Histological Evaluations Following 1,064-nm Nd:YAG Laser Resurfacing

Steven Dayan, MD,1,* John F. Damrose, MD,1 Tapan K. Bhattacharyya, PhD,1 Steven Ross Mobley, MD,2 Minu K. Patel, MS,1 Kevin O'Grady, BS,1 and Steven Mandrea, MD1
1Division of Facial Plastic and Reconstructive Surgery, Department of Otolaryngology, Head and Neck Surgery, University of Illinois at Chicago, Chicago, Illinois 60612
2Division of Otolaryngology, Department of Surgery, University of Utah, Salt Lake City, Utah 84132
3Department of Biostatistics and Research Resources Center, University of Illinois at Chicago, Chicago, Illinois 60612

Background and Objectives: The long pulse 1,064-nm Nd:YAG laser is used clinically to decrease rhytids formation. The dermal level at which this change occurs has not been established. This study attempts to answer these questions using a porcine skin model.

Study Design/Materials and Methods: Non-randomized prospective experimental trial involving the domestic piglet treated serially with the long pulse 1,064-nm Nd:YAG laser.

Results: Collagen formation occurred at the level of the reticular dermis. After one laser treatment, a significant level of collagen formation was induced in the reticular dermis compared to controls. The greatest gain was observed after four laser treatments. Energy levels of 20, 30, 40, and 50 J/cm² were evaluated. Although not statistically significant, 30 J/cm² had the greatest effect on collagen formation. However, at 50 J/cm², marked ablative changes to the epidermis were observed.

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Key words: Nd:YAG laser; laser resurfacing; collagen formation

INTRODUCTION

Rejuvenating facial skin through non-ablative means is an area of ongoing investigation. Ultrasound, intense pulsed light, pulsed dye lasers, and Nd:YAG lasers represent technologies intended to smooth the appearance of wrinkled skin without damaging its most superficial layers [1–5]. This latter feature is important to minimize patient recovery time and to prevent undesirable complications. Most outcome research in this area is based upon subjective assessments of cosmetic appearance. While the rhytid reduction results reported from studies on non-ablative methods of facial rejuvenation are generally positive, there are a subset of patients who are treated that either have no appreciable improvement or are disappointed with the limited results. This may be an overlooked part of the published reports but is a critical element to the success of these procedures in a clinical practice. Prognosticating factors for determining good outcomes are still yet to be clearly identified. Identifying the optimal parameters for stimulating collagen production may lead to greater patient satisfaction.

Histological studies have documented rejuvenating changes that occur at the dermal level [6–8]. Reported changes include increase in epidermal keratinocytes, collagen, elastin, and glycosaminoglycans. Additionally age related dermal products are displaced such as elastic dermal connective tissues [9]. The histological data that is reported in the literature is often based upon qualitative assessments of changes in dermal thickness or collagen formation. Studies attempting to quantitatively describe changes occurring in cutaneous elements following non-ablative treatment are few.

In our clinical practice, we have found evidence of clinical improvement in the appearance of facial skin treated in series with a 1,064-nm Nd:YAG laser [10]. We theorized that the reduction in the appearance of rhytids and the improvement in skin laxity were due to heat deposition into the reticular and papillary dermis stimulating collagen formation. However, histological evidence supporting our theory was not obtained. In addition, the operating parameters chosen were based on theory rather than empirical evidence.

The objective of this study is to quantitatively evaluate changes in dermal structures of in vivo porcine skin following non-ablative therapy using the long pulse 1,064-nm Nd:YAG laser at variable energy levels.

MATERIALS AND METHODS

Institutional guidelines regarding animal experimentation were followed as established at the University of Illinois at Chicago. The subject was a 50-pound domestic piglet. The animal was anesthetized with ketamine 20 mg/
kg intramuscularly (IM) and xylazine 2 mg/kg IM. An intravenous (IV) line was placed in a dorsal auricular vein for pre-operative administration of kefzol 1 mg/kg IV and fluid maintenance during surgery. For the duration of the procedure (approximately 1.0 hour), inhalational anesthesia (isoﬂurane) was administered to effect.

The animal was placed on its ventral surface, a ﬂank was randomly chosen (the left), and ﬁve separate grids were tattooed onto the side. The energy level to be used on each grid was assigned as shown in Figure 1 with a control grid being randomly assigned. The animal was then subjected to laser treatments using a 10 mm hand piece (Laserscope Corporation, San Jose, CA) combined with a chill-tip skin cooling device set at 1.5°C (Model T-502P, ThermoTek Corporation, Carrollton, TX). Clear ultrasound gel was applied to the skin prior to treatment to facilitate movement of the hand piece. Treatments were conducted weekly for four consecutive weeks.1 The energy levels investigated ranged from 20 to 50 J/cm² depending on group assignment. All other laser settings for each group were identical (spot size of 10 mm, pulse duration of 50 milliseconds, pulse rate of one per second for 5 seconds). The same experimenter operated the laser in all cases. Punch biopsies (3.0 mm) were taken immediately after the ﬁrst treatment (B1), 1 week after the ﬁrst treatment (B2), 1 week after the third treatment (B3), 1 week after the fourth treatment (B4), and 8 weeks after completion of the fourth treatment (B5). Control biopsies were obtained in the same fashion and time course as the experimental biopsies.

The biopsy areas were closed with 3.0 nylon suture. The animal was fed a normal-grower ration throughout the experiment and was sacriﬁced in a humane and standard manner.

Specimens were ﬁxed in 10% neutral buffered formalin. They were then embedded in parafﬁn and 8 μm sections were obtained perpendicular to the skin surface and stained by either the hematoxylin–eosin–phloxine sequence or the Verhoeff-van Gieson (VVG) technique for differentiation of collagen and reticular ﬁbers. Morphometric data were collected using ocular micrometry and a point-counting method as described previously [11]. Briefly, a 10 × 10–division square grid (105 × 105 μm) with 121 intersecting points is placed over the ﬁeld of interest and studied at a magniﬁcation of 400 power. The number of points overlying a given dermal element (e.g., collagen bundles or elastic ﬁbers) is then divided by the total number of intersecting points (121). This number is referred to as the “area fraction” and gives the relative fraction of space occupied by the given dermal element.

The depths of the epidermis and papillary dermis, and the collagen bundle widths in the reticular dermis, were recorded in micrometers. The area fractions of collagen, elastic ﬁbers, capillaries, and remaining tissue space (which contains nerve ﬁbers and other cellular elements) were determined for both the papillary dermis and the reticular dermis.

1None of the authors listed has a commercial or proprietary interest in the product or company.

All experimental data were analyzed using two-way analysis of variance (ANOVA) with SPSS 10.0 statistical software. The assumptions of homogeneity of variances and normality of the error terms were satisﬁed. Results were considered statistically signiﬁcant at a P-value of less than 0.05.

RESULTS

The various histological parameters measured in the control and experimental groups are shown in Tables 1 and 2. The individual values obtained for the control biopsies were averaged together. No signiﬁcant differences were found between them prior to averaging.

As noted in table one, epidermal thickness, papillary dermal thickness, and collagen bundle width all increased 8 weeks following the fourth treatment. While this trend was consistent it did not meet statistical signiﬁcance.

As demonstrated in the Table 2, compared to controls, a signiﬁcant increase in the area fraction of collagen in the reticular dermis was noted beginning 1 week following the ﬁrst laser treatment. Collagen formation increased with each successive treatment. The greatest amount of collagen formation (81%) was seen after four laser treatments although this was not signiﬁcantly different from the group treated only three times (79%). Eight weeks following cessation of treatment, the area fraction of collagen was signiﬁcantly greater than that of controls. Figure 2 depicts photomicrographs taken of collagen at the level of the reticular dermis from a control specimen and a specimen treated with four laser treatments at 30 J/cm². Note that there is an increased density of collagen in the treatment group.

Energy level had no signiﬁcant effect on the degree of collagen formation. Although, on average, a setting of 30 J/cm² induced collagen formation in the reticular dermis to a greater extent than the other energy levels studied, these observed differences were not statistically signiﬁcant. Of note, skin treated at a level of 50 J/cm² resulted in ablative changes to the epidermis (see Fig. 3) characterized by separation of the keratinized and non-keratinized layers of the epidermis, increased vacuolization of the cells of deeper epidermis, and disruption of the epidermal–dermal junction. Epidermal thickness was also noted to increase in this group, a change which was still observed 8 weeks after cessation of treatment (Fig. 4). Compared to controls, however, this increased thickness was not statistically signiﬁcant.
TABLE 1. Thickness of the Epidermis, Papillary Dermis, and Collagen Bundle Widths in the Control and Treatment Groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Epidermis (µm)</th>
<th>Papillary dermis (µm)</th>
<th>Collagen bundle width (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>83</td>
<td>32</td>
<td>24</td>
</tr>
<tr>
<td>Immediately after first treatment (B1)</td>
<td>94</td>
<td>52</td>
<td>30</td>
</tr>
<tr>
<td>One week after first treatment (B2)</td>
<td>102</td>
<td>39</td>
<td>32</td>
</tr>
<tr>
<td>One week after third treatment (B3)</td>
<td>94</td>
<td>44</td>
<td>31</td>
</tr>
<tr>
<td>One week after fourth treatment (B4)</td>
<td>99</td>
<td>39</td>
<td>26</td>
</tr>
<tr>
<td>Eight weeks after fourth treatment (B5)</td>
<td>98</td>
<td>51</td>
<td>51</td>
</tr>
</tbody>
</table>

No significant differences were noted between the experimental groups and the controls for the remaining histological variables studied.

DISCUSSION

The long pulse 1,064-nm Nd:YAG laser stimulates collagen formation at the level of the reticular dermis and clinically represents an effective, non-ablative treatment method for improving the appearance of facial rhytids. Non-selective heat deposited into the dermis creates a subclinical wound response and fibroblast stimulation. The properties of this laser, when combined with a chilling device, allow for energy to effectively bypass the epidermis in patients of all skin types with minimal risk for superficial thermal injury. Within the dermis, Nd:YAG laser energy is weakly absorbed by melanin and water. Dermal components targeted in non-ablative rejuvenating efforts reside in the papillary and reticular dermis approximately 100–500 µm below the skin surface. It has been postulated that this is the area in which an ideal laser for non-ablative dermal remodeling would deposit its energy [12]. Although, a specific dermal zone receiving the heat in order to maximally stimulate collagen formation has yet to be definitively proven [9]. Laser light and tissue interaction is based on many factors but with the interpreting benefits of Beer’s law, the depth of a particular laser penetration and conversion of laser energy into heat can be predicted. Beer’s law states that the depth of penetration of the laser light is equal to the reciprocal of the absorption coefficient at the selected optical wavelength [12]. Because the infrared laser’s chromophore is tissue water, the absorption coefficient of water and the hydration level of the dermis will inversely affect the depth of penetration and need to be taken into consideration. The epidermis and superficial dermis is less hydrated then the deeper dermis resulting in less laser energy absorption superficially and greater absorption deeper in the dermis. The greater depth of penetration achieved by the Nd:YAG lasers is illustrated in part by Beer’s law but its penetrating depth it also dependant on the scattering coefficient. The Nd:YAG lasers are recognized to have a low scattering coefficient [9]. Based on these dynamics it has been suggested that the 1,450 Nd:YAG laser penetrates to the an ideal targeted dermal depth for non-ablative treatments [12]. However, there are many difficult to control factors, which may at any-time affect laser depth of penetration and subsequent heat production. Tissue hydration can vary and be affected by topical placement of anesthetic creams, scattering coefficients are not standardized, and variations in pulse width and repetition rate can affect depth of laser light penetration.

All the infrared lasers heat a tissue block within the dermis and may stimulate collagen deposition. Histological studies of patients treated in clinical trials with 1,320;

TABLE 2. Average Area Fraction of Collagen, Elastic Fibers, Capillary Space, and Tissue Space by Dermal Location in the Control and Treatment Groups

<table>
<thead>
<tr>
<th></th>
<th>Collagen</th>
<th>Elastic fibers</th>
<th>Capillary space</th>
<th>Tissue space</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Papillary dermis</td>
<td>63</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Reticular dermis</td>
<td>73</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Immediately after first treatment (B1)</td>
<td>Papillary dermis</td>
<td>63</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Reticular dermis</td>
<td>74</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>One week after first treatment (B2)</td>
<td>Papillary dermis</td>
<td>63</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Reticular dermis</td>
<td>76</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>One week after second treatment (B3)</td>
<td>Papillary dermis</td>
<td>69</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Reticular dermis</td>
<td>79</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>One week after fourth treatment (B4)</td>
<td>Papillary dermis</td>
<td>68</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Reticular dermis</td>
<td>81</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Eight weeks after fourth treatment (B5)</td>
<td>Papillary dermis</td>
<td>66</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Reticular dermis</td>
<td>78*</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

*Denotes statistical significance.
Nd:YAG LASER RESURFACING

1,450; 1,540 nm; and Q-switched 1,064 Nd:YAG lasers have documented increases in dermal collagen [13–15], in the homogenization of collagen [16], and in the papillary dermis density [17]. In particular, punch biopsies taken from the pre-, post-auricular and arm sites on patients treated non-ablative in series with a long pulse 1,064-nm Nd:YAG and KTP lasers revealed increase collagen and elastin formation throughout the papillary and reticular dermis [6]. The key is to find the right parameters for each laser in which to maximize the collagen production yet without excess heat that may injure surrounding structures.

Clinical studies have shown the 1,064 nm Nd:YAG laser used in a series to be a well tolerated and effective method for modestly improving the appearance of facial rhytids [10,18]. The properties of the longer wavelength 1,064-nm Nd:YAG, which is less absorbed by water than other Nd:YAG lasers, allows it to penetrate deeper into the dermis, therefore, the epidermis is particularly protected from heat injury and discomfort is minimal. However, higher fluences are needed to reach a denaturing dermal temperature and the targeted zone of heat deposition may be deeper than the other Nd:YAG lasers.

Therefore, questions remain as to the 1,064-nm Nd:YAG laser's optimal operating parameters for positively effecting dermal structures. A significant barrier to answering these questions has been the impracticality of performing multiple skin biopsies in the same human patient. Porcine skin has shown to have many similarities to human skin and has proven a valid alternative model in answering just such questions [19].

Based on the theory of selective photothermolysis [20], if the energy deposited is sufficient and within an appropriate time frame (thermal relaxation time), chromophore is heated beyond its destructive temperature threshold. Heat is contained within the chromophore and not dissipated to surrounding tissues, therefore, preventing local thermal injury. However, we theorized that if the energy level was below the chromophore's threshold and the pulse width was extended beyond the thermal relaxation time, the heat would be absorbed by the chromophore but then dissipated to surrounding tissues before reaching a destructive threshold level. Therefore, energy settings in our study were below that which are used for hair or vein removal.

Following our intervention, the greatest increase in collagen formation was seen at the level of the reticular dermis. The 1,064-nm wavelength of the Nd:YAG laser extends to a deeper depth than other lasers with shorter wavelengths and may explain why the greatest change was

Fig. 2. Photomicrographs of biopsies obtained from a control sample (left: control biopsy, VGG stain, 20X.jpg) and 1 week after four laser treatments at 30 J/cm² (right: B4, 30 J treatment, VGG stain, 20X.jpg) (VGG stain, original magnification ×20). Note the increased collagen formation in the treated group. [Color figure can be viewed in online issue via www.interscience.wiley.com.]

Fig. 3. Photomicrographs of biopsies obtained immediately after treatment at a level of 50 J/cm² (right: B1, 50 J treatment, H&E stain, 20X.jpg). (Hematoxylin–eosin–phloxine stain, original magnification ×20). Following treatment, the epidermis is thicker with vacuolization of cells of its deeper layer. [Color figure can be viewed in online issue via www.interscience.wiley.com.]
seen in the reticular dermis and not the papillary. Although it never reached statistical significance, epidermal thickness increased throughout the study. Initial increases in epidermal depth may have been secondary to edema formation. Increased epidermal thickness may explain our clinical experience of an immediate reduction in the appearance of fine wrinkles noted by our patients.

Reticular collagen formation was increased at all energy settings evaluated 20, 30, 40, and 50 J/cm². Although 30 J/cm² resulted in the greatest amount of collagen formation it was not found to be statistically significant from the other energy settings. Ablative injury to the epidermis was noted when 50 J/cm² was utilized. However, treatments at the other energy settings caused no immediate or delayed overt injury to the epidermis or dermis. This has been consistent with our clinical practice in which the long pulse Nd:YAG laser used at modest settings of 22 J/cm² and 50 milliseconds has resulted in no evidence of injury in over 500 treatments. These treatments have been well tolerated with minimal to no discomfort in patients of skin types 1 through 5 [10].

Our study evaluation was limited to 8 weeks following the completion of treatment. At this time, we still noted an increase in collagen formation. However, there was a decreasing trend among all treatment groups. Whether the increased level of collagen is maintained for an extended period still yet to be determined. Further research should include a longer evaluation period to better assess the durability of collagen formation.

CONCLUSIONS

Our study demonstrates that porcine skin treated with the long pulse 1,064-nm Nd:YAG laser in series results in a statistically significant increase in reticular dermal collagen formation. The greatest increase in collagen formation was noted at 30 J/cm². Our findings provide histological evidence that supports subjective improvements noted in the appearance of facial skin established in clinical trials utilizing the long pulse 1,064-nm Nd:YAG laser at non-ablative levels. Non-ablative resurfacing represents the newest approach to improving photodamaged skin and is best suited for those patients who have a limited willingness for downtime. Patients must be counseled and expectations managed but with proper patient selection, and operating parameters patient satisfaction can be maximized.

REFERENCES


