
Effects of Intense Pulsed Light and the 1,064 nm Nd:YAG Laser on Sun-Damaged Human Skin: Histologic and Immunohistochemical Analysis

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BACKGROUND. Nonablative methods may produce collagen synthesis in sun-damaged skin.

OBJECTIVE. To study the effects of intense pulsed light (IPL) and 1,064 nm neodymium:yttrium-aluminum-garnet (Nd:YAG) laser: a histologic and immunohistochemical analysis of sun-damaged skin.

MATERIALS AND METHODS. Nine subjects participated. Five subjects received five-monthly treatments with IPL (560 nm cutoff filter, 8 × 35 mm spot size, pulse duration 2.4/4.2 milliseconds, pulse delay 15 milliseconds, fluence 28–35 J/cm²). Four subjects received treatment with a 1,064 nm Nd:YAG laser (130 J/cm², triple pulse, 7.0/7.0/7.0-millisecond pulse duration, 75-millisecond delay). Routine histology and immunohistochemistry on 2 mm punch biopsies were taken before treatment and then at 3

and 6 months. We quantified collagen in the upper dermis and expression of heat shock protein 70 and procollagen 1.

RESULTS. Pretreatment specimens contained solar damage. After treatment with the 1,064 nm Nd:YAG laser, the amount of collagen in the papillary dermis was slightly thicker than in those subjects treated with the IPL device (nonsignificant differences). Scattered dendritic cells in the papillary and upper reticular dermis expressed heat shock protein 70 and procollagen 1 after treatment with either light device.

CONCLUSION. Both the IPL and 1,064 nm Nd:YAG laser-induced heat activation of superficial dermal dendritic cells resulted in deposition of collagen in the papillary dermis without evident morphologic damage to the epidermis or dermis.

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SEVERAL METHODS have been used to improve the esthetic appearance of sun-damaged skin. Because laser skin resurfacing may result in a lengthy recovery period and persistent erythema following the procedure, nonablative laser technologies associated with no interruption of the epidermal integrity and only minimal post-treatment erythema have emerged. These include pulsed light, long-pulse 1,320 nm neodymium:yttrium-aluminum-garnet (Nd:YAG) laser, 1,450 nm diode laser, and 1,540 nm sapphire glass technologies.^{1–5}

Intense pulsed light (IPL) and the 1,064 nm Nd:YAG laser have been used for management of different conditions, including elimination of varices, photoepilation, and removal of vascular and pigmented lesions. IPL devices

contain a flashlamp that emits a broad wavelength spectrum of 550 to 1,200 nm, delivering high peak energy and short pulses. We have studied the changes induced by IPL treatment on sun-damaged skin.⁶ There is relatively little experience with the use of the Nd:YAG laser for skin rejuvenation.^{4,7,8} This study compares the histologic changes induced by IPL and a Nd:YAG laser on sun-damaged skin and examines the possible role of heat shock proteins in the tissue response to these devices.

Research Design

Nine women (mean age 52 years) with Fitzpatrick skin types I to IV, wrinkling elastosis class I to III, gave informed consent to participate in this study. Five subjects received five-monthly treatments with the following settings: 560 nm cutoff filters, spot size 8 × 35 mm, 28 to 35 J/cm², pulse duration 2.4 to 4.2 milliseconds, pulse delay 15 milliseconds, and application of a thin coat of water-

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based cooling gel. Four subjects received five treatments with a 1,064 nm Nd:YAG laser, 6 mm spot size, fluence of 130 J/cm², triple pulse, pulse duration 7.0/7.0/7.0 milliseconds, pulse delay 75 milliseconds, and application of a thin coat of water-based cooling gel. The end point of therapy for both devices was mild erythema. Two-millimeter punch biopsies were taken from each subject from both treated (at 3 and 6 months after the treatment) and untreated (control) areas. In three of the subjects, the last biopsy at 6 months was not available. The specimens were fixed in formalin and processed in a routine manner using hematoxylin-eosin. Special techniques were used to study collagen (Masson trichrome), elastic tissue (van Gieson), and reticulin fibers (reticulin). All specimens were analyzed without knowledge of the time before or after treatments. All specimens were studied for the presence or absence of solar elastosis, inflammatory infiltrates, fibrin, extravasation of red blood cells, and vacuolization of epithelial cells in the epidermis, skin adnexa, and vessels. To evaluate the presence of new collagen in the papillary dermis, the band of collagen and elastosis immediately beneath the epidermis was measured in a fashion similar to the measurement of the Breslow thickness, that is, from the top of the granular layer of the epidermis.

Expression of heat shock protein 70 (hsp70) and of procollagen 1 was performed by immunohistochemistry using a standard technique. Briefly, slides were deparaffinized and treated for antigen retrieval by incubation in citrate buffer with microwave heating for 1 minute at full power followed by 14 minutes at 30% power. Then the slides were incubated overnight at 4°C with a monoclonal antibody (Santa Cruz, Santa Cruz, CA, USA) at 1:100 dilution. After incubation with the ABC complex, the slides were lightly counterstained with hematoxylin.

Student's *t*-test was employed to analyze possible differences before and after treatment and between the two techniques. A *p* value smaller than .05 was considered to be significant.

Results

Analysis of untreated skin confirmed the presence of mild to moderate degrees of sun damage, mainly characterized by solar elastosis deposited in the papillary dermis. There were no differences in the amount of collagen, elastosis, or

reticular fibers between biopsies from subjects treated with IPL versus those treated with the 1,064 nm Nd:YAG laser: the average thickness from the epidermal surface was 0.2 mm versus 0.21 mm, respectively (Table 1 and Figures 1 and 2). At 3 and 6 months after treatment, with routine histology, there were no statistically significant differences with the control biopsies. However, when examining the tissue with special stains, there was slightly increased thickness of the collagen located immediately below the epidermis. The differences appeared to be more pronounced for the biopsies from subjects treated with the Nd:YAG laser (0.28 mm post- versus 0.18 mm pretreatment for the 1,064 nm laser) (see Figures 1 and 2). However, the differences were not statistically significant. Examination with elastic tissue stains revealed a slightly smaller amount of elastotic material in the papillary dermis (see Figures 1 and 2).

Regarding the immunohistochemical studies, epithelium and smooth muscle expressed hsp70 in the pretreatment biopsies. Procollagen 1 was expressed mainly in the subepithelial region of the epidermis and adnexa. In the specimens after treatment with IPL or Nd:YAG laser, there was expression of hsp70 and procollagen 1 in dendritic cells in the papillary dermis and upper reticular dermis. When comparing specimens treated with IPL or Nd:YAG, there appeared to be a slightly higher expression in those specimens treated with Nd:YAG (Figure 3).

Clinically, all five patients in the IPL-treated group showed improvement of pigmentation and vascular abnormalities, whereas three of four patients in the 1,064 nm Nd:YAG subset showed one grade of clinical improvement in their wrinkling based on physician evaluation and the Fitzpatrick Wrinkling Elastosis Scale.

Discussion

Several devices have been used to try to stimulate collagen production in sun-damaged skin, with the goal of improving the skin's appearance (rejuvenation). Regarding IPL, there have been reports of collagen deposition⁹⁻¹¹; in our experience, in addition to collagen deposition, some of the clinical improvement seen after these treatments may be secondary to a reduction in inflammatory perifollicular infiltrates.⁶ The long-pulsed 1,064 nm Nd:YAG laser has been primarily used for treatment of leg veins and hair

Table 1. Comparison of IPL- and Nd:YAG-Treated Areas

	IPL			Nd:YAG			p Value
	Pretreatment	At 3 mo	At 6 mo	Pretreatment	At 3 mo	At 6 mo	
Elastosis	0.22	0.26	0.20	0.24	0.27	0.28	NS
Collagen	0.16	0.18	0.23	0.19	0.23	0.25	NS

IPL = intense pulsed light; Nd:YAG = neodymium:yttrium-aluminum garnet; NS = not significant.

All measurements are in mm from the top of the granular layer.

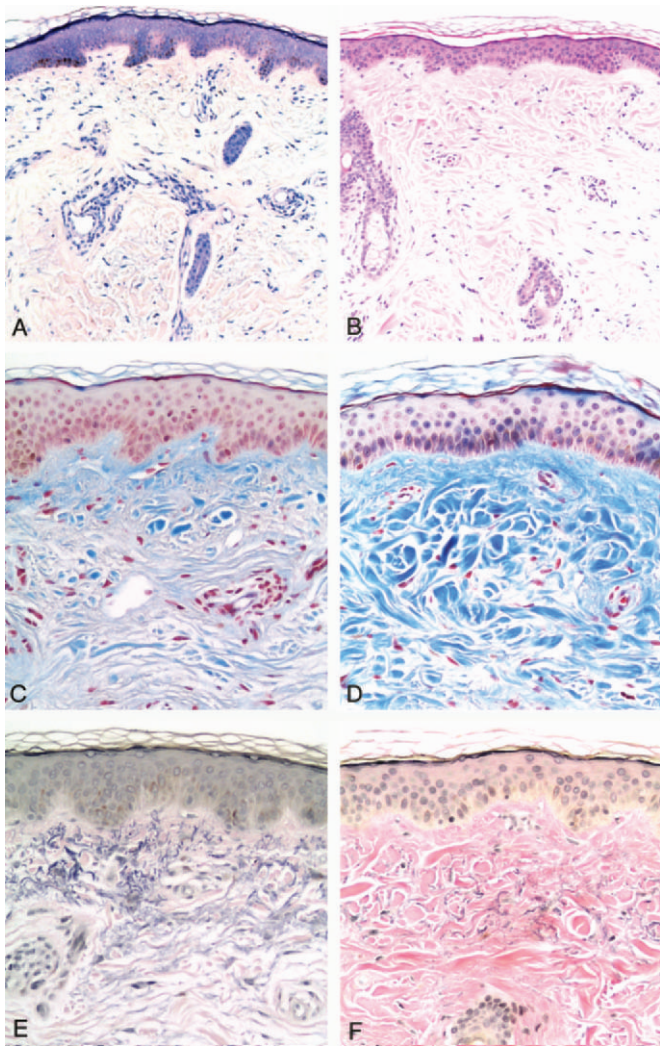


Figure 1. Effect of intense pulsed light on sun-damaged skin. (A, C, E) Pretreatment skin. Notice the presence of moderate solar elastosis (best evidenced on the elastic tissue technique of F). (B, D, F) Last follow-up biopsy after 6 months. Apparently increased collagen in the subepidermal region (best seen on the Masson technique of D) and slightly decreased amount of elastotic material (best seen on the elastic tissue technique of F). A and B: hematoxylin-eosin stain; $\times 10$ original magnification; C and D: Masson technique; $\times 20$ original magnification; E and F: elastic tissue technique, van Gieson's stain; $\times 20$ original magnification.

removal, but some authors have reported increased homogenization of the superficial collagen with a decrease in solar elastosis.^{7,12} Along these lines, our study has also detected a mild decrease in elastotic material in the dermis. A study on pigs reported increased collagen in the reticular dermis after using the 1,064 nm Nd:YAG laser with energy levels of 20 to 50 J/cm². Regarding humans, a previous study compared the efficacy of IPL and the Nd:YAG laser in the treatment of facial rhytids.⁴ Both treatments resulted in clinical improvement, and the authors suggested the possibility of collagen remodeling. However, the study did not include a histologic examination.

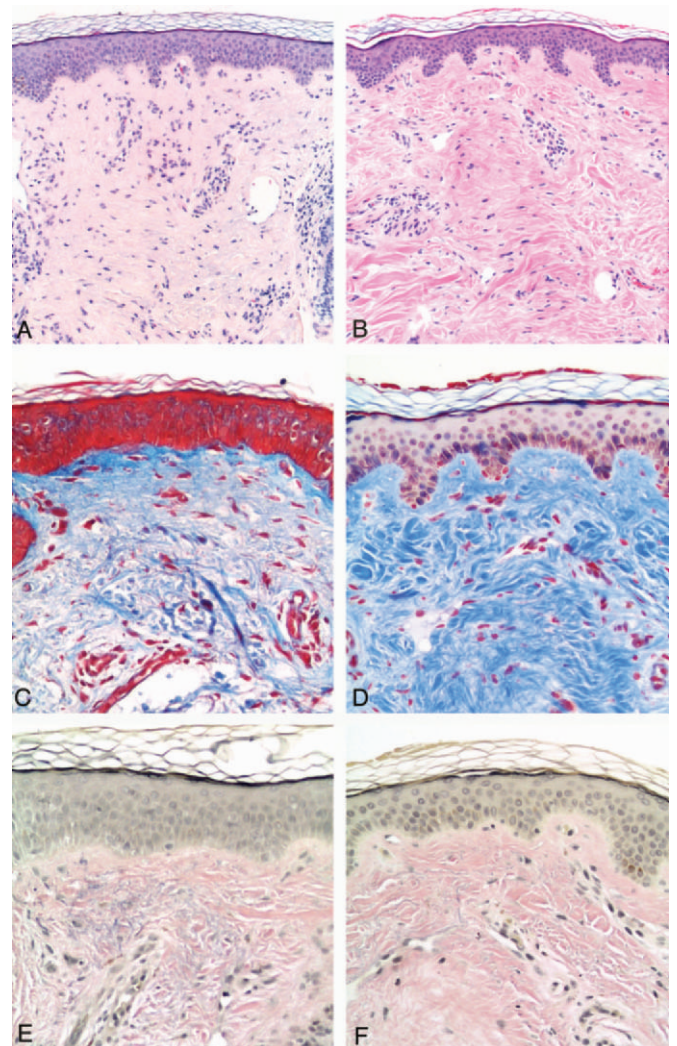


Figure 2. Effect of the Nd:YAG laser on sun-damaged skin. (A, C, E) Pretreatment skin. Notice the presence of moderate solar elastosis (best evidenced on the elastic tissue technique of F). (B, D, F) Last follow-up biopsy after 6 months. Apparently increased collagen in the subepidermal region (best seen on the Masson technique of D) and slightly decreased amount of elastotic material (best seen on the elastic tissue technique of F). A and B: hematoxylin-eosin stain; $\times 10$ original magnification; C and D: Masson technique; $\times 20$ original magnification; E and F: elastic tissue technique, Verhoeff-van Gieson; $\times 20$ original magnification.

The present study shows that IPL used in this setting induces no detectable damage to facial, sun-damaged skin. Epithelial and dermal structures appear to be preserved at both follow-up biopsies. Therefore, any cosmetic benefits are probably not related to destruction of preexisting dermal structures.

Some reports have indicated some degree of formation of new collagen after use of these two nonablative techniques.^{11,12} Although we have not seen statistically significant differences regarding the amount or quality of collagen between treated and nontreated areas, there appeared to be a trend for increased collagen in the upper dermis. In

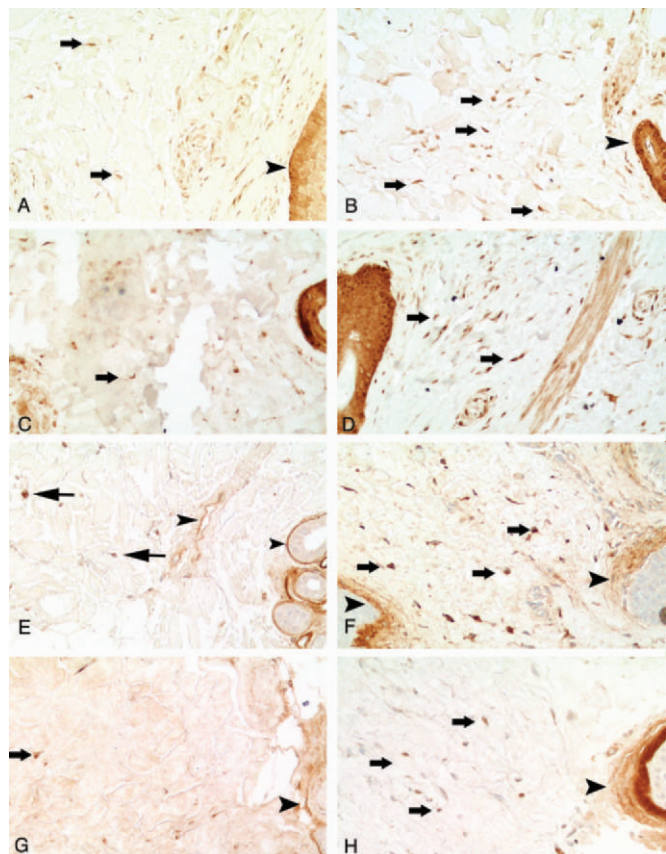


Figure 3. Expression of heat shock protein 70 (hsp70) and procollagen 1 before and after treatment with intense pulsed light (IPL) and the Nd:YAG laser. Pretreatment biopsies showed expression of hsp70 (A, C) and procollagen 1 (E, G) in rare dendritic cells in the dermis (arrows). Also notice the expression of these markers in the epithelium, endothelium, and basement membrane (arrowheads). After treatment with IPL (B, D) or the 1,064 nm device (F, H), there was expression of both markers in multiple dermal dendritic cells. A–D: anti-hsp70; E–H: antiprocollagen 1; all at 1:100 dilution and light hematoxylin counterstain; $\times 20$ original magnification.

the settings employed in this study, the end point selected for the application of the device was erythema rather than pinpoint bleeding. Because pinpoint bleeding likely represents vascular damage, the mechanism of collagen formation is likely to also be related to subclinical wound formation.

Previous studies have shown that laser treatment induces expression of several heat shock proteins.¹³ Our data show for the first time that both IPL and Nd:YAG induce expression of hsp70 by dermal dendritic cells; activation of these cells may be the underlying mechanism of collagen deposition. This may be similar to the mechanism proposed in radiation-induced fibrosis. In a study performed using skin from patients with breast carcinoma treated with radiation therapy, there was increased expression of early collagens I and III, as well as metalloproteinase 2 and the tissue inhibitor of the metalloproteinase

1.¹⁴ Dermal fibroblasts were considered to be the source of this increased expression. Supporting this hypothesis, our study has also shown that both devices tested resulted in deposit of procollagen 1, a precursor of collagen deposition, in the subepidermal region.

In summary, in the settings employed, there is no obvious morphologic damage to the epithelial and mesenchymal structures in the skin. Our data indicate that treatment with both IPL and Nd:YAG may result in increased collagen deposition in the papillary and upper reticular dermis, which might explain, in part, the clinical improvement seen with these techniques. Expression of the heat-induced protein hsp70 and procollagen 1 by dermal dendritic cells suggests that these cells may participate in the deposition of dermal collagen after treatment.

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