Opportunities for laser-assisted drug delivery in the treatment of cutaneous disorders

Emily Wenande, MD; Andrés Már Erlendsson, MD, PhD; and Merete Haedersdal, MD, PhD, DMSc

Abstract

Fractional laser-assisted drug delivery (LADD) is increasingly finding its way into clinical practice as a new means to enhance topical drug uptake and improve treatment of cutaneous disorders. To date, LADD has been used for a wide range of conditions, including photodamaged skin, neoplastic lesions, scars, cutaneous infections, and vitiligo as well as for topical anesthetic and aesthetic procedures. Substantiated by randomized controlled clinical trials, strong evidence is available for LADD's usefulness for photodynamic therapy (PDT), for which improved efficacy using laser-assisted photosensitizer treatment is established for actinic keratosis compared with conventional PDT. Over time, the modality has undergone increasing refinement and offers the potential advantages of reduced treatment durations, shortened incubation times, and the replacement of cumbersome, patient-dependent treatment regimens with quick, in-office procedures. Notwithstanding, LADD is still a new enhancement technique, and risks of both local and systemic adverse events are insufficiently explored. With conscientious development, however, LADD promises to improve existing regimens and make new pharmacological treatments a reality for a wide range of cutaneous disorders.

Semin Cutan Med Surg 36:192-201 © 2017 Frontline Medical Communications

Topical therapies form the cornerstone of dermatological treatment. Therapeutic efficacy hinges not only on pharmacological potency, but also penetrative ability through the different skin layers. To reach their target, topical medications must thus diffuse down concentration gradients via intercellular, transcellular, or appendageal pathways. In general, the major rate-limiting step in this process is passage through the epidermis' outermost layer, the stratum corneum (SC). Comprising a "brick and mortar-like" structure of densely packed corneocytes within a hydrophobic lipid-enriched extracellular matrix, the SC not only provides the primary defense against external insult, but also constitutes an exceedingly effective barrier to drug delivery.[2] As a result, topical agents generally demonstrate poor absorption, with only 1% to 5% of an applied dose penetrating into intact skin.[3]

Department of Dermatology, Bispebjerg Hospital, University of Copenhagen, Copenhagen, Denmark.

Disclosures: Dr. Haedersdal reports other from Ellipse, grants from Galderma, grants from Leo Pharma, grants from Procter & Gamble, grants and other from Sciton, grants from Sebacia, other from Syneron-Candela, grants from Lumenis, outside the submitted work. In addition, Dr. Haedersdal has a patent PA 2013 00195 pending. Dr. Erlendsson and Dr. Wenande have nothing to disclose.

Acknowledgement: The contents of this work are adapted in part from Erlendsson et al. Transepidermal Drug Delivery: Overview, Concept, and Applications. In: M.C.A. Issa, B. Tamura, eds. *Lasers, Lights and Other Technologies, Clinical Approaches and Procedures in Cosmetic Dermatology*, Springer International Publishing AG 2016, written by the same authors.[1]

Correspondence: Emily Wenande; ewenande@mgh.harvard.edu

Drug properties and the stratum corneum

While the SC is relatively permeable to low molecular weight (<500 Da), hydrophobic and uncharged compounds, drugs that are hydrophilic, charged, or of higher molecular weight (>500 Da) only sparingly penetrate the skin barrier.[4,5] Depending on their physicochemical properties therefore, many topical medications are restricted in their ability to reach their intended target at therapeutic levels. In consequence, the development of delivery methods to increase cutaneous as well as transdermal drug uptake remains an area of continued research.

Chemical drug-delivery techniques

Topical drug-delivery strategies that disrupt or interact with the skin barrier are broadly divided into biochemical and physical techniques.[4,5] Mechanisms of chemical modulation are numerous and range from disruption of cutaneous intercellular lipid or protein organization, displacement of skin-bound water, loosening of corneocytes and SC delamination, to increased drug solubility/partitioning into SC, and keratin denaturation. These effects can be achieved using various strategies, including penetration enhancers, supersaturated systems, prodrugs, liposomes, nanoparticles, and other carrier systems.[6-8] Though long established, chemical biomodulation is, however, not without limitations. Few chemical enhancers possess the ideal dual properties of efficacy and tolerability, and compared with physical enhancement techniques, chemical drug delivery shows limited success in increasing skin penetration of high molecular weight molecules.[9]

Physical drug-delivery techniques

In order to enhance local or transdermal uptake of topically applied drugs using physical delivery techniques, an external energy is applied to disrupt the skin barrier. Thus far, a multitude of approaches have been introduced, including iontophoresis, electroporation, curettage, microdermabrasion, microneedles, pressure waves, sonophoresis, radiofrequency, and lasers (Table). In contrast to the aforementioned chemical enhancement techniques, compounds successfully delivered using physical modalities range from small topical drugs (eg, aminolevulinic acid [ALA][22,25,42]) to large systemic macromolecules (eg, human growth hormone [hGH][27,46,47] and stem cells[48]). Though most evidence remains experimental with results primarily derived from in vitro settings, lasers in particular are gaining clinical impact as a promising new means of drug delivery into the skin.

1085-5629/13$-see front matter © 2017 Frontline Medical Communications
DOI: https://10.12788/j.sder.2017.046

TABLE Different types of physical enhancement techniques to enhance skin permeability, their proposed MoA, and examples of delivered compounds.[a]

Type	Technique external driving energy force	Proposed MoA	Examples of delivered compound
AFL	i) Fractional tissue ablation ii) Photothermal effects	i) Thermal removal of SC and cutis	5-Fluorouracil (130 Da)[10] ALA/MAL (177-182 Da)[11] Lidocaine (234 Da)[12] Methotrexate (455)[13]
NAFL	i) Photothermal effects ii) Photomechanical waves	i) Thermal exposure primarily affecting the epidermis and dermis below SC ii) Light energy converted to mechanical energy with formation of epidermal vacuoles and dermal-epidermal junction disruption	ALA/MAL (177-182 Da)[14,15] Tretinoin (300 Da)[16] Botulinum toxin (150 kDa)[17] Tacrolimus (804 Da)[18,19]
Microdermabrasion	Mechanical abrasion	Exfoliative crystals or sandpaper, mechanically removing SC	5-Fluorouracil (130 Da)[20] Ascorbic acid (176 Da)[21] ALA (177)[22] Insulin (5.8 kDa)[23]
Microneedles	Mechanical introduction of a needle array	Physical disruption of skin barrier with vertical microchannels through the skin	Ascorbic acid (176 Da)[24] ALA/MAL (177-182)[25] Tretinoin (300)[26] hGH (22.1 kDa)[27]
Curettage	Mechanical debridement of the upper skin layers	Surgical scraping causes physical disruption of skin barrier	ALA/MAL (177-182 Da)[28] 5-Fluorouracil (130 Da)[29] Imiquimod (240 Da)[30]
Iontophoresis	Low-level electric current (max 0.5 mA cm2)	Active ion flow driven by an applied electric field	ALA (177 Da)[22] Lidocaine (234 Da)[31] Methotrexate (455 Da)[32] Botulinum toxin (150 kDa)[33]
Electroporation	High-voltage (>100 V) electric pulses	Formation of transient transmembrane pores and disruption of cell membranes	ALA (177 Da)[22] Methotrexate (455 Da)[34] Bleomycin (1,500 Da)[35] Vaccines[36]
Pressure	Mechanical pressure force	External pressure	ALA/MAL (177-182 Da)[37] Caffeine (194 Da)[38] Polyethylene glycol (400 Da)[39]
Radiofrequency	High-frequency alternating current (~100 kHz)	Ionic vibrations within cells, causing localized heating and ablation.	ALA (177 Da)[40] hGH (22.1 kDa)[41]
Sonophoresis	Ultrasound. Most often low-frequency waves are used in the range of 20-100 kHz. High-frequency sonophoresis may also be used (>3 MHz).	Primary mechanism is considered transient cavitation in intercellular lipids. Also thermal effects, induction of convective transport, and mechanical effects due to pressure variation.	ALA (177 Da)[42] Diclofenac (296 Da)[43] Hydrocortisone (363 Da)[44] EPO (48.0 kDa)[45]

[a] Table adapted with permission from Erlendsson et al.[1]

Abbreviations: AFL, ablative fractional laser; ALA, aminolevulinic acid; EPO, erythropoietin; hGH, human growth hormone; MAL, methyl aminolevulinate; MoA, mechanism of action; NAFL, nonablative fractional laser; SC, stratum corneum.

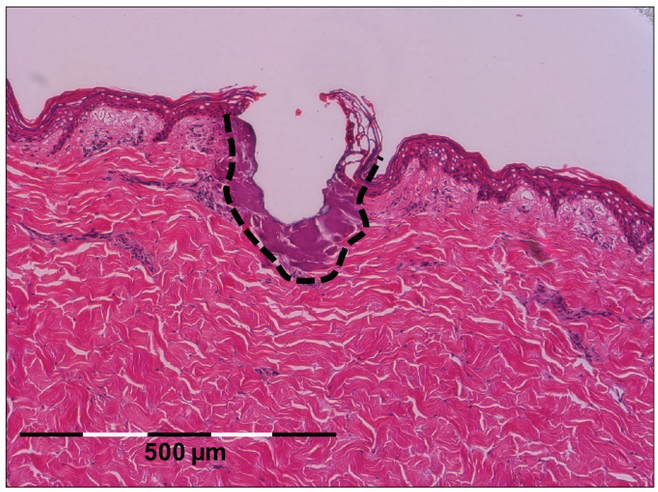

FIGURE 1. H&E-stained skin section illustrating a single MAZ with surrounding CZ, generated by AFL exposure using a 10,600-nm CO_2 laser at 17.5 mJ/microbeam and spot size of 200 µm. AFL, ablative fractional laser; CZ, coagulation zone; H&E, hematoxylin and eosin; MAZ, microscopic ablation zone.

Laser-assisted drug delivery: past to present

First developed in 1987, laser-assisted drug delivery (LADD) was initially performed using fully ablative lasers capable of removing the upper layers of the skin en face.[49] Fractional photothermolysis was introduced in 2004, utilizing an array of laser microbeams to generate localized columns of thermal injury while sparing surrounding skin.[50] These early fractional lasers emitted light at nonablative wavelengths, creating nonspecific tissue coagulation below an intact SC.[51] With the subsequent development of ablative fractional lasers (AFLs) in 2007, the ability to create ablated laser channels through the skin's surface became possible.[11,52] The pretreatment of skin with AFL as a means to enhance topical drug uptake was later reported in 2009, introducing the concept of AFL-assisted drug delivery.[11]

Ablative fractional lasers

As a drug-delivery technique, AFL offers the feature of quick, sterile treatment for large skin areas, with controlled and relatively predictable tissue responses.[53,54] The most commonly applied ablative fractional devices comprise the carbon dioxide (CO_2; λ = 10,600 nm) and erbium-doped yttrium aluminum garnet laser (Er:YAG: λ = 2940 nm), both of which operate in the absorption spectrum of water (>1000 nm). Depending on pulse energy and wavelength, residual thermal damage may vary; CO_2 lasers create greater residual thermal damage than Er:YAG lasers because of lower water absorbance at 10,600 nm (800 cm^{-1})[55] compared with 2940 nm (12,800 cm^{-1}).[56] The histological response to CO_2 laser exposure is illustrated in Figure 1.

Ablative fractional laser-assisted drug delivery: theoretical concepts

A simplified way to characterize AFL's impact on drug delivery can be made using Fick's first law, which describes the passive diffusion of a substance through a homogenous planar medium. Assuming steady-state conditions of stable drug concentrations in vehicle and negligible concentrations at the bottom of the dermal layer, flux (J) will remain constant, as illustrated by the equation $J = D \times K\frac{\Delta C}{L}$. Drug delivery will then depend on four parameters: (2) drug diffusivity in the skin (D), (2) partition coefficient between the vehicle and skin (K), (3) concentration gradient ($\mathbf{\Delta C}$), and (4) distance of diffusion (L).[57] Upon fractional removal of the SC by AFL, direct access to underlying hydrophilic, viable epidermal and dermal layers is achieved. Partitioning (K) to these skin compartments by hydrophilic compounds in particular, is thereby improved. These underlying skin layers additionally exhibit higher general drug diffusivity (D) than the SC, resulting in a rapid, more extensive drug distribution around the AFL channels. By taking advantage of the full length of laser channels, diffusion distance (L) is further minimized, theoretically aiding delivery to deeper skin layers.

Ablative fractional laser-assisted drug delivery: laser settings

AFL-assisted drug delivery has the potential to advance topical dermatological therapy by improved cutaneous uptake and treatment efficacy, expanding the number of deliverable drugs, targeting drug deposition to specific skin layers, and modulating the drug delivery rate. In AFL-assisted delivery, the two factors of primary importance are laser channel depth and density. Mainly determined by pulse energy, channel depth relates to how deeply ablated laser channels extend into the skin. Density, on the other hand, represents the total surface area of ablated tissue and depends on both laser spot size and channel number per unit skin area. By modifying these main parameters, total drug amount as well as delivery rate can be increased, with the potential for improved efficacy and shortened incubation times.

In theory, laser channel depth can be regulated to target specific skin compartments. In studies examining the relationship between channel depth and drug deposition, however, results are incongruous. Channel depth-dependent uptake is described for hydrophilic drugs, eg, 5-fluorouracil (5-FU) (logP −0.89),[10] methotrexate (MTX; logP −1.85),[58] and polyethylene glycols (logP <0).[59] In contrast, intracutaneous delivery of compounds of greater hydrophobicity, including lidocaine (logP 2.44),[12] ingenol mebutate (logP 2.5),[60] and imiquimod (logP 2.7),[59] does not appear to be enhanced by increasing laser channel depth. This discrepancy may be explained by differences in the drug's hydrophilicity/hydrophobicity ie, degrees of partitioning from vehicle into the medium-occupying channels shortly after laser treatment (thought to be interstitial fluid or fibrin), as well as from the medium to surrounding tissue.[39,61] Taking advantage of channel depth to increase total accumulation may thus rely on the properties of the individual drug, and various methods to overcome differences in solubility by actively filling channels are currently under investigation.[39,62,63]

Like channel depth, drug delivery can similarly be modified by adjusting laser channel density. In accordance, increasing laser channel width and number will enhance cutaneous drug deposition until saturation, after which point additional gains in uptake subside. The relationship between laser density and drug uptake is best described for methyl aminolevulinate (MAL), where densities of up to 5% coverage result in increasing skin deposition. The use of densities higher than 5%, however, is not associated with further enhancement in delivery.[64] MAL diffuses up to 1.5

mm beyond individual laser channels, explaining why low laser densities may suffice.[11] Corresponding findings are reported for other low molecular weight drugs, including ingenol mebutate (431 Da),[60] diclofenac (296 Da),[65] and tretinoin (300 Da),[66] as well as the macromolecule hepatitis B surface antigen (HBsAg; 23-27 kDa).[67] Thus, the use of laser densities much greater than 5% may be unwarranted for AFL-assisted drug delivery, although more information is needed on the parameter's impact on individual drug diffusion patterns.[60,68,69]

While the importance of laser channel depth and density is well described, other aspects that may merit consideration during AFL-assisted drug delivery include drug vehicle type, timing of topical drug application, and the extent of laser-mediated thermal damage.[70-72] Termed the laser channel coagulation zone (CZ), AFLs induce varying degrees of damage to the tissue immediately surrounding channels (Figure 1). The impact of CZ thickness on the rate and extent of cutaneous drug delivery constitutes an area of ongoing investigation, and preliminary results indicate diffusion to be delayed by CZs as compared with normal skin.[72] Future studies will determine whether the CZ can be used to modulate drug delivery.

Ablative fractional laser-assisted drug delivery: time-related uptake

AFL induces a wound-healing response, which may influence the duration of enhanced drug delivery. Laser channels heal quickly without scarring, potentially due to AFL-induced thermal damage, causing an inherent stress response with up-regulated cell-protective and protein-stabilizing proteins such as heat shock proteins.[73] Keratinocytes migrate into the wound shortly after laser treatment, and defects are reepithelialized within the first 48 hours.[48,74] Rekeratinization, measured by transepidermal water loss, is complete around day 4, with channels clinically resolved by 14 days.[65,75] The first LADD study to examine the window for optimal drug delivery noted enhanced skin deposition following drug application up to 6 hours after laser treatment.[70] Maximum uptake was achieved when application took place within the first 30 minutes of laser exposure, while no enhancement was observed 24 hours after AFL.[70] Going forward, the specifics of time-related uptake of individual drugs warrant further investigation.

Ablative fractional laser-assisted drug delivery: basics to clinical application

The vast majority of compounds previously studied show successfully enhanced cutaneous deposition following AFL-assisted drug delivery.[76] Investigated drugs range in molecular weight from 130 to 27,000 Da and display significant variation in water solubility, charge, and overall polarity. In preclinical settings, examined compounds include ALA, MAL,[11,64,77-81] 5-FU,[10] imiquimod,[59] ingenol mebutate,[60] diclofenac,[65] methotrexate,[13,58] cisplatin,[82] prednisone,[83] tranexamic acid,[84] tretinoin,[66] tetracycline,[66] ascorbic acid,[85,86] lidocaine,[12,69] minoxidil,[87] sulconazole nitrate,[88] hGH,[47] diphencyprone,[87] small interfering RNA,[89] HBsAg,[67] ovalbumin-containing liposomes,[90,91] and polymeric microparticles containing triamcinolone acetonide.[92]

In the clinical setting, AFL-assisted drug delivery has been associated with enhanced therapeutic efficacy for a range of cutaneous disorders, including actinic keratosis (AK),[93-100] actinic cheilitis,[101,102] nonmelanoma skin cancer (NMSC),[103-109] scars,[110-116] rhytides, photoaging and dyspigmentation,[63,117,118] onychomycosis,[88,119-121] warts,[122] hemangiomas,[123] vitiligo,[116,124-129] and for topical anesthetic treatment.[130-132] An overview of findings related to the most frequently described indications is summarized below.

Dysplastic lesions

Photodynamic therapy

The best evidence on AFL-assisted drug delivery is available for topical photodynamic therapy (PDT) for AKs (Figure 2), where studies confirm improved efficacy as compared to conventional PDT with ALA or MAL alone. AK clearance rates in randomized controlled trials thus range from 87% to 92% for AFL-assisted PDT, compared with 61% to 67% for PDT at 3-month follow-up.[93,96,97] For other dysplastic lesions, advantages of AFL-assisted PDT versus conventional PDT are similarly noted, with clearance rates of 85% versus 29% and 79% versus 45% for actinic cheilitis and Bowen Disease (BD), respectively.[101,109]

In addition to improved efficacy, prolonged remission is described after AFL-assisted PDT, with lower reported AK recurrence rates of 8% to 10% versus 22% to 27% for conventional treatment at 12-month follow-up.[76] Shortened PDT incubation times also appear possible when combined with AFL. Thus, AK clearance rates after AFL-assisted PDT using 2- (77%)[96] and 1.5-hour (71.4%)[98] incubations have been found comparable to standard 3-hour PDT alone (64.7%-66%). Recently, ultrashort incubation times of 15 and 30 minutes following AFL-assisted ALA treatment were further associated with 86% to 90% AK clearance, compared with the notably lower efficacy of 69% to 71% after 8 weeks without AFL delivery.[99]

Particularly for immunocompromised patients and individuals with fields of severe actinic damage, AFL-assisted drug delivery in combination with both conventional or daylight PDT may provide a more potent treatment strategy, requiring fewer courses than PDT alone.[94,95]

In contrast with AK treatment, clinical studies investigating AFL-assisted PDT for NMSC are few and present more varied results. A study on nodular basal cell carcinoma (nBCC) initially found 12-month clearance rates of 93% and 82% after AFL- and

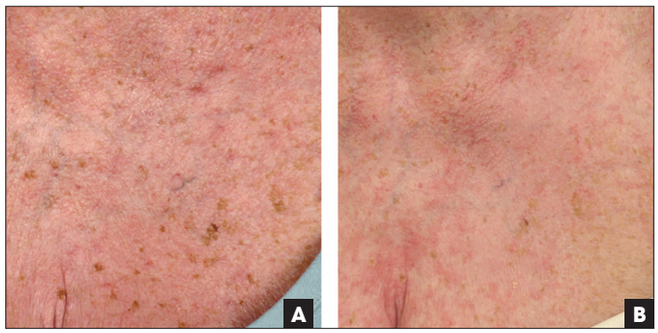

FIGURE 2. Patient with photodamage and multiple AKs treated with AFL-assisted daylight PDT using topical MAL cream. **(A)** Clinical appearance prior to AFL-assisted PDT treatment. **(B)** Treatment outcome after a single 2940-nm fractional Erbium YAG Er:YAG laser treatment using 5-10 mJ/pulse and 2.4% density, followed by MAL application and daylight exposure for 2 hours. AFL, ablative fractional laser; AKs, actinic keratoses; MAL, methyl aminolevulinate; PDT, photodynamic therapy.

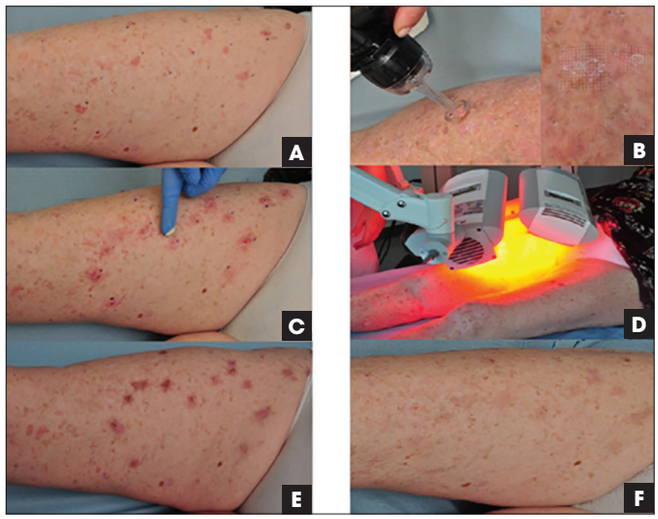

FIGURE 3. A 72-year-old woman with multiple AKs on her thigh receives targeted AFL-assisted PDT using topical MAL cream. **(A)** Prior to PDT treatment. **(B)** During fractional CO_2 laser exposure of AKs at 20-40 mJ/microbeam, depending on degree of hyperkeratosis. Close-up illustration of laser grid at 5% density. **(C)** Topical application of MAL cream on AK lesions, left under occlusion for 3 hours. **(D)** Illumination of treatment area by using a red light source (630 nm, 37 J/cm^2, 8 minutes). **(E)** Local skin reactions demonstrated at 14 days post treatment. **(F)** Treatment effect demonstrated at 10 weeks post treatment. AFL, ablative fractional laser; AK, actinic keratosis; MAL, methyl aminolevulinate; PDT, photodynamic therapy.

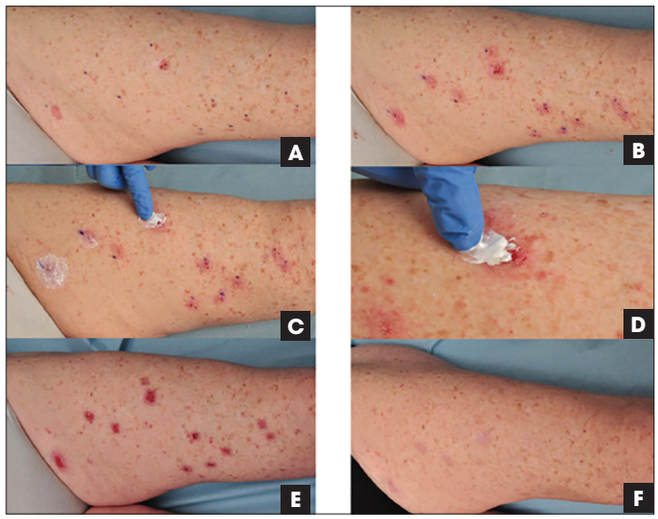

FIGURE 4. A 72-year-old woman with multiple AKs on her thigh receives targeted AFL-assisted treatment with 5% 5-FU cream. **(A)** Prior to treatment. **(B)** After fractional CO_2 laser exposure of AKs at 5% density. **(C)** and **(D)** Topical application of 5-FU cream on AK lesions, left under occlusion for 5 days. **(E)** Local skin reactions demonstrated at 14 days post treatment. **(F)** Treatment effect demonstrated at 10 weeks post treatment. AFL, ablative fractional laser; AKs, actinic keratoses; 5-FU, 5-Fluorouracil.

curettage-assisted MAL-PDT, respectively.[103] A subsequent study comparing two courses of AFL-assisted PDT with conventional PDT for high-risk nBCC conversely found no difference between AFL- and conventional PDT, with histological cure rates of 63% and 56% at 12 months.[104] More recently, differences in treatment efficacy were again shown for thin nBCCs, with complete response rates of 79% versus 22% following a single AFL-PDT session, compared to two sessions of PDT with no skin pretreatment at 12 months, respectively.[105] The same authors also reported superior efficacy using AFL-assisted PDT for microinvasive squamous cell carcinoma, observing a 68% complete response at 24 months post-treatment versus 14% for PDT performed without skin pretreatment.[106] Overall, however, given the limited evidence presently available as well as the potential for insufficient efficacy, AFL-assisted PDT may require further improvement to warrant recommendation for NMSC lesions.

5-Fluorouracil

AFL-assisted delivery of the topical chemotherapeutic agent 5-FU has previously been investigated for BD and superficial basal cell carcinoma (sBCC).[107,108,133] Following a single laser treatment with subsequent application of 5-FU under occlusion for 7 days, histological clearance rates of 92% for BD and 67% for sBCC were noted at 9 months after treatment.[108] Like AFL-assisted PDT, however, more studies examining treatment efficacy for dysplastic cutaneous lesions are needed before clinical implementation of topical delivery of 5-FU is justified. To conclude, a stepwise illustration of AFL-assisted PDT by using MAL (Figure 3) and 5-FU (Figure 4) for the treatment of AK lesions is provided.

Scars

Preliminary evidence on AFL-assisted steroid delivery for scars appears encouraging. For keloids, an initial retrospective study found clinical improvement in scar appearance following topical AFL delivery of betamethasone, although recurrences were commonly noted.[110] More recently, a prospective split-scar study found that four treatment sessions with AFL-assisted topical desoximethasone offered similar clinical outcomes and comparable patient-reported satisfaction versus intralesional triamcinolone acetonide injection, while causing significantly less treatment-related pain.[113] Notwithstanding, additional prospective studies are still necessary before AFL-assisted drug delivery's efficacy for keloid treatment can be definitively established.

The treatment of hypertrophic and atrophic scars using AFL-assisted drug delivery is also reported in the literature. For hypertrophic scars, improved appearance with superior scar texture and reduced hypertrophy and dyschromia are noted after AFL-assisted triamcinolone acetonide (average improvement 2.73, 0-3 scale), and the combination of AFL with stem cell therapy for the same indication is forthcoming.[111,114] For atrophic scars, topical AFL delivery of poly-L-lactic acid provides a reported average clinical enhancement of 2.18 (scale 0-3),[112] while improvement following AFL+ autologous platelet rich plasma has been found comparable to intralesional injection for the same indication.[115] AFL-assisted drug delivery thus appears to offer clinical benefit for a multitude of scar types. Nevertheless, randomized controlled trials are needed before broader recommendations on scar treatment can be made.

Anesthetics

When applied prior to topical anesthetics, AFL is reported to provide enhanced pain reduction as compared with topical anesthetics alone.[130-132] Offering further indication that vehicle type impacts

AFL-assisted drug delivery, a study described greater reduction in pain using liquid articaine hydrochloride + epinephrine solution, as opposed to lidocaine + prilocaine cream formulations.[131]

Onychomycosis

Preliminary evidence on the usefulness of AFL-assisted drug delivery in the context of clearing cutaneous infections has now been established.[88,119-122,134,135] For onychomycosis, AFL-assisted topical amorolfine led to a 50% clinical and mycological cure rate for *Trichophyton rubrum*-, *T. mentagrophytes*-, and *Epidermophyton floccosum*-infected nail plates at 12 weeks after 3 treatment sessions.[119] In combination with terbinafine, AFL-assisted drug delivery also resulted in a 92% negative culture rate at 3 months and an 80% cure rate at 6 months after 3 treatment sessions.[120] Finally, in a randomized clinical trial of 60 patients treated with AFL-assisted luliconazole, clinical and mycological cure rates were 70% and 57% respectively after 6 months, as compared with 51% and 39% by using laser alone.[121] AFL-assisted drug delivery of antimycotic drugs thus appears to be a promising new treatment alternative for onychomycosis.

Aesthetics

In the field of aesthetic medicine, AFL-assisted drug delivery provides not only a new administration strategy but also the potential for enhanced therapeutic outcomes for aesthetic and antiaging agents.[63,117,118,136] An initial indication of its applicability, topical AFL-assisted botulinum toxin A delivery for periorbital rhytides was associated with superior clinical efficacy as compared with AFL-assisted drug delivery of normal saline, which in turn showed no significant change from baseline following 2 treatment sessions.[117] Topical drug delivery has also been examined in combination with AFL resurfacing, for which stepwise application of cosmeceutical formulations is reported to provide both 69% enhancement on the global aesthetic improvement scale as well as a reduction in pigmentation, fine lines, wrinkles, and overall aging after 6 months.[118] A similar study exploring AFL-assisted drug delivery of the antiaging and antipigment agents correspondingly noted reduced rhytide severity (3.25 to 2.60 on a 4-point scale), lower degrees of redness and pigmentation, and an increased lightness in patients with photoaging, dyschromia, and acne scarring.[63] Finally, the short-term benefits of AFL-assisted drug delivery on postprocedural healing following AFL resurfacing are also described, and beginning immediately after laser exposure, daily application of vitamin C, E, and ferulic acid serum has been correlated with more rapid healing versus AFL and vehicle alone.[136] While encouraging, AFL-assisted drug delivery within the field of aesthetic medicine still remains new, and future studies examining both the technique's therapeutic potential and safety for this indication are necessary.

Ablative fractional laser-assisted drug delivery: comparison to other physical enhancement techniques

In parallel with the introduction and refinement of other physical enhancement techniques, studies comparing their relative effects on drug uptake, therapeutic efficacy, and safety versus AFL are increasingly relevant. A first head-to-head comparison of AFL, nonablative fractional laser (NAFL), curettage, microdermabrasion, and microneedling was recently reported for MAL in a clinical randomized controlled trial.[137] Study results revealed that AFL, microdermabrasion, microneedling, and curettage all led to enhanced protoporphyrin IX (PPIX) accumulation and PDT reactions in normal skin, while NAFL pretreatment did not improve drug uptake when compared to MAL alone. Notably, pretreatment with AFL was associated with the highest and most uniform PPIX deposition as well as more intensified local skin reactions. Microdermabrasion, microneedling, and curettage were meanwhile found comparable in both respects, despite differing physical impacts on skin (Table).[137]

In diseased skin, the relative potential of the aforementioned physical enhancement techniques remains to be systemically examined. A well-established and common pretreatment, curettage is inexpensive and simple to perform. However, the technique's effect on topical treatment outcomes appears inconsistent, potentially due to a high degree of operator dependence.[138] Furthermore, a direct comparison with AFL-assisted drug delivery has yet to be made in diseased skin, although previous reports postulate that curettage-associated oozing and/or bleeding may, for some indications, compromise subsequent uptake of topically applied drugs.[103,105]

Increasingly, microneedling is being implemented as a novel means of cutaneous drug delivery. While preclinical trials have demonstrated lower uptake with microneedle pretreatment as compared with AFL,[71,137] no studies have evaluated the modality's clinical treatment efficacy relative to laser delivery. On the other hand, direct comparison with curettage investigated in AK patients in a small, split-face study of 10 participants found similar clearance rates using both microneedle- and curettage-assisted PDT.[139] Going forward, additional clinical studies in diseased skin are desirable before conclusions concerning the efficacy of microneedles versus AFL pretreatment can be drawn.

Microdermabrasion is another new physical pretreatment strategy shown to enhance uptake of topical drugs. The modality employs impingement of microparticles from an abrasive pad or tip over the skin's surface.[20,21] Like curettage, however, the technique is operator dependent, and a randomized side-by-side trial in severely photodamaged AK patients recently demonstrated an enhanced impact of AFL-assisted daylight PDT compared with pretreatment with electrode pad microdermabrasion, noting intensified, AFL-mediated local skin reactions, improved AK clearance rates and cosmetic outcomes, and superior patient preference.[140]

In normal skin, NAFL seems less effective than AFL in enhancing topical drug uptake as NAFL-assisted MAL delivery has been found comparable to the application of MAL alone.[137] Nevertheless, whether this finding translates into lower treatment efficacy of NAFL-assisted drug delivery has yet to be investigated in clinical trials, and additional studies are needed before NAFL can be discounted as a useful enhancement strategy for dermatological application.

Overall, individual physical pretreatment strategies present with their unique advantages and drawbacks. Easily accessible and associated with relatively mild skin reactions, curettage, microdermabrasion, and microneedling are nevertheless highly user dependent and may prove less effective in enhancing drug uptake than AFL in diseased skin. In contrast, laser procedures including AFL require costly equipment and are potentially associated with greater risks of adverse events.[93,94] Still, the potential for highly

customizable, operator-independent, and predictable laser-tissue interactions is a compelling argument for the continued application and development of AFL-assisted drug delivery.

Safety of ablative fractional laser-assisted drug delivery

Topical AFL-assisted drug delivery remains a relatively new technique, and safety profiles in combination with individual drugs are inadequately explored. AFL-assisted drug delivery causes a breach in the skin's natural barrier, resulting in access not only to the viable cutis but also underlying vascular plexuses.[12] Thus, the introduction of pathogens or unsterile drug formulation components represents legitimate concerns associated with the modality.[12,93] Enhanced laser-mediated drug diffusion to proximal vascular structures may additionally increase the risk of systemic toxicity, particularly when treating large skin surface areas. Mounting evidence also suggests that local skin responses can be aggravated as a result of the combined action of laser pretreatment and topical drugs.[93,94] Thus, adverse events, including intensified drug side effects, infection, systemic toxicity, and hypersensitivity reactions all constitute potentially undesirable consequences of disrupting the cutaneous barrier. AFL-assisted drug delivery should therefore be performed with caution and be limited only to well-controlled settings. The use of drug doses no higher than what would be suitable for local injection is furthermore advisable. Providers must be aware of known, laser-related side effects, signs of infection, systemic toxicity, hypersensitivity, and the potential for not previously described adverse events. Ultimately, more clinical studies are, at present, needed to establish AFL-assisted drug delivery's safety profile in combination with individual drugs.

Ablative fractional laser-assisted drug delivery: future perspectives

Fractional LADD has until now shown significant promise as a means to enhance cutaneous uptake of topical drugs, with the ultimate goal of improved clinical outcomes. Perspectives of AFL-assisted drug delivery further include significantly shortened treatment durations and incubation times as well as the replacement of cumbersome, patient-dependent treatment schedules with convenient, in-office procedures. Beyond the benefits for existing regimens, topical application of systemic drugs not previously deliverable through skin, including the antiproliferative agents methotrexate and cisplatin, is also possible, paving the way for new pharmaceutical options and administration routes for the management of cutaneous disorders.[13,82] The combination of AFL-assisted drug delivery with other enhancement techniques, such as iontophoresis and electroporation, as well as chemical enhancers, including liposomes and nanoparticles, is increasingly used to enhance and accelerate drug uptake in order to provide more potent treatment strategies.[90,91,141] More sophisticated visualization of AFL-assisted drug delivery and its clinical effects is also possible with the concurrent development of noninvasive, real-time imaging techniques such as optical coherence tomography and confocal reflectance microscopy.[70,88] Finally, in the arena of transdermal delivery, AFL pretreatment has successfully been applied for a range of exciting new applications, including the use of antibodies, vaccine antigens, nucleic acids, allergens, growth factors, scaffold materials, and cells.[48,67,89,142-147] While still in its infancy, AFL-assisted drug delivery thus represents a promising, widely applicable and minimally invasive delivery system useful in dermatology and beyond.

References

1. Erlendsson AM, Wenande E, Haedersdal M. Transepidermal Drug Delivery: Overview, Concept, and Applications. In: Issa MCA, Tamura B, eds. *Lasers, Lights and Other Technologies, Clinical Approaches and Procedures in Cosmetic Dermatology*. Switzerland: Springer International Publishing AG; 2016.
2. Elias PM, Menon GK. Structural and lipid biochemical correlates of the epidermal permeability barrier. *Adv Lipid Res*. 1991;24:1-26.
3. Surber C, Davis AF. Bioavailability and Bioequivalence Dermatological Formulations. In: Walters KA, ed. *Dermatological and Transdermal Formulations*. Vol. 119. Boca Raton, FL: CRC Press; 2002:401-474.
4. Morrow DI, Garland MJ, McCarron PA, Woolfson AD, Donnelly RF. Innovative drug delivery strategies for topical photodynamic therapy using porphyrin precursors. *J Environ Pathol Toxicol Oncol*. 2007;26(2):105-116.
5. Govil S. Transdermal drug delivery systems. In: Tyle P, ed. *Drug Delivery Devices: Fundamentals and Applications*. 1st ed. New York: Marcel Dekker; 1988:385-420.
6. Pathan IB, Setty CM. Chemical Penetration Enhancers for Transdermal Drug Delivery Systems. *Trop J Pharm Res*. 2009;8(2):173-179. http://dx.doi.org/10.4314/tjpr.v8i2.44527.
7. Brown MB, Martin GP, Jones SA, Akomeah FK. Dermal and transdermal drug delivery systems: current and future prospects. *Drug Deliv*. 2006;13(3):175-187. https://doi.org/10.1080/10717540500455975.
8. Benson HA. Transdermal drug delivery: penetration enhancement techniques. *Curr Drug Deliv*. 2005;2(1):23-33.
9. Paudel KS, Milewski M, Swadley CL, Brogden NK, Ghosh P, Stinchcomb AL. Challenges and opportunities in dermal/transdermal delivery. *Ther Deliv*. 2010;1(1):109-131.
10. Wenande E, Olesen UH, Nielsen MM, et al. Fractional laser-assisted topical delivery leads to enhanced, accelerated and deeper cutaneous 5-fluorouracil uptake. *Expert Opin Drug Deliv*. 2017;14(3):307-317. https://doi.org/10.1080/17425247.2017.1260119.
11. Haedersdal M, Sakamoto FH, Farinelli WA, Doukas AG, Tam J, Anderson RR. Fractional CO(2) laser-assisted drug delivery. *Lasers Surg Med*. 2010;42(2):113-122. https://doi.org/10.1002/lsm.20860.
12. Oni G, Brown SA, Kenkel JM. Can fractional lasers enhance transdermal absorption of topical lidocaine in an in vivo animal model? *Lasers Surg Med*. 2012;44(2):168-174. https://doi.org/10.1002/lsm.21130.
13. Taudorf EH, Lerche CM, Vissing AC, et al. Topically applied methotrexate is rapidly delivered into skin by fractional laser ablation. *Expert Opin Drug Deliv*. 2015;12(7):1059-1069. https://doi.org/10.1517/17425247.2015.1031216.
14. Ruiz-Rodriguez R, López L, Candelas D, Zelickson B. Enhanced efficacy of photodynamic therapy after fractional resurfacing: fractional photodynamic rejuvenation. *J Drugs Dermatol*. 2007;6(8):818-820.
15. Lim HK, Jeong KH, Kim NI, Shin MK. Nonablative fractional laser as a tool to facilitate skin penetration of 5-aminolaevulinic acid with minimal skin disruption: a preliminary study. *Br J Dermatol*. 2014;170(6):1336-1340. https://doi.org/10.1111/bjd.12817.
16. Prens SP, de Vries K, Neumann HA, Prens EP. Non-ablative fractional resurfacing in combination with topical tretinoin cream as a field treatment modality for multiple actinic keratosis: a pilot study and a review of other field treatment modalities. *J Dermatolog Treat*. 2013;24(3):227-231. https://doi.org/10.3109/09546634.2012.687088.
17. Fan X, Yin Y, Wang S, et al. Clinical Assessment of the Safety and Effectiveness of Nonablative Fractional Laser Combined with Transdermal Delivery of Botulinum Toxin A in Treating Periocular Wrinkles. *Plast Reconstr Surg Glob Open*. 2016;4(8):e1004. https://doi.org/10.1097/GOX.0000000000001004.
18. Chitvanich S, Rerknimitr P, Panchaprateep R, Pongprutthipan M, Asawanonda P. Combination of non-ablative fractional photothermolysis and 0.1% tacrolimus ointment is efficacious for treating idiopathic guttate hypomelanosis. *J Dermatolog Treat*. 2016;27(5):456-460. https://doi.org/10.3109/09546634.2015.
19. Wolfshohl JA, Geddes ER, Stout AB, Friedman PM. Improvement of erythema dyschromicum perstans using a combination of the 1,550-nm erbium-doped fractionated laser and topical tacrolimus ointment. *Lasers Surg Med*. 2017;49(1):60-62. https://doi.org/10.1002/lsm.22567.
20. Lee WR, Tsai RY, Fang CL, Liu CJ, Hu CH, Fang JY. Microdermabrasion as a Novel Tool to Enhance Drug Delivery via the Skin: An Animal Study. *Dermatol Surg*. 2006;32(8):1013-1022. https://doi.org/10.1111/j.1524-4725.2006.32224.x.
21. Lee WR, Shen SC, Kuo-Hsien W, Hu CH, Fang JY. Lasers and microdermabrasion enhance and control topical delivery of vitamin C. *J Invest Dermatol*. 2003;121(5):1118-1125. https://doi.org/10.1046/j.1523-1747.2003.12537.x.
22. Fang JY, Lee WR, Shen SC, Fang YP, Hu CH. Enhancement of topical 5-amino-

laevulinic acid delivery by erbium:YAG laser and microdermabrasion: a comparison with iontophoresis and electroporation. *Br J Dermatol*. 2004;151(1):132-140. https://doi.org/10.1111/j.1365-2133.2004.06051.x.
23. Andrews S, Lee JW, Choi SO, Prausnitz MR. Transdermal insulin delivery using microdermabrasion. *Pharm Res*. 2011;28(9):2110-2118. https://doi.org/10.1007/s11095-011-0435-4.
24. You SK, Noh YW, Park HH, et al. Effect of applying modes of the polymer microneedle-roller on the permeation of L-ascorbic acid in rats. *J Drug Target*. 2010;18(1):15-20. https://doi.org/10.3109/10611860903115274.
25. Mikolajewska P, Donnelly RF, Garland MJ, et al. Microneedle pre-treatment of human skin improves 5-aminolevulininc acid (ALA)- and 5-aminolevulinic acid methyl ester (MAL)-induced PpIX production for topical photodynamic therapy without increase in pain or erythema. *Pharm Res*. 2010;27(10):2213-2220. https://doi.org/10.1007/s11095-010-0227-2.
26. Kim JH, Park HY, Jung M, Choi EH. Automicroneedle therapy system combined with topical tretinoin shows better regenerative effects compared with each individual treatment. *Clin Exp Dermatol*. 2013;38(1):57-65. https://10.1111/j.1365-2230.2012.04405.x.
27. Fukushima K, Ise A, Morita H, et al. Two-layered dissolving microneedles for percutaneous delivery of peptide/protein drugs in rats. *Pharm Res*. 2011;28(1):7-21. https://doi.org/10.1007/s11095-010-0097-7.
28. Christensen E, Mørk C, Foss OA. Pre-treatment deep curettage can significantly reduce tumour thickness in thick Basal cell carcinoma while maintaining a favourable cosmetic outcome when used in combination with topical photodynamic therapy. *J Skin Cancer*. 2011;2011:240340. https://doi.org/10.1155/2011/240340.
29. Jambusaria-Pahlajani A, Ortman S, Schmults CD, Liang C. Sequential Curettage, 5-Fluorouracil, and Photodynamic Therapy for Field Cancerization of the Scalp and Face in Solid Organ Transplant Recipients. *Dermatol Surg*. 2016;42 Suppl 1:S66-S72. https://doi.org/10.1097/DSS.0000000000000589.
30. Zeichner JA, Patel RV, Birge MB. Treatment of Basal cell carcinoma with curettage followed by imiquimod 3.75% cream. *J Clin Aesthet Dermatol*. 2011;4(5):39-43.
31. Marro D, Kalia YN, Delgado-Charro MB, Guy RH. Optimizing iontophoretic drug delivery: identification and distribution of the charge-carrying species. *Pharm Res*. 2001;18(12):1709-1713.
32. Alvarez-Figueroa MJ, Blanco-Méndez J. Transdermal delivery of methotrexate: iontophoretic delivery from hydrogels and passive delivery from microemulsions. *Int J Pharm*. 2001;215(1-2):57-65.
33. Pacini S, Gulisano M, Punzi T, Ruggiero M. Transdermal delivery of Clostridium botulinum toxin type A by pulsed current iontophoresis. J Am Acad Dermatol. 2007;57(6):1097-1099. https://doi.org/10.1016/j.jaad.2007.08.037.
34. Lee WR, Shen SC, Fang CL, Zhuo RZ, Fang JY. Topical delivery of methotrexate via skin pretreated with physical enhancement techniques: low-fluence erbium:YAG laser and electroporation. *Lasers Surg Med*. 2008;40(7):468-476. https://doi.org/10.1002/lsm.20655.
35. Gothelf A, Mir LM, Gehl J. Electrochemotherapy: results of cancer treatment using enhanced delivery of bleomycin by electroporation. *Cancer Treat Rev*. 2003;29(5):371-387.
36. Sardesai NY, Weiner DB. Electroporation delivery of DNA vaccines: prospects for success. *Curr Opin Immunol*. 2011;23(3):421-429. https://doi.org/10.1016/j.coi.2011.03.008.
37. Doukas AG, Kollias N. Transdermal drug delivery with a pressure wave. *Adv Drug Deliv Rev*. 2004;56(5):559-579. https://doi.org/10.1016/j.addr.2003.10.031.
38. Treffel P, Panisset F, Humbert P, Remoussenard O, Bechtel Y, Agache P. Effect of pressure on in vitro percutaneous absorption of caffeine. *Acta Derm Venereol*. 1993;73(3):200-202.
39. Erlendsson AM, Doukas AG, Farinelli WA, Bhayana B, Anderson RR, Haedersdal M. Fractional laser-assisted drug delivery: Active filling of laser channels with pressure and vacuum alteration. *Lasers Surg Med*. 2016;48(2):116-124. https://doi.org/10.1002/lsm.22374.
40. Park JM, Jeong KH, Bae MI, Lee SJ, Kim NI, Shin MK. Fractional radiofrequency combined with sonophoresis to facilitate skin penetration of 5-aminolevulinic acid. *Lasers Med Sci*. 2016;31(1):113-118. https://doi.org/10.1007/s10103-015-1835-1.
41. Levin G, Gershonowitz A, Sacks H, et al. Transdermal delivery of human growth hormone through RF-microchannels. *Pharm Res*. 2005;22(4):550-555. https://doi.org/ 10.1007/s11095-005-2498-6.
42. Krishnan G, Grice JE, Roberts MS, Benson HAE, Prow TW. Enhanced sonophoretic delivery of 5-aminolevulinic acid: preliminary human ex vivo permeation data. *Skin Res Technol*. 2013;19(1):e283-e289. https://doi.org/10.1111/j.1600-0846.2012.00640.x.
43. Rosim GC, Barbieri CH, Lanças FM, Mazzer N. Diclofenac phonophoresis in human volunteers. *Ultrasound Med Biol*. 2005;31(3):337-343. https://doi.org/10.1016/j.ultrasmedbio.2004.11.012.
44. Griffin JE, Echternach JL, Price RE, Touchstone JC. Patients treated with ultrasonic driven hydrocortisone and with ultrasound alone. *Phys Ther*. 1967;47(7):594-601.
45. Mitragotri S, Blankschtein D, Langer R. Ultrasound-mediated transdermal protein delivery. *Science*. 1995;269(5225):850-853.
46. Ameri M, Kadkhodayan M, Nguyen J, et al. Human Growth Hormone Delivery with a Microneedle Transdermal System: Preclinical Formulation, Stability, Delivery and PK of Therapeutically Relevant Doses. *Pharmaceutics*. 2014;6(2):220-234. https://doi.org/10.3390/pharmaceutics6020220.
47. Song Y, Hemmady K, Puri A, Banga AK. Transdermal delivery of human growth hormone via laser-generated micropores [published online March 20, 2017]. *Drug Deliv Transl Res*. 2017. https://doi.org/10.1007/s13346-017-0370-y
48. Oni G, Lequeux C, Cho MJ, et al. Transdermal delivery of adipocyte-derived stem cells using a fractional ablative laser. *Aesthet Surg J*. 2013;33(1):109-116. https://doi.org/10.1177/1090820X12469222.
49. Jacques SL, McAuliffe DJ, Blank IH, Parrish JA. Controlled removal of human stratum corneum by pulsed laser. *J Invest Dermatol*. 1987;88(1):88-93.
50. Manstein D, Herron GS, Sink RK, Tanner H, Anderson RR. Fractional photothermolysis: a new concept for cutaneous remodeling using microscopic patterns of thermal injury. *Lasers Surg Med*. 2004;34(5):426-438. https://doi.org/10.1002/lsm.20048.
51. Laubach HJ, Tannous Z, Anderson RR, Manstein D. Skin responses to fractional photothermolysis. *Lasers Surg Med*. 2006;38(2):142-149. https://doi.org/10.1002/lsm.20254.
52. Hantash BM, Bedi VP, Chan KF, Zachary CB. Ex vivo histological characterization of a novel ablative fractional resurfacing device. *Lasers Surg Med*. 2007;39(2):87-95. https://doi.org/10.1002/lsm.20405.
53. Hantash BM, Bedi VP, Kapadia B, et al. In vivo histological evaluation of a novel ablative fractional resurfacing device. *Lasers Surg Med*. 2007;39(2):96-107. https://doi.org/ 10.1002/lsm.20468.
54. Taudorf EH, Haak CS, Erlendsson AM, et al. Fractional ablative erbium YAG laser: Histological characterization of relationships between laser settings and micropore dimensions. *Lasers Surg Med*. 2014;46(4):281-289. https://doi.org/10.1002/lsm.22228.
55. Marini LG, Krunic AL. *European Handbook of Dermatological Treatments*. 3rd ed. (Katsambas A, ed.). Berlin, Heidelberg: Springer Verlag; 2015.
56. Walsh JT Jr, Deutsch TF. Er:YAG laser ablation of tissue: measurement of ablation rates. *Lasers Surg Med*. 1989;9(4):327-337.
57. Franz TJ. Kinetics of Cutaneous Drug Penetration. *Int J Dermatol*. 1983;22(9):499-505.
58. Taudorf EH, Lerche CM, Erlendsson AM, et al. Fractional laser-assisted drug delivery: Laser channel depth influences biodistribution and skin deposition of methotrexate. *Lasers Surg Med*. 2016;48(5):519-529. https://doi.org/10.1002/lsm.22484.
59. Lee WR, Shen SC, Al-Suwayeh SA, Yang HH, Yuan CY, Fang JY. Laser-assisted topical drug delivery by using a low-fluence fractional laser: imiquimod and macromolecules. *J Control Release*. 2011;153(3):240-248. https://doi.org/10.1016/j.jconrel.2011.03.015.
60. Erlendsson AM, Taudorf EH, Eriksson AH, et al. Ablative fractional laser alters biodistribution of ingenol mebutate in the skin. *Arch Dermatol Res*. 2015;307(6):512-522. https://doi.org/10.1007/s00403-015-1561-3.
61. Kositratna G, Evers M, Sajjadi A, Manstein D. Rapid fibrin plug formation within cutaneous ablative fractional CO2 laser lesions. *Lasers Surg Med*. 2016;48(2):125-132. https://doi.org/10.1002/lsm.22412.
62. Waibel JS, Rudnick A, Nousari C, Bhanusali DG. Fractional Ablative Laser Followed by Transdermal Acoustic Pressure Wave Device to Enhance the Drug Delivery of Aminolevulinic Acid: In Vivo Fluorescence Microscopy Study. *J Drugs Dermatol*. 2016;15(1):14-21.
63. Alexiades M. Randomized, Double-Blind, Split-Face Study Evaluating Fractional Ablative Erbium:YAG Laser-Mediated Trans-Epidermal Delivery of Cosmetic Actives and a Novel Acoustic Pressure Wave Ultrasound Technology for the Treatment of Skin Aging, Melasma, and Acne Scars. *J Drugs Dermatol*. 2015;14(11):1191-1198.
64. Haak CS, Christiansen K, Erlendsson AM, et al. Ablative fractional laser enhances MAL-induced PpIX accumulation: Impact of laser channel density, incubation time and drug concentration. *J Photochem Photobiol B*. 2016;159:42-48. https://doi.org/10.1016/j.jphotobiol.2016.03.021.
65. Bachhav YG, Heinrich A, Kalia YN. Using laser microporation to improve transdermal delivery of diclofenac: Increasing bioavailability and the range of therapeutic applications. *Eur J Pharm Biopharm*. 2011;78(3):408-414. https://doi.org/10.1016/j.ejpb.2011.03.006.
66. Chen WY, Fang CL, Al-Suwayeh SA, Yang HH, Li YC, Fang JY. Risk assessment of excess drug and sunscreen absorption via skin with ablative fractional laser resurfacing : optimization of the applied dose for postoperative care. *Lasers Med Sci*. 2013;28(5):1363-1374. https://doi.org/ 10.1007/s10103-012-1257-2.
67. Scheiblhofer S, Strobl A, Hoepflinger V, et al. Skin vaccination via fractional infrared laser ablation - Optimization of laser-parameters and adjuvantation. *Vaccine*. 2017;35(14):1802-1809. https://doi.org/10.1016/j.vaccine.2016.11.105.
68. Haak CS, Bhayana B, Farinelli WA, Anderson RR, Haedersdal M. The impact

of treatment density and molecular weight for fractional laser-assisted drug delivery. *J Control Release*. 2012;163(3):335-341. https://doi.org/10.1016/j.jconrel.2012.09.008.
69. Bachhav YG, Summer S, Heinrich A, Bragagna T, Böhler C, Kalia YN. Effect of controlled laser microporation on drug transport kinetics into and across the skin. *J Control Release*. 2010;146(1):31-36. https://doi.org/10.1016/j.jconrel.2010.05.025.
70. Banzhaf CA, Thaysen-Petersen D, Bay C, et al. Fractional laser-assisted drug uptake: Impact of time-related topical application to achieve enhanced delivery. *Lasers Surg Med*. 2017;49(4):348-354. https://doi.org/10.1002/lsm.22610.
71. Haak CS, Hannibal J, Paasch U, Anderson RR, Haedersdal M. Laser-induced thermal coagulation enhances skin uptake of topically applied compounds. *Lasers Surg Med*. 2017;49(6):582-591. https://doi.org/ 10.1002/lsm.22642.
72. Erlendsson AM, Karlsson E, Doukas AG, et al. Thermal damage impedes fractional laser-assisted drug delivery [abstract 62]. *Lasers Surg Med*. 2016;48(S27):22.
73. Helbig D, Bodendorf MO, Grunewald S, Kendler M, Simon JC, Paasch U. Immunohistochemical investigation of wound healing in response to fractional photothermolysis. *J Biomed Opt*. 2009;14(6):064044. https://doi.org/10.1117/1.3275479.
74. Banzhaf CA, Wind BS, Mogensen M, et al. Spatiotemporal closure of fractional laser-ablated channels imaged by optical coherence tomography and reflectance confocal microscopy. *Lasers Surg Med*. 2015;48(2):157-165.
75. Grunewald S, Bodendorf M, Illes M, Kendler M, Simon JC, Paasch U. In vivo wound healing and dermal matrix remodelling in response to fractional CO(2) laser intervention: clinicopathological correlation in non-facial skin. *Int J Hyperthermia*. 2011;27(8):811-818.
76. Haedersdal M, Erlendsson AM, Paasch U, Anderson RR. Translational medicine in the field of ablative fractional laser (AFXL)-assisted drug delivery: A critical review from basics to current clinical status. *J Am Acad Dermatol*. 2016;74(5):981-1004. https://doi.org/10.1016/j.jaad.2015.12.008.
77. Haedersdal M, Katsnelson J, Sakamoto FH, et al. Enhanced uptake and photoactivation of topical methyl aminolevulinate after fractional CO2 laser pretreatment. *Lasers Surg Med*. 2011;43(8):804-813. https://doi.org/10.1002/lsm.21096.
78. Haak CS, Farinelli WA, Tam J, Doukas AG, Anderson RR, Haedersdal M. Fractional laser-assisted delivery of methyl aminolevulinate: Impact of laser channel depth and incubation time. *Lasers Surg Med*. 2012;44(10):787-795. https://doi.org/10.1002/lsm.22102.
79. Haedersdal M, Sakamoto FH, Farinelli WA, Doukas AG, Tam J, Anderson RR. Pretreatment with ablative fractional laser changes kinetics and biodistribution of topical 5-aminolevulinic acid (ALA) and methyl aminolevulinate (MAL). *Lasers Surg Med*. 2014;46(6):462-469. https://doi.org/10.1002/lsm.22259.
80. Forster B, Klein A, Szeimies RM, Maisch T. Penetration enhancement of two topical 5-aminolaevulinic acid formulations for photodynamic therapy by erbium:YAG laser ablation of the stratum corneum: continuous versus fractional ablation. *Exp Dermatol*. 2010;19(9):806-812. https://doi.org/10.1111/j.1600-0625.2010.01093.x.
81. Huth S, Marquardt Y, Amann PM, et al. Ablative non-sequential fractional ultrapulsed CO2 laser pretreatment improves conventional photodynamic therapy with methyl aminolevulinate in a novel human in vitro 3D actinic keratosis skin model. *Exp Dermatol*. 2016;25(12):997-999. https://doi.org/10.1111/exd.13068.
82. Wenande E, Olesen UH, Boesen M, Sturup S, Gammelgaard B, Haedersdal M. Fractional laser-assisted topical delivery of the anticancer agent cisplatin [abstract 92]. *Lasers Surg Med*. 2017;49(S28):22.
83. Yu J, Bachhav Y, Summer S, et al. Using controlled laser-microporation to increase transdermal delivery of prednisone. *J Control Release*. 2010;148(1):e71-e73. https://doi.org/10.1016/j.jconrel.2010.07.032.
84. Hsiao CY, Sung HC, Hu S, Huang CH. Fractional CO2 Laser Treatment to Enhance Skin Permeation of Tranexamic Acid with Minimal Skin Disruption. *Dermatology*. 2015;230(3):269-275. https://doi.org/10.1159/000371386.
85. Hsiao CY, Huang CH, Hu S, et al. Fractional carbon dioxide laser treatment to enhance skin permeation of ascorbic acid 2-glucoside with minimal skin disruption. *Dermatol Surg*. 2012;38(8):1284-1293. https://doi.org/10.1111/j.1524-4725.2012.02454.x.
86. Hsiao CY, Sung HC, Hu S, Huang YL, Huang CH. Fractional CO2 Laser Pretreatment Facilitates Transdermal Delivery of Two Vitamin C Derivatives. *Molecules*. 2016;21(11): pii: E1547. https://doi.org/10.3390/molecules21111547.
87. Lee WR, Shen SC, Aljuffali IA, Li YC, Fang JY. Erbium-yttrium-aluminum-garnet laser irradiation ameliorates skin permeation and follicular delivery of antialopecia drugs. *J Pharm Sci*. 2014;103(11):3542-3552. https://doi.org/10.1002/jps.24143.
88. Tsai MT, Tsai TY, Shen SC, et al. Evaluation of Laser-Assisted Trans-Nail Drug Delivery with Optical Coherence Tomography. *Sensors (Basel)*. 2016;16(12): pii: E2111. https://doi.org/10.3390/s16122111.
89. Lee WR, Shen SC, Chen WY, Aljuffali IA, Suen SY, Fang JY. Noninvasive delivery of siRNA and plasmid DNA into skin by fractional ablation: erbium:YAG laser versus CO_2 laser. *Eur J Pharm Biopharm*. 2014;86(3):315-323. https://doi.org/10.1016/j.ejpb.2013.08.006.
90. Fujimoto T, Wang J, Baba K, et al. Transcutaneous drug delivery by liposomes using fractional laser technology. *Lasers Surg Med*. 2017;49(5):525-532. https://doi.org/10.1002/lsm.22616.
91. Fujimoto T, Ito M, Ito S, Kanazawa H. Fractional laser-assisted percutaneous drug delivery via temperature-responsive liposomes. *J Biomater Sci Polym Ed*. 2017;28(7):679-689. https://doi.org/10.1080/09205063.2017.1296346.
92. Singhal M, Del Río-Sancho S, Sonaje K, Kalia YN. Fractional Laser Ablation for the Cutaneous Delivery of Triamcinolone Acetonide from Cryomilled Polymeric Microparticles: Creating Intraepidermal Drug Depots. Mol Pharm. 2016;13(2):500-511. https://doi.org/10.1021/acs.molpharmaceut.5b00711.
93. Togsverd-Bo K, Haak CS, Thaysen-Petersen D, Wulf HC, Anderson RR, Hædersdal M. Intensified photodynamic therapy of actinic keratoses with fractional CO2 laser: a randomized clinical trial. Br J Dermatol. 2012;166(6):1262-1269. https://doi.org/10.1111/j.1365-2133.2012.10893.x.
94. Helsing P, Togsverd-Bo K, Veierød MB, Mørk G, Haedersdal M. Intensified fractional CO2 laser-assisted photodynamic therapy vs. laser alone for organ transplant recipients with multiple actinic keratoses and wart-like lesions: a randomized half-side comparative trial on dorsal hands. *Br J Dermatol*. 2013;169(5):1087-1092. https://doi.org/10.1111/bjd.12507.
95. Togsverd-Bo K, Lei U, Erlendsson AM, et al. Combination of ablative fractional laser and daylight-mediated photodynamic therapy for actinic keratosis in organ transplant recipients - a randomized controlled trial. *Br J Dermatol*. 2015;172(2):467-474. https://doi.org/10.1111/bjd.13222.
96. Choi SH, Kim KH, Song KH. Efficacy of ablative fractional laser-assisted photodynamic therapy with short-incubation time for the treatment of facial and scalp actinic keratosis: 12-month follow-up results of a randomized, prospective, comparative trial. *J Eur Acad Dermatol Venereol*. 2015;29(8):1598-1605. https://doi.org/10.1111/jdv.12953.
97. Ko DY, Jeon SY, Kim KH, Song KH. Fractional erbium: YAG laser-assisted photodynamic therapy for facial actinic keratoses: a randomized, comparative, prospective study. *J Eur Acad Dermatol Venereol*. 2014;28(11):1529-1539. https://doi.org/10.1111/jdv.12334.
98. Song HS, Jung SE, Jang YH, Kang HY, Lee ES, Kim YC. Fractional carbon dioxide laser-assisted photodynamic therapy for patients with actinic keratosis. *Photodermatol Photoimmunol Photomed*. 2015;31(6):296-301. https://doi.org/ 10.1111/phpp.12184.
99. Alexiades M. Randomized, Controlled Trial of Fractional Carbon Dioxide Laser Resurfacing Followed by Ultrashort Incubation Aminolevulinic Acid Blue Light Photodynamic Therapy for Actinic Keratosis. *Dermatol Surg*. 2017;43(8):1053-1064. https://doi.org/10.1097/DSS.0000000000001117.
100. Jang YH, Lee DJ, Shin J, Kang HY, Lee ES, Kim YC. Photodynamic therapy with ablative carbon dioxide fractional laser in treatment of actinic keratosis. *Ann Dermatol*. 2013;25(4):417-422. https://doi.org/10.5021/ad.2013.25.4.417.
101. Choi SH, Kim KH, Song KH. Efficacy of ablative fractional laser-assisted photodynamic therapy for the treatment of actinic cheilitis: 12-month follow-up results of a prospective, randomized, comparative trial. *Br J Dermatol*. 2015;173(1):184-191. https://doi.org/10.1111/bjd.13542.
102. Dinani N, Topham E, Derrick E, Atkinson L. Ablative fractional laser assisted photodynamic therapy for the treatment of actinic cheilitis. *Br J Dermatol*. 2015;173(1):15. https://doi.org/10.1111/bjd.13911.
103. Lippert J, Smucler R, Vlk M. Fractional carbon dioxide laser improves nodular basal cell carcinoma treatment with photodynamic therapy with methyl 5-aminolevulinate. *Dermatol Surg*. 2013;39(8):1202-1208. https://doi.org/10.1111/dsu.12242.
104. Haak C, Togsverd-Bo K, Thaysen-Petersen D, et al. Fractional laser-mediated photodynamic therapy of high-risk basal cell carcinomas – a randomized clinical trial. *Br J Dermatol*. 2015;172(1):215-222. https://doi.org/10.1111/bjd.13166.
105. Choi SH, Kim KH, Song KH. Er:YAG ablative fractional laser-primed photodynamic therapy with methyl aminolevulinate as an alternative treatment option for patients with thin nodular basal cell carcinoma: 12-month follow-up results of a randomized, prospective, comparative trial. *J Eur Acad Dermatol Venereol*. 2016;30(5):783-788. https://doi.org/10.1111/jdv.13453.
106. Choi SH, Kim KH, Song KH. Effect of Methyl Aminolevulinate Photodynamic Therapy With and Without Ablative Fractional Laser Treatment in Patients With Microinvasive Squamous Cell Carcinoma. *JAMA Dermatol*. 2017;153(3):289-295. https://doi.org/10.1001/jamadermatol.2016.4463.
107. Glenn CJ, Parlette EC, Mitchell C. Fractionated CO_2 Laser-Assisted Delivery of Topical 5-Fluorouracil as a Useful Modality for Treating Field Cutaneous Squamous Cell Carcinomas. *Dermatol Surg*. 2015;41(11):1339-1342. https://doi.org/10.1097/DSS.0000000000000473.
108. Hsu SH, Gan SD, Nguyen BT, Konnikov N, Liang CA. Ablative Fractional Laser–Assisted Topical Fluorouracil for the Treatment of Superficial Basal Cell Carcinoma and Squamous Cell Carcinoma In Situ. *Dermatol Surg*. 2016;42(9):1050-1053. https://doi.org/10.1097/DSS.0000000000000814.
109. Ko DY, Kim KH, Song KH. A randomized trial comparing methyl aminolaevulinate photodynamic therapy with and without Er:YAG ablative fractional laser treatment in Asian patients with lower extremity Bowen disease: results from a 12-month follow-up. *Br J Dermatol*. 2014;170(1):165-172. https://doi.org/10.1111/bjd.12627.

110. Cavalié M, Sillard L, Montaudié H, Bahadoran P, Lacour JP, Passeron T. Treatment of keloids with laser-assisted topical steroid delivery: a retrospective study of 23 cases. *Dermatol Ther*. 2015;28(2):74-78. https://doi.org/10.1111/dth.12187.
111. Waibel JS, Wulkan AJ, Shumaker PR. Treatment of hypertrophic scars using laser and laser assisted corticosteroid delivery. *Lasers Surg Med*. 2013;45(3):135-140. https://doi.org/10.1002/lsm.22120.
112. Rkein A, Ozog D, Waibel JS. Treatment of atrophic scars with fractionated CO2 laser facilitating delivery of topically applied poly-L-lactic acid. *Dermatol Surg*. 2014;40(6):624-631. https://doi.org/10.1111/dsu.0000000000000010.
113. Park JH, Chun JY, Lee JH. Laser-assisted topical corticosteroid delivery for the treatment of keloids. *Lasers Med Sci*. 2017;32(3):601-608. https://doi.org/10.1007/s10103-017-2154-5.
114. Waibel J, Badiavas E, Davis S, Rodriguez-Menocal L. Hypertrophic burn scars modulation through laser assisted delivery of adipose derived stem cells vs bone-marrow mesenchymal stem cells with the CO2 vs Er:YAG laser [abstract 9]. *Lasers Surg Med*. 2017;49(S28):4.
115. Gawdat HI, Hegazy RA, Fawzy MM, Fathy M. Autologous platelet rich plasma: topical versus intradermal after fractional ablative carbon dioxide laser treatment of atrophic acne scars. *Dermatol Surg*. 2014;40(2):152-161. https://doi.org/10.1111/dsu.12392.
116. Kanokrungsee S, Chanprapaph K, Chaiyabutr C, Vachiramon V. A comparative study of combined treatment with fractional carbon dioxide and targeted ultraviolet B phototherapy for facial vitiligo. *Lasers Med Sci*. 2016;31(7):1343-1349. https://doi.org/10.1007/s10103-016-1982-z.
117. Mahmoud BH, Burnett C, Ozog D. Prospective randomized controlled study to determine the effect of topical application of botulinum toxin A for crow's feet after treatment with ablative fractional CO2 laser. *Dermatol Surg*. 2015;41 Suppl 1:S75-S81. https://doi.org/10.1097/01.DSS.0000452642.83894.ab.
118. Trelles MA, Leclère FM, Martínez-Carpio PA. Fractional carbon dioxide laser and acoustic-pressure ultrasound for transepidermal delivery of cosmeceuticals: a novel method of facial rejuvenation. *Aesthetic Plast Surg*. 2013;37(5):965-972. https://doi.org/10.1007/s00266-013-0176-3.
119. Lim EH, Kim H, Park YO, et al. Toenail onychomycosis treated with a fractional carbon-dioxide laser and topical antifungal cream. *J Am Acad Dermatol*. 2014;70(5):918-923. https://doi.org/10.1016/j.jaad.2014.01.893.
120. Bhatta AK, Keyal U, Huang X, Zhao JJ. Fractional carbon-dioxide (CO2) laser-assisted topical therapy for the treatment of onychomycosis. *J Am Acad Dermatol*. 2016;74(5):916-923. https://10.1016/j.jaad.2015.12.002.
121. Zhou BR, LU Y, Permatasari F, et al. The efficacy of fractional carbon dioxide (CO2) laser combined with luliconazole 1% cream for the treatment of onychomycosis. *Medicine (Baltimore)*. 2016;95(44):e5141. https://doi.org/10.1097/MD.0000000000005141.
122. Park SM, Kim GW, Mun JH, et al. Fractional Laser-Assisted Topical Imiquimod 5% Cream Treatment for Recalcitrant Common Warts in Children: A Pilot Study. *Dermatol Surg*. 2015;42(12):1304-1346. https://doi.org/10.1097/DSS.0000000000000885.
123. Ma G, Wu P, Lin X, et al. Fractional carbon dioxide laser-assisted drug delivery of topical timolol solution for the treatment of deep infantile hemangioma: a pilot study. *Pediatr Dermatol*. 2014;31(3):286-291. https://doi.org/10.1111/pde.12299.
124. Vachiramon V, Chaiyabutr C, Rattanaumpawan P, Kanokrungsee S. Effects of a preceding fractional carbon dioxide laser on the outcome of combined local narrowband ultraviolet B and topical steroids in patients with vitiligo in difficult-to-treat areas. *Lasers Surg Med*. 2016;48(2):197-202. https://doi.org/10.1002/lsm.22389.
125. Yuan J, Chen H, Yan R, et al. Fractional CO2 lasers contribute to the treatment of stable non-segmental vitiligo. *Eur J Dermatol*. 2016;26(6):592-598. https://doi.org/10.1684/ejd.2016.2875.
126. Cunha PR, Scabine Pessotti N, Bonati Mattos C, Salai AF. New approach in the treatment of refractory vitiligo: CO_2 laser combined with betamethasone and salicylic acid solution. *Dermatol Ther*. 2017;30(1):e12410. https://doi.org/10.1111/dth.12410.
127. Li L, Wu Y, Li L, et al. Triple combination treatment with fractional CO_2 laser plus topical betamethasone solution and narrowband ultraviolet B for refractory vitiligo: a prospective, randomized half-body, comparative study. *Dermatol Ther*. 2015;28(3):131-134. https://doi.org/10.1111/dth.12202.
128. Mohamed HA, Mohammed GF, Gomaa AH, Eyada MM. Carbon dioxide laser plus topical 5-fluorouracil: a new combination therapeutic modality for acral vitiligo. *J Cosmet Laser Ther*. 2015;17(4):216-223. https://doi.org/10.3109/14764172.2014.1003241.
129. Komen L, Vrijman C, Wietze van der Veen JP, de Rie M, Wolkerstorfer A. Observations on CO_2 laser preparation of recipient site for noncultured cell suspension transplantation in vitiligo. *J Cutan Aesthet Surg*. 2016;9(2):133-135. https://doi.org/10.4103/0974-2077.184055.
130. Oni G, Rasko Y, Kenkel J. Topical Lidocaine Enhanced by Laser Pretreatment: A Safe and Effective Method of Analgesia for Facial Rejuvenation. *Aesthetic Surg J*. 2013;33(6):854-861. https://doi.org/10.1177/1090820X13496248.
131. Meesters AA, Bakker MM, de Rie MA, Wolkerstorfer A. Fractional CO 2 laser assisted delivery of topical anesthetics: A randomized controlled pilot study. *Lasers Surg Med*. 2016;48(2):208-211. https://doi.org/10.1002/lsm.22376.
132. Tian T, Luo Y, Jiang T, et al. Clinical effect of ablative fractional laser-assisted topical anesthesia on human skin: A randomized pilot study. *J Cosmet Laser Ther*. 2016;18(7):409-412. https://doi.org/10.1080/14764172.2016.1197404.
133. Nguyen BT, Gan SD, Konnikov N, Liang CA. Treatment of superficial basal cell carcinoma and squamous cell carcinoma in situ on the trunk and extremities with ablative fractional laser-assisted delivery of topical fluorouracil. *J Am Acad Dermatol*. 2015;72(3):558-560. https://doi.org/10.1016/j.jaad.2014.11.033.
134. Gupta AK, Studholme C. Novel investigational therapies for onychomycosis: an update. *Expert Opin Investig Drugs*. 2016;25(3):297-305. https://doi.org/10.1517/13543784.2016.1142529.
135. Basnett A, Nguyen TA, Cannavino C, Krakowski AC. Ablative fractional laser resurfacing with topical paromomycin as adjunctive treatment for a recalcitrant cutaneous leishmaniasis wound. *Lasers Surg Med*. 2015;47(10):788-791. https://doi.org/10.1002/lsm.22426.
136. Waibel JS, Mi QS, Ozog D, et al. Laser-assisted delivery of vitamin C, vitamin E, and ferulic acid formula serum decreases fractional laser postoperative recovery by increased beta fibroblast growth factor expression. *Lasers Surg Med*. 2016;48(3):238-244. https://doi.org/10.1002/lsm.22448.
137. Bay C, Lerche CM, Ferrick B, Philipsen PA, Togsverd-Bo K, Haedersdal M. Comparison of Physical Pretreatment Regimens to Enhance Protoporphyrin IX Uptake in Photodynamic Therapy. *JAMA Dermatol*. 2017;153(4):270-278. https://doi.org/10.1001/jamadermatol.2016.5268.
138. Moseley H, Brancaleon L, Lesar AE, Ferguson J, Ibbotson SH. Does surface preparation alter ALA uptake in superficial non-melanoma skin cancer in vivo? *Photodermatol Photoimmunol Photomed*. 2008;24(2):72-75. https://doi.org/10.1111/j.1600-0781.2008.00338.x.
139. Torezan L, Chaves Y, Niwa A, Sanches JA Jr, Festa-Neto C, Szeimies RM. A Pilot Split-Face Study Comparing Conventional Methyl Aminolevulinate-Photodynamic Therapy (PDT) With Microneedling-Assisted PDT on Actinically Damaged Skin. *Dermatol Surg*. 2013;39(8):1197-1201. https://doi.org/10.1111/dsu.12233.
140. Haedersdal M, Wenande E, Bay C, Karmisholt KE, Togsverd-Bo K. Comparison of tailored pretreatment regimens with microdermabrasion versus ablative fractional laser prior to daylight PDT: A randomized trial. [abstract 59]. *Lasers Surg Med*. 2017;49(S28):21.
141. Choi SH, Kim TH, Song KH. Efficacy of iontophoresis-assisted ablative fractional laser photodynamic therapy with short incubation time for the treatment of actinic keratosis: 12-month follow-up results of a prospective, randomised, comparative trial. *Photodiagnosis Photodyn Ther*. 2017;18:105-110. https://doi.org/10.1016/j.pdpdt.2017.01.184.
142. Yu J, Kalaria DR, Kalia YN. Erbium:YAG fractional laser ablation for the percutaneous delivery of intact functional therapeutic antibodies. *J Control Release*. 2011;156(1):53-59. https://doi.org/10.1016/j.jconrel.2011.07.024.
143. Lee WR, Shen SC, Al-Suwayeh SA, Yang HH, Li YC, Fang JY. Skin permeation of small-molecule drugs, macromolecules, and nanoparticles mediated by a fractional carbon dioxide laser: the role of hair follicles. *Pharm Res*. 2013;30(3):792-802. https://doi.org/10.1007/s11095-012-0920-4.
144. Bachhav YG, Heinrich A, Kalia YN. Controlled intra- and transdermal protein delivery using a minimally invasive Erbium:YAG fractional laser ablation technology. *Eur J Pharm Biopharm*. 2013;84(2):355-364. https://doi.org/10.1016/j.ejpb.2012.11.018.
145. Bach D, Weiss R, Hessenberger M, et al. Transcutaneous immunotherapy via laser-generated micropores efficiently alleviates allergic asthma in Phl p 5-sensitized mice. *Allergy*. 2012;67(11):1365-1374. https://doi.org/10.1111/all.12005.
146. Lee YB, Lee KJ, Park HJ, Cho BK. Topical application of growth factors after carbon dioxide fractional laser therapy: a randomized controlled split-face study. *J Cosmet Laser Ther*. 2011;13(1):38-40. https://doi.org/10.3109/14764172.2011.552610.
147. Chen X, Shah D, Kositratna G, Manstein D, Anderson RR, Wu MX. Facilitation of transcutaneous drug delivery and vaccine immunization by a safe laser technology. *J Control Release*. 2012;159(1):43-51. https://doi.org/10.1016/j.jconrel.2012.01.002.