

Nonablative Facial Remodeling

Erythema Reduction and Histologic Evidence of New Collagen Formation Using a 300-Microsecond 1064-nm Nd:YAG Laser

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Background: A variety of nonablative lasers have been used to improve skin color and toning. Evidence of new collagen has been seen. Using blinded observer analysis of electron microscopic changes, we have documented the effect of a nonablative Nd:YAG laser on collagen production and its relationship to patient age.

Observations: Ultrastructural analysis of 9 patients showed a decrease in overall collagen fiber diameter in the papillary dermis at 1 month and 3 months after 3 treatment sessions. This is consistent with the formation of new collagen. Younger patients had a greater decrease in collagen fiber diameter compared with older patients. The change in collagen fiber diameter with time as well as the relationship between that change and the

patient's age were statistically significant ($P < .001$). Photographic evaluation showed that those patients with pre-existing erythema showed improvement in erythema along with an associated improvement in skin quality. There were no adverse events.

Conclusions: Microsecond Nd:YAG lasers appear to be safe for nonablative laser remodeling. Our study indicates that microsecond Nd:YAG lasers can produce new collagen formation in the papillary dermis. In addition, the condition of patients with erythema may be improved. Younger patients may form more new collagen compared with older patients with photodamage.

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BOTH NANOSECOND (Q-switched) and millisecond (long-pulsed) Nd:YAG lasers are currently used for nonablative dermal remodeling.¹⁻⁷ They are thought to stimulate new collagen production by producing a thermal injury to the dermis that initiates a wound-healing response.⁸ During wound healing, procollagen and type III collagen fibers are produced initially and have a small diameter. Later in the wound-healing process, thicker type I collagen fibers are made and cross-linking occurs, leading to an increase in the average diameter of collagen fibers in the dermis.⁹ Collagen fiber diameter can be measured via electron microscopy (EM). Electron microscopy studies of nonablative lasers such as the 585-nm flashlamp pulsed dye laser have shown a decrease in diameter of dermal collagen fibers after nonablative laser therapy.¹⁰ A decrease in collagen fiber diameter has been associated with production of new collagen,^{8,9} which is thought to increase skin firmness and improve skin texture in patients after treatment.

Recently, intermediate pulsed Nd:YAG lasers have been developed with pulse durations in the microsecond range. We investigated one of these new microsecond Nd:YAG lasers to determine its safety

and efficacy in nonablative dermal remodeling by using clinical photographs and EM analysis of dermal collagen fibers.

METHODS

Ten women aged 28 to 67 years with erythema and/or fine lines were enrolled in a study approved by the Pascack Valley Hospital (Westwood, NJ) institutional review committee. Subjects had Fitzpatrick skin types I through III. After signing a consent form, all subjects' faces (excluding the periorbital area) and jawline were treated 3 times at 2-week intervals with a 1064-nm Nd:YAG laser (CoolGlide Vantage; Cutera, Brisbane, Calif).

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Prior to treatment, the skin was cleansed with a standard bacteriostatic soap and water. Subjects' eyes were protected with stainless steel external ocular shields, and laser pulses were applied on the skin adjacent to but outside the orbital rim. The laser parameters were set to a fluence of 13 J/cm^2 , a pulse duration of 300 microseconds, and a spot size of 5 mm. A smooth, rapid painting motion was used to administer treatment, with the tip of the instrument 2 to 4 cm above the skin surface. Based on this separation from the skin, the actual laser beam at the skin surface is 6 to 7 mm in diameter, with corresponding fluences from 9 to 7 J/cm^2 , respectively. No cooling was performed.

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Figure 1. A, Erythema of the face before treatment with a 300-microsecond 1064-nm Nd:YAG laser; B, 3 months after final treatment.



Figure 2. A, Erythema of the face before treatment with a 300-microsecond 1064-nm Nd:YAG laser; B, 3 months after final treatment.

The face was treated in 4 sections, with a combined total of 12 000 to 14 700 light pulses applied per treatment. The pulses were applied at a rate of 7 Hz with a continuous motion of the handpiece at approximately 8 cm/s such that the pulses had little, if any, overlap. This was accomplished through a back and forth motion in 1 direction, combined with gradual motion in the perpendicular direction. Within each section of the face, repeated passes were made. The end point was determined based on the number of pulses. Each entire treatment consisted of approximately 30 minutes.

Two-millimeter punch biopsy specimens were obtained at baseline and at 1 and 3 months after final treatment. Biopsy specimens were taken from the infra-auricular sun-exposed area. Sequential biopsy specimens were taken nearly adjacent to each other. Collagen fibers from the papillary dermis were visualized and photographed via EM by previously described methods.^{11,12} Collagen fiber diameter of longitudinally cut fibers was measured directly on the photographs in millimeters, then adjusted for magnification to give a diameter in nanometers. For each biopsy specimen, a blinded physician observer measured and recorded the diameter of collagen fibers from 5 photographs. Ten fibers were measured per photo for a total of 50 collagen fiber measurements per biopsy specimen. The measurements for each specimen were averaged and then a mean fiber diameter for all patients was calculated for each time point.

Statistical analysis of the collagen fiber measurements was performed using SAS, version 8.2 (SAS Inc, Cary, NC), on a UNIX platform. The fiber diameter reduction from baseline to 1 and 3 months was tested using the 1-sided paired *t* test. Linear regression analyses were performed, and Pearson correlation coeffi-

cients were calculated for the correlations between age and change in fiber size from baseline to 1 and 3 months. An analysis of variance (ANOVA) was performed to analyze the relationship between the fiber size distributions and the time point.

In addition to the ultrastructural measurements, subjects were photographed at baseline, prior to each treatment, and at 1 and 3 months after final treatment. Digital photography with identical lighting conditions was used for all photographs. Photographs were evaluated by 2 nonblinded physicians, and changes in erythema and skin quality were assessed at each time point as compared with baseline. At each visit, patients were assessed for adverse effects, including erythema, edema, purpura, blistering, pigmentary changes, and scarring. At each visit, all subjects were asked if they had any adverse events between visits.

RESULTS

There were no adverse events reported in this study. Treatments were well tolerated by all patients, with minimal discomfort. Transient erythema occurred after almost all treatments but disappeared within hours of treatment.

One patient was lost to follow-up after the first treatment and was excluded from all analysis. One patient was excluded from clinical analysis owing to photographic errors but was still included in the EM collagen fiber analysis.

Four patients had noticeable erythema prior to treatment. All 4 of these patients had a reduction in erythema at 1- and 3-month follow-up visits as evidenced by pho-

Table. Mean Diameters of Collagen Fibers

Patient No. /Age, y	Baseline Fiber Diameter, nm	1-mo Diameter, nm	3-mo Diameter, nm	Change (Decrease) in Diameter, nm	
				At 1 mo*	At 3 mo†
1/52	60.7	60.0	55.0	0.8	5.8
2/35	71.9	61.5	64.7	10.4	7.2
4/58	47.8	47.8	51.7	<0.1	-3.9
5/31	77.6	60.2	53.6	17.4	24.0
6/44	53.9	62.8	49.8	-9.0	4.1
7/67	55.6	62.8	66.1	-7.2	-10.5
8/32	67.2	56.8	53.2	10.4	14.1
9/43	62.3	55.7	52.6	6.6	9.7
10/38	67.6	52.7	52.5	14.9	15.2
Mean/40	62.7	57.8	55.4	4.9	7.3

*P=.08

†P=.03

tographic evaluation. The improvement was greatest at 3 months. Skin texture was also improved in areas of erythema reduction as evidenced by photographic evaluation (**Figure 1** and **Figure 2**).

Mean collagen fiber diameter decreased compared with baseline at 1 and 3 months after the final treatment (**Table**) (**Figure 3**) in 7 of 9 patients. This difference approached statistical significance at 1 month ($P=.08$) and was significant at 3 months ($P=.03$). The relationship between fiber width distribution and the 3 time points (Figure 3) was significant ($P=.02$) by the ANOVA. An example set of EM photographs demonstrating the decrease in fiber size is shown in **Figure 4**.

The decrease in mean collagen fiber diameter appeared to be related to age. Younger patients appeared to have a greater decrease in collagen fiber diameter at 3 months (**Figure 5**). The linear regression analysis showed that for regression between subject age and change in fiber size from baseline to 1 month, the P value is .01 (Pearson correlation coefficient, -0.78), and to 3 months the P value is $<.001$ (Pearson correlation coefficient, -0.91).

COMMENT

The microsecond Nd:YAG laser investigated in this study appears to be safe for Fitzpatrick skin types I through III when used within the parameters studied and may be useful for reducing facial erythema and improving skin texture. The significant decrease in collagen fiber diameter seen by EM analysis at 3 months after treatment suggests that new collagen is being produced.^{8,9} This is consistent with an early wound-healing response in which thin procollagen and/or collagen III fiber production is increased in the papillary dermis. This new collagen production may be responsible for the improvement in skin quality seen after nonablative treatments.

Older patients appeared to have little to no decrease in collagen fiber diameter after treatment. This may suggest that older patients do not produce new collagen in response to nonablative laser therapy. The oldest patient in this study (patient 7; age, 67 years) actually showed an increase in collagen fiber diameter. Marked solar elastosis was

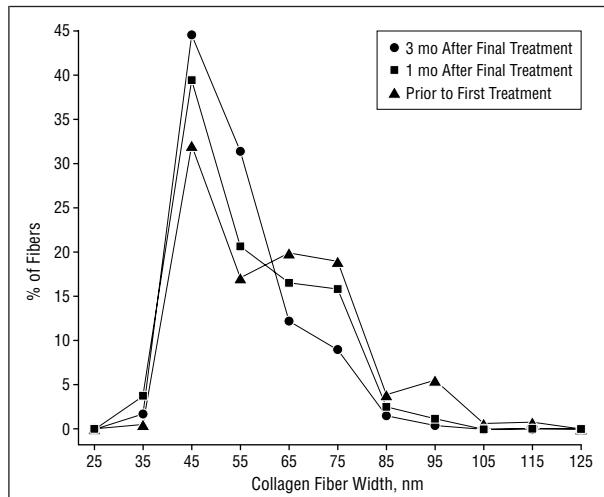


Figure 3. Mean percentage of collagen fibers of varying sizes before and 1 and 3 months after treatment.

present microscopically in all 3 biopsy specimens from this patient. Elastosis can impede accurate measurement of collagen fiber diameters, making data analysis difficult to interpret by our methods. When the data for patients 58 years and younger ($n=8$) were analyzed, the significance of the decrease in mean collagen fiber diameter was more evident with baseline mean diameter of 63.6 nm, 1-month diameter of 57.2 nm, and 3-month diameter of 54.0 nm. The difference between baseline and 1-month diameters (6.4 nm) was statistically significant ($P=.04$), as was the difference between baseline and 3-month diameters (9.5 nm) ($P=.007$).

It is possible that elastosis can block laser energy from hitting its targets and thereby inhibit its effectiveness in stimulating dermal remodeling. This may be why the 2 oldest patients in our study did not show a decrease in collagen fiber diameter. Older patients with photodamage may have minimal new collagen production in response to therapy. Anecdotal reports from clinicians indicate that younger patients with less photoaging have the greatest improvement from nonablative laser therapies. This study in-

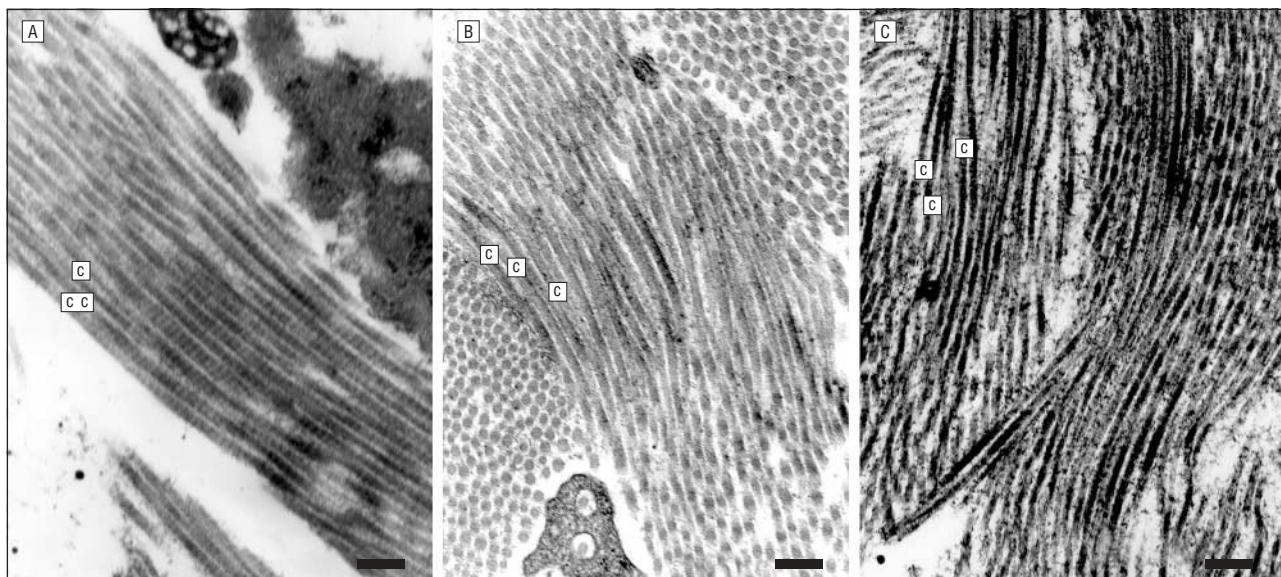


Figure 4. Electron micrographs of collagen fibers in the papillary dermis in 1 patient (A, pretreatment; B, 1 month after treatment; and C, 3 months after treatment [reticular background represents either proteoglycans or glycosaminoglycans]). The collagen fibers show typical periodicity and are cut longitudinally and transversely. There is gradual decrease in average width from pretreatment to 3 months (scale bar, 320 nm; original magnification $\times 40000$). Typical collagen fibers are labeled "C."

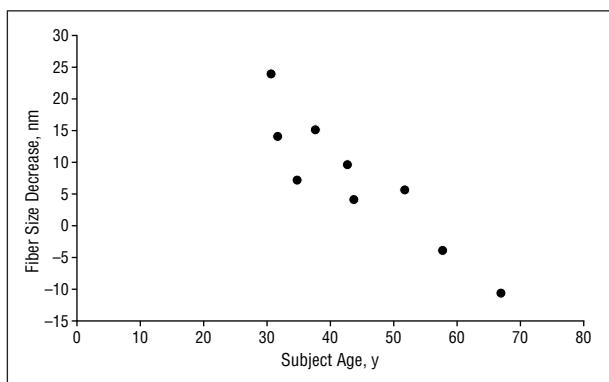


Figure 5. Mean decrease in collagen fiber diameter 3 months after final treatment.

dicates that microsecond Nd:YAG lasers can reduce facial erythema and improve skin texture. The skin textural changes may be similar to those reported with a variety of nonablative lasers.¹³⁻¹⁶ A statistically significant reduction in collagen fiber diameter after treatment indicates formation of new collagen. This may be 1 mechanism by which microsecond Nd:YAG lasers exert their clinical effects. Older patients with photodamage had less of a reduction in collagen fiber diameter. This may reflect less new collagen formation, which may lead to reduced clinical effects in such patients.

Further work remains to determine the role that age and photodamage may play in dermal remodeling and clinical effectiveness after nonablative laser resurfacing. Studies are also needed to better define the mechanisms by which nonablative laser resurfacing exerts effects on collagen and other components of the dermal matrix.

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