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Effects of Common Laser Treatments on Hyaluronic Acid Fillers in a Porcine Model

Jordan P. Farkas, MD; James A. Richardson, DVM, PhD; Spencer Brown, PhD; John E. Hoopman; and Jeffrey M. Kenkel, MD

BACKGROUND: Injectable hyaluronic acid fillers (HAFs) and laser/light procedures have become increasingly popular for noninvasive facial rejuvenation in many cosmetic practices. However, the effect of laser/light treatments on HAFs is unknown.

OBJECTIVE: Our objective was to examine the effect of laser/light treatments on HAFs in a porcine model.

METHODS: The abdomens of 6 Yorkshire pigs were injected with 3 different HAFs: Restylane (Medicis, Scottsdale, AZ), Perlane (Medicis), and Juvéderm (Allergan, Santa Barbara, CA). Two weeks after injection, the injection sites were treated with 1 of 7 common laser/light ablative or nonablative devices. Following laser treatment, 8-mm punch biopsies were collected from the treated tissue and fixed for histopathologic evaluation. Sections were stained with hematoxylin–eosin and alcian blue stains for identification of the preinjected HAF.

RESULTS: The filler was identified in different areas of the dermis in different sections. The Sciton intense pulsed 560 nm filter (Sciton, Palo Alto, CA), the Sciton Nd: YAG, Lux1540 (Palomar Medical Technologies, Burlington, MA), or ActiveFX (Lumenis, Yokneum, Israel) treatments showed no sign of interaction with superficial or deep dermal filler. No evidence of morphologic changes to the filler or the surrounding tissues was observed. Obvious interaction between the HAFs and the laser injury was demonstrated in sections treated with the deep ablative systems fractional erbium 2940 (Profractional; Sciton) and DeepFX CO₂ (Lumenis). However, no uncharacteristic tissue injury or morphologic change in the filler was appreciated in any of the preinjected specimens.

CONCLUSIONS: Injected HAFs were unaffected by the nonablative laser/light and superficial ablative treatments. The more aggressive deeper laser treatments demonstrated laser/filler interaction and may have a clinical effect on the longevity of the filler and/or efficacy of laser treatments. Novel ablative fractional lasers have the capability of deep dermal penetration, and this should be taken into consideration when planning to use them in combination with soft tissue fillers for noninvasive facial rejuvenation. (*Aesthetic Surg J* 2008;28:503–511.)

Noninvasive facial rejuvenation procedures have become increasingly popular in many cosmetic surgery practices. Soft tissue fillers and/or laser/light therapy are attractive options for many patients who desire improvement of facial contour and skin rejuvenation without surgical intervention. It is estimated that more than half of patients treated by aesthetic specialists undergo multiple procedures within the same year, with soft tissue filler injections ranking amongst the most common and fastest growing of these procedures.¹

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Soft tissue fillers such as hyaluronic acid (HA), calcium hydroxylapatite, and poly-L-lactic acid are used to improve facial volume and rhytids by direct filling of facial troughs and “furrows,” stimulating surrounding fibroblasts for new collagen production, and/or acting as a scaffold or framework for new collagen.^{3–5} Cross-linked HA fillers (HAFs) are biocompatible, immunologically inert, naturally occurring polysaccharides extracted from the nonpathogenic bacteria *Streptococcus equi*. HAFs are reported to have tissue longevity for 3 to 9 months following injection.^{5–8} Currently, HAFs are approved in the United States for the correction of moderate to deep wrinkles such as the nasolabial folds.^{5,6,9} Even with experience and accomplished injection techniques, precise positioning of the filler within the targeted region of the dermis is quite difficult. Differences in skin laxity, hydration, and

Table 1 Laser systems and parameters

| Laser systems | Parameters | |
|------------------------------------|-----------------------------------|--|
| Sciton IPL (560 nm filter) | 10 J/20 msec, 11 J/20 sec | 12 J/20 msec, 10 J/ 30msec |
| Sciton Nd: YAG 1064 | 60 mJ/20 msec | 80 mJ/20 msec |
| Lux1540 (Er:glass) | 50 mJ/10 msec, 58 mJ/10 msec | 60 mJ/15 msec, 65 mJ/15 msec |
| Profractional | 400 μ m; 5% coverage | 600 μ m; 5% coverage |
| ActiveFX | 125 mJ/75 Hz | 150 mJ/100 Hz |
| DeepFX | 10 mJ/D = 4 (single pulse) | 20 mJ/D = 4 (single pulse) |
| | 10 mJ/D = 4 (double pulse) | 20 mJ/D = 4 (double pulse) |
| | 15 mJ/D = 4 (single pulse) | 30 mJ/D = 4 (single pulse) |
| | 15 mJ/D = 4 (double pulse) | 30 mJ/D = 4 (double pulse) |
| Palomar Er:YAG 2940 (500 optic) | 0 mJ/2 ms/4 mJ/mb (double pulse), | 5 mJ/0 ms/0 mJ/mb (double pulse), |
| | 0 mJ/2 ms/4 mJ/mb (four pulses), | 5 mJ/0 ms/0 mJ/mb (four pulses), |
| | 2 mJ/2 ms/4 mJ/mb (double pulse), | 5 mJ/2 ms/4 mJ/mb (double pulse), |
| | 2 mJ/2 ms/4 mJ/mb (four pulses) | 5 mJ/2 ms/4 mJ/mb (four pulses). |
| | | 5 mJ/5 ms/5 mJ/mb (double pulse), 5 mJ/5 ms/5 mJ/mb (four pulses) |

The Profractional and the Sciton IPL and 1064 systems are manufactured by Sciton (Palo Alto, CA).

The Lux1540 and Palomar Er:YAG 2940 systems are manufactured by Palomar Medical Technologies (Burlington, MA).

The ActiveFX and DeepFX systems are manufactured by Lumenis (Yokneum, Israel).

epidermal/dermal thickness from patient to patient can also cause potential variability in filler placement.^{5,6}

Fractional photothermolysis causes skin remodeling and regeneration by creating microscopic thermal or ablative injury to the underlying epidermis or dermis.^{10,11} Multiple common laser/light devices are marketed for skin improvement and rejuvenation. Pulsed light therapy focuses its treatment to the epidermis and dermal–epidermal junction, penetrating 125 to 150 μ m from the surface to improve dyschromias, pigment, lentigines, and epidermal architecture. Nonablative devices treat the papillary and superficial reticular dermis for collagen remodeling using various cooling methods to protect the epidermal surface. Clinically, nonablative laser devices target 400 to 600 μ m deep to the stratum corneum. More aggressive fractional ablative devices use microscopic beams of energy to mechanically remove photodamaged epidermis and older disorganized collagen matrix and stimulate the surrounding uninjured fibroblasts, creating a new full-bodied epidermal layer and robust dermal collagen matrix.¹² Some of these new fractional short pulse ablative carbon dioxide and erbium devices are capable of impressive injury and penetration into the deep reticular dermis, up to 3 to 4 mm from the epidermal surface.

With the rising popularity of fractional laser treatments and soft tissue fillers, the interaction between laser/light treatments and soft tissue fillers is an area of considerable interest. Because HAFs and laser therapy share a common target—the skin—combination treatments for noninvasive facial rejuvenation are being used and can be a valuable tool. However, the effect of common laser treatments over skin that has been injected with HAF has not been clearly elucidated in the literature.¹³

In this study, the histologic effect of 7 common laser treatments over previously injected HA tissue fillers in an abdominal porcine model was explored, with specific attention being paid to any morphologic changes to the filler or surrounding tissue following laser/light treatment.

METHODS

Six female Yorkshire pigs were used for the study. Animal surgery, anesthesia, and euthanasia protocols were all reviewed and approved by the University of Texas Southwestern Medical Center Institutional Animal Care and Use Committee. One pig was injected at a time. The animal was intubated and placed under general anesthesia. The animals were placed supine on the operating room table and prepped, shaved, and draped in a sterile fashion. A predesigned template was placed over the animal's abdomen and areas planned for injection were tattooed for later identification. Each template consisted of 30 or 40 1.5-cm \times 1.5-cm spots divided into 3 or 4 rows of 10.

Each 1.5-cm spot was injected with 0.1 to 0.2 cc of either Juvéderm, Restylane, or Perlane using the linear threading injection technique with a 30-gauge needle. The animals were extubated, recovered, and maintained for 2 weeks following injection. After 2 weeks, the animals were again placed under appropriate anesthesia and prepped and draped in a sterile fashion. The template was replaced over the abdomen of each animal using the tattoos to accurately identify the previously injected sites. Injection areas were then treated with the various laser/light systems. The systems examined were the Sciton intensified pulsed light (IPL) with a 560 filter (Sciton, Palo Alto, CA), Sciton Nd: YAG 1064 device (Sciton), Profractional (Sciton), Lux1540 Er:glass,

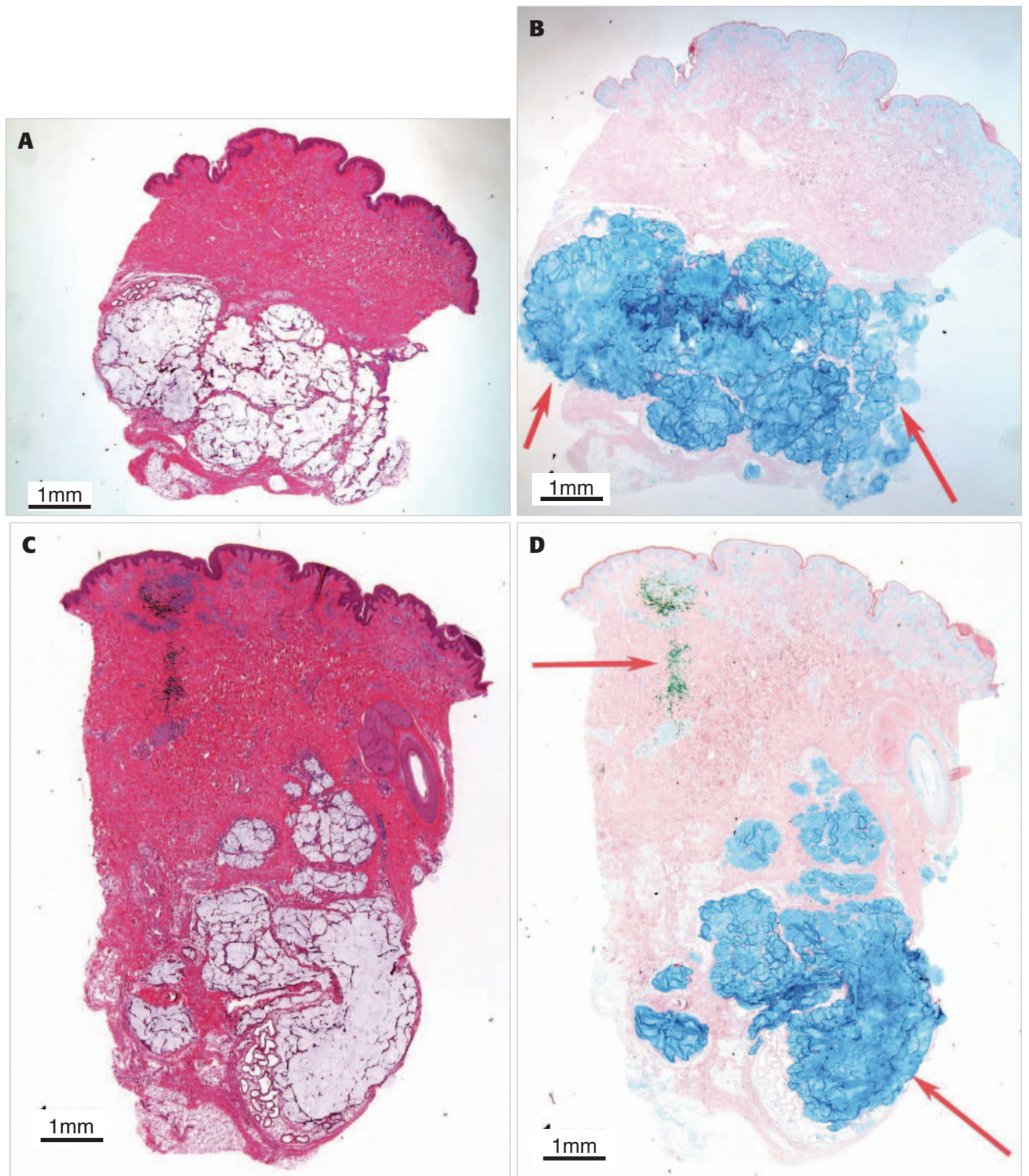


Figure 1. Contiguous histopathologic skin sections injected with Restylane (**A** and **B**) and Juvéderm (**C** and **D**) and stained with hematoxylin–eosin and alcian blue stains. Note the rich blue staining of the hyaluronic acid (parts **B** and **D**, *bottom arrows*) and the green tattoo ink (part **D**, *top arrow*). No difference in the staining of the hyaluronic acid filler was appreciated with the different fillers. (Original magnification: $\times 1.25$.)

Palomar erbium 2940 (Palomar Medical Technologies, Burlington, MA), and the ActiveFX and DeepFX (Lumenis, Yokneum, Israel). Parameters for a given device were selected based on clinical experience of the senior author (JMK). The settings explored are outlined in [Table 1](#). At least 6 injected spots were treated with

each device. Only single-pass treatments were evaluated. Each setting for a given device was performed in triplicate. Immediately following treatment, the animals were euthanized and biopsies of the treated areas were taken for histologic processing and evaluation.

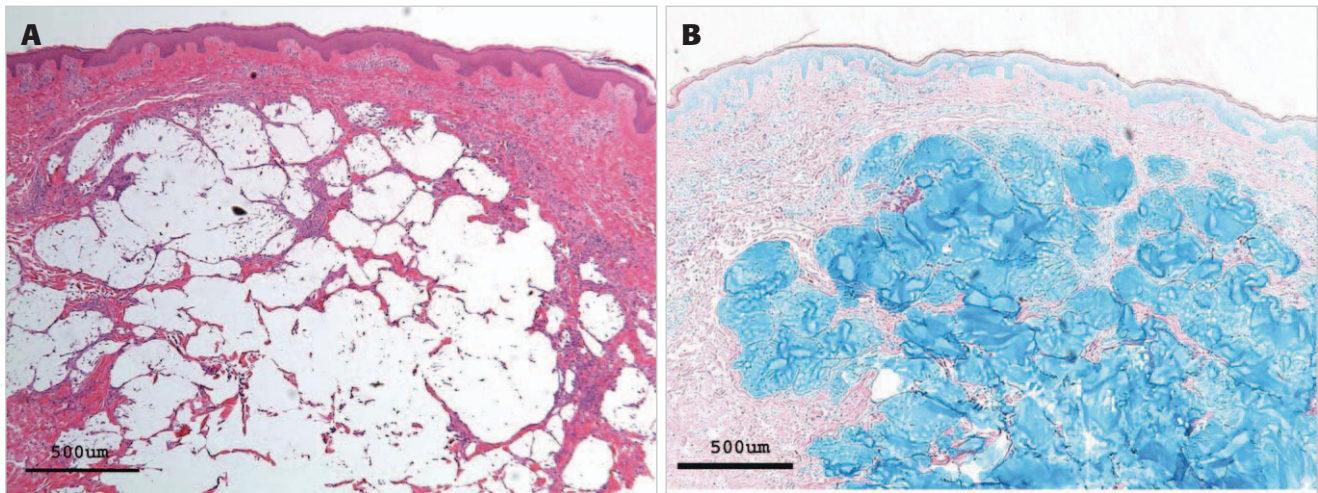


Figure 2. Contiguous histopathologic skin sections treated with Sciton intense pulsed light 560 filter (12 J/20 msec) and stained with hematoxylin–eosin (A) and alcian blue (B) stains. There was no evidence of any morphologic changes to the filler itself or to the surrounding tissue. (Original magnification: $\times 4$.)

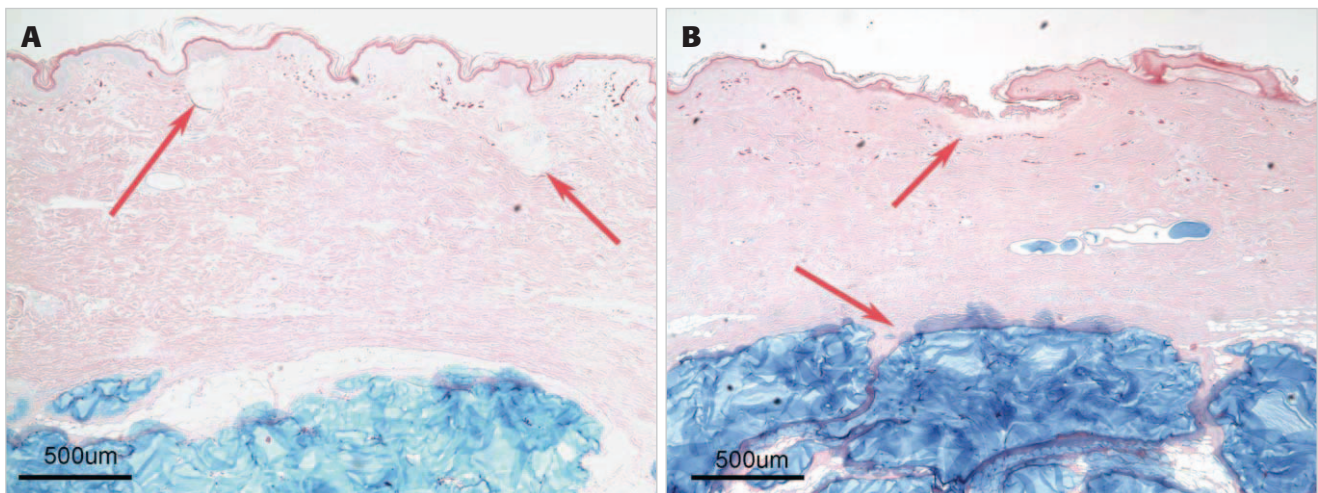


Figure 3. Sections of skin treated with the Lux1540 (65 mJ/15 ms) (A) and ActiveFX (150 mJ/100 Hz) (B) and stained with alcian blue. The arrows point to the superficial injury created by each of laser treatments. Note the distance between the laser-induced tissue injury and underlying soft tissue filler. (Original magnification: $\times 4$.)

Sections were placed in 10% neutral buffered formalin, and placed on a shaker for 24 hours. After rinsing in 70% ethanol solution, the biopsies were processed, embedded in paraffin, cut in serial longitudinal sections (4–6 μm) and mounted on poly-L-lysine slides. Multiple serial sections (5–10) of each specimen were processed in order to obtain accurate representation of the laser/filler interaction in each treatment sample.

All biopsy specimens were stained with hematoxylin–eosin stain, and contiguous sections stained with alcian blue stain to highlight the HA within the section. All specimens were reviewed by a board-certified pathologist. Three spots on each animal were treated with the HAF only and used as controls.

RESULTS

There was no sign of gross injury, blistering, oozing, or leakage of the filler following any of the laser treatments. In addition, histologically, no evidence of coagulation or tissue injury surrounding the filler in any of the preinjected treated specimens was observed.

Each of the different HA soft tissue fillers was identified in either the dermis or subcutis of each of the histologic biopsy specimens. There was no correlation between the type of filler and the depth of the HA identified in the skin specimens. Alcian blue stain assisted in clearly identifying the filler from the surrounding dermal collagen and subcutaneous adipose cells. Each of the fillers was identified as a discrete mass within the dermis or subcutaneous tissue that stained a rich blue with the alcian blue (Figure 1). The location of the HAF within the dermis of each section was variable. Encapsulation and incorporation of the HAF into the surrounding tissues were observed in all sections.

The injected areas treated with the Sciton IPL or 1064 did not cause any morphologic change to the soft tissue filler (Figure 2). Coagulated microcolumns of collagen were observed tapering from the epidermis into the papillary dermis following treatment with the Lux1540 system. Following treatment with the Active FX device, superficial craters of ablation were evident, and they extended from

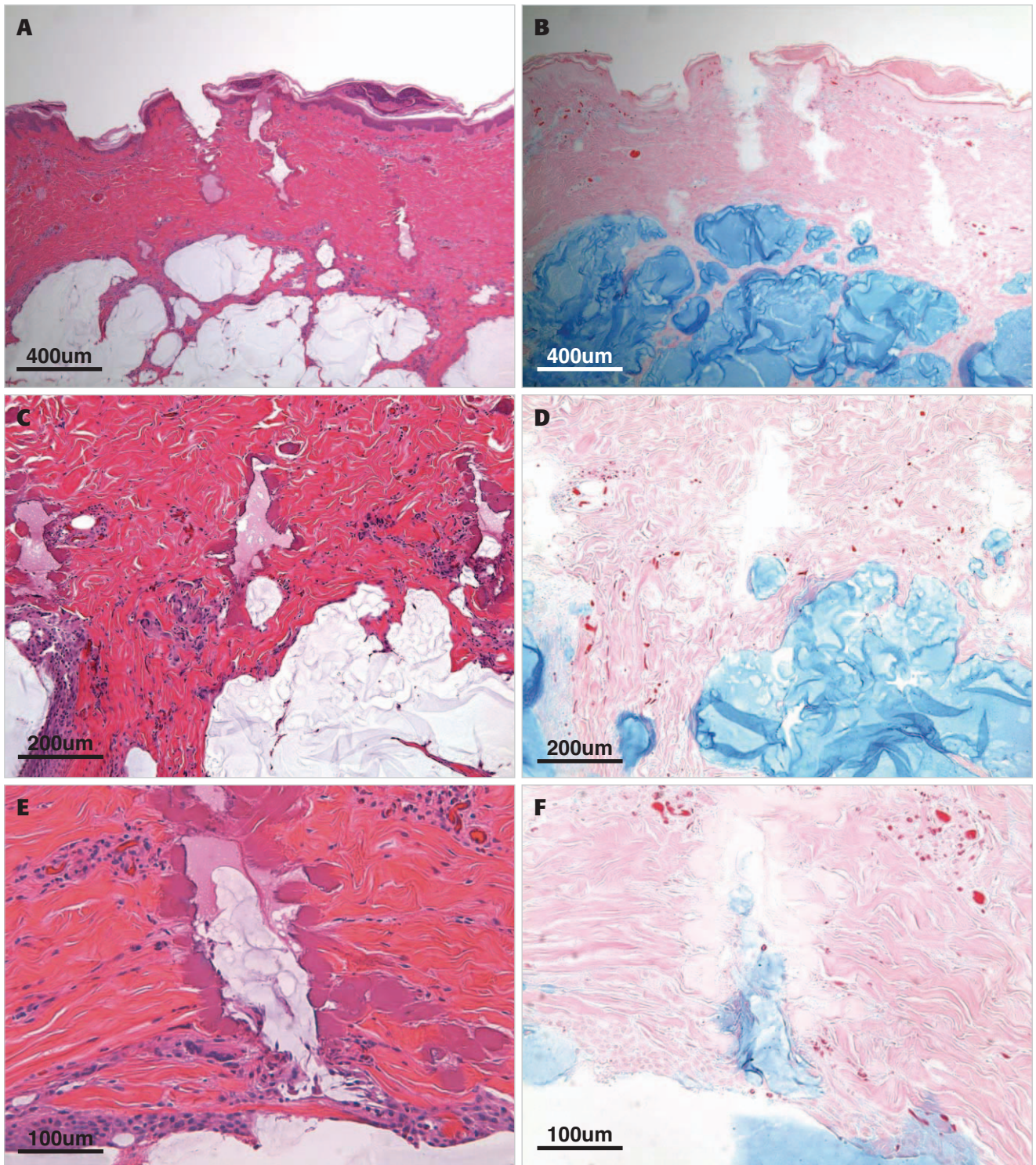


Figure 4. Corresponding histopathologic skin sections treated with the Palomar 2940 fractional erbium ablative laser (5 mJ short pulse/4 mJ/mb/2 ms long pulse) stained with hematoxylin–eosin (**A, C, E**) and alcian blue (**B, D, F**) stains shown at increasing magnifications. Note the microcolumns of ablation coming in direct contact with the preinjected hyaluronic acid filler within the reticular dermis. Hyaluronic acid filler was identified within some of the collapsed ablation channels in the reticular dermis.

the epidermis into the papillary dermis. The superficial damage and alteration to the epidermis and papillary dermis seen with these devices at the different energy settings did not penetrate deeply enough into the dermis to come in contact with the HAF (Figure 3).

Following treatment with the fractional Er: YAG devices (Profractional, Palomar 2940) microablation

columns of injury penetrating from the epidermis into the underlying papillary and reticular dermis to various depths in the dermis were observed. Some of the microablation columns were in direct contact with the HAF in the papillary and reticular dermis. A few microablation columns surrounded and trapped the HAF (Figure 4).

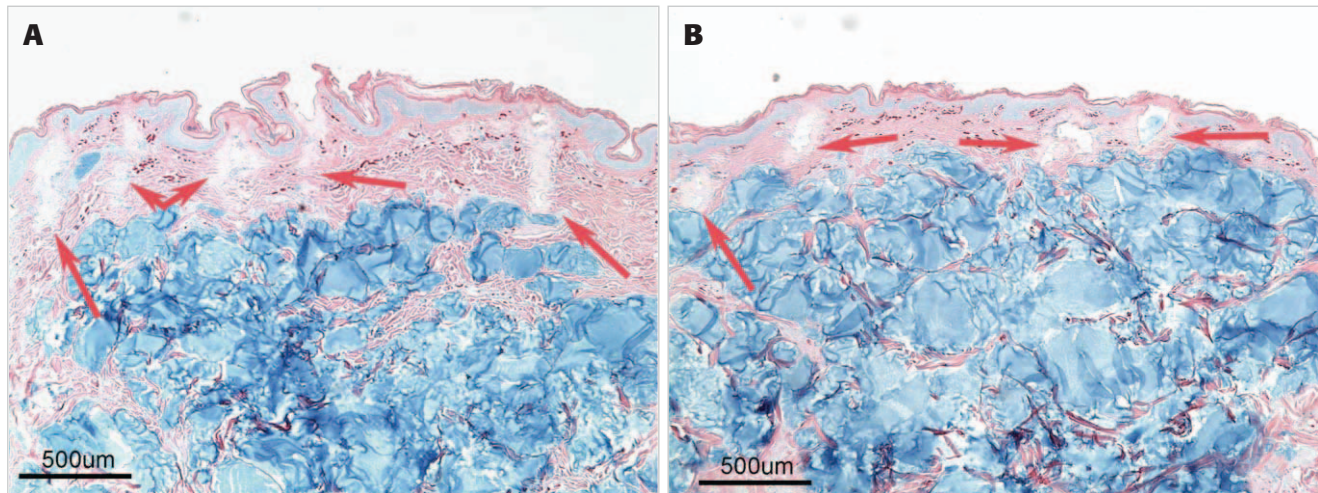


Figure 5. **A** and **B**, Adjacent histopathologic skin sections treated with the DeepFX CO₂ laser (15 mJ/density 4/single pulse) and stained with alcian blue. Note the relationship between the filler and microablation columns penetrating through the epidermis and into the papillary and reticular dermis (arrows). No ablated or morphologically altered filler was observed. Filler was identified within some of the collapsed and open microablation columns within the dermis. Any ablated filler is most likely masked by the immediate replacement of these voids with surrounding hyaluronic acid filler. (Original magnification: $\times 4$.)

Evidence of deep dermal injury was also evident following treatment with the Deep FX CO₂ device (Lumenis). The microablation columns were interspersed among the injected pool of HAF in certain biopsy specimens that came in direct contact with pockets of the filler and demonstrated evidence of migration of the filler into the ablated microchannels. In some areas, the HA was trapped within the collapsed microablation columns (Figure 5). Higher energy settings caused deeper tissue injury and increased penetration into the reticular dermis but did not show any obvious morphologic changes or denaturation of the preinjected filler. Ablated areas of filler were not appreciated (Figure 6).

DISCUSSION

The popularity of nonsurgical facial rejuvenation using soft tissue augmentation and ablative/thermal remodeling from laser/light procedures has resulted in a significant shift in many cosmetic surgery practices. Both procedures aim to improve facial skin contour and rhytids using significantly different approaches. Bioengineered HAFs, such as Restylane and Juvéderm, cause an immediate improvement by augmenting the volume of the mid-deep dermis. Superficial laser treatments target the epidermis or dermal-epidermal junction to affect pigment, lentigines, or epidermal damage. More aggressive ablative laser resurfacing procedures mechanically remove photodamaged or actinic skin while stimulating production of new deeper collagen from the surrounding uninjured tissue. As opposed to the immediate gratification seen with filler injections, skin remodeling and rejuvenation following laser procedures is a gradual process and can take up to a year before the final result. Combination procedures are now a topic of interest and increasing in popularity.¹³⁻¹⁵ Anecdotal reports have alleged that,

when used in combination, laser/light procedures may substantially reduce the effect and longevity of HAFs.¹³ Interestingly, it is known that the shrinkage effect of collagen from laser treatments occurs at approximately 65°C.^{14,15} The stability of HA products to such extreme heating has come into question. However, these products are sterilized at temperatures of approximately 120°C.¹² Our results failed to show any adverse acute effect on the filler following treatment with any of the laser modalities.

The filler was identified within some of the ablation microchannels when coming in direct contact with the preinjected filler. The histology re-emphasizes the importance of injection depth. This is vital when considering the use of laser/light procedures and filler injections in combination for nonsurgical rejuvenation. When placing filler in the papillary or superficial reticular dermis, which is recommended for some of the more fluid fillers with a lower concentration of HA, it is important to be cognizant of the targeted depth of laser treatment with a respective laser system (Figure 7).

Overall, the deep ablative laser systems were the only devices that demonstrated mid-deep dermal penetration following treatment and had any significant interaction with the injected filler. The wavelengths of the Er:YAG and CO₂ devices target intracellular water, and depth and efficacy of treatment may be compromised when coming in contact with the hydrophilic water-based HAF. That being said, these laser treatments also have the potential to affect the longevity of the fillers by decreasing filler water content and potentially decreasing filler volume, and vice versa. It must be noted that filler placed in the deep dermal or subcutaneous tissue was too far from the skin surface to be affected by any of the laser treatments, especially the IPL or epidermal/superficial papillary dermal treatments like the Active FX.

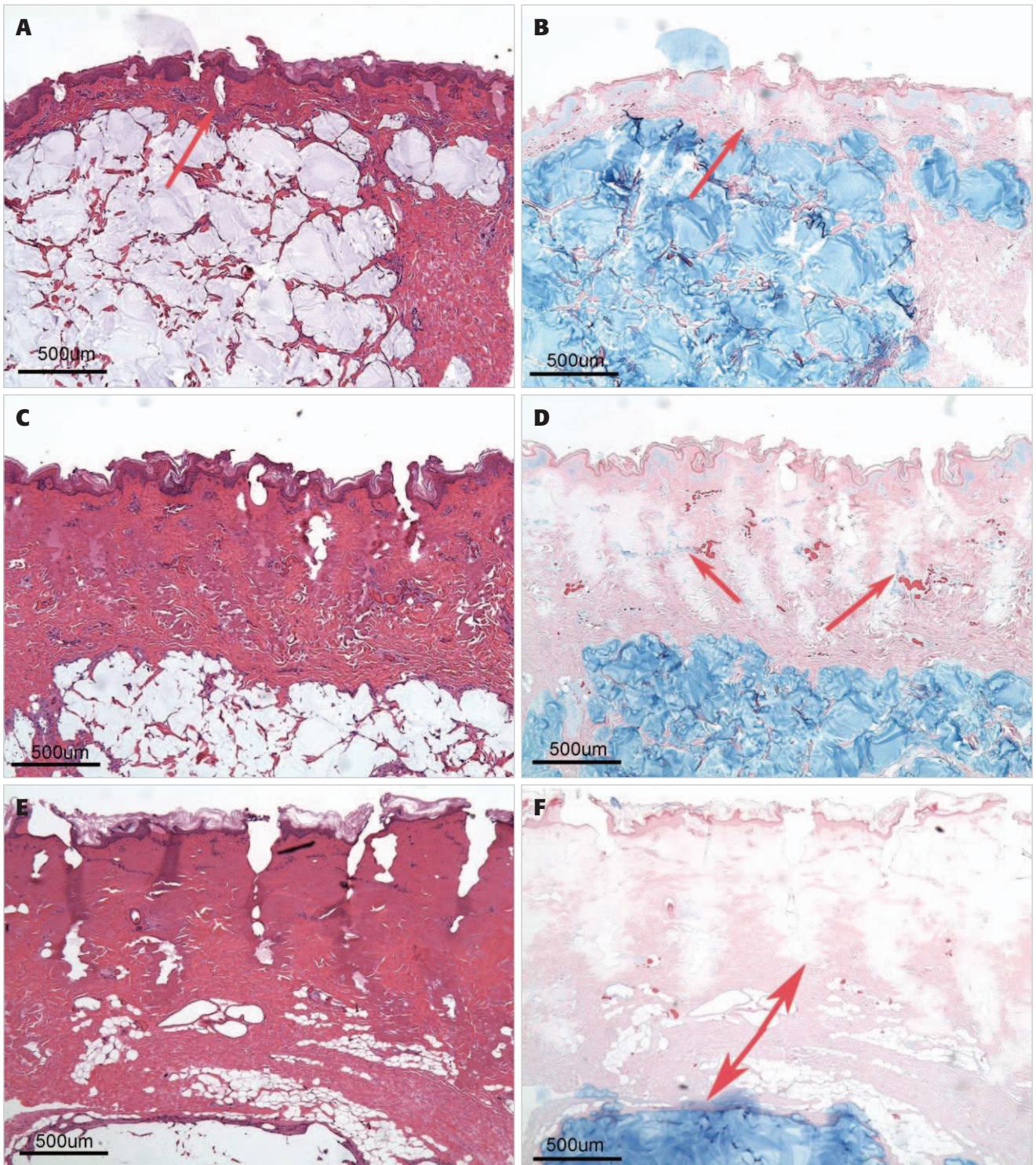


Figure 6. Contiguous preinjected histopathologic skin sections treated at increasing energies (10 mJ/density 4/double pulse; 15 mJ/density 4/double pulse; 30 mJ/density 4/double pulse) with the DeepFX CO₂ laser and stained with hematoxylin–eosin (**A, C, E**) and alcian blue (**B, D, F**) stains. Aggressive energy fluences are capable of deep dermal penetration and will inevitably interact with preinjected mid-deep dermal hyaluronic acid filler.

Although this pilot study has interesting implications, it is not without limitations and is not a clinical study. It is, however, the first substantial acute histologic evaluation of the effect of multiple novel laser treatments on preinjected HAFs in an *in vivo* porcine model. Because these combination procedures are used regularly in aes-

thetic surgery practice, more scientific data dealing with laser/filler interaction are needed.

A delayed inflammatory reaction in the skin by the different laser treatments is possible, and further study evaluating the changes in the treated tissue over a longer time course may be valuable. Allowing for a

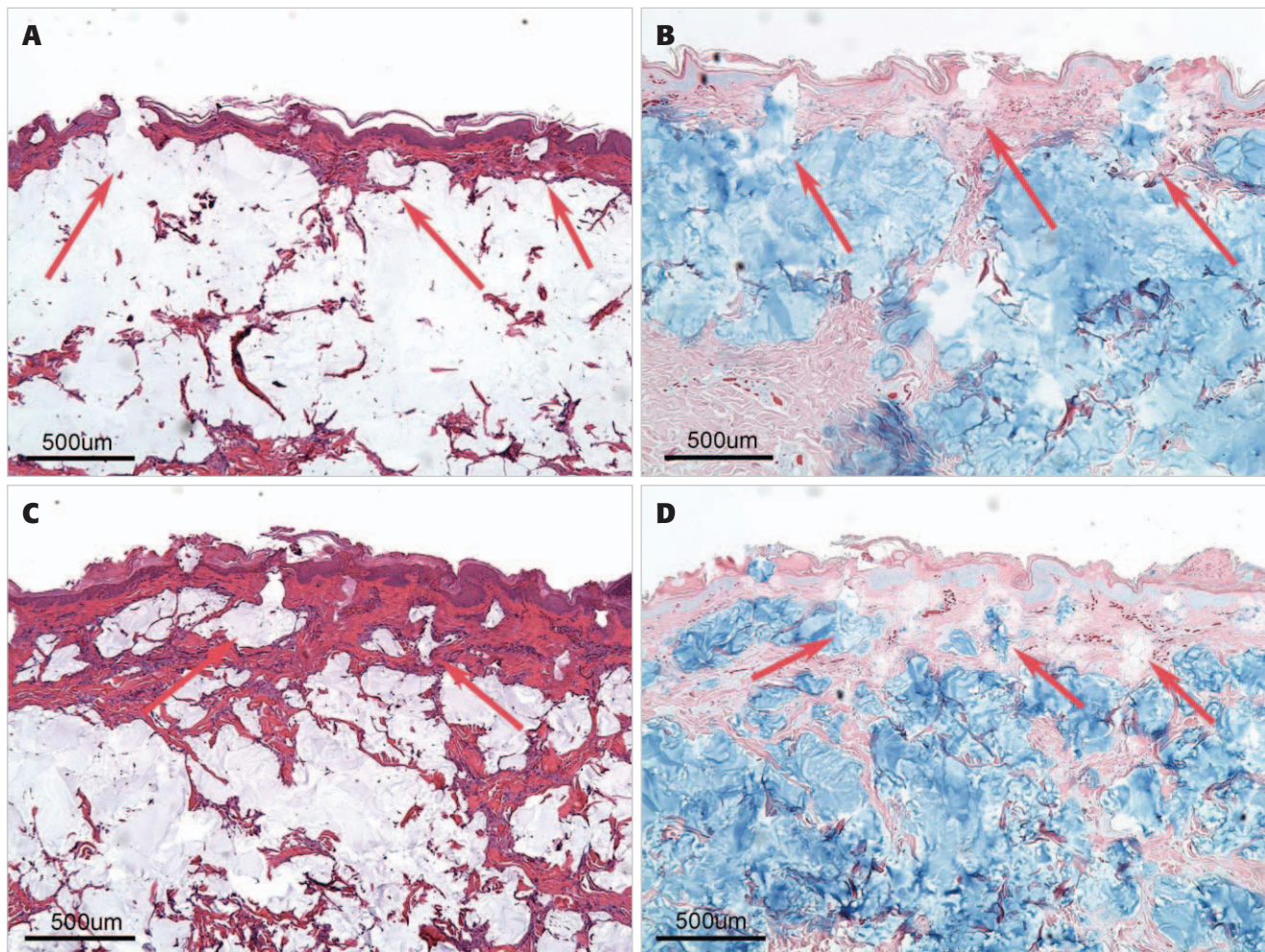


Figure 7. Contiguous preinjected histopathologic skin sections treated with the DeepFX CO₂ laser (15 mJ/density 4/single pulse) and stained with hematoxylin-eosin (A, C) and alcian blue (B, D), demonstrating the importance of depth of filler placement in the location of the filler when planning on treating preinjected areas with a deep ablative device. Arrows indicate interaction between the laser injury/ablated microcolumns and the filler.

longer time interval following injection for incorporation or absorption of filler may also be beneficial. A few reported studies in the literature had difficulty with the histologic sampling and identification of the filler in the dermis of the biopsied specimens.^{13,14} The microtattooing and predesigned template was imperative in order to accurately identify and treat the preinjected regions. Future study into evaluating volume loss of the filler following ablative laser treatments is currently being investigated.

The abdominal skin of the Yorkshire pig has been cited as an appropriate model and is histologically similar to abdominal human skin.^{15,17} However, it should be noted that laser resurfacing and IPL treatments are commonly instituted in facial skin, which contains a much higher density of dermal adnexal and has a thinner epidermis and dermis than abdominal skin. We recognize that *in vivo* human facial tissue is the optimal skin model, but is also quite difficult to obtain. An understanding of the difference in skin thickness across the face is an important point to consider when using soft tissue fillers and laser treatments.¹⁸

CONCLUSIONS

Noninvasive facial rejuvenation procedures are an exciting alternative for patients seeking nonsurgical methods of treatment. It is important to scientifically evaluate new technologies and modalities to ensure patient safety before performing such procedures. Certain novel laser/light technologies have the capability of deep tissue penetration. If planning superficial filler placement with aggressive deep resurfacing, it may be best to treat with the laser before the soft tissue filler injections and to stage these procedures in order to maximize the treatment effect of each of the modalities.

Following laser/light treatments, there was no sign of abnormal tissue damage or injury, or alteration of the filler, grossly or histologically, in the preinjected abdominal sites. However, this is not a clinical report, and it would therefore be inappropriate to comment on the clinical interaction between the HAF and the various laser treatments based on our results. Future clinical studies are needed regarding the safety of combination filler/laser treatments. It is important to be cognizant of the location of filler placement within the tissue if plan-

ning on using concomitant laser therapy for noninvasive skin rejuvenation. ■

DISCLOSURES

Research grants were received from Palomar Medical Technologies and Lumenis Inc. Devices used in the study were donated by Lumenis, Palomar Medical Technologies, and Sciton. Hyaluronic acid fillers were supplied by Medicis and Allergan.

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