Treatment of Scars with Laser-Assisted Delivery of Growth Factors and Vitamin C: A Comparative, Randomised, Double-blind, Early Clinical Trial

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ABSTRACT

Background: Scarring can jeopardize the final result of plastic surgeries. Deep dermal injuries activate dermal fibroblasts that produce excessive amount of collagen and

inflammatory cytokines and growth factors, which contributes to increased fibrous tissue and scarring tissue formation.

Objectives: The aim of this double-blind, prospective, randomised clinical trial was to investigate the use of laser-assisted drug delivery (LADD) for scar improvement to support the establishment of LADD as standard therapy modality and to indicate suitable drugs for dermal administration.

Material and Methods: In total, 132 patients seeking scar treatment were consented and randomised. The control group (64 patients) received laser resurfacing immediately followed by skin surface application of Vitamin C and 68 patients received laser treatment followed by skin surface application of a cosmeceutical containing growth factors (GFs) and Vitamin C. Photographs were obtained before and three months after the procedure and submitted to three-dimensional reconstruction by the software Dermapix®. Objective measurements provided by the software were statistically analysed and established the differences in the treatment result between the two groups.

Results: There was a significant reduction in scar roughness and volume in both groups (p<0.01). Mann-Whitney test confirmed that the group treated vitamin C and GFs presented significantly better results than the group treated with vitamin C alone (p<0.01).

Conclusion: LADD has proven efficient as scars were reduced in both study groups. Furthermore, the addition of growth factors provided statistically significant better outcomes and resulted in more inconspicuous scars. No adverse reactions were observed.

Keywords: Laser-assisted drug delivery; scars; scar treatment; growth factors; vitamin C.

Evidence-Based Medicine
Level I: Evidence obtained from at least one properly designed randomized controlled trial.

Declarations

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Conflicts of interest/ Disclaimer: The authors have no financial interest or support related to this study. The three-dimensional stereophotogrammetry system and drugs have been fully paid by the first author as a part of a PhD study.

Ethics approval: This study was approved by the XXXXXX Ethics Committee of XXXXX, XXXXXXX, XXXX. It was also approved by the Faculty Research Ethics Panel (FREP) at XXXXXXXX University and followed the principles of the World Medical Association Declaration of Helsinki (2013). Participants received a comprehensive explanatory information sheet about the research and signed a consent form to engage in the study.

Clinical trial registration: Plataforma Brasil under the number CAAE:63710716.2.0000.5664.

Consent to participate: All patients have consented to participate and have signed a consent form that has been submitted to both Ethics committees.

Consent for publication: The photographs are property of the first author and can be published.

Availability of data and material: Raw data, additional tables and graphics not included in this version are available for consultation.

Authors' contributions: XXXX, XXXX contributed to the study conception and design. Material preparation, data collection and analysis were performed by XXXX. XXX performed the review of the statistical analysis. XXX and XXXX performed the manuscript review. All authors read and approved the final manuscript.

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1 Introduction

Skin trauma induced by physical agents or burn injuries immediately triggers a universal phenomenon called wound healing. This synchronised reaction is guided by the intercellular communication signalled by growth factors (GFs) and cytokines [1-4]. Disruptions affecting the pathways guided by these endogenous proteins, the depth of the lesion, the healing time, and ethnic factors can result in scarring tissue formation. Scarring refers to abnormality in scar tissue colour, contour, roughness or texture.

Scars are clinically classified as normotrophic, atrophic, hypertrophic, and keloid [5] (Figure 1). As common features, scars lack skin appendages and do not exhibit the flexibility or

strength of the original tissue [1]. Hypertrophic scars exhibit increased cellularity, vascularity and connective tissue. Keloids arise spontaneously or after major or minimal cutaneous injury and are related to significant morbidity, social stigma, psychological distress, cosmetic disfigurement, local pain and pruritus. They present lateral expansion of the scar beyond the boundaries of the original wound, and do not regress spontaneously [2,5-7]. At a molecular level, GFs such as TGF-β1 (transforming growth factor beta1) and VEGFs (vascular endothelial growth factor) increase the proliferation of fibroblasts and the collagen synthesis 6 to 20 times more in keloids than in healthy skin [7].

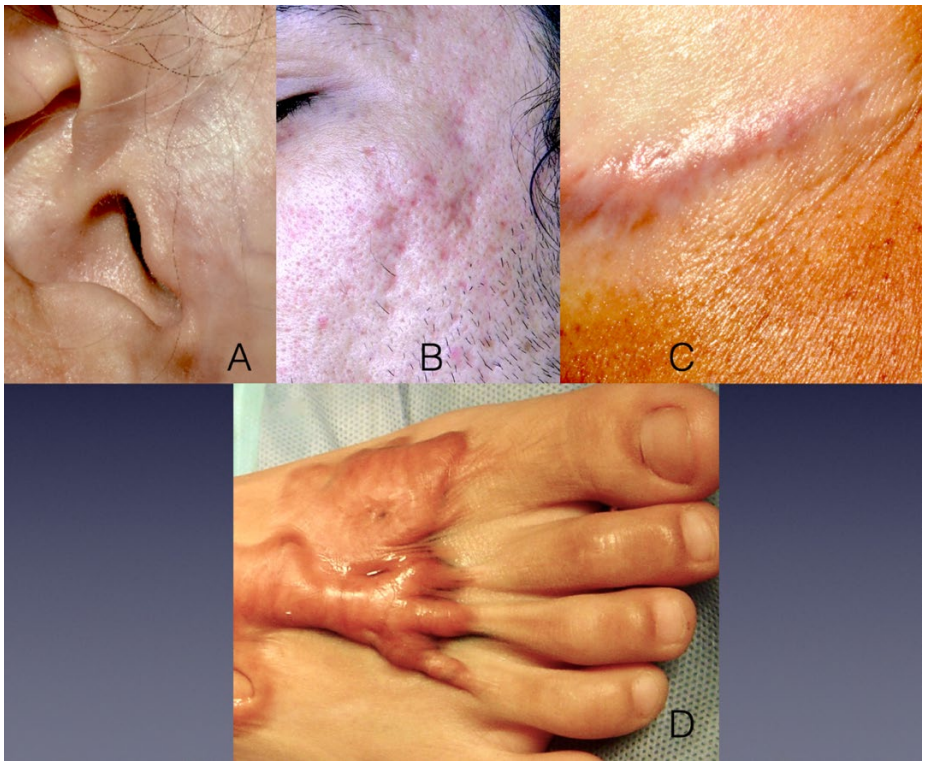

Figure 1: (A) A normotrophic pre-auricular scar, (B) an atrophic scar consequent to acne sequelae, (C) a post-mammoplasty hypertrophic scar, and a keloid (D) that developed as a burn sequelae.

Patients presenting hypertrophic scars and keloids usually seek scar treatment to achieve aesthetic improvement and to relieve pain, itching or functional restriction. The therapeutic armamentarium to improve scarring includes over-the-counter products, cosmetic camouflage, compression therapy, dermabrasion, microneedling, laser therapy, surgical intervention followed by immediate radiation therapy, and intralesional injections of chemical substances or fat into the scar tissue [2,3,8-12]. These treatments aim at reducing or preventing scarring as

opposed to simply managing scar symptoms. Nonetheless, some therapies are related to significant morbidity or have low efficacy.

Ablative fractional lasers (AFXLs) have become the standard of care of scars in many burn centers. They produce uniform microchannels into the dermis, irrespective of the scar contour. These devices increase the pliability and improve the colour and textural properties of scars, decrease scar volume and height, and reduce local symptoms such as pruritus and pain. AFXLs can (i) break down the disorganised collagen fibrils that create the scar contracture, (ii) stimulate the remodelling of collagen into a more orderly and parallel arrangement, (iii) induce mature hypertrophic scar regression by suppressing the deposition of collagen types I and III [9] and (iv) ameliorate the scar roughness and the tension in the upper dermis of the scars [3,13]. And finally, the laser-induced physical trauma and microthermal injury caused by AFXLs are supposed to reproduce the same signalling pathways that are involved in the phases of wound healing, and have to ability to reduce the expression of GFs, namely TGF-family, and FGF-β (fibroblast growth factor) [3].

The laser-induced microchannels are theorised to improve drug diffusivity inside deeper layers of the scars. Nonetheless, *in vitro* studies on laser-assisted drug delivery (LADD) aiming at scar improvement are inadequate because abnormal scar formation is unique to humans [14], and research investigating therapeutic doses of GFs in a clinical setting aiming at LADD are scarce [15-18].

2 Objectives

This double-blind, prospective, randomised clinical trial aimed at quantifying the scar modification after treating scar surface with an Er:YAG laser resurfacing immediately followed by topical application of growth factors contained within a cosmeceutical (TNS Recovery Complex®, SkinMedica) and/or vitamin C (ascorbic acid - Vitasantisa®, Brazil) for injectable use. According to the fabricant, the GFs and cytokines contained in the cosmeceutical are VEGF, HGF, IL-6 and IL-8, TGF-β1 and PDGF [19]. The primary endpoint was the quantification of the change in scar roughness (Rgh_{DS}) and scar volume (V_{DS}) between baseline and three months after the procedure. The efficacy of the treatment was statistically analysed. The secondary endpoint was the investigation of any local or systemic event that could accrue from LADD.

3 Study design

The sample size calculation was based on a pilot study performed in 2017 as part of the PhD studies of the first author. That study estimated the *n* of 47 patients for each study group to have 80% power and a significance level of 5% to detect the mean difference of 0.5624 between the two related samples (SD = 1.335). The *n* should exceed the sample size to prevent from lack of data which would reduce the power of the study.

From a total of 176 patients searching for scar improvement, 132 patients met the inclusion criteria (Fitzpatrick skin type I to IV, between 18 and 70 years old, presenting scars) and were recruited between September 2018 and September 2019. Exclusion criteria included pregnancy; patients who could not present for follow-up; patients with history of recent cutaneous allergies involving the cicatricial area; and/or those were subject to corticosteroid topical injections, laser, micro-needling or dermabrasion in the scar to be treated within 6 months pre-treatment.

All enrolled patients signed the consent form and were photographed with a 3D-SPM camera (LifeViz^{TM} Micro, Quantificare, France). A laser tape measure was used to define consistent anatomical landmarks across the topography of the scar. After photographic documentation, an anesthetic ointment composed of lidocaine 7% and tetracaine 7% was topically applied to the scar for 30 minutes.

Before entering the treatment room, each patient was randomised into groups DS-C and DS-CGF. They picked a paper from a container indicating the description of the treatment to be delivered.

The scar was cleaned, and patients were subjected to one session of fractional ablative laser treatment by the first author (Starlux® 500 Palomar Inc., Burlington, MA). Standardised laser settings were used for all patients regardless of the scar elevation. The blue optic 6x6 mm handpiece was employed to deliver a dual pulse mode. The short pulse, which targets cutaneous ablation was set to 9 mJ/µb and the pulse width was pre-set to 250 µs. The energy of the long pulse, which promotes tissue coagulation and scar contraction was set to 8 mJ/µb with a duration of 5 ms. Four passes were conducted to produce equal distribution of microchannels in all patients.

After laser treatment, the researcher's assistant who was not linked to the research applied the chemical substances on the scar surface according to the branch of the study. The researcher

and the patient were blinded to the medication applied. Patients of group DS-CGF received the cosmeceutical containing GFs associated with 200 mg of vitamin C; 2 ml sterile solution containing 200 mg of vitamin C and 1ml of the patented formula was spread over the scar area regardless of the scar size. Group DS-C was the control group and received 200 mg of vitamin C only. The medications were kept under occlusion and protected from light exposure for 30 minutes. Figure 2 is a flowchart of the study.

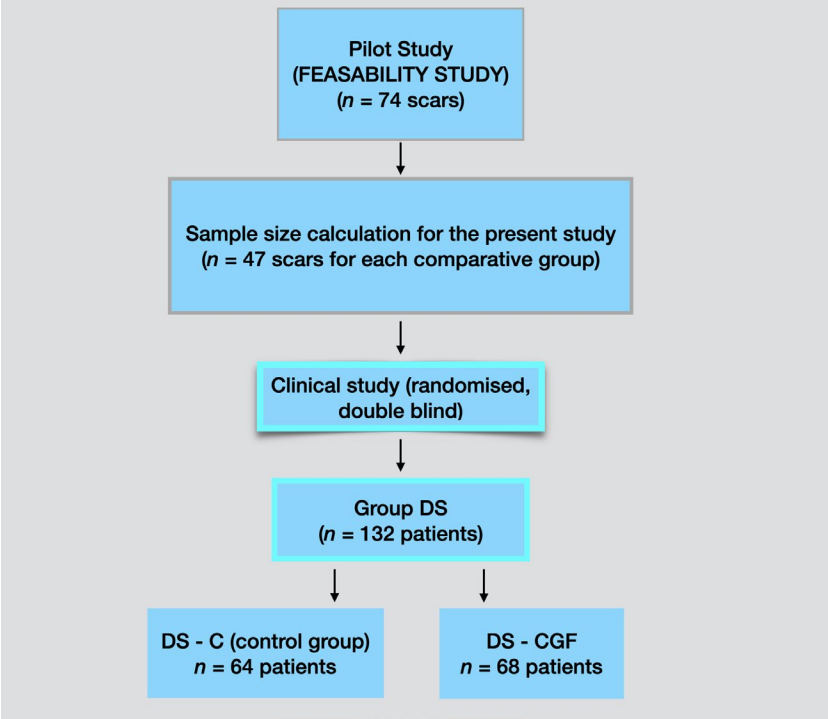

Figure 2: Flowchart of the study.

No anti-herpetic viral prophylaxis was prescribed and all patients were instructed to cover the scar with dexpanthenol (Bepantol® - Bayer) four times a day until the cutaneous debris have disappeared.

Photographs post-procedure were taken at three months. The images were transferred to the software Dermapix® and synchronised to permit comparing the same cicatricial area pre- and post-procedure. A contour comprising the scar area exhibiting significant scar irregularity was drawn in the pre-procedure image and replicated to the post-procedure image. The software performed the 3D reconstruction and the contour was transposed to the 3D images. The software calculated the measurements of scar volume (V_{DS}) and scar roughness (Rgh_{DS}) contained within the limits of the marked area being investigated.

4 Results

A pilot study was conducted prior to this clinical study (data not shown) to detect and anticipate potential failures that could lead to drop out or withdrawal. During this study no withdrawal/dropout was reported. Furthermore, no complications were detected for both study groups.

Every patient who was admitted to this research was monitored for adverse events systematically and analysed in terms of outcomes during the study period. Data obtained via the Dermapix® software were analysed by the package software SPSS IBM (Version 26.0 IBM Corp© for Mac, Armonk, New York, USA). The objectives were to compare the efficacy of each treatment regimen and to confirm if there was a significant difference in the variables Rgh_{DS} and V_{DS} between the two study groups. The data were not normally distributed and non-parametric tests were applied. Mean, standard deviation (SD), median and interquartile range (IQR) from 25^{th} to 75^{th} percentile were provided. The criterion for determining significance was set at 5% and findings were considered significant with a p-value < 0.05.

4.1 Group DS-C (control group)

Sixty-four patients composing group DS-C were treated with LAM followed by topical application of vitamin C. Participants were aged from 19 to 62 years old (median 39 years and IQR 29.5 – 44.8 years-old); 53 patients (82.8% of the cases) were female. The median scar age was 6 months (IQR 4 – 11.8 months).

In group DS-C, Rgh_{DS} decreased in 81.3% of the patients, whereas 92.2% of the patients presented V_{DS} reduction. The pre-procedure Rgh_{DS} median was 0.21 with an IQR of 0.13 – 0.32 (mean 0.13 ± 0.32) compared to 0.18 and IQR of 0.1 – 0.28 post-procedure (mean 0.2 ± 0.12). The median V_{DS} was 17.3 mm^3 with an IQR of 8.3 – 35.1 mm^3 (22.2 ± 16.1 mm^3) pre-procedure compared to 14.4 mm^3 with an IQR of 6 – 27.8 mm^3 (16.9 ± 13.1 mm^3) post-procedure. The Wilcoxon signed-rank test has showed a $p < 0.01$ meaning that the reduction of scar volume and roughness were statistically significant.

The percentual variation between the pre- and post-procedure measurements is a statistical measure called ∂ reduction, and is calculated by the formula:

Percentage of parameter modification (∂ reduction) = $\frac{\text{(pre – post measure) x 100}}{\text{pre-measure}}$

The median of Rgh_{DS} ∂ reduction was 13.3% (IQR: 3.5 – 25%) (mean14.5 ± 20.6) and the median V_{DS} ∂ reduction was 20.5% (IQR:11.4 – 36.4%) (mean 23.3 ± 22.0 mm^3). The simultaneous computation of both parameters showed a median skin modification of 37.8% with an IQR of 18.9 – 59.6% (mean 35.0 ± 37.4%).

Figure 3 illustrates a patient in group DS-C who underwent an abdominoplasty 13 months before being treated with LSR followed by topical application of vitamin C.

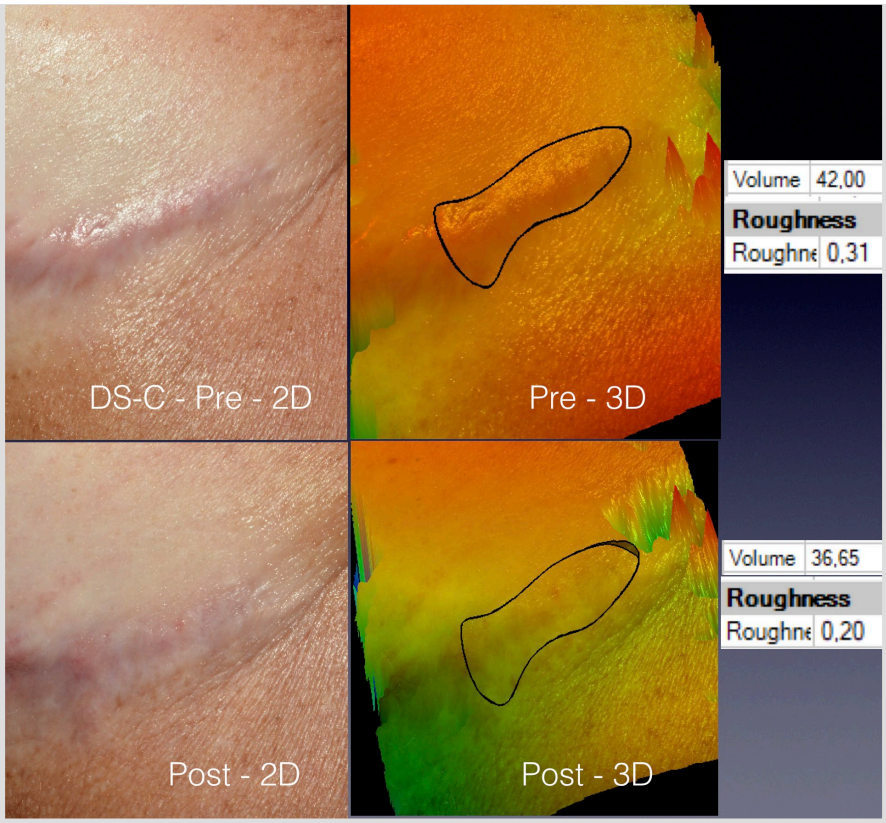

Figure 3: Pictures of a 32-year-old patient subjected to LAM with vitamin C. Data displayed on the right were obtained on the 94th day. The ∂ reduction of Rgh_{DS-C} was 35%, whereas V_{DS-C} decreased by 12.7%.

4.2 Group DS-CGF

A total of 68 patients comprised group DS-CGF and were treated with LAM followed by topical application of the cosmeceutical containing GFs and vitamin C. Participants were aged from

20 to 68 years (median 52 years and IQR of 39 – 58 years-old). Fifty-seven patients (83.8% of the cases) were female. The mean age of scars pre-study was 7.0 months (IQR 5 – 10.8 months).

Both $Rgh_{DS\text{-}CGF}$ and $V_{DS\text{-}CGF}$ decreased in 98.5% of the cases. The pre-procedure Rgh_{DS} median was 0.3 with an IQR of 0.19 – 0.42 (mean 0.33 ± 0.18) compared to 0.17 with an IQR of 0.12 – 0.23 (mean 0.19 ± 0.11) post-procedure. The V_{DS} median was 28.8 mm^3 with an IQR of 11.8 – 39.9 mm^3 (mean 27.9 ± 16.1 mm^3) pre-procedure compared to 8.3 mm^3 with an IQR of 4.5 – 17.1 mm^3 (mean 11.8 ± 10.1 mm^3) post-procedure. The Wilcoxon signed-rank test confirmed statistical significance for the reduction concerning both parameters ($p < 0.01$).

The median of $Rgh_{DS\text{-}CGF}$ ∂ reduction was 36% with and IQR of 24.4 – 53.8% (mean 37.5 ± 18.8) and the median $V_{DS\text{-}CGF}$ ∂ reduction was 54.9 with an IQR of 40.7 – 74.0% (mean 56.2 ± 23.4%). The median of 89% and IQR of 71.8 – 118.4% (mean 93.7 ± 35.5%) related to the simultaneous computation of ∂ reduction of the parameters Rgh_{DS} and V_{DS} represented the visual scar relief improvement. Some patients treated with GFs also experienced a reduction of visible vessels (Figure 4) .

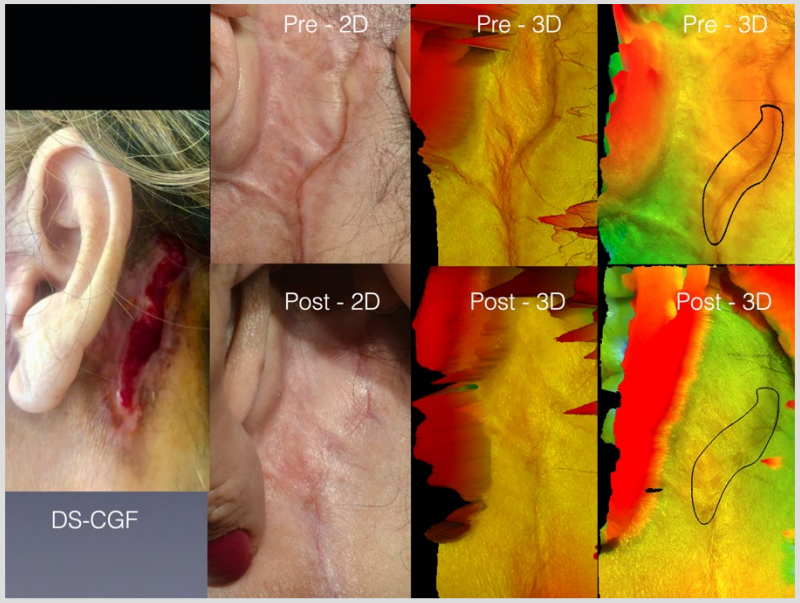

Figure 4: The pictures of a 63-year-old patient exhibiting a scar resulting from a retroauricular flap necrosis post-facelift 6 months before. The noticeable scar improvement was confirmed by the data obtained on the 97th day post-procedure provided by the 3D SPM system. The modification inside the contours drawn on the photographs on the right showed that the ∂ reduction of $V_{DS\text{-}CGF}$ was 61% and $Rgh_{DS\text{-}CGF}$ decreased by 42.5%.

4.3 Comparison between groups DS-C and DS-CGF

Table 1 shows the population distribution is terms of scar classification, aetiology, skin type according to Fitzpatrick classification and the treated scar area. The treatment area of the scars surface varied from 4 to 35 cm^2. The smallest scars were located mainly on the face while the largest ones were verified on the abdomen.

Table 1 – Distribution of study groups concerning scar classification, scar ethiology, Fitzpatrick skin type and scar area

	Group DS-C		**Group DS-CGF**	
Scar Classification				
	n	%	*n*	%
keloid	2	3.1	5	7.4
hypertrophic	60	93.8	57	83.8
Atrophic	2	3.1	6	8.8
Aetiology				
	n	%	*n*	%
Burn	2	3.1	5	7.4
Surgical incision	61	95.3	59	86.7
Trauma (car accident)	1	1.6	3	4.4
Healing by secondary intention	0	0	1	1.5
Fitzpatrick Skin Type				
	n	%	*n*	%
I	4	6.2	2	2.9
II	17	26.6	10	14.8
III	41	64.1	47	69.1
IV	2	3.1	9	13.2
Scar area in cm^2				
	n	%	*n*	%
4 - 15	12	18.7	10	14.7

15 - 30	39	61.0	49	72.1
30 - 35	13	20.3	9	13.2

Figure 5 shows the boxplots representing the variation of the scar volume and scar roughness in both study groups.

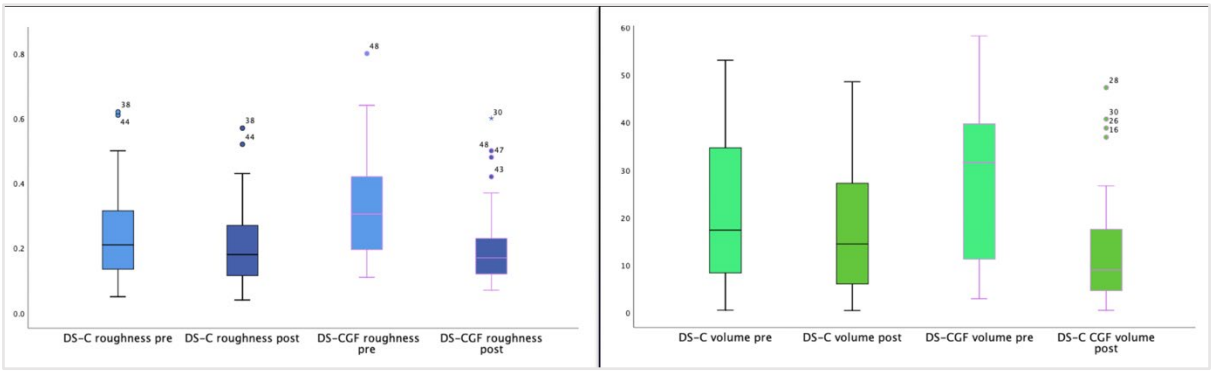

Figure 5: Boxplots representing the variables scar roughness (blue) and volume (green) pre and post-treatment in groups DS-C and DS-CGF. The y-axis could not be equally represented because the range was different for both variables. Volume is given in mm^3 and roughness is unitless.

A Mann-Whitney test (Table 2), and a Fisher's test (Table 3), established comparisons between groups DS-C and DS-CGF. The differences between both study groups concerning the age of the scars ($p = 0.53$) and the age of the patients ($p = 0.57$) (Table 2), the anatomical site of the scars ($p = 0.94$) and the gender variation ($p = 0.87$) (Table 3) were not significant. Those statistical analysis demonstrated the randomisation process did not lead to possible bias accruing from any heterogeneity between both groups.

Table 2 - Age of the patients (years) and age of the scars (months) : differences between groups C and DS-CGF - Mann-Whitney U test

	Group DS-C					Group CGF					Mann-Whitney U test
Variable	**Mean ± SD**	**Median**	**IQR**	**Min**	**Max**	**Mean ± SD**	**Median**	**IQR**	**Min**	**Max**	***p*-value**

Age (years)	38.9 ± 11.2	39.0	29.5 – 44.8	19	62	48.5 ± 13.9	52	39 – 58	20	68	0.57
Age of the scars (months)	7.9 ± 5.5	6.0	4.0 – 11.8	2.0	28	7.8 ± 4.1	7.0	5.0 – 10.8	2	17	0.53
IQR: interquartile range (25th - 75th percentile); Min: Minimum: Max: Maximum; n/a : not applicable											

Table 3 - Group DS - Fisher's exact test

Variable	**Group DS-C (n = 64)**		**Group DS-CGF (n = 68)**		**p-value**
	n	**%**	**n**	**%**	
Gender					0.87
male	11	17.2	11	16.2	
female	53	82.8	57	83.8	
Anatomical site					0.94
face/neck	20	31.3	23	33.8	
abdomen/flanks	23	35.9	24	35.3	
breast/thorax	21	32.8	21	30.9	

The Mann-Whitney test showed that patients in group DS-CGF presented scars with more roughness and more volume than patients in group DS-C ($p < 0.05$). Yet, this group presented more RghDS ∂ reduction and VDS ∂ reduction and the simultaneous computation of ∂ reduction of VDS + RghDS than group DS-C ($p < 0.01$) (Table 4). These findings demonstrated that scars treated with growth factors (GFs) combined with vitamin C after superficial LSR presented a favourable outcome over controls.

Table 4 - Mann-Whitney test regarding the variables in groups DS-C and DS-CGF

	Group C (*n* = 64)		**Group CGF (*n* = 68)**		**MWT**
Variable	**Median**	**IQR (25th – 75th percentiles)**	**Median**	**IQR (25th – 75th percentiles)**	***p*-value**
Rgh_{DS} pre-procedure	0.21	0.13 – 0.32	0.3	0.19 – 0.42	0.002
Rgh_{DS} post-procedure	0.18	0.11 – 0.28	0.17	0.12 – 0.23	0.77

V_{DS} pre-procedure	17.3	8.3 – 35.1	28.8	11.8 – 39.9	0.04
V_{DS} post-procedure	14.4	6 – 27.8	8.3	4.5 – 17.1	0.03
Rgh_{DS} ∂ reduction	13.3	3.5 – 25.0	36.0	24.4 – 53.8	< 0.01
V_{DS} ∂ reduction	20.5	11.4 – 36.4	54.9	40.7 – 74.0	< 0.01
Simultaneous computation of Rgh_{DS} + V_{DS} ∂ reduction	35.0	18.9 – 59.6	89.0	71.8 – 118.4	< 0.01
IQR: interquartile range (Q1-Q3). MWT: Mann-Whitney test.					

Spearman correlation coefficient (Sig 2-tailed) established the correlation between scar roughness and volume. In group DS-C, *rho* was 0.417 pre-procedure and 0.387 post-procedure, whereas in group DS-CGF, *rho* was 0.306 pre-procedure and 0.280 post-procedure ($p < 0.01$). The correlation between the variables was not strong (Figure 6) which suggested that RghDS and VDS responded to LADD with different intensity.

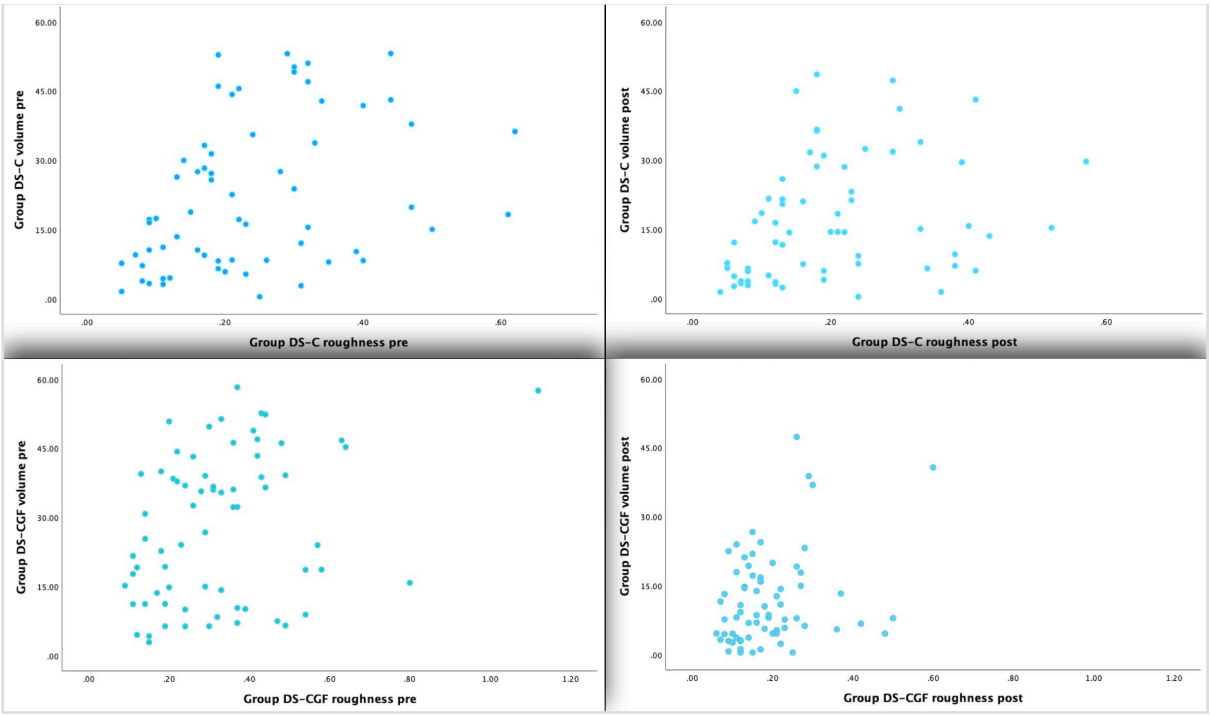

Figure 6: The scatterplots comparing both groups demonstrate that the correlation between Rgh_{DS} and V_{DS} was not strong in the two groups, in spite of the fact that the parameters decreased and converged towards zero.

A Mann-Whitney test showed that the scars responded to treatment regardless of the anatomical location ($p < 0.01$) (Table 5). Nonetheless, the group DS-CGF presented ∂ reduction of RghDS and VDS significantly higher and up to 3 times more than the control group (DS-C) ($p < 0.01$).

Table 5 - Mann-Whitney test regarding the anatomical location of scars in groups DS-C and DS-CGF

Variable	Group DS-C			Group DS-CGF			*p*-value
	n	Median	IQR	*n*	Median	IQR	
Site: face/neck							
Rgh_{DS} ∂ reduction	20	16.0	9.5 – 27.7	23	37.5	23.3 – 50.0	0.002
V_{DS} ∂ reduction	20	22.3	17.8 – 33.8	23	59.3	39.6 – 72.8	0.01
Site: abdomen/flanks							
Rgh_{DS} ∂ reduction	23	11.1	0 – 27.8	24	35.2	18.9 – 57.0	< 0.01
V_{DS} ∂ reduction	23	16.2	6.0 – 44.3	24	58.7	38.7 – 80.6	0.0003
Site: breast/thorax							
Rgh_{DS} ∂ reduction	21	11.1	3.6 - 25.0	21	35.5	27.9 - 52.9	< 0.01
V_{DS} ∂ reduction	21	18.4	10.8 - 36.3	21	53.0	41.3 - 70.5	< 0.01

IQR: interquartile range (25^{th} – 75^{th} percentiles)

5 Discussion

After skin trauma, the body launches a series of dynamic and physiological events mediated by GFs that result in wound healing. GFs bind to their specific receptors on the cell surface and this interaction control vital inflammatory responses, and regulates the growth, differentiation, proliferation, and migration of cells involved in wound healing [4,8,18-21]. The major GF families involved in wound healing and tissue repair are described in Table 6.

Table 6 – Major growth factor families involved in wound healing and tissue repair

Transforming growth factors beta (TGF-βs)	• Mediate the cutaneous immune response, and the crosstalk between the dermis and epidermis [3,9].
Vascular endothelial growth factors (VEGFs)	• Regulate angiogenesis and vascular permeability; • Stimulate cell survival, proliferation and migration [14,22]; • Stimulate granulation tissue formation; • Deregulation of VEGFs has been associated with nonhealing wounds, tumours and intraocular neovascular disorders [21,23].
Epidermal growth factors (EGFs)	• Stimulate keratinocyte proliferation and migration, differentiation and re-epithelialization. • Increase the number of fibroblasts in the wound and augment the expression of keratins involved in the proliferative signalling pathway. • Deregulation of EGFs is associated with tumourigenesis. • EGF improved acne scars, brown spotting, skin texture, pore size, red spotting, stretch marks and wrinkles [18,24,25].
Platelet-derived growth factors (PDGFs)	• PDGFs stimulate macrophages to secrete TGF-βs. • Essential for fibroblast proliferation, production of ECM, wound healing and angiogenesis. • Abnormal regulation and production of PDGFs may cause tumours and fibrotic disease.
Fibroblast growth factors (FGFs)	• Angiogenic factors involved in morphological (embryonic) processes, keratinocyte organization and wound healing [21].
Insulin-like growth factors (IGFs)	• IGF-1 stimulates both hypertrophy (increase in cell size) and hyperplasia (increase in cell number) in most tissues. • Induce neuron survival, stimulate wound re-epithelialization and fibroblast proliferation.
Hepatocyte growth factor (HGF).	• Regulates cell growth, cell motility, wound healing and morphogenesis (embryonic organ development and adult organ regeneration). • Stimulate mitogenesis and matrix invasion;

	• Central role in angiogenesis and tumourigenesis.

A meta-analysis concerning published randomised controlled trials investigating patients receiving GFs in the management of partial-thickness burns demonstrated that the topical application of GFs significantly reduced healing time, scarring pigmentation, scar pliability, height and vascularity [26]. No significant adverse events were reported. Dose-dependent, repeated treatment with EGF increased the epithelial cell proliferation and accelerated the wound-healing process. Conversely, the use of EGF alone as a treatment had no noticeable effect [22,27].

Studies show that inordinate TGF-β1 activity leads to excessive myofibroblast activation, increased tissue tension, stretching of the scar tissue and increases the likelihood of hypertrophic scar formation. The intradermal injection of TGF-β3 or inhibitors of TGβ-1accelerates wound healing and can produce less noticeable scars. A depletion of TGF-β2 receptor results in reduced granulation tissue formation and decreased scar size [3].

Disphanurat et al. (2020) recently published one of the few studies involving the LADD of GFs in humans [25]. They treated 24 participants for striae alba (stretch marks) with three sessions of ablative fractional carbon dioxide laser at 4-week intervals followed by topical application of recombinant human epidermal growth factor (rhEGF) or Aloe Vera gel. After the laser resurfacing, all patients received both medications, i.e. each side of the body received either rhEGF or Aloe Vera gel treatment. However, some areas were left untreated for comparison, and this might have been a source of bias because it is challenging to establish landmarks to identify which stretch marks have been treated. Both substances provided statistically significant improvement of the striae surface texture but no statistical significance was found between the rhEGF- and Aloe Vera-treated sides. They instructed the patients to apply the same substances in a gel preparation on the skin surface for one month, twice a day, after the last laser session. It is possible that the gel may have hydrated the SC and interfered with the analysis of results. Furthermore, patients may not have uniformly complied with this treatment step. The number of laser passes in each treatment session was not stated and the multiple laser sessions is a hindrance to determine whether the improvement resulted from the laser treatment itself or from the LADD. Finally, the 3D SPM system used in that study was a scanner, which required skin contact and a slight pressure on the skin surface to obtain skin analysis.

By contrast, the present randomised clinical study consisted of one laser treatment session, which reduced the laser interference from overpassing and stifling the action of the medication. All patients received the same laser protocol so that the laser treatment could not be an additional variable to interfere with the treatment outcomes [18]. The application establishment of a control group was essential and no area of the scars was left without treatment which reduced the possibility of bias. The 3D SPM system chosen for this research was contactless to avoid interference with the readouts [18, 28]. Photographs of the scar post-treatment were taken at a consistent angle, position and background lighting as the pre-treatment images.

Finally, some authors have reported the inefficiency of the application of one GF only [8,29]. To avoid inefficacy of the treatment due to the lack of interaction among GFs, the patients treated in group DS-CGF received a blend of GFs. Although it is not possible to confirm which GFs were responsible for the response to the treatment, the combination of GFs may have permitted for satisfactory intercellular communication and resulted in the overall satisfactory result.

The effect of laser alone on scar roughness and volume was not investigated because several studies have already demonstrated the efficacy and safety of laser therapy on scar treatment. In addition, this study can be considered as an early report for hypertrophic and keloid types of scar. Longer term follow up studies are highly recommended to provide comprehensive analysis covering all types of scars.

For ethical reasons, it was not possible to use GFs as part of a composition with known concentrations and obliged the use of a blend of GFs contained in a patented formula. The absence of cutaneous or systemic reactions confirmed that the treatment was safe [18]. However, risk characterization may be necessary if new formulas with potential contribution to the pathways involved in wound healing and skin regeneration are to be clinically used. Nonetheless, tests aiming at quantifying the serum and cutaneous concentration of the applied substances would prove meaningless because GFs are produced endogenously and vitamin C undergoes dietary influences.

It is our belief that this study can contribute to translational medicine. As for future studies and to warrant the safety of the procedure, it is advisable to use sterile drugs suitable for intravenous application. This choice can minimize the risk of infection and inadvertent systemic absorption of drugs applied on the skin surface after laser treatment.

6 Conclusion

Fractional lasers have been efficiently used to improve the scar appearance. These devices produce microchannels that cause physical and thermal trauma on the skin surface and the response to this controlled aggression reproduces the signalling pathways that are involved in the phases of wound healing. Apart from collagen remodelling, fractional lasers can improve the transcutaneous delivery of medication.

This is one of the few studies involving human subjects that investigates LADD. The number of participants previously established by a pilot study and the comparison with a control group have reinforced the quality of the study. Although the results obtained could be somewhat weakened by the significant physical involvement of the laser treatment, the therapeutic response of the patients whose scars were treated with vitamin C and GFs was statistically significant in comparison with the control group, which was treated with laser and vitamin C only. This finding confirms the positive effect of adding GFs to scar treatment. As a secondary outcome, LADD has proven to be safe because no local adverse event or systemic reaction was detected.

Despite the satisfactory results seen in this study, scars presented a challenging architecture to work with. The age of the scars did not interfere with the result of the LADD but the low correlation between the variables confirms that reducing the scar roughness and the scar volume, at the same time, was challenging and this could be related to the disarranged structure of the skin post-trauma.

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