Research Article

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Regenerative Medicine

Comparing different nanofat procedures on scars: role of the stromal vascular fraction and its clinical implications

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Aim: Recently, a new fat grafting technique termed 'nanofat grafting' was proposed which improved tissue repair by the stem cells contained in the stromal vascular fraction (SVF) of nanofat. Here, we reported the clinical outcomes of different nanofat procedures in the treatment of scars in relation with SVF cell yield. **Methods:** Three different modified nanofat grafting procedures (supercharged-, evo- and centrifuged-modified nanofat) were compared with the classic nanofat method, and histological analysis was performed to assess skin regeneration. Residual nanofat samples were analyzed to determine SVF immunophenotype and yield from each procedure. **Results:** Supercharged-modified nanofat gave the best results in terms of clinical outcome and SVF yield. Histological analysis revealed similar skin regeneration in all treatments. **Conclusion:** This work suggested a positive correlation between SVF yield and clinical outcomes in the nanofat treatment of scars.

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Keywords: adipose-derived stem cells • clinical outcome • nanofat grafting • scars • skin regeneration • stromal vascular fraction

The adipose tissue is a highly complex tissue and consists of mature adipocytes, preadipocytes, fibroblasts, pericytes, vascular smooth muscle cells, endothelial cells, resident monocytes/macrophages [1–5] and lymphocytes [4]. This heterogeneous cell population, named stromal vascular fraction (SVF), has come more and more into the focus of stem cell research, since this compartment provides a rich source of multipotent adipose-derived stem cells (ASCs) [6–8]. Rodriguez *et al.* [9] described the isolation and culture of ASCs with their multipotent differentiation capacity. The presence of ASCs was reported not only in the adipose portion of liposuction aspirates (a major contributor of ASCs) but also in the infranatant liquid portion (liposuction aspirate fluid) [10]. Fat grafting and stem cells have been commonly used in plastic surgery for regeneration and rejuvenation purposes [11]. The authors have already published the results obtained from using of platelet-rich plasma mixed with fat grafting in the treatment of scars of the face [12], chronic lower-extremity ulcers [13], loss of substance on the lower limbs [14] and application of SVF in post-traumatic lower extremity ulcers [15,16]. These previous findings demonstrated that the application of SVF can improve tissue healing and maintenance of fat graft volume. In fact, by early methods of adipose tissue transplantation, significant portions of the transplanted tissue were often reabsorbed, perhaps for a poor blood supply due to insufficient neoangiogenesis [17]. It has been reported that ASCs and their secretome possess angiogenic properties [18,19]. Recently, a new fat grafting method, named 'nanofat grafting', was proposed by Tonnard *et al.* [20] for skin rejuvenation, especially to treat particular areas, such as eyelids, lips or fine rhytides. Nanofat is obtained after several steps of emulsification and filtration. The final liquid suspension, virtually devoid of mature adipocytes, can be injected in a more superficial plane through finer needles (27 gauge) and would

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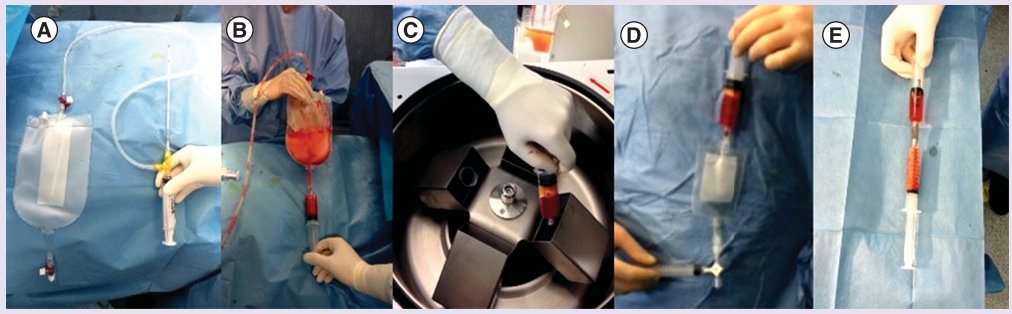

Figure 1. The method of supercharged-modified nanofat preparation. (A) Nano-FastKit® (Corios, Milan, Italy) system for nanofat and SVF isolation in a closed system. **(B)** Bag with the harvested fat. **(C)** Automatic filtration (120-μm filter) and centrifugation for SVF isolation and fat preparation. **(D)** At the end of this process, centrifuged fat is in the middle part of the syringe instead the suspension, containing SVF, in the lower part. **(E)** SVF suspension (40 ml) was collected and further filtered (120-μm filter) to obtain the final volume of 20 ml to enrich the emulsified fat (obtained only by automatic filtration and emulsification, see Materials & methods section). The authors added 0.2 ml of SVF suspension to each milliliter of fat obtained.
SVF: Stromal vascular fraction.

improve tissue repair because of the presence of SVF and therefore ASCs, without enzymatic digestion or *in vitro* cell expansion, as also reported by Bo *et al.* in liposuction aspirate fluid [10].

However, it has been supposed that SVF cell population may be lost during the nanofat procedure [17]. For this reason, the authors compared, in terms of clinical outcomes and SVF yield, three different modified nanofat procedures, using mechanical filtration and/or centrifugation step in a close system (Figure 1), with the classic nanofat by Tonnard. The findings achieved from this study demonstrated that clinical outcome based on skin improving depends in part on SVF amount contained in the fat. In addition, the authors demonstrated that each single step in the nanofat procedure can influence the final SVF yield.

Materials & methods

Patients

Between January 2014 and March 2016, 43 patients, between 20 and 73 years of age, affected by outcomes of burn or post-traumatic scars were treated, in a randomized manner, with classic [20] or modified nanofat grafting at the Plastic and Reconstructive Surgery Department of 'Tor Vergata' University of Rome. The treated scars ranged from at least 4 months from complete healing to 3-year old. In particular, 13 patients (six females and seven males), affected by outcomes of burn (n = 5) or post-traumatic scars (n = 8), were treated with supercharged-modified nanofat. Ten patients (five females and five males), affected by outcomes of burn (n = 6) or post-traumatic scars (n = 4), were treated with evo-modified nanofat. Ten patients (five females and five males), affected by outcomes of burn (n = 6) or post-traumatic scars (n = 4), were treated with centrifuged-modified nanofat. Clinical outcomes of these different nanofat procedures were compared with those of classic nanofat, according to Tonnard [20], in a group of ten patients (five females and five males), affected by outcomes of burn (n = 5) or post-traumatic scars (n = 5). The exclusion criteria were uncompensated diabetes, sepsis and cancer. Tobacco use and genetic disorders were not considered.

Modified nanofat procedures

To obtain modified nanofat, the lipoaspirate (80 ml, obtained with luer-look syringes and Coleman cannula 3 mm-diameter) was mechanically emulsified after rinsing. Emulsification of the fat was achieved by shifting the fat between two 10-cc syringes connected to each other by a female-to-female Luer-Lok connector. After 30 passes, the fat changed into an emulsion. At the end of the fragmentation process, the fat became liquid and took on a whitish appearance. After this emulsification process, the fatty liquid was again filtered over the sterile nylon cloth and the effluent was collected in a sterile recipient to remove the connective tissue remnants that would block the fine needles. Tonnard [20] called this product 'nanofat'. For modified nanofat, the authors did not apply high-negative-pressure liposuction and not use a standard 3 mm Mercedes-type liposuction cannula with large side holes

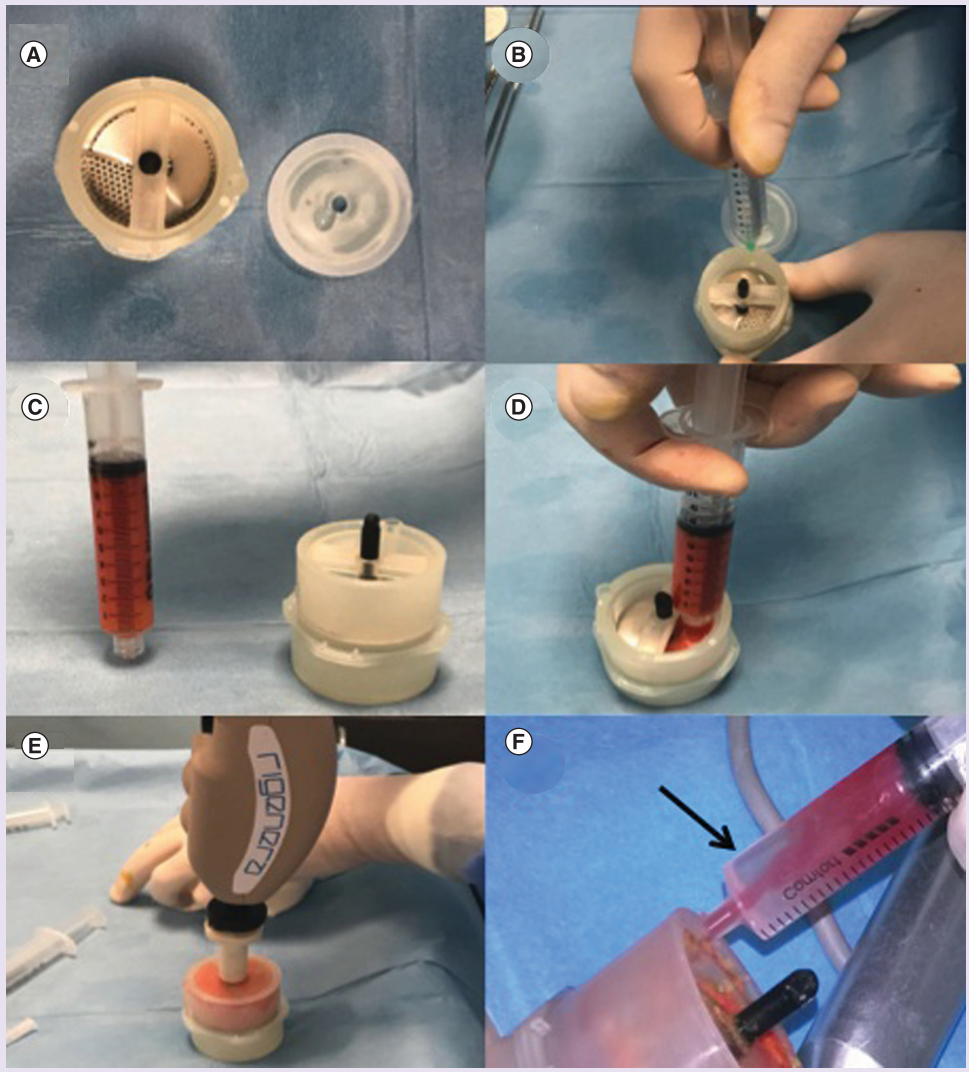

Figure 2. The method of evo nanofat-modified procedure. (A) Adipecons kit according to Rigenera procedure; **(B)** addition of 16ml of saline solution in Adipocons; **(C)** decanted fat graft; **(D)** addition of 16 ml of decanted fat graft in Adipecons; **(E)** slow centrifugation at 80 rpm x 3 min; and **(F)** 10 ml of stromal vascular fraction suspension was collected and emulsified.

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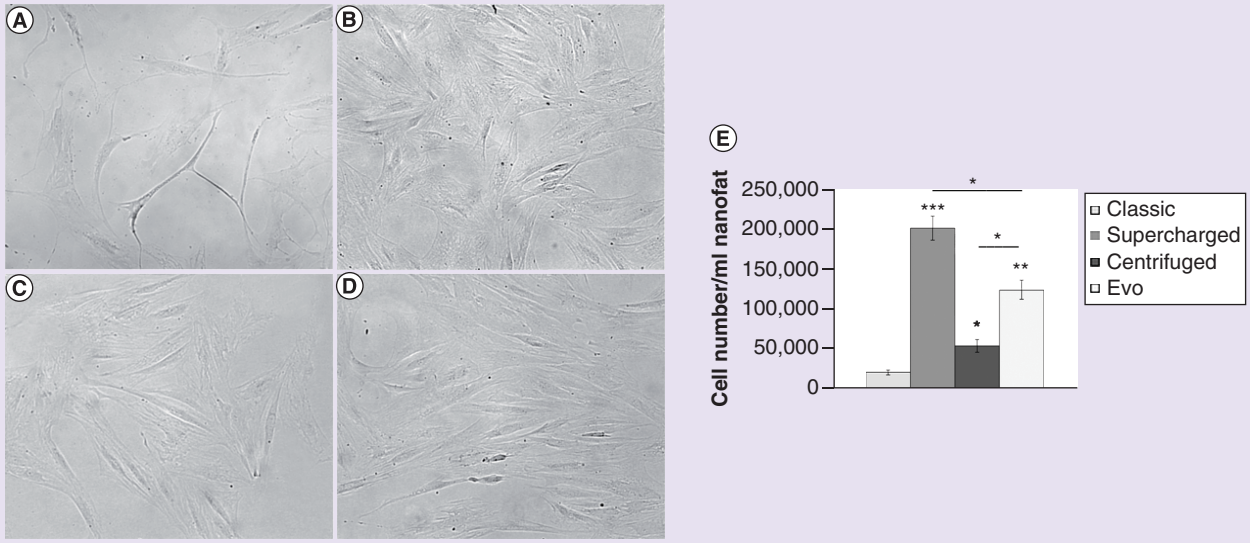

Figure 3. Stromal vascular fraction cell culture and yield from different nanofat procedures. (A–D) Representative images of cultured stromal vascular fraction obtained by different nanofat samples. **(E)** Bar graph showing stromal vascular fraction cell yield per milliliter of nanofat. Results were reported as the mean of five samples/group. *t*-test: *p < 0.05, **p < 0.01 and ***p < 0.001.

Table 1. Immunophenotypic characterization of stromal vascular fraction by flow cytometry analysis.

Antigen	Supercharged-modified nanofat	Evo-modified nanofat	Centrifuged-modified nanofat	Classic nanofat
CD44	71.2 ± 8.0	65.6 ± 9.1	68.3 ± 7.8	69.4 ± 9.0
CD90	62.8 ± 7.2	59.7 ± 6.9	56.9 ± 6.1	60.5 ± 8.2
CD73	30.1 ± 5.4	27.2 ± 4.8	32.3 ± 5.5	28.1 ± 5.2
CD34	58.1 ± 6.3	55.3 ± 7.5	50.4 ± 5.2	52.6 ± 5.8
CD31	19.9 ± 4.4	21.5 ± 5.1	17.8 ± 4.8	20.3 ± 4.2
CD146	22.1 ± 4.5	19.7 ± 3.9	20.7 ± 5.1	23.0 ± 5.2

Data (percentages) are presented as the mean ± standard error of the mean obtained from three different patients for each experimental group. No significant difference was observed between different groups.

(2 × 7 mm) but a 3 mm multiport cannula, containing several sharp side holes of 1 mm diameter. In particular, to obtain supercharged-modified nanofat, the lipoaspirate was divided in two part. The first part (80 ml), harvested in a closed system with 20 ml Luer-Lok syringe and collected in a bag (Nano-FastKit® System, Corios, Milan, Italy; see Figure 1A & B), was subjected to an automatic filtration (120-µm filter) and centrifugation at 1300 rpm for 10 min (Nano-FastKit System, Figure 1C). At the end of this process, centrifuged fat is in the upper part of the syringe instead the pellet, containing SVF [21–23], in the lower part. Only the SVF pellet was collected and its suspension (40 ml) further filtered (Figure 1D) to obtain the final volume of 20 ml. The second part of lipoaspirate (80 ml) was only shifted into two 10-ml Luer-Lok syringes, connected to each other by a female-to-female Luer-Lok connector, for emulsification (30 passes). Subsequently, the SVF suspension, previously obtained in the first part, was mixed to the emulsified fat. The authors added 0.2 ml of SVF suspension to each milliliter of fat obtained (Figure 1E). For centrifuged-modified nanofat, the lipoaspirate (80 ml) was subjected to centrifugation (skipping the filtration step) and at the end of this process, only the centrifuged fat was collected (the SVF pellet was discarded) and shifted into two 10 ml Luer-Lok syringes for emulsification (30 passes). To obtain evo-modified nanofat, the authors processed the lipoaspirate (80 ml) performing only slow centrifugation 80 RPM x 3min according to Rigenera procedure using Adipecons [Adipecons Rigenera System, HBW, Turin, Italy] (skipping the filtration and SVF discard) 16 ml at a time and emulsification step. There was no SVF-enrichment. At the end of these different preparation procedures, nanofat was aseptically injected for correcting burn or post-traumatic scars. Intradermal injections were performed with a 27-gauge needle (Figure 2).

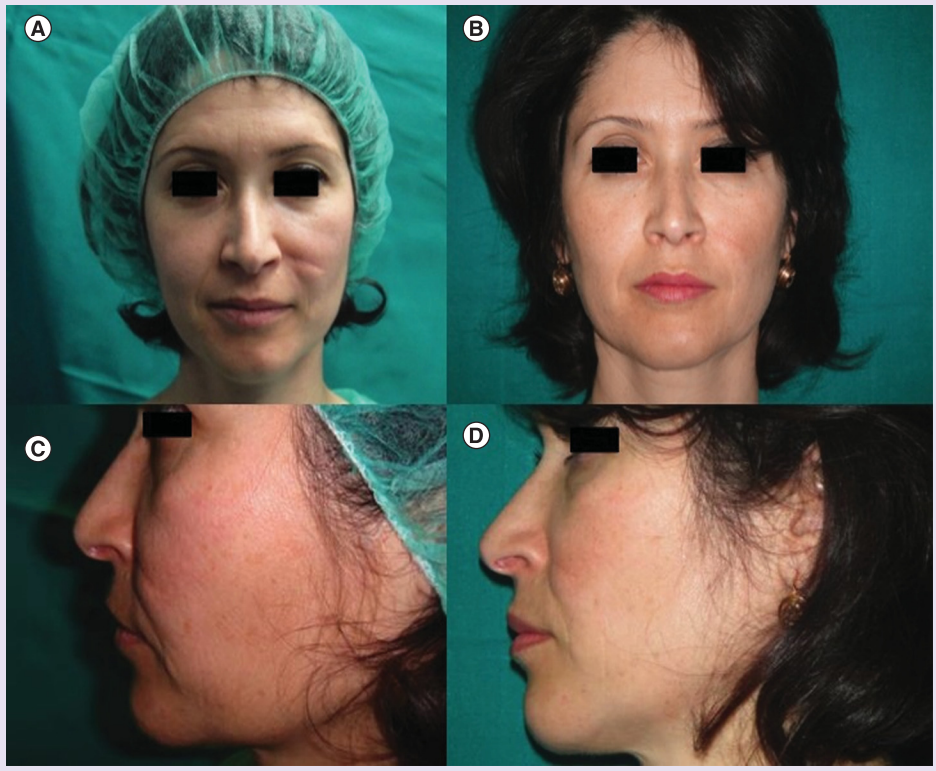

Figure 4. Clinical case of a patient affected by outcomes of scars in left nasolabial fold treated with supercharged-modified nanofat. (A) Pre-operative situation in frontal projection with outcomes of scars in the left nasolabial fold region. **(B)** Post-operative situation in the left nasolabial fold region at 6-month post-treatment with an increased skin thickness. **(C)** Pre-operative situation in lateral left projection. **(D)** Post-operative situation in lateral left projection at 6 month post-treatment with an increased skin thickness.

SVF isolation & counting

For SVF isolation, residual volumes of processed fat were washed three-times with phosphate-buffered saline (PBS) and suspended in an equal volume of PBS and 0.1% collagenase type I (C130; Sigma-Aldrich, Milan, Italy) prewarmed to 37°C [24]. Adipose tissue was placed in a shaking water bath at 37°C with continuous agitation for 60 min and centrifuged for 10 min at 600 ×*g* at room temperature. The supernatant, containing mature adipocytes, was aspirated. The SVF pellet was resuspended in erythrocyte lysis buffer (155 mM NH4Cl, 10 mM KHCO3 and 0.1 mM EDTA) and incubated for 5 min at room temperature. After centrifugation at 1100 rpm for 5 min, the pellet was resuspended in few microliters of growth medium and passed through a 100 µm Falcon strainer (Becton and Dickinson, CA, USA) and cellular population was counted using hemocytometer with trypan blue staining exclusion [25]. Cell yield was reported as cell number per milliliter of nanofat. Results represented the mean of five samples per group.

Flow cytometry

Flow cytometry was performed on SVF harvested by the different nanofat procedures. The primary antibodies were mouse monoclonal anti-human CD90-PE, CD73-FITC, CD34-PE, CD31-FITC, CD44-FITC and CD146-PE (BD Biosciences, NJ, USA). The cells were washed once in PBS containing 0.5% BSA and 0.1% sodium azide, resuspended in wash buffer with 25 µg/ml mouse IgG, and incubated for 10 min on ice. A volume of 100 µl for each cell suspension (~5 × 10^5 cells) was aliquoted per tube and labeled with monoclonal antibodies on ice, protected from light for 30 min. At least 10,000 events for each antibody were acquired on FACS-Caliber flow cytometer using CELLQuest acquisition software (BD Biosciences). Positive cell populations (percentages) were presented as the mean ± standard error of the mean (SEM) obtained from three different patients for each experimental group.

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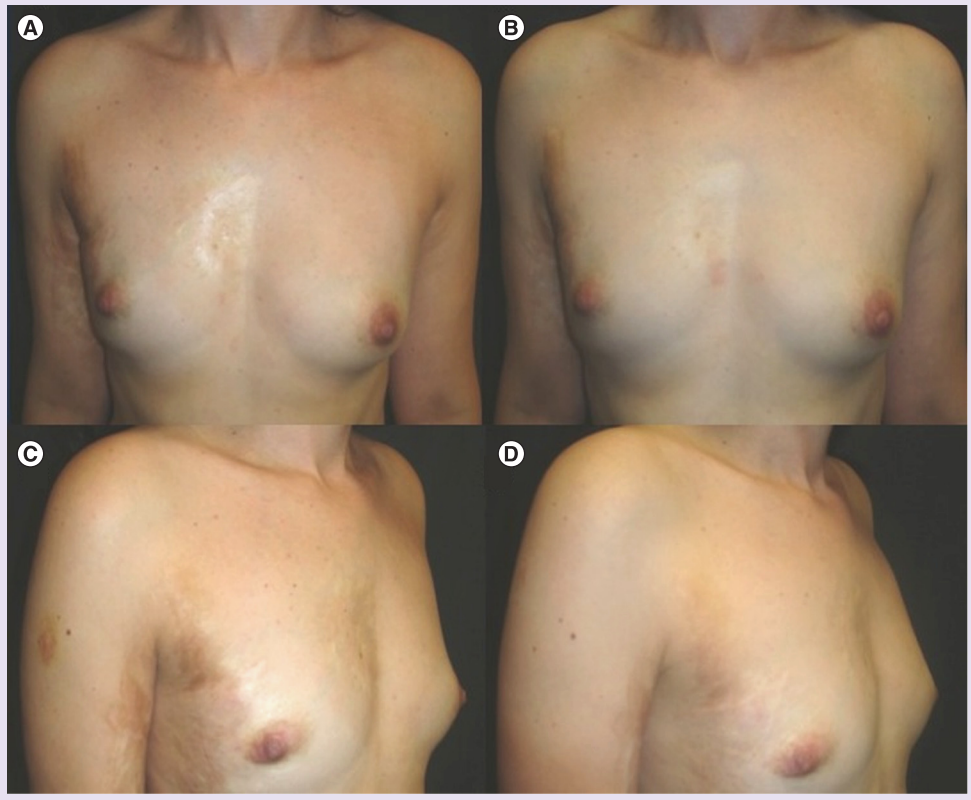

Figure 5. Clinical case of a patient affected by outcomes of burn in right breast treated with evo-modified nanofat. **(A)** Pre-operative situation in frontal projection with outcomes of burn in the upper and lower external quadrant of the right breast. **(B)** Postoperative situation in frontal projection at 6 months post treatment with an increased skin thickness. **(C)** Pre-operative situation in three-fourth right projection. **(D)** Postoperative situation in three-fourth right projection at 6 months post treatment with an increased skin thickness and quality.

Clinical evaluation methods

The preoperative and postoperative study was carried out through a complete clinical examination, a photographic assessment and histological analysis for a small sample of patients. Postoperative follow-up took place after 3, 4, 6 and 12 months. Through the analysis of preoperative and postoperative photos, the authors were able to evaluate the defect correction and skin quality improvement. Photos were taken with the same magnification, resolution, brightness and contrast to facilitate the comparison. In addition, two methods for the outcome evaluation were used: team (operator) evaluation and patient self-evaluation. Team evaluation was based on clinical observation, using a scale of six scores (excellent = 5, good = 4, discreet = 3, enough = 2, poor = 1, inadequate = 0). The patient self-evaluation used the same six scores mentioned previously. Additional factors/variables taken also into account were pigmentation, vascularization, pliability, thickness, itching and pain. Finally, the mean between patient and team evaluation was made.

Histological assessment

Incisional punch biopsies (2 mm diameter) were obtained from outcomes of burns or post-traumatic scars treated with different nanofat procedures (n = 4 each group, randomly selected patients) at baseline and after 6 months post treatment [15]. Microscopic evaluation of routinary hematoxylin & eosin-stained sections, from paraffin-embedded skin tissue, was performed to assess skin regeneration [26]. Briefly, measurements of epidermal and dermal thickness were taken using the ImageJ image processing and analysis software. Using the ImageJ measure tool, all epidermal and dermal thicknesses were measured and the mean was calculated, for each sample, from at least ten different slides.

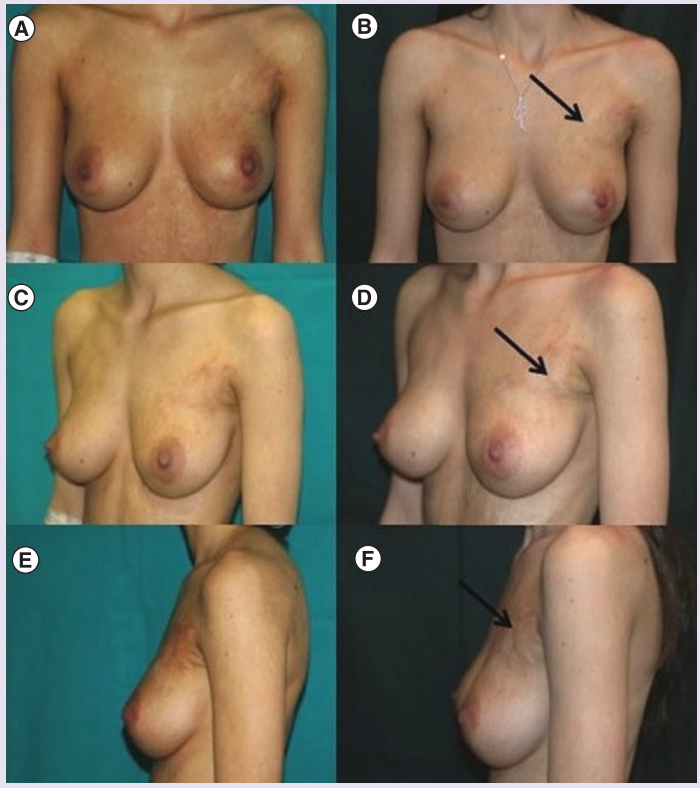

Figure 6. Clinical case of a patient affected by outcomes of burn in left breast treated with centrifuged-modified nanofat. (A) Pre-operative situation in frontal projection with outcomes of burn in the upper external quadrant of the left breast. **(B)** Postoperative situation in frontal projection at 6 months post treatment with an increased skin thickness and quality. **(C)** Pre-operative situation in three-fourth left projection. **(D)** Postoperative situation in three-fourth left projection at 6 months post treatment with an increased skin thickness. **(E)** Pre-operative situation in lateral left projection. **(F)** Postoperative situation in lateral left projection at 6 months post treatment with an increased skin thickness and quality.

Statistical analysis

Data were expressed as the mean ± SEM, and one-way analysis of variance (Bonferroni correction) was used for multiple comparisons; Student's *t*-test was used for the comparison between only two groups. Values of $p < 0.05$ were considered statistically significant.

Results

Immunophenotypic characterization of SVF & cell yield from different nanofat samples

Flow cytometry analysis (Table 1) of SVF from different nanofat samples showed the expression of specific mesenchymal stromal stem cell markers (CD44, CD90, CD73), pericyte marker (CD146), endothelial marker (CD31) and hematopoietic/endothelial stem cell marker (CD34) in all experimental groups. No significant difference was observed between groups.

Representative microphotographs of cultured SVF obtained from different nanofat samples are shown in Figure 3A–D. From classic nanofat, about 20,000 (±) 3000 nucleated cells/ml of nanofat were obtained, whereas from supercharged-modified nanofat about 200,000 (±) 15,000 nucleated cells/ml. For centrifuged-modified nanofat about 53,334 (±) 8000 nucleated cells/ml were isolated and for evo-modified nanofat about 125,000 (±) 12,000 nucleated cells/ ml (Figure 3E). Classic nanofat showed the lowest SVF content while supercharged-modified nanofat the highest.

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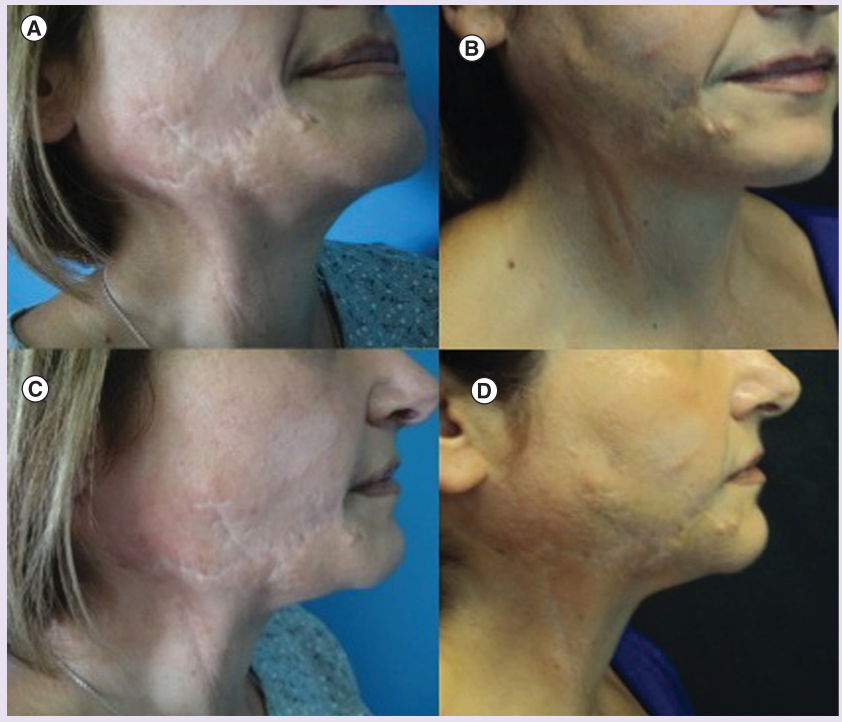

Figure 7. Clinical case of a patient affected by scars right cheeck and emimandibular area treated with classic nanofat. (A & C) Pre-operative situation in three-fourth and lateral right projection with outcomes of scars in the right neck region. **(B & D)** Postoperative situation in three-fourth and lateral right projection at 6 months after treatment with the increase of skin quality.

Clinical outcomes from different nanofat procedures

The end point of nanofat grafting was correcting the skin defects and improving the quality in the damaged area (outcomes of burn and post-traumatic scars). This effect generally compared after 3 months from the injection and gradually improved, for all treatments, over time peaking between 4 and 6 months (Figure 4–7). No significant complications, infections, fat cysts, granulomas or other unwanted side effects were observed in patients. However, team evaluation and patient self-evaluation (Tables 2 & 3) indicated that supercharged-modified nanofat ($p < 0.05$ vs all treatments) gave the best results followed by evo ($p < 0.05$ vs centrifuged and classic nanofat), centrifuged ($p < 0.05$ vs classic nanofat) and last the classic nanofat. In addition, we did not observed any significant difference in quality improvement between recent scars compared with older ones.

Histopathological evaluation

A representative microscopic evaluation of supercharged-modified nanofat grafting in a patient affected by outcomes of burns. Images showed a skin regeneration process, starting from baseline (T0) biopsy to 6 months post-treatment (T6) biopsy (Figure 8), in which a normal epithelium and newly deposed dermal collagen fibers as well as vessels were evident. Total epidermal and dermal thickness, measured for each experimental group ($n = 4$ patients/group), revealed a significant increase in skin thickness at 6 months post treatment ($p < 0.05$; Table 4). No substantial difference in skin thickness and regeneration between the different nanofat procedures was observed.

Discussion

Fat grafting and stem cells have been commonly used in plastic surgery for regeneration and rejuvenation purposes [11]. The authors have already published the results obtained by using platelet-rich plasma mixed with fat grafting in the treatment of scars of the face [12], chronic lower-extremity ulcers [13], loss of substance on the lower limbs [14] and application of the SVF in post-traumatic lower extremity ulcers [15,16]. These previous findings demonstrated that the application of SVF can improve tissue healing and maintenance of fat graft volume. In

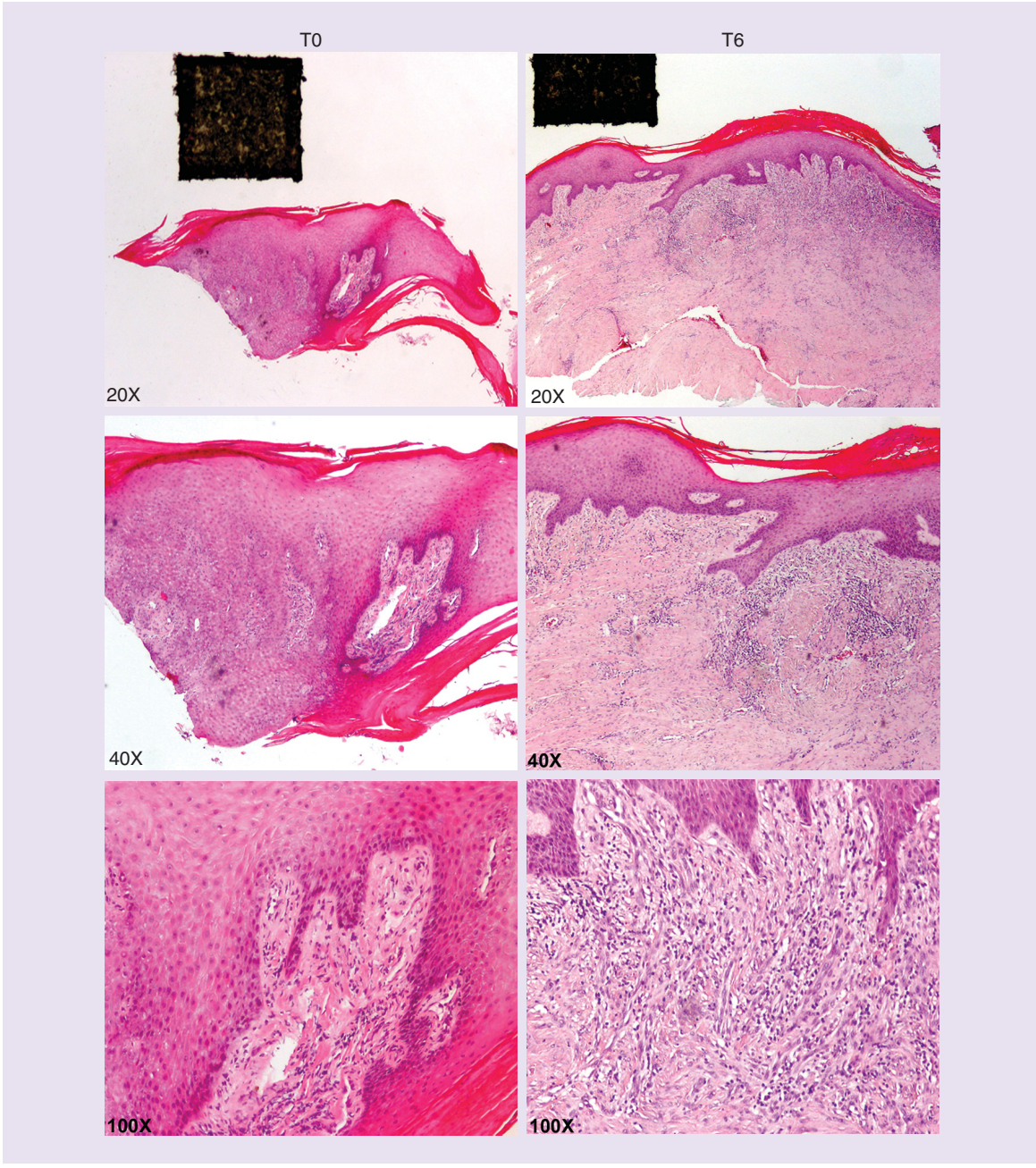

Figure 8. Histopathological findings of the outcomes of a burn before and after treatment with supercharged-modified nanofat. Baseline evaluation of a skin punch biopsy of a burn revealing a hyperkeratotic skin with a dermal thinning (T0). At 6 months post treatment (T6), skin regeneration with normal epithelium with newly deposed dermal collagen fibers and vessels (hematoxylin & eosin staining. Magnification: 20×, 40× and 100×, respectively).

fact, by early methods of adipose tissue transplantation, significant portions of the transplanted tissue were often reabsorbed, perhaps for a poor blood supply due to insufficient neoangiogenesis [17]. Therefore, to decrease graft failure rate different strategies have been developed in the last years to obtain fat grafts as rich as possible of mesenchymal stem cells, exploiting their regenerative capacities. In fact, it has been reported that ASCs and their secretome possess angiogenic properties [18,19]. Recently, a new fat grafting method, named 'nanofat', has been proposed by Tonnard *et al.* [20] for skin rejuvenation, especially to treat particular areas, such as eyelids, lips or fine rhytides. Several steps of emulsification and filtration of fat are necessary to obtain nanofat. The final liquid

Table 2. Team evaluation scale (6 months post treatment).

Nanofat kits/evaluation	Excellent = 5	Good = 4	Discreet = 3	Enough = 2	Poor = 1	Inadequate = 0
Supercharged-modified nanofat (n = 13 patients)						
Color/pigmentation	8	4	1	0	0	0
Vascularization	6	3	4	0	0	0
Pliability	6	4	3	0	0	0
Thickness	6	4	3	0	0	0
Itching	7	4	2	0	0	0
Pain	5	5	3	0	0	0
Total patients	38	24	16	0	0	0
Total score	190	96	48	0	0	0
Mean value				25.7		
Evo-modified nanofat (n = 10 patients)						
Color/pigmentation	5	3	2	0	0	0
Vascularization	4	4	1	1	0	0
Pliability	4	5	1	0	0	0
Thickness	4	4	2	0	0	0
Itching	4	3	3	0	0	0
Pain	5	3	1	1	0	0
Total patients	26	22	10	2	0	0
Total score	130	88	30	4	0	0
Mean value				25.2		
Centrifuged-modified nanofat (n = 10 patients)						
Color/pigmentation	4	3	2	1	0	0
Vascularization	3	4	2	1	0	0
Pliability	4	4	2	0	0	0
Thickness	4	4	2	0	0	0
Itching	3	4	2	1	0	0
Pain	3	2	3	2	0	0
Total patients	21	21	13	5	0	0
Total score	105	84	39	10	0	0
Mean value				23.8		
Classic nanofat (n = 10 patients)						
Color/pigmentation	3	3	3	1	0	0
Vascularization	2	2	3	3	0	0
Pliability	2	4	3	1	0	0
Thickness	4	2	4	0	0	0
Itching	2	4	3	1	0	0
Pain	3	2	2	3	0	0
Total patients	16	17	18	9	0	0
Total score	80	68	54	18	0	0
Mean value				22.0		

suspension, virtually devoid of mature adipocytes, can be injected in a more superficial plane through finer needles (27 gauge) and would improve tissue repair because of the presence of SVF and therefore ASCs [17], without enzymatic digestion or *in vitro* cell expansion. Because of the reduced number of viable adipocytes, destroyed during the emulsification process [17], the filling capacity of nanofat is obviously very limited [20]. In some cases, lipofilling was used for skin regeneration in the treatment of radiotherapy ulcers or scars [27]. One study reported a statistically significant improvement in dermal elasticity after injection of facial scars in 14 patients [28]. These effects are presumably due to the increased collagen and elastin synthesis and remodeling, likely triggered by stem cells rather than by grafted adipocytes [28,29]. However, it has been supposed that also a part of SVF can be lost during

Table 3. Patient evaluation scale (6 months post treatment).

Nanofat kits/evaluation	Excellent = 5	Good = 4	Discreet = 3	Enough = 2	Poor = 1	Inadequate = 0
Supercharged-modified nanofat (n = 13 patients)						
Color/pigmentation	8	5	0	0	0	0
Vascularization	6	4	1	2	0	0
Pliability	6	3	3	2	0	0
Thickness	7	3	1	1	0	0
Itching	7	5	1	0	0	0
Pain	7	5	0	0	0	0
Total patients	41	25	6	5	0	0
Total score	205	100	18	10	0	0
Mean value				25.6		
Evo-modified nanofat (n = 10 patients)						
Color/pigmentation	6	3	0	0	0	0
Vascularization	4	5	0	1	0	0
Pliability	4	5	0	3	0	0
Thickness	4	2	2	2	0	0
Itching	5	3	1	0	0	0
Pain	6	3	1	0	0	0
Total patients	29	21	4	6	0	0
Total score	145	84	12	12	0	0
Mean value				25.3		
Centrifuged-modified nanofat (n = 10 patients)						
Color/pigmentation	6	4	0	0	0	0
Vascularization	3	4	0	3	0	0
Pliability	2	5	0	3	0	0
Thickness	2	3	3	2	0	0
Itching	5	3	2	0	0	0
Pain	4	5	1	0	0	0
Total patients	22	24	6	8	0	0
Total score	110	96	18	16	0	0
Mean value				24.0		
Classic nanofat (n = 10 patients)						
Color/pigmentation	4	5	0	0	0	0
Vascularization	3	3	0	4	0	0
Pliability	2	4	0	4	0	0
Thickness	3	0	4	3	0	0
Itching	4	3	2	2	0	0
Pain	4	4	2	0	0	0
Total patients	20	19	8	13	0	0
Total score	100	76	24	26	0	0
Mean value				22.6		

Table 4. Total epidermal and dermal thickness (6 months post treatment).

Nanofat procedures	Baseline (mm)	6 month post-treatment (mm)
Supercharged-modified nanofat	1.43 ± 0.15	2.70 ± 0.31*
Evo-modified nanofat	1.11 ± 0.09	2.15 ± 0.25*
Centrifuged-modified nanofat	1.65 ± 0.20	3.02 ± 0.33*
Classic nanofat	1.74 ± 0.21	3.13 ± 0.28*

Values are means of four different patients for each experimental group. *t*-test: *$p < 0.05$, baseline versus 6 months post treatment. No significant difference was observed between different groups.

10.2217/rme-2017-0076

the nanofat procedure [17]. A study conducted by Mashiko *et al.* demonstrated that mechanical micronization can condense fat tissue removing adipocytes without damaging key components, such as adipose-derived stromal cells and endothelial cells [30].

In this study, we compared, in terms of clinical outcomes, different nanofat procedures in order to evaluate the influence of each processing step in the final result. In addition, we evaluated, for each procedure, the SVF yield and correlated it with the clinical outcomes obtained from each method. Data from flow cytometry analysis of SVF obtained by different nanofats revealed the expression of specific mesenchymal stromal stem cell markers (CD44, CD90, CD73), pericyte marker (CD146), endothelial marker (CD31) and hematopoietic/endothelial stem cell marker (CD34), according to literature [5,31]. The percentages of positive cell populations were similar in all experimental groups. So, SVF cellular composition was similar between the procedures, the only difference was represented by the cell yield obtained. This comparison was performed in order to detect which step or protocol modification can be applied to nanofat procedure to optimize the SVF yield and obtain the best possible clinical result. Different-modified nanofats (supercharged, evo and centrifuged) were obtained by using mechanical filtration and/ or centrifugation steps of the fat in a close system, and compared with the classic nanofat by Tonnard. In all treated cases, we observed a gradual improvement of skin quality and the correction of the defect that compared after 3 months from the injection and peaked between 4 and 6 months. No significant complications, infections, fat cysts, granulomas or other unwanted side-effects were observed in patients. Histologic analysis evidenced skin regeneration and new dermal formation in all treated cases with no significant differences. However, the clinical assessment, performed by our observations of preoperative and postoperative photographs and by team evaluation/patient self-evaluation scores, indicated that supercharged-modified nanofat gave the best results followed by evo, centrifuged and the classic nanofat. No significant difference in quality improvement was found between recent scars compared with older ones. In addition, the clinical outcomes correlated with the SVF cell yield obtained in the different nanofat procedures; in fact, the highest SVF cell content was found in supercharged-modified nanofat followed by evo, centrifuged and the classic nanofat. The results obtained in this study were in line with those of Tonnard [20] and with the modifications applied to the nanofat procedure. In particular, supercharged-modified nanofat was an emulsified fat enriched with SVF, instead no SVF enrichment was performed in evo- and centrifuged-modified nanofat. Moreover, in centrifuged-modified nanofat the SVF was partially removed by centrifugation, whereas in evo-modified nanofat the filtration step preserved the SVF content. These modified nanofat procedures showed a higher SVF yield than the classic method by Tonnard. This finding demonstrated that clinical outcome based on skin improving depends in part on SVF amount contained in the fat. In addition, the authors demonstrated that each single step in the nanofat procedure, including fat harvesting, can influence the final SVF yield.

Conclusion

We reported the clinical outcomes of different nanofat procedures in the treatment of scars in relation with SVF cell yield. SVF enrichment of nanofat (supercharged-modified nanofat) offered the highest concentration of cells and the best clinical evaluation scores; on the other hand, nanofat performed by slow centrifugation (evo-modified nanofat) was the best procedure compared with the regular centrifugation (centrifuged-nanofat) and classic nanofat. Histological assessment revealed a skin regeneration process, but no significant difference between the procedures. This work reported, for the first time, a positive correlation between SVF yield and clinical outcomes in the nanofat treatment of scars.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Summary points

- Fat grafting and stem cells have been commonly used in plastic surgery for regeneration and rejuvenation purposes.
- Previous studies demonstrated that the application of stromal vascular fraction (SVF), containing adipose-derived stem cells, can improve tissue healing and maintenance of fat graft volume.
- Recently, a new fat grafting method, named 'nanofat', has been proposed by Tonnard *et al.* for skin rejuvenation, especially to treat particular areas, such as eyelids, lips or fine rhytides.
- Several steps of emulsification and filtration of fat are necessary to obtain nanofat.
- We reported the clinical outcomes of different nanofat procedures in the treatment of scars in relaion with SVF cell yield.
- We compared three different modified nanofat procedures (supercharged-, evo- and centrifuged-modified nanofat) with the classic nanofat by Tonnard *et al.*
- Clinical assessment by team evaluation and patient self-evaluation scale was performed as well as histological analysis on cutaneous biopsies to assess skin regeneration. In addition, residual nanofat samples were analyzed by flow cytometry to characterize SVF immunophenotype, and cell counting performed to estimate SVF cell yield from each different procedure.
- Supercharged-modified nanofat gave the best results in terms of clinical outcome and SVF cell yield. Anyway, histological analysis revealed a similar skin regeneration in all treatments.
- This work suggested a positive correlation between SVF yield and clinical outcomes in the nanofat treatment of scars.

References

1. Franchini M. [Mesenchymal stem cells: from biology to clinical applications]. *Recenti. Prog. Med.* 94(11), 478–483 (2003).
2. Kern S, Eichler H, Stoeve J *et al.* Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. *Stem Cells* 24(5), 1294–1301 (2006).
3. Weisberg SP, McCann D, Desai M *et al.* Obesity is associated with macrophage accumulation in adipose tissue. *J. Clin. Invest.* 112(12), 1796–1808 (2003).
4. Xu H, Barnes GT, Yang Q *et al.* Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J. Clin. Invest.* 112(12), 1821–1830 (2003).
5. Gimble JM, Katz AJ, Bunnell BA. Adipose-derived stem cells for regenerative medicine. *Circ. Res.* 100(9), 1249–1260 (2007).
6. Katz AJ, Tholpady A, Tholpady SS *et al.* Cell surface and transcriptional characterization of human adipose-derived adherent stromal (hADAS) cells. *Stem Cells* 23(3), 412–423 (2005).
7. Izadpanah R, Trygg C, Patel B *et al.* Biologic properties of mesenchymal stem cells derived from bone marrow and adipose tissue. *J. Cell. Biochem.* 99(5), 1285–1297 (2006).
8. Zuk PA, Zhu M, Mizuno H *et al.* Multilineage cells from human adipose tissue: Implications for cell-based therapies. *Tissue Eng.* 7(2), 211–228 (2001).
9. Rodriguez AM, Elabd C, Amri EZ *et al.* The human adipose tissue is a source of multipotent stem cells. *Biochimie* 87(1), 125–128 (2005).
10. Doi K, Kuno S, Kobayashi A *et al.* Enrichment isolation of adipose-derived stem/stromal cells from the liquid portion of liposuction aspirates with the use of an adherent column. *Cytotherapy* 16(3), 381–391 (2014).
11. Kemaloğlu CA. Nanofat grafting under a split-thickness skin graft for problematic wound management. *Springerplus* 5, 138 (2016).
12. Gentile P, De Angelis B, Pasin M *et al.* Adipose derived stromal vascular fraction cells and Platelet rich plasma: basic and clinical evaluation for cell based therapies in patients with scars on the face. *J. Craniofac. Surg.* 25(1), 267–272 (2014).
13. Cervelli V, Gentile P, Grimaldi M. Regenerative surgery: use of fat grafting combined with platelet-rich plasma for chronic lower-extremity ulcers. *Aesthet. Plast. Surg.* 33(3), 340–345 (2009).
14. Cervelli V, De Angelis B, Lucarini L *et al.* Tissue regeneration in loss of substance on the lower limbs through use of platelet- rich plasma, stem cells from adipose tissue, and hyaluronic acid. *Adv. Skin Wound Care* 23, 262–272 (2010).
15. Cervelli V, Gentile P, De Angelis B *et al.* Application of enhanced stromal vascular fraction and fat grafting mixed with PRP in post-traumatic lower extremity ulcers. *Stem Cell Res.* 6(2), 103–111 (2011).
16. Gentile P, Orlandi A, Scioli MG *et al.* A comparative translational study: the combined use of enhanced stromal vascular fraction and platelet-rich plasma improves fat grafting maintenance in breast reconstruction. *Stem Cells Transl. Med.* 1(4), 341–351 (2012).
17. Lo Furno D, Tamburino S, Mannino G *et al.* NANOFAT 2.0: experimental evidence for a fat grafting rich in mesenchymal stem cells. *Physiol. Res.* 66(4), 663–671 (2017).

10.2217/rme-2017-0076

18. Kalinina N, Kharlampieva D, Loguinova M *et al.* Characterization of secretomes provides evidence for adipose-derived mesenchymal stromal cells subtypes. *Stem Cell Res. Ther.* 6, 221 (2005).
19. Charles-de-Sá L, Gontijo-de-Amorim NF, Maeda Takiya C *et al.* Antiaging treatment of the facial skin by fat graft and adipose-derived stem cells. *Plast. Reconstr. Surg.* 135(4), 999–1009 (2015).
20. Tonnard P, Verpaele A, Peeters G *et al.* Nanofat grafting: basic research and clinical applications. *Plast. Reconstr. Surg.* 132(4), 1017–1026 (2013).
21. Oberbauer E, Steffenhagen C, Wurzer C *et al.* Enzymatic and non-enzymatic isolation systems for adipose tissue-derived cells: current state of the art. *Cell Regen. (Lond.)* 4, 7 (2015).
22. Domenis R, Lazzaro L, Calabrese S *et al.* Adipose tissue derived stem cells: *in vitro* and *in vivo* analysis of a standard and three commercially available cell-assisted lipotransfer techniques. *Stem Cell Res. Ther.* 6, 2 (2015).
23. Gentile P, Scioli MG, Orlandi A *et al.* Breast reconstruction with enhanced stromal vascular fraction fat grafting: what is the best method? *Plast. Reconstr. Surg. Glob. Open* 3(6), e406 (2015).
24. Cervelli 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* 15(4), 625–634 (2009).
25. 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.* 1(3), 206–220 (2012).
26. Agabalyan NA, Su S, Sinha S *et al.* Comparison between high-frequency ultrasonography and histological assessment reveals weak correlation for measurements of scar tissue thickness. *Burns* 43(3), 531–538 (2017).
27. Rigotti G, Marchi A, Galiè M *et al.* Clinical treatment of radiotherapy tissue damage by lipoaspirate transplant: a healing process mediated by adipose-derived adult stem cells. *Plast. Reconstr. Surg.* 119(5), 1409–1422 (2007).
28. Sardesai MG, Moore CC. Quantitative and qualitative dermal change with microfat grafting of facial scars. *Otolaryngol. Head Neck Surg.* 137(6), 868–872 (2007).
29. Gentile P, Scioli MG, Bielli A *et al.* Concise review: the use of adipose-derived stromal vascular fraction cells and platelet rich plasma in regenerative plastic surgery. *Stem Cells* 35(1), 117–134 (2017).
30. Mashiko T, Wu SH, Feng J *et al.* Mechanical micronization of lipoaspirates: squeeze and emulsification techniques. *Plast. Reconstr. Surg.* 139(1), 79–90 (2017).
31. Mitchell JB, McIntosh K, Zvonic S *et al.* Immunophenotype of human adipose-derived cells: temporal changes in stromal-associated and stem cell-associated markers. *Stem Cells* 24(2), 376–385 (2006).