Prevention of postoperative scars in dark skin types using a fractional carbon dioxide laser: a clinical and histopathological split-scar study

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Background

Treatment of mature postoperative scars is frustrating for both patients and doctors. Accordingly, a fractional carbon dioxide (CO_2) laser could be used during the early postoperative period as a prophylaxis against scarring. However, its safety in patients with dark skin types and the histopathological changes during early treatment remain controversial.

Objective

To evaluate the efficacy and safety of a fractional CO_2 laser in the prevention of postoperative scars in patients with dark skin types, with assessment of its histopathological effect.

Patients and methods

Six fractional CO_2 laser sessions were performed at 2-week intervals on one part of early postoperative wounds of 20 patients in comparison with other untreated parts 3 months after the last session using the Vancouver scar scale (VSS). Using histopathological and computerized morphometric analysis, morphological changes in collagen and elastic fibers were assessed and a quantitative evaluation of epidermal thickness was performed 3 months after the last session.

Results

In treated parts, there was a significant improvement in pliability, height, and total VSS score ($P \leq 0.05$), with no significant difference in vascularity and pigmentation (>0.05), compared with untreated parts. Hypertrophic scars appeared in untreated parts of 40% of the patients. Histopathologically, collagen fibers were fine and well organized in treated parts (75%); however, they were arranged as fairly thickened hyaline bundles (75%), either disorganized (35%) or randomly, within nodules with some parallel fibers to the epidermis (40%) in untreated parts. Dense elastotic tissues, in untreated biopsies (80%), became less with the appearance of fine and well-organized fibers in treated biopsies (70%). There was a significant increase in the epidermal thickness of treated parts compared with untreated ones ($P<0.05$).

Conclusion

A fractional CO_2 laser was effective, tolerable, and safe in the prevention of postoperative scars, especially the hypertrophic type, in patients with dark skin types. Histopathologically, remodeling of collagen and elastic fibers occurred with an increase in the epidermal thickness after its use.

Keywords:

CO_2 laser, fractional, postoperative, scar

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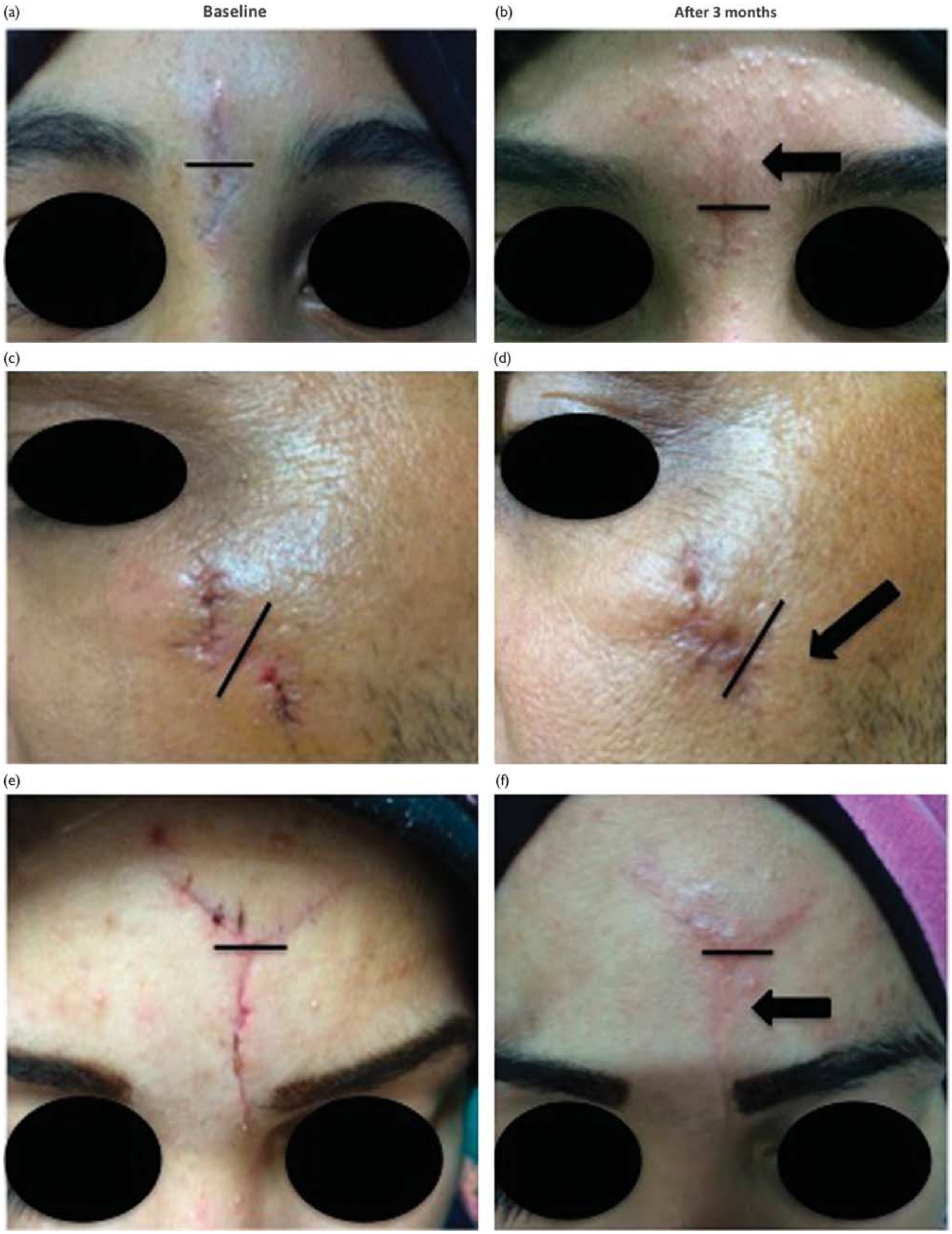
Introduction

Patients undergo various kinds of surgery for therapeutic or cosmetic reasons [1]. Of these,-15% have excessive scar formation, especially in populations with Fitzpatrick skin type III or higher skin pigmentation [2]. However, surgical reconstruction can result in disfiguring of these postoperative scars, which causes considerable distress to patients [1]. Accordingly, various types of lasers have been used to treat scars [3].

Ablative lasers used for skin resurfacing, such as the carbon dioxide (CO_2) and erbium-doped yttrium aluminum garnet (Er:YAG), lead to the greatest improvement in scars with a single treatment [3]. However, a long downtime and a high incidence of adverse effects have limited its use [4]. Moreover, it can destabilize a healing tissue bed or disrupt the epidermal barrier before sealing of the incision. Therefore, the optimal time for treatment is during the scar formation phase. Meanwhile, nonablative pulsed or

Figure 1.



Clinical evaluation of an early postoperative wound in response to the use of a fractional CO_2 laser. Representative photographs of the face (a–f) before (a, c, e) and after laser (b, d, f). There was a significant improvement in the treated parts of scars (arrows) (b, d, f) compared with the untreated parts (b, d, f). Note the appearance of hypertrophic scars only in the untreated parts, with no occurrence in the treated areas of the same patient (f).

Table 1. Comparison between untreated and treated parts of postoperative scars using the Vancouver scar scale

Scare scales	Untreated parts	Treated parts	*P* value
Vascularity			
Range	0–3	0–1	0.08
Mean ± SD	1.1 ± 0.9	0.7 ± 0.5	
Pigmentation			
Range	0–3	0–2	0.1
Mean ± SD	1.5 ± 0.9	1.1 ± 0.8	
Pliability			
Range	0–3	0–2	< 0.001
Mean ± SD	2 ± 0.8	0.95 ± 0.5	
Height			
Range	0–4	0–1	0.01
Mean ± SD	1.5 ± 1.5	0.25 ± 0.4	
Total score			
Range	1–10	1–6	< 0.001
Mean ± SD	6.1 ± 2.6	3 ± 1.2	

fractionated lasers place minimal mechanical stress on tissues, making an argument for earlier treatment [5].

A fractional ablative 10 600 nm CO_2 laser has gained popularity in the treatment of scars because it targets the tissue producing microscopic thermal zones of injury surrounded by areas of untreated skin and intact epidermis [6,7], enabling much deeper treatment with fast healing and reduction of side effects [4], which in turn stimulates progressive collagen remodeling [6].

A fractional ablative CO_2 laser has been used for mature scars for at least 2 months in most previous studies [1]. Meanwhile, few reports have focused on early treatment of postoperative scars using fractional CO_2 [1,8] to prevent or at least reduce the apparition of scars [9]. Meanwhile, on review of the literature, there were no studies discussing the safety of a fractional ablative CO_2 laser and the treatment guidelines in patients with dark skin types. Moreover, there were no evidence-based publications discussing its effect at the histopathological level. Accordingly, the aim of the present study was to evaluate the clinical efficacy and safety of a fractional ablative CO_2 laser in the prevention of postoperative scars in patients with dark skin types with assessment of its histopathological effect.

Patients and methods

Patients

This study included 20 patients with early postoperative facial wounds of 2–3 weeks' duration. These patients were recruited from the out-patient Clinics of Dermatology and Plastic and Reconstructive Surgery departments, Minia University. An informed written consent was obtained from each patient for treatment and to obtain skin biopsies. The study was approved by the Committee for Postgraduate Studies and Research of Minia University.

Patients with Fitzpatrick skin types III or more were included in this study. The patients had not been treated with topical and systemic medication or laser before starting the study. Patients with a history of keloidal tendency, isotretinoin use, pregnancy, immunosuppressive drug use, and postoperative infection of the wound were excluded from the study.

Treatment protocol

One part of the wound was subjected to six sessions using a fractional CO_2 laser [Multixel, DS-40U (B), 10 600 nm; Deashin Enterprise (D.S.E) group, Seoul, Korea]; however, the other part was left untreated, serving as a control. A 2.5% lidocaine/prilocaine cream (EMLA cream; AstraZeneca AB, Södertälje, Sweden) was used as a topical anesthetic and applied to the treated areas under occlusion 1 h before treatment. Three passes in each session, with a 2-week interval between sessions [1], were performed. Laser parameters were established according to the device manufacturer using a fluence of 30 mJ, depth of level 3 (1.5–2 mm), a spot density of 100 dots/cm^2, and a pulse duration of 900 ms. The scanner size was adjusted according to the length and width of the scar, with an increase in its size by 2 mm all around the scar.

After treatment, the treated areas were cooled with ice packs for 10 min to protect the spared epidermis. Patients were instructed to use a moisturizer to both the treated and the untreated areas for a week after the treatment with application of a full-spectrum sunscreen (> SPF 50) in between sessions up to the clinical assessment. Photographs were taken before, at every laser session, and after 3 months of the last session.

Clinical assessment of the response

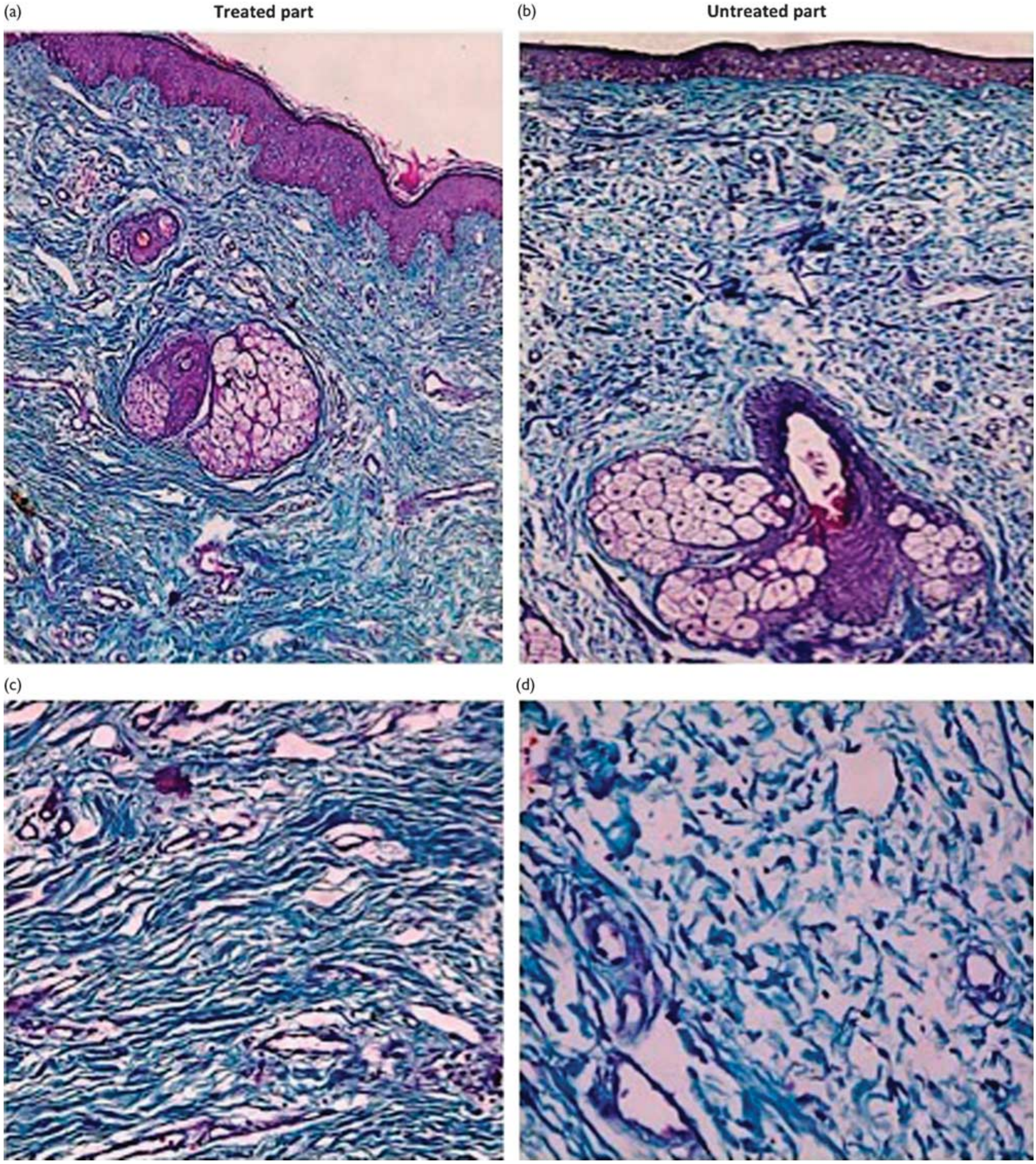
Assessment of the response was performed 3 months after the last laser session through grading of both treated and untreated parts by two blinded independent dermatologists using the Vancouver scar scale (VSS), where the average of their evaluation was calculated. This scale included pigmentation (0 = normal, 1 = hypopigmented, 2 = mixed pigmentation and 3 = hyperpigmented), pliability (0 = normal, 1 = supple, 2 = yielding, 3 = firm, 4 = ropes and 5 = contracture), height (0 = flat, 1 is < 2 mm, 2 is 2–5 mm, and 3 is ≥ 5 mm), and vascularity (0 = normal, 1 = pink, 2 = red and 3 = purple). The score for each parameter was assessed separately and all four scores were added and averaged [10]. The scores range from 0 to 14. The maximum score is 14, indicating the worst result. The minimum score is 0, indicating the best result, that is, normal skin.

Three months after the treatment sessions, the patients were surveyed about their overall level of satisfaction using quartile-grading scale as follows: very satisfied, satisfied, slightly satisfied, and unsatisfied [1]. The patients were asked about sequels and side effects observed during every session and after 3 months of the last laser session, namely, erythema, edema, pain, crusts, hyperpigmentation, hypopigmentation, and hypertrophic scars.

Skin biopsy

Skin biopsy specimens were taken 3 months after the last laser session, using 2-mm punch probes, from both treated and untreated parts of the scar. All biopsies were fixed in 10% buffered formalin, embedded in paraffin, and sectioned into 5 μm thick sections. These sections were subjected to a histopathological examination using Hematoxylin and Eosin, Masson trichrome, and Orcein stains. A light microscope [Accu-Scope # 3025 five

Figure 2.



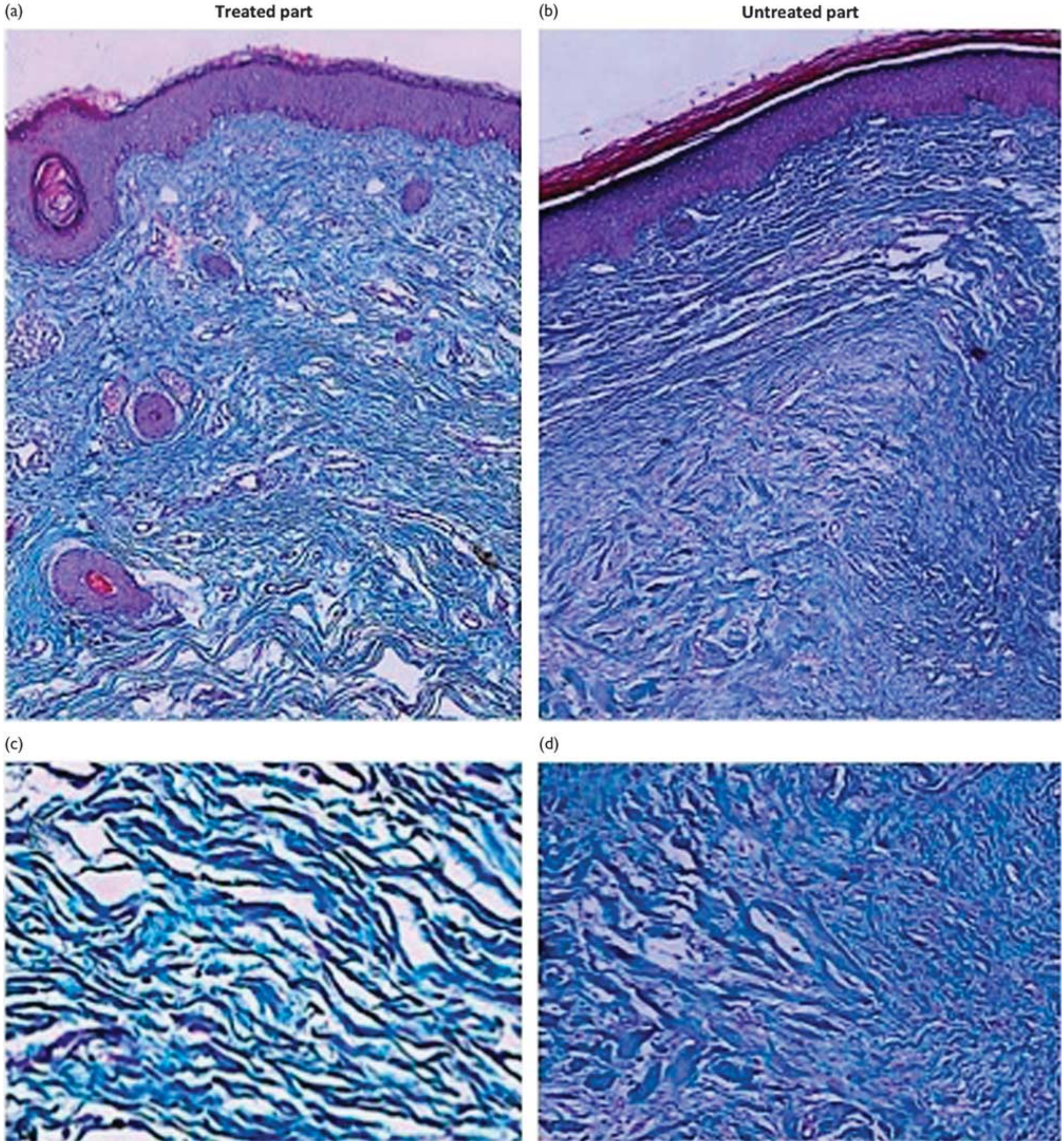
Skin biopsy specimens after the use of a fractional CO_2 laser. The treated specimen shows fine and well-organized collagen fibers with parallel orientation to the epidermis and decreased interfibrillary spaces in the entire dermis (a, c). The untreated specimen shows disorganized thickened hyaline-like bundles of collagen with increased interfibrillary spaces in the entire dermis (b, d) (Masson trichrome; a, b ×100 and c, d ×400).

headed (A3025-5); Olympus, Tokyo, Japan] with a built-in camera (digital camera E-330 SLR; Olympus) was used to examine and photograph the sections.

Histopathological assessment of the response

The histopathological evaluation of both treated and untreated parts, 3 months after the last session, included examination of the epidermis (normal, flattened or hyperplastic) and the dermis [collagen; site (papillary or reticular), arrangement (disorganized, nodules or parallel to the skin surface) and thickness (thickened or fine fibers) of elastin, and site (papillary or reticular) and appearance (thickened elastotic tissues or fine fibers)].

Figure 3.



Skin biopsy specimens after the use of a fractional CO_2 laser. The treated specimen shows fine and well-organized collagen fibers with a parallel orientation to the epidermis and decreased interfibrillary spaces in the entire dermis (a, c). The untreated specimen from hypertrophic scars shows that collagen fibers are fairly thick (cigar-shaped) and have a random distribution within nodules in the middle and deep dermis and with parallel orientation to the epidermis outside the nodule in the superficial dermis (b, d) (Masson trichrome; a, b $\times$100 and c, d $\times$400).

Histometric evaluation of epidermal thickness

A computer-assisted program (analySIS Five; Olympus Soft Imaging Solutions GmbH, Munster, Germany) was used to measure the epidermal thickness in hematoxylin and eosin-stained sections from the two biopsy specimens 3 months after the last laser session. The mean epidermal thickness was determined after measuring the distance between the outermost surface of the epidermis excluding the stratum corneum and the dermoepidermal junction at five points through the entire length of the three examined sections.

Statistical analysis

Data were statistically analyzed using SPSS for Windows (version 16.0.1; SPSS Inc., Chicago, Illinois, USA). Quantitative data were expressed as range and mean ± SD, whereas qualitative data were expressed as number and percent. Statistical analysis included the Mann–Whitney test for nonparametric quantitative

Table 2. Comparison between histopathological changes in the untreated and treated parts of postoperative scars

	Treated parts	Untreated parts	P value
Collagen [n (%)]			
Improved biopsies	15 (75)	5 (25)	0.002
Not improved biopsies	5 (25)	15 (75)	
Elastin [n (%)]			
Improved biopsies	14 (70)	4 (20)	0.001
Not improved biopsies	6 (30)	16 (80)	
Epidermal thickness (μm)			
Range	65.19–79.99	46.13–62.99	<0.001
Mean ± SD	70.81 ± 3.32	55.15 ± 4.76	

data and Chi-square test for qualitative data. Significance was expressed in terms of the *P*-value, which was considered significant when it was 0.05 or less.

Results

The study included 20 patients [eight females (40%) and 12 males (60%)] with early facial postoperative wounds. The age of these patients ranged from 10 to 51 years, with a mean of 22.45 ± 11.46 years. In terms of Fitzpatrick skin types, three (15%), 15 (75%), and two (10%) patients had skin types III, IV, and V, respectively.

Clinical assessment

Vancouver scar scale

Three months after the last laser session, there was a significant improvement in pliability, height, and the total VSS score ($P \leq 0.05$) in treated parts, with no significant difference in vascularity and pigmentation (>0.05) compared with untreated parts (Fig. 1 and Table 1).

Patients' satisfaction

The patients themselves assessed the improvement in the treated part as follows: very satisfied in 10 (50%) patients, satisfied in eight (40%) patients, and slightly satisfied in two (10%) patients. For the control part, two (10%) patients were satisfied, seven (35%) patients were slightly satisfied, and 11 (55%) patients were unsatisfied. Accordingly, there was significant patient satisfaction in the treated parts compared with the untreated ones (<0.001).

Safety and tolerability

Post-treatment side effects after each session included erythema, edema, and dark crusts, which resolved within 7–10 days after treatment with removal of the dark crusts. There was mild pain during the laser session, which subsided shortly after the session in 18 (90%) out of 20 cases and lasted several hours to 2 days in the remaining two (10%) cases. Hyperpigmentation and hypopigmentation occurred in two (10%) patients and one (5%) patient, respectively, after the last session and disappeared 3 months after the last laser session. No side effects were noted 3 months after the last session in the treated parts. However, only in the untreated areas, hypertrophic scars were apparent in eight (40%) patients (Fig. 1).

Histopathological evaluation

Epidermis

In untreated parts, flattening of the epidermis was prominent in 18 (90%) patients. Meanwhile, treated parts showed a normal epidermis with well-developed rete ridges in 15 (75%) patients (Figs 2–4).

Collagen fibers (Masson trichrome)

There was a significant improvement in collagen fibers (0.002), which became fine and well organized and parallel to the epidermis with decreased interfibrillary spaces in the entire dermis in the treated parts of 15 (75%) patients and the untreated parts in five (25%) patients. The rest of the untreated parts (15 biopsies, 75%) showed fairly thickened hyaline bundles, either disorganized (seven biopsies, 35%) with increased interfibrillary spaces in the entire dermis or randomly within nodules (cigar-shaped) in the middle and deep dermis with some parallel fibers to the epidermis outside the nodule in the superficial dermis (eight biopsies, 40%) (Figs 2 and 3, Table 2).

Elastic fibers (Orcein stain)

There was dense elastotic material throughout the entire dermis in 16 (80%) untreated parts and six (30%) treated parts. Meanwhile, the treated areas of 14 (70%) patients showed a significant improvement (0.001) with less elastotic material and appearance of newly synthesized elastic fibers, which became finer and well organized (Fig. 4 and Table 2).

Histometric evaluation of epidermal thickness

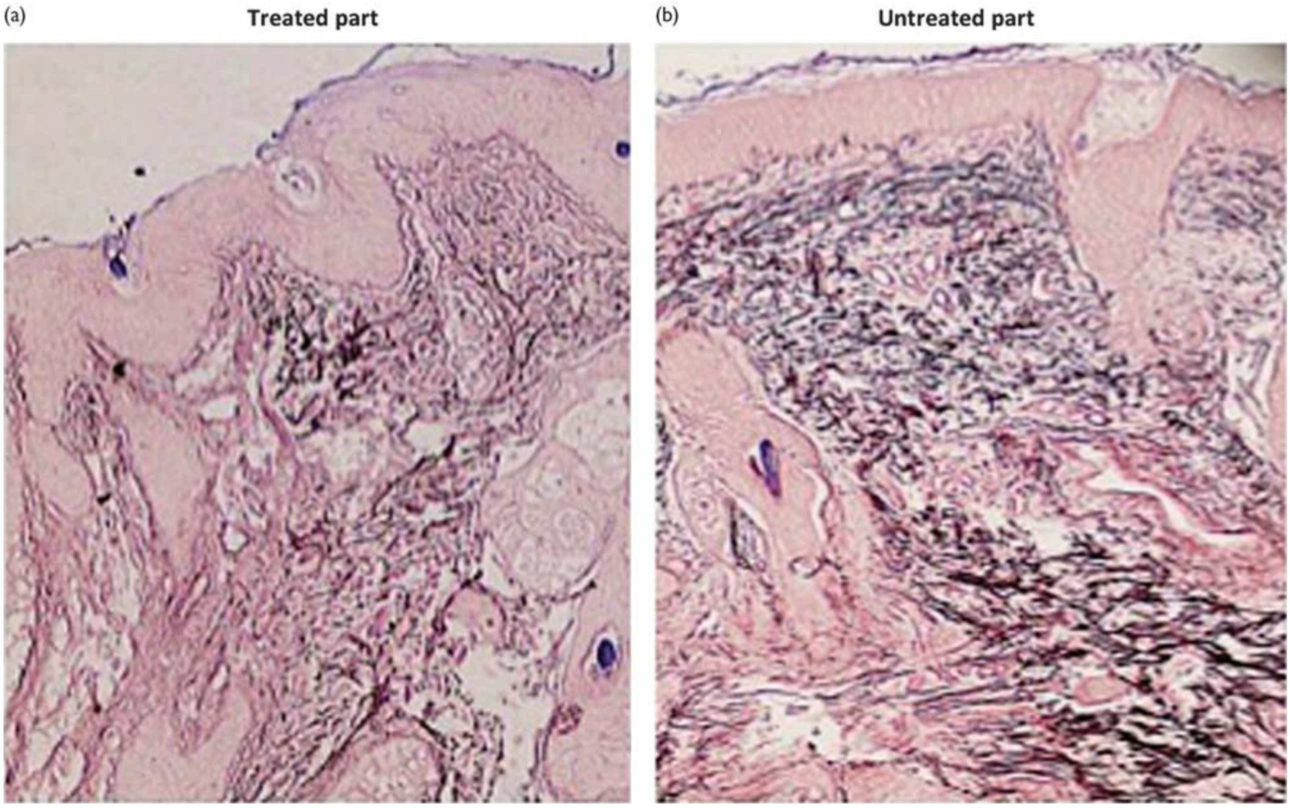
After treatment, the epidermal thickness of the treated part ranged from 65.19 to 79.99 (mean, 70.81 ± 3.32 μm), with a significant increase compared with the untreated part (range, 46.13–62.99; mean, 55.15 ± 4.76; $P < 0.001$) (Fig. 5 and Table 2).

Discussion

Skin injuries because of trauma or surgery are relatively common, and patients are very concerned about scar formation. Although normal wound healing and scar remodeling continue to occur until 2–3 years after surgery [11], many patients prefer treatment of scars as soon as possible [1], especially when they are located on visible sites [7]. Moreover, focus has recently been directed toward early laser treatment for scar prevention to yield better cosmetic results [1].

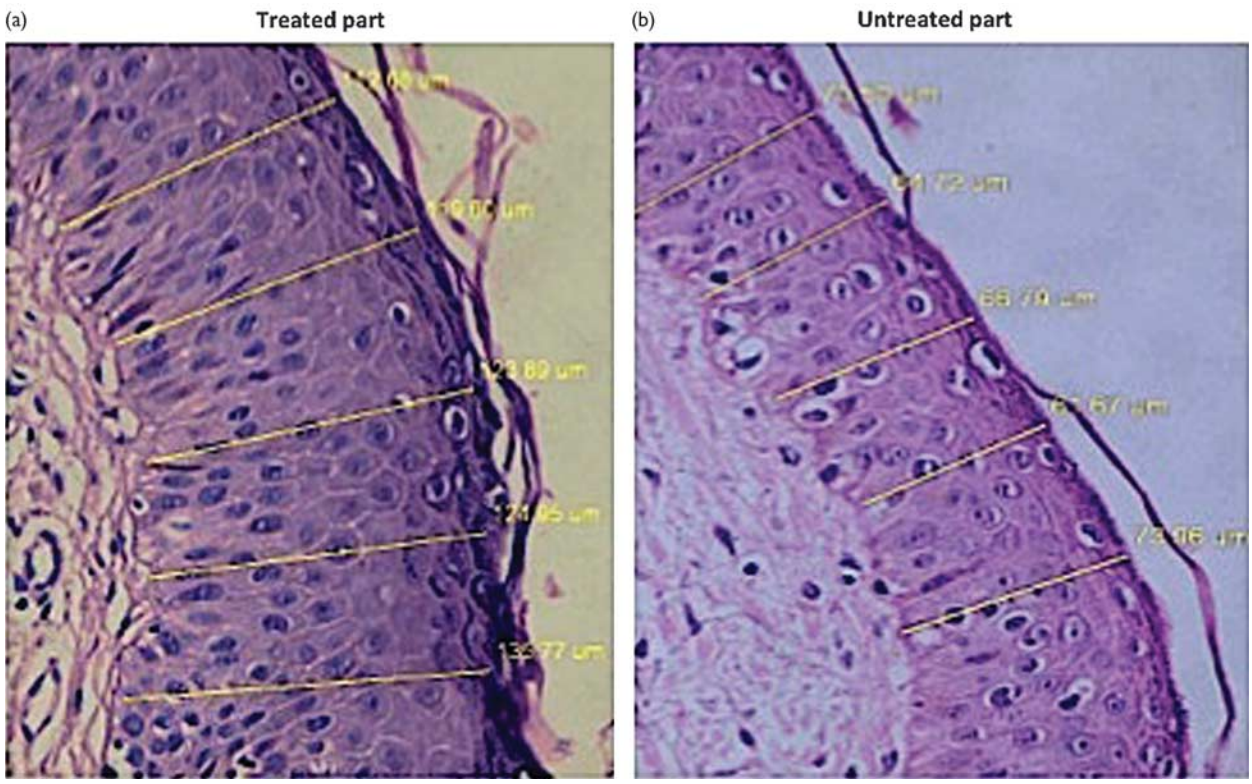
Specifically, early surgical scars have been treated successfully using a pulsed dye laser (PDL) on the day of suture removal [12], a 1550-nm fractional Erbium glass laser on new thyroidectomy scars [13], fractional ablative 2940 nm Er-YAG in combination with PDL [14], and fractional ablative 10 600 nm CO_2 [1,7,8]. Meanwhile, on review of the literature, there were no studies discussing the safety of a fractional ablative CO_2 laser and the treatment guidelines in patients with dark skin types. Moreover, there were no evidence-based publications discussing its effect at the histopathological level in terms of tissue remodeling during the early postoperative period. Accordingly, the aim of the

Figure 4.



Skin biopsy specimens after the use of a fractional CO_2 laser. The treated areas show less dense elastotic tissue with the appearance of the newly synthesized elastic fibers, which became finer and well organized (a). The untreated biopsies show dense elastotic material throughout the entire dermis (b) (Orcein, ×200).

Figure 5.



Histometry of skin biopsy specimens after the use of a fractional CO_2 laser showing a significant increase in the epidermal thickness in the treated part (a) compared with the untreated part (b) (H&E, ×400).

present study was to assess the clinical efficacy and safety of a fractional ablative CO_2 laser in the prevention of postoperative scars in dark skin types, with evaluation of its histopathological effect.

In the present study, a fractional ablative CO_2 laser was used during the early postoperative period (2–3 weeks after surgery) on one part of the wound, leaving the rest untreated, serving as a control. We chose this timing of treatment because re-epithelialization would be complete at this point and treatment initiation at this point of time was shown to yield positive results in previous reports [1,8]. Moreover, this timing occurred after the end of the inflammation phase of wound healing (4–6 days) and was either in the late proliferative phase of wound healing (4–14 days), where fibroblasts migrate into the wound site and start producing collagen [15], or in the early remodeling phase, which starts roughly at day 8 after the injury and lasts for over a year [16], and during which the extracellular matrix is dynamic, balanced between synthesis and degradation [17].

Six fractional CO_2 laser sessions, at 2-week intervals [1], were performed using a pulse energy of 30 mJ. Meanwhile, Lee *et al.* [1] performed two sessions using a fractional CO_2 laser, but with a high pulse energy of 80 mJ. Moreover, Jung *et al.* [8] performed one session of fractional CO_2 with a pulse energy of 50 mJ on patients with thyroidectomy scars. These previous two studies were carried out on Asian patients with Fitzpatrick skin types III and IV. Meanwhile, this study applied more number of sessions with low fluence on facial wounds of Egyptian patients with Fitzpatrick skin types III to V to ensure efficacy while avoiding prolonged erythema and permanent pigmentation as Egypt is characterized by a sunny climate. Moreover, the scanner size was adjusted according to the length and width of the scar, with an increase in its size about 2 mm all around the scar to stimulate collagen remodeling from healthy skin around wounds.

After 3 months of the last laser session, there was a significant improvement in the total VSS score of the treated parts in addition to significant patient satisfaction compared with the untreated ones. This is in agreement with Lee *et al.* [1] Moreover, Hong *et al.* [7] and Jung *et al.* [8] found a significant decrease in the mean total VSS after treatment with a fractional CO_2 laser compared with the baseline state only without involvement of the control untreated part.

In the subset analysis of VSS, there was a significant improvement in pliability and height scores in treated parts, with no significant difference in vascularity and pigmentation compared with the untreated parts. This is in agreement with Lee *et al.* [1]. Meanwhile, Kim *et al.* [14], who used both PDL and an Er-YAG laser, found that the best response was found in pliability. Meanwhile, Hong *et al.* [7] and Jung *et al.* [8], using fractional CO_2 in early scars, reported a total VSS score without describing its four components separately.

A fractional ablative CO_2 laser enables treatment of scars with fewer side effects and less downtime than traditional CO_2 lasers [1,6,8,18]. This was evident in the present study, where post-treatment mild pain, erythema, edema, and dark crusts were transient and resolved within 7–10 days after each session with removal of the dark crusts. The pain subsided shortly after the session in 90% of the patients and lasted several hours to 2 days in 10% of the patients.

In terms of the pigmentation abnormalities detected in treated parts of the scars in this study, hyperpigmentation and hypopigmentation were observed in two (10%) patients and one (5%) patient, respectively, after the last session and disappeared spontaneously 3 months after the last session. This is similar to Lee *et al.* [1] and Jung *et al.* [8], who observed transient post-treatment hyperpigmentation in one patient and two patients, respectively, on the treated area of the scar. Meanwhile, Chapas *et al.* [19] detected permanent delayed-onset hypopigmentation, which can markedly detract from overall clinical outcomes.

In this study, hypertrophic scars were apparent in the untreated parts only in eight (40%) patients. Meanwhile, Lee *et al.* [1] observed hypertrophic scarring in one patient on both treated and untreated parts of the scar. Therefore, it is difficult to state that the fractional laser was the sole cause of the scar elevation, although the treated side became thicker and wider than the untreated side, and thus the risk of developing hypertrophic scarring after fractional CO_2 treatment cannot be excluded. They recommended using low power (< 80 mJ).

Generally, collagen fibers form a network with elastic fibers resulting in a highly ordered structure in the dermis. Meanwhile, collagen is considered the main structural and most abundant extracellular matrix component of the dermis, and plays an essential role in the strength and elasticity of healthy skin and scar tissue [20]. In contrast, elastic fibers compose only 1–2% of the dermis and they are critical to the ability of the skin to stretch and recoil [21]. Therefore, it is important to study the remodeling of collagen and elastic fibers during wound healing after early laser therapy.

In this study, there was a significant improvement in collagen fibers, which became fine and well organized and parallel to the epidermis with decreased interfibrillary spaces in the entire dermis in the treated parts of 15 (75%) patients and the untreated parts in five (25%) patients.

The rest of the untreated parts (15 biopsies, 75%) showed fairly thickened hyaline bundles, either disorganized (seven biopsies, 35%) with increased interfibrillary spaces in the entire dermis or randomly within nodules (cigar-shaped) in the middle and deep dermis, with some parallel fibers to the epidermis outside the nodule in the superficial dermis (eight biopsies from hypertrophic scars, 40%). On review of the literature, Oliae *et al.* [22] described the scar histopathologically after 4 months of incision without treatment; the collagen fibers became thicker and denser. Moreover, the state of collagen in untreated hypertrophic scars in this study was similar to that described by Linares and Larson [23], Lee *et al.* [24], and Verhaegen *et al.* [25].

For the elastic fibers, dense elastotic material was found throughout the entire dermis in 16 (80%) untreated parts and six (30%) treated parts. Meanwhile, the treated areas of 14 (70%) patients showed a significant improvement, with less elastotic material and appearance of newly synthesized elastic fibers, which became finer and well organized.

Accordingly, it is important to differentiate between normal healing and abnormal scarring. Normal healing occurs when the newly synthesized collagen is not excessive [26] and organized in a well-organized fine network [17] as a natural process of the remodeling stage of wound healing and the skin has sufficient elasticity not to restrict movements. Accordingly, areas of normal scarring should not be distinct from the surrounding skin [26].

For the epidermis, there was a significant increase in the mean epidermal thickness of the treated parts compared with the untreated parts. Accordingly, the interaction between epidermal keratinocytes and dermal fibroblasts could play an important role in regulating tissue homeostasis and processing scar formation [27].

To the best of our knowledge, this study was the first to examine the state of remodeling of collagen and elastin and epidermal thickness following an early intervention of wound during the early postoperative period.

Some authors have hypothesized about how the laser affects the scar-remodeling phase after trauma or surgery. One hypothesis involves thermal injury to the dermis, where neocollagenesis and remodeling occur [8]. Other theories focus on the stimulation of growth factors and cytokines by lasers [28] or the initiation of collagen signaling cascades [29]. Meanwhile, earlier intervention can theoretically alter the inflammatory phase of wound healing and alter fibroblast migration, leading to a reduction in the appearance of scars [5].

Conclusion

The fractional CO_2 laser was effective, tolerable, and safe in the prevention of postoperative scars, especially hypertrophic types, in dark-skinned patients. Histologically, the collagen fibers became fine and well organized, with rectification of the elastic fiber degeneration in addition to increased epidermal thickness. Accordingly, fractional CO_2 could act through remodeling of the dermis to activate deposition of its normal architecture (collagen and elastin) in addition to increasing the epidermal thickness.

Conflicts of interest

There are no conflicts of interest.

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