derived-stem cell-mediated regulation. Expert Opin Biol Ther. 2010;10(11):1529-1537.
49. Kakudo N, Tanaka Y, Morimoto A, et al. Adipose-derived regenerative (ADRC)-enriched fat grafting" optimal cell concentration and effects on grafted fat characteristics. J Transl Med. 2013;11:254-262.
50. Halme DG, Kessler DA. FDA regulation of stem cell-based therapies. The New England Journal of Medicine. 2006;355:1730-1735.
51. Yu JM, Jun ES, Bae YC, et al. Mesenchymal stem cells derived from human adipose tissues favor tumor cell growth *in vivo*. Stem Cells and Development. 2008;17:463-473.
52. Muehlberg FL, Song YH, Krohn A, et al. Tissue-resident stem cells promote cancer growth and metastases. Carcinogenisis. 2009;30:589-597.
53. Kuhbier JW, Bucan V, Reimers K, et al. Observed changes in the morphology and phenotype of breast cancer cells in direct co-culture with adipose-derived stem cells. Plast Reconstr Surg. 2014;134:414-423.
54. Casiraghi F, Remuzzi G, Abbate M, et al. Multipotent mesenchymal stromal cell therapy and risk of malignancies. Stem Cell Rev. 2013;9:656-79.
55. Centeno CJ, Fuerst M, Faulkner SJ, et al. Is cosmetic platelet-rick plasma a drug to be regulated by the Food and Drug Administration? J Cosmet Dermatol. 2011;10:171-173.

LEGENDS

Figure 1. Panel A (left). Diagrammatic location and outline of the three key deep compartments (deep medial cheek fat compartment, medial sub-orbicularis fat compartment and lateral sub-

orbicularis fat compartment) and two superficial compartments (nasolabial compartment, medial fat compartment) of the anterior mid-face. Panel B (right). Preoperative outlines of the deep and superficial compartments as a practical treatment map based on the assessed topographical volume depletion within each compartment.

Figure 2. Panel A (left). This 50 year old presented with a residual left Bell's palsy, brow ptosis, pseudo-herniated fat to lower lids, mid-face fat atrophy, tear-trough and nasojugal hollows, marionette folds, jowls and platysmal neck bands. The patient underwent a Baker-designed mid-face lift with lateral SMAS resection, palmaris longus tendon sling to the left oral commissure, and total neck lift with medial and lateral platysmaplasty. After skin closure, a total of 10ml of processed fat was distributed in 2ml allotments into each of the five key compartments of each anterior mid-face. Thereafter, 0.4ml of concentrated PRP was depot-deposited into each of the five fat compartments. Panel B (right). At one year, the patient demonstrated improved elevation and symmetry to the brow complexes, smooth contours to the lower lids, maintenance of symmetrical fullness within the mid-face facial fat compartments, and correction of the ptotic face and neck.

Figure 3. Panel A (left). This 71 year old presented with significant anterior mid-face fat atrophy, pronounced nasojugal, nasolabial and marionette folds with SMAS laxity to the face and neck. The patient underwent a Baker-designed limited SMASectomy mid-face lift and total neck with medial platysmaplasty and lateral resection of the platysmal muscles. Five ml of SVF was uniformly mixed with 20ml of processed fat by the Coleman technique after skin closure. A total of 12.5ml of SVF-assisted fat was lipotransferred into each anterior mid-face by distributing 2.0-2.5ml portions into each of the five key compartments of each anterior mid-face. Panel B (right). At one year, the patient showed volume maintenance to the mid-face fat compartments in addition to SMAS-skin correction to her face and neck.

Figure 4. Panel A (left). This 59 year old sought improvement to her moderate mid-face fat atrophy and naso-jugal folds, moderate ptosis to her nasolabial and marionette folds, and SMAS laxity to the plastysma neck muscles. The patient had a Baker-designed lateral SMASectomy mid-face lift and total neck with medial platysmaplasty and lateral resection of the platysmal muscle, including and upper and lower lid blepharoplasty. Prior to skin closure, 5ml of SVF was uniformly mixed with 20ml of processed fat by the Coleman technique. A total of 12.5ml of SVF-assisted fat was injected into each of five fat compartments of the anterior mid-face. Thereafter, 0.4ml of concentrated PRP was depot-deposited into the same five fat injected compartments. Panel B (right). At one year, demonstrated volume retention to the mid-face fat compartments in addition to SMAS-skin adjustment to her face and neck.

Table 1. Demographic data of number and sex of patients, average and range distribution of ethnic backgrounds, ages, weights, heights and BMIs within each of the four treatment groups.

Table 2. The average and range of total processed fat, PRP and SVF injected into each mid-face and average fat distributions within individual deep and superficial compartments for each of four treatment groups.

Table 3. The mean ± SD and range of volumes of processed fat, collagenase reagent, and SVF, including the average number of live nucleated cells, percent viabilities and SVF/Fat ratios in Group 3 and Group 4.

Table 4. The mean ± SD and range of baseline numbers of platelets in whole blood and in 4ml of platelet concentrates, including final PRP volumes, number of platelets/ml injected and PRP/Fat ratios in Group 2 and Group 4.

Table 5. The mean percentage ± SD and range of volume maintenance, compared to baseline values, by 3D Vectra Analyses over 1 year follow up after traditional Coleman fat grafting to the five compartments in the anterior mid-face.

Table 6. The mean percentage ± SD and range volume maintenance, compared to baseline values, by 3D Vectra Analyses over 1 year follow up after traditional Coleman fat grafting and depot PRP injections to five fat compartments in the anterior mid-face.

Table 7. The mean percentage ± SD and range of volume maintenance, compared to baseline values, by 3D Vectra Analyses over l year follow up after traditional Coleman fat grafting mixed with SFV to five fat compartments in the anterior mid-face.

Table 8. The mean percentage ± SD and range of volume maintenance, compared to baseline values, by 3D Vectra Analyses over 1 year follow up after traditional Coleman fat grafting mixed with SFV and depot PRP injections into five fat compartments in the anterior mid-face.

ABSTRACT

Background: Numerous methodologies and algorithms have been suggested to enhance fat graft survival, including the usage of stromal vascular fraction (SVF) and platelet-rich plasma (PRP), but no long-term studies are available.
Objectives: This is a single-center prospective, case-controlled study investigated the safety and efficacy of combining a modified Baker-designed lateral SMASectomy or plication face-lift with simultaneous anterior mid-face grafting into site-specific compartments by 1) conventional Coleman's technique or 2) Yoshimura's cell-assisted lipografting (CAL technique.
Methods: On the voluntary principle, candidates selected one of four techniques for volumization of their mid-face: conventional fat grafting; PRP-assisted fat grafting; SVF-assisted fat grafting; and PRP/SVF- assisted fat grafting. For comparison data, comparable fat volumes, SVF volumes and nucleated cells, and PRP volumes and platelet concentrations were injected into each designated

group. Indirect volume retentions were determined by standardized Vectra 3D analyses up to 1 year.
Results: PRP, SVF and PRP/SVF cell supplementation of processed fat resulted in statistically significant percent mean graft retention over their baseline control at 12 months ($p < 0.01$). The use of either PRP or SVF alone resulted in almost equal outcomes. Combining cell populations provided no additional advantage over single cellular therapy. Complications were negligible.

Conclusion: Autologous fat grafting continues to be a viable adjunct in facial aesthetic surgery. With refinements in the entire grafting process and the potential benefits of autologous cell approaches with stromal vascular fractions and platelet-rich plasma, future evidence-based controlled studies under regulatory approval may improve graft survival in a safe and effective manner.

Level of Evidence: 3

Keywords: autologous fat transfer, stromal vascular fraction, adipose-derived stem cells, platelet-rich plasma

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