Lasers and Light Sources to Activate Fibroblasts

Christopher Spock, MD; Andrei I. Metelitsa, MD, FRCPC; Joely Kaufman, MD; Jeremy B. Green, MD

There is a decrease in normal collagen in aged and sun-damaged skin. Neocollagenesis can promote a more youthful appearance. A key target in the mitigation of the pathophysiology of photoaging is fibroblasts. Various therapeutic modalities can be employed, including topical retinoids, dermabrasion, radiofrequency or ultrasound energy, and filler injections. This review focuses on the molecular-level effects of lasers and light sources to activate fibroblasts. Fractional ablative and nonablative resurfacing lasers are the gold standard for stimulating aged fibroblasts and inducing neocollagenesis; however, other laser and light modalities can be employed to activate quiescent fibroblasts.

fibroblast. Neocollagenesis can promote a more youthful appearance. Specifically, dermal remodeling is thought to occur via increased deposition of types I and III collagen, with collagen reorganization into parallel arrays of compact fibrils.

Dermatologists employ diverse therapeutic modalities, including topical retinoids, dermabrasion, radiofrequency or ultrasound energy, and filler injections, to stimulate collagen production; this review focuses on the molecular-level effects of lasers and light sources utilized to achieve this goal.

**ABLATIVE LASERS**

Ablative laser resurfacing entails vaporization of the superficial layers of the skin and coagulative necrosis. The 10,600-nm CO\textsubscript{2}, 2940-nm erbium:YAG (Er:YAG), and 2790-nm erbium:YSrG lasers are the most commonly used ablative lasers. Traditional continuous-wave ablative lasers have given way to short-pulsed, high-energy fractionated systems. Ablative laser resurfacing elicits notable improvement in dyspigmentation, rhytides, and atrophic scars; however, there is a prolonged postoperative recovery period and potentially permanent adverse sequelae, such as scarring. Following ablative laser procedures, patients also can experience prolonged erythema, edema, milia formation, pigment alterations, and delayed wound healing.

**10,600-nm CO\textsubscript{2} Laser**

Despite the potential risks of ablative laser resurfacing, the CO\textsubscript{2} laser remains one of the most effective modalities available for facial rejuvenation. Pulsed CO\textsubscript{2} lasers have been shown to vaporize epidermal and dermal cells and cause coagulative necrosis of the underlying cell layers. These lasers act further to denature extracellular proteins in adjacent zones.

The seminal work by Orringer et al\textsuperscript{13} quantified the molecular-level effects of a CO\textsubscript{2} laser (Ultrapulse, Coherent, Inc) by analyzing messenger RNA (mRNA) transcripts of types I and III collagen. A total of 28 patients with photodamage of the forearms were treated with 2 passes of the CO\textsubscript{2} laser at 60 W in a single treatment session. Tissue biopsies of the treatment area were obtained at baseline and 5 additional times following treatment. Production of types I and III procollagen mRNA peaked 21 days posttreatment and remained elevated for at least 6 months. Increases in several cytokines (IL-1\textbeta, tumor necrosis factor \textalpha, and transforming growth factor \textbeta1) preceded and/or accompanied changes in collagen levels. Marked increases in mRNA levels of MMP-1, MMP-3, MMP-9, and MMP-13 also were noted. The increased expression of MMPs preceded the increased production of types I and III procollagen.

Based on their findings, Orringer et al\textsuperscript{13} proposed the following model: Proinflammatory cytokines (IL-1\textbeta, tumor necrosis factor \textalpha) induce the expression of MMPs. The MMPs, including collagenase (MMP-1 and MMP-3) and gelatinase-B (MMP-9), break down existing collagen. Levels of MMP-9 remain elevated in the postoperative period, evidence of continued collagen removal. The degradation of photodamaged collagen facilitates replacement by new, well-organized collagen bundles.

Orringer et al\textsuperscript{13} also found that collagenase-3 (MMP-13), known to have a role in the collagen remodeling of fetal wounds\textsuperscript{14} but not normally healing adult wounds,\textsuperscript{15} was maximally expressed on postoperative day 14. Interestingly, the upregulation of MMP-13 may occur in a temperature-dependent fashion, as it also is seen in human skin that has been treated with fractional radiofrequency energy to a temperature of 72°C.\textsuperscript{16}

In a similar report, Reilly et al\textsuperscript{17} examined the molecular effects of a fractional CO\textsubscript{2} laser (Pixel Perfect, Alma Lasers, Ltd) in 9 patients on infra-auricular skin that was sampled preoperative, immediately postoperative, and at 2 of 3 other time points (days 7, 14, or 21). They found that MMP-1, MMP-3, MMP-9, and MMP-13 also were upregulated; however, upregulation of 2 additional MMPs (MMP-10 and MMP-11) was demonstrated after treatment with the fractional CO\textsubscript{2} laser.\textsuperscript{17} Based on these findings, the mechanisms by which fractional and full-field CO\textsubscript{2} lasers induce neocollagenesis appear to be similar.

**2940-nm Er:YAG Laser**

The Er:YAG laser has a greater water absorption coefficient than the CO\textsubscript{2} laser and a subsequently more shallow absorption depth. In one study, 10 patients with clinically evident photodamage of the forearms were treated with a single pass (750 mJ; 5-mm spot size) of the full-field Er:YAG laser (SmoothPeel, Candela Corporation); then skin biopsies were taken at 1, 3, 7, and 14 days posttreatment.\textsuperscript{18} Molecular studies following treatment with the Er:YAG laser revealed induction of epidermal expression of keratin 16, a marker of epidermal injury; however, immunostaining with laminin \gamma2 confirmed that, despite epidermal damage, the basement membrane remained intact following treatment. Interestingly, heat shock protein 70 (Hsp70), which is thought to participate in procollagen synthesis in normal wound healing, was not shown to be elevated.

As with CO\textsubscript{2} lasers, resurfacing with the Er:YAG laser increases MMP-1, MMP-3, MMP-9, and types I and III
collagen, a process that has been clinically shown to improve mild to moderate atrophic acne scars. According to the authors, the evidence that epidermal injury with the Er:YAG laser significantly induces dermal neocollagenesis could shift the paradigm for activating quiescent fibroblasts to less-invasive interventions. Of note, the 2790-nm erbium:YSGG laser was introduced in 2007 (Pearl, Cutera, Inc). Its water absorption coefficient is one third that of the Er:YAG laser and 5 times that of the CO₂ laser. Although there are studies evaluating the clinical and histologic efficacy of this device, we were unable to find any published evidence of its molecular effects.

**NONABLATIVE LASERS AND LIGHT SOURCES**

Nonablative lasers can be classified into 3 main groups: near-infrared lasers, such as the 1550-nm and 1540-nm erbium glass fractional resurfacing lasers and 1320-nm and 1064-nm Nd:YAG lasers; visible light lasers such as the pulsed dye laser (PDL) and the pulsed potassium titanyl phosphate (KTP) laser; and broadband intense pulsed light (IPL) sources.

Traditional nonablative laser treatments are effective for improving rhytides and scars, but the results of a single nonablative session are less impressive than a single treatment with an ablative counterpart. Nonablative lasers function to stimulate dermal collagen formation without visible epidermal wounding. The concept of fractional photothermolysis (FP) that was first published in 2004 revolutionized resurfacing lasers. During nonablative FP procedures, infrared laser irradiation is delivered in microthermal zone columns, sparing intervening areas of skin while the stratum corneum remains intact. The undamaged surrounding skin rapidly repopulates the injured tissue columns, resulting in less downtime and risk for scarring than traditional homogenous laser interventions.

**1550-nm Erbium Glass Laser**

The near-infrared 1550-nm erbium glass laser’s primary chromophore is intracellular water, and it penetrates to a depth of up to 2 mm. Compared to shorter-wavelength, 1320-nm and 1450-nm infrared laser systems, the 1550-nm wavelength induces the least amount of melanin and hemoglobin absorption. In 2010, Orringer et al published their findings of a 1550-nm erbium-doped fiber laser (Fraxel SR1500/Fraxel re:store, Solta Medical, Inc) used to treat the actinically damaged forearms of 20 patients and performed analyses similar to those used in their prior study of CO₂ lasers. Histologic examination 24 hours after treatment revealed thermally altered columns in the epidermis and dermis of treated skin. Despite epidermal damage, the basement membrane remained intact as indicated by laminin γ2 staining.

Following treatment, the investigators found an acute inflammatory response in the form of neutrophil infiltration and elevation of proinflammatory cytokines IL-1β and tumor necrosis factor α. They also found that FP induced rapid and transient expression of Hsp70, which potentiates procollagen synthesis by protecting against misfolding of proteins during heat stress. Production of types I and III collagen also was increased, and elevated mRNA levels of MMP-1, MMP-3, and MMP-9 were observed. With respect to molecular mediators, minimal differences were observed between lower and higher microbeam energy settings (15 vs 70 mJ) and collagen production was not statistically different between the two. Based on these findings, the authors suggested that lower microbeam energy and higher microbeam density treatment parameters might be optimal for achieving the best combination of patient tolerability and clinical outcome. The molecular changes observed after nonablative FP were remarkably similar to those observed after traditional ablative resurfacing.

**1540-nm Erbium Glass Laser**

The 1540-nm fractional erbium glass laser is similar in nature to the 1550-nm device. Lupton et al evaluated 24 female patients with mild to moderate periorbital and perioral rhytides following a series of 3 monthly treatments with a near-infrared 1540-nm erbium-doped phosphate glass laser (Aramis, Quantel Medical). Skin biopsies were obtained at baseline, immediately following laser irradiation, and at 1 and 6 months posttreatment. Six months after the final treatment, a mild but noticeable increase in dermal fibroplasia was evident. Molecular analysis was not performed on the specimens.

Fournier et al used ultrasonography to quantify the posttreatment changes. Sixty patients with perioral and periorbital rhytides were treated 4 times over 6-week intervals with a 1540-nm erbium-doped phosphate glass laser (Aramis). Participants were evaluated using photographs, histology, ultrasound imaging, and profilometry using silicone imprints. Ultrasound imaging demonstrated a 17% increase in dermal thickness and biopsy specimens qualitatively showed new collagen formation.

In a recent study by de Angelis et al, a 1540-nm erbium glass laser (Lux1540, Palomar Medical Technologies, Inc) was used to treat striae distensae in 51 patients and demonstrated clinical improvement as well as histologic evidence of dermal thickening, neocollagenesis, and increased elastin deposition 1 month after a series of 2 to 4 treatments. Although there was no molecular analysis of the posttreatment...
wound-healing process, one would expect that it would be similar to that observed following treatment with the 1550-nm laser.

### 1320-nm Nd:YAG Laser

The 1320-nm Nd:YAG laser for nonablative dermal remodeling has a high scattering coefficient. Consequently, the laser energy largely is confined to the dermis. This wavelength is coupled with cryogen cooling so that dermal damage can be induced with less risk of epidermal injury. This device has been used for the treatment of photoaging as well as acne scarring.30

Goldberg30 qualitatively assessed the efficacy of this laser in a study of 10 female participants with photoaging. Unique to this analysis was the use of a thermal sensor to measure epidermal temperature. Peak surface therapeutic temperatures, as measured by a thermal sensor contained within the laser handpiece, ranged from 40°C to 48°C, which corresponded to a dermal temperature of 60°C to 65°C.30

The clinical improvements noted by Goldberg30 were more remarkable than a prior study by Menaker et al32 in which improvement was noted in only 4 of 10 patients and pitted scarring was found in 3 participants during this 3-month study; however, in the Menaker et al32 study, all participants were treated with a constant set of parameters, as opposed to Goldberg’s30 study in which treatment fluences varied.

Histologic analysis of nonablative remodeling using a microsecond-pulse, 1320-nm Nd:YAG laser (Cool Touch I, CoolTouch Corporation) demonstrated epidermal spongiosis and basal cell layer edema visible 1 hour after treatment with 3 passes of the laser.33 Three days following treatment, there was evidence of dermal microthrombi, sclerosis of blood vessels, and neutrophilic infiltration. These findings correlated with clinical improvement, suggesting that epidermal and vascular injury may play a role in inducing neocollagenesis.33

### 1064-nm Nd:YAG Laser and 532-nm KTP Laser

The 1064-nm Nd:YAG laser in the near-infrared spectrum offers greater cutaneous depth of penetration than the KTP laser or PDL, with capabilities reaching 5 to 10 mm when used with epidermal cooling. A variety of Nd:YAG lasers are available with pulse widths ranging from the Q-switched nanosecond lasers to long-pulsed millisecond devices. Oxyhemoglobin absorbs 1064-nm light 10 times more effectively than water, which makes this device an ideal choice for darker skin types because 1064-nm light is poorly absorbed by melanin. The nonselective thermal damage induced by the Nd:YAG laser stimulates dermal remodeling.34

In a split-face study of 11 participants receiving treatment of rhytides in the periorbital or perioral regions, Goldberg and Whitworth34 compared the resurfacing capability of the Q-switched Nd:YAG versus 2 CO2 lasers (Silk Touch, Sharplan, Inc; UltraPulse, Coherent, Inc). Although all 11 (100%) participants treated with both CO2 lasers showed clinical improvement, only 9 (82%) participants treated with the Q-switched Nd:YAG laser showed improvement, which was notably less impressive.34 In a follow-up study, Goldberg and Silapunt35 conducted a histologic analysis of sun-damaged, infra-auricular skin specimens 3 months following a single treatment with the 1064-nm Q-switched Nd:YAG laser, which revealed inconsistent papillary dermal collagen formation. Four of 6 (67%) participants demonstrated increased fibrosis in the papillary dermis, which is evidence of collagen remodeling, 3 months following treatment; however, the authors noted that histologic changes are not always directly correlated with clinical improvement.35

Using a submillisecond (300-microsecond pulse duration) 1064-nm Nd:YAG laser (CoolGlide, Cutera, Inc), Schmults et al36 examined the effects on dermal remodeling using histology and electron micrography. Nine female patients were treated at 2-week intervals for a total of 3 treatment sessions. Electron micrograph analysis showed a decrease in overall collagen fiber diameter in the papillary dermis at 1 and 3 months after treatment, indicating the formation of new collagen; however, no quantitative analysis of collagen production was performed.37

When 1064-nm light passes through a KTP crystal, it is frequency doubled (wavelength halved) to 532-nm green light. The strong melanin absorption level of this wavelength limits its depth of penetration to 1 to 2 mm. The long-pulsed KTP is an excellent vascular laser and its Q-switched variant is effective at removing the pigment of lentigines and tattoos. Reported clinical improvement in scars and rhytides after treatment with KTP and Nd:YAG lasers suggest their effectiveness at delivering heat to induce neocollagenesis.37

A study by Dang et al39 elucidated the in vitro effects of the 532-nm KTP and 1064-nm Q-switched Nd:YAG lasers on human skin fibroblasts. The investigators found increased expression of types I and III procollagen after irradiation with Q-switched KTP or Nd:YAG lasers. Both lasers markedly downregulated the expression of MMP-1 and MMP-2 and upregulated the expression of tissue inhibitors of metalloproteinases. Interestingly, the authors contended that the increased tissue inhibitors of metalloproteinases and reduced expression of collagen-digesting MMPs facilitates neocollagenesis,39 whereas the aforementioned Orringer et al13 study with ablative...
lasers found elevated MMPs and theorized that they were necessary to clear actinically damaged collagen prior to fibroblast deposition of new collagen. Dang et al.\(^1\) found that the KTP laser induced IL-6, a potent mediator of fibroblast proliferation that decreases with aging, and Hsp70. In contrast, they found that the Nd:YAG laser induced transforming growth factor \(\beta\), a major inducer of procollagen synthesis in wound healing, suggesting a different mechanism of action for neocollagenesis for each laser.\(^2\) A more recent study by this same group found that in vitro irradiation of human skin fibroblasts by both the KTP and Nd:YAG lasers led to upregulation of the major antioxidant enzymes superoxide dismutase and glutathione peroxidase, demonstrating that reduced oxidative stress potentiates fibroblast neocollagenesis.\(^3\) The contributions of these investigators have remarkably advanced the understanding of the molecular mechanisms driving the activation of fibroblasts by KTP and Nd:YAG lasers.

### 585-nm to 595-nm PDL

Pulsed dye lasers emit yellow light (585–595 nm) that is well absorbed by blood vessels in the dermis. Prior investigations of PDL that demonstrated its ability to improve scars and striae distensae clinically and histologically by inducing neocollagenesis form the basis for its use in activating aged fibroblasts.\(^4\)–\(^6\) An earlier investigation by Zelickson et al.\(^7\) evaluated the effects of PDL therapy in 10 patients with mild to moderate perioral or periorbital wrinkling and 10 patients with moderate to severe perioral or periorbital wrinkling after a single PDL (Photogenica V, Cynosure, Inc) treatment session (585-nm wavelength; 3.0–6.5\(\text{J/cm}^2\); 450-microsecond pulse duration; 7–10-mm spot size). Fourteen months after the purpura-inducing, short-pulsed PDL treatment, 90% (9/10) of patients with mild to moderate rhytides and 40% (4/10) with moderate to severe rhytides demonstrated clinical improvement. Histologic analysis of treated areas demonstrated increased staining of elastin and collagen fibrils in the papillary dermis as well as increased mucin.\(^8\)

In a study by Rostan et al.,\(^9\) 15 women who received 4 monthly, long-pulsed, 595-nm PDL treatments demonstrated a modest 18% clinically graded improvement in dyspigmentation and erythema are accompanied by histologic changes indicative of a dermal remodeling effect, such as an increase in extracellular matrix proteins and neocollagenesis.

In a study by Goldberg,\(^10\) 5 patients underwent 4 IPL treatment sessions followed by a biopsy 6 months after the final treatment. Histologic analysis demonstrated new collagen formation in the papillary dermis that was associated with clinical improvement.\(^11\) It has been theorized that the effect of IPL on dermal collagen is caused by heat diffusion from the vasculature with release of inflammatory mediators that are stimulated by vessel heating.\(^12\) Another study by Feng et al.\(^13\) evaluated 4 patients after 2 to 4 IPL treatments (Lumenis One, Lumenis Aesthetic) and found an increase in types I and III collagen with immunohistochemistry. Transmission electron microscopy revealed “younger” fibroblasts and increased activity of cellular organelles.\(^14\)

Compared to ablative and nonablative resurfacing, IPL offers more modest clinical improvement in rhytides and texture and is the most beneficial for pigmented and vascular targets.\(^15\) Nonetheless, there is histologic and molecular evidence that the dermal heat generated by IPL treatments induces neocollagenesis.

### CONCLUSION

The dermatologic laser surgeon possesses an ever-expanding armamentarium of lasers and light-based devices to improve the appearance of photoaged skin. Fractional ablative and nonablative resurfacing lasers are the current gold standard for stimulating aged fibroblasts and inducing neocollagenesis; however, as this review has illustrated, other laser and light modalities are capable of delivering energy to the dermis to activate quiescent fibroblasts and improve the appearance of aged skin.

### REFERENCES

ActiVating Fiборblasts


