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Original Research

Selective non-ablative wrinkle reduction by laser

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BACKGROUND AND OBJECTIVES: Skin resurfacing and wrinkle removal is a large medical laser market. However, the rate of undesirable side effects is high and sometimes is not warranted by the aesthetic improvement observed. The authors have evaluated the potential benefits of an approach to selective non-ablative wrinkle reduction.
MATERIALS AND METHODS: This technique selectively targets the microvasculature which plays a key role in the stimulation of enhanced collagen production.
RESULTS: The study reported shows that application of the laser parameters described enhances collagen production by an average of 84%, measured 72 hours after a single laser treatment. This is achieved whilst leaving the skin barrier intact and with no adverse pigmentary changes. The study further shows that a cosmetic improvement is observed with an average value of 1.88 reduction in wrinkle appearance as measured on the Fitzpatrick Wrinkle Severity scale. This improvement was achieved with one brief treatment and no reported incidence of side effects.
CONCLUSION: In conclusion, the treatment modality described may be a new approach to the treatment of wrinkles.
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Introduction

It is estimated that of the order of 300 000 laser resurfacing procedures were carried out during 1999 in the USA alone.[1] This ablative technique utilizes either a CO_2 or Er:YAG laser to remove the epidermis and upper dermal layers and promote the growth of new, fresher looking skin. Several authors have reported good results. However, the post-treatment care required is substantial and the incidence of side effects is relatively high.[2]

A typical full-face skin resurfacing procedure requires the patient to be under general anaesthesia, with the inherent risks involved, for up to 2 hours. For 7–14 days post-procedure, the skin barrier is disrupted, requiring significant care and regular changing of dressings.[3] As the skin heals, the transparent epidermis in combination with

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increased blood flow results in a pink appearance for up to 3–6 months during which the patient is required to avoid direct exposure to sunlight.[4] The most common side effect associated with the procedure is hyperpigmentation, with a reported incidence of between 30–40%.[2] Other side effects such as hypertrophic scarring, late onset hypopigmentation and an inability of the skin to tan normally are also reported.[5] There have been reports of ablative skin resurfacing reactivating dormant herpes simplex virus.[6]

CO_2 and Er:YAG resurfacing initiate stimulation of new collagen growth in the dermis as replacement for the ablated superficial layers. Histologically, it has been shown that these new collagens replace the elastotic, disorganized collagen/elastin connective tissue matrix associated with wrinkled or photodamaged skin in the upper dermis. However, since CO_2 and Er:YAG wavelengths target water as the chromophore, they ablate the full epidermis before reaching the target papillary dermis. Having ablated the epidermis and part of the papillary dermis, a thin layer of heat-denatured dermal tissue remains. It is believed that during the repair processes this heat damaged layer releases mediators which induce formation of the new and improved collagen matrix with an improved aesthetic appearance.

The authors hypothesized that it is possible to achieve the same effect in a selective manner without the need to ablate the surface tissue. By selectively targeting a chromophore in the dermis, in theory it is possible to selectively deposit energy mimicking the effect of the residual heat-damaged layer associated with CO_2 resurfacing. The natural chromophores to be targeted in the dermis are oxyhaemoglobin and deoxyhaemoglobin in the blood vessels. These blood vessels can be less than 15 μm in diameter with thermal relaxation times of less than 100 μs. Optical energy must be deposited in these blood vessels quickly but in such a way as to insult but not injure the vessel. Ideally, the effect should be achieved without purpura and with the minimum of pain and discomfort.

The key laser parameters that produce the desired result are:

- Wavelength: to ensure the selective targeting of the appropriate chromophore
- Pulse duration: optimized to achieve the desired effect without damaging the microvasculature
- Temporal profile: should be tailored to match the thermal relaxation time of the target microvasculature.

These three parameters are essential; however, energy stability coupled with a uniform spatial profile are also of critical importance.

Theory predicted the following laser parameters:

Wavelength 585 nm
Pulse duration 250–400 μsec
Energy density 2–4 J/cm^2
Temporal profile matched to thermal characteristics of the microvasculature.

The technique reported here achieves the desired clinical effect of stimulating the growth of new dermal collagen, and the subsequent cosmetic improvement in the appearance of wrinkles, without the side effects and post-treatment care associated with ablative wrinkle removal procedures.

Materials and methods

Two groups of volunteers, 40 subjects in total, were recruited to evaluate the proposed non-ablative wrinkle removal procedure. One group of 10 subjects was selected for biochemical analysis of the laser treatment. The second group of 30 subjects was treated with identical laser parameters on facial wrinkles to determine the cosmetic outcome of the procedure.

Biochemical analysis

Ten subjects, with an average age of 38 years (range 20–50 years) were selected for biochemical analysis of the effect of the laser procedure. Six areas, three on the medial dorsal aspect of the non-dominant forearm and three on the inner medial aspect of the non-dominant upper arm, were selected. The forearm was selected to represent typical sun-damaged skin whilst the inner medial aspect of the upper arm normally has minimal exposure to sunlight.

Each area measured 6 cm by 6 cm: one area was irradiated with a single pass of the laser, one area irradiated with two passes at an interval of 5 minutes and the third area remained untreated as a control. Nomination of treatment sites was via a randomization table. The laser parameters used for all treatments were a wavelength of 585 nm, pulse duration of 350 μsec, energy density of 2.4 J/cm^2 with a 5 mm diameter spot (laser: Model NLite, SLS Ltd, Llanelli, Wales, UK). The entire treatment area was covered evenly with laser pulses, care being taken to ensure minimum overlap of adjacent pulses. No pre- or post-treatment preparations were used.

Prior to laser irradiation, the integrity of the skin barrier of all the selected areas was measured using the Trans Epidermal Water Loss (TEWL) technique. The procedure was undertaken in accordance with the guidelines stipulated by the Standardisation Group of the European Society of Contact Dermatitis, utilizing a TEWL monitor (model: Dermalab, Cortex Technology, Hadsund, Denmark). Immediately post treatment, the skin barrier integrity was again measured to determine the effect of the treatment.

Seventy-two hours post treatment, suction blisters were raised in all areas, including the control, and the interstitial fluid collected was stored at -18°C for analysis. The fluid was analysed to determine the concentration of the aminoterminal propeptide of type

III procollagen (PIIINP), this concentration level being indicative of the skin type III collagen production rate.[7] The levels of the PIIINP concentration in the treated and untreated areas were compared to determine the effect of the laser irradiation.

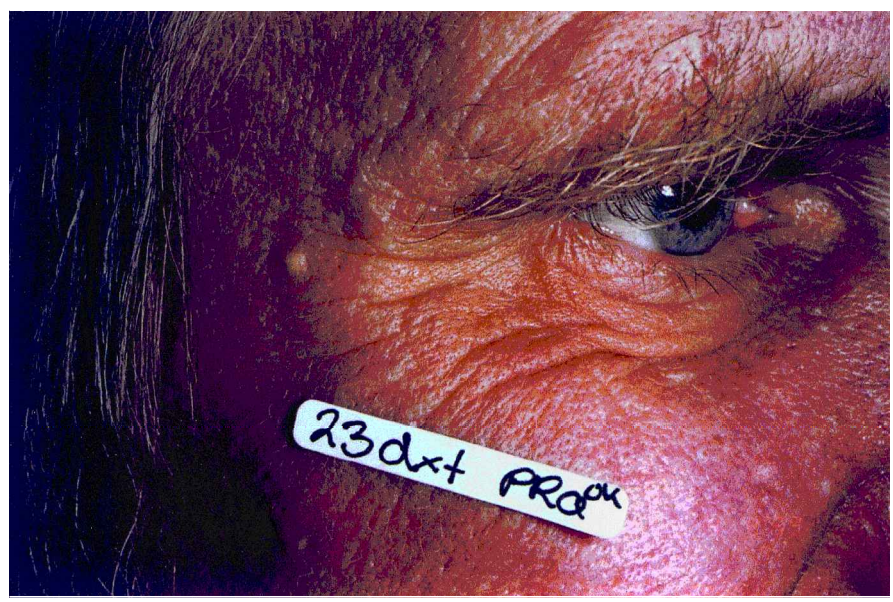
The statistical analyses were performed with the Jandel Scientific statistical package SigmaStat, and due to the relatively low number of observations, non-parametric statistics were used. Statistical significance was accepted at the standard 5% level.

Cosmetic efficacy study

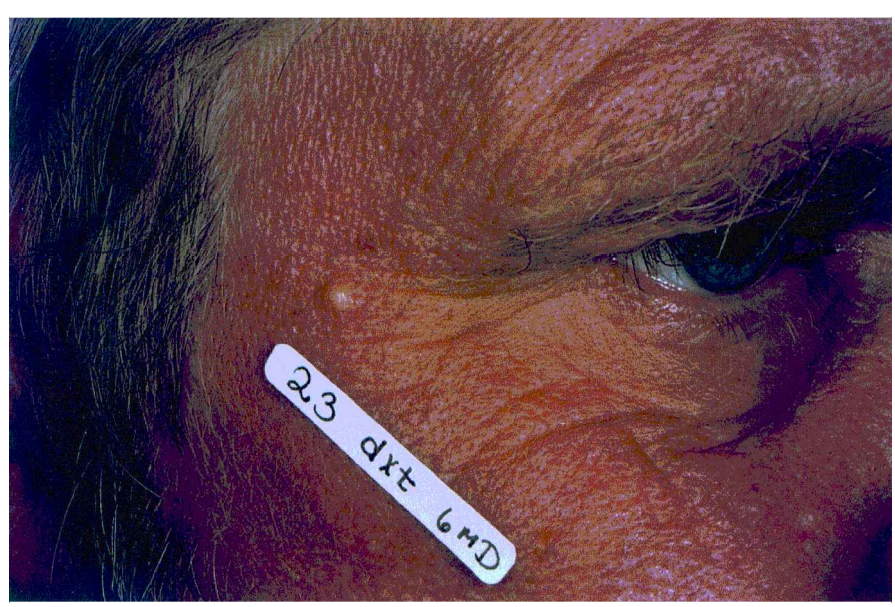
Thirty subjects, average age of 46 (range 31–60), were recruited for irradiation with laser parameters identical to those used in the biochemical analysis trial. In all cases, the periorbital region was selected as the treatment site.

The pre-treatment regime was minimal. The treatment site, either right side or left side, was allocated via a randomization table. Prior to treatment, any cosmetics were removed and photographs taken. The treatment site required no preparation or application of any topical or general anaesthetic. The subject and treating physician were required to wear suitable eye protection.

Once the treatment site had been selected, the laser energy was applied evenly over the site with less than 10% overlap of the individual laser pulses. Following treatment, the subjects were monitored for 1 hour to



(A)



(B)

Figure 1
(A) 58-year-old male pre-treatment.
(B) 6 months post single laser treatment.

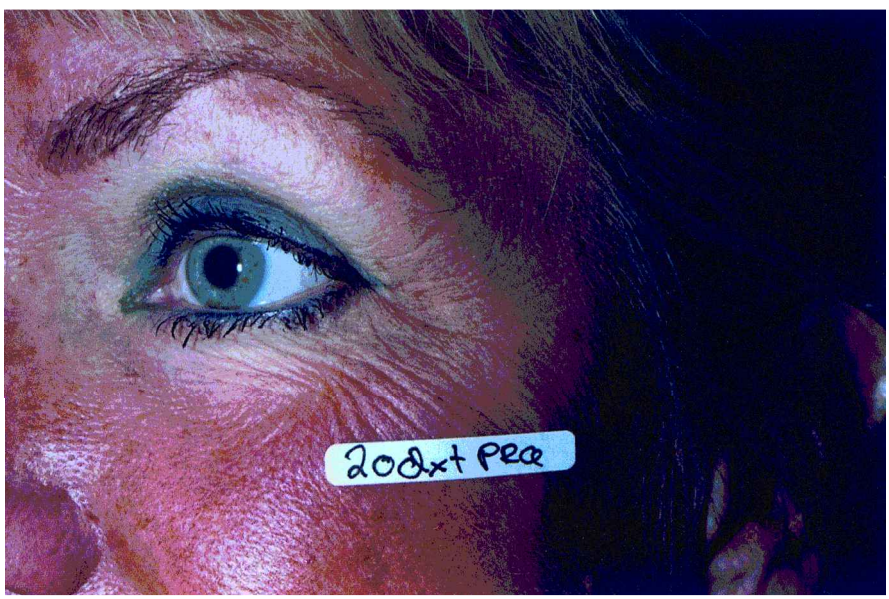
Control sites	Single treatment sites	Multiple treatment sites
61.5 μg/l	113.5 μg/l	95.5 μg/l

Table 1
Level of PIIINP measured from extracted dermal interstitial fluid.

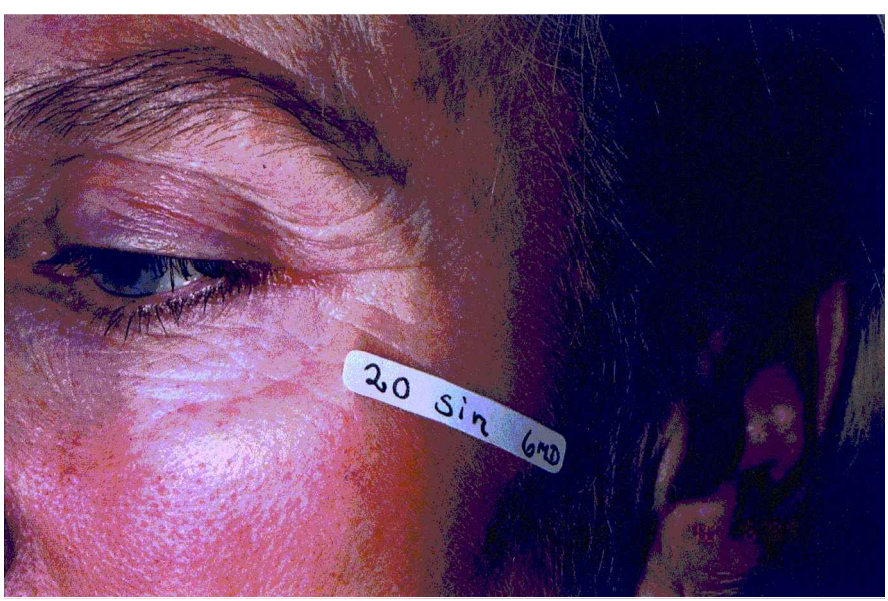
determine if any immediate side effects, such as bruising or erythema, developed.

The patients were reviewed after 7, 30, 90 and 180 days. At the predefined review points, the subjects were photographed and indications of side effects were recorded. At 180 days, final photographs were taken for comparison with the pre-treatment appearance.

Evaluating the cosmetic improvement in photoaged skin can be a difficult procedure. Fitzpatrick et al[8] proposed a methodology which has become an established approach to overcoming this challenge. A nine-point clinical scoring system was devised for the evaluation of the degree of wrinkling and photodamage present. The clinical scoring system was based on blinded evaluations by two dermatologists and a plastic surgeon, and classified reference photographs were used to orient the physicians to the grading system. Pre-operative and post-operative photographs were compared to evaluate treatment response. Photographs were randomized and the independent reviewers rated the photographs on a numerical scale of 1–9 by means of a blinded format. The present clinical study was assessed in the same manner as prescribed by Fitzpatrick et al.



(A)



(B)

Figure 2
(A) 44-year-old female pre-treatment.
(B) 6 months post single laser treatment.

Wrinkle class	Number of subjects	Pre-treatment rating	Post-treatment rating	Average reduction
		Reviewer 1		
Class I	10	2.7±0.48	2.0±0.0	0.7
Class II	16	4.86±0.77	2.43±0.65	2.43
Class III	4	7.5±0.58	4.0±0.82	3.5
Combined	30	4.46±1.73	2.5±0.84	1.96
		Reviewer 2		
Class I	5	3.0±0.0	2.0±0.0	1.0
Class II	16	4.95±0.73	2.56±0.73	2.39
Class III	9	7.5±0.53	5.25±0.71	2.25
Combined	30	5.11±1.73	3.25±1.46	1.86
		Reviewer 3		
Class I	7	2.43±0.53	1.71±0.49	0.72
Class II	16	4.47±0.64	2.4±0.74	2.07
Class III	7	7.5±0.55	5.0±1.1	2.5
Combined	30	4.61±1.85	2.79±1.24	1.82

Table 2
Results of the blinded review of pre- and post-treatment photographs.

Results

Biochemical analysis

The TEWL values measured pre- and post-treatment showed no increase in the level of the vapour-pressure gradient above the skin surface.

The measured PIIINP values were as shown in Table 1. The statistical analysis showed statistically significant differences between control and single treatment sites, $p=0.011$, between the single and multiple treatment sites, $p=0.011$. However, between the control and multiple treatment sites, no significant difference was seen ($p=0.317$).

Cosmetic efficacy study

No significant post-treatment pain was recorded; the subjects reported only a slight warming sensation. During treatment, a very slight 'sting' was felt. Immediately post treatment, there were no clinical signs of skin damage. Bruising, or purpura, indicative of vessel damage was not seen. At all post-treatment review points, no side effects were reported. Hyperpigmentation and hypopigmentation was not observed in any of the subjects.

Traditionally, patients undertaking laser procedures of the skin are advised to avoid excessive exposure to sunlight for 6–12 months. No restrictions regarding sun exposure were imposed. Those subjects exposed to sunlight post treatment experienced no side effects, and their skin tanned as normal. Textural changes, such as hypertrophic or atrophic scarring, were not reported in any subjects.

Figures 1 and 2 are representative of the results obtained in the 30 patients treated. Both show pre-treatment and 6 months post-treatment. Table 2 shows the results of the evaluation of three independent 'blinded' reviewers.

The three evaluations of the pre-treatment periorbital wrinkles showed means of 4.46 ± 1.73, 5.11 ± 1.73 and 4.61 ± 1.85 while the post-treatment scores were 2.5 ± 0.84, 3.25 ± 1.46 and 2.79 ± 1.24, resulting in mean improvements of 1.96, 1.86 and 1.82 respectively. The follow-up period was 6 months. None of the subjects experienced hyperpigmentation and no post-treatment care was required. None of the patients experienced erythema and the skin tanned as normal.

Subgroup analysis showed statistically significant decreases of 0.7, 1 and 0.72 for Class I wrinkles, 2.43, 2.39 and 2.07 for Class II wrinkles and 3.5, 2.25 and 2.5 for Class III wrinkles respectively.

The cosmetic improvement achieved was assessed

Wrinkle class	CO_2 resurfacing (Fitzpatrick et al)	Non-ablative technique (Bjerring et al)	Non-ablative technique as % of CO_2 resurfacing
Class I	1.57	0.81	52%
Class II	2.57	2.29	89%
Class III	2.57	2.75	79%
Combined	2.25	1.88	84%

Table 3
Comparison of the non-ablative technique to CO_2 resurfacing.

following as closely as possible the protocol outlined by Fitzpatrick et al.[8] The blinded evaluations gave results that may be compared with those achieved by CO_2 resurfacing. Table 3 details that comparison.

The results in Table 3 show that the treatment protocol described here provides good comparison to the cosmetic improvement achieved using the CO_2 resurfacing technique. This improvement was achieved with one brief treatment and no reported incidence of side effects.

Discussion

The study reported shows that application of the laser parameters described enhances collagen production by an average of 84% measured 72 hours after a single laser treatment. This is achieved whilst leaving the skin barrier intact and with no adverse pigmentary changes. The study further shows that a cosmetic improvement is observed with an average value of 1.88 reduction in wrinkle appearance as measured on the Fitzpatrick scale.

The biochemical analysis showed that at the relatively low light levels used for the trial, a considerable increase in the production of type III procollagen was induced. The lower increase in collagen production at the double treated sites indicates that the additional energy dosage has a negative physiological effect. This may account for the fact that improvement in the cosmetic appearance of wrinkles has not been reported as a side-effect of pulsed dye laser treatment of vascular lesions, typically performed at significantly higher energies than those used in this trial. However, this is not the only factor; it is the opinion of the authors that the temporal profile of the laser is of critical importance. In order to initiate an inflammatory response, and hence stimulate enhanced collagen production, the primary target must be those vessels that allow the transfer of mediators that trigger fibroblast activity. These vessels are less than 15 μm in diameter and have thermal relaxation times less than 100 μsec.

The increase in the collagen production rate has to be a secondary effect, as the fibroblasts responsible for producing the collagen have minimal interaction with the incident laser energy. It is postulated that the following physiological process is taking place. The laser light is specifically absorbed in the blood vessels of the upper dermal vascular plexus, after passing through the epidermis with negligible interaction. The light reaching the plexus is such that there is insufficient intensity to cause vessel rupture or coagulation, common with vascular lesion treatment, hence there is no purpura or vessel damage. The level of light interaction within the vessel is such that a low grade inflammatory/growth response is induced. Inflammatory mediators are released, presumably from the endothelial cells through the vessel wall and into the dermal interstitium where they stimulate fibroblast activity. The fibroblasts effectively start tissue repair mechanisms, which include enhanced new collagen production.

Recent studies reported utilizing pulsed dye lasers have demonstrated both a clinical and a histological improvement.[9] The level of cosmetic improvement reported is significantly less than documented here and may be associated with the difference in laser parameters, particularly pulse duration, temporal profile and treatment fluence. It is therefore apparent that precise selection of laser parameters is of critical importance in achieving the maximum cosmetic benefit.

The level of post-treatment purpura and swelling, lasting for 1–2 weeks and probably resulting from the larger spot size and higher treatment fluences, may prove unacceptable for the patients.

Other techniques utilizing near infrared lasers and high intensity pulsed white light sources have demonstrated minimal cosmetic effects.[10–15] As the wavelength of light used in these studies has significantly higher direct absorption in collagen, it indicates that the mechanism of dermal rejuvenation is not purely a function of direct collagen stimulation and that an interaction with the vasculature is of critical importance. The level of cosmetic efficacy and the reported incidence of complications severely limits the acceptability of this treatment modality.

In conclusion, it appears that there is significant preliminary evidence that the treatment modality described herein may be a new approach to the treatment of wrinkles. Further work is currently underway to investigate the effect on other anatomical locations and in particular, the possibilities of improving the cosmetic results with follow-up treatments.

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