A Systematic Histologic Analysis of Nonablative Laser Therapy in a Porcine Model Using the Pulsed Dye Laser

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Background: To our knowledge, no systematic analysis of nonablative laser therapy has been performed.

Objective: To alter the parameters (fluence, spot size, pulse duration, and use of cooling spray) for the pulsed dye laser to determine the precise settings that would yield the most favorable dermal remodeling in a porcine model.

Methods: Research was conducted in an animal laboratory at Albany Medical College. An anesthetized Yu-catan miniature pig was subjected to a pulsed dye laser at various parameters. After 10 weeks, the laser-treated areas were harvested and processed for blinded, randomized, histologic evaluation. Negative (nontreated skin) and positive (ablative carbon dioxide laser–treated skin) controls were compared with the nonablative pulsed dye laser–treated areas.

Main Outcome Measures: Quantitative assessment of collagen band width and cells per high-power field and qualitative assessment of epidermal and dermal changes.

Results: A significant difference (P<.001) in collagen band width was evident when nonablative laser–treated skin and carbon dioxide ablative laser–treated skin specimens were compared with untreated skin specimens, but no significant (P=.18) difference existed between the nonablative and ablative modalities. Similarly, cellular hypertrophy, as measured by high-power field, corroborated the previous findings. Furthermore, a higher fluence, a larger spot size, and a longer pulse duration proved statistically significant for increased collagen band width (P=.01, P<.001, and P<.001, respectively), and a larger spot size and a longer pulse duration exhibited significance for cells per high-power field (P=.02 and P=.009, respectively), with a trend toward significance for higher fluence (P=.09). Overall, the dermis was considerably thicker for nonablative and ablative laser–treated areas compared with untreated skin, but this could not be quantified because the depth exceeded the punch biopsy instrument. The epidermis remained unchanged.

Conclusions: The nonablative pulsed dye laser has demonstrated favorable histologic evidence of dermal remodeling, and its effects were similar to histologic changes seen with the carbon dioxide ablative laser, both of which were statistically significant compared with untreated skin, as seen in this preliminary animal model.

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Traditionally, cutaneous rejuvenation has been achieved with ablative methods (chemical exfoliation, laser therapy, and mechanical abrasion).1-4 All of these modalities deliberately efface the epidermis and partially the upper dermis to effect the desired collagen remodeling and the formation of a new epithelial covering. However, ablative techniques produce significant postoperative morbidity, including risk of hyperpigmentation and hypopigmentation; dermatitis; perioperative edema; protracted erythema; milia, herpetic, and acne outbreaks; physical discomfort; an involved cleaning and occlusive regimen; need for solar protection; and the most dreaded outcome, potential scar-
age to the overlying epidermis (dyschromias and severe rhytidosis) cannot be readily addressed with this modality because no epidermal effacement occurs. Furthermore, dermal remodeling is purportedly more limited with nonablative therapy than what may be achievable with ablative therapy. In select patients with mild to moderate rhytidosis who have relatively little photodamage to the epidermis, nonablative therapy may provide an effective and convenient treatment method for facial rejuvenation.

Many laser and broad-spectrum light devices have been applied for nonablative therapy, including the Q-switched 1064-nm Nd:YAG laser, the erbium:glass laser, the 980-nm diode laser, the 595-nm wavelength. Besides the pulsed dye laser, many laser companies have also promoted their pulsed dye laser for nonablative therapy; the Vbeam (Candela Corp) has recently received Food and Drug Administration approval for nonablative use. Allegedly, the pulsed dye laser effectively stimulates collagen synthesis by virtue of the nonspecific thermal injury caused in the dermis when the adjacent dermal blood vessels are targeted by the 595-nm wavelength. Besides the pulsed dye laser, many lasers that have been used for other medical purposes have also been applied for nonablative therapy, including the 1320-nm Nd:YAG laser, the 980-nm diode laser, and the erbium:yttrium-aluminum-garnet laser, and intense pulsed light (which is admittedly not a laser). Treatment protocols usually involve multiple sessions to effect a noticeable change in skin appearance, and results may begin to develop in 4 to 6 weeks after the final treatment.

Despite promising clinical trials that have investigated the benefit of the nonablative laser, to our knowledge, no systematic research has been conducted to evaluate the optimal parameters to achieve the maximal outcome in dermal remodeling for the various laser types. Ideally, human skin would be the favored tissue model for histologic analysis. However, harvesting a large series of punch biopsy specimens from a human patient is impractical and ethical considerations. The pig model has been proved the most similar to human epidermal and dermal structure in terms of thickness, response to injury, and collagen synthesis. Therefore, this study endeavors to rely on a porcine model to evaluate in a prospective, randomized, blinded, and controlled fashion the exact parameters that may be most favorable for achieving collagen remodeling in nonablative resurfacing.

### METHODS

Before the execution of this project, all research protocols were submitted to and approved by the Albany Medical College Animal Care and Use Protocol under the auspices of the Albany Medical College Institutional Animal Care and Use Committee. The principal investigators (S.M.L. and R.D.) involved in this study underwent training in animal care and handling through attendance of laboratory didactic lectures, practical laboratory exercises, and requisite examinations, per the Albany Medical College Animal Resource Facility.

The entire study was performed in the animal laboratory of Albany Medical College. A Yucatan miniature pig was procured as the subject of this study and allowed to mature to adult size before initiation of the first part of the study. The pig was administered a preanesthetic regimen of ketamine hydrochloride, a combination of tiletamine hydrochloride and zolazepam hydrochloride (Telazol), and xylazine hydrochloride, 0.5 mL/20 kg; and was then intubated and kept sedated with 1% to 3% isoflurane as the general inhaled anesthetic. Trained veterinary technicians monitored the animal throughout the procedure. After adequate anesthesia was achieved, the pig was placed in the left lateral decubitus position to be shaved and prepared with povidone-iodine solution and draped with sterile surgical towels. An aseptic technique was used to mark 2-cm distances horizontally (16 marks across) and vertically (10 marks per column) with a surgical marking pen, for a total of 160 marked points on the pig’s left dorsal side. Then, 0 silk sutures were secured at the marked points to determine the exact laser-treated areas for histologic retrieval at the conclusion of this study. The pig’s left side was then cleaned and sterilized, and the negative control were applied similarly (ie, each control and the positive control, the carbon dioxide laser at 7 J/cm² (Surgilase; Lumenis Ltd, Yokneam, Israel), and the control was applied similarly (ie, each control was also applied 10 times to increase statistical power). After the pig’s left side was effectively treated with the laser device according to the outlined parameters, the pig was repositioned onto its right side, and the same technique was undertaken to shave and sterilely prepare and drape the pig for histologic analysis. However, harvesting a large series of punch biopsy specimens from a human patient is impractical and ethical considerations. The pig model has been proved the most similar to human epidermal and dermal structure in terms of thickness, response to injury, and collagen synthesis.

**Unique Combinations of Pulsed Dye Laser Parameters Used in This Study**

<table>
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<tr>
<th>Fluence, J</th>
<th>Spot Size, mm</th>
<th>Cooling Spray</th>
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*The carbon dioxide laser (the positive control) was set at the recommended start-safe parameter (6 W and 18 J/cm²). The minus sign indicates that no coolant spray was used; plus sign, coolant spray was used.

After appropriate protective goggles were donned, the laser device (Vbeam) used for this study was applied 1 cm to the right of the placed silk sutures according to predetermined parameters. A total of 24 unique combinations of parameters (fluence, spot size, pulse duration, and coolant spray), as outlined in the Table, were evaluated, with each combination applied 10 times to increase statistical power. The positive control, the carbon dioxide laser at 7 J/cm² (Surgilase; Lumenis Ltd, Yokneam, Israel), and the negative control were applied similarly (ie, each control was also applied 10 times to increase statistical power). After the pig’s left side was effectively treated with the laser device according to the outlined parameters, the pig was repositioned onto its right side, and the same technique was undertaken to shave and sterilely prepare and drape the pig for histologic analysis.
suture placement. The pig was again cleaned of all povidone-iodine solution before the initiation of laser treatment on the right side. The laser treatment on the right side followed the predetermined parameters designed for that side.

After the completion of the first part of this study, the pig was assigned to its individual housing, where twice-daily measurements of its vital signs (temperature, pulse, and respiratory rate) were recorded and any signs of distress, discomfort, or other impairment were checked for the first 3 postoperative days, in accordance with animal care protocols. The animal recovered well and had no obvious sequelae from the initial treatment.

After 10 weeks were allowed to transpire, during which the pig was kept housed and fed in the animal care facility, the animal was returned to the operating suite for tissue retrieval. Of the sutures placed during the initial portion of this study, 97.3% were maintained at the time of the second part of the study. The sites where the remaining sutures had unraveled were fortunately identifiable by a postinflammatory hyperpigmented mark imparted by the sutures. No infected suture material was encountered during this study. After the animal received the same preanesthetic and anesthetic regimen as outlined previously, the left side of the pig was prepared and draped steriley. Punch biopsy specimens (3 mm) were taken centered 1 cm to the right of the sutures, which corresponded to the laser-treated areas. These punch biopsy specimens were placed into individual containers holding formaldehyde. The sites where the biopsy was performed were closed with 3-0 chromic sutures, and the silk sutures were removed to minimize the subject’s discomfort after awakening from general anesthesia. The untreated skin specimens were also harvested adjacent to a prescribed column of sutures to avoid criticism that any tissue reaction that the suture material may engender would have contributed to the dermal remodeling seen in the laser-treated specimens. The animal was not killed, but adopted by a humane farmstead at the conclusion of the study.

All the tissue samples were assigned a random number between 1 and 320 according to a computer program to blind the pathologist’s analysis of the specimens. Each specimen was evaluated microscopically at ×400 magnification using hematoxylin-eosin staining. The main outcome measures included quantitative assessment of collagen band width and cells per high-power field (HPF) and qualitative assessment of epidermal and dermal changes. These measures were carefully chosen after a review of the literature and in consultation with a dermatopathologist. In much of the literature,1-5,8 even objective outcomes tend not to be quantified; rather, histologic descriptions are provided. Most of these studies focus on collagen deposition and/or architecture. Our first outcome measure quantitatively evaluated collagen deposition. For each slide (corresponding to a specimen from 1 treated spot), 3 individual collagen bands were measured in micrometers. For each laser parameter, 3 measurements of collagen band width were made and averaged to provide 10 mean widths (1 for each treated spot in a given parameter). The second outcome measure is based on the concept that if there is more collagen in a given segment of dermis, the nuclei of the fibroblasts should be more spread out. Thus, we measured the number of nuclei in a given HPF 3 times for each slide. The outcome of this grading was subjected to statistical analysis using computer software (Minitab, Inc, State College, Pa). The data collected underwent a 1-way analysis of variance and the Tukey pairwise comparison test.

RESULTS

Quantitative assessments of mean collagen band width and mean cells per HPF were undertaken to evaluate the effect that nonablative and ablative (positive control) laser therapy had on dermal remodeling compared with untreated skin (negative control). A significant difference (P < .001) in collagen band width was evident when nonablative laser–treated skin (13.9 µm) and carbon dioxide ablative laser–treated skin (14.9 µm) specimens were compared with untreated skin specimens (10.5 µm). However, no significant (P = .18) difference existed in the extent of collagen remodeling between the nonablative and ablative modalities, as revealed by a Tukey pairwise comparison test (confidence interval, −2.01 to 0.03). Similarly, cellular hypertrophy, as measured by cells per HPF—which displays an inverse relationship (ie, greater cellular hypertrophy is evident with fewer cells per HPF)—was observed at ×400 magnification corroborated the previously described findings: 25.47 cells per HPF in nonablative laser–treated skin, 25.60 in ablative laser–treated skin, and 34.70 in untreated skin. The difference between the laser-treated skin specimens (ablative and nonablative) and untreated skin specimens proved statistically significant (P < .001). No significant (P = .82) difference arose in cells per HPF between the ablative and nonablative laser–treated skin, as indicated by a Tukey pairwise comparison test (confidence interval, −2.27 to 2.20). Furthermore, a higher fluence, a larger spot size, and a longer pulse duration all proved statistically significant for increased collagen band width (P = .01, P < .001, and P < .001, respectively), and a larger spot size and a longer pulse duration exhibited significance for cells per HPF (P = .02 and P = .009, respectively), with a trend toward significance for higher fluence (P = .09) (Figure 1). In addition to increased collagen remodeling in the laser-treated skin, the collagen bundles tended to be configured more densely together within the dermis and had an accompanying increase in interstitial fluid. The use of cooling spray, however, did not significantly (P = .08) influence dermal remodeling.

Qualitative assessments of epidermal and dermal changes were also undertaken to evaluate the effect that nonablative and ablative laser therapy had on dermal remodeling compared with untreated skin. The dermis in ablative and nonablative laser–treated skin appeared considerably thicker compared with the dermis in untreated skin, but this could not be accurately quantified because the laser-treated skin thickness exceeded the capacity of the punch biopsy instrument. In contrast, every specimen of untreated skin had an underlying cuff of subcutaneous adipose tissue, because the dermis was consistently thinner. Again, because the full thickness of the dermis could not be measured in the treated specimens, an exact ratio between the treated and control groups cannot be derived. However, we know that in the untreated specimens, the subcutaneous fat was on average 2 to 3 mm thick. This layer was not seen in the treated specimens; thus, we know that the dermis expanded by at least this measure. When a ratio is made between this and the average dermal thickness, we can conclude that the dermis had to increase by a minimum of 30% to 50%. The epidermis, on the other hand, remained the same thickness for all specimens. However, the epidermis did show signs of melanocyte incontinence, with spillage of melanin granules throughout all the epidermal layers in nonablative laser–treated skin.
when no cooling spray was used and in ablative laser–
treated skin—which was consistent with clinical evi-
dence of postinflammatory hyperpigmentation in these
groups (Figures 2, 3, and 4).

COMMENT

Nonablative laser and light therapy has enjoyed success as a clinical tool for facial rejuvenation with limited scientific support. A paucity of studies documents the histologic and clinical changes afforded by this technology, and no study, to our knowledge, has investigated the histologic effects in a prospective, randomized, blinded, controlled fashion using systematic and exten-
sive laser parameters. The lack of scientific research may be partly attributed to the nascent aspect of nonablative therapy. However, given the generally subtle clinical improvements that are effected and the lack of any discernible tissue reaction observed during the procedure, a rigorous scientific inquiry should be undertaken to determine the validity of this type of therapy and to ensure that nonablative treatments are not merely a product of hype. Furthermore, this study has aimed to investigate the exact parameters that may be best suited to effect dermal remodeling without epidermal damage and to compare those histologic outcomes with traditional ablative cutaneous therapy (positive controls) and untreated skin (negative controls).

Figure 1. Photomicrograph of porcine dermis (hematoxylin-eosin, original magnification ×400) showing increased collagen band width, cellular hypertrophy with decreased cells per high-power field, and increased dermal thickness in a nonablative laser–treated specimen (7 J/cm², 10-mm spot size, and cooling spray) (A) and a carbon dioxide ablative laser–treated specimen (6-mm spot size and 18 W) (B) compared with untreated skin (C).

Figure 2. Photomicrograph of porcine epidermis (hematoxylin-eosin, original magnification ×400) in a nonablative laser–treated specimen with cooling spray (A) and without cooling spray (B) and in ablative laser–treated skin (C), showing melanocyte incontinence (consistent with clinical evidence of postinflammatory hyperpigmentation) in B and C and similar epidermal thickness in all groups (A–C).
Given the scale of this project, a human model would have been impractical and unethical. We recognize the inherent limitations of any animal vehicle and the precarious nature of extrapolating from animal-derived data. However, many studies have supported the remarkable cutaneous histologic similarity between human and pig and the comparable wound-healing capacities of both. Small mammals (rabbits, guinea pigs, rats, and mice) have been used for wound-healing studies, but diverge radically from their human counterparts in many fundamental aspects. These diminutive mammals exhibit different anatomic features (dense body hair coverage and an attenuated epidermis and dermis) and differ in their response to tissue injury (healing by wound contraction as opposed to reepithelialization).14

The pig, on the other hand, shares many similar cutaneous traits of the human. Epidermal thickness and dermal-epidermal thickness ratios are similar in humans and pigs.15-18 The caliber and distribution of porcine dermal vasculature are mimicked in humans. The fact that the pulsed dye laser targets the dermal vascular network to effect dermal collagen remodeling further supports the use of a porcine model. Both species heal wounds via a physiologically similar mechanism (reepithelialization, as opposed to wound contraction), which is accomplished by similar adnexal structures and lack of a pancilicus carnosus found in smaller mammals.19 Overall, pigs and humans have analogous body physiological features with comparable key organ systems in anatomic features and function.14,20,21 Beyond the inherent risks of extrapolating data from a porcine model, this study also does not purport that histologic changes necessarily translate into a clinically apparent outcome.

Despite these limitations, the study demonstrates conclusively that nonablative and ablative laser therapies yield significant dermal changes (increased dermal thickness, collagen band width, and cellular hypertrophy) compared with untreated skin. Surprisingly, these histologic measurements were extremely similar for ablative and nonablative laser therapies. It may not be entirely unreasonable to speculate from these results that the effect of nonablative therapy may be quite similar to ablative laser therapy in regard to dermal changes. However, whether this similarity is clinically apparent is yet to be determined. Clearly, nonablative therapy, unlike ablative therapy, offers no hope in effacing unfavorable epidermal dyschromia and texture.

Another important finding of this article is that a significant difference arose in almost all laser parameters, resulting in increased collagen band width and cellular hypertrophy with higher fluences (7 vs 4 J/cm²), larger spot sizes (10 vs 7 mm), and longer pulse durations (20.0 vs 1.5 milliseconds). Despite the use of higher fluences, we did not observe any immediate tissue purpura or long-term hyperpigmentation except in those cases in which the cooling spray was not used. The lack of a cooling spray uniformly created tissue reaction and subsequent hyperpigmentation. However, the pig used in this study had a darker complexion (Fitzpatrick grade IV) and, therefore, was potentially more susceptible to postinflammatory hyperpigmentation. Perhaps if a lighter-colored animal were recruited as the subject, the results of the study

One study that examined a relatively small number of patients in a prospective double-blinded fashion has shown favorable clinical efficacy of the pulsed dye laser in nonablative therapy. Raston and colleagues treated 15 patients who exhibited moderate to marked rhytidosis with a pulsed dye laser and cooling spray on one hemiface and cooling spray alone as a placebo on the contralateral side. The study noted that 11 of the 15 patients improved on the treated side, and only 3 of the 15 improved on the placebo-treated side, through the use of blinded photographic analysis—a difference that was significant (P= .004). All patients underwent 4 treatment sessions at 1-month intervals, and clinical grading was performed at the completion of all treatments. In addition, a histologic evaluation revealed significantly increased activated fibroblasts, collagen, and dermal thickness on the treated side—a finding that corroborates our results in this porcine model. It is unclear, however, whether the histologic analysis that was undertaken in the study was conducted in a blinded manner. Interestingly, our study demonstrates signs of the dramatic histologic remodeling engendered by the pulsed dye laser even after only 1 treatment session.
would have been somewhat different. Moreover, the dermal thickness differs to some extent from the dorsal to the ventral region of the pig and must be accounted for in a histologic analysis. The study was designed to accommodate this anatomic feature, and each identical cluster of parameters was distributed in a column traversing from the dorsal to the ventral aspect. Furthermore, no significant difference was noted in histologic features from specimens harvested from the dorsal to the abdomen.

Future studies that compare various carbon dioxide laser parameters (single vs multiple passes, fluences, and spot sizes) with those of nonablative lasers may serve to elucidate more effectively the differences that these two modalities have on dermal remodeling. Similarly, a broader study using various laser and light devices aimed at nonablative therapy may test the efficacy of these different models in cutaneous rejuvenation. Also, a study that compares multiple-treated skin over several sessions vs a single treatment may more accurately reflect guidelines for nonablative therapy. Finally, rigorous, prospective, blinded, large-scale, clinical trials are ultimately needed to verify the utility of this type of therapy.

In conclusion, nonablative therapy has gained popularity as a viable method of facial rejuvenation for selected patients with minimal to moderate rhytidoses. Rapidity, painlessness, lack of morbidity, and the absence of any discernable recovery time make this noninvasive technology seemingly the modality of choice for many patients who cannot tolerate traditional ablative resurfacing. This preliminary animal study demonstrates the histologic basis for the benefits derived from nonablative therapy and further refines our understanding of what parameters would be ideal to achieve that objective.

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