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The Addition of Tissue Stromal Vascular Fraction to Platelet-Rich Plasma Supplemented Lipofilling Does Not Improve Facial Skin Quality: A Prospective Randomized Clinical Trial

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Abstract

Background: Lipofilling has become popular as a treatment to improve aging-related skin characteristics (eg, wrinkles, pigmentation spots, pores, or rosacea). Different additives such as platelet-rich plasma (PRP) or stromal vascular fraction (SVF) have been combined with lipofilling to increase the therapeutic effect of adipose-derived stromal cells (ASCs).

Objectives: The aim of this study was to examine the hypothesis that mechanically isolated SVF augments the therapeutic effect of PRP-supplemented lipofilling to improve facial skin quality.

Methods: This prospective, double-blind, placebo-controlled, randomized trial was conducted between 2016 and 2019. In total, 28 female subjects were enrolled; 25 completed the follow-up. All patients received PRP-supplemented lipofilling with either mechanically isolated SVF or saline. SVF was isolated by fractionation of adipose tissue (tSVF). Results were evaluated by changes in skin elasticity and transepidermal water loss, changes in skin-aging-related features, ie, superficial spots, wrinkles, skin texture, pores, vascularity, and pigmentation, as well as patient satisfaction (FACE-Q), recovery, and number of complications up to 1 year postoperative.

Results: The addition of tSVF to PRP-supplemented lipofilling did not improve skin elasticity, transepidermal water loss, or skin-aging-related features. No improvement in patient satisfaction with overall facial appearance or facial skin quality was seen when tSVF was added to PRP-supplemented lipofilling.

Conclusions: In comparison to PRP-supplemented lipofilling, PRP-supplemented lipofilling combined with tSVF does not improve facial skin quality or patient satisfaction in a healthy population. PRP-supplemented lipofilling combined with tSVF can be considered a safe procedure.

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Lipofilling has rapidly become a popular treatment modality for facial rejuvenation to restore loss of volume and to decrease aging-related changes to the skin (eg, wrinkles, pigmentation spots, pores, or rosacea).¹ In the literature, these effects are mainly ascribed to the presence of adipose-tissue-derived stromal cells (ASCs) which reside in the stromal vascular fraction (SVF) of adipose tissue. The precursors of (cultured) ASCs are attached around vessels as perivascular cells and pericytes.^{2,3} ASCs secrete a plethora of growth factors, cytokines, and proteins which can enhance tissue regeneration based on angiogenesis and matrix remodeling.^{4,5} In this way, autologous lipofilling might reverse loss of facial skin elasticity.

To enhance the regenerative effects of lipofilling, different additives have been advocated, including platelet-rich plasma (PRP) or SVF to increase the number of ASCs. SVF can be isolated by means of enzymatic or mechanical isolation. Enzymatic dissociation yields a single-cell suspension of SVF (cSVF) without cell-cell communications and extracellular matrix, whereas mechanical dissociation results mainly in a SVF with intact cell-cell interactions including extracellular matrix (tissue-SVF [tSVF]).^{6,7} The use of tSVF might be advantageous over cSVF because intact cell-cell interactions warrant retention of ASCs after injection. Additionally, an intact native network of extracellular matrix binds and releases cells as well as trophic factors and thus preserves the regenerative function of tSVF. In contrast to mechanical isolation, enzymatic isolation is time consuming and expensive, and clinical use of enzymes is forbidden by law in an increasing number of countries.⁶

PRP is defined as a portion of blood plasma having a platelet concentration above baseline. Platelets serve as a source of regenerative growth factors and cytokines.⁸ These regenerative factors have been shown to influence ASCs in a dose-dependent fashion in both animal and in vitro studies.⁹⁻¹¹ A concentration of platelets above baseline results in increased cell proliferation and RNA expression of genes related to angiogenesis, matrix remodeling, and wound healing.⁹⁻¹¹ To date, clinical studies investigating the combination of PRP and autologous lipofilling have indicated reduced postoperative recovery time and showed preliminary evidence of increased dermal wound healing.¹²⁻¹⁵ Plasma also contains fibrinogen, which forms fibrin fibers after activation with thrombin. Fibrin clots entrap platelet-released trophic factors and also serve as transient scaffolds for tissue repair. We hypothesized that tSVF augments

the therapeutic benefit of PRP-supplemented lipofilling in treatment of aged facial female skin.

METHODS

Study Overview

This study was conducted from July 2016 to November 2019 as a single-center, double-blind (patient as well as investigator), randomized, placebo-controlled clinical trial with a standard follow-up of 1 year at Bergman Clinics, The Hague, the Netherlands. The study protocol was carried out in compliance with the Declaration of Helsinki and was approved by the national medical ethics committee (CCMO) of the Netherlands (national legislator trial code, NL54409.000.15; Dutch trial register code, NTR5703). All subjects provided written informed consent prior to the start of the study.

Patient Population and Randomization

A power calculation was performed to calculate the total number of subjects required for this study ($n = 64$). Subjects were randomly divided into 2 groups: the experimental group received subcutaneous lipofilling with additional transcutaneous PRP and tSVF injections, whereas the control group received subcutaneous lipofilling with additional transcutaneous PRP as well as sterile saline injections, to serve as placebo. Randomization was performed with the randomization tool on <http://www.randomization.com>. Inclusion and exclusion criteria are listed in Table 1. Patient enrollment started in June 2016 and ended prematurely in November 2019 because the required pace of inclusion was not met, endangering completion of the study. After enrollment, subjects were asked to refrain from undergoing any facial rejuvenation procedure and from starting smoking. Any subjects who failed to comply were excluded from the study.

Harvesting and Injection of Condensed Lipoaspirate

Liposuction, processing, and deep and superficial lipofilling was performed with Sorenson harvesters (2.4×22 cm) and smaller, curved lipofilling cannulas (0.9×5 cm) (Tulip Medical Products, San Diego, CA).¹⁶⁻¹⁸ In short, 100 mL of

Figure 1. Locations of skin Cutometer and Tewameter measurements used for this 58-year-old female patient. Evaluation zone I: 2 cm lateral and 2 cm caudal from the lateral canthus. Evaluation zone II: 2 cm lateral from the lateral commissure.

adipose tissue was harvested from the upper legs, centrifuged, and 15 to 18 mL was injected immediately after processing against the inside of the skin to each side of the face. The part of the injection side that was used for assessment was outlined by the mandible bone, the nostril, lower eyelid, and the corner of the eyebrow up to the earlobe. In this particular area 6 mL of condensed lipoaspirate was injected as superficial lipofilling, keeping the round tip of the curved canula upwards against the inside of the skin. Remaining condensed lipoaspirate was used as deep lipofilling. All patients were treated under sedative anesthesia with intravenous propofol and remifentanyl.

Table 1. Inclusion and Exclusion Criteria of Subjects Participating in This Study

Inclusion criteria	Exclusion criteria
Females	Facial surgical intervention 1 year prior start of the study
Age 35-60 years	Any oncologic event in history
Stable BMI (20-15 kg/m ²) at least 1 year prior start of the study	Known psychiatric condition
	Known systemic disease that will impair wound healing
	Smoking
	Pregnancy or active child wish
	Frequent exposure to known carcinogenic substances (eg, work related)
	Active or previous use of hormone replacement therapy

BMI, body mass index.

PRP and tSVF Preparation

Prior to the surgery, 62 mL of whole blood was drawn from each subject in this study. PRP was prepared by adding 8 mL of anticoagulant citrate dextrose solution A (ACD-A) to 52 mL of whole blood and then following the Arthrex Angel system instructions. This resulted in 6 mL of nonactivated PRP with a platelet concentration of 4 times the baseline. The other 10 mL of whole blood was collected in an ACD-A syringe and analyzed for the number of platelets.

Of the 100 mL adipose tissue harvested, 60 mL was used to create three 1-mL portions of tSVF according to the previously described fractionation of adipose tissue procedure.¹⁹ In short, all harvested adipose tissue was decanted and centrifuged at 3000 rpm for 2.5 minutes with a 9.5-cm-radius fixed-angle rotor (Medilite, Thermo Fisher Scientific, Waltham, MA) at room temperature. After centrifugation, three 10-mL portions of adipose tissue were mechanically dissociated with the use of a fractionator (a Luer-to-Luer transfer with three 1.4-mm holes inside; Tulip Medical Products) by pushing the lipoaspirate forward and backward 30 times. Then, the dissociated adipose tissue was again centrifuged at 3000 rpm for 2.5 minutes with a 9.5-cm-radius fixed-angle rotor (Medilite) at room temperature. Then, 1 mL of tSVF was mixed with 2.5 mL of PRP and injected transcutaneously against the inside of the skin with the use of a 23G needle (BD Microlance, blue) to each side of the face. The ratio of 1:2.5 tSVF/PRP was chosen for practical reasons as a consequence of the standardized techniques used to create the PRP and tSVF. As a control, 1 mL of sterile saline was mixed with 2.5 mL of PRP

and used as a placebo. For all subjects in this study, 1 mL of tSVF was sent to the laboratory for analysis. All procedures were performed by the senior author (H.P.S.).

Immunohistochemical and Immunocytochemical Analysis of tSVF

Samples of tSVF were formalin-fixed and embedded in paraffin. Then, 4 μ m slides were cut, deparaffinized, and stained following the protocol previously published by van Dongen et al.¹⁹ Samples were stained for α -smooth muscle actin (α -SMA, 1:200; Abcam, Cambridge, UK) to stain smooth muscle cells, von Willebrand factor (vWF, 1:200; DAKO, Glostrup, Denmark) to stain endothelial cells, and perilipin A (1:200, Abcam) to stain adipocytes. As secondary antibodies, polyclonal rabbit anti-mouse for α -SMA, polyclonal swine anti-rabbit for vWF, and polyclonal goat anti-rabbit for perilipin A were used in 1:100 (DAKO). A third antibody was used for the α -SMA staining (polyclonal, swine anti-rabbit, 1:100; DAKO). Masson's trichrome staining was used to stain extracellular matrix deposition.

Measurement Outcomes

All measurements of every patient were performed by the first author (J.A.D.), who was blinded for treatment, at predetermined time points: preoperative, 6 weeks postoperative, 3 months postoperative, 6 months postoperative, and 12 months postoperative. After 1 week postoperative, early recovery was examined using FACE-Q questionnaires. Subjects did not apply any skin products on the day of surgery as well as on the day of the measurements. Only the right side of each subject's face was measured.

Skin Elasticity and Transepidermal Water Loss Measurements

Local skin elasticity was measured with a noninvasive validated Cutometer MPA580 elasticity probe (Courage + Khazaka electronic GmbH, Cologne, Germany).²⁰⁻²³ Local transepidermal water loss (TEWL) was measured with a noninvasive validated Tewameter TM300 (Courage + Khazaka electronic GmbH).^{24,25} Measurements were performed at standardized evaluation zones (Figure 1). True skin elasticity was defined as the ratio of elastic recovery to total deformation (R7 parameter). In addition, net elasticity (R5 parameter) and the ratio of viscoelasticity to elastic extension (R6 parameter) were measured. TEWL is expressed in grams per square meter per hour (g/m²/h). Data were corrected for humidity, room temperature, and age.

Figure 2. Mask used for the VISIA analysis for this 34-year-old female patient. All masks were created according to the same anatomic landmarks, and encompassed the medial canthus, around the eyelashes to the lateral canthus, lateral side of the eyebrow, 0.5 cm in front of the hairline to the height of the earlobe, perpendicular line to the mandible line ending 0.5 cm above the mandibula and nostril.

Skin Quality Measurements

Local skin quality was measured with a noninvasive VISIA camera (Canfield Imaging Systems, Parsippany, NJ). The VISIA camera containing the VISIA Complexion analysis software measures several skin parameters: superficial spots, wrinkles, skin texture, pores, vascularity, and pigmentation. Results are given as an absolute total number of occurrences of a particular parameter being evaluated and a relative score that combines the size and intensity of a parameter. Prior to surgery, a predetermined evaluation mask was created with the use of fixed anatomic marks (Figure 2).

FACE-Q

The following FACE-Q parameters were examined at the predetermined time points except for 3 months postoperative: satisfaction with facial appearance overall, satisfaction with facial skin, appraisal of lines overall, appraisal of crow's feet lines, age appraisal on a visual analog scale (VAS), aging appearance appraisal, psychological

Table 2. Age and Platelet Count at Time of Surgery of All Included Subjects in Both Groups

	Group 1: Lipofilling + PRP + tSVF (n = 14)			Group 2: Lipofilling + PRP (n = 14)			P value
	Mean	SD	Range	Mean	SD	Range	
Age at time of surgery	51.64	4.24	45-58	48.93	7.08	38-60	0.23
Platelet count at time of surgery	213.93	45.76	142-280	202.38	35.82	145-263	0.89

No significant difference between the two groups. PRP, platelet-rich plasma; tSVF, tissue stromal vascular fraction.

well-being, social function, and satisfaction with outcome. Recovery from early symptoms and recovery from early life impact were only examined at a 1-week postoperative follow-up. All questionnaires were validated and translated into Dutch using a linguistic validation. Raw FACE-Q scores were converted to Rasch scores according to the protocol of the FACE-Q editorial board.²⁶ VAS scores of age appraisal were not converted to Rasch scores. FACE-Q scores of 155 Caucasian Dutch women between 18 and 80 years old who have never received any aesthetic facial procedure where acquired to complete the baseline FACE-Q modules and served as control. All FACE-Q modules were distributed blind on paper by the first author (J.A.D.).

Statistical Analysis

Statistical analysis was performed under the supervision of an independent statistician (K.M.V.) who received blinded data from the first author (J.A.D.) and the randomization from the senior author (H.P.S.). All analysis was performed with SPSS 20 (IBM, Chicago, IL). Prism 6 was used to design the graphs (GraphPad, La Jolla, CA). Visual inspection revealed normally distributed data for the Cutometer and VISIA analyses, and thus a paired-samples *t* test and regular *t* test were used to analyze the outcomes of these analyses. A Wilcoxon signed rank test and Mann-Whitney test were used for the FACE-Q data. A 2-sided $P < 0.05$ was considered statistically significant.

RESULTS

Demographics and Platelet Counts

A total of 28 subjects were enrolled in this study; 25 subjects completed the entire study (tSVF = 14, control = 11). Only 28 of the calculated 64 subjects were included because the required pace of inclusion was not met, endangering completion of the study. Four subjects failed to complete all the follow-up appointments: 1 subject underwent a facial aesthetic procedure after 6 months of follow-up; 2 subjects did not show up for the last follow-up;

and 1 subject missed the 6-month follow-up due to personal circumstances. Fourteen subjects were included in both study arms (Table 2). The mean [standard deviation] age of subjects in the experimental group was 51.64 [4.24] years (range, 45-58 years) and 48.93 [7.08] years (range, 38-60 years) in the control group ($P > 0.05$). No difference was seen in baseline platelet count between both groups ($P > 0.05$).

Immunohistochemical and Immunocytochemical Analysis of tSVF

Fractionation of adipose tissue to obtain tSVF was effective in all patients because almost all adipocytes were disrupted. Reduction of adipocytes resulted in an enrichment of small vessels (determined as mean α -SMA positivity of 21.24% [5.02%] and mean vWF positivity of 8.95% [3.26%]) and extracellular matrix per remaining 10% volume (Figure 3, Table 3). This study was not designed to focus on any correlation between clinical effect and histologic composition of tSVF.

PRP-Supplemented Lipofilling With tSVF Does Not Improve Skin Elasticity or Reduce TEWL

The addition of tSVF produced no difference in total deformation (R7) between both groups at any moment for both evaluation zones except for evaluation zone I at 12 months postoperative (Figure 4). Evaluation zone I showed a higher skin elasticity in the control group with a mean of 2.7 [0.01] vs 2.4 [0.03] in the experimental group ($P < 0.05$). On the other hand, the ratio of viscoelasticity to elastic extension (R6) was increased in the treatment group compared with controls but only for evaluation zone II at 6 months postoperative ($P < 0.05$) (Figure 4). Treatment did not influence net elasticity (R5). No difference in TEWL was seen between both groups at any follow-up for both evaluation zones (Figure 5). Within each group, the effect of lipofilling and PRP with or without tSVF on R5, R6, and R7 was negligible compared with preoperative values.

Figure 3. Light microscope images of (A) α -smooth muscle actin staining of tSVF, (B) von Willebrand factor staining of tSVF, (C) Masson's trichrome staining of tSVF, and (D) perilipin staining of tSVF. tSVF, tissue stromal vascular fraction.

Table 3. Quantification of α -SMA and vWF Staining of tSVF

Staining results (mean % area)		
Patient	vWF (n = 3)	α SMA (n = 3)
1	14.40	18.26
2	8.13	11.51
3	10.10	22.82
4	7.79	25.33
5	13.71	16.23
6	9.12	23.56
7	8.07	16.16
8	7.58	28.79
9	5.86	20.99
10	12.48	23.91
11	3.40	16.50
12	4.23	24.76
13	11.00	28.23
14	9.46	20.34

α -SMA, α -smooth muscle actin; vWF, von Willebrand factor.

PRP-Supplemented Lipofilling With tSVF Does Not Improve Skin Quality

The addition of tSVF caused no difference in the number of superficial spots and pores, or in skin texture, vascularity, and pigmentation for both absolute and relative scores between both groups at any time point during follow-up (Figure 6). The absolute number of wrinkles was reduced in the control group compared with the experimental group at 12 months postoperative ($P < 0.05$). In contrast, the relative score of wrinkles did not differ between groups. Within each group, PRP-supplemented lipofilling with or without tSVF did not reduce the value of any parameter when preoperative and postoperative values were compared.

PRP-Supplemented Lipofilling With tSVF Does Not Improve Patient Satisfaction

No difference was seen in satisfaction with overall facial appearance or facial skin quality between both groups at any follow-up time point (Figure 7). In comparison with the control group of women who never sought aesthetic facial procedures, pre- and post-operative satisfaction rates were lower. All other FACE-Q modules regarding subject satisfaction with skin characteristics revealed no difference between

Figure 4. Changes in true skin elasticity as ratio of elastic recovery to total deformation (R7 parameter) for (A) evaluation zone I and (B) evaluation zone II, ratio of viscoelasticity to elastic extension (R6 parameter) for (C) evaluation zone I and (D) evaluation zone II, and net elasticity (R5 parameter) for (E) evaluation zone I and (F) evaluation zone II are presented between both the experimental and control group for all time points. *In (A) a higher R7 parameter was shown for evaluation zone I in the tSVF- group than in the tSVF+ group at 12 months postoperative ($P < 0.05$). *In (D) a higher R6 parameter was shown in the tSVF+ group in comparison with the tSVF- group at 6 months postoperative ($P < 0.05$). No difference was shown for the R5 parameter. tSVF, tissue stromal vascular fraction.

both groups at any follow-up time point (Table 4). Psychological well-being, social function, and recovery were similar for both groups pre- and postoperatively. Subjects in both groups had comparable satisfaction rates regarding the final outcome of the treatment (Table 4).

Lipofilling and PRP with or without tSVF did not improve any FACE-Q modules regarding subject satisfaction. However, only overall facial appearance in the control group was improved at 12 months postoperative ($P < 0.05$)

(Figure 8). Psychological well-being and social function did not improve as well. No major complications occurred in either group.

DISCUSSION

The addition of tSVF to autologous facial lipofilling with PRP did not show benefits for skin quality for aesthetic reasons, recovery, or subject satisfaction in this study.

Figure 5. Changes in local TEWL for (A) evaluation zone I and (B) evaluation zone II are presented between both the experimental and control group for all time points. No difference was shown. TEWL, local transepidermal water loss; tSVF, tissue stromal vascular fraction.

Figure 6. Changes in VISIA data, ie, absolute numbers of (A) superficial spots, (B) wrinkles, (C) texture, (D) pores, (E) pigmentation, (F) vascularization and relative scores of (G) superficial spots, (H) wrinkles, (I) texture, (J) pores, (K) pigmentation, and (L) vascularization are presented between both the experimental and control group for all time points. *In (B) a higher absolute number of wrinkles was shown in the tSVF+ group than in the tSVF- group at 12 months postoperative ($P < 0.05$). tSVF, tissue stromal vascular fraction.

Figure 6. Continued.

Lipofilling with PRP alone did not improve skin quality compared with preoperative values, whereas patient satisfaction for overall facial appearance was improved. In the literature, clinical reports studying autologous lipofilling or any component of adipose tissue, eg, cSVF or tSVF, as a treatment to improve facial skin quality show different results compared with this prospective randomized controlled trial.²⁷⁻³¹ Those reports mentioned improvements in skin quality, eg, skin texture, wrinkles, and pore size. However, the level of evidence in those clinical studies does not allow strong conclusions to be drawn,¹ and thus, those results should be interpreted with caution. To date, only 1 well-designed, prospective, double-blind, randomized controlled trial showed no increase in facial skin elasticity after treatment with autologous lipofilling with or without PRP.¹² However, a regression analysis

of true skin elasticity, ie, R7 parameter, as a function of age showed a negative correlation with the preoperative condition. After treatment with lipofilling, this correlation reversed to a positive correlation, especially in the PRP group. The change from negative to positive correlation was not significant ($P = 0.056$), probably due to the high number of dropouts. On the other hand, postoperative recovery in this study was decreased by the addition of PRP to lipofilling.

To date, 4 studies have used histologic outcomes to evaluate the effect of autologous lipofilling on facial skin.^{27,32-34} Three studies only subjectively described the histologic outcomes and failed to correlate the histologic outcomes with clinical outcomes.³²⁻³⁴ It is well known that changes in histology after application of a treatment do not always result in significant observable clinical effects.

Figure 7. Changes in FACE-Q assessment of facial skin quality are presented between the experimental and control groups for all time points. No significant difference is apparent. tSVF, tissue stromal vascular fraction.

None of the histology studies used a control group with microneedling alone. Several clinical and animal studies have shown that microneedling alone has substantial effects on the skin, eg, epidermal thickening and increased dermal density.³⁵⁻³⁸ The described effects in the 4 studies examining histologic outcomes after autologous lipofilling for facial skin rejuvenation might largely be caused by needling.

The definition of “skin rejuvenation” is broad and difficult to describe, leading to noncomparable results between different clinical studies. In the literature, a clear definition of skin rejuvenation is lacking, which contributes to a large number of publications stating that autologous lipofilling improves skin quality, eg, disappearance of wrinkles or dark infraorbital circles.^{39,40} These observed effects are, in our opinion, volumetric effects rather than due to “true” skin rejuvenation. Lipofilling causes an increased subcutaneous volume at the site of injection. Increased subcutaneous volume stretches the overlying skin, thereby reducing wrinkle formation. Moreover, dark infraorbital circles are caused by increased transparency of the orbicularis oculi muscle due to an aging-related decrease in subcutaneous volume and thinning of the skin. Increasing subcutaneous volume by transplantation of adipose tissue results in a less visible orbicularis oculi muscle and thus decreased dark infraorbital circles. In our opinion,

the definition of skin rejuvenation should only be used when ordinary aging-related improvements of the skin without any volumetric component are being described, eg, improvements in thickness, texture, elasticity, pore size, or pigmentation. There should also be a clear distinction between ordinary (physiologic) aging-related changes of the skin and changes due to pathologic processes such as traumatic scars, fibrotic diseases, or wound healing.

All the aforementioned clinical studies used lipofilling as a treatment to enhance skin rejuvenation in patients with ordinary aged skin. In our opinion, aging of the skin is a physiologic process where elastin gradually degrades due to ultraviolet exposure and wear-and-tear with no substantial changes in a short period of time. These changes over time cause no significant release of cytokines, and thus transplanted ASCs are probably not sufficiently triggered by the host environment to regenerate tissue. To induce a regeneration trigger, the host environment must be damaged deliberately prior to treatment by trichloroacetic peeling. Aging-related skin modifications cannot be considered damaged tissue and therefore lipofilling for skin rejuvenation is not a suitable indication and should only be used for a volumizing effect of the facial fat compartments in the aging face. In contrast, pathologic processes are accompanied by a burst release of growth factors and cytokines that initiates inflammation, disturbs extracellular matrix remodeling, and reduces angiogenesis. These growth factors and cytokines trigger ASCs to release anti-inflammatory and proangiogenic cytokines as well as metalloproteinases to remodel extracellular matrix.⁴¹ To date, several clinical studies have shown that autologous lipofilling might increase scar remodeling.⁴²

Although PRP contains a large number of proangiogenic growth factors and metalloproteinases, clinical data on skin rejuvenation remain lacking.^{8,43} A systematic review by Maisel-Campbell et al evaluated studies that used PRP as a monotherapy for skin rejuvenation.⁴³ In total, 10 studies treating 180 patients with PRP showed improvements in satisfaction, skin texture, hydration, and pigmentation. However, almost all these studies used nonvalidated and subjective outcome measurements and most failed to report the concentration of platelets in PRP or baseline blood samples. It is well known that platelet concentrations are highly variable during the day and most PRP preparation devices use the relative increase in the number of platelets compared with baseline at the time of preparation. Studies have shown that high concentrations of PRP increase proliferation of ASCs but may also induce undesirable differentiation.^{10,11} High concentrations of PRP may change ASCs towards a more fibrotic phenotype with a lower angiogenic capacity as well.⁹ Hence, the concentration of PRP was reduced from 6 to 8 times above threshold in our previous study to 4 times above threshold

Table 4. Patient Satisfaction and Psychological Well-being as Well as Social Function Measured by Different FACE-Q Modules at Different Time Points

Time Point	N	Mean	Standard deviation	P value
Appraisal of lines overall				
tSVF+/tSVF–				
Preoperative	14/14	29.00/37.64	16.72/20.57	0.27
6 weeks	14/14	44.86/46.14	21.58/26.61	0.89
6 months	13/13	36.54/44.85	17.88/25.02	0.27
12 months	14/11	42.64/39.45	25.83/19.77	0.74
Appraisal of crow's feet lines				
tSVF+/tSVF–				
Preoperative	14/14	42.43/48.29	16.08/26.93	0.85
6 weeks	14/14	56.64/56.57	25.06/33.06	0.98
6 months	13/13	46.54/55.23	18.99/23.22	0.43
12 months	14/11	47.79/40.73	22.29/20.00	0.36
Age appraisal (VAS)				
tSVF+/tSVF–				
Preoperative	14/14	3.21/2.14	6.48/6.33	0.62
6 weeks	14/14	1.71/1.21	4.85/5.95	0.72
6 months	13/13	2.62/1.38	5.71/5.58	0.47
12 months	14/11	2.21/1.55	4.85/4.54	0.48
Age appearance appraisal				
tSVF+/tSVF–				
Preoperative	14/14	34.50/51.14	21.62/25.83	0.06
6 weeks	14/14	46.43/52.36	23.94/25.23	0.52
6 months	13/13	42.46/60.54	21.59/28.80	0.16
12 months	14/11	46.36/46.64	23.09/26.90	0.72
Psychological well-being				
tSVF+/tSVF–				
Preoperative	14/14	59.43/65.5	15.73/22.84	0.30
6 weeks	14/14	61.93/60.00	14.36/24.96	0.80
6 months	13/13	62.92/68.85	20.11/17.45	0.72
12 months	14/11	65.07/64.73	18.29/16.29	0.91

in this study.¹² However, the optimum PRP concentration to maximize tissue regeneration by ASCs remains unknown in vivo, which is one of the limitations of this study as well.

Table 4. Continued

Time Point	N	Mean	Standard deviation	P value
Social function				
tSVF+/tSVF–				
Preoperative	14/14	68.36/66.71	19.58/25.59	0.89
6 weeks	14/14	70.71/63.07	19.75/16.46	0.36
6 months	13/13	68.23/71.08	21.02/17.85	0.86
12 months	14/11	68.79/68.82	20.84/19.45	0.72
Satisfaction with outcome				
tSVF+/tSVF–				
6 weeks	14/14	36.14/37.79	26.68/30.09	0.98
6 months	13/13	32.08/36.85	24.66/35.39	0.88
12 months	14/11	33.57/34.90	28.80/25.27	0.81
Recovery of early symptoms				
tSVF+/tSVF–				
1 week	14/14	32.64/31.86	10.43/7.96	0.96
Recovery early-life impact				
tSVF+/tSVF–				
1 week	14/14	48.93/45.43	15.48/10.84	0.76

No significant differences between the 2 groups. tSVF, tissue stromal vascular fraction; VAS, visual analog scale.

Nevertheless, the baseline concentration of platelets was comparable between both study groups.

Similarly, the ratio of SVF/lipofilling remains a matter of debate. In this study, a tSVF/superficial lipofilling ratio of 1:6 was used for pragmatic reasons. Ni et al showed, in a rabbit model, that a cSVF/lipofilling ratio of 1:3 was ideal in terms of volume maintenance 3 months after injection in comparison with lower ratios.⁴⁴ Other studies confirmed the aforementioned results and showed that a cSVF/lipofilling ratio higher than 1:1 did not increase the survival rate in animal models.^{45,46} A higher survival rate of transplanted SVF-enriched fat grafts might also result in a greater regenerative effect on the overlying skin. Compared with our study, these animal models used a significantly higher ratio of cSVF/lipofilling and therefore patients might be undertreated in this study. However, all the other studies used only animal models and did not investigate the effect of SVF-enriched lipofilling on skin quality. Moreover, the other studies all used enzymatically isolated SVF instead of mechanically isolated SVF, making it difficult to compare their results with clinical outcomes of this study.

Figure 8. Changes in FACE-Q regarding satisfaction with facial appearance overall are presented between the experimental and control groups for all time points. *A higher satisfaction with facial appearance overall was shown in the control group than in the experimental group at 12 months postoperative ($P < 0.05$). tSVF, tissue stromal vascular fraction.

A limitation of this study is the absence of cell yield and viability analysis for the mechanically isolated tSVF. These data might have given detailed information about the quality of tSVF, although the cellular and tissue composition of tSVF determined by histologic staining is more important regarding the potential regenerative effect of tSVF. Another limitation of this study is the use of VISIA analysis to evaluate skin quality because this device is not validated; for that reason, the VISIA analysis was only used as secondary outcome, with the validated Cutometer data serving as the primary outcome. Moreover, only the right side of each subject's face was measured, based on the assumption that the skin quality of both facial sides was comparable. If measurements were performed on both sides of the face, more data would be available for analysis. However, the quality of the data might be compromised because even performing measurements only of the right side of the face was time consuming at each follow-up time point, and taking measurements from both facial sides might have resulted in a higher number of drop-outs. There is also a lack of power in this study due to a low sample size with only 14 subjects in each group and 5 subjects not completing the entire study. Furthermore, this study was terminated prematurely because the required pace of inclusion was

not met, endangering completion of the study. Designing a well-designed prospective randomized controlled trial with a strong power and bringing it to completion is challenging in regenerative aesthetic surgery, but definitely necessary to further develop the field.

CONCLUSIONS

The addition of tSVF to PRP-supplemented lipofilling did not improve skin elasticity, recovery, or subject satisfaction in a healthy population in this study. PRP-supplemented lipofilling with tSVF can be considered a safe procedure. However, some controversy remains regarding tSVF/lipofilling ratios, PRP concentration, and the optimal procedure to isolate cSVF or tSVF, and these aspects require further elaboration in the future.

Disclosures

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