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Carbon dioxide laser treatment in burn-related scarring: A prospective randomised controlled trial☆

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Summary *Aim:* To investigate the effect of ablative fractional CO_2 laser ($AFCO_2L$) on burns scar appearance and dermal architecture at 6 weeks and up to 3-years post-treatment.
Methods: Twenty adult patients with a burn-related scar were recruited. Inclusion criteria were a minimum scar area of 10×10 cm and Vancouver scar scale (VSS) score of >5 and ≥ 6 months since the time of injury. The region of scar was randomised to treatment/control zones. Treatment zones received 3 standardised laser treatments at 4- to 6-week intervals. All areas of scar received standard scar care. Outcome measures were recorded at baseline, 6-weeks post final treatment and up to 3 years post-treatment. Measures included blinded assessor VSS, Patient Scar Assessment Scale and histological tissue analysis.
Results: Nineteen and nine patients completed the short- and long-term studies, respectively. Clinical results revealed improvement in all scar areas over time. There was a statistically significant improvement in pain and itch in the treatment zone compared to the control zone

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at 6 weeks. Histological data revealed a significant increase in medium-sized collagen fibres at 6 weeks relative to the control site. Sub-group analysis according to scar age revealed greater histological improvement following laser treatment in immature scars relative to more mature scar.

Conclusions: Results demonstrate that 3 treatments of $AFCO_2L$ significantly improve scar pain, itch and dermal architecture at 6 weeks post-treatment. Histological results suggest greater potential in treating immature scar. Further investigation into the timing of laser treatment could help assist treatment protocols.

Introduction

Problematic scarring following burn injury remains a significant burden for patients and a challenge for clinicians.[1,2] Many factors increase the risk of problematic scarring, including prolonged healing, increased depth of injury, wound complications and prolonged hospital stay.[2] Strategies to reduce scar severity include accurate fluid resuscitation, timely and effective surgery and early scar management.[3,4] Standard scar management involves scar massage, hydration, silicone and pressure garments.[3-5] Pressure garments are considered standard of care in most developed countries but are time-consuming, expensive and can be socially restricting.[5,6] Despite good scar management, hypertrophic scars do occur and symptoms may include pain, allodynia, pruritus and restricted range of motion. Burn scars can cause significant functional, aesthetic and psychological problems[7,8] and can affect growth and alter appearance to the extent that patients can feel disfigured, depressed and attempt suicide.[8,9]

Reconstructive surgical options include surgical release of scar contractures and reconstruction with a cell suspension, skin graft, flap or tissue-expanded skin.[3] This surgery can be effective but is not without risk, requires further donor sites and can involve significant recovery time.[3]

Ablative fractional CO_2 laser ($AFCO_2L$) is emerging as a promising scar treatment. CO_2 laser treatment for scarring is not a new concept; however, in the past, the higher risk profile of unfractionated ablative laser delivery (permanent pigmentation changes, higher rates of infection and scarring) limited its application in the burn population.[10] Fractionated delivery of laser energy has reduced risks and recovery time by leaving undamaged columns of skin between micro-thermal treatment zone (MTZ's) resulting in rapid re-epithelialisation.[11] The mechanisms of CO_2 laser action are unclear but likely involve combinations of macroscopic ablative fenestration, microscopic thermal collagen alteration and molecular profile alterations. These alterations include changes in expression of transforming growth factor beta, matrix metalloproteinases and vascular endothelial growth factor.[12-15] Use of $AFCO_2L$ for scar management is increasing amongst burn clinicians; consensus opinion and several large series have demonstrated safe and effective results.[16-20] However, robust randomised controlled evidence for the efficacy of CO_2 laser on burns scarring is limited. As a result, a prospective randomised controlled study on the effects of $AFCO_2L$ on burn-related scarring was undertaken.

The aims were to assess the impact of $AFCO_2L$ on clinical outcomes and collagen matrix of burn scars. Primary outcome measures included Vancouver scar scale (VSS), patient scar assessment scale (POSAS), collagen orientation and collagen fibre size. In addition, patients were invited to participate in a long-term follow-up trial to investigate whether changes seen were sustained over a 2–3 year period.

Methods

Ethical approval

The study was conducted in accordance with the NHMRC statement on ethical conduct in human research (2007) and was approved by the Human Research Ethics Committees of Royal Perth Hospital (2013/135) and Monash University (2014/000989). All eligible subjects were given a patient information sheet, verbal explanation of the study and written consent obtained.

Inclusion and exclusion criteria

Inclusion criteria were

- Minimum burn injury scar area of 10×10 cm
- VSS score of >5
- $\geq$6 months following injury
- Patient age 18+ years

Exclusion criteria were

- Current pregnancy/lactation
- Patients unable to consent (any cognitive dysfunction)
- Non-English-speaking patients
- Scars on the face or hand

Study design

Twenty adult patients were recruited to the initial short-term outcomes trial. The process of randomisation was conducted by a clinician not involved with the trial using a random number generator to assign a closed envelope to each patient. The envelope contained a 10×10 cm square vector 'map', which split the scar into a control and treatment half along one of 4 vectors: vertical, horizontal, and along both diagonals. The envelope revealing the vector map and treatment zones was opened by the treating laser clinician immediately prior to the first treatment. The same clinician performed all laser treatments. Patients were offered laser

treatments to other areas of scarring not adjacent to study scar areas.

Each patient received three standardised $AFCO_2L$ treatments using the Deep FX setting hand piece (UltraPulse, Lumenis), under general anaesthetic at 4–6 week intervals. All treatments consisted of a single pass of 300 Hz, 5% density and 50 mJ energy with minimal overlapping. Postoperatively, all laser treatment and control zones had emollient and silicone dressings applied, which were removed at 48 h. Further emollient was applied twice daily for 2 weeks to all scar areas. Standard care (silicone, massage and pressure garments) directed by burn occupational therapists was continued for all scar areas. Blinded VSS and patient POSAS scar assessments were measured at baseline and 6 weeks post-final treatment.

Tissue histology

Punch biopsies (3 mm diameter) of both control and treated scar segments were taken at baseline, 48–72 h after the first treatment and 6 weeks post-final treatment. Tissue samples were fixed in 4% paraformaldehyde for 24 h at 4 °C and paraffin embedded. Three 5 μm sections were cut using a microtome and collagen stained using picrosirius red. Slides were imaged using an Olympus IX81 inverted microscope (Olympus, Tokyo, Japan) and polarising filters at the Centre for Microscopy, Characterisation and Analysis (CMCA), UWA. The use of polarised light and picrosirius red stain causes collagen fibres to appear in different colours depending on their relative thickness, with green representing the thin fibres through yellow, orange and finally red fibres that represent the thickest collagen strands. Superficial and deep dermal images were captured and analysed using ImageJ[21]. Detailed analysis methodology is included in Supplementary Methods.

Long-term follow-up

All patients who completed the initial trial were contacted regarding the long-term follow-up trial. Information regarding any further laser treatments to the control zone or adjacent areas was recorded. Clinical assessment involving photographs, blinded VSS and patient POSAS was completed as per initial trial protocols. Control and treatment zone 3 mm punch biopsies were taken, processed, imaged and analysed as per initial trial protocols. Size of effect was compared for treatment and control zones.

Statistical analysis

The Wilcoxon signed-rank test was selected for paired analysis of primary outcomes. Size of effect was compared for treatment and control zone.

Results

Nineteen of 20 recruited patients completed the initial laser trial and were included in analysis. Patients had a mean age of 29 years and a mean TBSA of 40%. Mean scar age was 84 months, and median scar age was 17 months (range 6–341). Patient demographics are presented in Table 1.

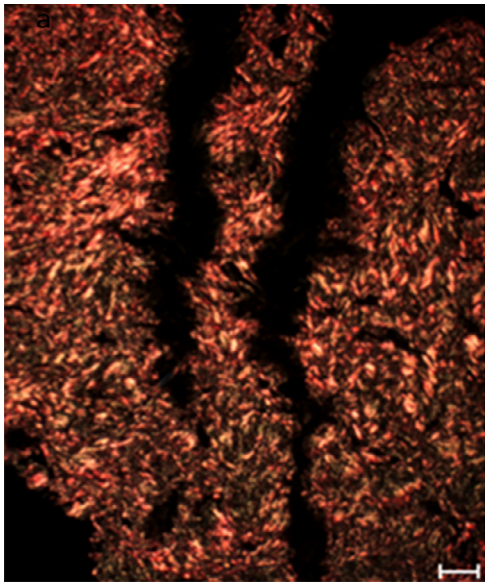

Figure 1 Microthermal zones. Microthermal zones (MTZ) observed at 48–72 h after laser treatment.

Microthermal zones (MTZ) observed at 48 h post-treatment

At 48 h post-injury, biopsies showed the presence of microthermal zones (Figure 1), observed by polarised light microscopy of picrosirius red-stained tissue sections.

Clinical changes at 6 weeks post-treatment

Clinical images of two patients who completed the initial and long-term follow-up study are shown (Figure 2). Patients D and F (Figure 2a–b and Figure 2c–d, respectively) had immature scars and the inferior right triangle of their scar treated. Both patients with immature scars have improved globally, with changes specific to the treatment area difficult to observe.

No significant difference in any domain of the VSS score was observed between treated and control tissue areas when compared to pre-treatment scores (Supplementary Figure 2a–d). Using the patient elements of the POSAS score, there was significant improvement in all domains after treatment (Supplementary Figure 2e-l). Significant improvement in scar pain ($p = 0.047$) and itch ($p < 0.01$) was noted from the patient element of the POSAS scar score only in the treated areas when compared to pre-treatment control (Supplementary Figure 2e and f).

Changes in histology at 6 weeks post-treatment

Changes in collagen were assessed by polarised light microscopy (Figure 3). There was a significant decrease in

Table 1 Patient demographics.

Patient	Age	M/F	%TBSA burn	Scar age (months)	Comorbidities	Location of trial scar area	Follow-up (months)
A	23	M	38.5	6	Nil	Left lower leg	35
B	27	M	30	6	Nil	Left thigh	36
C*	55	M	70	6	Polyneuropathy	Abdomen	33
D*	54	M	39	6	Polyneuropathy	Left lower leg	33
E	23	M	30	7	Nil	Right lower leg	32
F*	23	M	55	7	Polyneuropathy	Abdomen	33
G	33	M	12	8	Nil	Right thigh	34
H*[2]	25	M	27	8	Nil	Left upper arm	32
I	24	M	40	11	Nil	Left upper arm	34
J	18	M	30	13	Nil	Right forearm	37
K	18	F	20	15	Nil	Left upper arm	35
L	30	F	45	18	Nil	Left upper arm	36
M*[3]	21	F	31	33	Nil	Right lower leg	36
N	19	M	55	121	Nil	Left upper arm	34
O*[4]	36	M	60	140	Nil	Left upper arm	33
P*[2]	41	M	30	248	Nil	Right upper arm	31
Q	33	F	20	284	Nil	Left flank	31
R*	36	M	70	328	Nil	Left knee	34
S*[1]	18	M	47	341	Nil	Right lower leg	34

(*= patients who completed the long-term follow-up study).
([n]= patients who received additional laser treatments to the study area after the initial trial: *n* = number indicates number of treatments received).

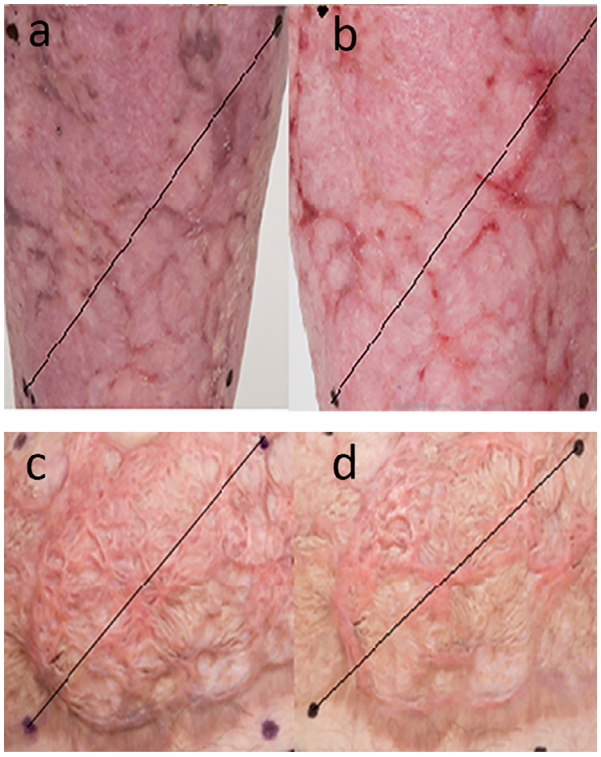

Figure 2 Changes in scar appearance at 6 weeks post-treatment. Clinical images of two immature scars before treatment (a, c) and 6 weeks post-treatment (b, d). Treatment areas are lower right diagonal areas shown in insets. Scars visibly improve.

the percentage of the biopsy area containing orange fibres in the superficial dermis in both treated and control biopsies at 6 weeks post-treatment compared to pre-treatment (Figure 3a). There was also a significant decrease in the area of yellow fibres in the superficial dermis of the treated sample only when compared to pre-treatment control (Figure 3b). No significant changes were observed in the deep dermis tissue sections (data not shown). There was no significant difference in collagen orientation measures between treated, non-treated and pre-treatment tissue sections (Supplementary Figure 3).

Changes in patients with immature or mature scars when treated

The recruited patients were divided into two subgroups (Table 1). One group was considered to have scars that would be undergoing remodelling when treated (6–18 months post-injury, (immature scars)), whilst a second group had mature scars (33–341 months post-injury). Analysis of changes in these two subgroups showed no significant difference in VSS domains (data not shown). Analysis of tissue biopsies showed significant differences in the deep dermis in the 'immature' scar group (Figure 4b–d). There was also a trend for reduced area of orange fibres in the superficial dermis of treated samples compared to pre-treatment controls (Figure 4a). No significant differences were observed in these parameters in the 'mature' treatment group. Coherency analysis showed a trend for reduced coherency in the deep dermis of treated samples in the 'immature' group (Figure 4e). No significant differences were observed in the 'mature' treatment group (Figure 4a–e).

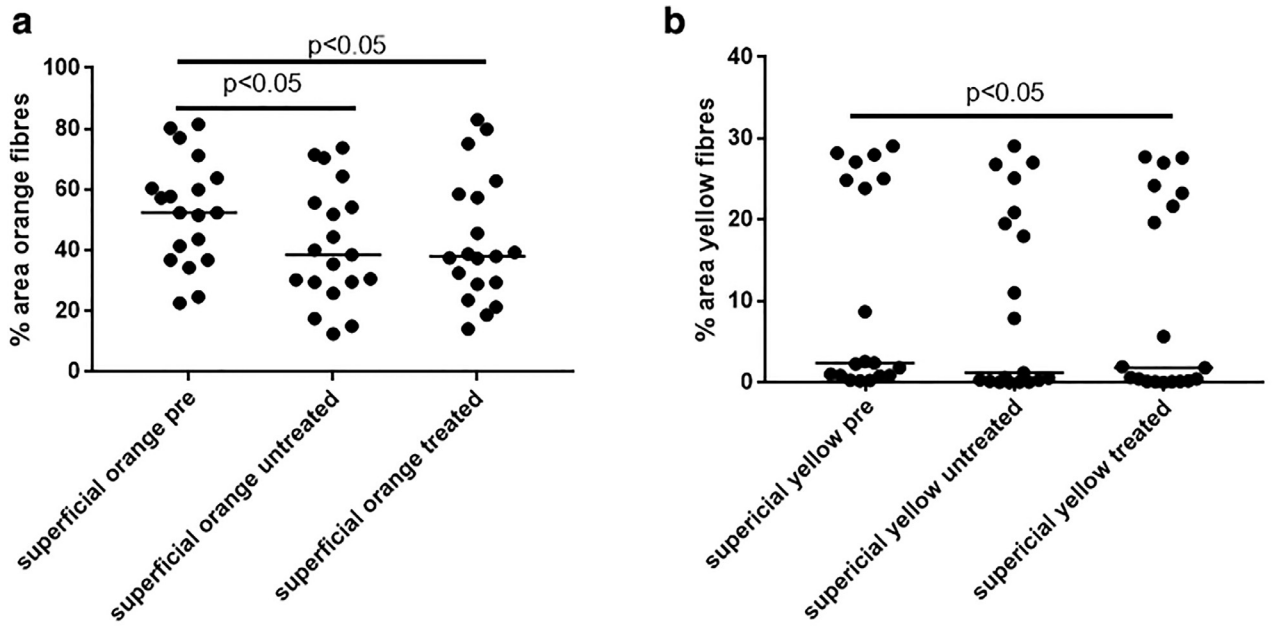

Figure 3 Changes in collagen fibre size and orientation at 6 weeks after initial treatment. A reduction in orange and yellow fibres determined by polarised light microscopy and picrosirius red staining is observed at 6 weeks post-treatment in treated zones in the superficial dermis (a, b). Polarised light and picrosirius red staining make collagen fibres appear red, orange, yellow and green, representing the thickest to thinnest fibres, respectively (detailed in supplementary methods). No significant changes in the deep dermis are observed at this time-point (data not shown).

Long-term follow-up of patients

Nine patients participated in the long-term follow-up study. These patients had an average age of 34 years, with a mean TBSA burn injury of 48%. Mean scar age was 127 months post-injury, and median scar age was 33 months (range 6–341). These patients are highlighted with an asterisk in Table 1. Five of these 9 patients had received additional laser treatments (range 1–4) to the study areas since the initial trial.

VSS scores from pre- to post-treatment showed no significant difference (data not shown). The total patient POSAS scores improved significantly from pre-treatment scores in both treatment and control segments, although no long-term differences were seen between treated and control segments (Figure 5a). No significant differences were observed in collagen by polarised light microscopy between pre-treatment and long-term samples (data not shown). However, coherency analysis showed an increase in coherency in superficial and deep dermis of treated samples at this time-point compared to pre-treatment ($p = 0.01$, $p = 0.02$, respectively, Figure 5b and c) and between treated and non-treated samples in the deep dermis ($p = 0.01$). Clinical images of two patients (D and F) with immature scars and two with mature scars (patients M and P) who completed the initial and long-term follow-up study are shown (Supplementary Figure 1). Patients D and F showed global improvement at both 6 weeks and at the long-term follow-up (Supplementary Figure 1a–c and d–f show pre-treatment, 6 week post-treatment and long-term follow-up, respectively). Patients with mature scars also showed improved scar appearance at the long-term follow-up (Supplementary Figure 1g and h show pre-treatment and Supplementary Figure 1i and j at long-term follow-up).

Discussion

This study is one of few randomised controlled clinical trials in the literature analysing efficacy of $AFCO_2L$ on burn-related scarring. It also contains long-term follow-up data of 2–3 years to assess long-term impacts.

The initial post-treatment biopsies taken at 48–72 h after the first treatment showed MTZs within the scar together with localised inflammation in the same regions. This is consistent with the early 'immediate release' described by patients undergoing $AFCO_2L$ who state that their scar feels less tight and more pliable immediately, despite no visible macroscopic change other than localised redness. This effect is often claimed by patients with mature burn scars, and perhaps these micro-damaged zones provide the initial relief that is described.

VSS and POSAS changes

The photographs of patients with immature scars clearly show a global improvement in all areas of scar at 6 weeks post-treatment, although subtleties of difference between treatment and control segments of scar are more difficult to determine.

The patient POSAS scores showed significant improvement in scar pain and itch at 6 weeks post-treatment,

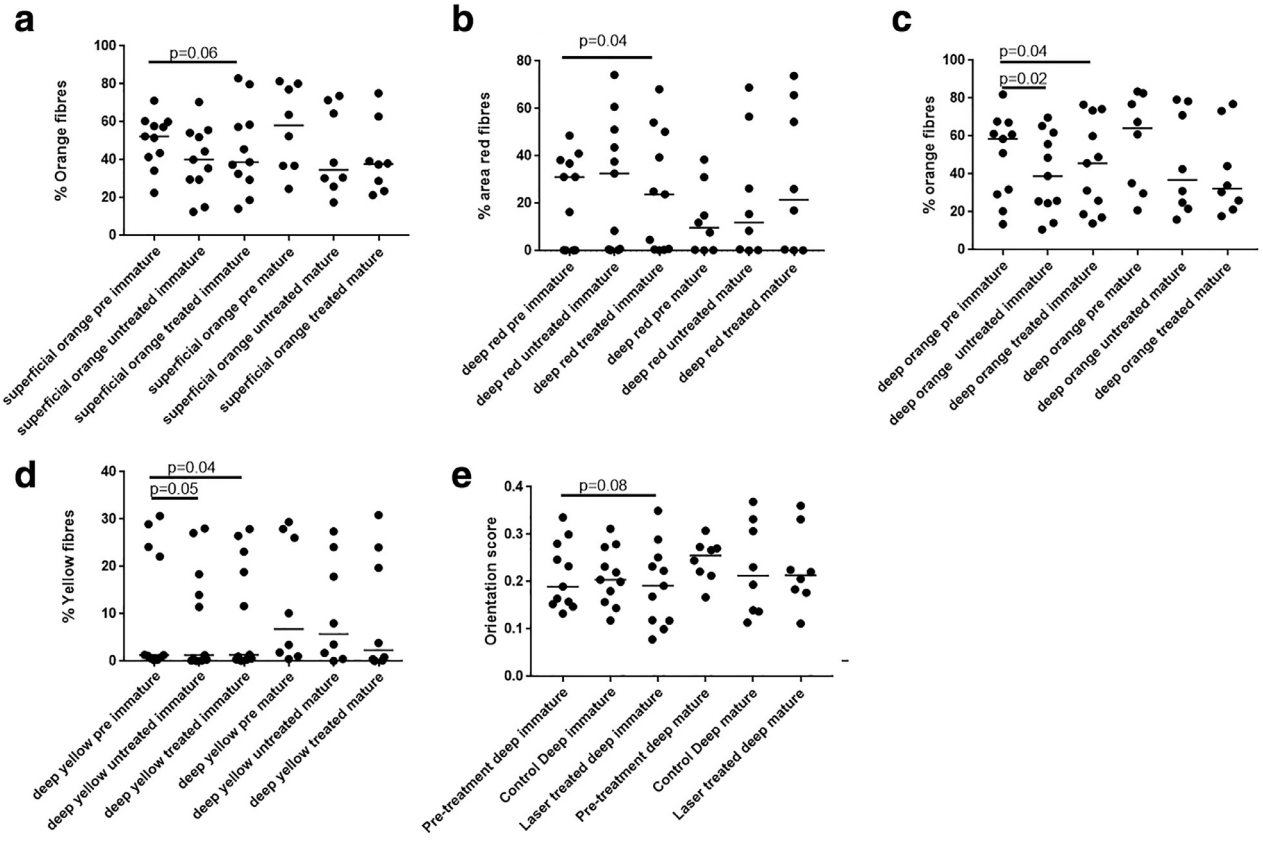

Figure 4 Changes in Immature and Mature scars at 6 weeks after initial treatment. Immature scars showed a tendency to reduced orange fibre types (a) in the superficial dermis and significantly reduced red, orange and yellow fibre types in the deep dermis (b, c, d). Polarised light and picrosirius red staining make collagen fibres appear red, orange, yellow and green, representing the thickest to thinnest fibres, respectively (detailed in supplementary methods). Coherency in the immature group appeared reduced but did not reach statistical significance ($p = 0.08$, e). No significant changes were observed in the mature scar group (data not shown).

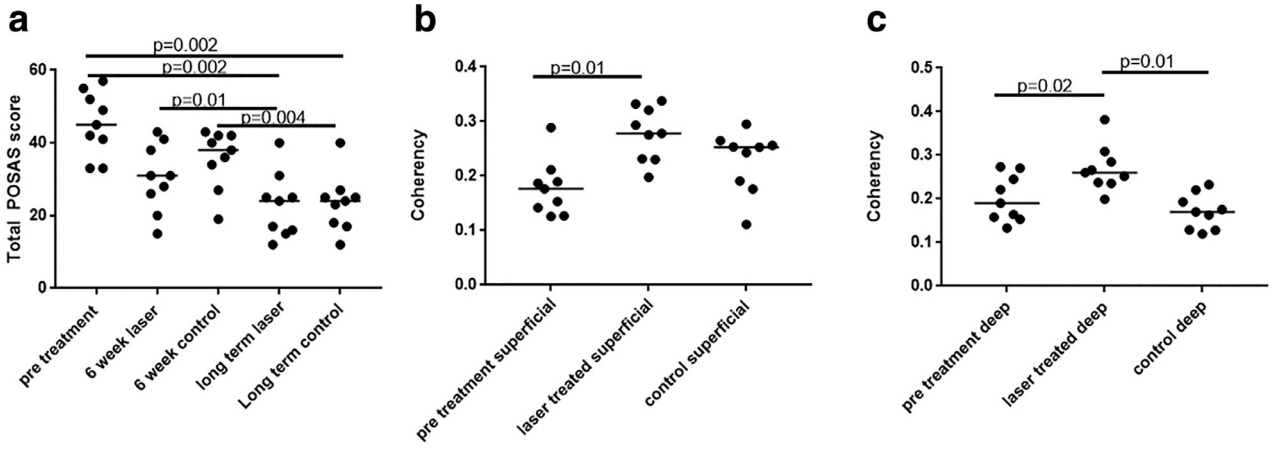

Figure 5 Changes observed in the long-term follow-up patients. Significant improvement in POSAS score was observed in all sites (a). Coherency of collagen significantly increases in laser-treated biopsies in both superficial (b) and deep dermis (c).

supporting previous findings.[19] This is interesting as previously most evidence regarding the reduction of itch in laser-treated burn scars relates to the use of pulsed dye laser or combination laser therapy.[16,22] VSS assessments did not show any significant difference between treatment and control segments compared to pre-treatment at 6 weeks or after 2–3 years. As shown in the clinical photographs, whether the laser-treated segment had improved most or not, the immature scars appear to have improved visibly in appearance globally. Although other researchers have shown significant VSS improvements in burn scars treated with AFCO^{2}L[13], in this study, the use of VSS as a measure

of outcome appears to be deficient in the light of the clinical photos of the scars showing clear improvement. Possible reasons for the difficulties using VSS include subjectivity of the tool, commented on by other researchers[19,20], and that this research spanned several years, during which time different occupational therapists assessed the scars. In view of this variability, more objective measures of scar thickness analysis, including ultrasound, 3D imaging or optical coherence tomography (OCT), are likely to be increasingly important in assessing scar outcomes in the future of scar research.[20,23]

Histology changes after treatment

A significant change in collagen matrix was observed 6 weeks after the initial treatment, with a significant decrease in orange and yellow fibre type density on imaging analysis. This was observed in superficial dermis only. Interestingly, when patients were categorised as 'immature' and 'mature' scars, only immature scars appeared to show changes at 6 weeks, with these changes reaching significance in deep dermal layers and trending towards significance in superficial layers. In contrast, no significant changes were observed in these measures in mature scars. This suggests that the impact of laser may be different when treating scars that are in the remodelling phase or those that are established. Improvements in scars treated with $AFCO_2L$ have been reported by other groups experienced with laser treatment of burn scars. Blome-Eberwein et al., in their prospective randomised study of mature, matched scars, found significant objective scar improvements after three treatments of $AFCO_2L$, and Issler-Fisher et al., in their prospective study of burns-related scarring, found scars improved significantly with $AFCO_2L$ treatment irrespective of scar age.[20,19] The patients in these two studies had scar ages of over 6 months since wound healing and between 10 and 43 months, respectively, compared to our 'immature' scar range of 6–18 months and 'mature' scar range of 121–341 months. Therefore, it is possible that changes observed in the previous studies reflect the use of less mature scars than that in the current investigation, suggesting diminished benefit from using the laser on established scars.

Long-term follow-up

The interesting observation that there is increased coherency in laser-treated tissue at this time in both superficial and deep dermis requires further investigation. It would be expected that after laser-induced damage and remodelling, the coherency, or orientation of collagen, would become more random and reflected in a lower coherency score. This would align with the increased pliability of scars reported after laser treatment. The increased score suggests the laser treatment in the long-term stimulates remodelling of collagen resulting in greater fibre alignment. However, there are limitations with regard to the use of the coherency coefficient to assess collagen alignment. Whilst the measure provides an indication of dominant fibre direction and unidirectional, parallel fibre arrangements are identified accurately, and the directional arrangement of fibres can be underestimated if there are large bundles of collagen arranged in a perpendicular fashion in the scar. Multiple sampling and assessing large regions of interest reduce this potential impact. However, further work and improved methodology of assessment are critical to understand this finding and its relationship with the long-term physical properties of the scars.

Whilst the long-term data are interesting, it is based on very limited numbers that we were able to follow through from the original patient cohort, which limits the ability to interpret the data. Whilst no significant differences were observed in collagen fibre types at this time, this may be due to the fact that only those treated with immature scars ($n = 5$) appeared to benefit significantly at the 6-week time point. Therefore, with proportionally fewer of these patients in the long-term follow-up, these differences may be masked by the presence of those patients ($n = 4$) with mature scars that were treated. Sub-group analysis in this smaller group was not possible due to the already small numbers of patients attending for long-term follow-up, and therefore, further work will be critical to understand the long-term impact of laser intervention.

Further investigation is needed to clarify the optimal timing of $AFCO_2L$ intervention. With this in mind, the Early Laser Intervention Promotes Scar Evolution (ELIPSE) study has been designed to assess early $AFCO_2L$ treatment at 12–16 weeks post-injury to bilateral non-contiguous burn scars (Australian New Zealand Trial Registry: ACTRN12616000343404p). By measuring clinical scar scores, scar thickness and histology on treatment and control scars, we aim to deduce whether early $AFCO_2L$ on these immature scars can reduce time spent in pressure garments and improve scar outcome.

Limitations

This study was designed in 2012, and since then, our knowledge of the molecular effects of CO_2 laser on scars has increased. It is possible that changes in growth factors and signalling molecules caused by the laser positively influenced the maturation of the adjacent control zone, thereby reducing our measurement of the effect. Site-matched controls in our current ELIPSE trial will eliminate this risk. However, it is also possible that these effects are systemic, mediated by inflammation and potentially influencing scar maturation at distant sites.

Only 9 patients were recruited for long-term follow-up. A selection bias may exist within this self-selected subgroup, those still attending the burns unit, with worse scars or stronger motivation for improvement. In addition, further laser treatment to sites in 5 patients is a limitation of data collected at long-term follow-up. Finally, the natural maturation of the scars over this long period and the variability of this process between patients make interpretation of the long-term data more difficult.

Conclusion

In this group of patients, $AFCO_2L$ significantly improved scar itch, pain and dermal architecture after 3 treatments. Data from initial long-term follow-up show a trend of sustained

improvement. The effects of $AFCO_2L$ were seen more significantly in immature scars than in mature scars; therefore, it is possible to intervene with $AFCO_2L$ earlier after injury may be beneficial.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.bjps.2019.01.027.

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