Collagen Remodeling After 585-nm Pulsed Dye Laser Irradiation: An Ultrasonographic Analysis

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BACKGROUND AND OBJECTIVES. **Nonablative dermal remodeling is an evolving technology that has generated great interest among both laser surgeons and patients. Evidence indicates that dermal collagen formation is the key mechanism of action for the nonablative techniques. We studied, with ultrasound, new collagen formation after nonablative laser irradiation.**

METHODS. **Ten patients with facial rhytids underwent a single treatment with a 585-nm pulsed dye laser. The patients were all female, ranging in age from 47 to 67, and were Fitzpatrick skin types I–III. Laser parameters were as follows: an energy fluence of 2.4 to 3.0 J/cm^2, a pulse duration of 350 μsec, and a spot size of 5 mm with no overlap. Ultrasonographic assessments of dermal collagen were taken at baseline and at 30 and 90 days after treatment.**

RESULTS. **Ultrasonography demonstrated an increase in dermal collagen after a single treatment with the 585-nm pulsed dye laser. The greatest degree of neocollagenesis occurred periocularly.**

CONCLUSION. **A single treatment with a 585-nm pulsed dye laser appears to increase dermal collagen. This increase in dermal collagen can be assessed with noninvasive cutaneous ultrasound.**

THE ULTRASOUND DEVICE AND LASER WERE LOANED FOR THIS STUDY.

THERE IS increasing interest in nonablative facial rejuvenation. This comes at a time when the number of ablative resurfacing procedures continues to decline.[1] A variety of nonablative techniques have been proposed. Broadly speaking, these include pulsed laser at wavelengths of 585, 1064, 1320, and 1450 nm, as well as intense pulsed light and radiofrequency devices.[2–6] These technologies are relatively new, and further understanding of their effects is needed. We describe the use of cutaneous ultrasound to demonstrate the dermal changes induced by irradiance with a 585-nm pulsed dye laser. Additionally, traditional photographs are often unable to reproducibly demonstrate changes after nonablative remodeling. This limitation of photography makes the use of a measurement tool such as ultrasound valuable to determine cutaneous changes objectively. The use of ultrasound in measuring dermal collagen has been well validated in studies that included histologic correlation.[7–9]

Methods

Ten patients with facial rhytids underwent a single treatment with the NLite 585-nm pulsed dye laser (ICN Photonics, Costa Mesa, CA). The patients were all female, ranging in age from 47 to 67, and were Fitzpatrick skin types I–III. Laser parameters used included an energy fluence of 2.4–3.0 J/cm^2, a pulse duration of 350 μsec, and a spot size of 5 mm with no overlap. The energy fluence was selected to be subpurpuric. The desired clinical endpoint was transient (less than 10 seconds), darkening at the laser impact site. No epidermal cooling is employed with the NLite laser. Ultrasonographic assessments of dermal collagen were taken at baseline and at 30 and 90 days after treatment. Ultrasound images were obtained with the Episcan I-100 (Longport Inc., Swarthmore, PA).

Images from all patients at all treatment locations were not recorded; rather, representative images were obtained. Ultrasound is primarily used as a comparative rather than an absolute imaging tool. For each site evaluated, the system settings were kept constant throughout the study. Some variation in these settings among patients and treatment sites was necessary to compensate for different characteristics of the dermal regions studied. Differences in any of the image sequences are the result of dermal changes rather than system settings. Computer-generated quantification of the ultrasound images was obtained. The increase in signal reflection correlates with increased dermal collagen.

Figure 1 represents the color palette applied to the reflected signal to allow for image analysis. Red presents the largest reflected signal, and the image

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Figure 1. Color palette employed for ultrasound images. The largest reflected signal results in a red image, and zero signal generates black. Each color merges into the next, and red is maintained even if the amplifiers are saturated.

remains red with signal saturation. Black represents no signal reflection. There was no clinical endpoint in this study, and clinical data were not recorded.

Results

All patients had an increase in dermal collagen from day 0 to day 90. The periorbital region appears to have the greatest increase in collagen. Computer-generated quantification of the ultrasound images was performed, and individual images were analyzed and scored. The value generated represents a quantification of the image intensity. That is, a measurement of the reflected echogenic signal and its color components (red, green, blue) was obtained. Although the images can be compared visually, all data acquisition and analyses were done via computer.

Data were then analyzed and subjected to statistical measure. Raw data for each color component (red, green, blue) were normalized against the red, green, blue mean to offset any variation in overall image brightness achieved for any given image. Normalized red levels (which represent the greatest amount of reflected signal and collagen) increase from 0 to 30 days, from a mean of 0.73 to 0.88. The likelihood that this is random is negligible (P value of 4×10^{7}), as measured by a paired t-test. Normalized red levels increase further from 30 to 90 days from a mean of 0.87 to 0.92. The group that was measured at 0 and 30 is not quite identical with those measured at day 30 and day 90, hence the difference between 0.88 and 0.87 for the 30-day mean. The P value that this is caused by random fluctuations is very small (0.01). When analyzing the data from day 0 to day 90, the mean increases from 0.74 to 0.92 (P value of 10^{11}).

As would be expected, normalized blue levels move in the opposite direction. As blue images represents little reflected signal, as reflected signal (red) increases, blue should decrease. From 0 to 30 days, the normalized blue levels decrease from 1.71 to 1.21 (P value of 4×10^{10}). From day 30 to day 90, they decrease from 1.22 to 1.01 (P value of 0.004). From day 0 to day 90, they decrease from 1.76 to 1.01 (P value of 5×10^{10}).

Enough data points exist to perform subgroup analysis for the periocular treatment areas and the cheeks. From 0 to 90 days, the 14 periocular treatment sites had an increase in normalized red signal from 0.76 to 0.93 (P value of 3×10^{6}) and a normalized blue decrease from 1.77 to 1.14 (P value of 3×10^{5}). From 0 to 90 days, the four cheek readings had a normalized red increase from 0.72 to 0.90 (P value of 0.002) and a normalized blue decrease from 1.56 to 1.06 (P value of 0.0004).

Figure 2a–c illustrates a representative image series obtained. Red represents the most reflected signal, black a lack of reflected signal, and blue and green intermediate signal reflection (as illustrated in Figure 1).

Discussion

The NLite laser is a Food and Drug Administration–cleared modality for nonablative wrinkle reduction. The patient demand for minimally invasive rejuvenation techniques has grown rapidly. In this study, we found highly suggestive data showing that irradiance with a 585-nm pulsed dye laser increases dermal collagen. This increase in dermal collagen is the cornerstone of nonablative dermal remodeling with laser therapy. Furthermore, collagen was increased after only a single treatment with the laser. Currently, most laser surgeons undertake a series of treatments to effect a clinically noticeable improvement in rhytids. Our experimental protocol used similar (and in most cases identical) laser parameters as Bjerring et al.[4] used in their study of the NLite laser. They measured an increase in aminoterminal propeptide procollagen III concentration after a single treatment with the NLite

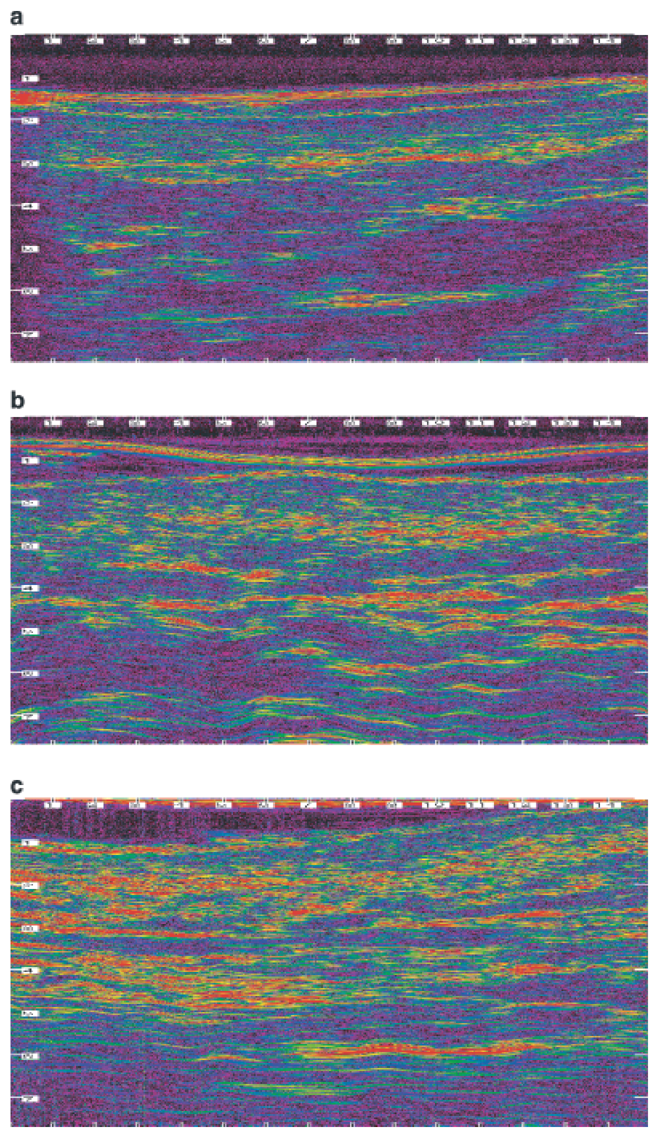

Figure 2. Representative images from left periorbital area (left to right): baseline, 30 days, 90 days. The normalized red values for this image series increase from 0.75 to 0.79 to 0.93 at 0, 30, and 90 days. Normalized blue values for this image series decrease from 1.77 to 1.44–1.07 at days 0, 30, and 90.

laser. An aminoterminal propeptide procollagen III concentration in suction blister fluid was used as a surrogate endpoint for new dermal collagen production. Our work builds on this finding by assessing actual dermal collagen as an endpoint. As in Bjerring et al.'s study, our patients experienced neither significant discomfort from treatment nor clinical evidence of overt skin damage. Bjerring et al. were also able to demonstrate a noticeable clinical improvement in facial rhytids. Our study was not designed to measure clinical end points.

One could speculate that the increase in ultrasound echogenicity that we detected could be related to factors extrinsic to the laser irradiation. The addition of a control group would have helped to avoid this concern. However, as nearly every subject had an increase in signal that was demonstrable on two separate occasions, it is unlikely that this change would be due to chance alone.

Conclusion

Irradiance with the NLite laser appears to result in increased dermal collagen. Additional studies to include histologic correlations would provide further assessment of the effects of nonablative irradiation.

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Commentary

It is cliché that the efficacy of nonablative treatments is difficult to measure. Blinded assessors evaluating before and after photographs can often not see a difference, even when investigators and patients are convinced that improvement has been achieved.

Moody et al. are to be commended for trying to interpret a standardized measurement instrument, in this case cutaneous ultrasound. In particular, their discussion of the technical aspects of this process is fascinating, if not entirely digestible, for the average dermatologist (myself included). Hopefully, more studies will further validate the use of this tool. Along with optical tomography,[1] the PRIMOS system,[2,3] evaluation of physical molds,[4,5] and the like, cutaneous ultrasound has a place in the objective evaluation of textural abnormality.

Of course, it is important to remember that the gold standard remains visual inspection by an unbiased, perceptive observer. People do not get nonablative resurfacing to impress

machines but rather to please themselves, their friends, their lovers, their coworkers, and other bystanders. To the extent that mechanistic algorithms can ascertain, with greater sensitivity than people, the same differences that people see, such approaches (e.g., cutaneous ultrasound) can be useful.

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