Dermal collagen production following irradiation by dye laser and broadband light source

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BACKGROUND: Improvement in the appearance of wrinkles has been observed following exposure to short-pulsed 585 nm laser light. The assumed effect is a specific absorption of light in the blood vessels of the superficial dermis, resulting in release of inflammatory mediators into the interstitium followed by stimulated fibroblast activity. The fibroblasts effectively initiate tissue repair mechanisms, which include enhanced new collagen production.

METHODS: Quantitative measures of collagen synthesis rate in the skin can be obtained from determinations of the aminoterminal propeptide of type III procollagen level in suction blister fluid using a radioimmunoassay.

RESULTS: A single laser treatment at subpurpura energy level showed that the 585 nm laser source induced an increase of 84% \((p<0.05)\) in the type III procollagen production rate compared with a non-treated control site. A broadband, pulsed, white light source at 4 J/cm\(^2\) showed no measurable increase, whilst the skin area treated with 7 J/cm\(^2\) increased the procollagen production rate by 17% \((NS, p>0.05)\). A second treatment 2 weeks later further improved the laser-induced increase in procollagen production rate to 148% \((p>0.05)\) compared with the control site. The broadband, pulsed, white light-irradiated skin sites showed that at 4 J/cm\(^2\) the procollagen production rate was increased by 21.4% and at 7 J/cm\(^2\) by 32.1% compared with the corresponding non-treated control site \((NS, p>0.05)\).

CONCLUSIONS: Irradiation by the haemoglobin-specific short-pulsed 585 nm laser induced a fivefold increase in procollagen production rate compared with a biologically comparable fluence delivered in a broadband spectrum. An additional treatment after 2 weeks further increased the effect of the short-pulsed 585 nm laser to 148% of the control. Vascular-specific light/tissue interactions seem to play a key role in stimulating skin collagen production.

Keywords: collagen remodelling – dye laser – intense pulsed light source – wrinkle reduction

Conflict of interest

MK is an employee of the laser company reporting this study. PB, MC, LH and HL have received research support from the laser manufacturer reporting this study.

Introduction

A recent paper\(^1\) reported that an improvement in the appearance of periorcular wrinkles was observed following exposure to 585 nm light. This was achieved when laser parameters such as energy density, temporal and spatial profile were tuned to target the appropriate vessel structures.

It was postulated that the following physiological
process occurred. The laser light was 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 was such that there was insufficient intensity to cause vessel rupture or coagulation, common with vascular lesion treatment. Hence, there was no purpura or functional vessel damage. The level of light interaction within the vessel was such that a low-grade inflammatory/growth response was induced. Inflammatory mediators were released, presumably from the endothelial cells through the vessel walls and into the dermal interstitium where they stimulated fibroblast activity. The fibroblasts effectively initiated tissue repair mechanisms, which included enhanced new collagen production.

This theory was underscored by a biochemical study, which showed conclusively that the laser exposure described introduced a significant increase in the rate of type III collagen production 72 hours after treatment. Type III collagen is present in most soft tissues in the body.2 When soft tissue is formed, specific collagen metabolites, called propeptides, are produced. PIIINP is one of the propeptides that are liberated during collagen synthesis.3 Synthesis of soft tissue can be demonstrated by measuring the concentration of PIIINP using a suitable radioimmunoassay (RIA).4

The biochemical study showed an 84% increase in the rate of collagen production leading to tissue remodelling and the consequent cosmetic improvement. Hypothetically, the higher the increase in collagen production rate after laser irradiation the better the cosmetic outcome.

Increasing the collagen production rate by utilizing higher treatment energies is not an option because this would lead to purpura and irreversible damage to the healthy microvasculature. An alternative mechanism is to undertake additional treatments.

In contrast to monochromatic laser sources, broadband white light sources contain a continuum of wavelengths from the visible to near infra-red region of the optical spectrum. By definition, the interaction of such light with tissues is non-selective since different wavelength components have different absorption characteristics in the various tissue structures. However, some clinicians have reported limited cosmetic benefit from exposure to such white light systems.5

This paper discusses two issues, namely:

1. Can an increase in collagen production rate be achieved using broadband (white) light sources?
2. Can an increase in collagen production rate be enhanced by repeat treatments?

**Materials and methods**

Two groups of volunteers, 19 in total, were selected for biochemical quantification of collagen production after treatment. One group of 10 subjects was irradiated with monochromatic laser light at 585 nm and the second group was irradiated with broadband (white) light. Both groups received two treatments with an interval of 14 days and the levels of PIIINP were measured 3 days after each treatment.

Enhanced fibroblast activity lasts for typically 30 days, reaching a peak approximately 10 days after treatment.6 Several days after this peak in collagen production was chosen as an appropriate time to undertake the repeat treatment.

**Laser irradiation**

Ten subjects, with an average age of 38 years, were selected for laser irradiation. Three areas on the medial dorsal aspect of the non-dominant forearm were selected. Each area measured 6 × 6 cm: two areas were irradiated with a single pass of the laser, 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 μs, energy density in the range of 2.4–3.0 J/cm² with a 5 mm diameter spot (laser: Model NLite, ICN Photonics Ltd, Llanelli, Wales, UK). Energy densities were selected slightly below the purpura threshold for the particular skin type in question (typically 10% below). 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.

At 72 hours after treatment, skin suction blisters were raised in the control and one treated area as previously described.7 Briefly, we applied a 250–400 mmHg negative air pressure through 6 mm holes in suction cups, with two holes for each test area. During a period of 40–270 minutes, epidermis and dermis split at the dermo-epidermal junction and dermal interstitial fluid accumulate in the space that slowly became a blister. When the suction blisters were fully developed, the fluid was collected immediately and stored at −18°C for analysis (Figure 1). Data on plasma proteins (albumin, transferrin, IgG, and alpha macroglobulin) indicate that suction blister fluid is not contaminated by plasma lost through damaged vessel walls, and that the levels of plasma proteins in suction blister fluid are comparable...
with those of average interstitial tissue fluid. The blister fluid was analysed for the concentration of the aminoterminal propeptide of type III procollagen (PIIINP), this level being indicative of the skin type III collagen production rate. The levels of the PIIINP concentration in the treated and untreated areas were compared to determine the effect of the different types of irradiation. The concentration of PIIINP in the suction blister fluid was analysed using a radioimmunoassay (Orion Diagnostica, Finland).

At 14 days after the initial treatment the second area received further laser irradiation at the same fluence as the first treatment. Suction blisters were raised on the second treated area following the same protocol.

**Broadband irradiation**

Nine subjects, with an average age of 33 years, were selected for broadband irradiation. Five areas on the medial dorsal aspect of the non-dominant forearm were selected.

The white light source used was specially made for the present study. Pulse duration was 15 ms and the wavelength band ranged from 450 nm to 950 nm. Spot size was 10 × 50 mm.

The treatment protocol was identical to that described for the laser treatment with the following modifications:

- A treatment fluence of 4 J/cm² was used to irradiate two of the selected areas. This fluence was chosen since it is comparable to the treatment fluences produced by the laser source.
- Two further areas were selected and treated with a fluence of 7 J/cm², this energy being selected since it is described by the subjects as the maximum tolerable without surface cooling.
- After 14 days, one of each treated area had a second irradiation with fluences identical to the initial treatment applied to that area.

Skin surface cooling was not utilized during this experiment because the purpose of the study was to compare, as closely as possible, two treatment modalities. The use of cooling would introduce a further variable, which may or may not have an impact on the physiological processes involved in stimulating collagen production.

**Statistics**

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.

**Results**

**Single treatment**

A single treatment at the described parameters showed that the 585 nm laser source induced an increase of 84% (p > 0.05) in the PIIINP level in the treated area compared to the control. The broadband white light source at 4 J/cm² showed no measurable increase, whilst the area treated with 7 J/cm² increased the PIIINP level by 17%. This was, however, not statistically significant (p > 0.05) (Figure 2).

**Double treatment**

Biochemical analysis 3 days after the second treatment showed that the 585 nm laser source increased the PIIINP level to 148% (p > 0.05) compared to the control site. Analysis on the broadband-irradiated sites showed that at 4 J/cm², PIIINP was increased by 21.4% and at 7 J/cm² the PIIINP was 32.1% compared to the corresponding control site. The data obtained from the broadband illuminated sites was not significantly different from the non-treated control sites (p > 0.05) (Figure 3).

**Discussion**

The two treatment modalities are differentiated by the fact the 585 nm light is selective in nature whilst the broadband white light has a non-specific tissue interaction.

The only dermal absorbing chromophore for the 585 nm light is the oxyhaemoglobin in the blood vessels, thus allowing the optical energy to be deposited in a controlled manner. White light on the other hand has only a

![Figure 2](image2.png)

*Figure 2* Percentage increase in collagen production 3 days after the initial treatment.

![Figure 3](image3.png)

*Figure 3* Percentage increase in collagen production 3 days after the second treatment.
relatively small component of delivered energy that is absorbed in the blood vessels. The remainder induces non-specific heating of the entire irradiated field.

There are three distinct phases of the wound repair process:10 the acute inflammation phase; the matrix and cellular proliferation phase; and the remodelling phase.

**Acute inflammation phase**
Immediately after injury, several vascular and cellular reactions initiate inflammation. The process begins with the release of chemical mediators from cells into the extracellular fluid. Histamine release from mast cells increases vascular permeability. Blood fluids and proteins leak from blood vessels causing oedema and consequent swelling leading to the classical signs of inflammation: redness, swelling, pain and heat. Inflammation sets the stage for tissue repair, lasts for 48–72 hours after injury and then gradually subsides as the repair process progresses.

**Matrix and cellular proliferation phase**
Chemical mediators released by inflammatory cells stimulate the migration and proliferation of fibroblasts that participate in the repair process. Fibroblasts secrete fibronectin, proteoglycans and collagen fibres.11 In the interstitial space, these freshly synthesized matrix molecules form granulation tissue which has little tensile strength at this stage.

**Remodelling phase**
This phase reshapes and strengthens damaged tissue by reforming the matrix and replacing cells. Inflammatory cells disappear as the repair progresses and the proportion of type I collagen to type III collagen increases, and collagen fibres are reoriented and mature as elastin forms and tensile strength increases.12 Typical timescales for the processes described above are shown in Figure 4.

The hypothesis regarding the mode of action from the Bjerring study as described earlier is a subset of this wound healing process. The microvasculature of the upper dermis is insulted through exposure to a carefully tailored short pulse of 585 nm light. These treatment parameters initiate modest inflammation leading to enhanced fibroblast activity followed by tissue remodelling.

It is evident from the present results that for a single treatment both laser and white light sources induced an increase in collagen production above the natural background level measured in the control area. However, the levels of PIIINP measured in the interstitial fluid was approximately seven times higher with the 585 nm laser source compared to the broadband source at both the 4 J/cm² and 7 J/cm² level. The additional treatment after 2 weeks further enhanced collagen production level.

Irradiation by a haemoglobin-specific light source, i.e. the 585 nm laser, induces a sixfold increase in collagen production compared with a biologically comparable fluence delivered in a broadband light spectrum. These results suggest that a vascular-specific interaction plays a key role in stimulating collagen production. However, the duration of the light pulse, which is 0.35 ms for the laser and 10 ms for the pulsed white light source, may also be important. Normal dye lasers intended for treatment of vascular malformations and haemangiomas are designed to target enlarged capillaries and larger post-capillary venules, whereas the laser used in the present investigation was constructed to target normal-sized capillaries. The white light source produced light pulses, which presumably were too long and of insufficient vascular-specific energy to obtain the required selective effect on normal capillaries.

It is possible that higher fluences of the IPL, e.g. 30 to 40 J/cm², different results may have been obtained. The fluence studied here with the IPL is much lower than that used clinically for photo-rejuvenation by most users.

**Conclusions**
Clinical data on wrinkle reduction using 585 nm short-pulsed laser light have been supported by biochemical analyses showing increased skin procollagen production levels which are fivefold higher after laser treatment than after skin irradiation with a pulsed broadband light source. An additional treatment after 2 weeks showed an even further increase of the type III collagen production rate by 148% after laser treatment and 32% after pulsed broadband light source treatment.

**References**
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