Research

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Autologous Lipofilling Improves Clinical Outcome in Patients With Symptomatic Dermal Scars Through Induction of a Pro-Regenerative Immune Response

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Abstract

Background: Autologous lipofilling is an emerging procedure to treat and possibly reverse dermal scars and to reduce scar-related pain, but its efficacy and mechanisms are poorly understood.

Objectives: The aim of this study was to test the hypothesis that repeated lipografts reverse dermal scars by reinitiation of wound healing.

Methods: In a prospective, non-placebo-controlled clinical study, 27 adult patients with symptomatic scars were given 2 lipofilling treatments at 3-month intervals. As primary outcome, clinical effects were measured with the Patient and Observer Scar Assessment Scale (POSAS). Scar biopsies were taken before and after treatments to assess scar remodeling at a cellular level.

Results: Twenty patients completed the study. Patients' scars improved after lipofilling. The total POSAS scores (combined patient and observer scores) decreased from 73.2 [14.7] points (mean [standard deviation]) pretreatment to 46.1 [14.0] and 32.3 [13.2] points after the first and second lipofilling treatment, respectively. Patient POSAS scores decreased from 37.3 [8.8] points to 27.2 [11.3] and 21.1 [11.4] points, whereas observer POSAS scores decreased from 35.9 [9.5] points to 18.9 [6.0] and 11.3 [4.5] points after the first and second treatment, respectively. After each lipofilling treatment, T lymphocytes, mast cells, and M2 macrophages had invaded scar tissue and were associated with increased vascularization. In addition, the scar-associated epidermis showed an increase in epidermal cell proliferation to levels similar to that normal in skin. Moreover, lipofilling treatment caused normalization of the extracellular matrix organization towards that of normal skin.

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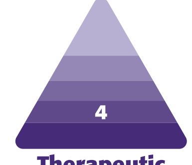
Conclusions: Autologous lipofilling improves the clinical outcome of dermal scars through the induction of a pro-regenerative immune response, increased vascularization, and epidermal proliferation and remodeling of scar tissue extracellular matrix.

Level of Evidence: 4



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Treatment of symptomatic dermal scars remains challenging and frequently does not sufficiently reduce scar visibility and associated pathologic symptoms.[1] Dermal scarring results from adverse wound healing, meaning it must always begin with damage to the skin. Upon progression through the distinct steps of wound healing (ie, inflammation, new tissue formation, and remodeling), this healing process resolves by fibrosis.[2] In normal physiology, the epidermis and dermis are subsequently restored.[3]

The resolution of wound healing results in a scar that might be indistinguishable from normal skin (normotrophic) or may acquire pathologic features such as in the case of hypertrophic and keloid scars,[4] which cause clinical symptoms.[1, 4] Although these pathologic scars give rise to complaints far more often than normotrophic scars, normotrophic scars may also cause symptoms. With regard to symptoms, we will use the terms physiologic vs symptomatic scar.

Symptomatic scars present several characteristics, including differences in color and texture compared to normal skin, which can lead to patients experiencing distress from their altered appearance and reduced aesthetics. In addition, volume defects may exist, eg, in burn wound scars or degloving injuries.[5] Scars may also be painful, itchy, and cause functional impairment by restricting movement.[1]

Lipofilling, the subcutaneous administration of processed autologous lipoaspirates, is a promising therapy for scars because it improves scar-tissue properties such as elasticity, while also resolving volume defects caused by scars.[6-8] Importantly, lipofilling reduces neuropathy via an unknown mechanism and also appears to reduce scar-related pain.[9] The existing published studies on the influence of lipofilling are often poorly controlled, and virtually all lack mechanistic insight. Therefore, the current study was undertaken to evaluate the clinical outcome of lipofilling on symptomatic scars that were resistant to conventional scar therapy and to understand the underlying histologic changes that may explain the mechanism underlying the effects produced by the treatment.

METHODS

Experimental Design

This study protocol was carried out in compliance with the Declaration of Helsinki and was approved by the medical ethics committees of both German centers involved (reference numbers 256/2014MPG23 and 167/2015MPG43). All patients who agreed to participate in this study gave their written informed consent prior to inclusion in the study (the patient information and consent forms were translated from English). Informed consent of the patients includes the use of the research data (including pictures) obtained in the course of the study. This was also approved by the ethics committees. Therefore, in the figures we anonymized the numbers assigned to the patients (which in principle are back-traceable) and replaced these with neutral numbering: patients 1, 2, and 3. The design was a prospective, non-placebo-controlled therapeutic study. Inclusion and exclusion criteria are listed in Table 1. Patients were included at the departments of Plastic, Reconstructive, Hand, and Burn Surgery, BG-Trauma Center, Eberhard Karls University, Tübingen, Germany, and the Department of Plastic, Aesthetic and Reconstructive Surgery, Ernst Von Bergmann Clinic, Potsdam, Germany. Outcome measures were recorded before lipofilling treatment and 3 months after the initial treatment. Then, another lipofilling treatment was performed and final evaluation took place 6 months after the initial treatment (the study outline is shown in Figure 1). Evaluations of biopsies and adipose tissue were performed at the Department of Pathology and Medical Biology, University Medical Center Groningen, Groningen, the Netherlands.

Lipofilling Treatment

The lipofilling treatment (minimally invasive scar release combined with water-jet-assisted autologous lipofilling) was performed as described previously[10,11] with modifications. All treatments were performed under general anesthesia and by the same surgeon (D.L.F.). The harvesting

Table 1. Inclusion and Exclusion Criteria

Inclusion criteria	Exclusion criteria
Age >18 years	Age <18 years
Symptomatic scars with complaints existing for >6 months and nonresponsive to conventional therapy[a]	Pregnancy or active wish for child
<6 months and >6 months: progressive scars with movement restrictions or contractures or both	Known psychiatric condition, including alcohol abuse
	Known cardiac conditions
	>5 kg weight change in past 2 months prior to treatment

[a]Silicon sheet treatment, compression therapy, scar creams, and operative scar revision.

and injection of lipoaspirate were performed in the same operative procedure. Lipoaspirates were harvested with a water-jet-assisted liposuction system (Human Med AG, Schwerin, Germany) from either the abdomen or inner thighs. To start the procedure, a modified Klein's solution was used as wetting solution, which consisted of isotonic saline solution supplemented with epinephrine (diluted 10^6-fold; Infectopharm, Heppenburg, Wedel, Germany), xylocaine (final concentration, 0.049%; AstraZeneca, Wedel, Germany), and sodium bicarbonate (final concentration, 1.48%; Fresenius Kabi, Bad Homburg, Germany). Lipoaspirates were harvested with liposuction cannulas (Human Med AG) and collected in a LipoCollector system (Human Med AG). To obtain pure adipose tissue aspirates, the superfluous infiltration fluid was removed from the collector system. Subsequently, the lipoaspirate was collected in 50-mL syringes and decanted for 10 minutes. Any remaining infiltration fluid was removed and the lipoaspirate was transferred to 10-mL syringes. Before injection of the lipoaspirate, percutaneous scar release was performed in a multiplanar, fan-shaped fashion with sharp cannulas ranging from 16G to 22G. The lipoaspirate was then injected by means of blunt cannulas into the scar area. The volume of lipoaspirate injected into each scar related to the surface area and depth of the scar, based on clinical judgment and experience. The scar area was immobilized when possible and a custom-made cushioning dressing was applied to decrease stress and pressure on the injected lipoaspirate. All patients received antibiotics for 5 days.

Clinical Assessment

As the primary outcome measure, we used the Patient and Observer Scar Assessment Scale (POSAS), which is a validated questionnaire (Supplemental Material) to evaluate the severity of scarring.[12] The POSAS questionnaire, ie, the patient and observer score, was downloaded from www.posas.org. For the convenience of the patients, it was translated to a German form. All forms were filled out on paper—by the patient for the patient score or by the clinician/surgeon for the observer score. The survey was distributed shortly before treatment in the hospital or during the outpatient visit for the final follow-up appointment. The clinician who performed the scoring was well-trained in scoring POSAS questions. The POSAS system has an inherent bias—namely, that clinicians tend to score differently than patients—which is why the developer of the POSAS, Professor van Zuijlen, combined both scores and refined the questions to achieve a scoring system that was as objective as possible .[12] To date, the POSAS has been widely adopted worldwide as a reliable scoring system for (burn) scars and to assess the influence of, for example, lipografting on (burn) scars. POSAS questionnaires (Appendix, available online at www.aestheticsurgeryjournal.com) were filled out before lipofilling, 3 months after the first lipofilling treatment and 3 months after the second lipofilling treatment. The observer scales were filled out by the same observer (D.L.F.). Total POSAS scores were calculated by summing the scores of all items of the patient and observer questionnaires, except for the item "overall opinion." Complications were also monitored during the entire follow-up period.

In addition, pain was scored on a visual analog scale (VAS). The VAS was determined prior to intervention, 3 months after the first intervention, and 3 months after the second intervention. The pain was scored between no pain (score of 0) and worst imaginable pain (score of 10) using numbers as well as textual explanations.

Tissue Collection and Preservation

Three consecutive scar biopsies were obtained from all patients: before first lipofilling treatment (intraoperative), 3 months after the first treatment, and 3 months after the second lipofilling treatment. Skin biopsies were obtained as excision biopsies or with a skin biopsy punch (size range, 2-5 mm). The incisions remaining from the biopsies were subsequently used as the entrance port for lipofilling. The final (third) biopsy was taken with a biopsy punch under local anesthesia. Normal human skin was obtained from anonymous surgical waste material (n = 5). Immediately after collection, tissues were formalin-fixed and then paraffin-embedded.

Scar Tissue Immunohistochemistry

Deparaffinized sections (5 µm) of scar tissue biopsies were stained with antibodies for Ki67 (clone 30-9), CD3 (clone 2GV6), tryptase (clone G3) (all from Roche Diagnostics, Mannheim, Germany), and CD163 (clone

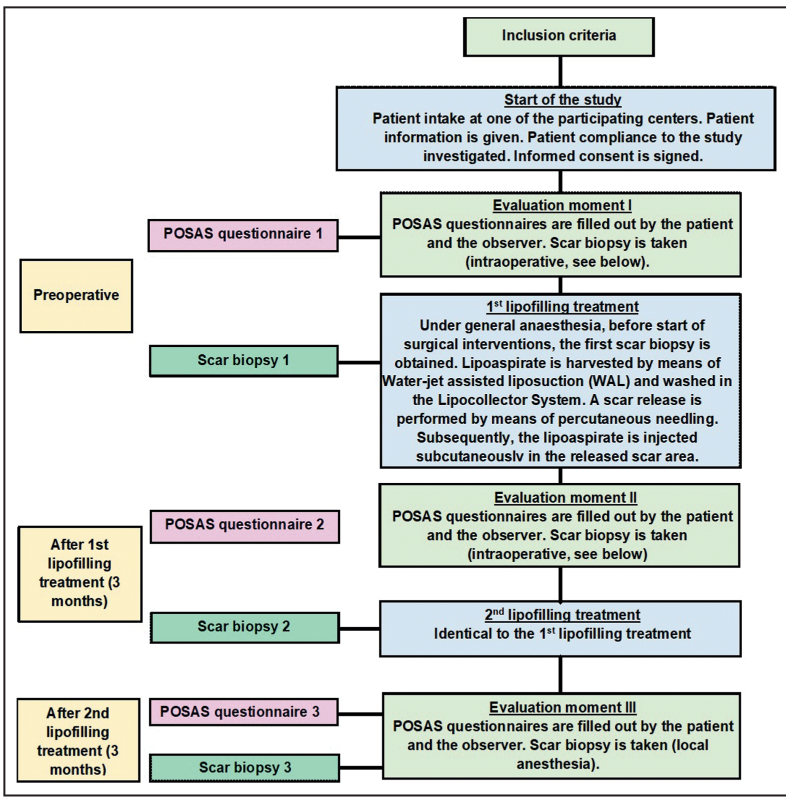


Figure 1. Study flow diagram.

MRQ-26) and α smooth muscle actin (αSMA; clone 1A4) (both from Cell Marque, Rocklin, CA). These were used to assess proliferation, CD3 T lymphocytes, mast cells, macrophages, and smooth muscle cells, respectively. All immunohistochemical stainings were performed with a Bench Mark Ultra automated immunostainer (Ventana Medical Systems, Tucson, AZ). Antigen retrieval was performed with UltraCC1 buffer. Slides were incubated with prediluted primary antibody solutions. For antigen detection, the OptiView IHC detection kit (Ventana Medical Systems) was used. For αSMA and CD163, this signal was amplified with the OptiView amplification kit (Ventana Medical Systems). All tissues were counterstained with hematoxylin and mounted with xylene and TissueTek film (Sakura Finetek, Alphen aan den Rijn, the Netherlands). For quantification, tissues were examined in a Leica DM2000 LED microscope (Leica Microsystems, Wetzlar, Germany). The number of positive cells and the number of vessels were scored on blinded samples in 4 randomly selected fields of view at ×40 magnification (combined surface area, 1 mm^2). For Ki67, only cells in the epidermis were scored.

Scar Tissue Extracellular Matrix Analyses

Scar tissues were sectioned at 3 μm and deparaffinized. Tissues were stained with Weigert's hematoxylin (Sigma Aldrich, St Louis, MO) and washed extensively with tap water. Afterwards, tissues were stained with Picrosirius Red Solution, consisting of 0.1% (w/v) Direct Red (Sigma Aldrich) in a saturated solution of 1.3% picric acid in water (Sigma Aldrich) for 10 minutes. Then, sections were washed with acidified water and dehydrated with 100% ethanol. Slides were examined in an Olympus BX50 (Olympus Optical Co., Hamburg, Germany) equipped with a linear polarization filter at ×10 magnification.

Conditioned Medium Collection

Conditioned medium for endothelial sprouting assays was collected from cultured primary human macrophages and from the human mast cell line HMC-1 clone 5C6. Human macrophages were isolated from buffy coats from 5 individual donors as described previously.[13] Extended methods are descriped in the Supplemental Material.

Endothelial Sprouting Assay

Human umbilical vein endothelial cells (Lonza) were used to assess the influence of M2 macrophage and mast conditioned medium on sprouting. Human umbilical vein endothelial cell culture and endothelial sprouting assays were performed as described previously.[14] An extended methodology can be found in the Supplemental Material.

Statistical Analysis

All data are represented as mean [standard deviation], unless stated otherwise. The normal distribution of data was tested using the Kolmogorov-Smirnov test. Normally distributed data were analyzed by a repeated-measures one-way analysis of variance (ANOVA) with Tukey multiple comparisons or mixed-effects ANOVA with Bonferroni post-hoc test and unpaired *t* tests where appropriate. Missing data were not imputed. For statistical analyses, GraphPad Prism version 9 (GraphPad Software Inc., La Jolla, CA) and IBM SPSS Statistics 22 (IBM Corp., Armonk, NY) were used. *P* values <0.05 were considered to indicate statistically significant differences.

RESULTS

Patient Inclusion

Twenty-seven patients were enrolled in this study between January 2015 and June 2018 (Table 1). Twenty patients (5 male, 15 female) completed all lipofilling treatments; 18 patients completed all POSAS questionnaires and the series of 3 scar biopsies was completed for 17 patients (2 patients refused the final biopsy; the second biopsy of 1 patient was too small for analyses). Seven patients quit the planned protocol because of traveling distance (n = 3), emergency treatment for a condition not related to the study (n = 1), or inability to comply with the study regimen (n = 3). Data of patients who dropped out of the study were not included in the analyses.

Patient Characteristics

Patient demographics for each individual patient are described in Table 2. The mean age was 49.5 [16.1] years (range, 25-83 years). The mean BMI was 25.4 [3.4] kg/m^2 (range, 20.5-30.5 kg/m^2). Scars were located in the head and neck area (n = 3), trunk (n = 8), upper extremities (n = 4), and lower extremities (n = 5). Scars were due to flap harvest (n = 4), burns (n = 1), necrotizing fasciitis (n = 1), a degloving injury (n = 2), or "other" surgical scars (n = 12). Prior scar treatments consisted of operative scar corrections, scar massage, compression, ergotherapy and physical therapy, corticosteroid injection, and scar creams.

Surgical Treatment Variables

Forty lipofilling treatments were performed in the course of this study (Figure 1). The mean operation time was 75 [30] minutes (range, 33-155 minutes). The average injected volume of lipoaspirate was 71.8 [74.3] mL per treatment (range, 4-355 mL). Injected volume depended primarily on clinical judgment and experience related to the surface area and depth of the scar. The interventions and biopsies were all taken within 1 week of the 3- and 6-month time points according to the scheduling of the protocol.

Complications

As expected, reported donor site complications after liposuction were pain, swelling, and hypo or- hyperesthesia. All donor site sequelae resolved spontaneously within 1 month. In the recipient (scar) area there was 1 major complication that required surgery in which a necrotic area of the skin was successfully treated with a skin graft. Two minor complications were reported: a wound-healing problem and a nerve compression in a radial forearm flap donor area; both were managed with conservative treatment. None of these complications adversely influenced the POSAS scores or immunohistologic results (repeated-measures ANOVA, $P > 0.05$).

Clinical Improvement of Scar Appearance After Lipofilling Treatment

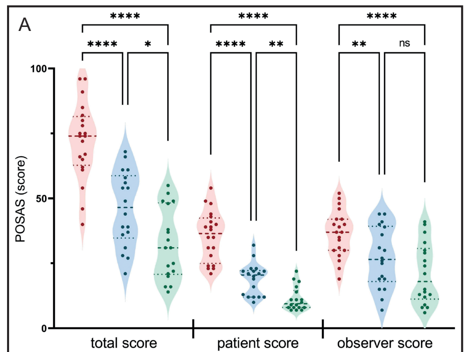
Macroscopically, the influence of repeated lipografting of large dermal scars showed as a resolution of the volume defect (Supplemental Figure 1) even after the first lipofilling. A second lipofilling had a further macroscopic effect, albeit less clearly discernible (Supplemental Figure 1). Preoperatively, the total POSAS score was 73.2 [14.7] points (range, 40-96 points). After the first and second lipofilling treatment, this decreased to 46.1 [14.0] points (range, 21-66 points) and 32.3 [13.2] points (range, 16-55 points), respectively ($P < 0.001$) (Figure 2A). The baseline POSAS observer score was 35.9 [9.5] points (range, 21-54 points), which decreased to 18.9 [6.0] points (range, 10-32 points) and 11.3 [4.5] points (range, 7-22 points) after the first and second lipofilling treatment, respectively ($P < 0.001$) (Figure 2A). For the POSAS patient scale, the preoperative score was 37.3 [8.8] points (range, 19-52 points), which decreased to 27.2 [11.3] points (range, 7-44 points) and 21.1 [11.4] points (range, 6-41 points) ($P < 0.001$) (Figure 2A) after the first and second lipofilling treatment, respectively. Although there was a difference between patient and observer POSAS scores— the mean observer score decreased by 68% whereas the patient scores decreased by 43% after the second lipofilling treatment vs preoperative values (repeated-measures ANOVA, $P < 0.001$)—all POSAS scores (total, patient, and observer) decreased between the first and second lipofilling treatments ($P < 0.05$) (Figure 2A).

The secondary measure, pain, was assessed via a VAS. The initial VAS score was 8.2 [1.3] (range, 6-10) which

Table 2. Demographics of the Study Patients

No.	Gender	Age (years)	Comorbidity[a]	BMI (kg/m²)	Scar location	Trauma/treatment indication	Scar age[b]	Previous scar therapy
1	Female	60	None	27.7	Back	Painful scar after lattisimus dorsi flap harvest	2 y 3 mo	SMT, creams
2	Female	60	HT	25.8	Knee, lower limb	Countour defect after degloving injury by accident	2 y 6 mo	SMT, creams, SST
3	Female	59	None	26.8	Abdomen	Painful scar after DIEP flap harvest	9 mo	
4	Female	52	None	28.4	Neck	Painful, tight scar after thyroidectomy	8 y	Operative scar corrections
5	Female	50	HT	32.9	Lower leg	Painful burn scar with movement restriction	2 y 7 mo	Operative scar corrections, creams, SST
6	Female	53	None	21.8	Lower arm	Painful, restrictive scar after fasciotomy for compartment syndrome	7 y 9 mo	Operative scar corrections, SMT, creams, SST
7	Female	25	None	23.7	Knee, lower limb	Painful scar with movement restriction after osteosarcoma extirpation	13 y	Operative scar corrections
8	Male	59	HT	28.9	Hand and wrist	Painful, restrictive scar after complex crush injury and amputation	1 y	Operative scar corrections, SMT, SST, PT
9	Female	65	None	28.5	Abdomen	Painful, tight scar after necrotizing fasciitis following abdominoplasty	11 mo	Operative scar corrections, creams, SST
10	Male	83	Pre-DM	30.5	Inguinal/ scrotal region	Painful, progressive scar contracture after scrotal unilateral orchiectomie	5 mo	None
11	Female	76	HT	23.1	Scalp	Painful, depressed scar after excision of basal cell carcinoma	11 y	Operative scar corrections, creams, SMT
12	Male	26	Smoker	21.2	Inner thigh	Painful scars with movement restriction after gunshot wounds	15 y	Operative scar corrections, creams
13	Female	55	None	21.6	Abdomen	Retracted scar after open appendectomy	33 y	None
14	Male	32	None	28.1	Lower arm	Painful, depressed, restrictive donor area (skin graft) of radial flap	2 y 9 mo	SMT, SST, creams
15	Female	53	HT	23.6	Abdomen	Painful scar after laparotomy	44 y	Operative scar corrections, creams, SMT, ET
16	Male	33	None	20.9	Lower arm	Painful, depressed, restrictive donor area (skin graft) of radial flap	3 y 8 mo	Operative scar corrections, creams, SMT, SST
17	Female	34	None	25.6	Temporal region	Painful scar and contour defect after craniotomy	2 y 7 mo	SMT
18	Female	42	Smoker	24.4	Sternum	Painful, itching scar after atheroma removal with subsequent infection and secondary healing	1 y 4 mo	Creams
19	Female	25	None	20.5	Clavicula	Painful, depressed scar after operative reposition of clavicula fracture	1 y 10 mo	Operative scar corrections, creams, CI, SST
20	Female	47	None	24.0	Hand, 4th ray	Painful and depressed scar after amputation following degloving injury	1 y	Operative scar corrections, creams, SMT, ET

CI, corticosteroid injection; DM, diabetes mellitus; ET, ergotherapy; HT, hypertension; mo, months; PT, physical therapy; SMT, scar massage therapy; SST, silicon sheet therapy; y, year. [a]Recorded comorbidities were hypertension, DM (prediabetic, type I or II) and smoking. [b]Time since original trauma.



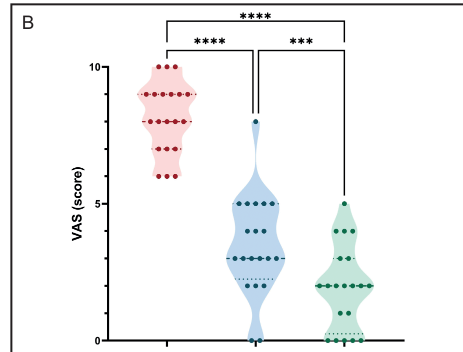


Figure 2. Lipofilling in symptomatic dermal scars improves clinical outcome, as measured by the POSAS questionnaire (n = 18). (A) Total POSAS scores—combination of the scores on all items of the patient and observer scales, except for the item "overall opinion" (maximum total score of 120 for the worst scar imaginable)—patient scores, and observer scores are plotted. The patient score combines scar pain, itch, color, stiffness, thickness, and irregularity. The observer score comprises the item's vascularity, pigmentation, thickness, relief, pliability, and surface area. (B) Visual analog scale scoring (pain) by patients postoperatively, after the first and second lipofilling. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{**}P < 0.001$, $^{****}P < 0.0001$ (POSAS: mixed-effects analysis of variance with Bonferroni post-hoc testing; visual analog scale: repeated-measures one-way analysis of variance with Tukey's multiple comparison testing). Preoperative data, red symbols; data after first lipofilling, blue symbols; data after second lipofilling, green symbols. POSAS, Patient and Observer Scar Assessment Scale.

decreased to 3.5 [1.8] (range, 0-8; $P < 0.0001$) 3 months after the first lipofilling (Figure 2B). A second lipofilling reduced the VAS score further to 1.9 [1.5] (range, 0-5; $P < 0.0001$ vs $t = 0$ and $P = 0.004$ vs $t = 3$ months).

Lipoaspirate Characteristics

From lipoaspirates of 5 study patients, adipose-derived stromal cells (ASCs) were isolated and cultured. After culture expansion, these ASCs were assessed for surface marker expression, and for differentiation and colony-forming unit potential. The ASCs were $CD90^+$ (99.70% [0.22%]), $CD44^+$ (98.9% [0.97%]), $CD105^+$ (96.95% [1.35%]), $CD29^+$ (99.62% [0.42%]), $CD45^-$ (99.79% [0.09]%), and CD31(99.56% [0.24%]) (Supplemental Figure 2). As for colony-forming unit capacity, after 14 days of culture with seeding densities of 10 or 100 cells/cm^2, ASCs covered 0.28% [0.26%] and 56.4% [22.40%] of the surface area, respectively (Supplemental Figure 2B). Furthermore, the ASCs harbored multipotency and differentiated into osteoblasts, adipocytes, and myogenic cells (Supplemental Figure 2C and E, respectively). Histologically, the main volume of lipoaspirate fragments comprised intact adipocytes (Supplemental Figure 3A) with the expected large variation in diameter that averaged around 100 µm (range, 20-180 µm) (Supplemental Figure 3B). The adipocytes were embedded in a highly vascularized stroma (Supplemental Figure 3C-E).

Increase in Epidermal Proliferation in Scar Biopsies After Lipofilling

In scar tissues, before and after lipofilling, proliferating epidermal cells resided in the basal layer, in a pattern similar to normal skin. Before lipofilling, the number of proliferating cells was 78 [51] (range, 9-219) positive cells/mm^2, which increased after the first treatment of lipofilling to 114 [48] (range, 6-207; $P = 0.0139$) and 124 [63] (range, 28-268) positive cells/mm^2 ($P = 0.0032$; Figure 3) after the second lipofilling, respectively.

Increase in Vessel Density in Scar Tissue After Lipofilling Treatment

Vascular density in scar tissue preoperatively was 53 [15] (range, 21-74) vessels/mm^2, which increased to 67 [21] (range, 21-86) and 71 [23] (range, 41-119) vessels/mm^2 ($P = 0.0312$ and $P = 0.0042$, respectively; Figure 4) after the first and second lipofilling treatment, respectively.

Increase in Immune Cells in Scar Tissue After Lipofilling Treatment

Numbers of T lymphocytes (CD3), (M2 polarized) macrophages (CD163), and mast cells (tryptase) in scars increased after both the first and the second lipofilling treatment compared with preoperative controls (Supplemental Figure 4). Before lipofilling, the number of T lymphocytes was 69 [46] per mm^2 (range, 15-194 per mm^2), which increased to 139 [88] per mm^2 (range, 23-429 per mm^2; $P = 0.0154$) and 166 [137] per mm^2 (range, 37-559 per mm^2; $P = 0.03$) after the first and second lipofilling treatment, respectively (Supplemental Figure 4). The number of macrophages was 183 [108] per mm^2 (range, 56-455 per mm^2) preoperatively, which increased to 260 [78] per mm^2 (range, 102-422 per mm^2; $P = 0.0105$) after the second lipofilling

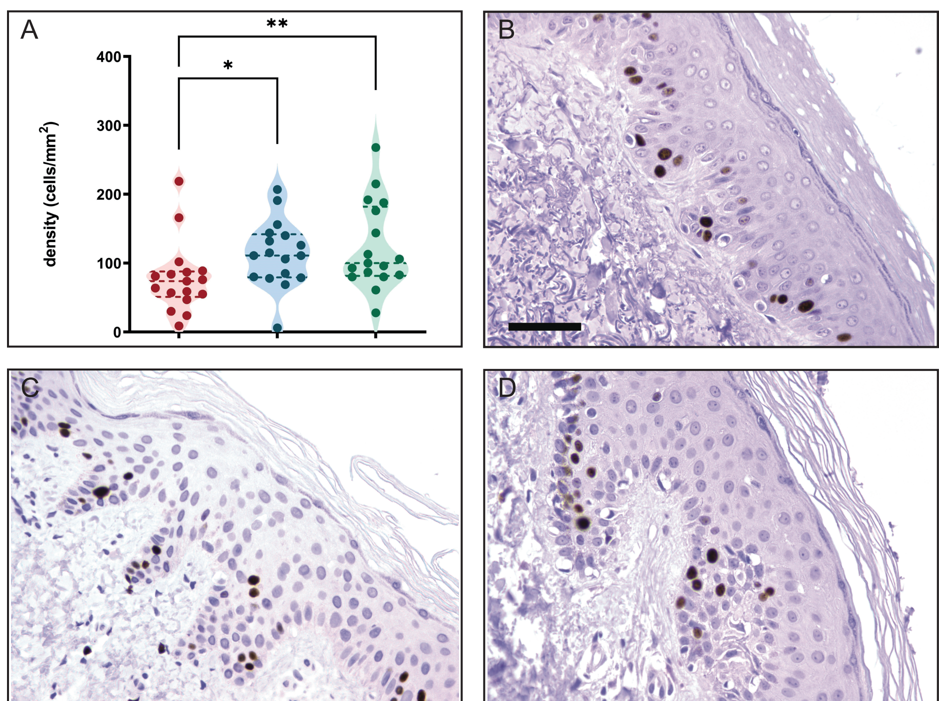


Figure 3. Epidermal proliferation is increased in scar tissues 3 months after the first lipofilling treatment and 3 months after the second lipofilling treatment compared with preoperative values. (A) Quantification of the number of Ki67-positive cells in the epidermis in 4 high-power fields (n = 17). (B-D) Representative images of immunohistochemical staining for Ki67 (brown) of biopsies taken prior to lipofilling (B), and after the first (C) and second (D) lipofilling. Scale bar, 50 µm. $^{*}P < 0.05$, $^{**}P < 0.01$ (repeated measures one-way analysis of variance with Tukey's multiple comparison testing). Preoperative data, red symbols; data after first lipofilling, blue symbols; data after second lipofilling, green symbols.

treatment (Supplemental Figure 4). The number of mast cells increased from 121 [56] per mm^2 (range, 26-237 per mm^2) preoperatively to 161 [56] per mm^2 (range, 65-246 per mm^2; $P = 0.0358$) and 160 [60] per mm^2 (range, 66-319 per mm^2; $P = 0.0308$) after the first and second lipofilling treatment, respectively (Supplemental Figure 4).

M2 Macrophage and Mast Cell Conditioned Medium Increase Endothelial Sprouting In Vitro

Both mast cells and M2 macrophages are known to promote and regulate vessel formation in vivo.[15] To test whether the increased vessel density after lipofilling could be related to paracrine effects of the invaded immune cells, the proangiogenic potency of conditioned culture medium of M2 macrophages and mast cells was assessed in endothelial sprouting assays. In vitro sprouting was quantified as the number of junctions and the total branch length after 6 hours of culture on Matrigel. With M2 macrophage conditioned medium (Supplemental Figure 5A-D) and mast cell conditioned medium (Supplemental Figure 5E-H), the number of junctions was increased by 1.1- and 1.2-fold, respectively (2-tailed *t* test, $P = 0.0394$ and $P = 0.0361$, respectively) and the total branch length was increased 1.1- and 1.2-fold respectively (2-tailed *t* test, $P = 0.0388$ and $P = 0.0079$, respectively) compared with controls.

Perivascular Extracellular Matrix Remodeling in Scar Tissues After Lipofilling Treatment

Prior to lipofilling treatment, scar tissues showed classic scar tissue extracellular matrix (ECM) consisting of thick, parallelly aligned fibers, often parallel to the epidermis as shown by polarized light microscopy of Picrosirius Red–stained thin sections (Supplemental Figure 6). The ECM architecture of normal skin was distinctly different: it consisted of thinner fibers, oriented in a basketweave-like fashion (Supplemental Figure 6, lowest panels). After the first lipofilling treatment, mild changes in ECM structure became visible. Around the blood vessels (white asterisks), areas with thinner, nonparallel fibers appeared. However, the classic scar tissue ECM organization with thick, parallel fibers remained partially present (Supplemental Figure 6). After the second lipofilling treatment, an ECM structure with thinner fibers, oriented in a basketweave pattern, became visible in scar tissues of most patients especially in

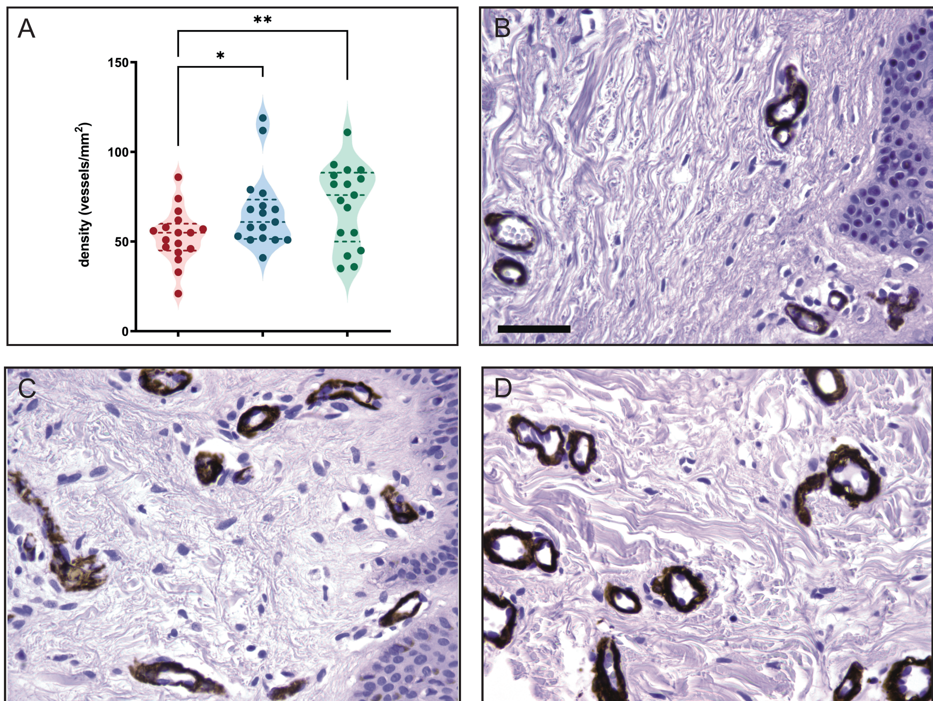


Figure 4. Vessel density increases in scar tissues 3 months after the first lipofilling treatment and 3 months after the second lipofilling treatment compared with preoperative values. (A) Quantification of the number of vessels per mm^2 of scar tissue (n = 17). (B-D) Representative images of immunohistochemical staining for α smooth muscle actin (brown) of biopsies taken prior to lipofilling (B), and after the first (C) and second (D) lipofilling. Scale bar, 50 µm. $^*P < 0.05$, $^{**}P < 0.01$ (repeated-measures one-way analysis of variance with Tukey's multiple-comparison testing). Preoperative data, red symbols; data after first lipofilling, blue symbols; data after second lipofilling, green symbols.

the vascularized areas (Supplemental Figure 6), whereas after both the first and second lipofillings vascularization had increased (Figure 4).

DISCUSSION

This is the first clinical study showing that consecutive sessions of autologous lipofilling to treat mature dermal scars resulted in a marked clinical improvement. First, the total POSAS score as well as the observer and patient POSAS scores were reduced (ie, improved) by up to 80%. This degree of improvement is unique; it is not achieved by any other current method for scar treatment. Second, clinical improvement was accompanied by physiologic and histologic changes in the scar's microenvironment that are reminiscent of normal wound healing. This includes vascularization, regeneration of the epidermis, and ECM remodeling. Furthermore, the increased invasion of T lymphocytes, mast cells, and $CD163^+$ (M2 polarized) macrophages indicates that these immune cells participate in remodeling scar and skin towards normal skin. Our POSAS-based findings fit well in the scope of a recent systematic review by Krastev et al[16] and other extensive reviews[17-19] which report that lipografting reduces scar stiffness, increases skin pliability and reduces scar-related pain. Krastev et al's review, however, did not assess the underlying mechanisms because the vast majority of the 45 eligible papers had reported only clinical outcomes. In general, evidence-based research that explains the clinical efficacy of lipofilling for scar treatment remains scarce. Besides POSAS scores, others showed that lipografting improves skin elasticity,[6] and improves aesthetics and local skin function in painful and depressed tracheostomy scars,[20-22] and that lipofilling of scars also reduced skin hardness.[7] We showed extensive ECM remodeling even after 1 lipofilling, which would corroborate previously mentioned macroscopic observations. In contrast, Gal et al reported no change in scar quality after lipofilling in burn scars.[23] Klinger et al, however, showed that in 3 case studies multiple lipofilling procedures resulted in visible reductions of facial burn scars, whereas incidental biopsies appeared to show increased vascularity and dermal hyperplasia and new collagen deposition.[24]

Normophysiologic remodeling of dermal scars requires reinstatement of physiologic wound healing while the rigid fibrotic ECM is simultaneously degraded and replaced by normal skin ECM. This would imply influx of immune cells with concurrent vascularization and reactivation of the epidermis while the ECM architecture alters too. We show that 2 consecutive lipofillings indeed result in an increased proliferation in the epidermis. This is in line with a report

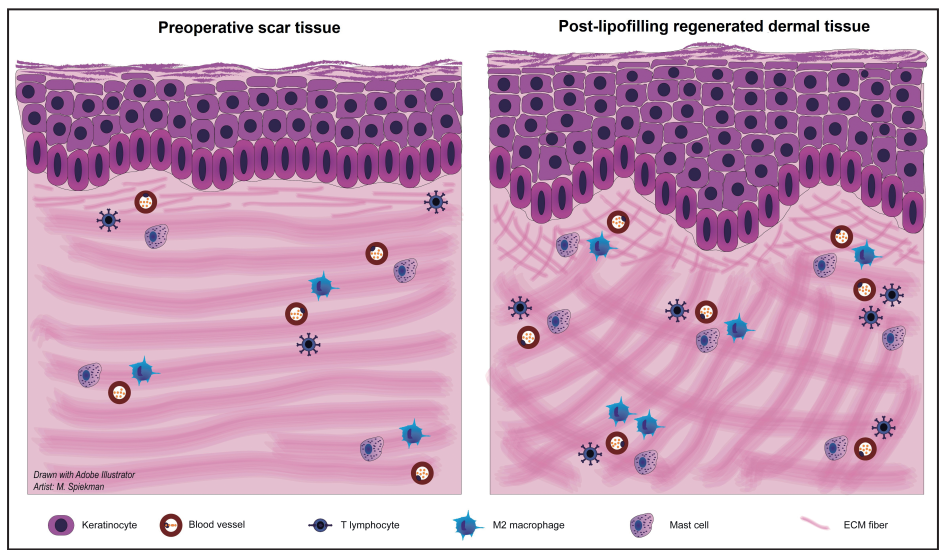


Figure 5. Schematic overview of histologic changes in scar tissues after 2 consecutive lipofilling treatments. Beneficial changes in the scar's microenvironment include increase in vascularization, regeneration of the epidermis, and extracellular matrix remodeling. Furthermore, invasion immune cells such as CD3 T lymphocytes, mast cells, and CD163^{+} (M2 polarized) macrophages point to a pathophysiologic explanation for scar release and skin remodeling.

of lipofilling of burn scars in which proliferation also increased[25] as did the POSAS scores. Unfortunately, the authors did not disclose the number of patients who were biopsied, ie, whether the histologic data were obtained from multiple patients. Several case reports and case series showed that lipofilling increased vascular density and epithelial hyperplasia in scars.[26,27] Our study corroborates and extends on these results. In their recent elegant randomized trial on 10 gender-biased patients Maricevich et al investigated the treatment of the relatively small scars that remain after cesarean section, and found that lipografting caused an influx of immune cells and vasculature while the epidermis gained thickness at 4 months postoperatively.[28] Unfortunately, the small size of the trial and the semiquantitative nature of the scoring did not show statistically significant differences, although our quantitative data and statistics corroborate these findings.[28] Our study population, however, differs due to inclusion of males and females and considers scars that were substantially larger.

We show that significant clinical improvement in patients with symptomatic scars is accompanied by histologic changes that suggest ongoing tissue remodeling and normalization (summarized in Figure 5). According to this concept multiple lipofilling treatments that are appropriately spaced in time could be superior to a single session.

An increase in epidermal proliferation and vessel density was observed after both the first and second lipofilling treatment. At 3 months after the second lipofilling treatment, remodeling of ECM structure had occurred. Typical scar tissue ECM, consisting of thick, parallelly aligned fibrils, was replaced by or transformed into thinner, smaller bundles with a more typical physiologic organization. These changes were most obvious in highly vascularized areas, where the influx of T lymphocytes, mast cells, and M2 macrophages had taken place. Thus, changes in immune balance may play an important role in the observed pro-regenerative effect of lipofilling. To date, limited studies in vitro and in vivo suggest both a stimulating as well as a suppressing effect for M2 macrophages and mast cells on scarring, which depends on time and the local microenvironment.[29-34] Compared to physiologic dermal wound healing, which completes within weeks, lipograft-induced scar reversal takes several months. It is remarkable that during all this time, the immune system, vascular system, and epidermal system remain activated as shown by the continuously increased numbers of cells. Whether this is the result of a sort of repeated kick-start initiated by lipografting, or the continuous influence of lipografted fat and the emigration of therapeutic cells, remains to be determined by future research.

The size of our biopsies was, unfortunately, too small to investigate the deeper layers of subcutaneous adipose tissue and the fate of lipografts. We do surmise that a fraction of the ASCs migrated from the lipograft to the scar. Chemokines MCP-1 and IL-8 by the ASCs, would attract macrophages and be responsible for the observed influx. Moreover, ASCs secrete proangiogenic growth factors such as vascular endothelial growth factor A and fibroblast growth factors, the latter of which also inhibit adverse myofibroblast differentiation. The lipoaspirates were autologous, which did not allow us to study the fate and

function of the administered cells with specific markers. Future animals studies with reporter-tagged lipoaspirates could shed light on the instructive and directive role of the administered cell preparations.

Study Limitations

A small bias in this study is the dropout of 7 of the 27 included patients. The randomized controlled trial protocol did not allow us to investigate these patients' reasons for dropping out (eg, lack of satisfaction with the lipofilling). Nevertheless, the correlation between POSAS scores and histologic observations remains strong. A limitation of our study is the lack of a placebo control group. We employed a treatment protocol that combines a scar release with lipofilling and did not compare this to only scar release without lipofilling or mock injection. We acknowledge and partially agree with the reviewer's feedback. Undercutting of scars causes bleeding and coagulation which is a first step in physiologic wound healing. Scars are the end-result of disturbed wound healing and the surgeons involved in the study have never observed that scar release alone suffices to reinitiate physiologic wound healing and/or reverse severe scars. Obviously, there are many surgical scar revision procedures that all rely on resection and/or repositioning local tissue. Thus, we did not expect a major contribution to scar reduction by undercutting the scars. In our current clinical trials where we use a derivative of whole fat (tissue stromal vascular fraction [tSVF]) to prevent but not reverse scarring after breast size reduction surgery, we treated 1 breast with saline and 1 with tSVF. The results showed that saline injection alone did not influence wound healing in any way. Others showed the beneficial effect of lipografting to improve large burn wound–associated scars which also suggests the scar release alone does not have a strong enough therapeutic effect, if any.[6] Moreover, the majority of included patients had already been (consecutively) treated, often with several different conventional therapies, without sufficient relief of their scar-related symptoms. The process of scarring was largely completed at the time that these patients were included in this study. In view of this, and according to best clinical experience, the improvements are unexpectedly high. Finally, this trial did not allow us to assess effect by skin type. The study was not designed to specifically register Fitzpatrick skin type, and the patients were too heterogeneous to allow for statistical analyses to compare Fitzpatrick type with scar severity (Table 2). Basically, the majority (approximately 18) of the patients were Caucasians of Northern and Central European origin and had Fitzpatrick scores of approximately I to III. The origin of 2 patients was unknown but they were dark skinned and likely had a Fitzpatrick score between IV and VI.

CONCLUSIONS

Treatment of large dermal scars with 2 consecutive lipografts benefits patients in terms of scar experience (POSAS) and pain relief (VAS). The scars underwent a physiologic remodeling process that was reminiscent of a regenerative wound-healing response. The epidermis normalized and the largely parallelly aligned collagenous bundles of the scars remodeled to the more physiologic, random, arrangement which probably caused the observed increase in local pliability and joint movability. These processes were driven by an influx of regenerative macrophages and mast cells that drove increased perfusion which was substantiated in vitro. Current research is dissecting the underlying mechanisms, for example, in appropriate in vitro 3-dimensional culture models of the relevant cellular players in skin ECM-derived hydrogels. In addition, this pilot study suggests that investigation of clinical aspects such as dose-response effects and the duration of the antiscarring effect is warranted, as well as assessment of the efficacy of smaller-volume components of fat such as the SVF, which are easier to administer.

Supplemental Material

This article contains supplemental material located online at www.aestheticsurgeryjournal.com.

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Disclosures

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