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# Tissue-Level Effects of Autologous Fat Grafting in Hypertrophic Scars—A Case Series Study

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ABSTRACT

*Introduction:* Fat grafting has antifibrotic effects and it improves scar quality. However, the biological mechanisms of fat grafts on scar healing are poorly understood.

*Methods:* This was a prospective study to identify differences in the epidermal and dermal structure, macrophage infiltration, or inflammatory and fibrotic markers in hypertrophic scars before and after fat grafting surgery compared to normal skin. Seven patients with hypertrophic scar completed the study. Biopsies from hypertrophic scars and normal skin were taken at the time of fat grafting surgery and follow-up biopsies 6 mo postoperatively. A clinical Patient and Observer Scar Assessment Scale was used to monitor the clinical aspects of the scars. Immunohistochemical stainings were performed to analyze the changes occurring in the hypertrophic scar tissue after fat grafting.

*Results:* Hypertrophic scars demonstrated decreased presence of rete ridges and increased levels of the profibrotic transforming growth factor beta-1 (TGF-β1) ($P < 0.05$) compared to normal skin. Fat grafting significantly increased the presence of rete ridges to the level of normal skin and reduced TGF-β1 expression (hypertrophic scars + fat) ($P < 0.05$). Fat grafting also increased the total macrophage count (CD68 pan-macrophage marker) ($P < 0.05$) and M1 macrophage count (inducible nitric oxide synthase M1 macrophage marker) ($P < 0.05$). The clinical evaluation of the scars (Patient and Observer Scar Assessment Scale) by the observer and patients improved after fat grafting ($P < 0.05$).

*Conclusions:* Our findings indicate that fat grafting promotes normalization of skin by improving epidermal structure and reducing TGF-β1 levels and favors less fibrotic healing by regulating macrophages levels.



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## Introduction

Autologous fat grafts are used for reconstruction of soft tissue defects, for example, softening and contour of hypertrophic scars and other fibrotic conditions.[1-4] Many of the effects of fat grafts are thought to be mediated by the immunomodulatory effects of adipose-derived stem cells (ADSCs).[5,6] ADSCs have been shown to reduce pro-inflammatory cytokine (interleukin [IL]-1, IL-17) levels and increase anti-inflammatory protein (IL-10) levels in mice.[7] Our group has shown that fat grafting increases IL-10 gene expression in mice.[8] IL-10 is also known to protect from transforming growth factor beta (TGFβ)-induced fibrosis and promote regenerative healing.[9] The antifibrotic effects of fat transfer and the basic biology are still poorly understood. Fat grafting has been shown to result in histological improvement of scars in terms of general structure, collagen remodeling, and vascularization[10,11] but quantitative data from human samples are very limited. Little is known about the role of macrophages during this process although they contribute to tissue remodeling and wound healing.[12] Furthermore, there is only little data of the effects of fat grafting to the epidermis, and knowledge of why some scars do not respond to fat grafting as well as others. This prospective study aimed at clarifying the biological effects of fat grafting on hypertrophic scars in terms of epidermal and dermal structure, and several factors previously associated with the wound healing process, including the number and type of macrophages, tissue vascularization and fibrosis, and inflammatory cytokines.[8] The Patient and Observer Scar Assessment Scale (POSAS) was used to monitor the clinical aspects of the scars. Immunohistochemical (IHC) stainings were performed to analyze the changes occurring in the hypertrophic tissue after fat grafting including TGFβ-1, IL-10, and different macrophage subtypes.

## Methods

### *Patients and samples*

Permission for collecting patient samples was approved by the Ethical Committee and institutional review board of the Turku University Hospital (ETMK: 78/180/2016) and institutional review board of Helsinki University Hospital. All patients signed an approval for sample collection and approved the use of their patient information in the study. Ten patients ($n = 10$) with symptomatic hypertrophic scars who were scheduled to have autologous fat grafting surgery at Jorvi hospital, Helsinki, Finland, were recruited in the study. Two patients were excluded due to missing follow-up samples (dropping out) and one due to inadequate sample orientation ($n = 3$). All together 7 of total 10 Caucasian patients completed the study ($n = 7$). All hypertrophic scars were a consequence of previous surgery. Previous treatment consisted of cortisone injections, photodynamic therapy, radiation therapy, and/or previous fat grafting 4-5 ys prior to the onset of this study. Postoperative treatment consisted of nonwoven taping.

Four mm diameter tissue punch biopsies of hypertrophic scars and control biopsies of healthy skin area (Nskin) were collected before fat grafting (0 mo samples) and follow-up samples 6 mo after the operation from the same area (hypertrophic scars + fat). Overview/timeline of the protocol is presented in Figure 1.

### *Surgery*

#### *Fat grafting details*

The lipofilling procedure was performed as previously described by Homsy *et al.*[13] The donor sites for fat grafting were the abdomen, the flanks, and the thighs. Each donor site was chosen based on the distribution of available fat and the patient preference. All fat grafting procedures were performed with WAL technique using the Body-jet system (Body-Jet, Human Med, Eclipse Ltd, Dallas, Texas, USA). Fat was separated from fluid in a LipoCollector (Human Med). Lipoaspirate was drawn into 50 mL syringes, held upright for decantation and the separated fat was then transferred to 10 mL syringes. Fat grafting was performed with 10 mL luer lock syringes on a Cytori cell brush (Cytori Therapeutics Inc, San Diego, CA, USA) connected to blunt 1,2-2 mm injection cannulas. In some cases where the scar was otherwise impenetrable, a sharp 18 G needle was used to inject the fat. Multiple retrograde passes from several different directions were done through

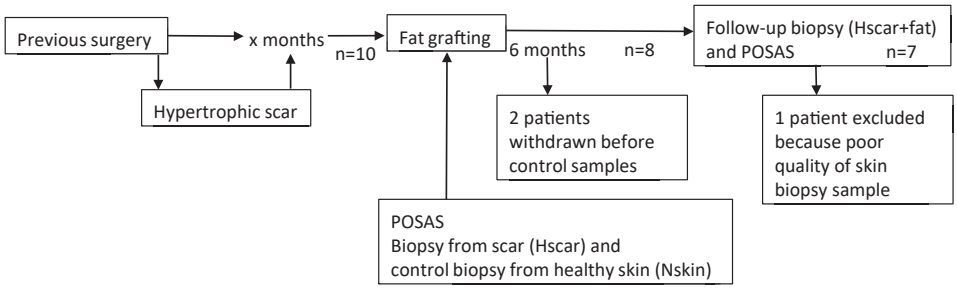


**Fig. 1 – Timeline of the protocol. All together 7 of total 10 Caucasian patients completed the study ($n = 7$). Four mm diameter tissue punch biopsies of hypertrophic scars and control biopsies of healthy skin area (Nskin) were collected before fat grafting (0 mo samples) and follow-up samples 6 mo after the operation from the same area (hypertrophic scars + fat). Hypertrophic scars were evaluated by both patient and plastic surgeon using a POSAS scale before fat grafting and 6 mo after the fat grafting.**

the scarred area, and the lipoaspirate was administered intradermally, subdermally, and to the subcutaneous planes. In some cases, the scar was also needled from the surface. In several of the cases, a volume correction was done in combination with the scar treatment, and treated surfaces varied from very limited to large. Thus the injected fat graft volumes varied between nine and 316 mL (mean 149 mL). Grafting was considered sufficient when the tissue turgor was clearly increased, or fat droplets started to evacuate from the injection sites.

### The Patient and Observer Assessment Scale

Hypertrophic scars were evaluated by both patient and plastic surgeon using a POSAS scale (The Patient and Observer Assessment Scale v2.0/EN) before fat grafting and 6 mo after the fat grafting. Patient scale was assessed by the patients and it was mainly focusing on the symptoms of the hypertrophic scar by measuring painfulness and itching of the scar as well as the color differences, stiffness, thickness, and irregularity of the scar. Observer scale was assessed by a plastic surgeon measuring vascularity, pigmentation, thickness, relief, pliability, and surface area of the scar. All items were scored from 1 (normal skin) to 10 (worst scar imaginable). The sum of these six items resulted in a total score. A lower score meant less scarring.

### Histology

Tissue samples were fixed in formalin for embedding in paraffin. Sections of 4 μm thickness were stained with hematoxylin and eosin, and Wright von Gieson to visualize the rete ridges and the quantity of connective tissue in the skin. Rete ridges are undulating epithelial extensions at the junction of the dermis and epidermis. The structure is essential to normal skin and the depth and architecture of rete ridges reflect the quality of wound healing.[14] The rete ridges were graded on a semiquantitative scale from 0 to 3 by evaluating the depth of the ridges (0 = absent, 1 = superficial, 2 = average, 3 = deep) and the frequency of their occurrence across the epidermal plane (1 = 0–24%, 2 = 25%-49%, 3 = 50%-74%, 4 = 75%-100%). The depth and the frequency of rete ridges were added to generate a total score. Epidermal thickness was quantified by counting the number of keratinocyte cell layers at six points of the epidermal plane (three measurements on random rete ridges and three on random non rete ridges). The histological scales were adapted from Limandjaja *et al.*[15]

### Immunohistochemical staining

Antibodies were selected to label vascularization (CD31), myofibroblast differentiation (αSMA), proliferation (Ki-67), and macrophage populations (CD68, inducible nitric oxide synthase [iNOS], mannose receptor [MRC]-1) of the hypertrophic scars. The expression levels of pro-inflammatory and profibrostic cytokine TGFβ-1 and an anti-inflammatory cytokine IL-10 in the hypertrophic scar was also of interest as both cytokines have been demonstrated to play an important role in the wound healing process.[8] The IHC staining protocol is presented in Table 1.

Immunohistochemical staining was performed on deparaffinized tissue sections with the antibodies listed in Table 1. The IL-10 and TGFβ-1 stainings were optimized separately using Vectastain Elite ABC-HRP kit (Peroxidase, Universal) and DAB Substrate Kit, Peroxidase (HRP). See supplementary data for specific protocol.

### Data analysis

Stained slides were scanned with Pannoramic P1000 slide scanner (3DHISTECH), and the images were analyzed using Fiji ImageJ software (version 1·50i; National Institutes of Health, Bethesda, MD, USA). The signals of the hematoxylin and DAB staining were first deconvoluted from each RGB image to enable quantitative image analysis. The images of each sample set were then thresholded equally to identify the positively stained components. The area of the positive staining was quantified for each section and divided by the total tissue area to obtain the percentage of positive tissue area.

**Table 1 – Immunohistochemical staining protocols.**

| Target protein | Antibody source | Marker for | Dilution of antibody |
|---|---|---|---|
| CD31 (platelet endothelial cell adhesion molecule-1) | Mouse | Endothelial cells | 1:100 |
| aSMA (α-smooth muscle actin) | Mouse | Myofibroblasts and vascular smooth muscle cells | 1:2000 |
| Ki67 (marker of proliferation ki-67) | Mouse | Cell proliferation | 1:500 |
| CD68 (cluster of differentiation 68) | Mouse | Macrophages | 1:200 |
| iNOS (inducible nitric oxide synthase) | Rabbit | M1 macrophages | 1:1000 |
| MRC-1 (mannose receptor-1) | Rabbit | M2 macrophages | 1:5000 |
| TGF-β1 (transforming growth factor beta-1) | Rabbit | TGF-β1 | 1:250 |
| IL-10 (interleukin 10) | Mouse | IL-10 | 1:250 |

### Statistical analysis

All statistical analysis were performed using GraphPad Prism, version 7.04 (GraphPad Software Inc, San Diego, CA, USA). Wilcoxon matched pairs signed rank test was used between-group comparisons ($P$ values $< 0.05$ were considered statistically significant). Data are presented as mean ± SD, $^{*}P < 0.05$, $^{**}P < 0.01$. Also due to a small sample size, the effect size and confidence intervals were calculated using SPSS, confidence interval type was related-samples Hodges–Lehman median difference, see Supplementary Material.

## Results

### Patient characteristics

All patients were Caucasian women. The mean age of patients was 53 ys and the duration of scar varied from 13 mo to 336 mo, the mean body mass index was 25. All the hypertrophic scars were a consequence of previous surgery. The characteristics of patients in this study are presented in Table 2. Patient 1, age 67 ys, had previously undergone a reduction mammoplasty which had resulted in a tight scar, scar duration was 18 mo. Injected fat graft volume was 80 mL. Six months after fat grafting the quality of the scar had improved; these findings are presented in Figure 2A. Patient 2, age 37, had an abdominoplasty which had resulted in a hypertrophic scar to the lower abdomen, scar duration 13 mo, and injected fat graft volume was 168 mL. Patient 3, age 51, had a breast reconstruction with DIEP (Deep Inferior Epigastric artery Perforator) flap which had led to a hypertrophic scar to the lower abdomen, scar duration 122 mo, and injected fat volume was 66 mL. Patient 3 was the only one smoking and had gotten cortisone injection as earlier treatment. Patient 5, age 55, had a mastectomy and breast reconstruction with LD (Latissimus Dorsi muscle) flap which had resulted in a scar to the right breast, scar duration was 20 mo and injected fat volume 125 mL. Patient 6, age 50 ys, had a mastectomy and implant reconstruction which had led to a scar to the left chest wall and had gotten radiation therapy as earlier treatment, scar duration 50 mo and injected fat volume was 150 mL. Patient 7, age 59, had a breast reconstruction with LD flap which had led to a hypertrophic scar on the upper back, scar duration 132 mo had received previous fat grafting in the scar 5 ys before this operation where injected fat volume was 139 mL. Patient 9, age 51, had a hypertrophic scar on sternum and on both axilla due to resection of hidradenitis, scar duration 336 mo and injected fat volume was 316 mL. Patients 1, 3, 6, and 9 got a second fat grafting later for further treatment.

### POSAS scale scoring of the hypertrophic scars improved after fat grafting

The POSAS total score of the hypertrophic scars significantly improved after fat grafting in both observer and patient scale ($P = 0.02$) (Fig. 2C, Table 3). The observer scale total score before fat grafting was 34.1 ± 11.1 (hypertrophic scars) and after 17.0 ± 6.2 (hypertrophic scars + fat) (Fig. 2C and D,

**Table 2 – Patient characteristics.**

| ID | Age | BMI | Scar location | Control location | Scar duration (months) | Smoking | Underlying conditions | Injected fat graft volumes (mL) | Scar treatment (needling, nanofat) | Etiology of hypertrophic scar |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 67 | 30.1 | Right breast | Breast | 18 | No | Hypertension, asthma, obesity, hypothyroidism | 80 | - | Reduction mammoplasty |
| 2 | 37 | 29.1 | Lower abdomen | Lower abdomen | 13 | No | Obesity | 168 | Needling | Abdominoplasty |
| 3 | 51 | 24.4 | Lower abdomen | Lateral abdomen | 122 | Yes | - | 66 | Needling | Breast reconstruction (DIEP flap)* |
| 5 | 55 | 22.9 | Right breast (LD) | Inner thigh | 20 | No | Breast cancer l.a. in remission | 125 | - | Mastectomy + breast reconstruction (LD flap) |
| 6 | 50 | 23.1 | Left chest wall | Outer thigh | 50 | No | Osteoporosis | 150 | - | Mastectomy + implant reconstruction |
| 7 | 59 | 20.1 | Upper back (LD) | Chest | 132 | No | Hypothyroidism, rheumatoid arthritis | 139 | Needling + nanofat 9 mL | Breast reconstruction (LD flap) |
| 9 | 51 | 27.0 | Sternum (+axilla l.a.) | Abdomen | 336 | No | Periductal mastitis, hidradenitis | 316 | Needling + nanofat 6 mL | Resection of hidradenitis |

BMI = body mass index; LD = latissimus dorsi muscle flap.
*DIEP = Deep Inferior Epigastric artery Perforator: blood vessels, fat, and skin from the lower belly are relocated to the chest to rebuild breasts after mastectomy.

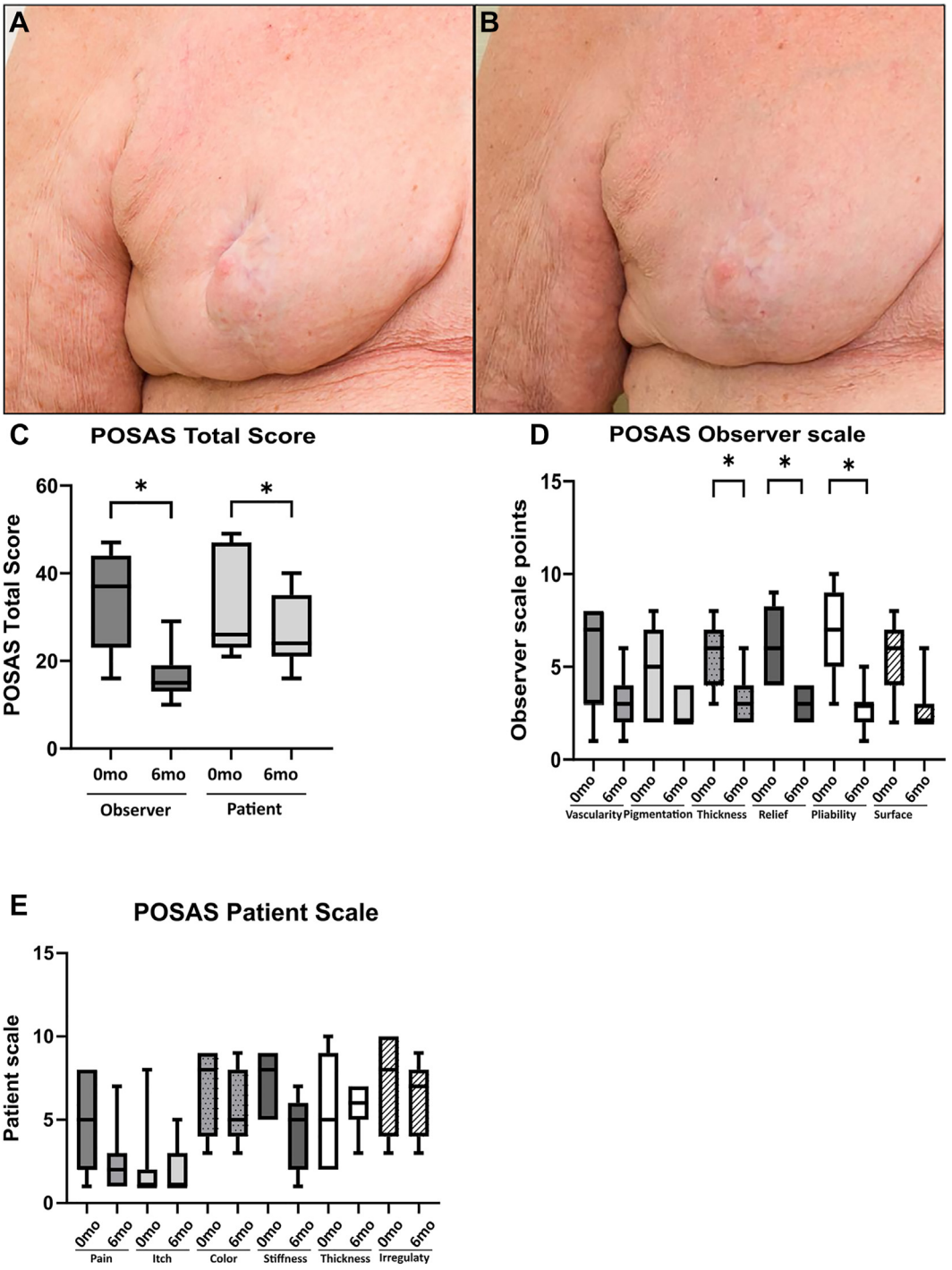


**Fig. 2 – (A and B) Patient 1 had previously undergone an upper lateral breast reduction surgery which had resulted in a tight scar (A). Six months after fat grafting, the quality of the scar had improved (B). (C, E) POSAS scale scoring of the hypertrophic scars improved after fat grafting. POSAS Total score pre and post (C). POSAS observer scale pre and post (D). POSAS patient scale pre and post (E). Data presented as box & whisker, plot Min to Max, median, Q1, Q3, *P < 0.05, $n = 7$.**

Table 3). The thickness, relief, and pliability of the hypertrophic scars all improved as separate parameters after fat grafting ($P = 0.03$). The patient scale total score before fat grafting was 33.7 ± 12.6 (hypertrophic scars) and after 26.7 ± 8.1 (hypertrophic scars + fat) (Fig. 2C and E, Table 3). The pain and the stiffness of the scar as separate parameters exhibited an improving but statistically not significant trend after fat grafting ($P = 0.06$).

**Table 3 – POSAS score.**

| Parameter | Hypertrophic scars | Hypertrophic scars + fat | P value |
|---|---|---|---|
| POSAS observer scale | | | |
| Vascularity | 5.9 ± 2.8 | 3.0 ± 1.6 | 0.06 |
| Pigmentation | 4.9 ± 2.5 | 2.6 ± 1.0 | 0.06 |
| Thickness | 5.6 ± 1.7 | 3.1 ± 1.5 | 0.03 |
| Relief | 6.2 ± 2.1 | 2.9 ± 0.9 | 0.03 |
| Pliability | 7.0 ± 2.4 | 2.7 ± 1.3 | 0.03 |
| Surface | 5.6 ± 2.1 | 2.7 ± 1.5 | 0.06 |
| Total score | 34.1 ± 11.1 | 17.0 ± 6.2 | 0.02 |
| POSAS patient scale | | | |
| Pain | 4.7 ± 2.8 | 2.6 ± 2.1 | 0.06 |
| Itch | 2.3 ± 2.6 | 2.0 ± 1.5 | 0.8 |
| Color | 6.6 ± 2.5 | 5.6 ± 2.2 | 0.3 |
| Stiffness | 7.3 ± 1.7 | 4.4 ± 2.1 | 0.06 |
| Thickness | 5.9 ± 3.2 | 5.6 ± 1.4 | 0.9 |
| Irregularity | 7.0 ± 2.9 | 6.3 ± 2.3 | 0.1 |
| Total score | 33.7 ± 12.6 | 26.4 ± 8.3 | 0.02 |

### *Fat grafting promotes epithelial healing and rete ridge formation*

Hypertrophic scars showed significantly decreased presence of rete ridges (rete ridges 2.0 ± 1.4 $P = 0.02$) and increased epidermal thickness (epidermal layers 7.4 ± 1.6, $P = 0.2$) compared to normal skin (Nskin) samples from a control skin area (5.1 ± 1.1 and 6.7 ± 1.1) (Fig. 3A and B). Six months after fat grafting (hypertrophic scars + fat), the presence of the rete ridges had increased to a similar level as in the normal control skin (5.0 ± 1.2, $P = 0.02$, $r = 0.91$) (Fig. 3A and B). Also, epidermal thickness was reduced after fat grafting (6.1 ± 0.4, $P = 0.1$, $r = 0.41$) (Fig. 3C).

### *Fat grafting reduces profibrotic TGF-β1 levels*

The IHC staining on TGF-β1 showed expression primarily in the epidermis (Fig. 4A). The stratum corneum and exfoliating dead cells were excluded from the quantitative image analysis. The hypertrophic scar samples showed significantly increased levels of the profibrotic TGF-β1 compared to controls skin (hypertrophic scars 1.3 ± 0.5 *versus* Nskin 0.7 ± 0.1 $P = 0.02$). Importantly, the expression of the TGF-β1 was significantly reduced after fat grafting (hypertrophic scars 1.3 ± 0.5 *versus* hypertrophic scars + fat 0.9 ± 0.4, $P = 0.02$, $r = 0.89$) (Table 4, Fig. 4A and B).

IL-10 was mostly expressed in the subcutaneous adipose tissue (Fig. 4A). However, there were no significant differences in the IL-10 expression in hypertrophic scars before and after fat grafting (hypertrophic scars 0.1 ± 0.09 *versus* hypertrophic scars + fat 0.06 ± 0.04, $P = 0.4$, $r = 0.35$) or compared to control skin (hypertrophic scars 0.1 ± 0.09 *versus* Nskin 0.2 ± 0.2, $P = 0.6$) (Table 4, Fig. 4C).

CD31 and aSMA were both localized to the vessels of the dermis (Fig. 4A). CD31 expression, but not aSMA, was elevated in hypertrophic scars compared to control skin (hypertrophic scars 0.6 ± 0.2 *versus* Nskin 0.3 ± 0.1, $P = 0.02$) and it remained elevated after fat grafting compared to control skin (hypertrophic scars + fat 0.7 ± 0.2 *versus* Nskin 0.3 ± 0.1, $P = 0.03$) (Table 4, Fig. 4A, D and E). There was no significant difference in CD31 staining between hypertrophic scars and hypertrophic scars + fat ($P = 0.9$, $r = 0.064$).

The cell proliferation marker Ki67 was mostly localized to the basal layer of epidermis (Fig. 4A). Ki67 expression was not significantly increased in hypertrophic scars compared to control skin (hypertrophic scars 0.2 ± 0.1 *versus* Nskin 0.1 ± 0.09, $P = 0.2$), and there was no statistically significant difference in proliferation before and after fat grafting although the effect size showed a potentially large effect (hypertrophic scars 0.2 ± 0.1 *versus* hypertrophic scars + fat 0.3 ± 0.2, $P = 0.2$, $r = 0{,}51$) (Table 4, Fig. 4F).

### *Fat grafting increased total macrophage count and M1 macrophage count*

The pan-macrophage marker CD68 was mainly expressed in the dermis. The total count of macrophages was similar hypertrophic scars and control skin (hypertrophic scars 0.07 ± 0.03 *versus* Nskin 0.07 ± 0.02, $P = 0.7$). Interestingly, fat grafting significantly increased total macrophage count (hypertrophic scars 0.07 ± 0.03 *versus* hypertrophic scars + fat 0.1 ± 0.05 $P = 0.03$, $r = 0.80$) (Fig. 5A and B).

The M2 macrophage marker MRC-1 was mostly detected from the dermis (Fig. 5A). Hypertrophic scars showed increased levels of M2 macrophages (MRC-1) compared to control skin (hypertrophic scars 1.2 ± 0.2 *versus* Nskin 0.9 ± 0.09, $P = 0.05$). However, there were no significant changes in the M2 macrophage count in hypertrophic scars before and after fat grafting although effect size showed a potentially large effect (hypertrophic scars 1.2 ± 0.2 *versus* hypertrophic scars + fat 1.4 ± 0.7, $P = 0.2$, $r = 0.51$) (Fig. 5A and C).

The M1 macrophage marker iNOS was localized in both epidermis and dermis, most likely detecting also the Langerhans cells of the epidermis (Fig. 5A). Hypertrophic scars showed no significant differences in the levels of M1 macrophages (iNOS) compared to control skin (hypertrophic scars 0.3 ± 0.1 *versus* Nskin 0.4 ± 0.08, $P = 0.2$). However, after fat grafting, the amount of M1 macrophages was significantly increased (hypertrophic scars 0.3 ± 0.1 *versus* hypertrophic scars + fat 0.5 ± 0.2, $P = 0.03$, $r = 0.83$) (Fig. 5D).

## Discussion

We provide the first evidence that fat grafting in hypertrophic scars leads to improved epidermal structure in terms of increased rete ridge formation and epidermal thickness. Our data also demonstrate that the levels of TGF-β1, a profibrotic cytokine, are normalized in hypertrophic scars after fat grafting. In addition, our results indicate that the infiltration

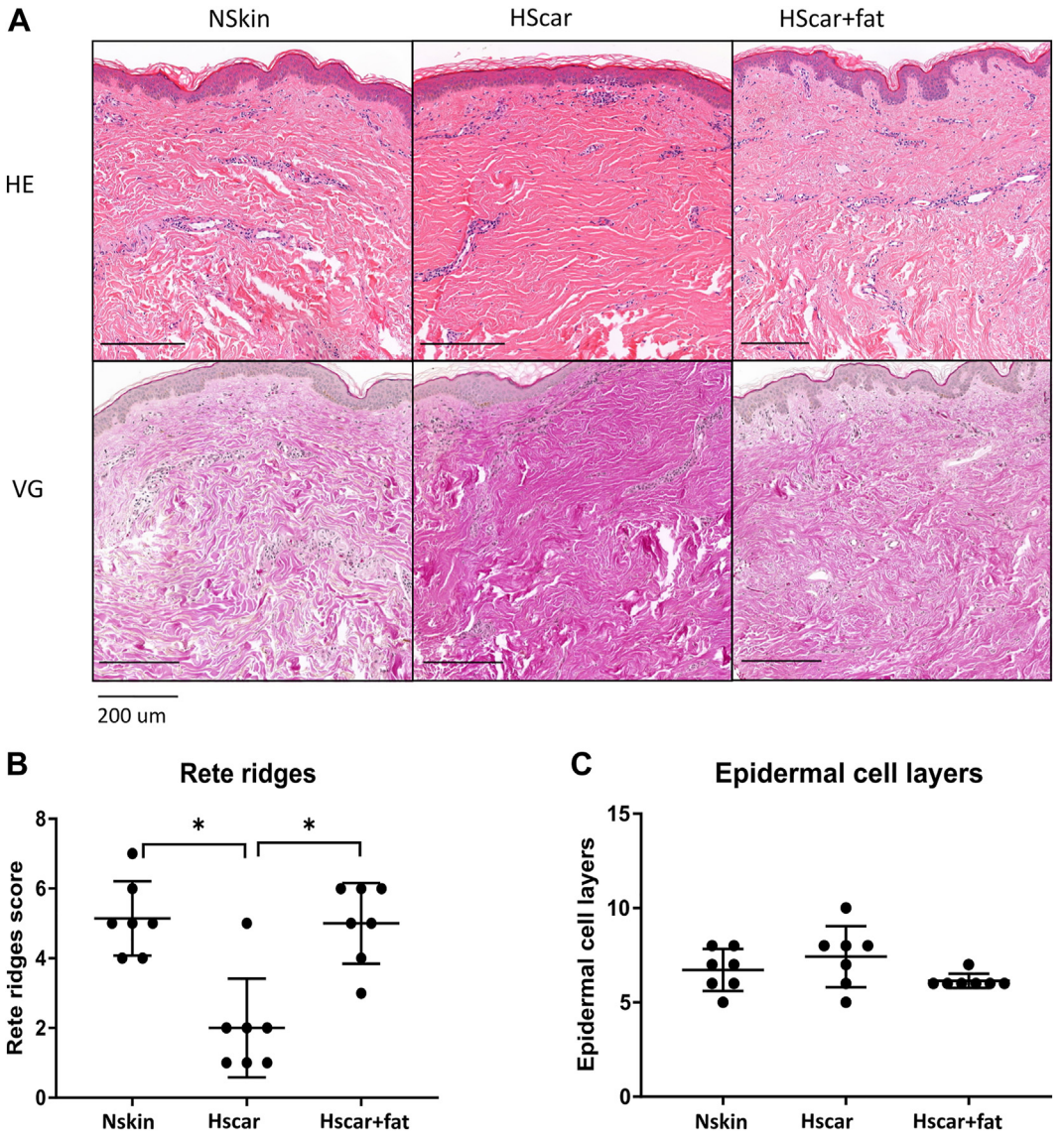


**Fig. 3 – (A). Overview of representative dermal staining for HE and Van Gieson (VG). The rete ridges and epidermal thickness were both evaluated from the HE staining. (B and C) Fat grafting promotes epithelial healing and rete ridge formation. The presence of rete ridges pre and post (B). Epidermal thickness (amount of epidermal cell layers) pre and post (C). Normal skin (Nskin), Hypertrophic scars and 6 mo after fat grafting (hypertrophic scars + fat), $n = 7$. Data presented as scatter plots mean with SD.**

and polarization of macrophages in the hypertrophic scars is affected by fat grafting, suggesting a potential mechanism for improved scar healing.

Our results support the previous findings that fat grafting improves the clinical features of hypertrophic scars according to the POSAS scale.[16,17] The effects were most prominent in thickness, relief, and pliability of hypertrophic scars. The patient scoring also suggests that pain and stiffness of the scar are reduced. Previous meta-analysis by Qurashi *et al.* shows that autologous fat grafting provides a beneficial effect on scar tissue improving significantly the vascularity, pigmentation, pliability, relief, and thickness of the hypertrophic scar on POSAS scale.[16] Krastev *et al.* also obtained similar results in their meta-analysis.[17] Their results are similar to our findings in that fat grafting has beneficial effects on hypertrophic scars.[17] However, both studies conclude that future randomized and mechanistical studies are needed to develop fat grafting treatment further.

Closer examination of the epidermal structure revealed that fat grafting has an effect on the epidermis by improving the rete ridge formation at the junction of dermis and epidermis. Rete ridges are an essential structure for skin's mechanical strength and homeostasis, and the quality of rete ridges reflects the quality of wound healing.[14] We found that hypertrophic scars have reduced rete ridge formation, which was improved after fat grating. A previous experimental porcine study investigating the preventive use of ADSCs on deep partial-thickness wounds, found more rete ridge formation at 6 mo after ADSC grafting compared to placebo group.[18] This observation was associated with normalized

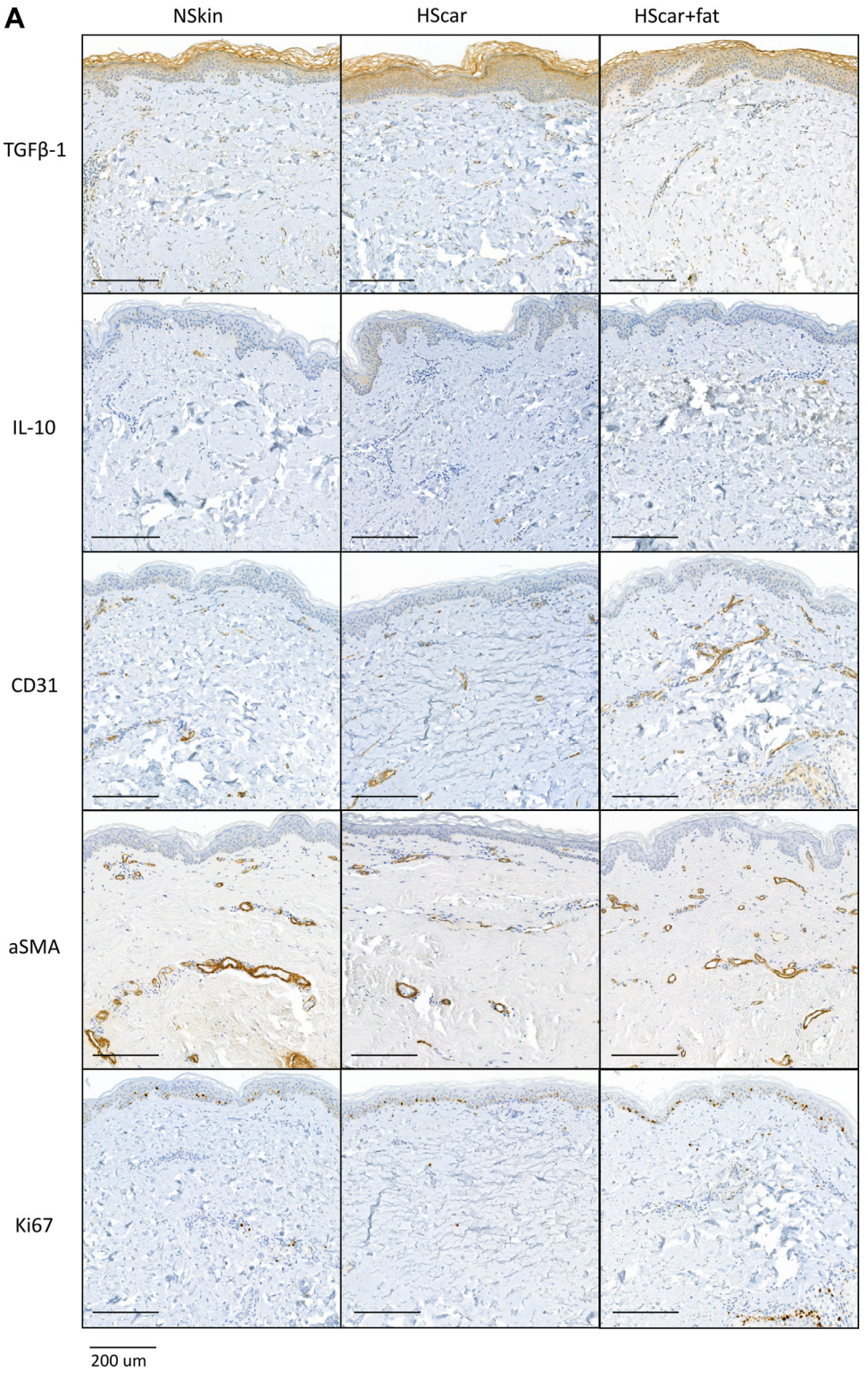


**Fig. 4 – (A) Overview of IHC staining/Expression of TGF-β1, IL-10, CD31, aSMA, and Ki67 pre and post. The IHC staining on TGF-β1 showed expression primarily in the epidermis, in the epithelium layer. IL-10 was mostly expressed in the subcutaneous adipose tissue (not seen in the figure) and partially in the epithelium layer. CD31 and aSMA were both localized to the vessels of the dermis. Ki67 was mostly localized to the basal layer of the epidermis. (B-F) Fat grafting reduces profibrotic TGF-β1 levels. Hypertrophic scars showed significantly increased levels of the profibrotic TGF-β1 compared to Nskin. The expression of the TGF-β1 was significantly reduced after fat grafting. CD31 expression was elevated in hypertrophic scars compared to Nskin. $^{*}P < 0.05$, $n = 7$.**

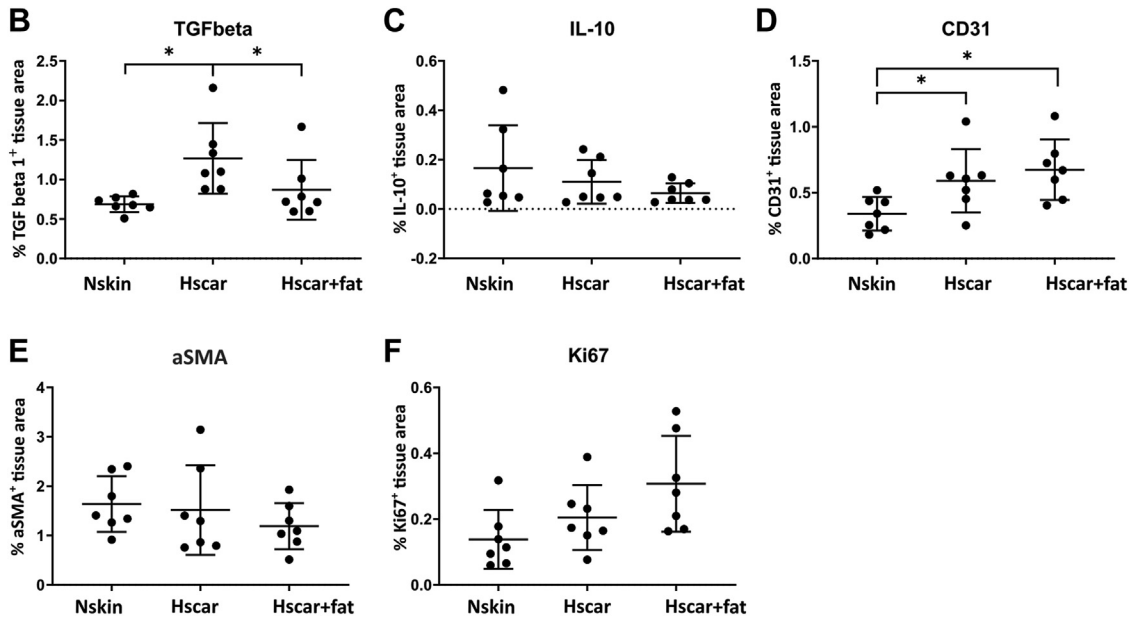


**Fig. 4 – (*continued*).**

**Table 4 – Summary of results.**

| Parameter | Nskin | Hypertrophic scars | *P* value Nskin *versus* hypertrophic scars | Hypertrophic scars + fat | *P* value hypertrophic scars *versus* hypertrophic scars + fat | *P* value hypertrophic scars + fat *versus* Nskin |
|---|---|---|---|---|---|---|
| Epidermal thickness (cell layers) | 6.7 ± 1.1 | 7.4 ± 1.6 | 0.2 | 6.1 ± 0.4 | 0.1 | 0.3 |
| Rete ridge formation (score) | 5.1 ± 1.1 | 2.0 ± 1.4 | 0.02 | 5.0 ± 1.2 | 0.02 | 0.6 |
| CD31 (% of positive tissue area) | 0.3 ± 0.1 | 0.6 ± 0.2 | 0.02 | 0.7 ± 0.2 | 0.9 | 0.03 |
| aSMA (% of positive tissue area) | 1.6 ± 0.6 | 1.5 ± 0.9 | 0.7 | 1.2 ± 0.5 | 0.7 | 0.6 |
| Ki67 (% of positive tissue area) | 0.1 ± 0.09 | 0.2 ± 0.1 | 0.2 | 0.3 ± 0.2 | 0.2 | 0.08 |
| CD68 (% of positive cells) | 0.07 ± 0.02 | 0.07 ± 0.03 | 0.7 | 0.1 ± 0.05 | 0.03 | 0.03 |
| iNOS (% of positive tissue area) | 0.4 ± 0.08 | 0.3 ± 0.1 | 0.2 | 0.5 ± 0.2 | 0.03 | 0.3 |
| MRC-1 (% of positive tissue area) | 0.9 ± 0.09 | 1.2 ± 0.2 | 0.05 | 1.4 ± 0.7 | 0.2 | 0.08 |
| TGF-β1 (% of positive tissue area) | 0.7 ± 0.1 | 1.3 ± 0.5 | 0.02 | 0.9 ± 0.4 | 0.02 | 0.7 |
| IL-10 (% of positive tissue area) | 0.2 ± 0.2 | 0.1 ± 0.09 | 0.6 | 0.06 ± 0.04 | 0.4 | 0.1 |

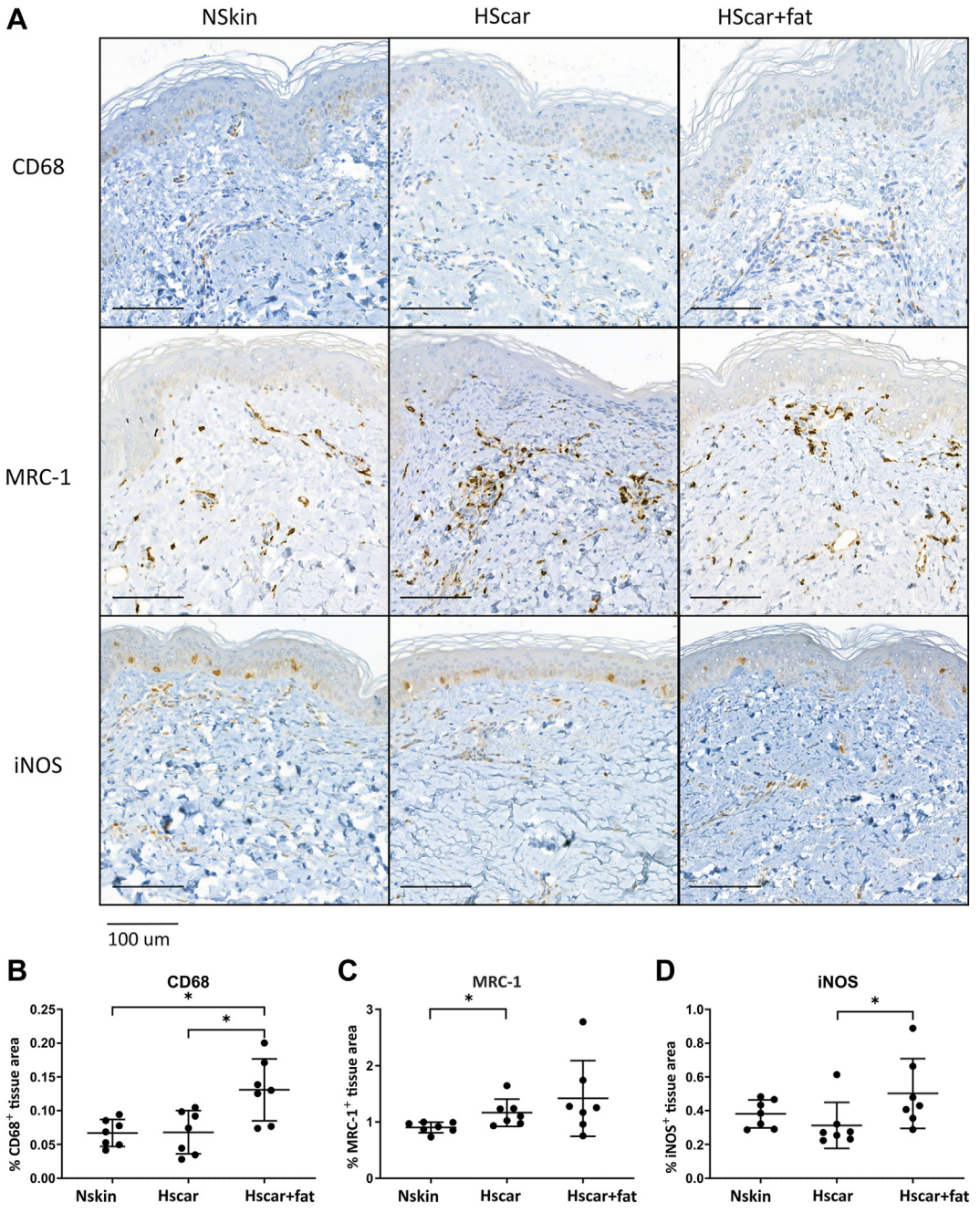


**Fig. 5 – (A) Overview of macrophage IHC staining. Expression of CD68, MRCI-1, and iNOS pre and post. CD68 and MRC-1 were mainly expressed in the dermis. iNOS was localized in both epidermis and dermis. (B-D) Fat grafting increased total macrophage count and M1 macrophage count. The CD68 expression was significantly increased after fat grafting. MRC-1 was elevated in the hypertrophic scars, indicating an elevated number of M2 macrophages. The expression iNOS (M1 macrophage marker) was significantly increased after fat grafting.**

collagen organization, softer skin, and lesser pigmentation in the scar area. Our results are in line with these data as we observed that the effects of fat grafting are mediated to the epidermal structure. Previous studies investigating the effect of fat grafting on epidermal thickness have obtained controversial results.[19,20] However, these studies did not include the comparison to healthy skin samples. Our results indicate a slight decrease of epidermal thickness in hypertrophic scars after fat grafting, toward the thickness of the normal skin. To conclude, this study is the first to show beneficial effects of autologous fat grafting on the epidermal structure in human skin and in a prospective manner.

Our results suggest that fat grafting also has several other effects on the epidermis of the hypertrophic scar. The epidermis of the hypertrophic scars showed increased levels of the profibrotic TGF-β1 which was significantly reduced 6 mo

after fat grafting. Previous studies have shown reduced TGF-β1 expression in the dermis in individual patient samples after fat grafting without quantification and statistical evaluation.[21,22] A few studies in mice have also shown that lipofilling can decrease TGFβ-1 levels.[23,24] Thus, this study is the first to quantitatively demonstrate that fat grafting significantly reduces TGF-β1 expression in hypertrophic scars. We also analyzed the expression of IL-10, an anti-inflammatory cytokine mainly expressed in the subcutaneous tissue, that has been shown to protect from TGF-β1-induced fibrosis[9] but observed no differences 6 mo after fat grafting. In our previous experimental studies, IL-10 expression was shown to be upregulated as early as 7 d after fat grafting and the expression was already decreased after 30 d.[8] As our postoperative samples were taken 6 mo after fat grafting, the potential earlier upregulation of IL-10 cannot be detected in these samples.

Macrophages respond to a variety of factors to change their phenotype and function, and also play a major role in the wound healing process. While the pro-inflammatory M1 macrophages are essential for initiating phases of wound healing, the immunosuppressive M2 macrophages participate in the proliferation and remodeling stage. Interestingly, our data show that fat grafting increases the total macrophage count and the M1 macrophage count in hypertrophic scars. Lindegren *et al.* have previously reported that autologous fat transplantation reduces macrophage density in the irritated adipose tissue.[25] They measured macrophage density from the fat, while we looked closer at the dermis and epidermis, and observed that the macrophage count increased in these areas after fat grafting, which may explain the difference in conclusions.

The high expression of M1 macrophages and low number of M2 macrophages is characteristic at the early stage of normal scarring. Typically, the level of M2 macrophages increases 4 wks postinjury and returns to baseline at 8 wks.[26] A sustained higher amount of M2 macrophages has been associated to pathological scarring in hypertrophic scars and keloids.[27] Xu, X. *et al.* also demonstrated a significantly lower number of M1 macrophages upon keloid formation.[27] These data are in line with our findings that an elevated M2 macrophage count is observed in the hypertrophic scars. Inhibiting M2-type macrophages is a promising target to inhibit fibrosis by impairing macrophage-induced fibroblast proliferation and migration.[28] In this study, we could not see a statistically significant effect of the fat graft on M2 macrophage numbers at 6 mo after grafting. This study also provides the first evidence showing that fat grafting increases M1 macrophage count in the epidermis. The current data does not reveal the origin of the macrophages, and they may have come from the transferred fat or have migrated from the bloodstream. Future studies will bring further information about the origin of elevated amount of macrophages in hypertrophic wounds after fat grafting. In future studies, the therapeutic potential of innate lymphoid cells (ILCs) and the interplay between ILCs and macrophage could be compared to that of the fat graft as ILCs are involved in wound healing and fibrosis.[29] Also the combination of fat grafting and topical antifibrotic treatments like hyaluronic acid hydrogels could be of interest.[30]

The study was limited by the small and heterogeneous number of patients as only seven patients completed the study. Also, due to the long follow-up time, some initial changes at the cellular level may have been missed. As we did not have a control group in this case series, further controlled trials are required to confirm that the grafting procedure itself, in the absence of autologous fat transfer, has no effects on the evaluated parameters.

To conclude, this study brings new biological insight into the tissue-level mechanisms of fat grafting in hypertrophic scars. The present study shows the impact of autologous fat grafting on the structural and cellular composition of hypertrophic scars, offering insights into the biological mechanisms underlying scar improvement post fat grafting up till 6 mo. Several patients received another round of fat grafting after 1-2 ys for volume contouring or persisting tightness of the scar but the decisions and procedures were made later than the end of the follow-up of this prospective study. We show for the first time that fat grafting significantly improves epidermal structure and decreases profibrotic TGF-β1 levels of hypertrophic scars. Hypertrophic scars also showed increased levels of M2 macrophages, and fat grafting increased the total and M1 macrophage count in the scar, thereby leading to a macrophage profile more beneficial for wound healing.

## Supplementary Materials

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jss.2024.11.039.

## Disclosure

Pauliina Hartiala received honoraria for participating in advisory boards of Herantis pharma. All other authors have no conflicts of interest to declare.

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## CRediT authorship contribution statement

**Mervi Laukka:** Writing – review & editing, Writing – original draft. **Susanna Kauhanen:** Writing – review & editing, Writing – original draft. **Anna Hockerstedt:** Writing – review

& editing. **Emilia Peuhu:** Writing – review & editing, Writing – original draft. **Pauliina Hartiala:** Writing – review & editing, Writing – original draft.

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