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REVIEW

Update of Ablative Fractionated Lasers to Enhance Cutaneous Topical Drug Delivery

Jill S. Waibel · Ashley Rudnick · Deborah R. Shagalov · Danielle M. Nicolazzo

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ABSTRACT

Ablative fractional lasers (AFXL) enhance uptake of therapeutics and this newly emerging field is called laser-assisted drug delivery (LAD). This new science has emerged over the past decade and is finding its way into clinical practice. LAD is poised to change how medicine delivers drugs. Topical and systemic application of pharmaceutical agents for therapeutic effect is an integral part of medicine. With topical therapy, the stratum corneum barrier of the skin impairs the ability of drugs to enter the body. The purpose of LAD is to alter the stratum corneum, epidermis, and dermis to facilitate increased penetration of a drug, device, or cell to its respected target. AFXL represents an innovative, non-invasive strategy to overcome the epidermal barrier. LAD employs three steps: (1) breakdown of the skin barrier with a laser, (2) optional use a laser for a therapeutic effect, (3) delivery of the medicine through laser channels to further enhance the therapeutic effect. The advantages of using lasers for drug delivery include the ease of accessibility, the non-invasive aspect, and its effectiveness. By changing the laser settings, one may use LAD to have a drug remain locally within the skin or to have systemic delivery. Many drugs are not intended for use in the dermis and so it has yet to be determined which drugs are appropriate for this technique. It appears this developing technology has the ability to be a new delivery system for both localized and systemic delivery of drugs, cells, and other molecules. With responsible development AFXL-assisted drug delivery may become a new important part of medicine.

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J. S. Waibel (✉) · A. Rudnick
Miami Dermatology and Laser Institute, Miami, FL, USA
e-mail: jwaibelmd@miamidermlaser.com

D. R. Shagalov
Dermatology, State University of New York, Downstate Medical Center, Brooklyn, NY, USA

D. M. Nicolazzo
Dermatology, Larkin Hospital/NSU-COM, South Miami, FL, USA

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INTRODUCTION

History of Laser-Assisted Delivery

Drug delivery is essential in the treatment of medical conditions. The efficacy of topical therapy is dependent on the ability of the therapeutic drug to reach its target.

Transcutaneous absorption is limited by the skin's inherent barrier properties, minimizing absorption of topically applied medications to only 1–5% [1]. Drugs which are semi-lipophilic (uncharged/non-polar) and small (<500 Da) may pass through the stratum corneum because the keratinocytes are embedded in a lipid matrix. Drugs that are lipophilic and large hydrophilic drugs are not able to traverse through normal intact skin. The molecular size of the drug is also important with larger molecules requiring a traditional drug delivery method including oral (pill), injectable (shot), intravenous (IV), transdermal patch, or systemic delivery. Strategies to enhance topical drug delivery include chemical (solvents and surfactants), biochemical (nanoparticles and lipid synthesis inhibitors), and physical methods (tape stripping, sonophoresis, and microneedling). The most commonly utilized approach in today's pharmaceutical industry is chemical modification.

Dr. Waibel has done clinical trials that are included in the references of this article. For example, reference [2].

Techniques of Laser Assisted Drug Delivery (LAD)

Since its initial publication in 2002 [3], the modality of LAD has continued to evolve, allowing better efficacy and precision in depth of penetration of drugs delivered transcutaneously. Ablative fractional lasers (AFXLs), either carbon dioxide (CO_2) or erbium:YAG (Er:YAG), provide a novel way to create a conduit in the stratum corneum, epidermal, and dermal layers, increasing penetration of topically applied molecules in a predictable and controlled pattern. AFXL creates vertical channels of ablation, opening a doorway into the human body. Both CO_2 and Er:YAG lasers are infrared lasers that heat skin tissue over 100 °C and cause vaporization. The Er:YAG laser has an absorption coefficient of 2×10^7/cm and owing to high absorption of water it takes less energy to ablate tissue. Less energy is needed to ablate channels with the Er:YAG systems and subsequent thermal damage around the channel is minimal. The CO_2 laser has an absorption coefficient of 2×10^6/cm and takes higher energies to ablate tissue, resulting in increased thermal damage (coagulation zone) around the ablated channels compared to Er:YAG (Fig. 1).

Topical drug delivery has many advantages over traditional oral administration. Benefits include easy accessibility, non-invasive and effective means of drug delivery, and limiting systemic toxicity. In addition, drug degradation by the gastrointestinal system and first-pass liver metabolism can be avoided with laser cutaneous delivery.

The objectives of a cutaneous delivery system include increasing access to therapeutic targets, decreasing the amount of drug needed, decreasing adverse events in other organs, and ease of use for patients. LAD is a new emerging concept bridging medicine with technology to improve health care [4].

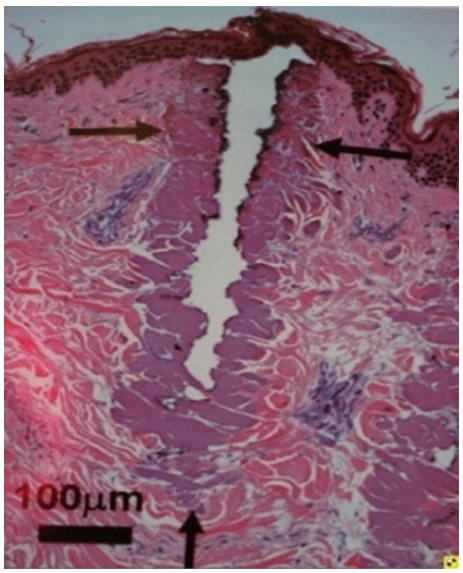

Fig. 1 H&E-stained histology 3 h after AFXL CO_2 laser exposure (50 mJ/microbeam) of porcine skin in vivo, showing a microscopic ablation zone extending to the mid-dermis. A cuff of coagulated tissue surrounds the laser channel, which is filled with a protein transudate. Such protein transudate is typically absent immediately after CO_2 laser exposure and it is unclear how it affects drug delivery

Methodology of LAD

LAD is technique that uses AFXL to deliver channels of different depths and, immediately following the procedure, that places a drug or molecule into the skin. This modality allows deeper drug penetration with minimal skin damage or discomfort. It is highly recommended to only deliver drugs or molecules that are FDA-approved or studied for the dermis. There need to be additional studies to determine the optimal treatment parameters, coagulation zones, and how long the channels of LAD remain open in the dermis.

Clinical Applications of LAD

Various medical conditions have been studied with LAD including dysplasia, non-melanoma skin cancer, psoriasis, inflammatory conditions, local anesthesia, and scars (Table 1) [5].

Multiple studies have demonstrated the benefit of LAD in the delivery of various drugs including lidocaine, 5-aminolevulinic acid (ALA), methyl-5-aminolevulinate (MAL), 5-fluorouracil, ascorbic acid, diclofenac, ingenol mebutate, imiquimod, methotrexate, and vaccinations. Specifically in the arena of LAD for scars, medications studied include corticosteroids, ascorbic acid, 5-fluoruracil, platelet-rich plasma, and stem cells [2, 6–18].

What Lasers Are Best for LAD?

Current laser choices for LAD include non-ablative fractional lasers, micro-ablative fractional lasers, and AFXL. Ablative lasers are generally considered superior to non-ablative lasers, though studies in this area are limited. The differences of these devices are best understood by histological differences between non-ablative fractional lasers and AFXLs. Non-ablative lasers disrupt the dermal epidermal junction but do not create an opening for larger molecules to gain access to the dermis [19, 20].

Haedersdal et al. used a variety of physical techniques in a preclinical model that disturbed the stratum corneum to study which modality best enhanced protoporphyrin IX accumulation. Modalities studied included non-ablative fractional laser, AFXL, microneedling, microdermabrasion, curettage, and control [18, 21]. Of these modalities, ablative laser therapy was

Table 1 Various medical conditions have been studied with LAD in the past years since this new clinical modality was introduced into medicine

Medical condition	Laser-assisted drug studied
Actinic keratosis, non-melanoma skin cancer	ALA, MAL, imiquimod, ingenol mebutate, methotrexate
Arthritis	Diclofenac, indomethacin
Hemangioma	Timolol
Hair, alopecia areata	Minoxidil, diphencyprone
Infectious disease	Leishmaniasis
Onychomycosis	Topical amorolfine
Photoaging	Botulinum toxin
Post inflammatory hyperpigmentation (PIH)	Corticosteroid
Psoriasis	Methotrexate
Scars	TAC, 5-FU, PLLA, Vit C, bimatoprost, stem cells
Vaccinations	Ovalbumin (OVA)

found to be statistically superior in enhancing protoporphyrin IX accumulation in the dermis.

Laser Treatment Recommendations for LAD

Laser Dosimetry for LAD

To understand how to best use lasers for LAD, it is necessary to understand basic LAD dosimetry strategies. By adjusting laser settings, it is possible to influence the quantity of drug delivered, drug delivery rate, and drug biodistribution. AFXL may be further tailored to specific laser settings including channel density, depth, and coagulation around the ablated channel to increase drug deposition into targeted cutaneous levels [22].

Channel Density and LAD

Channel density is defined as the ablative skin surface area which can be adjusted via laser spot size or the number of applied channels in a fixed scan pattern. Rate and extent of drug delivery are impacted by channel density as studied with lidocaine [6] and MAL by using fractional ablative lasers [9]. Bachhav et al. studied the effect of channel density on permeation of topical lidocaine in an in vitro porcine model using an Er:YAG fractional ablative laser. Their initial hypothesis was that by increasing the number of laser channels, drug delivery would be increased. Channel densities studied included 0 (control), 150, 300, 450, and 900 channels per 3 cm^2 with a fixed laser fluence at 24 h [23]. Contrary to what was expected, there was no statistically significant difference in cumulative permeation at 6 h between 450 and 900 channels or 300, 450, and 900 channels at 24 h. The authors therefore concluded that there is a minimum channel density to achieve maximum drug penetration but that increasing channel density beyond this point will not improve delivery.

Another study by Sakamoto et al. [24] showed no difference in drug penetration for either low channel densities or high channel densities. An in vivo porcine model with a CO_2 fractional ablative laser using 100, 200, and 400 MTZ/cm^2 densities for ALA in photodynamic therapy revealed that increasing channel density did not influence depth of ALA delivery to deeper skin layers and this may be unique to the chemical properties of ALA.

In both literature and clinical experience there appears to be a plateau around 100 channels/cm^2. Therefore, low channel density is favored in LAD.

Laser Channel Depth Effect on LAD

Early studies with lidocaine examined the effect of channel depth on rate and extent of drug delivery [6]. The depth of the laser channels can be adjusted by pulse energy. Higher pulse energies in fractional ablative lasers give deeper ablated laser channels. Deeper channels were initially proposed to result in greater dermal uptake of drug; however, the published literature has been inconsistent.

Using an in vitro porcine model with an Er:YAG laser, Bachhav et al. studied the effect of increasing laser fluences from 150 to 200 μm on channel depth and lidocaine permeation. This study found that with fixed channel density, a greater depth did not result in a statistically significant greater cumulative lidocaine permeation. The authors concluded that lidocaine delivery is enhanced with LAD, but its transport is independent of depth or fluence, suggesting that the main purpose of LAD in lidocaine delivery is disruption of the stratum corneum [23].

Similarly, in another well-performed porcine LAD study, Oni et al. hypothesized that greater channel depth would enhance transdermal absorption [6]. This experiment tested fractional ablative channels at 25, 50, 250, and 500 μm. This study revealed that maximum systemic absorption occurred at 250 μm depth. Oni concluded that this may be due to vascular plexus between 100 and 300 μm in porcine skin. Increasing the depth to 500 μm did not increase absorption.

Additional research is needed but it appears that various drug properties such as its hydrophobic or hydrophilic nature may determine the influence of channel depth on drug delivery.

In addition to drug properties, another vital consideration when choosing laser depth is the underlying condition being treated. LAD may be used solely to enhance drug delivery or may

work synergistically with the inherent therapeutic effects of the laser. For example, optimal laser channel depth for scars most likely relates to the thickness of the scar. Scars tend to have abnormal collagen mainly in the papillary and reticular dermis lying between 200 and 3000 μm in depth. When treating scars it is recommended to try to establish and treat the full depth of the scar with the laser, and adding a drug via LAD makes the treatment even more effective.

Coagulation Zone in LAD

The coagulation zone in a fractional ablative laser is defined by the thickness of coagulated tissue surrounding the ablative zone. This may be adjusted with some laser systems by the total energy delivered and turning coagulation on or off. The diffusion coefficient of coagulated tissue is less than that of normal tissue; thus, ablative zones with coagulated tissue have a lower diffusivity. This may have an effect on drug delivery in that a thick coagulation zone may serve as a secondary diffusion barrier. This also can create a drug reservoir in these channels which we may find to be a positive attribute if we are aiming to deliver drug to the dermis and do not want systemic delivery. Conversely, no coagulation zone may be better for systemic delivery goals. Further research is warranted to understand the in vivo ramifications of a coagulation zone [22].

LAD Drug, Molecule, and Stem Cell Considerations

Molecular Weight and Drug Diffusion in LAD

Using a fractional ablative CO_2 laser, Haak et al. evaluated the effect of channel density and molecular weight of polyethylene glycols (PEGs). PEGs of increasing molecular weights from 250 to 4000 Da were applied to the skin after AFXL at densities of 25, 100, 225, and 440 MTZ/cm^2 [25]. Mass spectrometry and nuclear magnetic resonance spectroscopy revealed that similar to findings in other studies, greater densities resulted in greater transdermal delivery with no statistically significant differences above 100 MTZ/cm^2. In addition, molecules with the lowest molecular weight had better uptake.

While the stratum corneum is the main barrier for classical topical drug delivery, in LAD the interstitial matrix consisting of collagen fibers, elastin, and hyaluronic acid can pose a significant diffusion barrier especially for drugs with higher molecular weights. Haedersdal et al. [24] studied diffusion distance from laser channels using an in vivo porcine model after CO_2 fractional ablative laser. They found that MAL diffused 1.5 mm from each laser channel. Learning about the various diffusion properties of each agent will allow for optimal channel density selection. The inherent physiochemical properties of a drug including molecular size, diffusion coefficient, and underlying skin disease will affect the ability of different compounds to transverse tissue.

Concentration of Drugs

Another factor requiring substantial consideration is drug concentration. Currently drugs have not been systematically studied or FDA indicated for laser delivery. One would hypothesize that as opposed to systemic delivery, a lower concentration of a drug would be required with LAD. This could potentially save health care dollars and also prevent many untoward adverse events such as gastrointestinal toxicities seen with many medications.

Timing of Delivery of Medication

The timing of drug application after AFXL is an important factor in maximizing LAD. Following laser exposure, the skin barrier is disrupted until re-epithelialization occurs 2–4 days later. After AFXL, tissue fluids, fibrin, plasma, and granulocytes presumably fill the ablative channels within hours. It is therefore thought that in order to utilize the channel depth, immediate application of the drug is necessary.

Techniques to Enhance LAD

AFXL pretreatment as described in the preceding sections is one of the latest and most promising of a variety of strategies used to enhance cutaneous delivery of topically applied agents. However, current LAD technique

generally relies on passive uptake into the newly ablated channels. While this has proven to increase the delivery of a variety of agents, passive uptake alone may not provide adequate access to deeper portions of the channel in part because of competing interstitial fluid and fibrin. As noted in prior discussion, increasing column depth has not been shown to lead to clear and reproducible increases in drug delivery beyond a certain threshold. Active filling of the channels to help optimize LAD with both novel and existing adjunctive methods (e.g., pressure and sonophoresis) has thus been studied.

Waibel et al. studied whether immediate transdermal acoustic wave air pressure after AFXL improved drug delivery [2]. Human subjects were treated and biopsied with four different modalities: (a) topical ALA, (b) AFXL + topical ALA, (c) AFXL + acoustic device, and (d) AFXL + topical ALA + acoustic device. Comparison of the difference of magnitude of diffusion, both lateral spread of ALA and depth diffusion of ALA, was measured by fluorescence microscopy. The results revealed that with AFXL + topical ALA + acoustic device the protoporphyrin IX lateral fluorescence was 0.024 mm on average versus 0.0084 mm observed with AFXL + topical ALA alone. The study concluded that combining AFXL with the acoustic device resulted in increased depth of penetration of the ALA.

Erlendsson et al. investigated a standardized method to actively fill laser-generated channels by altering pressure (compressed air), vacuum, and pressure (PVP) in a porcine model. The authors found that PVP after AFXL treatment induced faster, deeper, and increased delivery of the test drug than conventional LAD with passive filling [26].

Clinical Applications

LAD utilizing AFXL pretreatment can be applied to multiple medical conditions across many specialties.

Dysplasia and Non-melanoma Skin Cancer

Actinic keratosis (AK) is a premalignant, cutaneous lesion induced by ultraviolet radiation. Common treatments for AKs include 5-FU [27], imiquimod, ingenol mebutate, or photodynamic therapy (PDT). AFXL-assisted drug delivery of MAL, a topical agent used for PDT, was investigated by Haedersdal et al. They found a 10-fold increase in uptake and deeper penetration as measured by porphyrin fluorescence in epidermis and dermis. In a randomized controlled clinical trial, AFXL-assisted PDT enhanced treatment efficacy of AKs by 30% [28].

In another study, Lee et al. demonstrate the benefit of using LAD to decrease cutaneous side effects of topical application of imiquimod in the treatment of AKs. By stimulating innate immunity pathways, imiquimod effectively treats AKs with the unwanted side effects of application site dermatitis, crusting, and erosions. They demonstrated that the therapeutic dose of imiquimod was lowered 125-fold using AFXL [15]. The findings of this study suggest a benefit of using AFXL with ingenol mebutate, another topical immune stimulant with undesired cutaneous side effects. Patient compliance is enhanced by its brief application period (2–3 days) which can be further reduced with AFXL-assisted delivery. This concept has not yet been tested in clinical studies.

Scars

Hypertrophic scars pose a significant disease burden causing itching, pain, disfigurement, and functional impairment. Difficulty achieving deep and uniform drug distribution within a scar limit the use of commonly recognized treatments of topical and intralesional corticosteroids. Waibel et al. investigated treating hypertrophic scars using AFXL-assisted delivery of topical corticosteroid. Compared with AFXL alone, an improved response was noted when an injectable formulation of triamcinolone acetonide was applied immediately after AFXL (Figs. 2, 3) [17]. A common clinical concerns when using corticosteroids include its cutaneous side effects of dermal and fat atrophy, telangiectasia, and pigmentation alterations. Therefore, in an ongoing study, Waibel and colleagues are comparing AFXL-assisted delivery of 5-FU with triamcinolone acetonide for hypertrophic scar treatment. Preliminary results

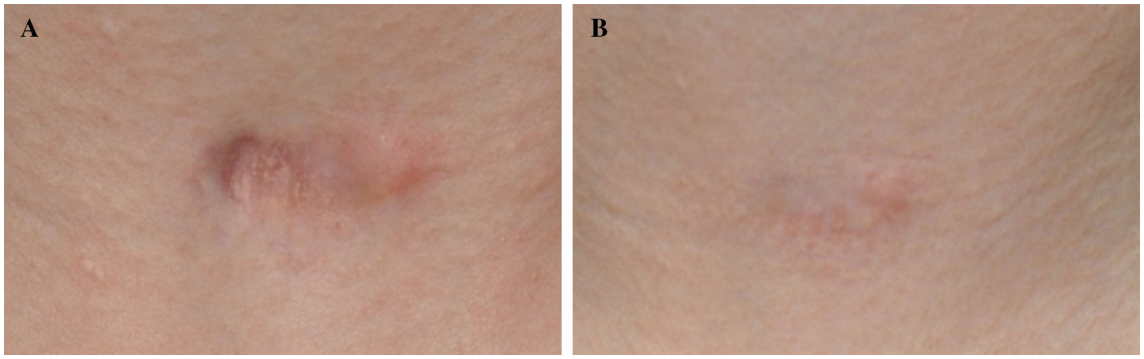

Fig. 2 Before (**a**) and 6 months after (**b**) AFXL, a series of CO_2 laser-assisted delivery of topical poly-L-lactic acid for an atrophic surgical scar. Thereafter, two treatments of AFXL-assisted delivery of topical poly-L-lactic acid decreased the atrophy and visible depression of the scar as well as the overall cosmetic appearance

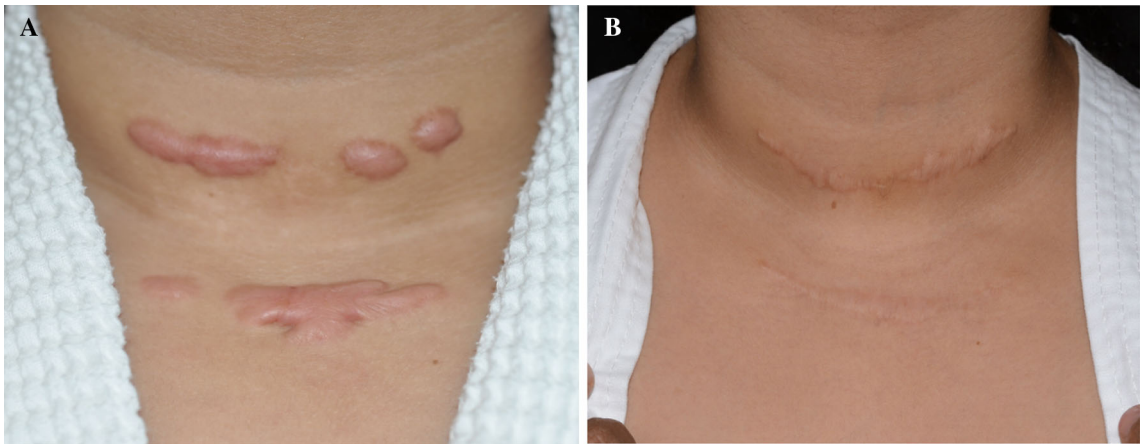

Fig. 3 Before (**a**) and 6 months after (**b**) AFXL, a series of CO_2 laser-assisted delivery of topical triamcinolone acetonide for a hypertrophic burn scar. Treatment began 5 years after the burn. Three non-ablative fractional treatments were initially administered with mild improvement. Thereafter, two treatments with AFXL (20 mJ at 10% density)-assisted delivery of topical triamcinolone acetonide led to impressive decrease in hypertrophy, improvement of dyschromia, and increased range of motion

reveal that both agents are equally effective in decreasing scar height, but that 5-FU resulted in less atrophy and telangiectasias. Comparisons of AFXL-assisted delivery versus standard injection have yet to be performed; nevertheless, it appears to be a promising adjunctive treatment.

Psoriasis

Another application of LAD that is currently being studied is psoriasis. Although localized cutaneous disease can be treated topically, severe disease frequently requires systemic drug therapy. These medications have potential systemic side effects requiring routine monitoring and follow-up. The optimal laser parameters for MTX delivery are currently being studied in vivo [28].

Inflammatory Conditions

Oral administration of non-steroidal anti-inflammatory drugs (NSAIDs) is used to treat inflammatory conditions but can result in gastrointestinal ulcers and bleeding. In an in vitro study, Bachhav et al. demonstrated a 13-fold

increase permeation of diclofenac after AFXL in both human and porcine skin [14]. The authors therefore postulate that AFXL may be used in the treatment of arthritis to increase uptake of diclofenac in synovial fluids.

Local Anesthesia

Topical and locally injected anesthetics are widely used in dermatology. Topical application is limited by minimal penetrance, while injections are painful. Successful delivery of lidocaine via AFXL has been demonstrated in vivo [6]. Pretreatment with a fully ablative laser has been shown to reduce the incubation period of topically applied lidocaine and this would likely translate to AFXL as well. Lidocaine toxicity is a significant concern and cautious use is recommended.

Other Applications

There are several other exciting potential applications of LAD. One such application is utilizing LAD to administer vaccines, providing an alternative to traditional injections especially for children and needle-phobic patients. Production of ovalbumin-specific antibodies increased 28–538 times after AFXL-assisted delivery of a model vaccine compared to intact skin [26]. While the safety and efficacy require further inquiry, AFXL-assisted vaccination may be a viable alternative.

In addition, AFXL is being studied to assist the delivery of stem cells for the treatment of chronic wounds. In an in vivo porcine model, AFXL successfully transplanted allogenic adipocyte-derived stem cells to the skin; at 48 h, 5.5% of applied stem cells were viable compared to 0% in intact skin [19].

LADS: Limitations and Potential Safety Concerns

Fractional ablative laser treatment alone is associated with a low rate of adverse events such as infection and new or worsening scarring for scar treatment and other applications, especially in contrast to non-fractionated treatments with the same laser wavelengths [29]. However, safety data for fractional ablative LAD is currently lacking and the concomitant risks of the drug, cosmeceutical, or other agent applied to the skin must also be considered. A wide array of agents may theoretically be delivered using LAD, though these have generally not been formulated nor FDA-approved for this route of delivery. Research in this area is in its infancy; with time the physiochemical properties of each drug, efficacy, safety, dosing, timing, and optimal procedural combinations and applications will begin to be elucidated. Because the dermal channels generate direct access to the cutaneous vascular system, there are concerns of potential systemic toxicity associated with LAD. For example, Oni et al. noted enhanced lidocaine absorption and detectable blood levels after LAD in a porcine model [6]. Potential toxicity is an especially important concept for children given their low overall body weight and high surface area to volume ratio.

Additional safety concerns include infection via the introduction of pathogens from the skin surface or from non-sterile topical preparations. In addition, application of other products by the patients after AFXL may result in absorption of molecules that were not intended to be applied to non-intact skin. At present, AFXL-assisted delivery should only be considered in well-controlled settings and clinical trials.

CONCLUSIONS

Ablative fractional lasers offer a unique and promising therapeutic modality to help bypass the epidermal barrier and enhance the delivery of a variety of topically applied agents for both local and systemic effects. Many questions remain unanswered, and a great deal of additional research is required to establish the efficacy and safety of LAD for particular indications, as well as the optimal formulations, dosing, timing, and laser platform and settings. LAD has the potential of opening the door to huge new advances in drug delivery in medicine.

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Compliance with Ethics Guidelines. This article is based on previously conducted studies and does not involve any studies of human or animal subjects performed by any of the authors.

Data Availability. The datasets generated during and/or analyzed during the current study are available via http://www.pubmed.com.

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