REVIEW ARTICLE

Minimal Invasive Cutaneous and Aesthetic Surgery

Histological Validity and Clinical Evidence for Use of Fractional Lasers for Acne Scars

Though fractional lasers are widely used for acne scars, very little clinical or histological data based on the objective clinical assessment or the depth of penetration of lasers on *in vivo* facial tissue are available. The depth probably is the most important aspect that predicts the improvement in acne scars but the studies on histology have little uniformity in terms of substrate (tissue) used, processing and stains used. The variability of the laser setting (dose, pulses and density) makes comparison of the studies difficult. It is easier to compare the end results, histological depth and clinical results. We analysed all the published clinical and histological studies on fractional lasers in acne scars and analysed the data, both clinical and histological, by statistical software to decipher their significance. On statistical analysis, the depth was found to be variable with the 1550-nm lasers achieving a depth of 679 μm versus 10,600 nm (895 μm) and 2940 nm (837 μm) lasers. The mean depth of penetration (in μm) in relation to the energy used, in millijoules (mj), varies depending on the laser studied. This was statistically found to be 12.9–28.5 for Er:glass, 3–54.38 for Er:YAG and 6.28–53.66 for CO_2. The subjective clinical improvement was a modest 46%. The lack of objective evaluation of clinical improvement and scar-specific assessment with the lack of appropriate *in vivo* studies is a case for combining conventional modalities like subcision, punch excision and needling with fractional lasers to achieve optimal results.

KEYWORDS: Acne scars, efficacy, fractional lasers, histological depth

INTRODUCTION

Fractional lasers, both ablative and nonablative [Figure 1], are based on the well-established concept of fractional damage to the skin which enable a rapid healing as compared to the conventional ablative lasers as the intervening skin is intact for the reparative process.[1-3] A secondary effect is the dermal remodelling induced in the dermis beyond the narrow zone of coagulation induced by the fractional lasers.[4-6] The carbon dioxide (CO_2) laser has a predominant coagulative and necrotic effect (horizontal effect) as compared to the erbium:yttrium–aluminium–garnet (Er:YAG) laser, which has a dose-dependent increase in depth with less necrosis or coagulative effect (vertical effect). Thus, resurfacing procedures may be thought of as having both horizontal (tightening effect) and vertical (depth) treatment vectors on the tissue. Probably, the CO_2 lasers have more of a horizontal effect while the erbium laser has a more vertical effect. For

Access this article online

Quick Response Code:

Website: www.jcasonline.com

DOI: 10.4103/0974-2077.99431

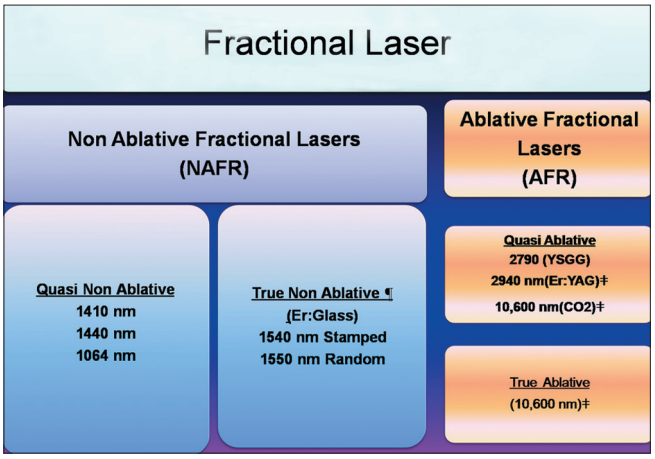

Figure 1: An overview of the fractional lasers: ¶Amongst the NAFR, the 1550/1540 nm laser has been used for acne scars. ‡Amongst the AFR, the 2940- and 10,600-nm laser has been used for acne scars

Kabir Sardana, Vijay K Garg, Pooja Arora, Nita Khurana[1]

Departments of Dermatology and [1]Pathology, Maulana Azad Medical College and Lok Nayak Hospital, New Delhi, India

Address for correspondence:

Dr. Kabir Sardana, 466, Sector 28, Noida - 201 303, Uttar Pradesh, India. E-mail: kabirijdvl@gmail.com

atrophic acne scars, it is important to choose a modality that reaches the appropriate depth to target the deep, boxcar and ice pick scars while concomitantly treating the superficial scars. The conjecture that fractional lasers are effective for acne scars is derived from the fact that the microcolumns (microscopic thermal zone) act akin to the effect of 'needling' which helps to detach the tethering of scars and creates sufficient 'collagen remodelling' to 'lift' up the atrophic scars. Resurfacing procedures are considered by some to be the gold standard for the treatment of postacne scarring, and the mechanism is probably a combination of vertical ablation and horizontal 'tightening' of the tissue. However, it has been proven by experience that no amount of stretching out or ablation of deep dermal and subcutaneous structural loss with resurfacing tools can completely ameliorate the deep scars. The effect of thermal remodelling works in consonance with the depth achieved and thus the histological confirmation of the depth of the microthermal zone (MTZ) is probably more important for scar-specific improvement. Interestingly, while the face is the focus of fractional laser in acne scars, the seminal studies used the forearm for histological assessment.[3-5,7,8] The paucity of appropriate histologically directed studies in relation to facial acne scars is probably because of a lack of volunteers for histological assessment, which contrasts with the plethora of clinical studies in acne scars where the evidence of improvement is usually based on subjective assessment.

METHODS

Our aim was to focus on the importance of histological assessment for evaluating the effectiveness of fractional lasers in acne scars and the lacunae that exist in the present published data. Our review was based on the published literature where fractional lasers used for acne scars have been mentioned. This was done by carrying out a PubMed search till October 2011 using the following terms 'fractional lasers, acne scars, histological assessment''. The doses used and the histological depth achieved with each laser were collated and analysed by Graph Pad Software (http://www.graphpad.com) and the statistical graphs were derived using Free statistics software (www.wessa.net). Also, all the clinical studies were analysed and a detailed analysis of the results achieved was done statistically. Tests of association, correlation and the *t*-test were used for analysing the data. In our review, we will first focus on the histological assessment of the fractional lasers relevant to facial skin histology. We will also discuss the histological and clinical variability induced by different laser settings. Based on the existing data and appropriate statistical analysis, we will try to arrive at the depth achieved by various fractional lasers and propose a dose–depth correlation for the different technologies. Last, we will analyse the existing studies of fractional lasers in acne scars and compare statistically the results between ablative fractional resurfacing (AFR) and nonablative fractional resurfacing (NAFR) lasers to arrive at an unbiased opinion on the superiority, if any, between the two technologies in relation to acne scars.

OVERVIEW OF FRACTIONAL LASERS

The fractional lasers [Figure 1] can be classified broadly into two types, the NAFR lasers and the AFR lasers. We will largely focus on three lasers commonly used, the Er:glass (1550 nm, 1540 nm), Er:YAG (2940 nm), and CO_2 (10,600 nm). It is largely believed, in spite of the lack of well-done comparative studies, that the AFR is better than NAFR for acne scars. On analysing the data [Table 1], it was obvious that the histological laser tissue dynamics is largely based on specimens that are derived from *ex vivo* tissues [Figure 2].[1,2,4-18] In studies

Table 1: Summary of studies with histological assessment of fractional lasers

Author	Dose	Depth	Tissue analysed
Er:glass (1540/1550 nm)			
Manstein *et al*.[3]	5 mJ energy/MTZ. Different MTZ densities (400, 1600 and 6400 per cm^2). These densities correspond to distances between MTZ centres of 500, 250 and 125 mm and an average fluence within the test site of 2, 8 and 32 J/cm^2	400 μm (maximum)	*In vivo* Forearm
Geronemus *et al*.[7]	5 mJ energy/MTZ, Density (400, 1600 and 6400 MTZ/cm^2)	560 μm (maximum)	*In vivo* Forearm
Bedi *et al*.[8]	4.5–40 mJ/MTZ	1000 μm (maximum)	*Ex vivo/in vivo*
Farkas *et al*.[9]	Energy of 90 mJ/MTZ	800 μm to 1 mm	*In vivo* Abdominoplasty
Cho *et al*.[19]	Energy of 25 mJ/MTZ	361.5 μm	*In vivo* Cheek
Walgrave *et al*.[10]	1550 nm (Fraxel)[a] 8–10 mJ/microbeam (MB) 1540 nm (Lux)[a] 15–30 mJ/MB	Maximum depth of 300–400 μm (width 95–100 μm) Depths of 400–600 μm (width 50–150 μm)	*Ex vivo* Yucatan black pig skin

(*Contd...*)

Table 1: *(Contd...)*

Author	Dose	Depth	Tissue analysed
Zelickson *et al.*[11]	Lux 1540[a] with a 10-mm tip Double pass mode : 84 mJ/MB, 15 ms (pulse width) and zoom mode 85 mJ/MB, 15 ms (pulse width)	700–750 μm (normal mode) 900–950 μm (zoom mode)	*Ex vivo* pig skin
Thongsima *et al.*[6]	SR750 laser 6 mJ 40 mJ SR1550 laser 6 mJ 100 mJ	 386 μm 826 μm 470 μm 1408 μm	*Ex vivo* human thigh temperature maintained
Er:YAG (2940 nm)			
Diceerx *et al.*[12]	Dose 1–12 mJ/MB Double pulse mode: 0.25–5 ms	450 μm (maximum at 0.25 ms)	*Ex vivo* pig *In vivo* abdominopalsty
Zelickson *et al.*[11]	Lux 2940[a]: 24 mJ/MB, 0.25 ms (pulse width) plus 21 mJ/MB, 5 ms (pulse width) 1–3 pulses	1400–2300 mm (1–3 pulses)	*Ex vivo* pig human abdominal skin
Farkas *et al.*[13]	Palomar 2940[a]=3–5 mJ/MB Profractional erbium device[a]=200–400 mJ/MB	275–300 μm 600–1100 μm	*Ex vivo* abdominoplasty
Kist *et al.*[17]	2940-nm device (Profractional)	25 and 1500 mm	*In vivo* abdominoplasty
CO_2 laser			
Hantash *et al.*[14]	8–23 mJ, 400 MTZ/cm^2	480–1000 μm	*Ex vivo* abdominoplasty
Saluja *et al.*[18]	ActiveFX[b] (90–100 mJ; density settings of 1, 2, 3)	90 mJ (density 1)=300 μm 90 mJ (density 2)=600 μm 90 mJ (density 3)=900 μm 100 mJ (density 1)=400/500 μm 100 mJ (density 2)=1000 μm 100 mJ (density 3)=1500-5000 μm	*Ex vivo* abdominoplasty
Sasaki *et al.*[15]	ActiveFX[b] 125–150 mJ/MTZ	145–450 μm	*Ex vivo* excised thigh skin
Farkas *et al.*[13]	ActiveFX[b], fluence 50–125 mJ/MTZ	100–140 μm	*Ex vivo* abdominoplasty
Farkas *et al.*[13]	Fraxel repair[a] Fluence 40–80 mJ/MTZ	800–1400 μm	*Ex vivo* abdominoplasty
Sasaki *et al.*[15]	DeepFX™[b] treatments (15 mJ, 20 mJ) at density 1	At 15 mJ, the depth is 700 μm (420 μm of ablation, 280 μm of thermal injury) At 20 mJ, the total penetration depth increased to about 1100 μm (660 μm of ablation, 440 μm of thermal injury)	*Ex vivo* excised thigh skin
Sasaki *et al.*[15]	Total FX[b] DeepFX™ (15 mJ, 20 mJ) followed by ActiveFX™ exposures at 100 mJ, 125-Hz frequency and density 1	800–1500 μm	*Ex vivo* excised thigh skin
Farkas *et al.*[13]	DeepFX[b], 5–20 mJ/MTZ	500–2000 μm	*Ex vivo* abdominoplasty
Skovbølling *et al.*[16]	Pulse duration of 2 ms, intensities varied from 1 to 18 W and resultant energies from 2 to 144 mJ/MB, based on single pulses and two, three and four stacked pulses	A low energy of 2 mJ penetrated the epidermis and reached the superficial dermis (median depth 41 μm, range 22–88); 144 mJ penetrated deeply into the reticular dermal compartments and approached the subcutaneous fat (median depth 1943 μm, range 1411–2146)	*Ex vivo* pig abdominal skin
Bailey *et al.*[29]	Fractional CO_2 laser with an energy setting of 15 mJ, 300 Hz at a density of 10	Face (mean depth 415 μm). Abdominal tissues (mean depth 582 μm)	15 patients were subjected to biopsy from the face and the abdomen (*in vivo* analysis)

[a]The brand names mentioned are to distinguish between the variable lasers used in the same studies. [b]In this, 10,600-nm Fractional laser variable settings can lead to markedly different dose penetration results

where an *in vivo* model was chosen,[3-5,7,8] only two studies had focussed on the face [Table 1].[19,20] For validation of fractional lasers, ideally the central face should be used but probably a more practical substrate would be the pre- and post-auricular skin.

ACNE SCAR TOPOGRAPHY AND FRACTIONAL LASERS

Though numerous classifications of acne scars have been proposed,[21-23] the simplest way is to divide them into

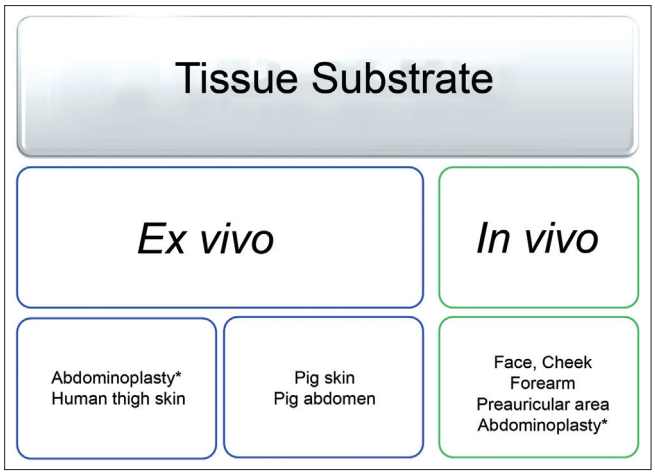

Figure 2: An overview of the various substrates used for histological studies: *Abdominal tissue can be used either intraoperatively or after harvesting it

hypertrophic and atrophic scars. The fractional lasers are predominantly used for the atrophic scars [Figure 3a]. The clinical implication of the depth of penetration of the lasers is particularly relevant in treating acne scars.[21-23] The rolling scars are a consequence of the destruction of the subcuticular fat,[21-23] which leads to abnormal fibrous anchoring of the dermis to the subcutis [Figure 3a].[21-23] Clinical experience suggests that they are amenable to lasers that penetrate up to the papillary dermis. Ice pick scars are narrow (<2 mm), deep, sharply marginated epithelial tracts that extend vertically to the deep dermis or subcutaneous tissue apex and it is believed that their depth is below than the depth of conventional skin resurfacing options and they rarely respond to fractional lasers.[21-23] Boxcar scars may be shallow (0.1–0.5 mm) or deep (≥0.5 mm) and are most often 1.5–4.0 mm in diameter [Figure 3a]. Logically, shallow boxcar scars and most deep boxcar scars are amenable to fractional lasers.

There are some basic tenets[21] in the treatment of acne scarring, the first being that there is no 'magic wand' therapy for all cases; each scar and each patient must be treated individually and the scar topography is usually the target of most interventions. The second tenet is that deep scars invariably require excisional surgery but even this leads to a modified contour while the scar is not completely effaced.

As acne scars are usually a mix of ice pick, boxcar and rolling scars, the final effect of fractional lasers would largely depend on the predominant scars and the type of laser used. The depth–width ratio (DWR) for most fractional lasers is about 4–5.[6] The higher the DWR, more the dermal volume that can be thermally damaged. As the width of most fractional lasers is almost the same due to the intrinsic quality of the fractional 'technology', the

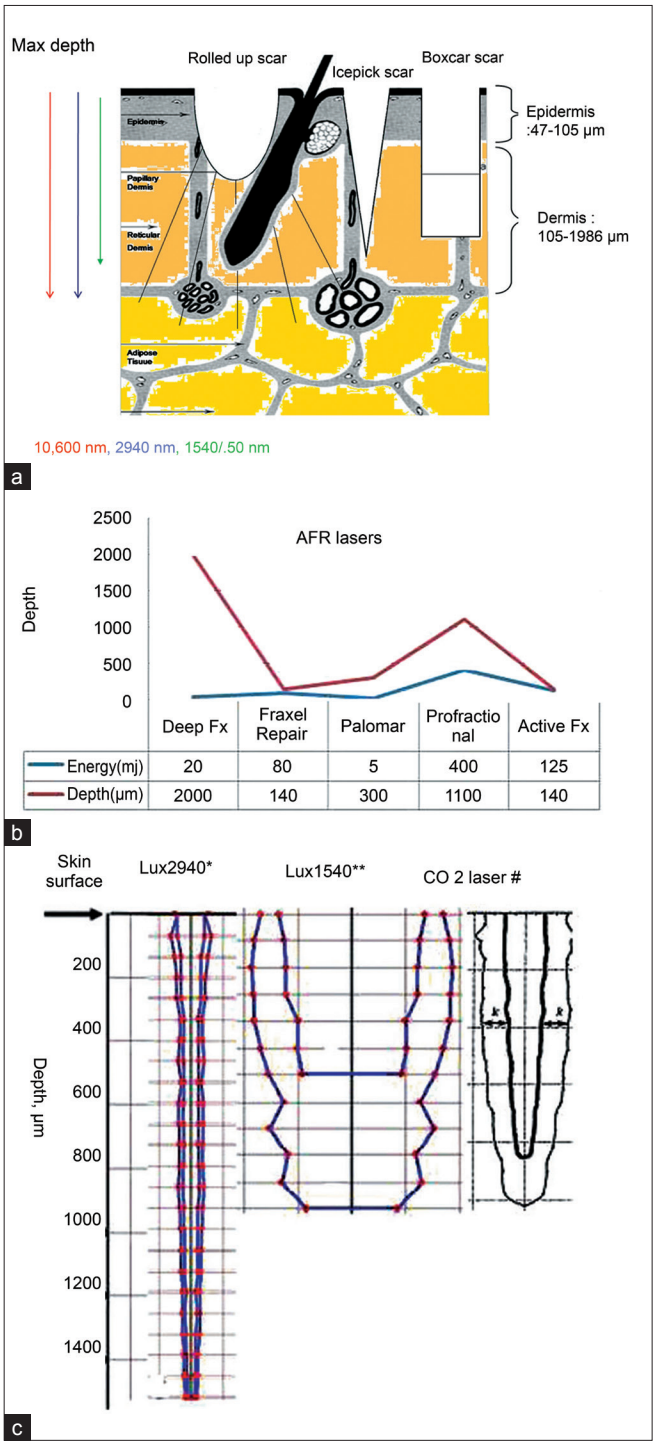

Figure 3: (a) A representation of the types of acne scars and the mean depth of penetration of fractional lasers on the facial skin (based on both *ex vivo* and *in vivo* data). (b) A comparison of the dose and depth achieved with AFR.[13] DeepFX, ActiveFX and Fraxel repair are fractional CO_2 lasers while the Palomar and Profractional are fractional Er:YAG lasers. (c) A comparative MTZ lesion reconstruction plots of three fractional lasers.[11,14] The plotted shapes have an outer and inner diameter corresponding to the zone of coagulation and ablation. *k* represents the zone of necrosis. *Lux 2940, 3 pulses, 24 mJ 0.25 ms and 21 mJ 5 ms,[11] **lux 1540 zoom 84 mJ, 15 ms),[11] #CO_2 23.3 mJ[14]

depth is the variable factor that may play a predominant role in fractional laser efficacy. Figure 3b gives an overview of the depth achieved but these data[1-20] are largely from *ex vivo* substrates.

INVASIVE (HISTOLOGICAL) ASSESSMENT OF FRACTIONAL LASER

The varied indications of the fractional lasers have not been consistently backed by site-specific histological studies.[24] Histological data are essential to arrive at proper dosimetry and various issues have to be ironed out with regard to histological analysis, some of which will be discussed in this review. Our focus will be in context to acne scars which is a useful template to extend the use of the fractional lasers for other indications. The data from the existing studies [Table 1] are variable and lack homogeneity as there is a marked variation in the histological parameters and laser settings used.

Substrate studied

Though fractional lasers are widely used for facial[25-28] dermatological indications, there are only two studies that studied the histological effects of lasers on the facial skin.[20,29]

The other studies where facial skin was studied were either not formally directed towards histological assessment[18] or could not demonstrate a marked clinical improvement[20] even though there was a histological increase in collagen and elastin.

Another issue is that the substrates used in studies are *ex vivo* models[1,2,4-6,8-18] and their replicability on facial skin is doubtful [Figure 2]. Extrapolating data from studies done for rhytides[25-27] are not useful as the dose and depth required are less as compared to acne scars. Also, in rhytides,[10,12,15] the periocular area is targeted, which is not the site for acne scars. Some studies employed a two-step procedure where the histology was done on *ex vivo* animal or *ex vivo* human model,[10,12,15] and the clinical evaluation was done on the *in vivo* facial skin. This is not scientifically replicable as there are differences in the tissue responses in different substrate models.[29] Some studies have tried to simulate the *in vivo* characteristics by maintaining the temperature of the *ex vivo* skin samples at 37°C but in spite of stringent quality controls, it is difficult to replicate a live human tissue.[6]

A commonly used substrate is the abdominoplasty tissue[9,12-14,16,29] which has been used in both *in vivo* and *ex vivo* settings. This tissue has anatomically a thick (almost 3 cm) fibroadipose tissue, which is not representative of the histology of the facial skin.[18] Thus the laser tissue dynamics cannot be extrapolated to the facial skin. The marked differences between the facial skin and abdominal skin in terms of moisture content, sebaceous glands and vascularity affect the histological depth achieved on the facial skin while using settings derived from the extrafacial skin. Instead of dispersing energy in a straight line from the epidermis to the dermis, the laser energy probably travels laterally along blood vessels, diverges around sebaceous glands, or possibly travels down a hair shaft without damaging the surrounding skin.[29] Thus the depth achieved on the facial skin is less as compared to the abdominal skin by a factor of 28%.[29]

The importance of this is that the depth of penetration provided by various laser manufacturers does not account for this and thus the dosimetry suggested is probably not reproducible for use in facial acne scars.

Even if we factor in the accepted variability of depth,[29] there is very little *in vivo* data on the histological depth of the normal facial skin which are crucial to extrapolate the variable depth generated by various fractional lasers.[1-20] It has been estimated that the facial skin depth (forehead, nose, medial and lateralcheeks, lips and chin) is about 2196 μm, which is composed of the epidermis (105 μm), papillary dermis (105 μm) and reticular dermis (1986 μm).[15] The predicted depth achieved (2–5 mm) with ablative fractional lasers[11,13,18,28] on abdominoplasty tissues may not be clinically applicable or relevant on the facial skin. Moreover, any AFR with a depth beyond 2200 μm (*ex vivo* substrate) will have no additional advantage in real *in vivo* scenarios, as on the face, it will exceed the total skin depth (2196 μm).

Time of biopsy

The time of biopsy also varies in studies ranging from zero to few hours to 24 h after the laser sittings. A few studies[4,5,20,26] have also assessed the tissue after 2–3 months when the collagen remodelling takes place which is more useful as it helps to assess the end result achieved. A recent study though has found that the histological changes can be seen up to 6 months to 1 year after the procedure.[30] Thus, ideally a biopsy at 6 or 9 months would give a more relevant picture of the extent of neocollagenisation which has a role in acne scar improvement. The drawback of repeated biopsies though is that the same area cannot be biopsied each time, and thus, the second biopsy can never replicate the exact tissue response. The laser that we use (Dermablate 2940, microspot mode Ascepelion) has a demonstrable effect on collagenisation and a tissue response up to a depth of 1500–2000 μm (data provided by the company) which is evident only after 2–3 months.

Storage

This varies from freezing to storing the tissue in formalin. Paraffin-embedded tissue sections require dehydration

of the tissue samples, which causes shrinkage. This is an important point to consider when evaluating the microcolumn lesion depth and width.[1-20]

Sectioning and depth assessment

The microcolumn injury produced has roughly a conical shape. Thus a difference in sectioning angles may change the interpretation of the depth.[11,13,16] To circumvent this, a laborious technique is to take multiple serial sections so that an average tissue depth/diameter can be assessed. Another way—by no means easy—is to perform treatments in triplicate to provide an accurate representation of the tissue injury at a given treatment parameter.[13] The depth/diameter of an MTZ has also variable assessment methods. In a study by Hantash *et al*.,[5,14] the maximum dermal ablation width of the laser channels was used whereas Skovbølling *et al*.[16] used the width at a defined localisation at the interface of the lower one-third and the upper two-thirds of the microcolumn. To complicate matters, another study[6] determined the depth of each MTZ by measuring the distance from the stratum corneum level to the deepest non-viable cell within the confines of any lesion. This lack of homogeneity makes comparison of studies difficult.

It is difficult by vertical sectioning to accurately locate the widest and deepest extent of an MTZ. A slight change in the orientation of the tissue and the sectioning angle can give variable tissue depths. Histologically, MTZ or microcolumns created are usually cylindrical, and conventionally, sectioning is done perpendicular to the surface (vertical sectioning).[2,3,4,12] This requires that the sample must be oriented to ensure that vertical sections are perpendicular to the skin surface, presuming that the MTZ have the same orientation. Presuming that the MTZ has a uniform diameter, which is not the case, the depth can be accurately measured with a single vertical section anywhere through the MTZ. However, logically the measurement of diameter requires vertical sectioning through the axis of the MTZ which can only be done with serial vertical sectioning where the section with the maximum width is presumed to be that closest to the axis. This process requires multiple sequential slides and most centres rarely employ this procedure which is a very laborious process with a high probability of human error.[11] This is also because most histopathological centres are burdened with routine pathology tests, and assessment by horizontal sectioning of fractional lasers may not be a priority. To circumvent most of these issues, Zelickson *et al*.[11] suggested replacing the conventional vertical method of sectioning with the horizontal sectioning. Though Zelickson *et al*.[11] have demonstrated that the horizontal sectioning method is superior, they were ably assisted by the semi-automatic fractional histology interpretation routine (SAFHIR) software, a luxury that most other centres are not endowed with. The use of a software-based analysis of images (J program) was used in another study[18] which makes comparison of data with the other studies difficult.[1-10,12-18,20]

Stains used

Apart from haematoxylin and eosin (H and E), the various stains employed include nitro blue tetrazolium chloride (NBTC), lactate dehydrogenase (LDH) and Masson's trichrome stains, and the demanding terminal deoxynucleotidyl transferase-mediated X-dUTP nick end labelling (TUNEL) stain which requires the fluorescence excitation microscopy for evaluation. If H and E is used as a marker for thermal damage, it is more likely to result in a relatively smaller width than NBTC-stained lesions. This is because the threshold to detect thermal cell damage is lower for the NBTC stain as compared to H and E staining. Some studies use specialised immunohistochemical stains for heat shock proteins and elastin (anti-HSP70, anti-HSP47 and anti-elastin).[30] Though there are merits of using stains like LDC and TUNEL stains, there is no homogeneity in the stains used in various studies which adds to the difficulty in comparing studies.[2-20,28]

NON-INVASIVE ASSESSMENT

Several theoretical and non-invasive biomedical, optical and acoustical methods have been used in the evaluation of tissue damage arising out of fractional lasers impacting the skin.[30-34] Most of these like optical coherence tomography and ultrasound-modulated optical tomography are expensive, require skills and have a limitation of depth up to 1.5 mm.[30-34] As the dermis scatters the light strongly, the signal to be detected is small; thus, biomedical optical engineering devices are difficult to standardise for skin pathologies. A recent study[33] has used high-resolution ultrasound imaging to evaluate the effect of the CO_2 fractional laser. It was found that while the dermal thickness increased, the epidermal thickness was unchanged which means that though dermal collagenosis might occur, the skin retains the sequelae of ageing.

LASER PARAMETERS

Wavelength

It has been observed that the optical window of 1.2 and 1.8 μm is optimal for skin treatments as the penetration depth of the laser is up to the upper dermis. On comparing three wavelengths, 1.32, 1.45 and 1.54 μm, it was found that 1.54 μm was well absorbed by water yet virtually not at all by melanin, allowing deep penetration into the skin with minimal side effects in the pigmented skin. Thus, this was the most commonly used wavelength initially. As ablative fractional lasers evolved, they were found to have a depth that was more than that for the nonablative fractional lasers [Table 1]. What is relevant to note is that on analysing the results of the various

histological studies,[1-20] it is obvious that as far as the facial skin is concerned, there are very little difference in depth if the right dose is used, and at least in theory, all the three wavelengths (1540, 2940 and 10600 nm) should be clinically effective in treating acne scars [Figure 3a].[35]

Pulse number/duration

It has been shown that both the DeepFX[14] and Lux 2940[11] achieve a greater depth if there is an increase in the number of pulses. It has been shown that[12,25] if the pulse width of the AFR (2940 nm) is increased to 2 ms, the heat impacting on the dermis increases whereas if it is lowered (0.25 ms), a deeper penetration can be achieved. In a study on an *ex vivo* model with the fractional erbium laser, reducing the pulse duration from 2–5 to 0.25 ms caused a 20–25% deeper penetration of the microbeams.[12] A similar effect has been noted with the fractional carbon dioxide laser[16] where the depth increased by 10-fold when the pulse duration was reduced from 5–10 to 2 ms.

Passes/energy density

The density of the 1550-nm lasers ranges from 100 to 1000 microbeam/probe.[9,6,36,37] Obviously, higher the density, more the dose (joule/cm^2) that can be achieved. By manipulating the density of the microcolumns, a more aggressive treatment may be achieved in a single treatment. This can be achieved by giving multiple passes. However, when retreating or passing over a treated area multiple times, a non-uniform damage is achieved.

Regardless of the mode of delivery, with repeated passes all devices lead to a randomly distributed placement of MTZs. Clustering of MTZs (i.e. placement of individual MTZs into a location that was already damaged) can occur with subsequent passes and lead to alterations in the MTZ size and fill factor. It can be conjectured that the approach of giving multiple passes to achieve a high density and energy is logical and in spite of numerous passes (1–30), the healing can still occur.

A study by Trelles *et al*.[25] using the Sciton Profile Er:YAG system showed that multiple passes produce photothermally related effects in the dermis. The repeated pulses first ablated the epidermis which created a 'window' that helped the dermis with its high water content to heat up adding to the residual thermal damage zone. This effect is useful for acne scars as there is collagen remodelling.

Contrary to this view, another study[9] pointed out that multiple-pass treatments, which are recommended for all fractional laser devices, created an increased broad-based destruction of the DEJ over a larger surface area. This was because the higher energy multiple-passtreatments demonstrated a more superficial treatment than anticipated for their respectivefluences that may be attributed to the potential absorption and concentration of energy or heat at the DEJ.

The balance between wound healing, neo-collagenesis, coagulation and remodelling for optimal skin tightening and rejuvenation with fractional technology warrants further investigations. Bulk damage can be caused with higher density settings at modest microbeam energies or, conversely, with higher microbeam energies at modest densities.[37] In Asian skin, it has been confirmed that it is better to increase the dose/microbeam than increase the density to achieve an equivalent energy.[37]

Histological depth of fractional lasers

The depth of penetration of fractional lasers is probably a crucial parameter for improving acne scars.[1-20] The most important factors predicting the depth are the density and the energy settings. An increased energy level leads to a non-linear increase in the depth of penetration. A study comparing two NAFR[6] lasers (Fraxel SR750 vs SR1500) found that the depth increased from 386±68 μm to 826±108 μm on increasing the dose from 6 to 40 mJ using the Fraxel SR750. The newer Fraxel SR 1500 achieved a deeper penetration (470±56 μm, 1408±53 μm) for proportionately higher energy (6–100 mJ). It is striking that for lower energies (up to 20 mJ), there was no difference or only a marginally significant difference in the lesion width and depth between the two devices. However, for higher energies (over 20 mJ), there was a statistically significant difference in diameter and depth. A study using the fractional CO_2 laser found a proportional increase in the depth and width depending on the density and dose (0.03–0.15 mm; dose 90–100 mJ).[18] This study used density levels 1–3 which predicted the overlap ranging from −10% to +10%. An increase in the density caused an increased depth of ablation while an increase in the dose (energy) affected the width of coagulation. Paradoxically, a study which used the fractional Er:YAG laser (2940 nm)[17] revealed that though the standard predicted depth of ablation ranged between 25 and 1500 mm, the actual achieved depth was much greater than the predicted.

Very few studies have formally compared the laser depth of fractional lasers. Farkas *et al*.[13] demonstrated that even within the same technology, AFR (ActiveFX, DeepFX, Fraxel repair), the depth of penetration may vary [Figure 3b]. It was initially believed that it is not possible to compare NAFR and AFR as the tissue responses would vary. This is not the case as studies with detailed tissue depth analysis[11,14] have shown [Figure 3c] that the tissue response is remarkably similar. All the three laser systems (2940, 1540 and 10,600 nm)

produce an MTZ which has an inner and outer diameter, a zone of ablation and a deeper zone of coagulation, and thus, probably have similar effects on the skin. The only difference is that the AFR lasers have less width of damage than the NAFR [Figure 3c].

The existing studies [Table 1] have not formally explored the correlation between depth and energy. For the 1550-nm fractional laser, it has been previously estimated that for every millijoule of increased energy, the depth of coagulation increased roughly by a factor of 10 μm (10 mJ/100–150 μm).[9] Similarly, for the fractional CO_2 (10,600 nm), a formula to estimate the depth has been proposed, where the ablation depth (μm)=12 μm/mJ×energy level (mJ).[16] The fractional erbium:YAG laser has a predicted ablation that starts above a defined threshold (ablation threshold), which is 1.6 J/cm². Energy densities less than 1.6 J/cm² heat up the tissue and may lead to thermal damages. Each subsequent increase in energy (J/cm²) ablates 5 μm of the tissue. Thus, the depth is predicted by the formula depth, D (μm)=5 μm/J/cm² × (F-N×1.5 J/cm²), where F is the fluence (J/cm²) and N is the number of stacked pulses (Dermablate microspot mode; Ascepelion).

On statistical analyses of the existing data [Table 1], we found that there was a statistical correlation between the dose and depth achieved by the three fractional lasers [Table 2 and Figure 4a–c]. As different fractional lasers have different settings (mJ/microbeam), it is practically useful to statistically analyse the mean depth (μm/mJ) achieved by the lasers used [Table 2]. The data given in Table 2 can give a rough assessment of the probable depth that can be achieved depending on the dose and laser used.

The two studies[11,14] where comparative data are available regarding the depth achieved show variable results. While Figure 3c reveals that the fractional Er:YAG laser is superior to other lasers, Figure 3b shows that the fractional CO_2 laser achieves a depth comparable to the fractional Er:YAG laser. Interestingly, Figure 3c shows that the NAFR (1440 nm) laser achieves a similar depth as the AFR (10600 nm) laser. These variations are probably because of the variable settings and substrates used. To overcome these variations, we statistically analysed the depth of penetration [Table 1] achieved by various fractional lasers, to average out the minor variations in doses. Our analysis revealed that the mean depth of penetration varied [Figure 5a–c], with NAFR laser (Er:glass, 679 μm) achieving a depth that was less than that of the AFR lasers (Er:YAG, 825 μm, and CO_2 895 μm; P<0.05). On the facial skin though, the difference in the histological depth of the three fractional lasers (1540, 2940 and 10600 nm) would probably be similar for superficial atrophic acne scars [Figure 3a]. For deeper scars, the AFR lasers, at least in theory, would be better as they penetrate deeper than NAFR lasers, but as seen in Figure 3a, the depth achieved is probably not deep enough to ameliorate the ice pick scars [Figure 3a].

Thus on statistical analysis of the existing data, we can conjecture that there is evidence, both mathematical and substrate based, which can help predict the depth achieved by a particular laser. But the extrapolation of existing data to *in vivo* facial skin would depend on replicating the exact settings (pulses, duration, passes, energy and density) which is rarely the case. This and the variability of the tissues studied [Figure 2] probably account partially for the disparity in subjective and objective clinical results for acne scars [Table 3].

CLINICAL RESULTS

A summary of the various salient studies using the AFR (carbon dioxide laser)[38-44] and NAFR (1550/1540 nm)[7,19,20,45-60] is given in Tables 3 and 4]. The apparent lack of satisfactory results with NAFR have prompted studies combining the 1550-nm erbium:glass laser with AFR,[54] long-pulse, 1064-nm neodymium-doped yttrium aluminium garnet (Nd:YAG) laser with AFR[53] or the consequent therapy of NAFR (1550 nm) with chemical reconstruction of skin scars (CROSS) and subcision.[56]

On the face of it, the most obvious drawback of the studies is the variation in the laser and settings used and the lack of histological data in relation to acne scars, except for three studies.[7,20,53] The most glaring drawback though is the non-uniformity of acne scar assessment scales. As has been seen in Figure 3, the success of the lasers largely depends on the type of scar (ice pick,

Table 2: Correlation between the energy and the depth of penetration of fractional lasers

Statistical parameters	Er:glass		Er:YAG		CO_2	
	Energy (mJ/MTZ)	Depth (μm)	Energy (mJ/MTZ)	Depth (μm)	Energy (mJ/MTZ)	Depth (μm)
Mean	40.57	637.28	110.83	837.5	74.21	895.6087
Spearman's correlation	0.77		0.82		0.17	
P value (two-sided)	0.001*		0.05†		0.42†	
Depth/mJ	Lux (12.9); Fraxel (28.5)		Lux (54.38); Profractional (3)		ActiveFX (6.28); DeepFX (53.66); Fraxel repair (20); MedArt (12)	

*P<0.005. †P<0.5

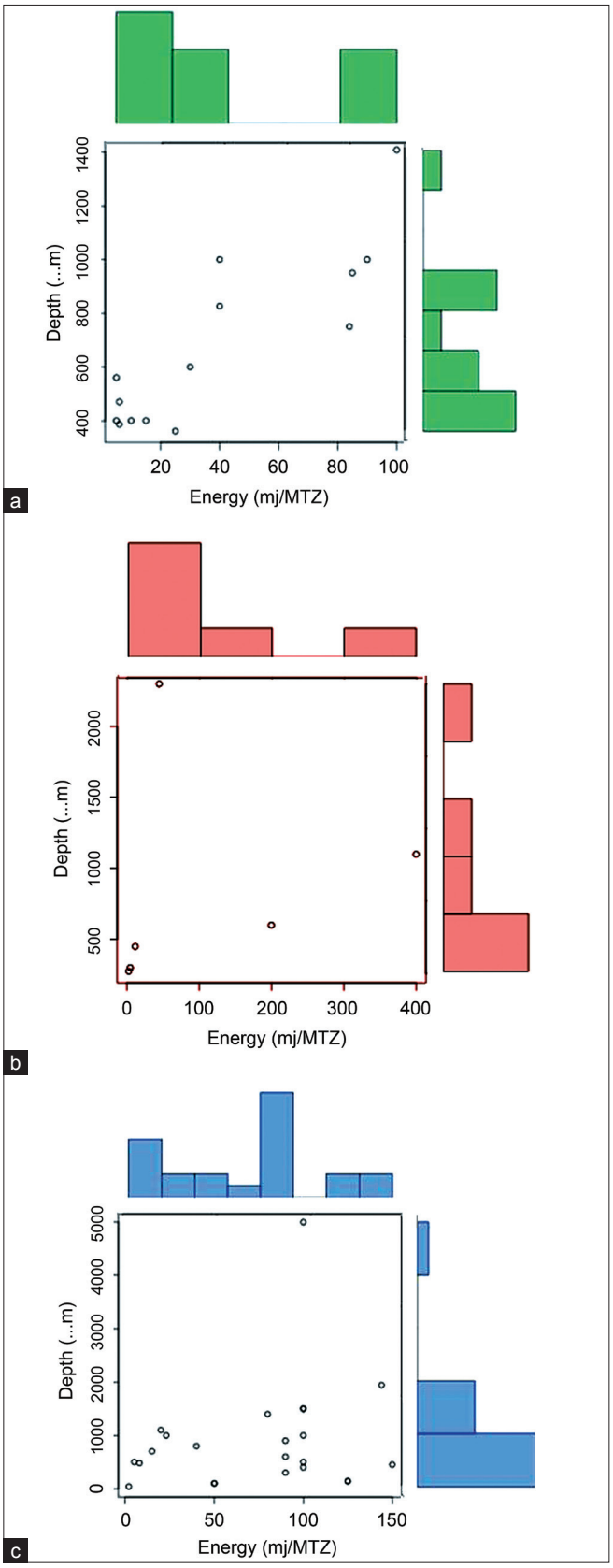

Figure 4: (a) An illustration of the correlation of the energy/depth of the Er:glass laser. (b) An illustration of the correlation of the energy/depth of the Er:YAG laser. (c) An illustration of the correlation of the energy/depth of the CO_2 laser

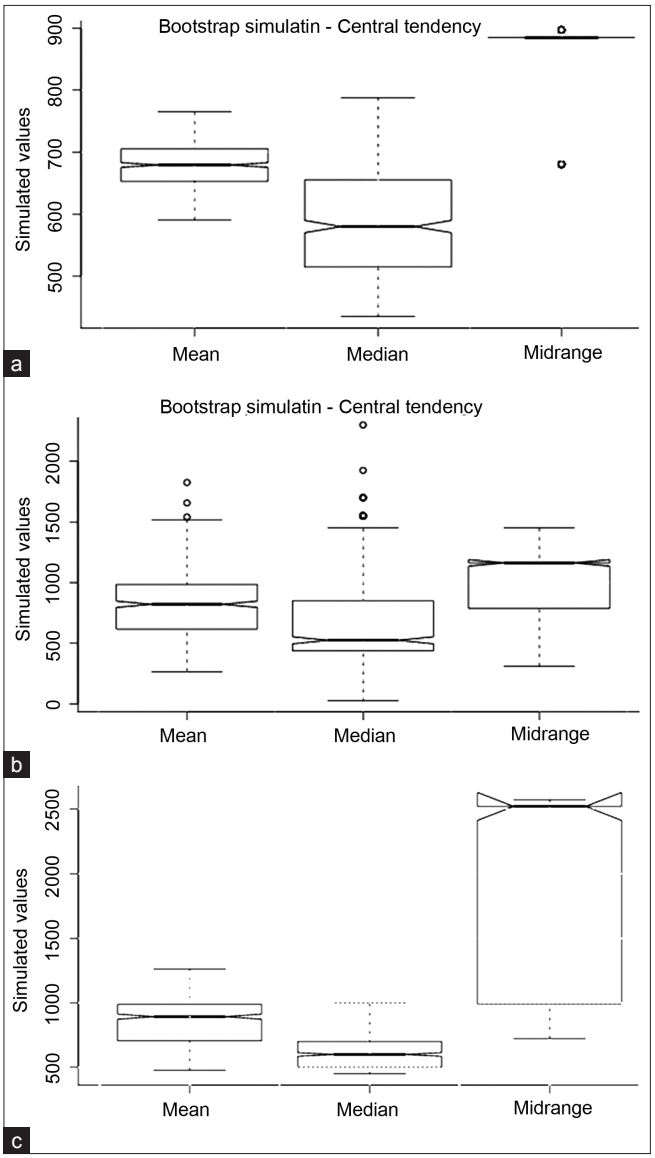

Figure 5: Bootstrap plot – central tendency analysis of the depth of penetration (*X*-axis, μm) of the (a) Er:glass laser (mean 679 μm, median 580 μm, mid-range 884 μm), (b) Er:YAG laser (mean 825 μm, median 525 μm, mid-range 1162.5 μm) and (c) CO_2 laser (mean 895 μm, median 600 μm, mid-range 2520 μm)

boxcar or rolling) and except for three studies[7,50,56] the other studies have not specified the scar studied. Studies that fail to distinguish between different subtypes of acne scars (e.g. rolling, boxcar, ice pick scars), have little objective value as the whole purpose of the fractional laser is to judge its use in specific scar subtypes. The only validated scoring scale (ECCA grading scale; échelle d'évaluation clinique des cicatrices d'acné) has not been used with NAFR/AFR lasers. Ice pick scars do not usually respond well to fractional laser therapy which has been echoed in a study by Geronemus.[7] It has been the experience of other authors[22,23] also that the depth of the ice pick scars is deeper than that reached with

Table 3: Summary of studies of AFR fractional laser in acne scars

Authors and number of patients studied	Scars treated	Laser	Dose	Assessment subjective	Assessment objective[a]	Results and improvement
Chapas *et al*.,[37] *n*=13	Moderate to severe acne scars	Fraxel repair	Energies of 20–100 mJ/pulse, spot size=120 mm, 10–400 MTZ/cm^2, total densities of 200–1200 MTZ/cm^2, 2–3 sittings	Quartile scale	Primos imaging	The result ranged from 43% to 79.9% with a mean level of improvement of 66.8%
Walgrave *et al*.,[38] *n*=30	Moderate to severe acne scars	Fraxel repair	20–100 mJ/pulse, 600–1600 MTZ/cm^2, 4 sittings	Quartile scale	Photography (but subjective observer evaluation)	1–25% at 3 months, 23/25 –improved
Weiss *et al*.,[39] *n*=15	Non-acne scars	Fraxel repair	20–100 mJ/pulse, 100–300 MTZ/cm^2 per pass, density of 100–900 MTZ/cm^2, 1–3 passes, 3 sittings	-	Yes, Primos	Volume improvement=26.8–57.5% Mean improvement in scar volume=38.0% Maximum depth of reduction 26.3–40.9%, with a mean reduction of 35.6% (at 6 months)
Cho *et al*.,[40] *n*=20	Acne scars	DeepFX ActiveFX	10–20 mJ, density 2, and 300 Hz using the DeepFX mode, 2 Sittings	Yes	-	1 pt =76–100% 9 pt =51–75% 7 pt =26–50% 3 pt =minimal to no improvements
Manuskiatti *et al*.,[42] *n*=13	Atrophic acne scars	Fraxel repair	3 sessions	Subjective (clinical evaluation by two blinded dermatologists)	Objective (ultraviolet A – light video camera)	85% of the subjects had 25–50% improvement in scars while 62% of subjects had a 50% improvement in their scars (6 months)
Chan *et al*.,[41] *n*=9	Acne scars	Fraxel repair	30–70mJ, Fraxel repair, 30–45% coverage, 1 sitting	-	Photographic evaluation	Only mild to moderate improvement after a single treatment sitting; 86% had a subjective improvement
Wang *et al*.,[43] *n*=5	Moderate to severe acne scars		Energy 28 J/cm^2; pulse width 2.5 ms; spot size 300 μm; penetration depth up to 500 μm; degree of skin coverage 20%; single pass; 2 treatment sittings	Quartile scoring	Photographic evaluation	At 2 months post-treatment, all five subjects showed some clinical improvement (four: Mild improvement; one: Moderate improvement)

[a]Though photography is touted as an objective measure, its evaluation by visual comparison is never accurate as depth analysis requires a 3D perspective. At present Primos is the only objective

conventional skin resurfacing options. Thus, ice pick scars with deep bases are ideally treated with punch excision. Another study[56] which compared NAFR with CROSS found that though the CROSS technique was marginally better for ice pick scars, statistically this was not significant. It was suggested that CROSS could be used in focal areas with the fractional laser for the whole face. In our experience, focal TCA in pigmented skin leads to marked hyperpigmentation which makes it, in our opinion, not a desired line of therapy. The third study[50] that subclassified the scar types used a quartile scale and reported patient assessment which is always superior to the physician assessment and thus has less validity.

Another problem is the lack of an objective assessment technique. As the acne scars have a three-dimensional quality, it is impossible to objectively assess it by visual comparison. The only objective tool is the software based on an optical profiling system (Primos Imaging; GFM; Tetlow, Germany),[61] which allows high-resolution topographical imaging of cutaneous scars and calculation of quantitative volumetric and depth changes in atrophic scar volumes before and after treatment. This has been used only in two studies[38,40] and one of them[40] did not study acne scars. All the other studies[7,19,20,39,41-60] use either a patient or observer-dependent quartile scoring which is not an accurate indicator of acne scars improvement. The use of photography is also meaningless as it is again assessed by a quartile or self-devised scoring pattern by an investigator wherein the assessment of the depth

Table 4: Summary of studies of fractional Er:glass (NAFR) lasers in acne scars

Study	No. of patients	Scars treated	Type of laser	Dose	Density	Total dose	No. of passes	Sittings	Results[e] (improvement/ assessment)
Geronemus[7][a]	17	Ice pick[d] box car rolling	Fraxel	5 mJ/MTZ	2000–3000/ cm^2	1.6–3 J/cm^2	10	3–5	Results: overall improvement 22–66% Ice pick, 25–50%; boxcar, 22–62%; rolling scars, 29–67%. Assessment: Digital photography, high-resolution typo-graphic imaging, and patient-completed questionnaires
Hasegawa *et al.*,[46] III/IV Skin type	10	Not stated	FraxelSR 1500 nm	6–30 mJ/MTZs	1000–1500/ cm^2	6–45 J	4	3	Results: 4 pts– excellent, 3 pts – good, 3 pts – fair. Assessment: Subjective
Alster *et al.*,[47]	53	Mild to moderate atrophic scar	FraxelSR 1500 nm (Ist generation)	Fluences of 8–16 J/cm	125–250/cm^2	1–4 J (4–6 kJ)	8–10	2–3	Results: 51–75%. Assessment: Subjective/ photography with quartile scoring)
Glaich *et al.*,[48]	2	Atrophic scars	FraxelSR 1500 nm	6–18 mJ/MTZ	1250–2000/ cm^2	NA	NA	5	Results: Physician clinical assessments revealed a moderate to marked improvement in atrophic acne scarring in all patients. Assessment: subjective
Lee *et al.*,[49] IV/V Skin type	27	Rolling,[d] box car, ice pick scars	1550–nm Fraxel	12–20 mJ/MTZ	750–500/cm^2	9–30 J	3–4	3–5	Results: All types of scars improved. Patient assessment: Excellent improvement in 8 (30%), significant improvement in 16 patients (59%) and moderate improvement in 3 patients (11%). Mean=51–75%. Assessment: Subjective/photography, quartile scoring)
Weiss *et al.*,[50]	500	Mild to moderate scars	1540-nm Lux Palomar	NA	100/cm^2	NA	3–5	NA	Results: Physician evaluation: 50–75% median improvement Patient evaluation: 85% of patients rated their skin as improved. Assessment: Subjective
Chrastil *et al.*,[51]	29	Mild to severe	IInd generation erbium-doped 1550-nm laser (Fraxel SR1500)	35–40 mJ/MTZ	Levels varied from 7 to 10 and 'advanced level 1'	NA	8–10	2–6	Results: 50–75% improvement in facial and back acne scarring 5 pts, >75% 5 pts, 25–50% and 1 pt <25% response Assessment: Photographic evaluation
Cho *et al.*,[54] IV	12	Mild/ moderate atrophic scars and pores	Mosaic LC 1550 nm	20–28 mJ/ MTZs	400–900/cm^2	8–25 J	Dynamic operating mode	3	Results: 3 pts, 76-100% 5 pts, 51–75% 2 pts, 26–50% 2 pt, 0% Mean improvement=26–50% Assessment: Subjective/ photography with quartile staging

(*Contd...*)

Table 4: *(Contd...)*

Authors	No. of patients	Scars treated	Type of laser	Dose	Density	Total dose	No. of passes	Sittings	Results[e] (improvement/ assessment)
Yoo *et al.*,[20][a]	16	Acne scars	Lux 1540 m	70 mJ/MTZ	Density, 100 MTZ/cm^2/pass	–	3	3	Mild to moderate improvement in acne scars Increase in collagen and elastin Assessment: Subjective
Hu *et al.*,[53] [b] Types III/IV Skin type	45	Mild to moderate atrophic scars	(1) Fraxel 750 (2) Fraxel 1500	(1) 15–20 mJ/ MTZ (2) 30–40 mJ/ MTZ	(1) 1000–2000/cm^2 (2) 392–520/ cm^2	11.5–40 J 11.7 –20.8 J	(1) 8 (2) 8	1	Results: 60% pts, good to excellent. 40% pts, none to fair. No significant d/fb/wtwo lasers Assessment: Subjective
Cho *et al.*,[54] [c]Types II/IV Skin type	1	Atrophic scars	Fraxel SR1500 Combined with AR (ultrapulse CO_2)	40 J/cm^2	(17% coverage/cm^2/ pass)	NA	8 passes	1	Scars improved
Kim *et al.*,[55][b] Types IV/V Skin type	20	Rolling and ice pick[d]	Mosaic LC, 1550 nm vs. CROSS	30–42 mJ/MTZ	300–350/cm^2	9–11.2 J	1	3	Results: Rolling and ice pick scars both improved by 25–75%. For rolling scars Er:glass is better. For Ice pick CROSS is better Assessment: Subjective/ photography, quartile
Kang *et al.*,[56] [c] Types IV/V Skin type	10	Atrophic scars	Mosaic LC1550 nm+ TCA+Subcision	25 mJ/MTZs	350–800/cm^2	8.9–20 J	4	4	Results: All subjects improved by 55% Assessment: Objective scar evaluation but subjective improvement scores
Cho *et al.*,[57][b]	8	Mild to severe scars	Fraxel SR1500 vs. fractional CO_2	40 mJ/cm^2 From 10–20 mJ to 50–100 mJ/ MTZ	17%/cm^2/pass	NA	NA	1	Results: Both lasers were equally good. All patients improved 26–50%. Assessment: Subjective
Hedelund *et al.*,[58](RCT split face study)	10	Atrophic scars	Star Lux 1540 nm	70–100 mJ/ MB, 10-mm handpiece (100 MB/cm^2), 15-ms pulse duration	–	21–40 J/ cm^2	3–4 passes	3	Results: Moderate –marked improvement in 50% pts. No PIH. The observer score came down from moderately even to mildly even scar texture Assessment: Subjective
Mahmoud, *et al.*,[59][b] Types IV–VI Skin type	15	Acne scars	1550-nm laser (Fraxel) with Trilumina cream	2 groups, 10 mJ, 40 mJ/ MTZ.	17% of the treated area	NA	8 passes	5	Results: Equal response in both groups, significant improvement seen by patients but with PIH. Observer improvement =1–25% No difference in doses Assessment: Photography, quartile

[a]In this study, histology was done. [b]This was a comparative study. [c]In this study, NAFR was combined with other modalities. [d]In this study, acne scars were subclassified as ice pick, boxcar and rolling. [e]Though photography is touted as an objective measure, its evaluation by visual comparison is never accurate as depth analysis requires a 3D perspective. Only Primos is a truly objective tool

of the scar is impossible. These factors combined with a lack of a proper acne scar type classification in most studies[7,19,20,38-45,47-49,51-55,57-60] makes the apparent wealth of data difficult to interpret *vis-à-vis* the type of acne scars. In spite of the disparate parameters,[7,19,20,38-60] the results are uniformly expressed as a percentage improvement. We statistically compared the improvement (in%) between the studies using NAFR and AFR lasers,

excluding the studies where various modalities were combined.[53,55,57] The mean improvement of NAFR (50.2%) was better than that of AFR (42.62%;. Figures 6a, b and 7). This paradoxical result with NAFR though is based on subjective assessment. The only study that evaluated objective improvement[40] with AFR showed a mean improvement in scar volume by 38% with a scar reduction of 35.6%. This gives a realistic improvement that can be achieved by probably the most effective fractional laser technology at present.

CONCLUSIONS

The clinicohistological correlation on the basis of the lasers used has numerous shortcomings and requires standardising in terms of histological evaluating and uniformity in laser parameters [Tables 5 and 6]. Also not all brands of lasers have studies (histological or clinical) to back their claims. The diversity in AFR (10600 nm, 2940 nm) in terms of spot sizes, pulse durations and intensity ranges makes histological data difficult to compare. The extrapolation of *ex vivo* data to standard clinical settings is not easy as the tissue response varies more so on the face.[29]

Comparative well-done studies with standard protocols are few[6,10,11,13,27,29,58] and have been rarely been done for acne scars.[20] Except for one study comparing AFR and NAFR[58] in acne scars that showed a marginal difference,

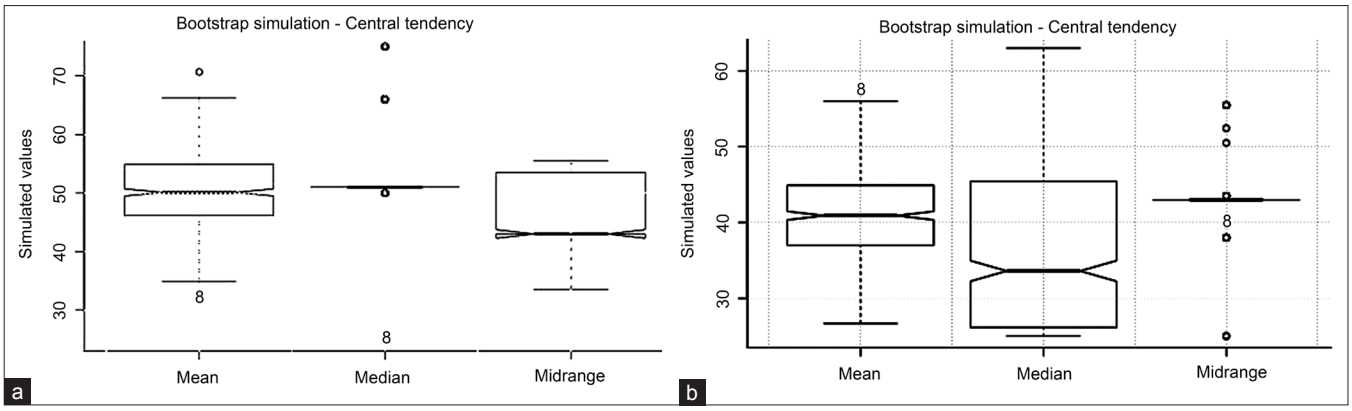

Figure 6: A figurative representation of the estimation results of bootstrap analysis of improvement (*Y*-axis) using the (a) Er:glass laser (mean 50.2, median 51, mid-range 43) and (b) CO_2 laser (mean 42.62, median 40.69, mid-range 43.45)

Table 5: Variations in published data due to histological assessment

Histological variables	Details	Comments
Sites of biopsy	Usually non-facial skin, on the face, commonly, the preauricular skin	Need for histological data from the face specially in patients with acne scars
Sample	Mostly *ex vivo* (animals/humans)	The lasers tissue dynamics vary across studies
Time of biopsy	Varies from immediately to a few hours to weeks	Ideally, apart from the immediate biopsy 2-, 3- and 6-month biopsies are needed to study the tissue response. The drawback is that it can never be done at the same site
Sectioning	Studies have used vertical and horizontal sectioning	Though horizontal sectioning is better, interpretation requires special skills
Stains	H and E, LDH, TUNEL staining	No standardised stains in studies

Table 6: Variations in publication data due to types of fractional lasers used

Laser variables	Description	Comments
Laser type	1540 m, 1550 nm, 2940 nm, 10600 nm	There is a difference between the laser tissue dynamics of different lasers
Pulses	The pulses used vary from 1 to 5	Not standardised for machines and it is important as there is a non-linear increase in depth depending on the pulses used
Pulse duration	For the 2940-nm laser, the pulse duration varies from 2–5 ms to 0.25 ms and for the 10,600-nm laser, from 10 ms to 2–5 ms	Smaller the pulse duration more the depth of the lasers. This makes comparisons between lasers difficult. Also the energy used is never uniform even with the same laser
Density (passes)	This is different in each machine. Also the number of passes used varies from 1 to 4 which impacts on the density	The density impacts on the side effects and too many passes makes it akin to a ablative laser resurfacing
Dose	Studies on NAFR (1550 nm) give doses in mJ/MB while for AFR either in mJ/MB or J/cm^2. Also, some studies use two lasers sequentially	The non-uniformity makes comparison impossible. It is advisable to use a uniform method of reporting either mJ/MB or J/cm^2 to make comparison possible
Depth of microcolumns	As is obvious, the variable settings and lasers give differing values of the depth	The depth in μm is usually in *ex vivo* models and makes its extrapolation to the *in vivo* tissue difficult

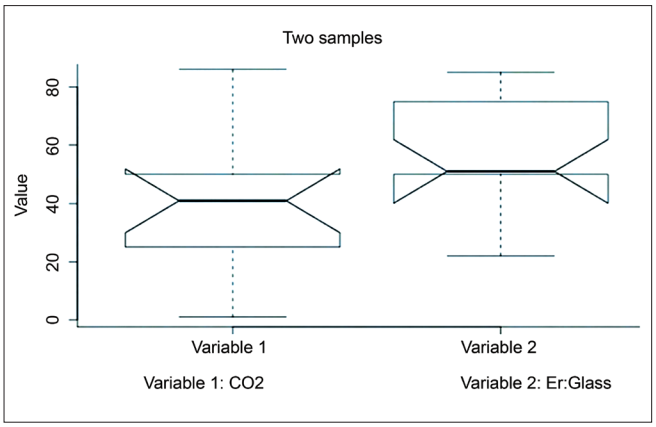

Figure 7: A comparison of the mean improvement (*X*-axis) with Er:glass and CO_2 using the Wilcoxon–Mann–Whitney test (value=60, *P*=0.22)

no other study has compared the two technologies for acne scars. Interestingly, most laser manufactures make more than one of the three major lasers (NAFR/AFR) and it would be of interest to us if the findings that they have are put in the public domain.

Thus, clinicians should avoid being influenced by promises and suggestions of the industry, which favour one device over the other.[61,62] It is thus important not to get swayed by data both published and unpublished as the non-uniformity of data collection and analysis is a hindrance for treating acne scars.[61,62] Probably, the manufacturers communicate data derived from *ex vivo* studies and it is up to the clinicians to interpret the data realistically with regard to the facial skin. It is desirable for the laser surgeons to standardise dose depth penetration by using appropriate substrates (*in vivo* facial skin) at various doses before considering interventions.

Our statistical analysis [Table 2, Figures 5–7] can enable the clinician to arrive at a reasonably accurate depth penetration to treat the individual scars. We have reliably shown that the results of the different studies are not as dramatic even by using subjective or objective analysis. Probably, the acne scars have a 'memory'[23] which is difficult to change by fractional lasers. It is likely that an appropriate balance between the depth and the collagen remodelling is necessary.[63] The underlying molecular changes are not fully understood but have been postulated to be induced by time-dependent changes in heat shock proteins, transforming growth factor β, matrix metalloproteinases, hyaluronic acid (HA) synthetases, hyaluronidases and HA, among others.[63] There is an emergent need for *in vivo* facial histological studies with objective acne scar evaluation after fractional lasers to settle this issue.

REFERENCES

1. Huzaira M, Anderson RR, Sink K, Manstein D. Intradermal focusing of near infrared optical pulses: A new approach for non-ablative laser therapy. Lasers Surg Med 2003;32:17-38.
2. Khan MH, Sink RK, Manstein D. Intradermally focused infrared laser pulses: Thermal effects at defined tissue depths. Lasers Surg Med 2005;36:270-80.
3. Manstein D, Herron GS, Sink RK. Fractional photothermolysis: A new concept for cutaneous remodeling using microscopic patterns of thermal injury. Lasers Surg Med 2004;34:426-38.
4. Laubach H, Chan HH, Rius F. Skin responses to fractional photothermolysis. Lasers Surg Med 2006;38:142-9.
5. Hantash BM, Bedi VP, Sudireddy V, Struck SK, Herron GS, Chan KF. Laser-induced trans-epidermal elimination of dermal content by fractional photo-thermolysis. J Biomed Opt 2006;11:041115.
6. Thongsima S, Zurakowski D, Manstein D. Histological comparison of two different fractional photothermolysis devices operating at 1,550 nm. Lasers Surg Med 2010;42:32-7.
7. Geronemus RG. Fractional photothermolysis: Current and future applications. Lasers Surg Med 2006;38:169-76.
8. Bedi VP, Chan KF, Sink RK, Hantash BM, Herron GS, Rahman Z, *et al*. The effects of pulse energy variations on the dimensions of microscopic thermal treatment zones in nonablative fractional resurfacing. Lasers Surg Med 2007;39:145-55.
9. Farkas JP, Richardson JA, Hoopman J, Brown SA, Kenkel JM. Micro-island damage with a nonablative 1540-nm Er:glass fractional laser device in human skin. J Cosmet Dermatol 2009;8:119-26.
10. Walgrave S, Zelickson B, Childs J, Altshuler G, Erofeev A, Yaroslavsky I, *et al*. Pilot investigation of the correlation between histological and clinical effects of infrared fractional resurfacing lasers. Dermatol Surg 2008;34:1443-53.
11. Zelickson BD, Walgrave SE, Al-Arashi MY, Altshuler GB, Yaroslavsky IV, Childs JJ, *et al*. Semi-Automated method of analysis of horizontal histological sections of skin for objective evaluation of fractional devices. Lasers Surg Med 2009;41:634-42.
12. Dierickx CC, Khatri KA, Tannous ZS, Childs JJ, Cohen RH, Erofeev A. Micro-fractional ablative skin resurfacing with two novel erbium laser systems. Lasers Surg Med 2008;40:113-23.
13. Farkas JP, Richardson JA, Burrus CF, Hoopman JE, Brown SA, Kenkel JM. *In vivo* histopathologic comparison of the acute injury following treatment with five fractional ablative laser devices. Aesthet Surg J 2010;30:457-64.
14. Hantash BM, Bedi VP, Chan KF, Zachary CB. *Ex vivo* histological characterization of a novel ablative fractional resurfacing device Lasers Surg Med 2007;39:87-95.
15. Sasaki GH, Travis HM, Tucker B. Fractional CO_2 laser resurfacing of photoaged facial and non-facial skin: Histologic and clinical results and side effects. J Cosmet Laser Ther 2009;11:190-201.
16. Skovbølling Haak C, Illes M, Paasch U, Hædersdal M. Histological evaluation of vertical laser channels from ablative fractional resurfacing: An *ex vivo* pig skin model. Lasers Med Sci 2010;26:465-71.
17. Kist DA, Elm CM, Eleftheriou LI, Studer JA, Wallander ID, Walgrave SE. Histologic analysis of a 2,940 nm fractional device. Lasers Surg Med 2011;43:79-91.
18. Saluja R, Khoury J, Detwiler SP, Goldman MP. Histologic and clinical response to varying density settings with a fractionally scanned carbon dioxide laser. J Drugs Dermatol 2009;8:17-20.
19. Cho SB, Lee JH, Choi MJ, Lee KY, Oh SH. Efficacy of the fractional photothermolysis system with dynamic operating mode on acne scars and enlarged facial pores. Dermatol Surg 2009;35:108-14.
20. Yoo KH, Ahn JY, Kim JY, Li K, Seo SJ, Hong CK. The use of 1540 nm fractional photothermolysis for the treatment of acne scars in Asian skin: A pilot study. Photodermatol Photoimmunol Photomed 2009;25:138-42.

21. Goodman GJ. Post-acne scarring: A review of its pathophysiology and treatment. Dermatol Surg 2000;26:857-71.
22. Jacob CI, Dover JS, Kaminer MS. Acne scarring: A classification system and review of treatment options. J Am Acad Dermatol 2001;45:109-17.
23. Goodman GJ. Treatment of acne scarring in Ethnic Skin. In: Alam M, Bhatia AC, Kundlu RV, Yoo SS, Chan HH, (editors). Cosmetic Dermatology for Skin of Colour, 3rd ed. New Delhi: Tata McGraw; 2009. p. 136-54.
24. Sherling M, Friedman PM, Adrian R, Burns AJ, Conn H, Fitzpatrick R, *et al* Consensus recommendations on the use of an erbium-doped 1,550-nm fractionated laser and its applications in dermatologic laser surgery. Dermatol Surg 2010;36:461-9.
25. Trelles MA, Vélez M, Mordon S. Correlation of histological findings of single session Er:YAG skin fractional resurfacing with various passes and energies and the possible clinical implications. Lasers Surg Med 2008;40:171-7.
26. Trelles MA, Mordon S, Velez M, Urdiales F, Levy JL. Results of fractional ablative facial skin resurfacing with the erbium:yttrium-aluminium-garnet laser 1 week and 2 months after one single treatment in 30 patients. Lasers Med Sci 2009;24:186-94.
27. Karsai S, Czarnecka A, Jünger M, Raulin C. Ablative fractional lasers (CO_2 and Er:YAG): A randomized controlled double-blind split-face trial of the treatment of peri-orbital rhytides. Lasers Surg Med 2010;42:160-7.
28. Farkas JP, Richardson JA, Hoopman JE, Brown SA, Kenkel JM. TUNEL assay for histopathologic evaluation of irreversible chromosomal damage following nonablative fractional photothermolysis. Plast Reconstr Surg 2008;122:1660-8.
29. Bailey SH, Brown SA, Kim Y, Oni G, Abtahi F, Richardson JA, *et al.* An intra-individual quantitative assessment of acute laser injury patterns in facial versus abdominal skin. Lasers Surg Med 2011;43:99-107.
30. Xu XG, Luo YJ, Wu Y, Chen JZ, Xu TH, Gao XH, *et al.* Immunohistological Evaluation of skin response after Treatment Using a Fractional Ultrapulse Carbon Dioxide Laser on Back Skin. Dermatol Surg 2011;37:1141-9.
31. Jacques S, Wang L. Monte Carlo modeling of light transport in tissue. In: Welsh AJ, van Gement MJC, editors. Optical-thermal response of laser-irradiated tissue, Chapter 4, New-York and London: Plenum Press; 1995. p. 73-99.
32. Karl A, Stepp H, Willmann E, Tilki D, Zaak D, Knuchel R, *et al.* Optical coherence tomography (OCT): Ready for the diagnosis of a nephrogenic adenoma of the urinary bladder? J Endourol 2008;22):2429-32.
33. Naouri M, Atlan M, Perrodeau E, Georgesco G, Khallouf R, Martin L, *et al.* High-resolution ultrasound imaging to demonstrate and predict efficacy of carbon dioxide fractional resurfacing laser Treatment. Dermatol Surg 2011;37:596-603.
34. Fournier N, Mordon S. Nonablative remodeling with a 1,540 nm erbium:glass laser. Dermatol Surg 2005;31(9 Pt 2):1227-35.
35. Manstein D, Zurakowski D, Thongsima S, Laubach H, Chan HH. The effects of multiple passes on the epidermal thermal damage pattern in nonablative fractional resurfacing. Lasers Surg Med 2009;41:149-53.
36. Kono T, Chan HH, Groff WF, Manstein D, Sakurai H, Takeuchi M, *et al*. Prospective direct comparison study of fractional resurfacing using different fluences and densities for skin rejuvenation in Asians. Lasers Surg Med 2007;39:311-4.
37. Chapas AM, Brightman L, Sukal S, Hale E, Daniel D, Bernstein LJ, *et al.* Successful treatment of acneiform scarring with CO_2 ablative fractional resurfacing. Lasers Surg Med 2008;40:381-6.
38. Walgrave SE, Ortiz AE, MacFalls HT, Elkeeb L, Truitt AK, Tournas JA, *et al.* Evaluation of a novel fractional resurfacing device for treatment of acne scarring. Lasers Surg Med 2009;41:122-7.
39. Weiss ET, Chapas A, Brightman L, Hunzeker C, Hale EK, Karen JK, *et al.* Successful treatment of atrophic postoperative and traumatic scarring with carbon dioxide ablative fractional resurfacing: Quantitative volumetric scar improvement. Arch Dermatol 2010;146:133-40.
40. Cho SB, Lee SJ, Kang JM, Kim YK, Chung WS, Oh SH. The efficacy and safety of 10,600-nm carbon dioxide fractional laser for acne scars in Asian patients. Dermatol Surg 2009;35:1955-61.
41. Chan NP, Ho SG, Yeung CK, Shek SY, Chan HH. Fractional ablative carbon dioxide laser resurfacing for skin rejuvenation and acne scars in Asians. Lasers Surg Med 2010;42:615-23.
42. Manuskiatti W, Triwongwaranat D, Varothai S, Eimpunth S, Wanitphakdeedecha R. Efficacy and safety of a carbon-dioxide ablative fractional resurfacing device for treatment of atrophic acne scars in Asians. J Am Acad Dermatol 2010;63:274-83.
43. Wang YS, Tay YK, Kwok C. Fractional ablative carbon dioxide laser in the treatment of atrophic acne scarring in Asian patients: A pilot study. J Cosmet Laser Ther 2010;12:61-4.
44. Katz B. Efficacy of a new fractional CO_2 laser in the treatment of photodamage and acne scarring. Dermatol Ther 2010;23:403-6.
45. Park GH, Rhee DY, Bak H, Chang SE, Lee MW, Choi JH, *et al.* Treatment of atrophic scars with fractional photothermolysis: Short-term follow-up. J Dermatolog Treat 2011;22:43-8.
46. Hasegawa T, Matsukura T, Mizuno Y, Suga Y, Ogawa H, Ikeda S. Clinical trial of a laser device called fractional photothermolysis system for acne scars. J Dermatol 2006;33:623-7.
47. Alster TS, Tanzi EL, Lazarus M. The use of fractional laser photothermolysis for the treatment of atrophic scars. Dermatol Surg 2007;33:295-9.
48. Glaich AS, Goldberg LH, Friedman RH, Friedman PM. Fractional photothermolysis for the treatment of postinflammatoryerythema resulting from acne vulgaris. Dermatol Surg 2007;33:842-6.
49. Lee HS, Lee JH, Ahn GY, Lee DH, Shin JW, Kim DH, *et al.* Fractional photothermolysis for the treatment of acne scars: A report of 27 Korean patients. J Dermatol Treat 2008;19:45-9.
50. Weiss R, Weiss M, Beasley K. Long-term experience with fixed array 1540 Fractional erbium laser for acne scars. Kissimmee, FL: Abstract presented at American Society for Laser Medicine and Surgery Conference; April 2008.
51. Chrastil B, Glaich AS, Goldberg LH, Friedman PM. Second-generation 1,550-nm fractional photothermolysis for the treatment of acne scars. Dermatol Surg 2008;34:1327-32.
52. Kim S, Cho KH. Clinical trial of dual treatment with an ablative fractional laser and a nonablative laser for the treatment of acne scars in Asian patients. Dermatol Surg 2009;35:1089-98.
53. Hu S, Chen MC, Lee MC, Yang LC, Keoprasom N. Fractional resurfacing for the treatment of atrophic facial acne scars in Asianskin. Dermatol Surg 2009;35:826-32.
54. Cho SB, Lee SJ, Kang JM, Kim YK, Oh SH. Combined fractional laser treatment with 1550-nm erbium glass and 10 600-nm carbon dioxide lasers. J Dermatolog Treat 2009;1:1-3.
55. Kim HJ, Kim TG, Kwon YS, Park JM, Lee JH. Comparison of a 1,550 nm Erbium: Glass fractional laser and a chemical reconstruction of skin scars (CROSS) method in the treatment of acne scars: A simultaneous split-face trial. Lasers Surg Med 2009;41:545-9.
56. Kang WH, Kim YJ, Pyo WS, Park SJ, Kim JH. Atrophic acne scar treatment using triple combination therapy: Dot peeling, subcision and fractional laser. J Cosmet Laser Ther 2009;17:1-4.
57. Cho SB, Lee SJ, Cho S, Oh SH, Chung WS, Kang JM, *et al.* Nonablative 1550-nm erbium-glass and ablative 10 600-nm carbon dioxide fractional lasers for acne scars: A randomized split-face study with blinded response evaluation. J Eur Acad Dermatol Venereol 2009;24:921-5.
58. Hedelund L, Moreau KE, Beyer DM, Nymann P, Haedersdal M. Fractional nonablative 1,540-nm laser resurfacing of atrophic acne scars. A randomized controlled trial with blinded response evaluation. Lasers Med Sci 2010;25:749-54.
59. Mahmoud BH, Srivastava D, Janiga JJ, Yang JJ, Lim HW, Ozog DM. Safety and efficacy of erbium-doped yttrium aluminum garnet fractionated laser for treatment of acne scars in type IV to VI skin. Dermatol Surg 2010;36:602-9.
60. Friedman PM, Skover GR, Payonk G, Kauvar AN, Geronemus RG.

3-D *in-vivo* optical skin imaging for topographical quantitative assessment of non-ablative laser technology. Dermatol Surg 2002;28:199-204.
61. Field LM. Laser resurfacing hysteria. The media, the marketers, the companies, and us. Dermatol Surg 1998;24:684-6.
62. Field LM. Never, ever a twinkle from billboard advertising—Nor from advertising budgets. Dermatol Surg 1999;25:828-9.
63. Helbig D, Paasch U. Molecular changes during skin aging and wound healing after fractional ablative photothermolysis. Skin Res Technol 2011;17:119-28.

How to cite this article: Sardana K, Garg VK, Arora P, Khurana N. Histological validity and clinical evidence for use of fractional lasers for acne scars. J Cutan Aesthet Surg 2012;5:75-90.

Source of Support: Nil. **Conflict of Interest:** None declared.

Author Help: Online submission of the manuscripts

Articles can be submitted online from http://www.journalonweb.com. For online submission, the articles should be prepared in two files (first page file and article file). Images should be submitted separately.

1) **First Page File:**
Prepare the title page, covering letter, acknowledgement etc. using a word processor program. All information related to your identity should be included here. Use text/rtf/doc/pdf files. Do not zip the files.
2) **Article File:**
The main text of the article, beginning with the Abstract to References (including tables) should be in this file. Do not include any information (such as acknowledgement, your names in page headers etc.) in this file. Use text/rtf/doc/pdf files. Do not zip the files. Limit the file size to 1 MB. Do not incorporate images in the file. If file size is large, graphs can be submitted separately as images, without their being incorporated in the article file. This will reduce the size of the file.
3) **Images:**
Submit good quality color images. Each image should be less than 4 MB in size. The size of the image can be reduced by decreasing the actual height and width of the images (keep up to about 6 inches and up to about 1200 pixels) or by reducing the quality of image. JPEG is the most suitable file format. The image quality should be good enough to judge the scientific value of the image. For the purpose of printing, always retain a good quality, high resolution image. This high resolution image should be sent to the editorial office at the time of sending a revised article.
4) **Legends:**
Legends for the figures/images should be included at the end of the article file.