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Fractional Laser-Mediated siRNA Delivery for Mitigating Psoriasis-like Lesions via IL-6 Silencing

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The poor permeability of topically applied macromolecules such as small interfering RNA (siRNA) has inhibited the translation to clinical application. In this study, the fractional CO_2 laser-assisted approach was developed to describe siRNA permeation enhancement mediated by the created microchannels for silencing the gene to treat psoriasiform lesions. *In vitro* permeation using Franz cell and *in vivo* interleukin (IL)-6 silencing using psoriasis-like plaque in mice were evaluated to verify the impact of the laser irradiation. Low-fluence laser exposure enabled a significant increase in skin transport of siRNA, peptide, and 5-fluorouracil (5-FU). The laser treatment resulted in the enhancement of siRNA flux by 33- and 14-fold as compared to the control in nude mouse and pig skin, respectively. The laser exposure also promoted siRNA penetration across psoriatic and photoaging skins with the deficient barrier, although the enhancement level was minor compared to that of intact skin. The 3D images of confocal microscopy revealed a diffusion of macromolecules into the laser-created microchannels; the radial and vertical distribution to the surrounding and deep tissues followed this. A single laser treatment and the following topical siRNA administration were able to reduce IL-6 expression by 64% in the psoriatic skin model. Laser assistance led to the marked improvement in the plaque and the reduction of specific cytokine expression, keratinocyte proliferation, and neutrophil infiltration. Our data support the use of the fractional laser for delivery of functional nucleic acid into the skin and the target cells.

INTRODUCTION

Small interfering RNA (siRNA) is a class of small double-stranded RNA silencing genes that interfere with the expression of specific genes by interrupting DNA translation into proteins. RNA interference (RNAi) provides the potential to treat an extensive variety of diseases. The targeting of a given gene mutation by siRNA design leads to a promising future for precision medicine. The US Food and Drug Administration (USFDA) approved the first RNAi therapy in August 2018. Patisiran, developed by Alnylam Pharmaceuticals, can silence the gene that underlies hereditary transthyretin amyloidosis.[1] Cutaneous disorders are the most common diseases, affecting >70% of the population worldwide.[2] siRNA therapeutics are effective in treating a number of skin conditions such as psoriasis, atopic dermatitis, epidermolysis bullosa, epidermolytic palmoplantar keratoderma, pachyonychia congenita, and skin cancers.[3] Topical administration is a suitable route for delivering siRNA into the skin due to the easy administration, accessibility of the target site, and the resulting avoidance of systemic adverse effects. Despite the possibility of using siRNA for skin-disorder treatment, the difficulty in siRNA delivery through the cutaneous barrier and insufficient cellular uptake limit the translation to clinical use.[4] Delivery of siRNA across the *stratum corneum* (SC) is a challenge because of the large molecular size, negative charge, and hydrophilic characteristic.[5] The rapid enzymatic degradation in the skin also hampers topical siRNA administration.[6] Currently, intradermal injection is used as an efficient delivery approach for siRNA to treat skin diseases. This method shows poor patient compliance because it causes severe pain, is invasive, and requires the assistance of medical professionals.

RNAi for cutaneous therapy can be achieved if its delivery hurdles can be conquered. In recent years, fractional laser ablation has been proven to magnify topical absorption of macromolecules by controlled and safe removal of the SC.[7,8] Fractional modality creates microscopic vertical channels on the cutaneous surface with unaffected viable tissue surrounding the microchannels. The ablated area can be recovered within 1 day.[9] Fractional laser treatment may be feasible for siRNA

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absorption, as it offers a strategy for delivering the polar macromolecules through the lipid-rich SC to a specific region and cellular population. Psoriasis is an inflammatory autoimmune skin disease affecting 125 million individuals each year.[10] It has a complex etiology involving genetic and environmental factors. siRNA has been proven to be useful for psoriasis treatment.[11–13] It is reported that the majority of psoriasis patients are not satisfied with the current topical therapy.[14] Fractional laser treatment may be useful for skin delivery of siRNA to manage psoriasis. Purposed with investigating this possibility, we used a low-fluence CO_2 ablative laser to assist skin permeation of siRNA for psoriasis mitigation in a mouse model.

Using interleukin (IL)-6 siRNA as the test permeant, three lines of experiments were performed. First, the appearance and histology of intact and psoriasis skins after laser irradiation were visualized. Second, we evaluated laser-mediated siRNA permeation by using various barrier-damaged skins. Topical therapy was proposed for use in diseased skin. The transport nature of siRNA in diseased skin is quite different from that in healthy skin.[15] Besides the psoriasis-like mouse skin, the photoaging mouse skin developed by UVB exposure was used as another diseased-skin model for siRNA absorption. The cutaneous transport is largely related to the molecular characteristics of the permeants. In addition to the DNA-based macromolecule, the peptide-based macromolecule and hydrophilic 5-fluorouracil (5-FU) with low skin permeability were employed to examine skin absorption for comparison. Finally, the therapeutic efficacy of laser-assisted siRNA treatment on psoriasis was assessed in the imiquimod (IMQ)-activated psoriasis-like mouse model. For the first time, we provided a basis for laser-enhanced siRNA delivery for psoriasis treatment.

RESULTS

The Appearance of Skin Surface

Figure 1A illustrates en face images of the mouse skin with and without photoaging or psoriasis-like inflammation. The laser-irradiated healthy nude and hairy mouse skins demonstrated an array of dark-colored micropores on the cutaneous surface. The created microchannels had a diameter of 200–300 μm, approximating the microscopic thermal zone (MTZ) of the fractional laser (300 μm). There were flaky scales and erythema on the UVB-treated nude mouse skin surface. The en face image of the psoriasiform skin exhibited silvery scales. The size of the scaly lesion created by IMQ was larger than that created by UVB. As with normal skin, CO_2 laser treatment produced discrete channels with a circular appearance on diseased skins. The light micrographs with higher magnification were taken as depicted in Figure 1B. The micropores caused by CO_2 laser were found in all skin types (arrows). The opening showed a round or ovary shape on the skin surface with a mean diameter of 150–200 μm. The dark region surrounding the pores could be the coagulation induced by the CO_2 laser.

The skin histology demonstrated more details about the microchannel structure as shown in Figure 1C. The healthy skin had all layers of the intact skin with no impairment. The laser exposure removed the SC and some epidermal layers (arrows). The holes were surrounded by the residual thermal coagulation extended down to the upper dermis. Examination of the photoaging skin section revealed the superficial scaling and epidermal hyperplasia. The immune cell infiltration was observed in the dermis, indicating an inflammatory condition. The laser irradiation on the UVB-treated skin penetrated the SC and extended into the epidermis. The H&E staining showed hyperkeratosis, epidermal thickening, and immune-cell infiltration in the psoriasis-like skin. The laser was effective for removing the SC and the upper epidermis of IMQ-treated skin. Some carbonization layers (coagulation) were visible around the ablated zone.

Permeation via Barrier-Defective Skins

We examined the flux of permeants across nude mouse and pig skins with pretreatment of SC stripping and removal of lipid, sebum, or protein. Because the nude mouse and baby pig skins were not as thick as human skin, the flux could be regarded as the permeant delivery to the deeper skin strata. Figure 2A summarizes the fluorescent-labeled siRNA flux across nude mouse and pig skins. Passive siRNA flux across the intact control skin indicated relatively low diffusion for both animal skins. The SC-stripped skin resulted in increased siRNA flux. This was the greatest permeation among the barrier-defective skins, followed by de-protein, de-lipid, and de-sebum skins. The laser was evaluated for the capability to promote fluorescently tagged siRNA penetration. The flux was 33- and 14-fold higher than across the untreated skin when the fractional ablation was employed in the case of intact mouse and pig skin, respectively. The laser did not greatly increase the flux in the case of SC stripping, suggesting that the laser had already diminished the barrier function of the SC. Contrary to this result, the laser greatly enhanced the flux in the case of sebum removal (>25-fold). The laser-assisted flux across de-protein skin was 5- to 7-fold higher than with the untreated control. The trend with nude mouse skin was similar to that of pig skin, except that the laser increased the siRNA flux across de-lipid pig skin by 7-fold but had no effect on de-lipid mouse skin.

Peptide represents a convenient and typical model macromolecule for a skin permeation test. As shown in Figure 2B, the flux of peptide across intact skin indicated low passive permeation in both animal skins. SC stripping led to a significant increase of flux, which was 22-fold higher than that with intact mouse skin. The peptide flux tendency of the barrier-defective skins was similar to that of siRNA flux. The laser exposure did not promote the peptide flux across SC-stripped skin. The laser-induced enhancement on de-lipid skin was also limited. Compared to de-sebum skin without laser irradiation, the peptide flux rose about 7-fold after fractional ablation. Laser application resulted in a 3-fold greater flux across de-protein skin compared to passive application for both animals. The permeation of the small-molecule drug 5-FU was also examined, as shown in Figure 2C. Both SC stripping and lipid removal increased the 5-FU flux across the mouse and pig skins at the same level. This indicates that the SC lipid bilayers were the predominant barrier for 5-FU transport. The laser did not facilitate 5-FU flux across the SC-stripped and de-lipid skins. Alternatively, in the case of intact skin, laser-mediated permeation was significantly 3-fold greater than in the untreated control. Laser

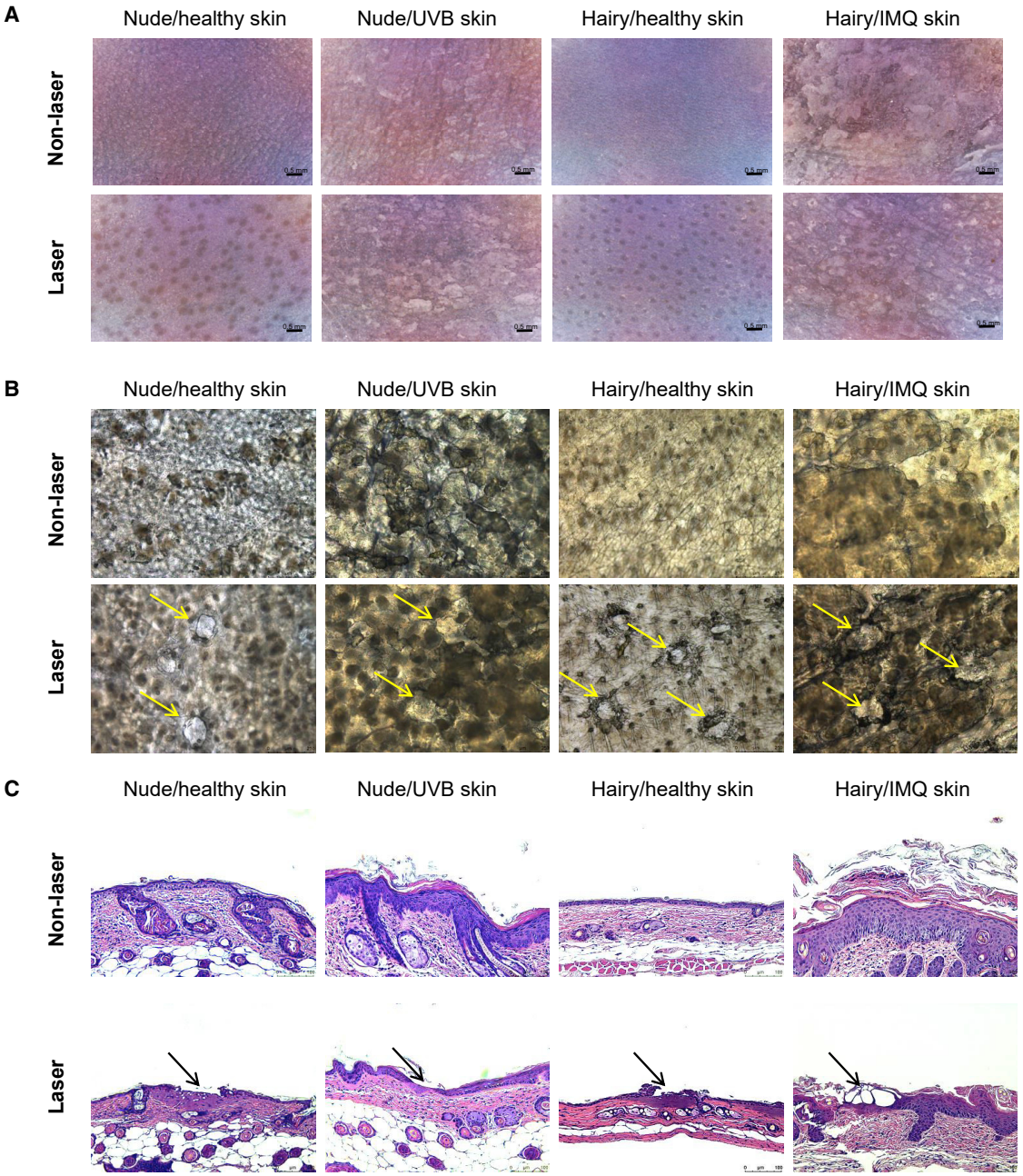

Figure 1. Macroscopic and Microscopic Observations of the Skin Treated by Fractional CO_2 Laser-Treated Skin as a Function of Different Recovery Times
(A) Skin surface imaging captured by handheld digital magnifier. (B) Skin surface imaging captured by optical microscopy. (C) H&E staining of the skin observed by optical microscopy. Arrows in the images indicate the micropores.

treatment on de-protein mouse skin increased 5-FU flux from 14 to 71 nmol/cm^2, which was a 13-fold enhancement. A 3-fold increase was observed in the case of de-protein pig skin.

Permeation via Diseased Skins

Photoaging and psoriasis-like skins were induced by treating UVB and IMQ in nude and hairy mice, respectively. The permeation of siRNA and 5-FU into (skin deposition) and across (flux) the diseased skin was evaluated as shown in Figure 3. The deposition in animal skin can be a predictor of permeant accumulation in a superior skin layer. As shown in Figures 3A and 3B, the laser was unable to enhance the flux and skin deposition of siRNA via photoaging skin. Fractional laser treatment led to increased siRNA flux across healthy BALB/c mouse skin, with a 35-fold enhancement versus the untreated control. The

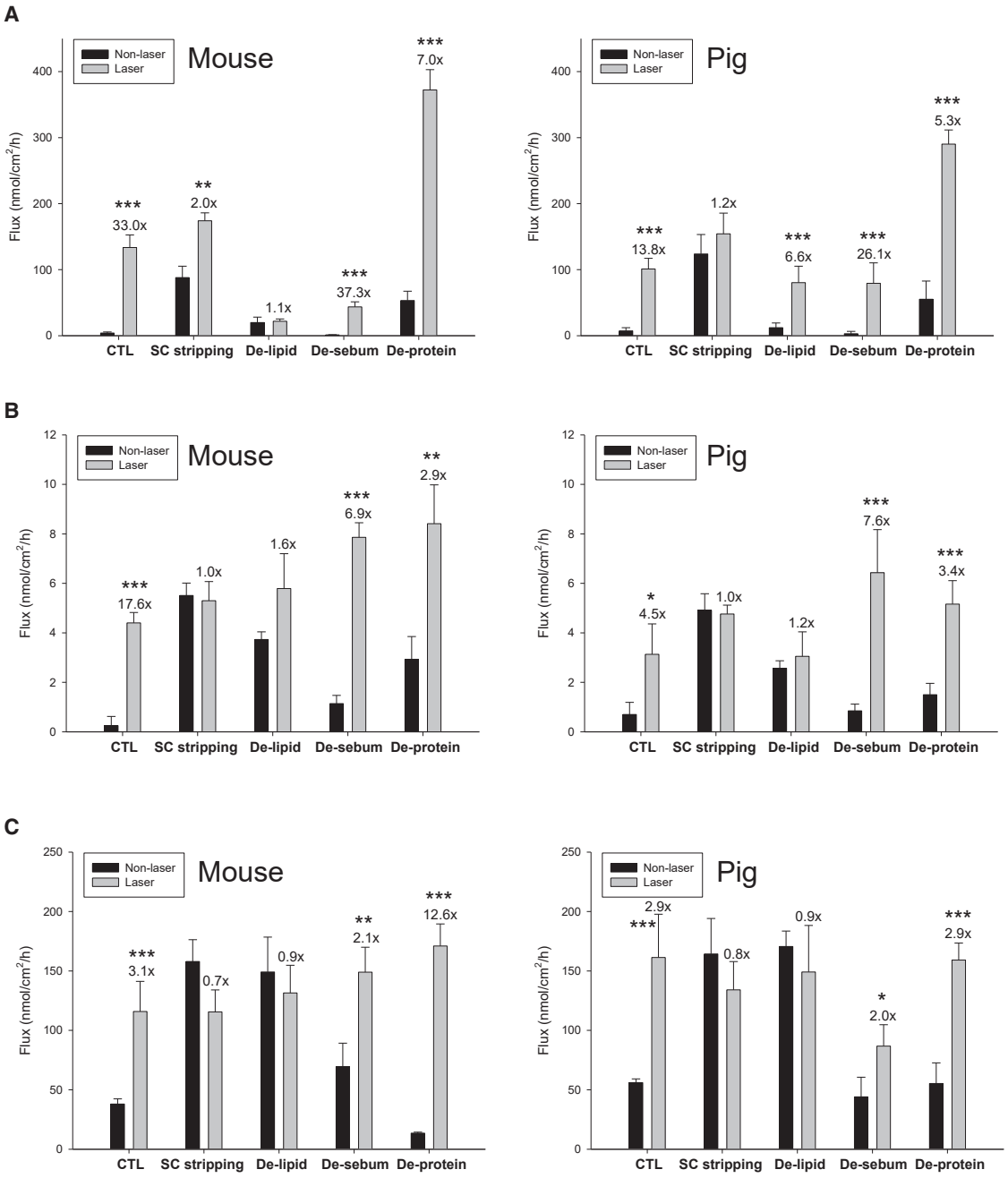

Figure 2. Flux (nmol/cm^2/h) of the Permeants via Intact and Barrier-Defective Skins with or without Fractional CO_2 Laser Treatment

(A) Flux of fluorescently labeled siRNA via nude mouse and pig skins. (B) Flux of fluorescently labeled peptide via nude mouse and pig skins. (C) Flux of 5-FU via nude mouse and pig skins. Data are presented as the mean of four experiments ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

BALB/c mouse skin permeability for siRNA significantly increased after IMQ treatment, indicating the loss of barrier function in the psoriasiform type. A similar trend was observed in the skin deposition of IMQ-treated skin versus normal skin, although the enhancement level was much lower than that of the flux. The laser intervention permitted a 3- and 2-fold increase in psoriasis-like skin flux and deposition, respectively. Figures 3C and 3D show the flux and skin deposition of 5-FU in the diseased skin. An 8-fold increase in 5-FU flux was found using the laser as compared to the passive permeation in the UVB-treated skin. However, the 5-FU deposition lessened after laser intervention on photoaging skin. A significant enhancement in hairy mouse flux and deposition with laser irradiation was achieved, with the

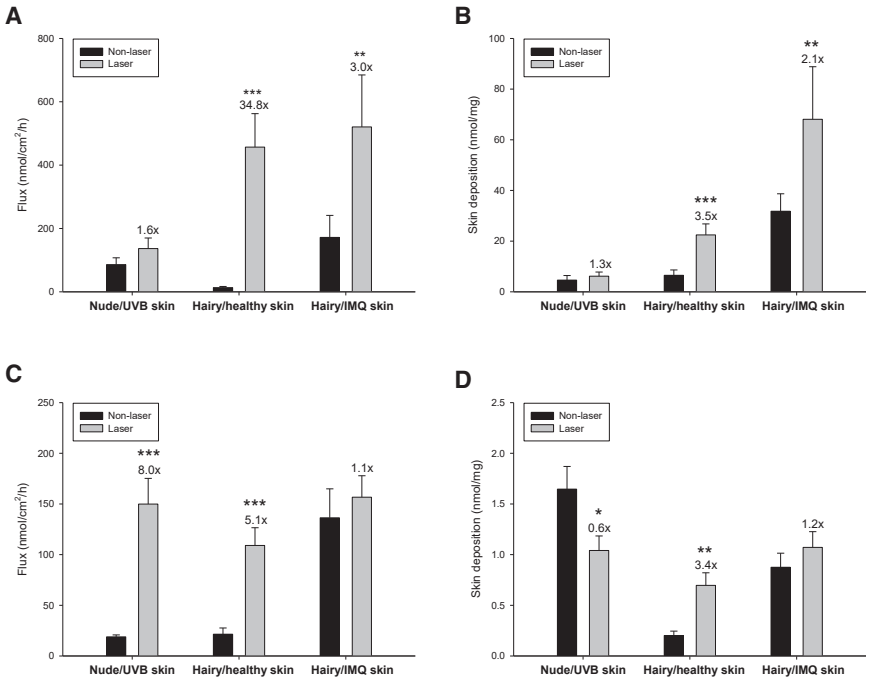

Figure 3. Flux (nmol/cm²/h) and Skin Deposition (nmol/mg) of the Permeants via Intact and Diseased Skins with or without Fractional CO_2 Laser Treatment

(A) Flux of fluorescently labeled siRNA via nude and hairy skins. (B) Skin deposition of fluorescently labeled siRNA in nude and hairy skins. (C) Flux of 5-FU via nude and hairy skins. (D) Skin deposition of 5-FU in nude and hairy skins. The data are presented as the mean of four experiments ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

enhancement being 5- and 3-fold greater than in the control group, respectively. 5-FU flux through laser-treated psoriatic skin was found to be 136 nmol/cm^2/h, which was comparable to the untreated control (157 nmol/cm^2/h). The laser also did not promote 5-FU deposition in psoriatic skin. This demonstrates the usefulness of CO_2 laser to assist 5-FU absorption in the IMQ-treated skin.

Distribution of siRNA and Peptide in the Skin

Cutaneous distribution of fluorescently labeled siRNA and peptide was acquired by confocal microscopy in a horizontal scanning fashion. Following the application of macromolecules using the laser approach, the skin sample was imaged as shown in Figure 4. The untreated skin served as a negative control, and no fluorescence was visualized in the skin tissue (data not shown). Figure 4A illustrates siRNA distribution in healthy and photoaging nude mouse skins with and without laser irradiation. The left panel of each group in the planar image is the summing of 15 separate sections. The right panel is the 3D skin structure seen in the photographs. A faint fluorescence distribution was observed in the healthy skin without laser treatment. UVB irradiation on the skin could increase siRNA distribution. After laser exposure, the distribution of siRNA fluorescence increased. The confocal images demonstrate a homogeneous siRNA distribution in the laser-treated photoaging skin. It was shown that laser-mediated siRNA could be delivered into the deeper layers of photoaging skin. In the case of psoriatic skin as shown in Figure 4B, the passive transport of siRNA into healthy BALB/c mouse skin has resulted in superficial distribution. Because of the barrier disruption by IMQ-induced inflammation, the fluorescence was increased. Following fractional ablation, there was pronounced siRNA distributed within the healthy and psoriatic skins with deeper penetration compared to the control. The green fluorescence was evident around the microchannels (arrows). An intense fluorescence was visualized at sites lateral to the channels, indicating a radial siRNA diffusion.

As seen in Figure 4C, the passive peptide application resulted in the distribution within the superficial layer of normal nude mouse skin. UVB exposure was able to increase peptide absorption. The laser further facilitated peptide delivery into the healthy and photoaging skins. We could not find the obvious microchannels in the laser-treated healthy skin. In the case of laser-treated photoaging skin, the peptide appeared to migrate along the microchannels and into the deeper skin strata (arrows). The openings of the micropores could be clearly visualized. The diameter of the peptide-stained area was larger than that of siRNA, suggesting a significant peptide spread from the channels via lateral diffusion. The same result was detected in the peptide application on psoriasiform skin, as shown in Figure 4D. Laser treatment created the microchannels to form the depots for peptide residence, followed by the perpendicular and lateral diffusion to increase the penetration depth and distribution.

Laser-Assisted siRNA Delivery for Silencing IL-6 in Psoriasiform Skin

A psoriatic skin model in mice was employed to examine the *in vivo* activity of IL-6 siRNA delivered by laser assistance. The capability of siRNA to induce a therapeutic response was first proved *in vitro* as revealed in Figure 5A. Incubation with siRNA resulted in 55% IL-6 knockdown relative to the control in 3T3-L1 cells. These data confirmed the IL-6 gene-silencing effect using siRNA. No significant cytotoxicity was detected following incubation with siRNA. To determine whether silencing IL-6 expression in psoriasiform skin by laser-assisted siRNA leads to therapeutic efficacy, we first assessed H&E-stained histology as shown in Figure 5B. The representative images exhibited the severity of hyperplasia and scaling in the order of IMQ ≥ IMQ+siRNA > IMQ+siRNA$^+$laser > untreated healthy control. The laser group proved to be more effective than the group without laser intervention. No microchannel was revealed in the laser-treated skin. This was because the micrographs were taken 3 days post-irradiation. The wounds were closed and

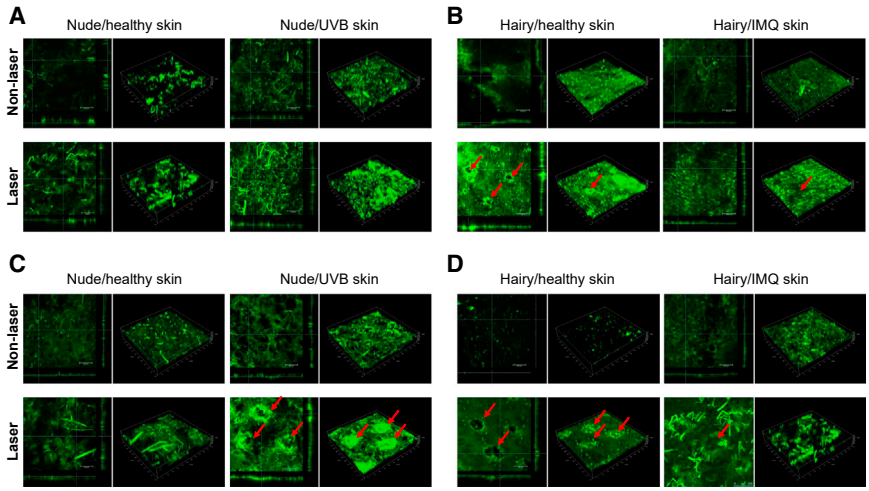

Figure 4. Biodistribution of the Permeants in the Fractional CO_2 Laser-Treated Skin Monitored by Confocal Microscopy

(A) Topical application of fluorescently labeled siRNA on nude mouse skin with or without UVB treatment. (B) Topical application of fluorescently labeled siRNA on hairy mouse skin with or without IMQ treatment. (C) Topical application of fluorescently labeled peptide on nude mouse skin with or without UVB treatment. (D) Topical application of fluorescently labeled peptide on hairy mouse skin with or without IMQ treatment. The left panel is the 2D (x-y axis) imaging of the skin structure. The right panel is the 3D (x-y-z axis) imaging of the skin structure.

recovered on the intact skin. In addition to H&E staining, specific psoriatic biomarkers were analyzed using immunohistochemistry. The increased IL-6 and IL-17A expression was observed for IMQ-treated skin compared to healthy skin as shown in Figures 5C and 5D, respectively. The immunohistochemical staining displayed a markedly decreased brown color for IL-6 and IL-17A by laser-assisted siRNA, signifying reduced inflammation and epidermal thickening.

The impact on keratinocyte proliferation was analyzed by Ki67, a proliferation marker highly expressed in psoriatic skin. Figure 5E represents a broad distribution of Ki67 throughout the basal layer of the epidermis following IMQ stimulation. Topically applied siRNA produced a decrease in Ki67 staining, with greater reduction by the laser group. Increased neutrophil infiltration in the dermis is profound in psoriatic lesions. The presence of brown staining by Ly6G in Figure 5F is considered a positive identification for neutrophils. A marked neutrophil migration was apparent in the dermis of the IMQ group. Laser treatment caused a significant decrease in neutrophils. Figure 5G shows the epidermal thickness calculated by histology. The thickness was increased from 12 to 164 μm by IMQ stimulation, while the thickness for siRNA treatment with and without laser was reduced to 75 and 146 μm, respectively. Figure 5H shows the microabscesses estimated by histopathology. The microabscesses were reduced by siRNA application with laser by 2-fold less than those in the IMQ group. Transdermal water loss (TEWL) as an indicator of skin-barrier function was determined as shown in Figure 5I. A 4-fold increase of TEWL was achieved in the psoriatic lesions as compared to normal skin. The application of siRNA did not improve the barrier function disrupted by IMQ. We used the ELISA method to quantify the psoriasis-related cytokines in the skin. These included IL-6, IL-17A, IL-23, and tumor necrosis factor alpha (TNF-α) (Figures 6A to 6D). IMQ activation resulted in the increase of all cytokines compared with the control. Topical siRNA administration without laser treatment was unable to reduce the protein expression. In contrast, laser-assisted siRNA reduced cytokine expression significantly compared to the IMQ group. The laser-mediated siRNA application could lower the interleukin family to the normal control baseline. The laser intervention induced a knockdown of siRNA with a 64% inhibition.

DISCUSSION

RNAi strategy is valid for treating inflamed skin diseases such as psoriasis, atopic dermatitis, and contact allergy. The conventional route of siRNA delivery for dermatological treatment is injection. However, the associated intense pain and difficult self-administration have limited the use of injection. The less invasive topical delivery is a focus for improving the siRNA delivery approach. The intrinsic barrier nature of the skin combined with the large molecule and negative charge leads to restricted siRNA skin permeation. Our previous work demonstrated the effective enhancement of siRNA absorption by low-fluence laser irradiation on the skin.[16,17] Nevertheless, whether this enhanced delivery can silence the gene to show the promising therapeutic benefit has not yet been established. In the present study, we used laser-assisted siRNA delivery for treating psoriasiform lesions via IL-6 silencing. The micropores formed by the laser ablation enabled the facile entry of siRNA and other low-absorption permeants into the skin. This effect still remained in the barrier-defective skins. The *in vivo* result demonstrated a significant improvement of the psoriatic lesions by laser-mediated siRNA administration. This is the first report of successful *in vivo* application of topical siRNA delivery using laser microporation.

Fractional laser illuminates the skin with a distinct microchannel array. The intact tissue around the microchannels accelerates the healing of the treated tissue, shortening the recovery time.[18] Our previous study suggested the closure of the CO_2 laser-treated micropore opening within 16 h.[19] The lining of coagulation surrounding the micropores was found after laser treatment. According to the microscopic observation, the micropores were smaller than the irradiated area of the fractional laser dot (300 μm). The effective treatment zone on the laser-treated skin may contain the micropores and thermal coagulation. The skin was artificially prepared to develop barrier-defective models for mimicking diseased skin in a controllable

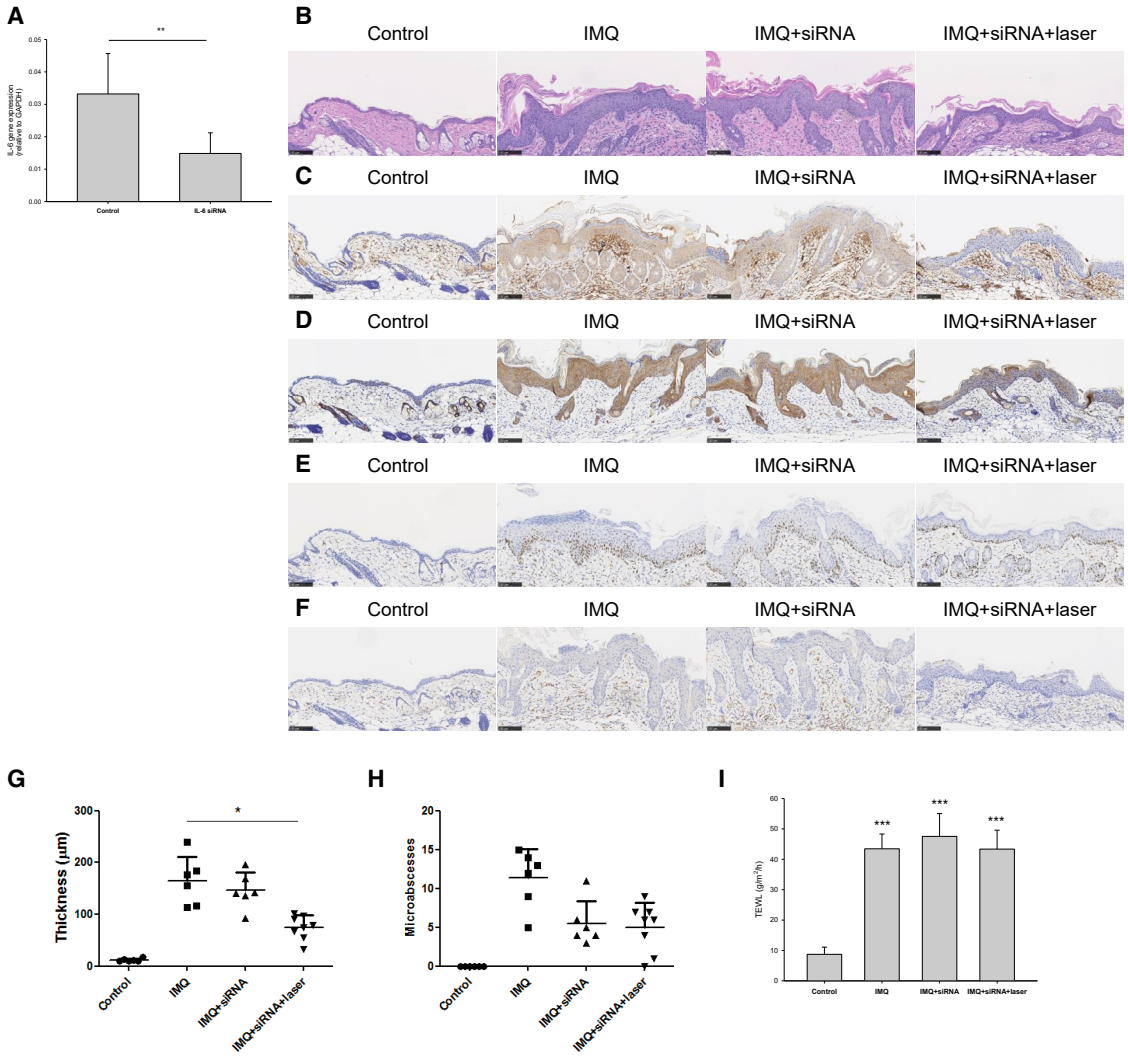

Figure 5. *In Vivo* Topical Application of IL-6 siRNA in IMQ-Induced Psoriasiform Lesion with or without Fractional CO_2 Laser Treatment

(A) IL-6 gene expression in 3T3-L1 cells with treatment by siRNA. (B) H&E staining of the skin observed by optical microscopy. (C) Immunohistochemical IL-6 staining of the skin observed by optical microscopy. (D) Immunohistochemical IL-17A staining of the skin observed by optical microscopy. (E) Immunohistochemical Ki67 staining of the skin observed by optical microscopy. (F) Immunohistochemical Ly6G staining of the skin observed by optical microscopy. (G) Epidermal thickness quantified by H&E staining. (H) Total counts of abscesses by H&E staining. (I) TEWL measurement. Data are presented as the mean of six to eight experiments ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

condition. These included SC stripping, lipid removal, sebum removal, and protein denaturalization.

The drugs should be lipophilic and small to achieve a meaningful passive permeation across intact skin. Healthy skin is almost impermeable for the charged, hydrophilic, and large molecules.[20] The permeants selected in this study are the molecules with poor skin penetration. Topical delivery of siRNA, peptide, and 5-FU remains a challenge. 5-FU was proved to be efficient for treating psoriasis, vitiligo, actinic keratosis, and skin tumors.[21] The CO_2 laser-assisted 5-FU delivery is evaluated in clinical trials for skin-cancer management.[22–24] Since the topical drug therapy is always applied on the barrier-defective diseased skin, we first prepared the impaired skin according to the standardized methods for examining permeation. The greater enhancement of siRNA flux by tape stripping indicated an important barrier of SC. It was possible that siRNA interacted with the SC and hence SC stripping dramatically elevated its delivery. The diffusion routes of the SC are mainly the lipid bilayers and intercellular proteins. The de-protein skin showed a greater siRNA permeability than did the de-lipid skin, demonstrating the stronger barrier of protein compared to lipid for siRNA transport. A contrary tendency was detected for peptide delivery across de-lipid and de-protein skins. The sebaceous glands produce sebum to cover the outermost skin surface. The permeant should partition into the sebum layer and then penetrate the SC.[25] Sebum removal significantly decreased siRNA

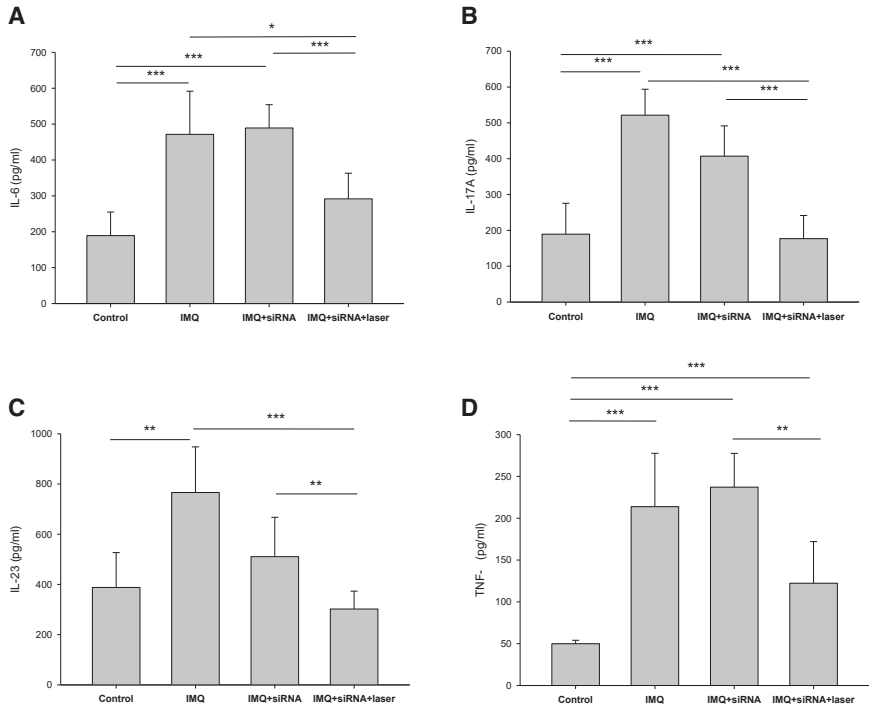

Figure 6. Cytokine Expression in the Skin after *In Vivo* Topical Application of IL-6 siRNA in IMQ-Induced Psoriasiform Lesion with or without Fractional CO_2 Laser Treatment

(A) IL-6. (B) IL-17A. (C) IL-23. (D) TNF-α. Data are presented as the mean of six to eight experiments ± SD. *p < 0.05, **p < 0.01, ***p < 0.001.

flux compared to intact skin, suggesting the importance of sebum partitioning for siRNA permeation.

A previous study[26] manifested the difficulty of peptide partitioning to a lipophilic skin surface. This difficulty has led to the higher peptide permeation via de-sebum skin than via intact skin. The sebum was not as significant for 5-FU partitioning due to the comparable flux between intact and de-sebum skins. The permeation enhancement of 5-FU by SC stripping was notably lower as compared to that for macromolecules. This indicates that the small molecules are still better able than macromolecules to pass across the SC. The cutaneous transport of hydrophilic small molecules is predominantly attributed to the polar pathway. This is why 5-FU flux was increased by protein removal but not lipid extraction.

Our results demonstrated that fractional laser treatment overcame the barrier of the SC to enhance the permeation of all permeants tested. This denoted the usefulness of laser-mediated delivery for the permeants with a molecular weight (MW) range from at least 130 to 8,490 g/mol. The SC is usually the main barrier for the delivery of macromolecules and hydrophilic molecules. The creation of microchannels by fractional laser treatment exposed the permeants to the underlying epidermis with a hydrophilic matrix.[27] The fractional pathway filled with aqueous permeant vehicle to produce hydrophilic channels in the viable tissue. The permeants facilely traversed into the channels to partition into the viable skin for broad biodistribution. The effortless delivery into the microchannels was evidenced by confocal imaging. The formation of microchannels increased the total contact surface area of the skin for drug delivery. In addition to vertical penetration into the deeper skin layer, the permeants in the microchannels diffused to the lateral region via radial permeation.[28] The confocal micrographs illustrated the possibility of horizontal diffusion from the channels. The smaller molecular size of the peptide compared to siRNA contributed to the larger diffusion radius of the horizontal penetration area. The irradiated area of the dot of the fractional laser was 300 μm, which was an overestimation of the created micropores (150–200 μm) according to microscopic imaging. This was probably due to the formation of thermal coagulation around the micropores by the fractional CO_2 laser dot.[29] The constituents of coagulation are mainly the platelets and fibrin fibers. The matrix serves as a protective shield against pathogenic microbes.[30] Hence, it is also the obstruction for drug delivery.[31] This layer prevented a fast release of macromolecules from the channels to the surrounding tissue, establishing a permeant reservoir inside the micropores for prolonged and sustained delivery. The filling of fluorescently labeled microchannels inside some pores could confirm this inference. The thermal denaturation hinders drug passage across the microchannels, especially for hydrophilic small molecules.[31–33] This could be the reason for the low enhancement level of 5-FU flux after fractional laser exposure.

The fractional laser was still effective in inducing the enhancement of permeant delivery in barrier-defective and diseased skin, although the enhancement level was lower than that in the intact skin. Tape stripping can be a robust model for dermatitis.[34] The laser did not promote the flux of all permeants across SC-stripped skin, except siRNA penetration via SC-stripped mouse skin. This indicates that the laser almost eliminated the barrier function of the SC. De-lipid skin mimics the diseased condition of psoriasis, xerosis, and ichthyosis.[35] The denaturation of protein in the SC resembles the model of atopic dermatitis and photoaging.[36] Our results demonstrated a lesser enhancement of laser-mediated delivery in de-lipid skin than in de-protein skin, suggesting a stronger laser impact on lipid bilayers than on proteins and corneocytes. The CO_2 laser can elicit a photomechanical wave for the enlargement of lacunar space in lipid bilayers, facilitating drug passage.[7] Both photoaging and psoriatic skins are characterized by an impaired barrier property. The fractional laser assisted the delivery of permeants into the diseased skins. The enhancement was lower compared to that of healthy skin because

of the already compromised barrier in the inflamed skin. The comparison of different permeants generally showed the greatest absorption enhancement for laser-assisted siRNA in all skin types. This suggests greater selectivity of siRNA to diffuse through the microchannels than for the other permeants.

An ideal delivery approach for topical siRNA therapy is to enhance nucleic acid transport across the SC and facilitate efficient uptake by target cells.[37] We further delivered IL-6 siRNA into the skin in a targeted fashion by using the fractional laser *in vivo*. IL-6 plays a critical role in the psoriasis triggering. We observed that IL-6 was significantly inhibited by laser-assisted siRNA. The result also demonstrated that the laser reduced cytokines, keratinocyte proliferation, and inflammation, which was less than with the passive siRNA delivery and IMQ-only stimulation. The silencing effect and anti-inflammatory ability in psoriasis-like plaque could be the result of laser ablation for enhancing siRNA permeation. In addition to IL-6, IMQ is reported to elevate cytokines IL-17 and IL-23.[38] IL-17 can synergize with TNF-α to activate psoriatic inflammation.[39] The neutralizing of TNF-α is an important indicator for therapeutic efficiency against psoriasis. All of these cytokines were downregulated by laser-mediated siRNA in our *in vivo* psoriasis-like model. The reduction of proinflammatory cytokines led to the repair of IMQ-induced skin inflammation and damage. No microchannel or coagulation was observed in the skin histology taken 3 days after laser irradiation, indicating a safe use of the fractional laser for psoriasis mitigation. The 3-(4,5-dimethylthiazol-2-yl)-2,5-dimethyltetrazolium bromide (MTT)-based assay in 3T3-L1 cells also revealed that IL-6 siRNA was nontoxic.

Once siRNA delivers into the skin, additional effort is necessary to reach the target cells and promote the cellular uptake. Transporting the nucleic acid family into the cells is problematic because of the large size and negative charge.[12] A previous study[40] has suggested a negligible knockdown of IL-6 by siRNA due to the insufficient cell uptake. In order to improve the nucleic acid uptake into the cells, an active approach is especially beneficial. Our data showed an efficient IL-6 silencing by laser treatment. A laser-enhanced cell uptake was possible. IL-17A is mainly released by neutrophils, T cells, and innate lymphoid cells. IL-6 is known as an upstream cytokine of IL-17A.[41] In psoriatic lesions, keratinocytes and dendritic cells produce a high level of IL-23.[39] The laser irradiation might increase the uptake in a variety of cells. Previous studies[26,42] have revealed that the fractional laser can act at a cellular level to increase the uptake of macromolecular vaccines and nanoparticles. Further study is needed to explore the detailed mechanisms of laser-assisted cell uptake. Cutaneous diseases related to inflammation are suitable for siRNA therapy because of the presence of determined molecular targets for silencing. Our results suggested that laser-assisted IL-6 siRNA could inhibit psoriatic plaque in a mouse model in the absence of transfection reagents. The enhanced knockdown was achieved with only one application of laser pulse and could be sustained over time. Some approaches, including ultrasound, iontophoresis, electroporation, and microneedles, are employed to facilitate nucleic acid permeation across the SC. Most of them need a prolonged period of application to obtain enhanced efficiency. Fractional laser is an ideal permeation-enhanced strategy with the advantages of a very short application time and avoidance of cross-contamination risk.

The present study had some limitations. We chiefly evaluated the skin delivery of the permeants by the *in vitro* Franz cell. The use of excised skin *in vitro* may exhibit some differences from the *in vivo* and clinical status. Although mouse and pig skins are extensively accepted as an alternative model for human skin, it is difficult to compare the cutaneous delivery in the different skin types. Further study is needed to clarify the laser-mediated siRNA absorption in humans. Another concern is that the laser-enhanced IL-6 siRNA transport could not completely reverse the psoriatic lesion to the normal baseline according to histology and TEWL. A complete silencing of the gene target is not required to accomplish the therapeutic benefit.[43] A previous study[3] suggests that a 50% expression reduction in the target gene via topical RNAi is adequate to treat keratinizing skin diseases. The experimental data showed a 64% decline of IL-6 expression by fractional CO_2 laser in the psoriasis mouse model, verifying the usefulness of the laser approach.

Conclusions

To date, several RNAi clinical trials have been conducted, but only one product (Patisiran) has obtained USFDA approval. RNAi has the potential for skin-disease treatment if the delivery approaches can be improved. Here, we develop fractional laser-mediated delivery for topical siRNA application. In this study, the macromolecules and hydrophilic small molecules were transported through the breached SC along the microchannels to reach the deeper skin strata. In the *in vivo* psoriasis-like lesion model, laser-assisted siRNA delivery was able to silence IL-6 in the epidermis. The psoriatic plaque was thus ameliorated. Laser-assisted siRNA permeation proved to be a potential therapeutic management for psoriasis. Further clinical study is warranted to verify the possible application of RNAi therapy by laser aid.

MATERIALS AND METHODS

Permeants

The siRNA containing fluorescein isothiocyanate (FITC) for the skin-permeation test exhibited the sequences of 5′-FITC-UGCUGACUCCAAAG-3′ and 3′-ACGACUGAGGUUUC-5′ (MW = 8,490 g/mol). The IL-6 siRNA sequences were 5′-UUCUCCGAACGUGUCACGUTT-3′ and 3′-ACGUGACACGUUCGGAGAATT-5′ (MW = 12,856 g/mol). Both siRNAs were synthesized by GenZyme (Miaoli, Taiwan). The FITC-conjugated peptide with the sequence of FITC-NH_2-Pro-Arg-Leu-Leu-Tyr-Ser-Trp-His-Arg-Ser-His-Arg-Ser-His-COOH was purchased from Biotools (New Taipei City, Taiwan). 5-FU was provided by Sigma-Aldrich (St. Louis, MO, USA).

Animals

The 8-week-old female nude and BALB/c mice were supplied by the National Laboratory Animal Center (Taipei, Taiwan). The pathogen-free 1-week-old pig was purchased from the Animal Technology

Institute of Taiwan (Miaoli, Taiwan). All experiments were conducted in strict accordance with the recommendations in the *Guidelines for the Care and Use of Laboratory Animals* of Chang Gung University.

Preparation of Barrier-Defective and Diseased Skins

Adhesive tape (Scotch, 3M) was applied to the surface of the excised nude mouse or pig skin and then removed, with the application/removal being done a total of 20 times to achieve SC-stripped skin. De-lipid skin was prepared by incubating the skin surface with chloroform/methanol (2:1) for 2 h. The de-sebum skin was obtained by a cold hexane (4°C) wash repeated five times.[44] We prepared the de-protein skin using ethanol/water (2:3) incubation on the skin surface for 2 h.[45] In the induction of photoaging skin, the back of the nude mice was exposed under UVB at 312 nm (Vilber Lourmat, Marne-la-Vallée, France).[46] The spectral irradiance was 175 mJ/cm^2. The dorsal area was exposed to UVB once a day for 5 days. The induction of a psoriasis-like lesion was performed according to the previous protocol.[47] In brief, the skin on the shaved back of the hairy mice was topically applied with IMQ cream (Aldara, 3M) at a dose of 62.5 mg each day for 6 consecutive days. The applied region was covered with Tegaderm and nonwoven cloth after the IMQ application.

Laser System

The fractional CO_2 laser (Mosaic eCO_2, Lutronic, San Jose, CA, USA) emitted a 10,600-nm irradiation with a scanning area of 14 × 14 mm. There were 400 spots in 1 cm^2 to produce a microscopic thermal zone (MTZ). The MTZ diameter was 300 μm with a fluence of 4 mJ and pulse duration of 80 μs.

The Appearance of the Skin Surface

The intact nude and BALB/c mice skins as well as UVB- and IMQ-treated skins were irradiated *in vivo* by CO_2 laser. The gross observation of the skin surface was accomplished using a handheld digital magnifier (Mini Scope-V, M&T Optics, Taipei, Taiwan). The microscopic observation of the skin surface was achieved by optical microscopy (DMi8, Leica, Wetzlar, Germany) with ×200 magnification.

H&E Staining

The excised skin with or without laser treatment was immersed in a 10% buffered formalin using ethanol embedded in paraffin wax; it was sliced at a thickness of 3 μm for H&E staining. The longitudinal section of the skin was visualized by optical microscopy.

Skin Permeation Assessment

Franz cell was used to assess the skin delivery of the permeants. The intact or barrier-defective skin with or without laser exposure was mounted between the donor and receptor with the SC facing toward the donor. The donor compartment was filled with 0.5 mL of FITC-conjugated siRNA (0.4 μM) in nuclease-free water, peptide (150 μM) in pH 7.4 buffer, or 5-FU (15 mM) in pH 7.4 buffer. The receptor medium was nuclease-free water for siRNA. The pH 7.4 buffer was the receptor solution for peptide and 5-FU. The effective permeation area was 0.789 cm^2. The stirring rate and receptor temperature were kept at 600 rpm and 37°C, respectively. The 300-μL aliquot in the receptor was withdrawn at the determined intervals. At the end of the experiment (24 h), the skin was removed to determine the skin deposition of the permeants. The skin was extracted by 0.1 N HCl in a homogenizer (MagNA Lyser, Roche). The homogenate was centrifuged at 10,000 × *g* for 10 min. The receptor amount and skin deposition of siRNA and peptide were quantified by fluorescence spectrophotometry. 5-FU was analyzed by high-performance liquid chromatography (HPLC) according to the previous condition.[48]

Distribution of siRNA and Peptide in the Skin

siRNA or peptide in the medium the same as the skin permeation assessment was applied in the Franz cell donor. The application duration was 6 h. Then, the skin was washed with water. The skin was positioned onto the stage plate of a confocal laser scanning microscope (TCS SP2, Leica). The skin thickness was scanned at 5-μm increments via z axis from the surface. The images were summed by 15 fragments. The 3D image of the skin was processed by confocal microscopy software.

IL-6 Silencing by siRNA in Cells

The mouse 3T3-L1 cell line was cultured in DMEM and was then supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin in 5% CO_2 at 37°C. The cells were transfected by IL-6 siRNA with Lipofectamine 2000 for 4 h. After an incubation of 24 h, the cells were collected to detect the mRNA level of IL-6. Total RNA was extracted by TRIzol (Thermo Fisher Scientific). cDNA was synthesized by reverse transcription with an iScript cDNA synthesis kit (Bio-Rad). Quantitative real-time PCR was carried out by iQ SYBR Green Supermix (Bio-Rad). The GAPDH gene expression was employed as a housekeeping gene to normalize IL-6 gene expression. The 3T3-L1 cell viability was analyzed by an MTT assay.

In Vivo Topical siRNA Administration on Psoriasiform Lesions

The BALB/c mice with IMQ treatment were enrolled in this experiment. IMQ cream was topically applied on the mouse back for 6 days. On day 3, a fractional laser was irradiated on the dorsal area. IL-6 siRNA at 20 μM (60 μl) was then applied on the mouse back. We applied siRNA each day for 3 days. TEWL was monitored by Tewameter TM300 (Courage and Khazaka, Köln, Germany). The animals were sacrificed on day 6 for the further examination of skin histology and cytokines.

Immunohistochemical Staining

The skin samples were fixed in 10% formalin buffered in phosphate saline for immunohistochemical staining. The detailed procedure was described in the previous study.[49] The skin sections were incubated with anti-IL-6 antibody, anti-IL-17A antibody, anti-Ki67 antibody, and anti-Ly6G antibody for 1 h, and subsequently incubated with biotinylated donkey anti-goat IgG for 20 min. Photomicrographs were taken using Leica DMi8 microscopy.

Cytokine Measurement

The number of cytokines in the skin was assessed by ELISA. The detailed process was shown in our previous work.[49] The level of

IL-6, IL-17A, IL-23, and TNF-α was measured using commercial kits (BioLegend, San Diego, CA, USA) according to the manufacturer's instructions.

Statistical Analysis

The statistical difference in the data of the different treatment groups was analyzed using a Kruskal-Wallis test. The *post hoc* test for checking individual differences was the Dunn's test. The 0.05, 0.01, and 0.001 levels of probability were taken as statistically significant.

AUTHOR CONTRIBUTIONS

J.-Y.F. initiated the study and drafted the manuscript. J.-Y.F. and W.-R.L. involved in the design of all experiments. W.-R.L., Y.-K.L., and P.-Y.L. carried out the experiments. Y.-K.L .and A.A. analyzed data and wrote the manuscript. J.-Y.F. supervised the entire project. W.-R.L. and P.-W.W. reviewed critically and approved the final manuscript. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare no competing interests.

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REFERENCES

1. Adams, D., Gonzalez-Duarte, A., O'Riordan, W.D., Yang, C.C., Ueda, M., Kristen, A.V., Tournev, I., Schmidt, H.H., Coelho, T., Berk, J.L., et al. (2018). Patisiran, an RNAi therapeutic, for hereditary transthyretin amyloidosis. N. Engl. J. Med. *379*, 11–21.
2. Hay, R.J., Johns, N.E., Williams, H.C., Bolliger, I.W., Dellavalle, R.P., Margolis, D.J., Marks, R., Naldi, L., Weinstock, M.A., Wulf, S.K., et al. (2014). The global burden of skin disease in 2010: an analysis of the prevalence and impact of skin conditions. J. Invest. Dermatol. *134*, 1527–1534.
3. Hegde, V., Hickerson, R.P., Nainamalai, S., Campbell, P.A., Smith, F.J., McLean, W.H., and Pedrioli, D.M. (2014). In vivo gene silencing following non-invasive siRNA delivery into the skin using a novel topical formulation. J. Control. Release *196*, 355–362.
4. Rosa, J., Suzuki, I., Kravicz, M., Caron, A., Pupo, A.V., Praça, F.G., and Bentley, M.V.L.B. (2018). Current non-viral siRNA delivery systems as a promising treatment of skin diseases. Curr. Pharm. Des. *24*, 2644–2663.
5. Ita, K. (2017). Dermal/transdermal delivery of small interfering RNA and antisense oligonucleotides- advances and hurdles. Biomed. Pharmacother. *87*, 311–320.
6. Aljuffali, I.A., Lin, C.F., Chen, C.H., and Fang, J.Y. (2016). The codrug approach for facilitating drug delivery and bioactivity. Expert Opin. Drug Deliv. *13*, 1311–1325.
7. Lin, C.H., Aljuffali, I.A., and Fang, J.Y. (2014). Lasers as an approach for promoting drug delivery via skin. Expert Opin. Drug Deliv. *11*, 599–614.
8. Zorec, B., Škrabelj, D., Marinček, M., Miklavčič, D., and Pavšelj, N. (2017). The effect of pulse duration, power and energy of fractional Er:YAG laser for transdermal delivery of differently sized FITC dextrans. Int. J. Pharm. *516*, 204–213.
9. Lee, W.R., Shen, S.C., Sung, C.T., Liu, P.Y., and Fang, J.Y. (2018). Is the fractional laser still effective in assisting cutaneous macromolecule delivery in barrier-deficient skin? Psoriasis and atopic dermatitis as the disease models. Pharm. Res. *35*, 128.
10. Greb, J.E., Goldminz, A.M., Elder, J.T., Lebwohl, M.G., Gladman, D.D., Wu, J.J., Mehta, N.N., Finlay, A.Y., and Gottlieb, A.B. (2016). Psoriasis. Nat. Rev. Dis. Primers *2*, 16082.
11. Desmet, E., Bracke, S., Forier, K., Taevernier, L., Stuart, M.C., De Spiegeleer, B., Raemdonck, K., Van Gele, M., and Lambert, J. (2016). An elastic liposomal formulation for RNAi-based topical treatment of skin disorders: Proof-of-concept in the treatment of psoriasis. Int. J. Pharm. *500*, 268–274.
12. Boakye, C.H.A., Patel, K., Doddapaneni, R., Bagde, A., Marepally, S., and Singh, M. (2017). Novel amphiphilic lipid augments the co-delivery of erlotinib and IL36 siRNA into the skin for psoriasis treatment. J. Control. Release *246*, 120–132.
13. Desmet, E., Van Gele, M., Grine, L., Remaut, K., and Lambert, J. (2018). Towards the development of a RNAi-based topical treatment for psoriasis: proof-of-concept in a 3D psoriasis skin model. Exp. Dermatol. *27*, 463–469.
14. Vaidya, T.S., and Alikhan, A. (2017). Treatment satisfaction among patients with psoriasis at a large academic center: an observational study. J. Dermatolog. Treat. *28*, 288–289.
15. Zakrewsky, M., and Mitragotri, S. (2016). Therapeutic RNAi robed with ionic liquid moieties as a simple, scalable prodrug platform for treating skin disease. J. Control. Release *242*, 80–88.
16. Lee, W.R., Shen, S.C., Zhuo, R.Z., Wang, K.C., and Fang, J.Y. (2009). Enhancement of topical small interfering RNA delivery and expression by low-fluence erbium:YAG laser pretreatment of skin. Hum. Gene Ther. *20*, 580–588.
17. Lee, W.R., Shen, S.C., Chen, W.Y., Aljuffali, I.A., Suen, S.Y., and Fang, J.Y. (2014). Noninvasive delivery of siRNA and plasmid DNA into skin by fractional ablation: erbium:YAG laser versus CO_2 laser. Eur. J. Pharm. Biopharm. *86*, 315–323.
18. Borges, J., Manela-Azulay, M., and Cuzzi, T. (2016). Photoaging and the clinical utility of fractional laser. Clin. Cosmet. Investig. Dermatol. *9*, 107–114.
19. Lee, W.R., Hsiao, C.Y., Huang, T.H., Wang, C.L., Alalaiwe, A., Chen, E.L., and Fang, J.Y. (2019). Post-irradiation recovery time strongly influences fractional laser-facilitated skin absorption. Int. J. Pharm *564*, 48–58.
20. Prausnitz, M.R., and Langer, R. (2008). Transdermal drug delivery. Nat. Biotechnol. *26*, 1261–1268.
21. Moore, A.Y. (2009). Clinical applications for topical 5-fluorouracil in the treatment of dermatological disorders. J. Dermatolog. Treat. *20*, 328–335.
22. Glenn, C.J., Parlette, E.C., and Mitchell, C. (2015). Fractionated CO_2 laser-assisted delivery of topical 5-fluorouracil as a useful modality for treating field cutaneous squamous cell carcinomas. Dermatol. Surg. *41*, 1339–1342.
23. Nguyen, B.T., Gan, S.D., Konnikov, N., and Liang, C.A. (2015). 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. *72*, 558–560.
24. Hsu, S.H., Gan, S.D., Nguyen, B.T., Konnikov, N., and Liang, C.A. (2016). Ablative fractional laser-assisted topical fluorouracil for the treatment of superficial basal cell carcinoma and squamous cell carcinoma in situ: a follow-up study. Dermatol. Surg. *42*, 1050–1053.
25. Schneider, D., Dennerlein, K., Göen, T., Schaller, K.H., Drexler, H., and Korinth, G. (2016). Influence of artificial sebum on the dermal absorption of chemicals in excised human skin: a proof-of-concept study. Toxicol. In Vitro *33*, 23–28.
26. Fujimoto, T., Wang, J., Baba, K., Oki, Y., Hiruta, Y., Ito, M., Ito, S., and Kanazawa, H. (2017). Transcutaneous drug delivery by liposomes using fractional laser technology. Lasers Surg. Med. *49*, 525–532.
27. Taudorf, E.H., Lerche, C.M., Erlendsson, A.M., Philipsen, P.A., Hansen, S.H., Janfelt, C., Paasch, U., Anderson, R.R., and Haedersdal, M. (2016). Fractional laser-assisted drug delivery: laser channel depth influences biodistribution and skin deposition of methotrexate. Lasers Surg. Med. *48*, 519–529.
28. Kumar, M.N.K., Zhou, C., and Wu, M.X. (2016). Laser-facilitated epicutaneous immunotherapy to IgE-mediated allergy. J. Control. Release *235*, 82–90.
29. Banzhaf, C.A., Lin, L.L., Dang, N., Freeman, M., Haedersdal, M., and Prow, T.W. (2018). The fractional laser-induced coagulation zone characterized over time by laser scanning confocal microscopy—a proof of concept study. Lasers Surg. Med. *50*, 70–77.
30. Minutti, C.M., Knipper, J.A., Allen, J.E., and Zaiss, D.M. (2017). Tissue-specific contribution of macrophages to wound healing. Semin. Cell Dev. Biol. *61*, 3–11.

31. Nguyen, H.X., and Banga, A.K. (2018). Delivery of methotrexate and characterization of skin treated by fabricated PLGA microneedles and fractional ablative laser. Pharm. Res. *35*, 68.

32. Choi, J.H., Shin, E.J., Jeong, K.H., and Shin, M.K. (2017). Comparative analysis of the effects of CO_2 fractional laser and sonophoresis on human skin penetration with 5-aminolevulinic acid. Lasers Med. Sci. *32*, 1895–1900.

33. Meesters, A.A., Nieboer, M.J., Kezic, S., de Rie, M.A., and Wolkerstorfer, A. (2018). Parameters in fractional laser assisted delivery of topical anesthetics: role of laser type and laser settings. Lasers Surg. Med. *50*, 813–818.

34. Abdel-Mottaleb, M.M.A., and Lamprecht, A. (2016). In vivo skin penetration of macromolecules in irritant contact dermatitis. Int. J. Pharm. *515*, 384–389.

35. Sahle, F.F., Gebre-Mariam, T., Dobner, B., Wohlrab, J., and Neubert, R.H. (2015). Skin diseases associated with the depletion of stratum corneum lipids and stratum corneum lipid substitution therapy. Skin Pharmacol. Physiol. *28*, 42–55.

36. Biniek, K., Levi, K., and Dauskardt, R.H. (2012). Solar UV radiation reduces the barrier function of human skin. Proc. Natl. Acad. Sci. USA *109*, 17111–17116.

37. González-González, E., Speaker, T.J., Hickerson, R.P., Spitler, R., Flores, M.A., Leake, D., Contag, C.H., and Kaspar, R.L. (2010). Silencing of reporter gene expression in skin using siRNAs and expression of plasmid DNA delivered by a soluble protrusion array device (PAD). Mol. Ther. *18*, 1667–1674.

38. Desai, P.R., Marepally, S., Patel, A.R., Voshavar, C., Chaudhuri, A., and Singh, M. (2013). Topical delivery of anti-TNFα siRNA and capsaicin via novel lipid-polymer hybrid nanoparticles efficiently inhibits skin inflammation in vivo. J. Control. Release *170*, 51–63.

39. Grine, L., Dejager, L., Libert, C., and Vandenbroucke, R.E. (2015). An inflammatory triangle in psoriasis: TNF, type I IFNs and IL-17. Cytokine Growth Factor Rev. *26*, 25–33.

40. Depieri, L.V., Borgheti-Cardoso, L.N., Campos, P.M., Otaguiri, K.K., Vicentini, F.T., Lopes, L.B., Fonseca, M.J., and Bentley, M.V.L.B. (2016). RNAi mediated IL-6 in vitro knockdown in psoriasis skin model with topical siRNA delivery system based on liquid crystalline phase. Eur. J. Pharm. Biopharm. *105*, 50–58.

41. Croxford, A.L., Karbach, S., Kurschus, F.C., Wörtge, S., Nikolaev, A., Yogev, N., Klebow, S., Schüler, R., Reissig, S., Piotrowski, C., et al. (2014). IL-6 regulates neutrophil microabscess formation in IL-17A-driven psoriasiform lesions. J. Invest. Dermatol. *134*, 728–735.

42. Chen, X., Shah, D., Kositratna, G., Manstein, D., Anderson, R.R., and Wu, M.X. (2012). Facilitation of transcutaneous drug delivery and vaccine immunization by a safe laser technology. J. Control. Release *159*, 43–51.

43. Lara, M.F., González-González, E., Speaker, T.J., Hickerson, R.P., Leake, D., Milstone, L.M., Contag, C.H., and Kaspar, R.L. (2012). Inhibition of CD44 gene expression in human skin models, using self-delivery short interfering RNA administered by dissolvable microneedle arrays. Hum. Gene Ther. *23*, 816–823.

44. Campbell, C.S., Contreras-Rojas, L.R., Delgado-Charro, M.B., and Guy, R.H. (2012). Objective assessment of nanoparticle disposition in mammalian skin after topical exposure. J. Control. Release *162*, 201–207.

45. Chuang, S.Y., Lin, Y.K., Lin, C.F., Wang, P.W., Chen, E.L., and Fang, J.Y. (2017). Elucidating the skin delivery of aglycone and glycoside flavonoids: how the structures affect cutaneous absorption. Nutrients *9*, 1304.

46. Hung, C.F., Chen, W.Y., Aljuffali, I.A., Lin, Y.K., Shih, H.C., and Fang, J.Y. (2015). Skin aging modulates percutaneous drug absorption: the impact of ultraviolet irradiation and ovariectomy. Age (Dordr.) *37*, 21.

47. van der Fits, L., Mourits, S., Voerman, J.S., Kant, M., Boon, L., Laman, J.D., Cornelissen, F., Mus, A.M., Florencia, E., Prens, E.P., and Lubberts, E. (2009). Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. J. Immunol. *182*, 5836–5845.

48. Fang, J.Y., Hung, C.F., Fang, Y.P., and Chan, T.F. (2004). Transdermal iontophoresis of 5-fluorouracil combined with electroporation and laser treatment. Int. J. Pharm. *270*, 241–249.

49. Lin, Z.C., Hsieh, P.W., Hwang, T.L., Chen, C.Y., Sung, C.T., and Fang, J.Y. (2018). Topical application of anthranilate derivatives ameliorates psoriatic inflammation in a mouse model by inhibiting keratinocyte-derived chemokine expression and neutrophil infiltration. FASEB J. *32*, fj201800354.