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Non-ablative fractional laser assists cutaneous delivery of small- and macro-molecules with minimal bacterial infection risk

Woan-Ruoh Lee [a,b], Shing-Chuan Shen [a], Ibrahim A. Aljuffali [c], Yin-Ku Lin [d,e], Chang-Wei Huang [f], Jia-You Fang [f,g,h,*]

[a] Graduate Institute of Medical Sciences, Taipei Medical University, Taipei, Taiwan
[b] Department of Dermatology, Taipei Medical University Shuang Ho Hospital, New Taipei City, Taiwan
[c] Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia
[d] School of Traditional Chinese Medicine, Chang Gung University, Kweishan, Taoyuan, Taiwan
[e] Department of Traditional Chinese Medicine, Chang Gung Memorial Hospital at Keelung, Keelung, Taiwan
[f] Pharmaceutics Laboratory, Graduate Institute of Natural Products, Chang Gung University, Kweishan, Taoyuan, Taiwan
[g] Department of Anesthesiology, Chang Gung Memorial Hospital, Kweishan, Taoyuan, Taiwan
[h] Research Center for Industry of Human Ecology, Chang Gung University of Science and Technology, Kweishan, Taoyuan, Taiwan

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ABSTRACT

Use of the ablative laser has been approved to enhance topical drug penetration. Investigation into the usefulness of the non-ablative laser for assisting drug delivery is very limited. In this study, we explored the safety and efficacy of the non-ablative fractional erbium:glass (Er:glass) laser as an enhancement approach to promote drug permeation. Both pig and nude mouse skins were employed as transport barriers. We histologically examined the skin structure after laser exposure. The permeants of 5-aminolevulinic acid (ALA), imiquimod, tretinoin, peptide, dextrans and quantum dots (QD) were used to evaluate in vitro and in vivo skin passage. The fractional laser selectively created an array of photothermal dots deep into the dermis with the preservation of the stratum corneum and epidermis. The barrier function of the skin could be recovered 8–60 h post-irradiation depending on the laser spot densities. The application of the laser caused no local infection of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Compared to intact skin, ALA flux was enhanced up to 1200-fold after laser exposure. The penetration enhancement level by the laser was decreased following the increase of permeant lipophilicity. The skin accumulation of tretinoin, an extremely lipophilic drug, showed only a 2-fold elevation by laser irradiation. The laser promoted peptide penetration 10-fold compared to the control skin. Skin delivery of dextrans with a molecular weight (MW) of at least 40 kDa could be achieved with the Er:glass laser. QD with a diameter of 20 nm penetrated into the skin with the assistance of the non-ablative laser. The confocal microscopic images indicated the perpendicular and lateral diffusions of dextrans and nanoparticles via laser-created microscopic thermal zones. Controlled Er:glass laser irradiation offers a valid enhancement strategy to topically administer the permeants with a wide MW and lipophilicity range.

1. Introduction

Topical/transdermal drug penetration offers an efficient method of administration because of the targeted delivery, avoidance of liver first-pass metabolism, and minimal invasiveness compared to hypodermic needles. Cutaneous delivery can be applied in different drug-treatment protocols, including cosmetology, dermatology, and oncology (Schoellhammer et al., 2014). The rigid structure of the skin, especially that of the stratum corneum (SC), makes the diffusion of many drugs difficult. A molecular weight (MW) 500 Da is regarded as the upper limit for passive drug permeation (Hwa et al., 2011). Many techniques are employed for enhancing drug permeation via SC. These include penetration enhancers, prodrugs, iontophoresis, electroporation, and ultrasound. The skin delivery of macromolecules such as proteins, DNA, small interfering RNA, and vaccines is particularly challenging. Currently, hypodermic needles are mostly used for delivering macromolecules into the skin. However, this method can produce pain, require regulated needle disposal, increase the risk of infection, and require professional assistance (Andrews et al., 2011). As a result of these drawbacks,

* Corresponding author at: Pharmaceutics Laboratory, Graduate Institute of Natural Products, Chang Gung University, 259 Wen-Hwa 1st Road, Kweishan, Taoyuan 333, Taiwan.
E-mail address: fajy@mail.cgu.edu.tw (J.-Y. Fang).

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interest in less-invasive approaches for topical/transdermal delivery has grown greatly. Recently, microneedles provide an assisted delivery for both small-molecular and macromolecular drugs with limited invasiveness (Cheung et al., 2015; Han and Das, 2015). Microneedles are able to permit the drug transport by bypassing SC after insertion of micron-sized needles that create channels through SC (Cheung and Das, 2015).

Recent advances have demonstrated a minimal invasive method of employing ablative fractional lasers to ameliorate topical application of drugs into the skin. The lasers can precisely control the ablation degree by adjustment of the energy and etched depth (Hædersdal et al., 2010; Yu et al., 2011; Oni et al., 2012; Lee et al., 2014a, 2014b, 2014c). The skin is exposed by the laser in a non-contact manner without the contamination risk (Chen et al., 2012). Fractional resurfacing is a laser modality to create arrays of microscopic thermal zones (MTZ) to reduce photothermal adverse responses and achieve fast epidermal healing by migration of the surrounding viable tissues (Prignano et al., 2011). Clinical trials have proved a successful drug delivery assisted by ablative fractional lasers for treatment of facial rejuvenation, actinic keratosis, and basal cell carcinoma (Trelles et al., 2013; Braun et al., 2015; Nguyen et al., 2015). Although the ablative laser offers an attractive strategy for cutaneous penetration enhancement, the superficial resurfacing may lead to skin irritation, erythema, and the possibility of infection since the SC is a vital barrier preventing the entrance of foreign microorganisms (Lee et al., 2010). Non-ablative fractional lasers produce tissue disturbance without significantly damaging the superficial skin layers. The SC remains intact by irradiation of the non-ablative modality, reserving the epidermal barrier function (Wanner et al., 2007). The benefits are the decreased risk of pigment change, short repair time, lower hypertrophic scarring, and less pain (Jung et al., 2011). As compared to studies of the ablative laser technique, few investigative studies are available on the non-ablative technique to assist drug delivery through the skin. A preliminary study (Lim et al., 2014) has indicated that the non-ablative fractional laser enhanced 5-aminolevulinic (ALA) permeation with minimal skin injury. The purpose of this work was to study the effect of the non-ablative fractional erbium:glass (Er:glass) laser on facilitating cutaneous penetration of small-molecule drugs, macromolecules, and nanoparticles. The Er:glass laser emits a near-infrared wavelength, allowing the option of interacting with skin tissues extending up to 1–2 mm from the surface (Farkas et al., 2009).

Both pig and nude mouse skins were employed as barrier membranes. The Franz diffusion cell was the in vitro skin permeation platform. The in vivo drug distribution in the skin was monitored by fluorescence and confocal microscopies for vertical and horizontal views, respectively. Several safety issues related to the laser were investigated. These included skin irritation, cutaneous barrier function, recovery period, and bacterial infection.

2. Materials and Methods

2.1. Materials

ALA, tretinoin, fluorescein isothiocyanate (FITC)-dextrans with MW of 4, 10, and 40 kDa (FD4, FD10, and FD40) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Imiquimod was supplied by LKT Laboratories (St. Paul, MN, USA). Fluorescein peptide (fluorescein-NH_2-Pro-Arg-Leu-Leu-Tyr-Ser-Trp-His-Arg-Ser- His-Arg-Ser-His-COOH) with an MW of 2335 Da was provided by Biotools (New Taipei City, Taiwan). The carboxylic acid quantum dots (QD) (Qdot® 525 ITK) were obtained from Invitrogen (Carlsbad, CA, USA).

2.2. Animals

One-week-old pathogen-free pigs were supplied by Animal Technology Institute Taiwan (Miaoli, Taiwan). Eight-week-old BALB/cByJNarl nude mice were purchased from National Laboratory Animal Center (Taipei, Taiwan). This study was carried out in strict accordance with the recommendation in the Guidelines for the Care and Use of Laboratory Animals of Chang Gung University of Science and Technology. All efforts were made to minimize suffering. The total number of the pigs and mice utilized in the present study was 8 and 54. The experimental design and animal number in each experimental group are outlined in Table 1 for a clear description of the experiments involved in animals.

2.3. Laser System

The non-ablative fractional Er:glass laser with a wavelength of 1550 nm was supplied by Dinona (Sellas evo®, Daejeon, Korea). The spot size of MTZ at the target was 100 μm. The spot densities used in this study were 256 and 529 spots/cm^2. The excised or lived skin was irradiated by the laser before histological/physiological examination and bacteria/permeant application. The pulse time was <1 ms with one pulse on the skin phase. The laser fluence of 30 mJ was generated on the animal skin with air cooling. The skin surface temperature before and after laser irradiation was monitored by TES-1326S infrared thermometer (TES Electrical, Taipei, Taiwan).

2.4. Macroscopic Visualization

The macroscopic images of the pig and nude mouse skins before and after laser exposure were observed using a handheld digital microscope (Mini Scope-V, M&T Optics, Taipei, Taiwan). A magnification of 100× was utilized to capture the images.

2.5. Microscopic Visualization

The surface of nude mouse dorsal skin was irradiated by the Er:glass laser. After the sacrifice of the mouse, the excised skin was immersed in a 10% buffered formaldehyde using ethanol, embedded in paraffin wax, and sliced at a thickness of 5 μm. The specimens were stained by hemoxylin and eosin (H&E) and viewed under light microscopy (IX81, Olympus, Tokyo, Japan).

2.6. Recovery of Laser-Treated Skin

The recovery of cutaneous physiology after laser irradiation was examined by transepidermal water loss (TEWL) and skin surface pH. A Tewameter® (TM300, Courage and Khazaka, Köln, Germany) was used for measuring TEWL (g/m^2/h). The pH was determined by Skin-pH-Meter® PH905 (Courage and Khazaka). The values were measured 72 h post-treatment. The temperature and relative humidity of the environment for measuring TEWL and pH was 25 ± 1 °C and 70 ± 5%, respectively.

2.7. In Vitro Permeation of Microorganisms

The in vitro permeation of microorganisms into the nude mouse skin was investigated using the Franz diffusion assembly. The excised skin with or without laser treatment was mounted between the donor and receptor compartments with the SC facing upward into the donor.

Table 1
The study design and animal number in each experimental group used in this work.

Experiment	Pig	Nude mouse
Macroscopic visualization	$n = 3$	$n = 3$
Microscopic visualization	–[a]	$n = 3$
Recovery of laser-treated skin	–	$n = 6$
In vitro permeation of microorganisms	–	$n = 4$
In vitro permeation of drugs	$n = 4$	$n = 4$
In vivo fluorescence microscopy	–	$n = 3$
In vivo confocal microscopy	–	$n = 3$

[a] –, not determined.