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Carbon dioxide laser treatment of burn-related scarring: Results of the ELIPSE (Early Laser Intervention Promotes Scar Evolution) prospective randomized controlled trial

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Summary *Aim:* To investigate the impact of ablative fractional carbon dioxide laser ($AFCO_2L$) on patient-reported outcomes measures, subjective scar appearance, dermal architecture, and gene transcription in early burn scars.
Methods: Fifteen adult patients with a burn-related scar were recruited. Inclusion criteria were two non-contiguous scar areas of 1% total body surface area, similar baseline Vancouver scar scale (VSS) score and 3 months since the time of injury. All participants acted as their own control. Scars were randomized to treatment or control. Treatment scars received three $AFCO_2L$ treatments at 6-week intervals. Outcome measures were recorded at baseline, 3, 6, and 1_2-months post-treatment. Measures included blinded VSS, Patient Observer Scar Assessment Scale (POSAS), Brisbane Burn Scar Impact Profile (BBSIP), blinded scar photo assessment, histological tissue analysis, and RNA sequencing analysis.

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Results: No significant difference was found in VSS, scar erythema, or pigmentation. Patient POSAS improved in scar thickness and texture following $AFCO_2L$. All elements of BBSIP improved in control and laser groups. $AFCO_{2L}$-treated scars were scored better than control scars by blinded raters. RNA sequencing illustrated that $AFCO_2L$ induced sustained changes in fibroblast gene expression.
Conclusions: $AFCO_2L$ treated scars had significantly altered scar thickness and texture 6 months post-laser and were rated better than controls on blinded photo analysis after 3 treatments. RNASeq results suggest laser treatment alters the transcriptome of treated fibroblasts for at least 3 months after treatment. Expansion of this research to study in more depth fibroblast changes in response to laser, as well as assessing the impact on daily activity and quality of life, will be beneficial.

Introduction

For burn survivors, the development of painful, restrictive, and disfiguring hypertrophic scarring has an incidence ranging from 30% to 70% in reviewed epidemiological data.[1] The consequences of scar formation are numerous and varied, including joint contractures, stiffness, chronic pain, itching, and anhydrosis.[2] Additionally, the emotional repercussions can be significant, with many patients reporting stigmatization and discrimination due to their burn scars.

Ablative fractional carbon dioxide laser ($AFCO_2L$) treatment is a widely accepted tool to improve burn scar quality and pliability,[2-5] but early laser treatment less than 3 months post-healing is not widely practiced. The mechanics of $AFCO_2L$ make it inherently suitable, as microscopic columns of scarred dermis are vaporized, increasing tissue pliability by creating channels through thick scar tissue. The microthermal zones (MTZs) created are between 70 and 100 micrometers in diameter, leaving intact epidermis between the channels and allowing faster healing,[6] usually within 48 h.

The reported biochemical and photomechanical processes associated with the clinical efficacy of $AFCO_2L$ include changes in the dermal framework,[7] collagen,[2,6] and elastin structure,[8] myofibroblast populations,[9] and alterations in epigenetic regulatory mechanisms.[10,11]

We have previously conducted a randomized controlled trial with $AFCO_2L$, and the results showed clinical improvement in pliability, pain, and itch.[2,7] This was evidenced by the significant improvement in collagen orientation of deep dermis in the treated tissues.[7] Subsequently, we performed another trial to investigate whether early laser intervention promotes scar evolution (ELIPSE) by assessing the impact of $AFCO_2L$ on early scar evolution, clinical outcomes, and scar biology.

Methods

A prospective, randomized, single-center, single blinded controlled trial of $AFCO_2L$ therapy application to immature burn scars was designed (ANZCTR trial registration number ACTRN12616000343404p).

Inclusion and exclusion criteria

Inclusion criteria:

- Adults over 18 years of age
- Two or more non-contiguous burn areas with similar modified Vancouver scar scores[8,12-16] (mVSS) at baseline
- Each burn area > 1% total body surface area (TBSA)
- Scars which would ordinarily be managed by pressure garments and laser treatment (those requiring operative intervention and/or taking more than two weeks to heal from point of injury)
- Scars > 3 months from burn injury

Exclusion criteria:

- Pregnant or lactating females
- Facial or keloid scars
- Inability to give informed consent
- Systemic glucocorticoid use

Study design

Fifteen adult patients were recruited six weeks following discharge. Each patient acted as their own control, with a whole scar site being randomly allocated to receive laser or control treatment. For patients with more than two eligible sites, the most closely matched pair in appearance (based on mVSS) was selected. Randomization was performed by a blinded closed envelope random number generator. Researchers and the treating team were blinded to allocation, except for the clinician performing the laser treatment and the patient.

All patients received $AFCO_2L$ treatment under general or topical local anesthetic using the Lumenis UltraPulse 10,600 nm laser (35-40 mJ; 300 Hz; 5% density; Deep FX setting). Three laser treatments were performed at the selected sites at four to six-week intervals.

Post-operatively, all laser-treated and control scars were treated with hydrocortisone acetate 10 mg/g ointment and silicone dressings, which were removed at 48 h. Further steroid ointment was applied twice daily for 2 weeks to all scar areas (both laser-treated and control). Standard care

Table 1 Study participant and scar demographics. Patient ID 15 (*italics*) was recruited but did not require the full study laser cycle of three sessions and was excluded from further analysis.

Patient	Age	M/F	FP skin type	% TBSA burn	Scar age (days)	Comorbidities	Control scar location	Laser scar location
1	31	M	2	6	91	Nil	Left shin	Right shin
2	62	M	2	18	95	Nil	Left shoulder	Left flank
3	39	M	2	5	88	Paraplegia	Right thigh	Left thigh
4	36	M	2	14.5	100	Asthma	Right forearm	Left forearm
5	43	M	2	26	119	Nil	Left foot	Right foot
6	28	F	1	18.5	88	Nil	Left hand	Right hand
7	33	F	2	6	108	Nil	Left shin	Right shin
8	20	M	2	5	108	Depression	Right forearm	Left forearm
9	34	F	2	15	129	Depression	Left shin	Right shin
10	70	M	2	23	86	Hypertension	Left shin	Right shin
11	39	M	4	22	99	Nil	Left calf	Right calf
12	61	M	2	8.5	98	Nil	Left shin	Right shin
13	27	M	2	27	91	Nil	Left calf	Right calf
14	49	M	2	9	108	Depression	Left thigh	Right thigh
*15**	*55*	*M*	*2*	*18*	*109*	*Nil*	*Right thigh*	*Left thigh*

(silicone, massage, and pressure garments) directed by burn occupational therapists was continued for all scar areas. During the trial period, no intralesional corticosteroid injections were performed on any scars in the study participants.

Clinical outcome measures

Clinical scar assessment was performed by an investigator blinded prior to treatment commencement and subsequently at 3, 6, and 12-months following treatment. The following blinded clinical outcome measures were taken:

- mVSS[8,12–16]
- Dermalab Combo® (scar erythema and melanin content)[17,18]
- Patient and Observer Scar Assessment Scale (POSAS)[19]
- Brisbane Burn Scar Impact Profile (BBSIP)[20–23]

Photographic scar quality assessment

Clinical photographs[12,19] were taken pre-treatment and at 3 months following completion of 3 laser treatments. Ten assessors (Burns Consultants, Fellow, and senior Plastic Surgery Registrars) blinded to treatment group were presented with 13 paired participant photographs of the same scar pre-treatment and 3 months following intervention (control or $AFCO_2L$ treated) and asked to rate the amount of scar improvement on a scale of 1–10, with a score of 1 suggesting minimal improvement and 10 suggesting excellent improvement (Supplementary Figure 1).

Histological scar assessment

Punch biopsies (3-mm diameter) of control and $AFCO_2L$-treated scars were taken 6 and 12 months following completion of treatment. Tissue samples were fixed in 4% paraformaldehyde and paraffin embedded. Tissue sections were cut and stained with haemotoxylin & eosin or Masson's trichrome to highlight αSMA and collagen fibers. The following parameters were assessed in all biopsy specimens using ImageScope software:

- Scar thickness
- αSMA positive cells/μm^2
- Collagen/μm^2

Further details on fibroblast culture, RNA extraction, and RNA sequencing analysis are available in the Supplementary Methods.

Statistical analysis

All scar histology measurements were performed in triplicate. Multiple paired *t*-tests with post-hoc Bonferroni correction were conducted. For the photographic scar improvement assessment, non-parametric analysis using the Wilcoxon signed-rank test was used. For these measures, alpha levels of $p \leq 0.05$ were considered significant.

Results

Fifteen patients were recruited (Table 1). Of these, fourteen participants completed three sessions of laser treatment and were included in subsequent analysis; one patient was deemed to only require one laser treatment and was excluded. The remaining patients had a mean age of 41 years and scars covering a mean TBSA of 15%. Mean scar age was 100 days and median scar age was 98 days (range 86–129) at the start of treatment.

$AFCO_2L$ impact on clinical outcome measures

The mVSS scores did not show any significant difference between treated and control scars (Supplemental Figure 2**A-D**). Dermalab Combo® found no significant difference for either erythema or pigmentation between scar groups (Supplemental Figure 2**E-F**).

Using the patient POSAS, there was no significant change in overall scar opinion score following treatment. However, further analysis illustrated a significant improvement in

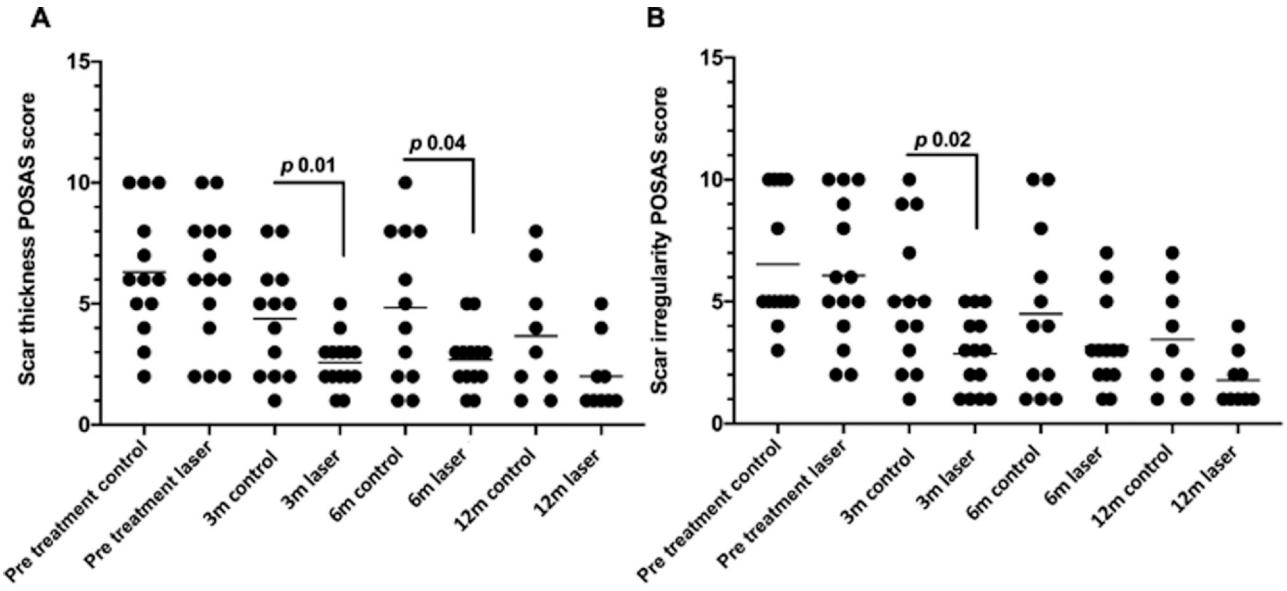

Figure 1 POSAS score for scar thickness and irregularity. (**A**) Significant improvement seen in the POSAS score for scar thickness at 3 and 6 months. There was a continuing improvement trend in scar thickness at 12 months, but this failed to reach significance. (**B**) Scar irregularity significantly improved 3 months following laser treatment versus control, with an improvement trend observed at 6 and 12 months, which failed to reach significance.

patient POSAS for scar thickness at 3 (p 0.01) and 6 months (p 0.04). We observed a continuing improvement trend in scar thickness at 12 months, but this failed to reach significance (Figure 1**A**). Scar irregularity significantly improved (p 0.02) 3 months following $AFCO_2L$ treatment (Figure 1**B**), with a continuing non-significant trend seen at 6 and 12 months. All other facets of patient POSAS showed no significant difference. POSAS did not distinguish any difference in overall score or in the individual elements.

The BBSIP illustrated significant improvement in the overall score and all individual tool facets (e.g., sensation, itch, appearance, mobility – Supplemental Figures 3 and 4).

$AFCO_2L$ impact on subjective scar appearance

The overall Wilcoxon signed-rank non-parametric test demonstrated that the scars for the areas treated by laser improved significantly more than controls ($p = 0.002$). For six patients, their laser-treated scars significantly improved more than their control scars ($p < 0.05$) (Figures 2, 3**A**-**D**). For one patient, their laser-treated scar was rated significantly (p 0.007) worse than their control scar following treatment (Supplemental Figure 5) and was subjectively noted to have hyperpigmentation and erythema both before and after control / laser treatment. For the remaining six patients, $AFCO_2L$-treated scars demonstrated no significant change compared with controls. There was no correlation between scar improvement following laser treatment in Fitzpatrick skin type.

Changes in scar histology following $AFCO_2L$

Nine out of 14 patients consented for scar biopsy and were included in histological analysis. Scar thickness was significantly (p 0.01) increased following $AFCO_2L$ at 6 months (1964 μm vs. 2157 μm), but there was no difference at 12

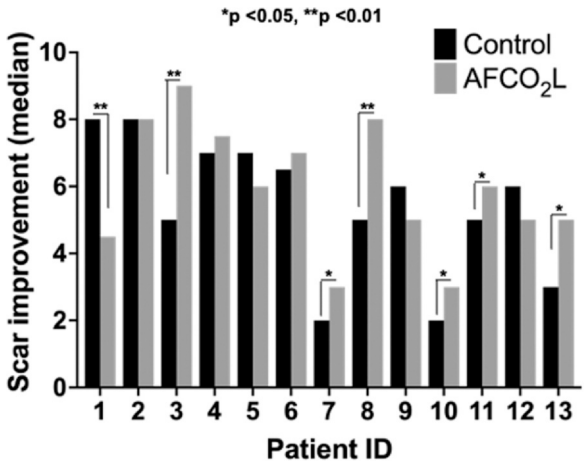

Figure 2 $AFCO_2L$ impact on subjective scar appearance. $AFCO_2L$-treated scars improved more than control scars in six participants ($p < 0.05$) and were rated significantly ($p < 0.001$) worse following laser treatment in one participant. For the remaining six, $AFCO_2L$-treated scars demonstrated no improvement.

months (Figure 4**A**). We found no difference in αSMA-positive cell density or collagen density between treatment groups at 6 or 12 months (Figure 4**B**-**C**).

Alterations in fibroblast gene transcription following $AFCO_2L$

RNA sequencing of fibroblasts cultured from 3-mm skin biopsies taken 1 month after treatment from control ($n = 2$) and treated sites ($n = 2$), as well as samples taken from control ($n = 1$) and treated ($n = 1$) sites at 3 months after treatment, showed significant differences in the transcriptome between

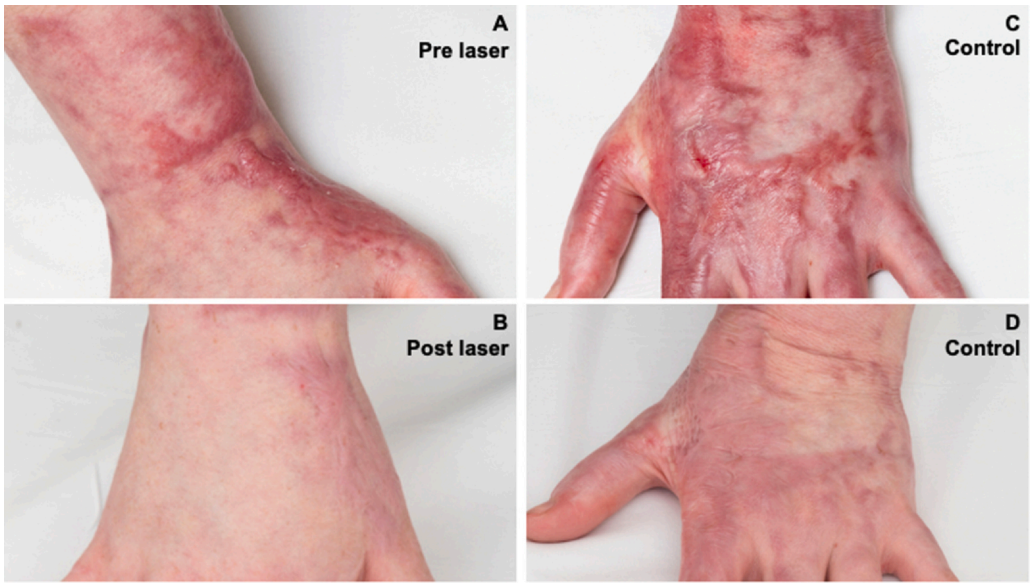

Figure 3 Changes in scar appearance following $AFCO_2L$ treatment versus controls. (**A, C**) Study scar appearances in same patient (right & left hand) prior to commencing study intervention. (**B, D**) Burn scars 3 months following (**B**) three sessions of $AFCO_2L$ or (**D**) control treatment, illustrating significantly improved scar texture, height, and appearance following laser intervention. All images seen were used for subjective scar assessment.

matched control and laser-treated fibroblasts, suggesting $AFCO_2L$ may induce sustained changes in the fibroblast transcriptome (Figure 5**A-D,** Supplemental Data 1). Pathway analysis showed that the pathways most affected included those involved in extracellular matrix/structure organization, connective tissue development (including genes *COL21A1*, *COMP*), and collagen metabolism (including *MMP* and *SERPINB7* genes).

Discussion

This study follows up and complements our previous early laser research, which reported significant improvements in pain and itch on the POSAS. The ELIPSE results revealed improvement in patient-reported scar thickness and irregularity.[7] Factoring in the other elements of the POSAS, however, did not illustrate a significant change overall. Similarly, as reported in our previous work, the VSS was unable to detect any improvement in scar outcome over the study period.[7]

Our results suggest that the POSAS identified early changes in scar thickness and texture following laser treatment, with significant early differences seen at 3 and 6 months, when scars are evolving. These subjective changes in scar appearance appear to then be lost as time-related scar progression catches up with laser-mediated changes in scar cosmesis.

It is possible that the early changes we observed in the POSAS reflect long-term alterations in scar architecture, although our histological analysis did not detect sustained differences in the limited parameters tested. Nevertheless, scar architecture is complex and involves collagen alignment, orientation, density, and other factors including collagen cross-linking that were not measured here. Therefore, we cannot determine from this data if there are sustained changes in the architecture attributable to laser treatment. More comprehensive histological analysis is a future avenue to explore to identify these changes long term.

Subjective scar assessment changes

In our study, patients served as their own control, which allowed identification of similar scars in terms of volume, location, baseline score, and previous treatment. For those patients with large TBSA burn injuries and scars, not all their scar volume was treated during the study. This may have contributed to the lack of significance seen in the VSS and some elements of POSAS, as there is inherent difficulty in commenting on improvements in the study-treated scars in a background of widespread scar burden. However, this method of self-controlled design is widely recommended for scar intervention trials, as it helps to reduce some of the many variables and heterogeneity that affect scar morphology.[24]

Although generally considered reliable and valid,[14,25] the POSAS has recently been updated to improve its global application and validity.[26,27] It should be acknowledged that in this study we applied the POSAS to early scars, and in this role, it did identify some changes in the earlier time assessment points. Whether it was less sensitive at detecting changes as scars mature is possible and is something that we are currently reviewing in a large data analysis of our laser-treated patients. Version 3 of the POSAS may be more sensitive in identifying small changes.

Similarly, the BBSIP focuses on assessing health-related quality of life in people with burn scars[20-23] and is non-discriminatory, focusing on global scar burden. Identifying the improvement in mobility, itch, and other daily activities was difficult for our patient cohort as only a percentage of their total scars had been treated; although significant improvements were

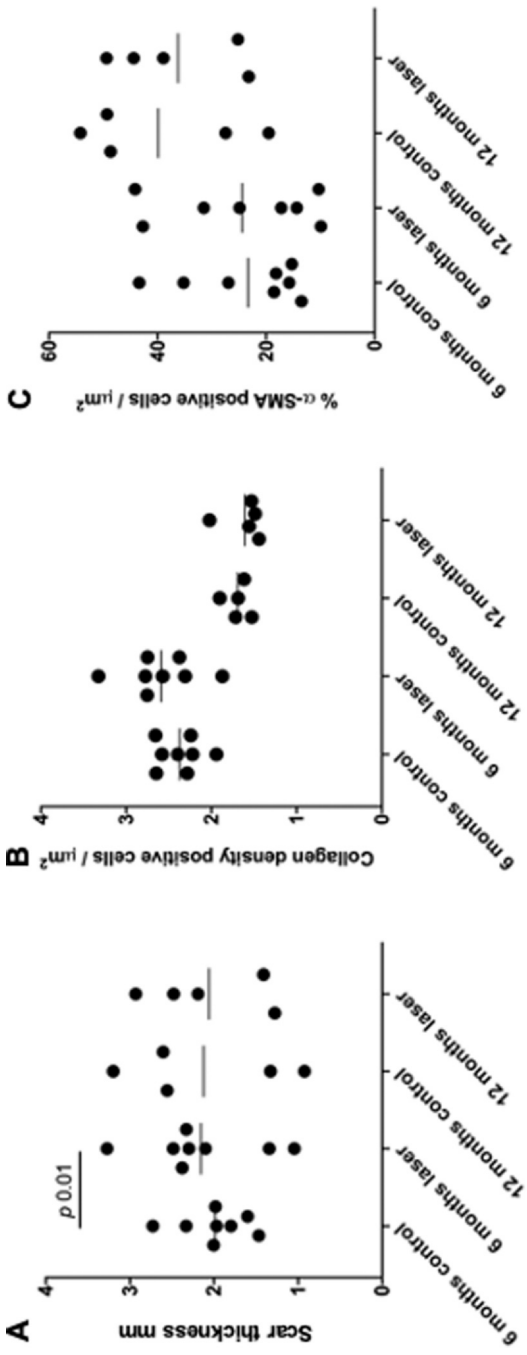

Figure 4 Changes in scar histology following $AFCO_2L$ treatment. (**A**) Scar thickness was significantly (p 0.01) increased following laser treatment at 6 months (1964 μm vs. 2157 μm). There was no difference in (**B**) collagen density or (**C**) αSMA-positive cell density between treatment groups at 6 or 12 months.

seen, it is not clear if this was purely due to laser treatment, time-related scar evolution, or most likely, a combination of both in a large scar.

This difficulty in separating time from treatment is an inherent problem with scar assessment. However, we believe that the presence of a matched control in our study increases the validity of observations made, both at a clinical and histological level. Scar assessment tools such as the VSS, POSAS, and BBSIP may not be sensitive enough to detect improvements in scarring at these early time points in scar evolution, illustrating that despite attempts to match scars as closely as possible, they are inherently heterogenous in the same patient. However, we identified changes that were evident at a cellular level, and scars were visually improved when rated by blinded clinicians.

Blinded scar assessment changes

Blinded scar assessment demonstrated an overall significant improvement in the $AFCO_2L$-treated scars compared with control scars 3 months following treatment. Only one patient was scored to have a worse scar appearance following laser treatment compared to their control. It is encouraging that analysis by experienced burn clinicians blinded to treatment group demonstrated a significant improvement in scar appearance following $AFCO_2L$.

The challenge of longitudinal assessment of all scar treatments is that scars will improve over time anyway. We have shown that early laser treatment significantly improves subjective scar appearance as early as 3 months post-treatment in comparison to standard scar care alone (Figure 3**A**-**D**). By introducing laser treatment early into our rehabilitation pathway, the scar evolution process can be modulated to significantly impact aesthetic outcome as well as improve patient-reported outcomes.

Notably, those participants whose scars rated no improvement after laser treatment had mostly flat scars that appeared to be hyperpigmented (Supplementary Figure 5**A**-**D**), which may account for their perceived lack of improvement as $AFCO_2L$ does little to address scar pigmentation. Post-inflammatory scar pigmentation can be difficult to treat and is multifactorial, based on individual genetic susceptibility and sun exposure.[28] Addressing the texture and redness can make pigmentation more obvious to the observer, which may account for our observations. We found no significant association between blinded scar assessment improvement and Fitzpatrick skin type, although we acknowledge that our study was not designed to investigate this link. This would be an interesting avenue to explore in future research.

Changes in scar histology and gene transcription

$AFCO_2L$-treated samples demonstrated no difference in melanin content or vascularity, which is to be expected as neither of these facets is directly targeted by $AFCO_2L$. However, it is surprising that erythema did not change in either group, as vascularity of time-mediated changes in scar maturation would typically be expected to improve irrespective of whether laser was administered. The relatively short 12-month follow-up of

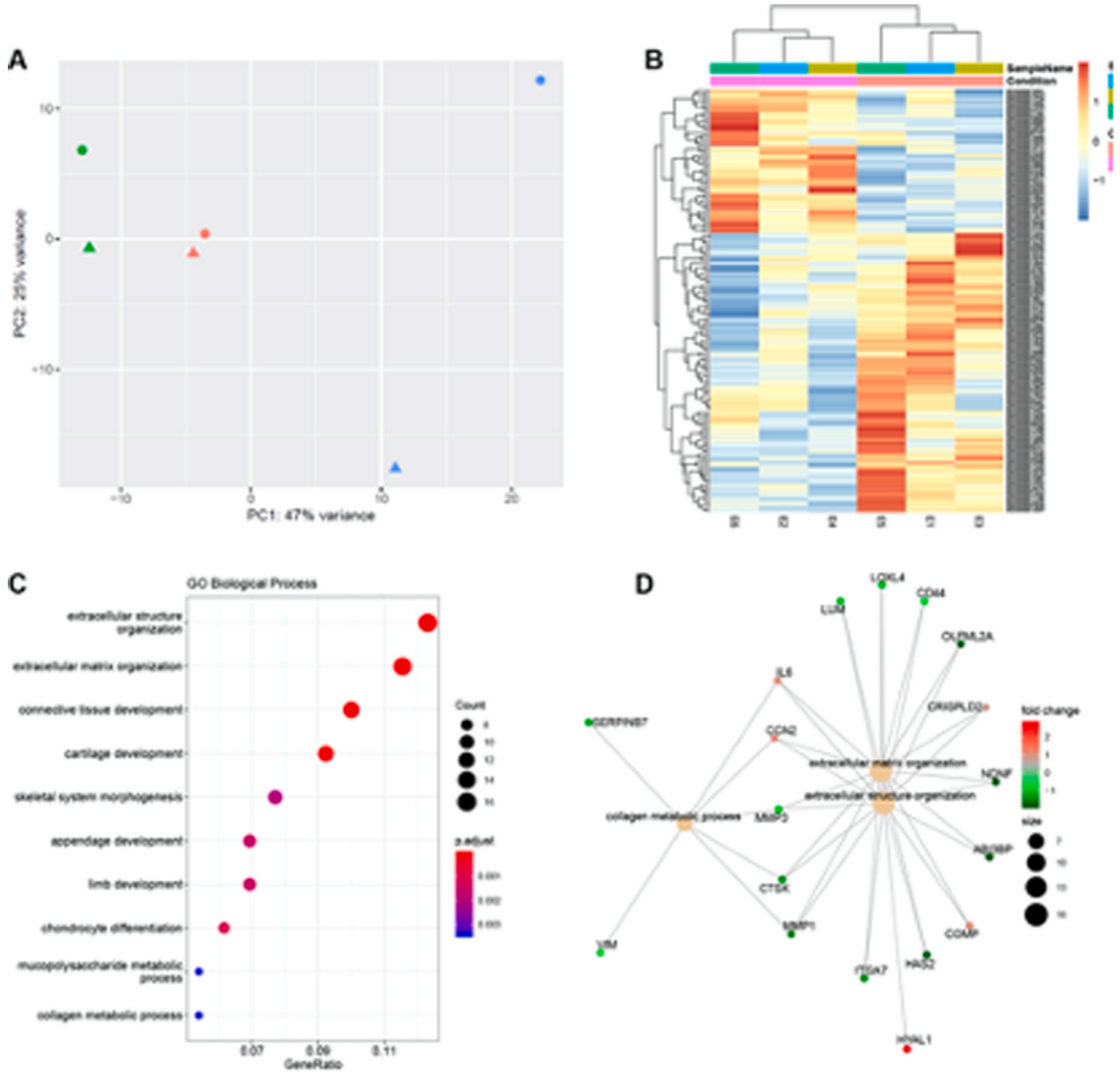

Figure 5 Differentially expressed genes identified in laser-treated fibroblasts compared with untreated scar fibroblasts. (**A**) Principal component analysis of the 1-month untreated (n = 2), 1-month post-treatment (n = 2), 3-month post-treatment (n = 1), and control (n = 1) fibroblasts (triangle is treated, circle untreated, blue 3-month samples). (**B**) Heatmap showing clustering of 150 significantly differentially expressed genes (DEGs) in treated fibroblasts compared with untreated fibroblasts. (**C**) Gene Ontology (CO) analysis identified over-represented GO terms for the DEGs in treated fibroblasts. The top 10 significantly enriched GO terms in the biological pathway are shown and include extracellular matrix and structure organization. (**D**) Concept network plot representing the expression of genes from functional categories associated with extracellular matrix. The selected genes were identified based on GO analysis.

participants may in part contribute to the lack of difference seen.

Our biopsy samples demonstrated a transient increase in scar thickness, but no change in αSMA or collagen density in laser-treated tissue. Our previous results[7] demonstrated a significant decrease in thick collagen fibers and significant increase in finer collagen fiber density after 6 weeks in laser-treated scars compared to pre-treatment in the deep and superficial dermis, respectively.

Other studies[8] have demonstrated a statistically significant decrease in type I collagen and a statistically significant increase in type III collagen in post-AFCO$_2$L specimens and an improvement in collagen structure toward a normal dermal framework. The increased scar thickness observed between control and laser-treated scars may well be post-inflammatory laser changes observed at 6 months, which plateaus within two months of treatment. Scars are highly heterogenous in their thickness and texture

and a small sample was taken from a large scar for analysis, which may not be representative. This could impact the observations in this study, especially with the relatively small sample size. However, changes in epidermal and dermal thickness following fractional treatment of scars have been described previously[11] within a similar timeframe. Objective scar thickness and volume assessment, for example using 3D camera, may be incorporated in our next study to investigate this further.

The transient increase in histological scar thickness contrasts with the POSAS results, which identified a significant improvement. The subjective nature of the POSAS is difficult to correlate with the objective scar architecture. $AFCO_2L$ is multifaceted in its ability to improve scar appearance, texture, and pliability, and subjective identification of one facet in the scale may be difficult for participants. Certainly, the newly released POSAS version 3 does not specifically ask about thickness. The newer iteration of this PROM will prove valuable in furthering subjective scar interpretation.

RNASeq analysis of the transcriptome of fibroblasts isolated before and after $AFCO_2L$ treatment demonstrated a significant alteration in gene transcription for pathways involved in extracellular matrix/structure organization, connective tissue development, and collagen metabolism. Interestingly, many of the genes involved in these pathways were downregulated after $AFCO_2L$ treatment, suggesting sustained changes in scar appearance may be due in part to similarly sustained changes in scar fibroblast gene expression. This suggests that laser ablation and perforation of a scar induces remodeling of the tissue matrix, and this likely leads to changes in scar tension, both are which are directly related to fibroblast function.[29] Therefore, it is possible that an indirect effect of the laser-reducing mechanical tension in the matrix is a change in fibroblast transcriptome and therefore a phenotype that further contributes to the scar changes observed. In light of the small numbers in this study, this interesting finding warrants further and more in-depth investigation.

$AFCO_2L$[19] has been shown to significantly reduce TGF-ß2/3 and bFGF expression in skin, while MMP-1 expression increases. This reiterates the potential for indirect effects of $AFCO_2L$ on cell activity in the scar.[12] $AFCO_2L$-induced inflammation stimulates MMPs and heat shock proteins to produce and reshape collagen,[8] improving scar architecture by decreasing the collagen type I to III ratio.[8] $AFCO_2L$ causes widespread changes to numerous inflammatory molecules, upregulating MMP-1 and downregulating FGF, TGF-b, and VEGF levels.[8,19] Together these changes alter the biological and physical properties of the extracellular matrix, and it appears from this study that this sustainably alters fibroblast phenotype.

Limitations

We acknowledge the small cohort size may have directly affected the significance of our results, particularly those obtained with the subjective scar assessment tools. The low numbers reflect the strict criteria adhered to by the research team to ensure the presence of two homogenous scars in patients that met the remaining inclusion criteria, which greatly restricted the number of eligible patients. A selection bias may exist with the selection of participants with a stronger motivation for improvement. Similarly, few samples were collected for transcriptome analysis, and the heterogeneity of scar tissue may have affected these results as well as the histological findings, particularly with limited sampling numbers. Further validation cohorts will be required to investigate in particular whether the transcriptome changes are observed after laser treatment. However, our participant and study numbers are in keeping with previous literature focused on laser impact on scars.[7,8,19]

ELIPSE specifically set out to ascertain the impact of early laser treatment on burn scars; hence, laser fluence was lower than that previously used in our previous research.[7] This was because high $AFCO_2L$ fluence is reserved for those scars that are thick and hypertrophic, and the most commonly encountered scars form after a burn injury. As ELIPSE focused on intervention in the evolution of scars, indeed aiming for prevention of the increased thickening of burn scars that is commonly seen between 3- and 6-months post-injury, the energy delivered to the scar was proportionate to the degree of hypertrophy based on our experience.

The addition of topical corticosteroid to the laser fenestrations to access the deeper scar tissue at the conclusion of $AFCO_2L$ administration is widely practiced as laser-assisted drug delivery. We acknowledge the potential impact of topical steroid application to the study scar, and for this reason, the control scar was treated with the same corticosteroid regime to negate any undue influence. Whether it was better absorbed by the scars that had been treated by laser, as they had microperforations, is unknown. However, these channels close rapidly following $AFCO_2L$ treatment, within 24-48 h.[30]

Conclusion

Early $AFCO_2L$ administration modulates burn scar evolution and impacts patient-reported outcomes, scar appearance, and fibroblast activity. Patients rated significant improvements in their POSAS score for scar thickening and texture following early $AFCO_2L$ treatment, and photographs demonstrated significant improvement in the appearance of laser-treated scars compared to controls at 3 months following treatment. $AFCO_2L$ treatment caused sustained changes in fibroblast gene transcription and appearance compared with standard care. Expansion of this research to increase participant numbers, assess scar volume, and assess impact on daily activity and quality of life measures following laser treatment will be beneficial.

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 Fiona Stanley Hospital (2013/135) and Monash University (2014/000989).

Funding

None.

Conflicts of interest

None.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bjps.2023.06.012.

References

1. Wallace HJ, Fear MW, Crowe MM, Martin LJ, Wood FM. Identification of factors predicting scar outcome after burn in adults: a prospective case-control study. *Burns* 2017;**43**:1271–83.
2. Zhang C, Yin K, Shen YM. Efficacy of fractional carbon dioxide laser therapy for burn scars: a meta-analysis. *J Dermatol Treat* 2021;**32**(7):845–50.
3. Issler-Fisher AC, Fisher OM, Clayton NA, et al. Ablative fractional resurfacing for burn scar management affects the number and type of elective surgical reconstructive procedures, hospital admission patterns as well as length of stay. *Burns* 2020;**46**:65–74.
4. Issler-Fisher AC, Fisher OM, Haertsch P, Li Z, Maitz PKM. Ablative fractional resurfacing with laser-facilitated steroid delivery for burn scar management: does the depth of laser penetration matter? *Lasers Surg Med* 2020;**52**:149–58.
5. Patel SP, Nguyen HV, Mannschreck D, et al. Fractional CO_2 laser treatment outcomes for pediatric hypertrophic burn scars. *J Burn Care Res* 2019;**40**:386–91.
6. Hultman CS, Edkins RE, Lee CN, Calvert CT, Cairns BA. Shine on: review of laser- and light-based therapies for the treatment of burn scars. *Dermatol Res Pract* 2012;**2012**:243651.
7. Douglas H, Lynch J, Harms KA, et al. Carbon dioxide laser treatment in burn-related scarring: a prospective randomised controlled trial. *J Plast Reconstr Aesthet Surg* 2019;**72**:863–70.
8. Ozog DM, Liu A, Chaffins ML, et al. Evaluation of clinical results, histological architecture, and collagen expression following treatment of mature burn scars with a fractional carbon dioxide laser. *JAMA Dermatol* 2013;**149**:50–7.
9. Borges J, Araujo L, Cuzzi T, et al. Fractional laser resurfacing treats photoaging by promoting neocollegenesis and cutaneous edema. *J Clin Aesthet Dermatol* 2020;**13**:22–7.
10. Jiang X, Ge H, Zhou C, Chai X, Deng H. The role of transforming growth factor beta1 in fractional laser resurfacing with a carbon dioxide laser. *Lasers Med Sci* 2014;**29**:681–7.
11. Makboul M, Makboul R, Abdelhafez AH, Hassan SS, Youssif SM. Evaluation of the effect of fractional CO_2 laser on histopathological picture and TGF-beta1 expression in hypertrophic scar. *J Cosmet Dermatol* 2014;**13**:169–79.
12. Bowen AC, Burns K, Tong SY, et al. Standardising and assessing digital images for use in clinical trials: a practical, reproducible method that blinds the assessor to treatment allocation. *PLoS One* 2014;**9**:e110395.
13. Thompson CM, Sood RF, Honari S, Carrougher GJ, Gibran NS. What score on the Vancouver Scar Scale constitutes a hypertrophic scar? Results from a survey of North American burn-care providers. *Burns* 2015;**41**:1442–8.
14. van der Wal MB, Tuinebreijer WE, Bloemen MC, et al. Rasch analysis of the Patient and Observer Scar Assessment Scale (POSAS) in burn scars. *Qual Life Res* 2012;**21**:13–23.
15. Vercelli S, Ferriero G, Sartorio F, Cisari C, Bravini E. Clinimetric properties and clinical utility in rehabilitation of postsurgical scar rating scales: a systematic review. *Int J Rehabil Res* 2015;**38**:279–86.
16. Brusselaers N, Pirayesh A, Hoeksema H, et al. Burn scar assessment: a systematic review of different scar scales. *J Surg Res* 2010;**164**:e115–23.
17. Gankande TU, Duke JM, Danielsen PL, et al. Reliability of scar assessments performed with an integrated skin testing device - the DermaLab Combo((R)). *Burns* 2014;**40**:1521–9.
18. Gankande TU, Duke JM, Wood FM, Wallace HJ. Interpretation of the DermaLab Combo(R) pigmentation and vascularity measurements in burn scar assessment: an exploratory analysis. *Burns* 2015;**41**:1176–85.
19. Qu L, Liu A, Zhou L, et al. Clinical and molecular effects on mature burn scars after treatment with a fractional CO(2) laser. *Lasers Surg Med* 2012;**44**:517–24.
20. Simons M, Kimble R, McPhail S, Tyack Z. The Brisbane Burn Scar Impact Profile (child and young person version) for measuring health-related quality of life in children with burn scars: a longitudinal cohort study of reliability, validity and responsiveness. *Burns* 2019;**45**:1537–52.
21. Simons M, Kimble R, McPhail S, Tyack Z. The longitudinal validity, reproducibility and responsiveness of the Brisbane Burn Scar Impact Profile (caregiver report for young children version) for measuring health-related quality of life in children with burn scars. *Burns* 2019;**45**:1792–809.
22. Tyack Z, Kimble R, McPhail S, Plaza A, Simons M. Psychometric properties of the Brisbane Burn Scar Impact Profile in adults with burn scars. *PLoS One* 2017;**12**:e0184452.
23. Tyack Z, Ziviani J, Kimble R, et al. Measuring the impact of burn scarring on health-related quality of life: development and preliminary content validation of the Brisbane Burn Scar Impact Profile (BBSIP) for children and adults. *Burns* 2015;**41**:1405–19.
24. Bush JA, McGrouther DA, Young VL, et al. Recommendations on clinical proof of efficacy for potential scar prevention and reduction therapies. *Wound Repair Regen* 2011;**19**(Suppl 1):s32–7.
25. Franchignoni F, Giordano A, Vercelli S, et al. Rasch analysis of the patient and observer scar assessment scale in linear scars: suggestions for a Patient and Observer Scar Assessment Scale v2.1. *Plast Reconstr Surg* 2019;**144**:1073e–1079ee.
26. Carriere ME, Mokkink LB, Tyack Z, et al. Development of the patient scale of the Patient and Observer Scar Assessment Scale (POSAS) 3.0: a qualitative study. *Qual Life Res* 2023;**32**(2):583–92.
27. van Zuijlen PPM, Mokkink LB, Hoogewerf CJ, de Vet HCW. The official update of the POSAS: an invitation to share experiences to improve the POSAS in 'Project POSAS 3.0'. *Burns* 2017;**43**:893–4.
28. Oosterhoff TCH, Beekman VK, van der List JP, Niessen FB. Laser treatment of specific scar characteristics in hypertrophic scars and keloid: a systematic review. *J Plast Reconstr Aesthet Surg* 2021;**74**:48–64.
29. Rippa AL, Kalabusheva EP, Vorotelyak EA. Regeneration of dermis: scarring and cells involved. *Cells* 2019;**8**(6):607.
30. Banzhaf CA, Thaysen-Petersen D, Bay C, et al. Fractional laser-assisted drug uptake: impact of time-related topical application to achieve enhanced delivery. *Lasers Surg Med* 2017;**49**:348–54.