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

Ravinder Dahiya, MD; Samuel M. Lam, MD; Edwin F. Williams III, MD

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 Yucatan mini-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). 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 scarring. Despite these limitations, ablative resurfacing achieves remarkable success in rejuvenating severely photodamaged skin and is still a mainstay of therapy for those patients who require intensive rejuvenation and who are willing to accept the expected recovery period.

Recently, preliminary clinical reports have suggested the potential efficacy of nonablative therapy, which targets the underlying dermis, with no ablative damage to the covering epidermis. Unlike traditional ablative methods, nonablative therapy offers the patient cutaneous rejuvenation without any discernible downtime. Because the epidermal integrity is maintained, all the sequelae and complications that are inherent to ablative techniques are avoided. However, solar dam-
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 recruited for nonablative facial rejuvenation. The pulsed dye laser, which is traditionally used to treat vascular lesions, has been successfully marketed as a nonablative laser, and the N-Lite (ICN Photonics Ltd, Orangeburg, NY) was the first pulsed dye laser approved for nonablative use in Europe and the United States. Other 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 Q-switched 1064-nm Nd:YAG laser, the 980-nm diode laser, the 1320-nm Nd:YAG laser, the erbium:glass laser, 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 manifest only 72 hours after initial 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 for histologic analysis is impractical and unethical 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 with isotonic sodium chloride solution to remove the povidone-iodine stain for improved laser absorption by the skin.

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 cooling 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. However, harvesting a large series of punch biopsy specimens from a human patient for histologic analysis is impractical and has practical 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.

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

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<th>Spot Size, mm</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 cooling spray was used; plus sign, cooling spray was used.
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
determined 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 mea-
surements 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
good 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 unrvolved were
fortunately identifiable by a postinflammatory hyperpig-
mented mark imparted by the sutures. No infected suture ma-
terial was encountered during this study. After the animal re-
ceived the same preanesthetic and anesthetic regiment as outlined
previously, the left side of the pig was prepared and draped ster-
ilely. 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 chronic su-
tures, and the silk sutures were removed to minimize the sub-
ject’s discomfort after awakening from general anesthesia. The
untreated skin specimens were also harvested adjacent to a pre-
scribed column of sutures to avoid criticism that any tissue re-
action that the suture material may engender would have con-
tributed to the dermal remodeling seen in the laser-treated
specimens. The animal was not killed, but adopted by a hu-
mane farmstead at the conclusion of the study.

All the tissue samples were assigned a random number be-
tween 1 and 320 according to a computer program to blind the
pathologist’s analysis of the specimens. Each specimen was
evaluated microscopically at X400 magnification using hema-
toxyl-eosin staining. The main outcome measures included
quantitative assessment of collagen band width and cells per
high-power field (HPF) and qualitative assessment of epider-
mal and dermal changes. These measures were carefully chosen
after a review of the literature and in consultation with a der-
matopathologist. In much of the literature,3-8 even objective out-
comes tend not to be quantified; rather, histologic descriptions
are provided. Most of these studies focus on collagen deposi-
tion and/or architecture. Our first outcome measure quantifi-
cably evaluated collagen deposition. For each slide (correspond-
ing to a specimen from 1 treated spot), 3 individual collagen
bands were measured in micrometers. For each laser parameter, 30
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 con-
cept 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 statis-
tical analysis using computer software (Minitab, Inc, State Col-
lege, Pa). The data collected underwent a 1-way analysis of vari-
ance 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) la-
sor therapy had on dermal remodeling compared with
untreated skin (negative control). A significant differ-
ce ($P<.001$) in collagen band width was evident when
nonablative laser–treated skin ($13.9 \mu m$) and carbon di-
oxide ablative laser–treated skin ($14.9 \mu m$) specimens
were compared with untreated skin specimens ($10.5 \mu m$).
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 $0.03$). Simi-
larly, cellular hypertrophy, as measured by cells per HPF—
which displays an inverse relationship (ie, greater cellular
hypertrophy is evident with fewer cells per HPF)—
observed at $\times400$ magnification corroborated the
previously described findings: 25.47 cells per HPF in non-
ablative laser–treated skin, 25.60 in ablative laser–
treated skin, and 34.70 in untreated skin. The differ-
ence 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 signi-
ficant 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 remodel-
ing in the laser-treated skin, the collagen bundles tended
to be configured more densely together within the der-
mis and had an accompanying increase in interstitial fluid.
The use of cooling spray, however, did not significantly ($P=.08$)
inefluence 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 remodel-
ing compared with untreated skin. The dermis in ablative
and nonablative laser–treated skin appeared con-
siderably thicker compared with the dermis in un-
treated skin, but this could not be accurately quantified
because the laser-treated skin thickness exceeded the ca-
pacity of the punch biopsy instrument. In contradistinc-
tion, every specimen of untreated skin had an underly-
ing 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, re-
mained the same thickness for all specimens. However,
the epidermis did show signs of melanocyte inconti-
nence, with spillage of melanin granules throughout all
the epidermal layers in nonablative laser–treated skin.

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when no cooling spray was used and in ablative laser–treated skin—which was consistent with clinical evidence 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 extensive 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).
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. Overall, pigs and humans have analogous body physiological features with comparable key organ systems in anatomic features and function. 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...
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 dorsum 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 rhytidosis. Rapidity, painlessness, lack of morbidity, and the absence of any discernible 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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Corresponding author: Edwin F. Williams III, MD, Williams Center for Facial Plastic Surgery, 1072 Troy Schenectady Rd, Latham, NY 12110 (e-mail: edwilliams@nelasersurg.com).

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