



# Lipofilling—A Regenerative Alternate for Remodeling Burn Scars: A Clinico-Immunohistopathological Study

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## Abstract

**Introduction** Any injury involving the dermis will lead to scarring. Scar tissue can cause functional limitations, cosmetic impairments, pain, and itch. Adipose-derived stem cells have also been shown to play a role in scar modulation. This study evaluates changes in lipofilled scar over the period of time and compares it with non-lipofilled scar tissue.

**Materials and Methods** A prospective case–control study with intraindividual follow-up was performed on 30 adult patients with post-burn scars from November 2016 to May 2019. Clinical, histopathological, and immunohistochemical parameters were assessed among the case and control regions of the scar.

**Results** Mean age of the study population was 30.6 years. The duration of the scar included in this study ranged from 1 to 28 years, with a mean duration of 5.91 years. There was a significant reduction in pain, itch, stiffness, and an increase in the pliability of the scar, and a substantial improvement in the modified Vancouver Scar Score in the lipofilled group. In histopathological analysis, the case group showed organized parallel collagen fibers, a significant reduction in melanocytes, improvement in vascularity, and a significantly increased amount of collagen fibers at the reticular dermis. Immunohistochemical analysis indicated new cell synthesis in the scar tissue and reduced melanocytes.

**Conclusion** The remodeling effect of adipocyte-derived stem cells is long-lasting, and there is a gradual improvement in most of the parameters. Lipofilling has regenerative capacity, which leads to the improved overall appearance of scar and improvement at the cellular level.

## Keywords

- burns
- scars
- lipofilling
- fat grafting
- immunohistopathology

## Introduction

Any injury involving the dermis will lead to scarring. Burn injury involving the dermis leads to scars involving large

areas. Scar tissue can cause functional limitations, cosmetic impairments, pain, and itch. It may also cause psychosocial and behavioral problems, mainly when it is located in exposed areas.<sup>1</sup> In the contemporary era, there is a

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significant improvement in survival of patients suffering from burn injuries. With ever-increasing patient survival more substantial number of burns, patients are presenting for secondary reconstruction to enhance the cosmetic appearance and to improve the function of the scarred, contracted area with the aim of bettering the overall quality of life.<sup>2</sup> Levine et al concluded that patients with facial scars report higher levels of depression and anxiety, and they feel dissatisfied with their body image.<sup>3</sup> Scars can also induce chronic pain, which may develop after acute pain, a symptom of normal wound healing. There is no standard treatment for scar, and a multimodal approach is recommended, which includes corticosteroids, silicone gels/sheets, pressure garments, emollient agents, and massages. To date, no gold standard exists for the treatment of scar tissue. Treatments are primarily based on the individual experience of clinicians, with varying degrees of success. Neuber enlightened a new approach to managing these scars by using autologous fat for scar remodeling. This technique was further refined and standardized by Coleman.<sup>4</sup> Adipose derived stem cells have also been shown to play a role in skin regeneration by forming tissue consisting of hypodermis, dermis, and epidermis. It is this regenerative capacity that is of particular interest in burn scar therapy. This study evaluates changes in lipofilled scars over a period of time and compares them with non-lipofilled scar tissue.

## Materials and Methods

### Study Design and Patients

A prospective case-control study with intraindividual follow-up was performed in 30 adult patients with post-burn scars from November 2016 to May 2019. Scar more than 20 cm squares in patients between 18 and 55 years were included in this study. They were post-burn scars that had healed by about a month from the burn injury, as elicited from the patient's history. Scars were divided into two homogenous parts. In the first half of the scar area, lipofilling was performed through subscar and intrascar infiltration; the second half of the scar area was the control one. The regional medical ethics committee approved the study protocol. As per the Declaration of Helsinki, written informed consent was obtained from all patients.

### Scar Evaluation

Clinical parameters were evaluated at 6-month intervals, 1 day before first sitting of lipofilling, 6 months later at second sitting of lipofilling, and at the end of 1 year.

Photographs were taken at the same focal length and exposure. The firmness of the scar evaluated with a Shore type A durometer. The measurements of each area were taken perpendicularly to the plane of the scar for 10 seconds after leaning the instrument to make the reading accurate. Baseline durometer reading was taken as the average of 10 readings at different areas of the scar and noted in the proforma by a single investigator who was not a part of the study group.

In addition, the entire scar area was subjected to the modified Vancouver scar scale (mVSS) and Patient and Observer Scar Assessment Scale (POSAS). Histopathological analysis, in the form of hematoxylin & eosin stain (H&E), Verhoeff elastin stain, and Masson trichrome (MT) stain, was done from biopsies taken before the first lipofilling and at the end of 1 year. Immunohistochemical analysis was done using p-53 and s-100 markers in the same manner. Points are given to different parameters in histology and immunohistochemistry with a maximum score of 18 in each category.

### Lipofilling

All the patients were operated under general anesthesia. Two sessions of lipofilling were done at 6 months intervals. We followed the standard Coleman technique for fat grafting. Fat was harvested using 3 mm Luer Lock-mounted cannulas. Manual suction was performed with gradual negative pressure. Compression dressing was applied to the donor site. Harvested fat was transferred in 10 mL syringes and centrifuged at 3,000 rpm for 3 minutes. Once centrifuged, harvested fat will divide into three layers. The upper oily layer was decanted using surgical cotton for 5 minutes. Then the lowermost layer was discarded, and the middle layer was transferred. Lipofilling was done at a subscar level using an 18G sharp needle and by a retrograde method in small pockets of 0.5 mL. Many radiating passages are made to distribute fat in different directions to form a lattice pattern. Approximately 2.5 mL of fat grafted for an area of 6.45 cm<sup>2</sup> of scar surface. Contour irregularities are minimized using digital pressure immediately after placement.

A small biopsy was taken at the preplanned site, and the biopsy site closed with nylon 5-0 suture and a noncompressive dressing applied. Both groups had scar massages and application of silicon sheets and pressure garments as per departmental protocol.

### Statistical Analysis

All the data were entered in an Excel spreadsheet (Microsoft Corp. 2013). All the data evaluated using SPSS version 25 (IBM Corp., Armonk, New York, United States). The statistical significance of quantitative variables between the two groups was determined by the unpaired t-test/nonparametric Mann-Whitney U test. The statistical significance of qualitative variables between the two groups was determined by the chi-squared test/Fisher's exact test. In both cases, the level of statistical significance was taken as a *p*-value less than 0.05.

## Results

The mean age of the study population was 30.6 years. The maximum number of patients was from the 18- to 25-year age group (60%). Out of 30 patients, 17 were female patients. The duration of scars included in this study ranged from 1 to 28 years, with a mean duration of 5.91 years. The thigh area was our donor area of choice, followed by the abdomen. Scars ranging from 28 cm<sup>2</sup> to 143 cm<sup>2</sup> in size was included in the study. The mean amount of fat injected was 14.85 mL (►Table 1; ►Figs. 1 and 2).

**Table 1** Summary of patient details

Sl. no.	Sex	Part of the body involving	Nature of burn	Duration of scar (years)	Scar area taken for study (cm <sup>2</sup> )	Lipofilled scar area (cm <sup>2</sup> )	The total volume of fat injected (mL)	Donor area
1	Female	Forearm	Scald	13	48	24	12	Abdomen
2	Male	Forearm	Thermal	12	105	52.5	17	Abdomen
3	Female	Forearm	Thermal	8	132	66	17	Abdomen
4	Female	Forearm	Scald	15	48	24	12	Abdomen
5	Female	Leg and Foot	Scald	3	32	16	4.5	Abdomen
6	Male	Neck	Scald	0.5	40	40	16	Abdomen
7	Male	Shoulder	Scald	0.5	42.25	22.75	6	Abdomen
8	Male	Face	Thermal	1	40	15	7	Thigh
9	Female	Face	Thermal	3	87	43.5	20	Thigh
10	Female	Chest wall	Thermal	28	37.5	35	20	Thigh
11	Male	Shoulder	Thermal	0.66	97.5	48.75	20	Thigh
12	Male	Abdomen	Thermal	0.66	99	49.5	18	Thigh
13	Male	Hand	Thermal	2	72	36	17	Thigh
14	Female	Face	Thermal	7	28	28	9	Thigh
15	Male	Neck	Thermal	10	143	71.5	25	Thigh
16	Male	Arm	Thermal	10	40	20	11	Thigh
17	Female	Face	Scald	1.5	42	21	12	Thigh
18	Female	Face	Scald	1.5	42	24	14	Thigh
19	Male	Forearm	Thermal	2.5	98	49	20	Thigh
20	Male	Arm	Thermal	2.5	30	18	5	Thigh
21	Male	Forearm	Thermal	2.5	120	60	18	Thigh
22	Male	Forearm	Thermal	2.5	112	56	20	Thigh
23	Female	Forearm	Scald	1	96	48	18	Thigh
24	Female	Forearm	Scald	1	44	22	8	Thigh
25	Female	Forearm	Thermal	0.5	40	18	12	Thigh
26	Female	Arm	Thermal	0.5	48	24	10	Thigh
27	Female	Forearm	Scald	9	140	84	30	Thigh
28	Female	Arm	Thermal	14	84	48	24	Thigh
29	Female	Face	Thermal	12	42	20	8	Thigh
30	Female	Forearm	Thermal	12	54	27.5	15	Thigh

**Durometer Reading**

The durometer reading in the lipofilled region showed significant changes as early as 6 months from first sitting, and the reading improves further as time passes over the first year. In control regions, the durometer reading changes were appreciable only at the end of the first year, and the 6 months readings showed no improvement (► **Graph 1**).

**Assessment of Scar-by-Scar Assessment Scales****Modified Vancouver Scar Scale**

In lipofilled areas, the mean score was 10.33, 9.73, and 8.37 at baseline, 6-month, and 1-year intervals. There was a significant improvement in mVSS scores as early as the initial

6 months and improved further over the first year. In control areas, the mean score was 10.66, 10.33, and 9.33 at baseline, 6-month, and 1-year intervals. The improvement was noticeable only at the end of the first year in control areas (► **Graph 2**).

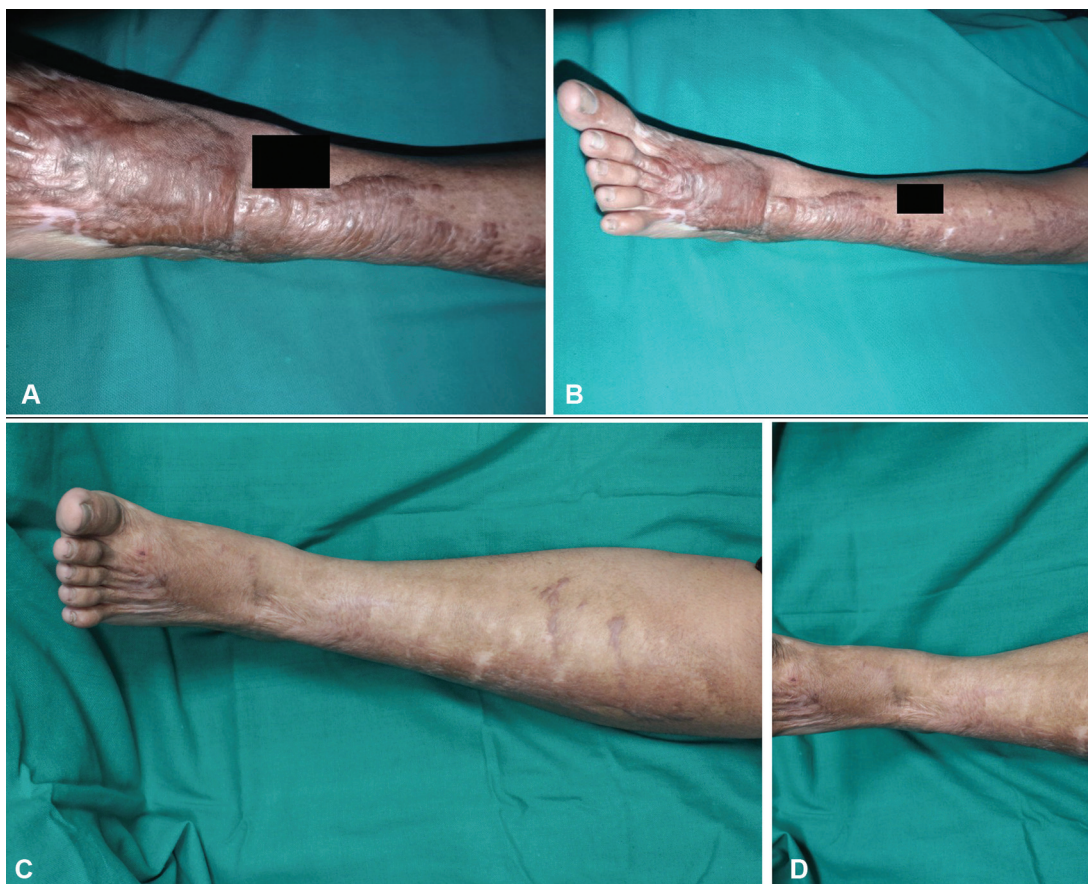
**POSAS Scale**

In lipofilled areas, improvement in all the parameters of the patient scale remains significant except for the thickness and surface area. Maximum improvement was noted in pliability relief, vascularity, and pigmentation. Most of the patients were able to appreciate these changes in the lipofilled areas as early as 6 months post-treatment. Patients did not appreciate the reduction in the thickness of the scar. In the control

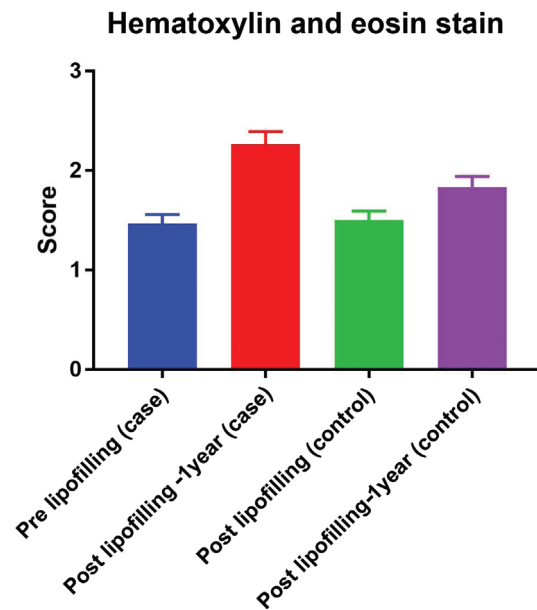




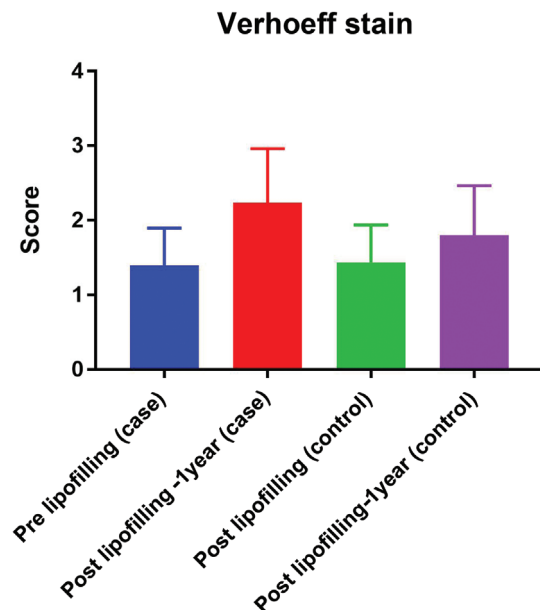
**Fig. 1** A 38-year-old woman with post-burn contracture neck released and grafted. (A–C) Pre-treatment photograph showing the hypertrophic post-burn scar over the chin sustained due to a thermal burn 3 years back. The right side of the scar was the case, and the left side of the scar was the control. (D–F) Post-treatment clinical photograph (1 year).



**Fig. 2** (A, B) A 18-year-old girl with a hypertrophic post-burn scar over the dorsum of foot and leg sustained due to a scald burn 3 years back. The dorsum of the foot scar was the case, and the leg scar was used as a control. (B) Post-treatment clinical photograph (1 year).



Graph 1 Hematoxylin and eosin stain comparison.



Graph 2 Verhoeff stain comparison.

**Table 2** POSAS overall trend

POSAS overall	Pre- and post-lipofilling at 1 year (difference in mean and SD)		
	Control	Case	p-Value
Vascularity	4.9 ± 2.175	4.5 ± 2.139	0.0005
Pigmentation	7.3 ± 1.619	7.1 ± 1.569	0.0022
Thickness	6.9 ± 1.482	6.7 ± 1.34	0.1698
Relief	6.9 ± 1.231	5.4 ± 1.048	<0.0001
Pliability	7.8 ± 1.046	6.8 ± 0.7916	<0.0001
Surface area	6.2 ± 1.4	6.1 ± 1.45	0.0733
Total	36.2 ± 5.192	30 ± 5.23	<0.0001

Abbreviations: POSAS, Patient and Observer Scar Assessment Scale; SD, standard deviation.

area, there was no improvement in any parameters at the end of 6 months. But there was an improvement in vascularity and pigmentation, at the end of 1 year (► **Table 2**).

## Histopathological and Immunohistochemical Markers Analysis

(► **Table 3**, ► **Figs. 3–7**, ► **Graphs 1–5**)

In H&E staining, after two sittings of lipofilling, there was a significant reduction in melanocytes. Collagen fibers that were irregular initially became parallel to the epidermis and finer. Improvement in collagen fibers was significant. In Verhoeff elastin stain, preoperative results show that 60% of cases have minimal elastin fibers in scar tissue, whereas 40% show a focal presence of elastin fibers. At the end of 1 year, in the lipofilled area, there is a significant improvement in elastin fibers. With respect to MT stain, preoperative results show 43.3% of scars had minimal and 56.6% of scars had focal collagen at the dermoepidermal junction. After

1 year of lipofilling, 20 out of 30 scars, the lipofilled regions showed significant improvement in collagen synthesis p-53 suggests the level of cellular activity in any tissue. In pre-lipofilling status, the mean p-53 score was 13.3, which improved significantly to 35.93 after 1 year of lipofilling s-100 stains the melanocytes. Pre-treatment level of the mean s-100 score was 102, which reduced to 66.4 after 1 year of treatment in the lipofilled region. An intergroup analysis of the parameters was done among the lipofilled and non-lipofilled region parameters using Tukey's multiple variable comparison test (H&E stain, Verhoeff stain, MT stain, p-53 and s-100). The comparison in the lipofilled regions pre- and post-intervention showed significant improvement in all the parameters analyzed. In the control regions, pre- and post-treatment analysis showed statistically significant improvement only in p-53 and s-100 scores. In the post-treatment analysis of case and control regions, the mean difference in all the parameters was statistically significant. Overall, the improvement in the parameters studied was much better in



**Table 3** Analysis of pre- and post-lipofilling changes in case and control group

Sl. no.	Category	Case			Control		
		Pre-lipofilling Mean $\pm$ SEM	Post-lipofilling (1 year) Mean $\pm$ SEM	p-Value	Pre-treatment Mean $\pm$ SEM	Post-treatment (1 year) Mean $\pm$ SEM	p-Value
1	Hematoxylin and eosin stain	1.467 $\pm$ 0.09	2.267 $\pm$ 0.12	<0.0001	1.5 $\pm$ 0.09	1.833 $\pm$ 0.10	0.0228
2	Verhoeff stain	1.4 $\pm$ 0.09	2.233 $\pm$ 0.13	<0.0001	1.433 $\pm$ 0.09	1.8 $\pm$ 0.12	0.0192
3	Masson trichrome stain	1.567 $\pm$ 0.09	2.267 $\pm$ 0.11	<0.0001	1.567 $\pm$ 0.09	1.8 $\pm$ 0.10	0.0923
4	p-53	13.13 $\pm$ 0.59	35.93 $\pm$ 1.04	<0.0001	12.93 $\pm$ 0.57	31.4 $\pm$ 1.60	<0.0001
5	s-100	102 $\pm$ 2.71	66.4 $\pm$ 1.57	<0.0001	102.2 $\pm$ 2.58	78.4 $\pm$ 2.65	<0.0001

Abbreviation: SEM, standard error of mean.

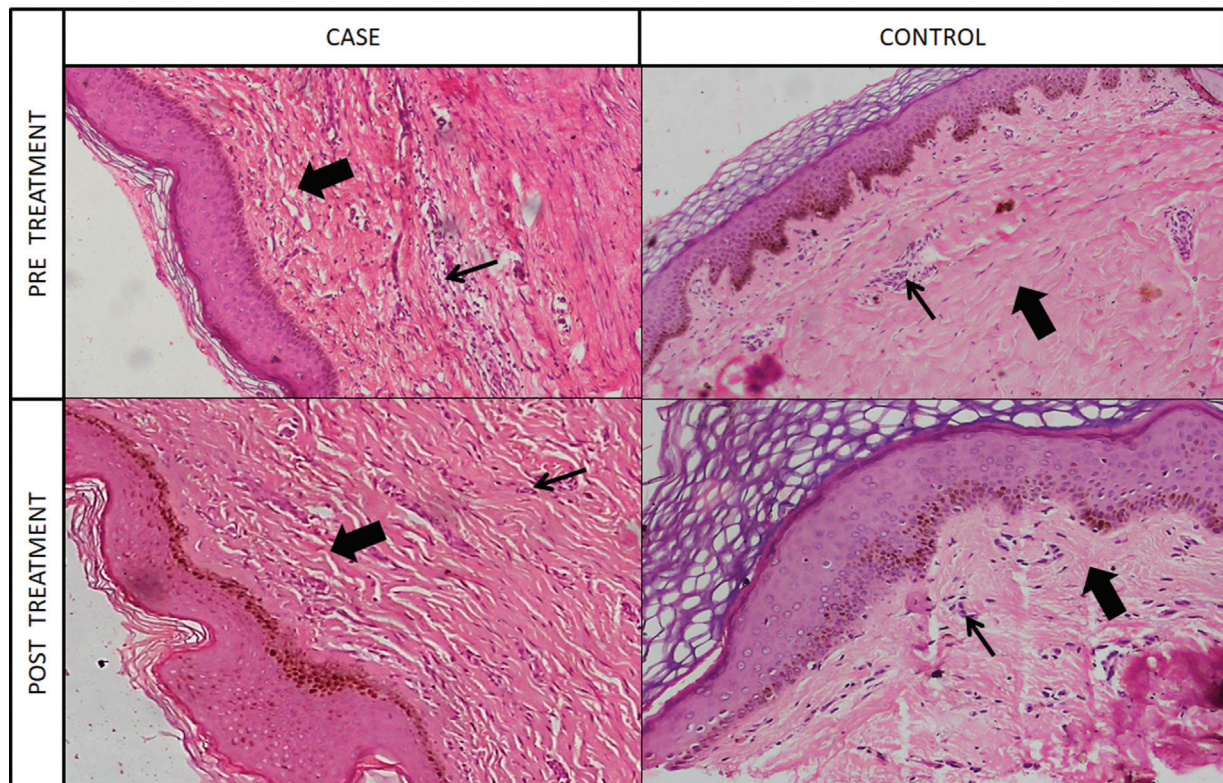
the lipofilled region when compared to the non-lipofilled areas.

## Discussion

Management of burn scars remains a challenge to date for treating surgeons as they involve large areas and have a tendency for hypertrophy and hyperpigmentations. For the treatment of scars, clinicians and researchers have described a variety of protocols. Yet, limited data about the effectiveness are derived

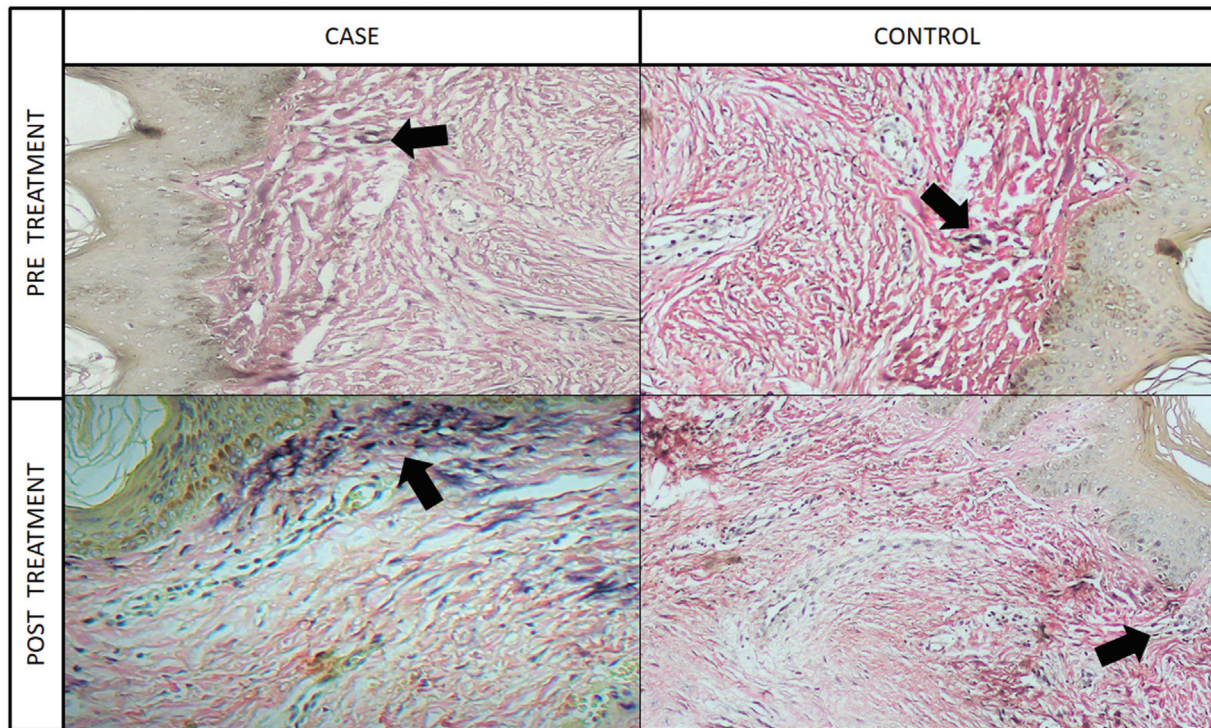
from well-designed, prospective, randomized, controlled clinical trials. To date, no gold standard exists for the treatment of scar tissue. Treatments are mainly based on the individual experience of clinicians, with varying degrees of success.

This study aimed to understand the remodeling effect of lipofilling in post-burn scars in patients of Indian origin. This study was conducted in a single center over a period of 30 months. A total of 30 scars in burned patients were included in this study and followed up for 12 months. The mVSS shows significant improvement in the score as early as

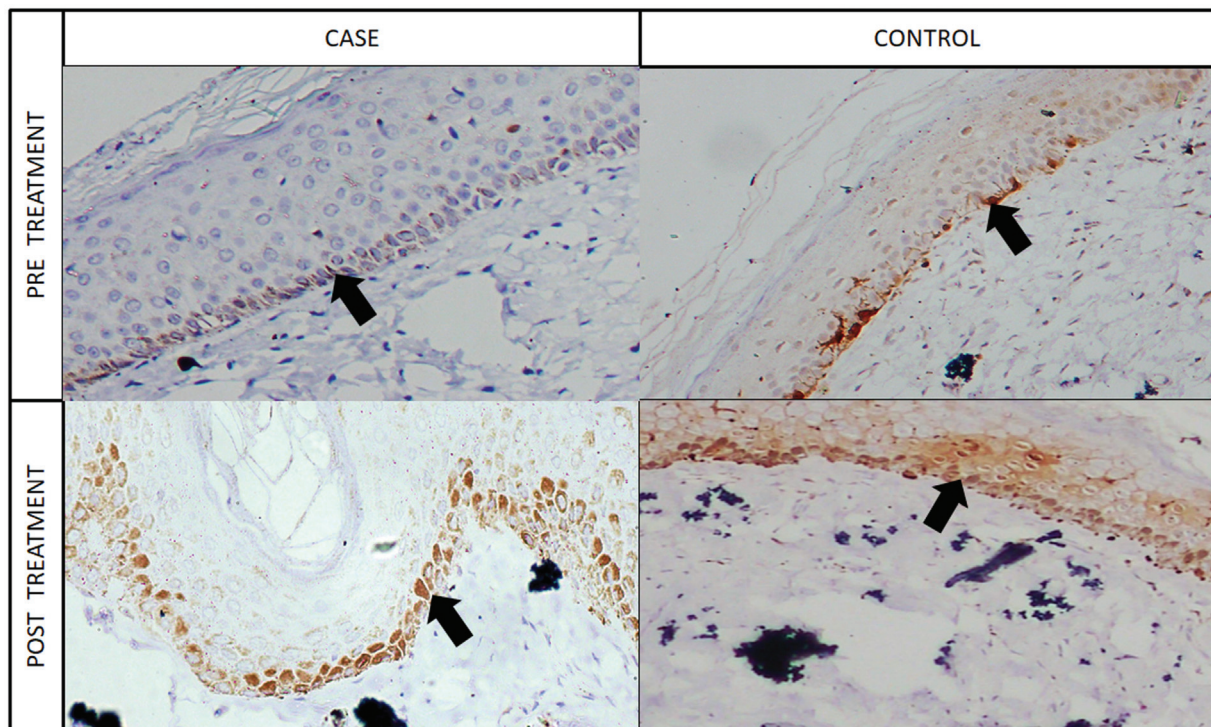


**Fig. 3** Hematoxylin and eosin stain (100x): Pre- and post-treatment histopathology in case and control groups. The bold arrow shows the collagen bundles and the thin arrow shows the cellular infiltrates. Pre-treatment slides show irregular and plump collagen fibers haphazardly arranged with prominent cellular infiltrates in the dermis. Post-treatment slides in the case group showing thin collagen fibers and better organization with reduced cellular infiltration in the dermis compared to the control group and pre-treatment slides.

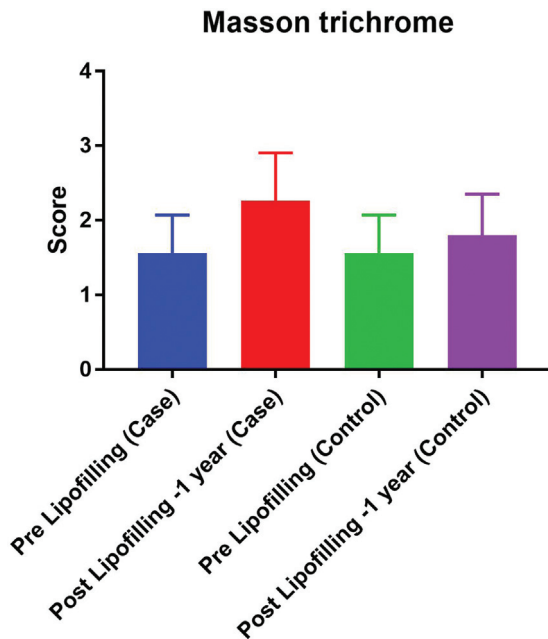




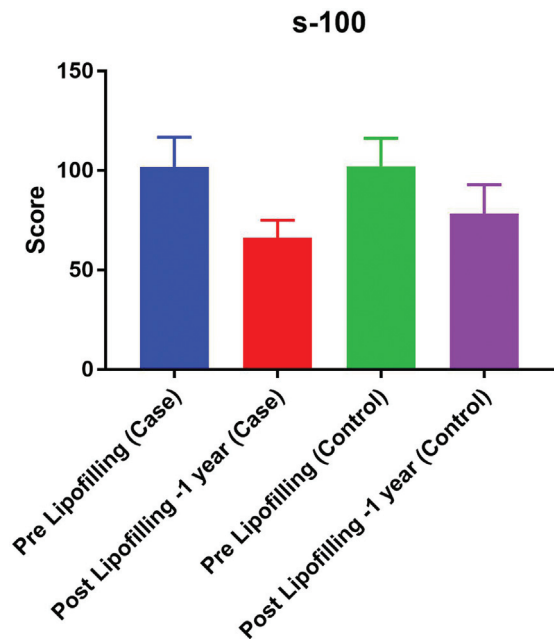
**Fig. 4** Masson trichrome (200x). Pre- and post-treatment histopathology in case and control groups. Bold arrow showing subepidermal collagen fibers. Pre-treatment slides and post-treatment control slides show the absence of subepidermal collagen deposition. Post-treatment slides in the case group showed significant deposition of fine collagen fibers at the dermoepidermal junction.



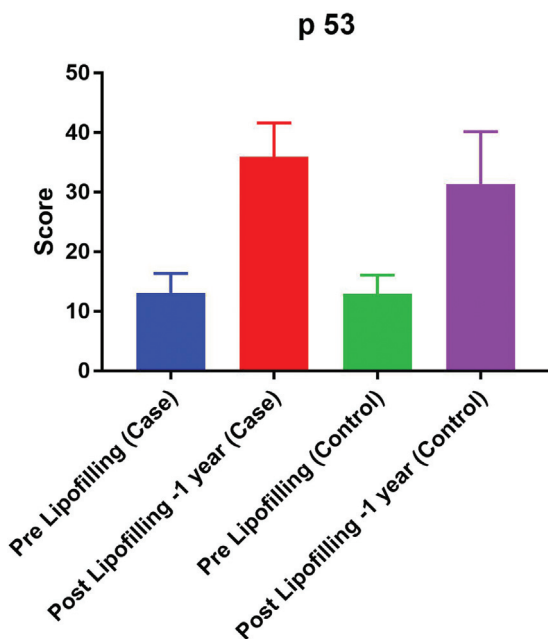
**Fig. 5** Verhoeff elastin stain (200x). Pre- and post-treatment histopathology in case and control groups. Bold arrow showing dermal elastin fibers. Pre-treatment slides and post-treatment control slides showed a near absence of elastin fibers in the dermis. However, post-treatment slides in the case group show a significant increase and better organization of elastin fibers in the dermis compared to the control and pre-treatment slides.



Graph 3 Masson trichrome stain comparison.



Graph 5 s-100 Immunohistochemistry comparison.



Graph 4 p-53 Immunohistochemistry comparison.

6 months postoperative follow-up; this trend of improvement is also present from 6 to 12 months of postoperative time ( $p < 0.05$ ). When we compare preoperative parameters with 1-year postoperative parameters among the POSAS, we found that improvement in vascularity, pigmentation, relief, and pliability shows significant improvement (→ Table 2).

Reduction in the thickness of the scar was noted but remains statistically insignificant. No reduction in surface area was noted at any time interval. Klinger et al reported significant improvement in all the parameters of the observer scale over 3 months.<sup>5</sup> Pallua et al reported significant improvement in pigmentation and pliability. Improvement

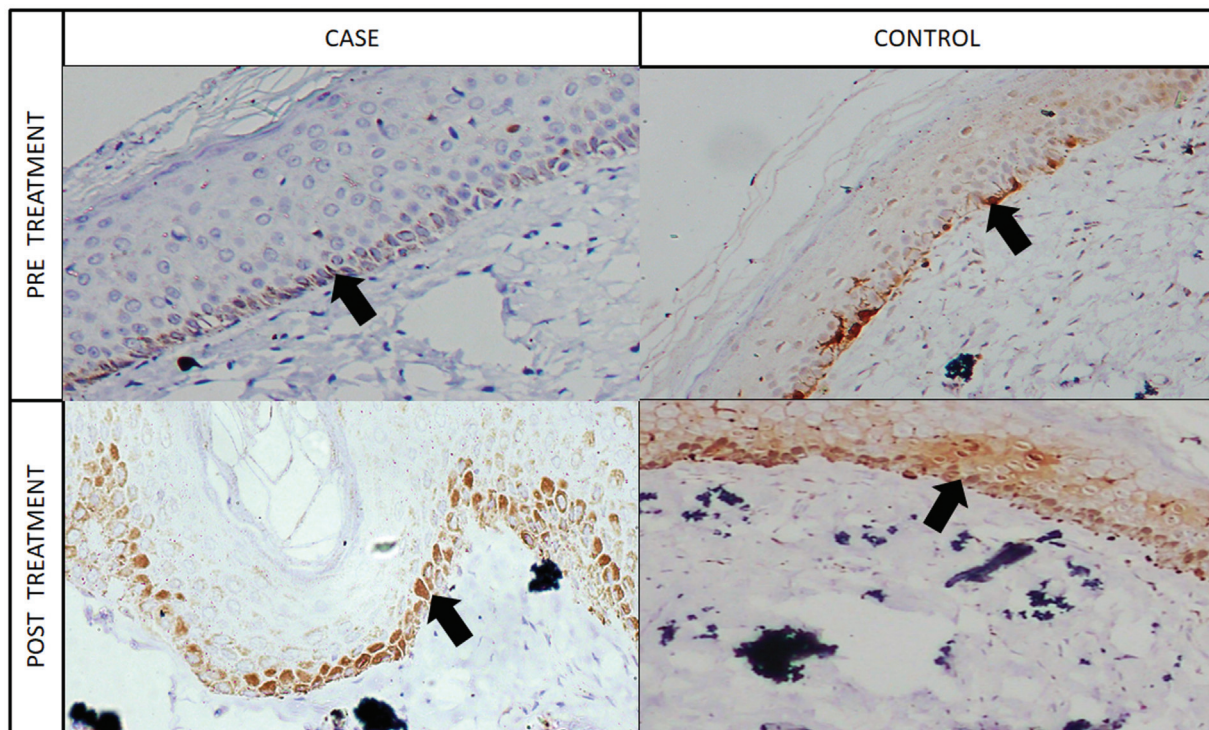
was also noted in the remaining parameters but remained statistically nonsignificant.<sup>6</sup> Caviggioli et al. reported significant improvement in all the parameters over 3 months.<sup>7</sup>

Over 1 year, there has been a significant improvement in collagen fiber arrangement and a reduction in melanocyte staining on H&E staining. Verhoeff stain shows a significant increase in elastin fibers in lipofilled areas after a 1-year interval. MT shows the laying of new collagen fibers at the dermoepidermal junction and reticular dermis. A significant improvement in the p-53 value suggests increased tissue regeneration in a quiescent scar. s-100 values reduce significantly over the period of 1 year, suggesting a reduction in the melanocytes in the scar. This finding also correlates with a reduction in melanocytes in H&E stains. A study by Bruno et al also shows improved vascularization of dermal papillae, better organization of collagen, a significant decrease in s-100-positive cells and Langerin-positive cells, and marked improvement in p-53 and Ki-67 expression.<sup>8</sup> Klinger et al show epithelial hyperplasia and neoangiogenesis in lipofilled scar tissue.<sup>5</sup> In their study, Brongo et al also reported histologic findings, which showed new collagen deposition, neoangiogenesis, and dermal hyperplasia in the context of new tissue, demonstrating tissue regeneration.<sup>9</sup> Lipofilling has efficacy in remodeling the scar, and the effect can be seen clinically as early as the initial 6 months. The remodeling effect of adipocyte-derived stem cells is long-lasting, and there is gradual improvement in most of the parameters. However, further study with a large sample size and long-term follow-up is required to confirm the remodeling capacity of lipofilling.

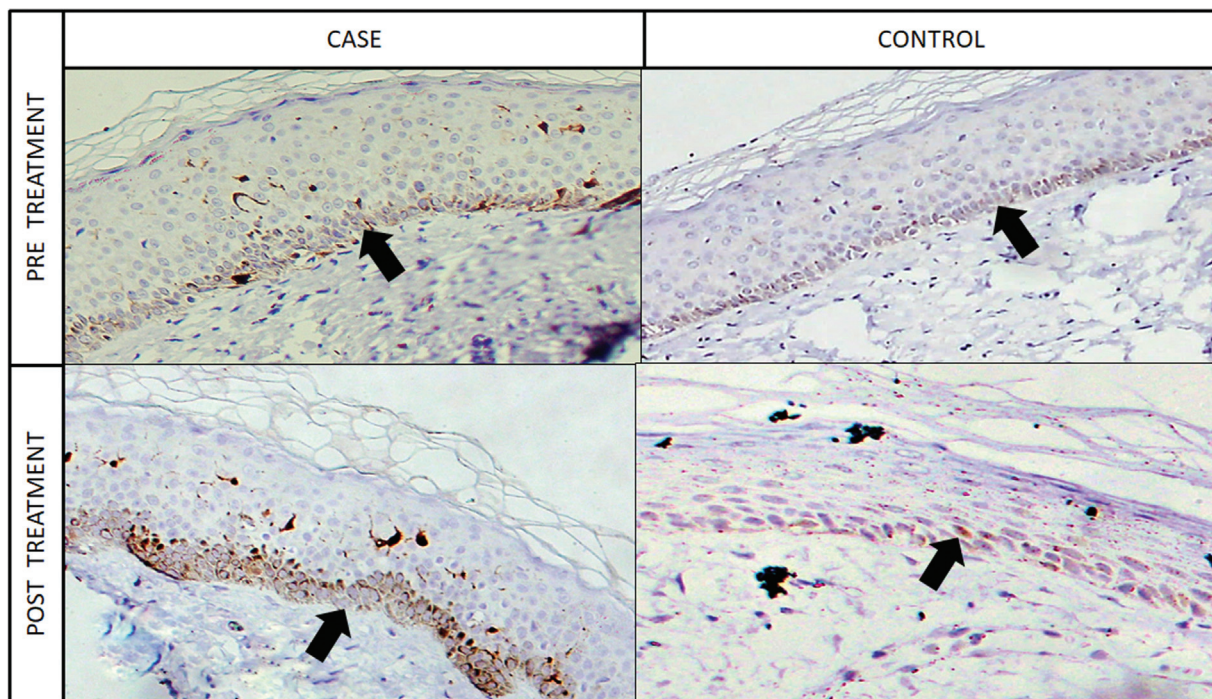
## Conclusion

Lipofilling is an emerging therapy that provides a new armamentarium to deal with burn scars. There is a significant reduction in pain, itch, and stiffness, an increase in the





**Fig. 6** p-53 (400x). Pre- and post-treatment immunohistopathology in case and control groups. Bold arrow showing p-53 in the basal layer of the epidermis. Post-treatment slides in both case and control groups showed a significant increase in basal p-53 activity post-treatment. However, the activity in post-treatment slides of the cases is more dominant.



**Fig. 7** s-100 (400x). Pre- and post-treatment immunohistopathology in case and control groups. Bold arrow showing s-100 activity in the epidermis. Post-treatment slides in both case and control groups showed a significant reduction in the expression of s-100 in the basal layer of the epidermis. However, the reduction in the activity is more in the case group compared to the control group when compared with the pre-treatment slides.

pliability of scar, and a significant improvement in mVSS score seen over 1 year. H&E staining shows organized parallel collagen fibers, a significant reduction in melanocytes, and an improvement in vascularity. Elastin fibers show a generalized and more organized distribution on the Verhoeff stain. MT stain shows a significantly increased collagen fiber at the reticular dermis. Immunohistochemical marker p-53 increases significantly, which indicates new cell synthesis in the scar tissue. The s-100 score reduces significantly over the lipofilling period, indicating a melanocyte reduction. We conclude that lipofilling has a regenerative capacity that leads to improvement in the overall appearance of scar and improvement at the cellular level over the follow-up period.

## Highlights

- This study evaluates changes in lipofilled burn scar over a period of time and compares them with non-lipofilled burn scar tissue.
- Significant reduction in pain, itch, stiffness, and increase in the pliability of burn scar and significant improvement in modified mVSS in lipofilled group.
- Immunohistochemical analysis indicated new cell synthesis in the burn scar tissue and reduced melanocytes.
- Lipofilling has regenerative capacity, leading to improved overall appearance of burn scar and improvement at the cellular level.

### Authors' Contribution

B.J., D.K. and C.A. were involved in conceptualization, designing, and drafting of the manuscript. V.M. and B.J.

helped in drafting of the manuscript, and collection and analysis of data. All authors read and approved the final manuscript.

### Conflict of Interest

None declared.

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