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ORIGINAL RESEARCH

High-Intensity Focused Ultrasound Enhances Drug Penetration into the Human Skin in the Franz Diffusion Cell

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Purpose: High-intensity focused ultrasound (HIFU)-assisted drug delivery is a non-invasive tool to deliver drugs to targeted areas, currently used mainly for treating cancer and cardiovascular diseases. However, in terms of transdermal drug delivery, HIFU technology is still poorly understood. Accordingly, this study sought to investigate the effectiveness of HIFU on drug penetration into the skin using human skin tissues.

Methods: Gel-type drugs whose ingredient is glutathione were labelled with fluorescein isothiocyanate, in turn the drugs were allowed to penetrate to the human skin tissue in the Franz diffusion cell for 24 hours in control and HIFU treatment groups, and their fluorescence intensity was measured using a multiple microplate reader at one, two, six, and 24 hours after drug application. In addition, tissue slice analysis was performed in each tissue slice at 24 hours post-drug application. The % area, fluorescence intensity per area, and penetration depth of the drug were measured using a fluorescence microscope.

Results: The fluorescence intensity increased with time in all groups. Specifically, at 24 hours after drug application, the fluorescence intensity (a.u). of the 10-shot HIFU treatment group was significantly enhanced compared to that of the control group ($p < 0.05$). The tissue slice analysis demonstrated that the % area of fluorescent drug and the fluorescence intensity per area (a.u.) were all significantly increased in both HIFU treatment groups compared to the control group ($p < 0.05$, $p < 0.001$). In addition, the penetration depth (μm) also markedly rose in both HIFU treatment groups compared to the control group ($p < 0.01$, $p < 0.05$).

Conclusion: It was demonstrated for the first time that HIFU significantly facilitated topical drug penetration into the human skin, strongly implying that HIFU can be a useful option for transdermal drug delivery.

Keywords: HIFU, transdermal drug delivery, sonophoresis, fluorescence analysis

Introduction

Skin is an effective physical barrier with a role in protecting organisms from a variety of environmental threats as the first line of defense, and which is therefore greatly helpful to preclude the invasion of foreign pathogens and particles into the body.[1,2] However, this skin property is also another barrier that must be surmounted to deliver drugs into or across the skin. Notably, the stratum corneum (SC) has a critical barrier function to hamper drug penetration into the skin.[3] In this respect, historically, a great many efforts have been devoted to developing and building techniques and modalities to effectively permeate drugs into the skin by passing through the SC.[4]

Transdermal drug delivery is a method of permeating drugs into or across the skin and is currently carried out by diverse modalities in clinical settings, typically including microneedle therapy system (MTS), jet injection, iontophoresis, electroporation, transdermal patches, and sonophoresis.[5–12] Many beneficial strategies are being investigated to improve the effectiveness and efficiency of transdermal drug delivery. These advances could be a driving force in reducing the prevalence of cardiovascular and central nervous system diseases, while simultaneously driving the advancement of vaccination and improving patient preferences. Therefore, researching and developing such systems is a timely task.[12]

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MTS and jet injection can be a useful option for transdermal drug delivery, but studies revealed that they had potential limitations such as pain and infection.[13,14] Iontophoresis and electroporation are generally safe and effective tools for enhancing skin and cellular permeability of drugs, which, however, have a risk of skin irritation and unnecessary cell damage or death.[15,16] Transdermal patches are used by attaching them to the skin surface and then release drugs by passive diffusion. They are non-invasive and safe, but less effective and can induce skin irritation and contact dermatitis.[17] Sonophoresis is characterized by the application of ultrasonic waves to enhance drug penetration into the skin, and a wide range of ultrasound frequencies, covering from low- (20–100 kHz) to high-frequency (2–16 MHz), are currently applied to increase skin or percutaneous permeability of drugs. Adverse events, such as thermal injury or burn, have been reported but were found to be minor and temporal. In general, it is believed that this modality is safe and highly effective.[18,19]

High-intensity focused ultrasound (HIFU) is a non-invasive therapeutic technique and has been much highlighted in recent years for cancer treatments such as tumor ablation and tumor-targeting drug delivery.[20,21] HIFU uses ultrasonic energy at the frequency range of 0.8–20 MHz and the intensity of 1000–25,000 W/cm^2.[22] Basically, HIFU has a crucial role as a cancer therapy in improving many types of cancers including brain, breast, kidney, liver, prostate, and rectal cancer. In this regard, HIFU was found to facilitate the accumulation and uptake of drug-loaded nanocarriers and release the drug at the target area.[20] However, regarding transdermal drug delivery, HIFU technology has been still poorly elucidated.

The cavitation and thermal effects are considered critical factors that can enhance the skin, cellular, and vascular permeability of drugs.[23,24] Accumulating data showed that low-to-intermediate range of ultrasound frequencies, 20–200 kHz, are more effective in sonophoresis, or transdermal drug delivery, due to their higher cavitation effects.[25,26] However, inconsistent with this report, previous experiments revealed that sonophoresis was markedly enhanced rather at higher frequencies (10 MHz, 16 MHz), and notably, ultrasonic waves were found to reduce more effectively skin barrier function at the higher frequencies.[27–29] In addition, high-intensity acoustic waves were found more suitable to induce temporal sonoporation in the cell membrane by creating micro jets and shockwaves, thereby promoting the permeability of tissue and cells.[25,30] Regarding thermal effects, HIFU creates a mild hyperthermia inside the skin by increasing the local temperature near to the target location and widens reversibly blood vessels by altering the pressure at the treated area, leading to vascular permeability.[18,21,24,31]

These results provide us with crucial clues and possibilities that HIFU can be another sonophoretic modality to enhance drug penetration into the skin. However, despite these advantages, HIFU has rarely been attracted in terms of transdermal drug delivery. Currently, a variety of HIFU procedures are being performed in cosmetic dermatological settings, and energy-based devices (EBDs) are commonly used in combination with injectables.[32,33] If effective ingredients that are good for the skin can be delivered to the dermis during HIFU treatment without using invasive injectables, this dual effect will further enhance clinical outcomes of HIFU in a simpler and less painful way.

Accordingly, in the present study, we attempted to show the possibility that HIFU could also be a useful tool for promoting transdermal drug penetration. In particular, a cartridge designed to irradiate HIFU to a limited area in a circular manner for the promotion of transdermal drug delivery was used. The cumulative amount of the fluorescein isothiocyanate (FITC)-labeled drug that penetrated into the skin was investigated by measuring the fluorescence intensity of the drug at each time point after drug application and HIFU treatment. In addition, tissue slice analysis was carried out in each tissue. The % area, fluorescence intensity per area, and penetration depth of fluorescent drug were measured under a fluorescence microscope.

Materials and Methods

Devices and Materials

Human-derived skin tissue [full skin 2 cm (W) X 2 cm (H)] with 5–6.5 mm thickness (Dermalab™, Wonju, Korea), Deep Synergy Booster (DSB) ampoule (Classys Inc., Seoul, Korea), a study drug whose ingredient is glutathione (molecular weight, 307.33 g/mol) and formulation is a gel-type product (semi-solid preparation), and a HIFU device (ULTRAFORMER MPT, Classys Inc., Seoul, Korea) and its cartridge (1.5-mm depth, 7 MHz, Energy 0.1 J/cm^2) (Booster cartridge, Classys Inc., Seoul, Korea) were used in the study. The study drug was used only to show how

much the drug penetrates into the skin after HIFU application to the skin, and the ingredient (glutathione) functions to protect the skin from the environmental and oxidative stress.[34]

Group Assignment and HIFU Irradiation

The study group was divided into a total of three groups. The control group is defined as a study drug application group without HIFU irradiation (n = 3). The experimental groups are defined as study drug application group with HIFU irradiation and are divided into two groups depending on the number of HIFU irradiation shots. The experimental group 1 is a group in which HIFU 10 shots were irradiated (n = 3), and the experimental group 2 is a group in which HIFU 20 shots were irradiated (n = 3).

After drug application to the tissue, a single session of 10- or 20-shot HIFU treatment was carried out on the tissue with the energy level of 0.1 unit, which corresponds to the energy of 0.1 J/cm^2. Shots refer to individual pulses or bursts of focused ultrasound energy delivered to the target tissue. In addition, each shot forms 16 thermal coagulation points (TCPs) beneath the skin and the time duration of each shot is approximately 1.04 sec (0.065 sec/TCP) (Figure 1).

Topical Drug Penetration

The equilibration of human-derived skin tissue was performed at room temperature 1 hour before the experiment. 5 µL of acetyl hexapeptide-FITC (5 mg/mL) (Peptron Inc., Daejeon, Korea) was added to 5 g of study drug, and they were thoroughly mixed by vortexing so that the weight ratio of FITC and study drug was 200,000:1. The human-derived skin tissue was placed in a static diffusion device (Franz diffusion cell) of a percutaneous absorption system (Diffusion Cell Drive Console, Labfine, Gunpo, Korea) with its SC facing upward (Supe & Takudage, 2020). 100 mg of the FITC-

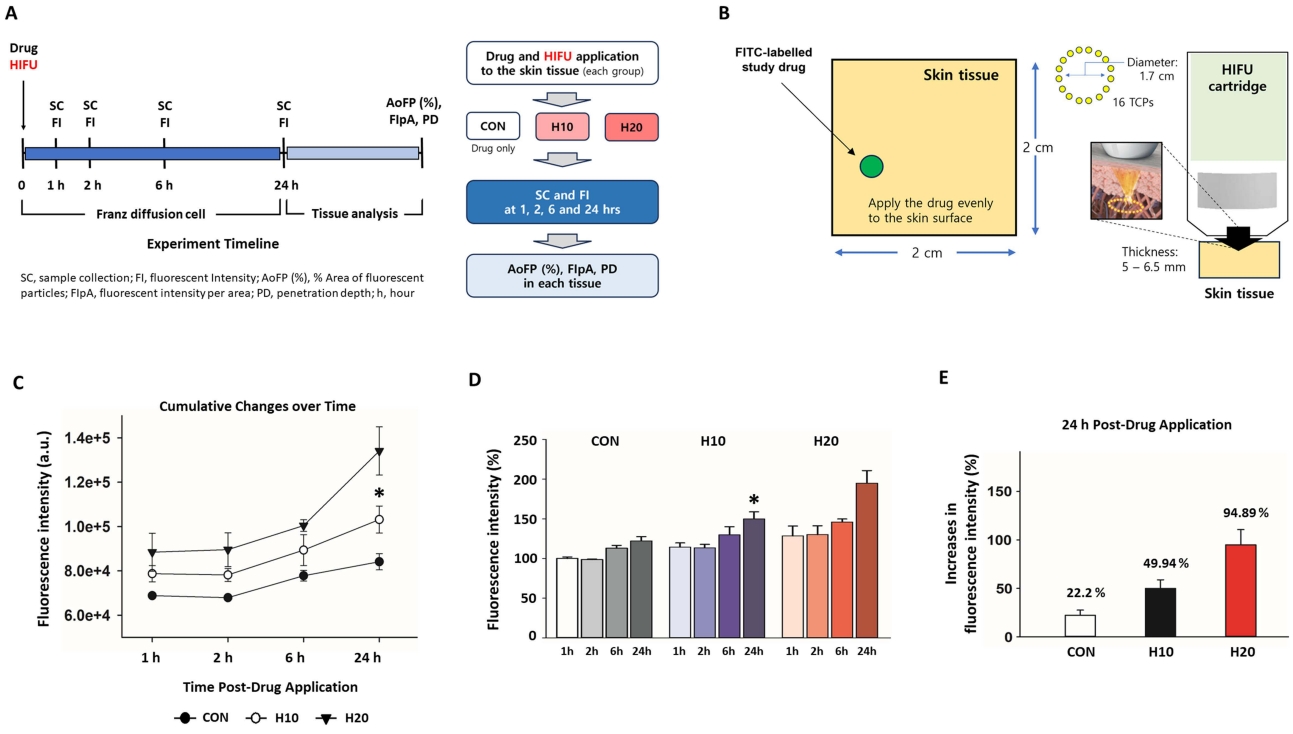

Figure 1 Enhanced transdermal drug delivery after HIFU treatment. (**A**) The Franz diffusion cell experiment and the tissue analysis were performed according to the experiment timeline. Drugs and a single session of HIFU treatment were applied on the skin tissue and then multiple measurements were carried out at each time point. (**B**) FITC-labelled study drug was applied to the tissue and a cylindrical-shaped cartridge was used to deliver HIFU energy to the tissue. (**C**) Changes in fluorescence intensity were investigated over time in all groups, finding that the H10 group showed a significant increase in fluorescence intensity (a.u.) ($p<0.05$). (**D**) And even regarding the increase rate in fluorescence intensity (%), there was a significant difference between the control and H10 groups. (**E**) Overall, at 24 hours after drug application, the increase rate in fluorescence intensity rose up to 22.2%, 49.94%, and 94.89% in the control, H10, and H20 groups, respectively.

Note: The symbol, *Indicates statistical significance between control group (**C**), or baseline (**D**), and H10 group at 24 hours (*$p < 0.05$).

Abbreviations: a.u., arbitrary unit; CON, control; H10, HIFU 10 shots; H20, HIFU 20 shots; TCP, thermal coagulation point.

labelled study drug was applied on the human-derived skin tissue through the donor chamber of the static diffusion device.

The tissue and the FITC-labelled study drug were allowed to react, and then the penetrated drug was collected from the receptor chamber of the static diffusion device at one, two, six, and 24 hours after drug application on the tissue. The fluorescence intensity of the penetrated drug was analyzed using a multimode microplate reader (SpectraMax iD3, Molecular Devices LLC., CA, USA) at each time point to investigate the degree of drug penetration into the tissue. For the analysis on the increase rate in fluorescent intensity (%), the baseline was defined as the 1-hour fluorescent intensity of the control group. Based on this baseline, we calculated the increase rate in all groups at each time point.

Tissue Slice Analysis

To explore the absorption of the FITC-labelled drug permeated into the human-derived skin tissue, the skin tissue was retrieved 24 hours after drug application on the skin. The retrieved skin tissue was fixed with 10% formalin and was made into a paraffin block. The block was sectioned to a thickness of 3–5 μm and was mounted onto a microscope slide. The slide was deparaffinized (xylene), hydrated (100%, 90%, 80%, and 70% EtOH), washed, and counterstained with DAPI (P36962, Invitrogen, CA, USA). Next, the slide was photographed using a fluorescence microscope (Axio Observer 7, Zeiss, Germany), and the fluorescence intensity and penetration depth were analyzed using the images obtained by the microscope.

The fluorescence intensity, % area of fluorescent drug, and fluorescent intensity per area were analyzed using the Image J program. For the fluorescence depth analysis, 10 large and dark fluorescence spots in the tissue image were selected, and their length was measured by vertically lowering them from the epidermis. Based on the scale bar, the length unit was converted to micrometers (μm).

Statistical Analysis

Statistical analysis was carried out using SPSS software ver. 26 (IBM, Armonk, NY, USA). All the values collected and produced from the study were expressed as mean ± standard errors of mean (SEM) and mean ± standard deviation (SD). For the cumulative changes in fluorescent intensity over time and the increase rate in fluorescence intensity, the comparison between groups was analyzed using a two-way repeated measures analysis of variance (ANOVA), followed by a post hoc Tukey's test or Holm-Sidak for multiple comparisons. In addition, concerning the fluorescence intensity per area, % area of fluorescent drug and penetration depth, the comparison between groups was analyzed using a one-way ANOVA, followed by a post hoc Tukey's test or Holm-Sidak for multiple comparisons. In particular, values, which were not evenly distributed or did not pass the normality test, were analyzed using the Friedman test with a post hoc Tukey's test for multiple comparisons. P-values of less than 0.05 were regarded statistically significant.

Results

HIFU Enhanced Topical Drug Penetration into the Skin

A single session of HIFU treatment was performed after topical drug application on the human-derived skin tissue. And sample collections and fluorescence measurements were also carried out at each time point in the Franz diffusion cell and in the tissue slice (Figure 1A). In addition, the HIFU cartridge has a cylindrical shape, fits almost to the size of the tissue, and is designed to irradiate HIFU pulses to a small and limited area in a circular irradiation manner. Each shot forms 16 TCPs in the target area and the diameter of circular-shaped 16 TCPs is approximately 1.7 cm (Figure 1B).

The amount of permeated drug was investigated by measuring the fluorescent intensity of the FITC-labelled drug at one, two, six, and 24 hours in the HIFU treatment (experiment) and non-treatment (control) groups. In all groups, the fluorescent intensity (a.u.) gradually rose over time, and these increasing trends were steeper in the two HIFU treatment groups compared to the control group. A significant difference in fluorescent intensity was found between the 10-shot HIFU treatment group (H10) and the control group at 24 hours after drug application ($p < 0.05$) (Figure 1C). In addition, the increase rate in fluorescence intensity (%) was also explored at each time point. Consistent with the result of Figure

1C, the increase rate over time was higher in the two HIFU treatment groups compared to the control group. Notably, there was a significant difference between H10 and control groups at 24 hours ($p < 0.05$) (Figure 1D) (Table 1).

As a result, it was found that the increase rate in fluorescent intensity rose up to 22.4%, 49.94%, and 94.89% at 24 hours-post drug application in the control group, H10, and 20-shot HIFU treatment (H20) groups, respectively (Figure 1E) (Table 1). Collectively, these results indicate that HIFU markedly facilitates drug penetration into the skin and this effect is proportional to the amount of HIFU energy irradiated.

HIFU Promotes More Fluorescent Drugs to Enter the Skin

Tissue slice analysis was performed in each tissue slice at 24 hours post-drug application. The FITC-labelled drug was permeated in the dermal area in all groups, and this compound was shown as green fluorescent particles under a fluorescence microscope. It was visually observed that the amount of the drug was greatest in the H20 group, followed by the H10 group and then the control group (Figure 2A). Importantly, the particle brightness was found to be stronger in the H10 and H20 groups, which, in contrast, was much faint in the control group that it was difficult to observe.

The quantitative investigation revealed that the % area of fluorescent drug accounted for 0.43%, 1.73%, and 4.35% of the total tissue area in the control, H10, and H20 groups, respectively (Figure 2B) (Table 2). There were significant differences between the control and H10 groups ($p < 0.05$), control and H20 groups ($p < 0.001$), and H10 and H20 groups ($p < 0.001$). Also, the two HIFU treatment groups showed higher fluorescence intensity per area (a.u.) than the control group ($p < 0.05$, $p < 0.001$), and moreover, this result increased with higher energy ($p < 0.001$) (Figure 2C) (Table 3). Collectively, these results indicate that HIFU is directly involved in transdermal drug penetration by enabling more fluorescent drugs to enter the skin in an energy-dependent manner.

HIFU Allows Fluorescent Drugs to Enter the Deeper Layer of the Skin

The second analysis was performed also in the tissue slices, showing the penetration depth of FITC-labelled drugs in each group. At 24 hours post-drug application, it was found that the drugs were permeated from the epidermis to the dermis in all groups (Figure 3A). The penetration depth was measured in each tissue slice (red arrows), indicating that the length of the arrows was longer in the two HIFU treatment groups compared to the control group.

Specifically, the mean penetration depth was 281.09 ± 5.93 μm in the control group, while being 322.05 ± 7.79 μm and 311.02 ± 2.10 μm in the H10 and H20 groups, respectively. These differences were found statistically significant

Table 1 Changes in Fluorescent Intensity (%) Over Time in Each Group

Group	HIFU	Time	Fluorescence Intensity (%)			Rate of Change (%) (vs Baseline)
			Mean	SEM	SD	
Control	No treat.	1 h	100[a]	1.84	3.19	0
		2 h	98.70	0.53	0.92	−1.3
		6 h	113.05	3.53	6.12	13.05
		24 h	122.24	5.31	9.21	22.24*
H10	10 shots	1 h	114.40	5.31	9.20	14.40
		2 h	113.58	4.29	7.43	13.58
		6 h	129.89	10.17	17.61	29.89
		24 h	149.94	8.86	15.34	49.94*
H20	20 shots	1 h	128.50	12.47	21.61	28.50
		2 h	130.16	11.09	19.20	30.16
		6 h	145.99	3.84	6.65	45.99
		24 h	194.89	15.81	27.38	94.89*

Notes: [a]Indicates the 1-hour fluorescent intensity of the control group and is defined as baseline. And *Indicates the increase rate of fluorescent intensity at 24 hours in each group compared to the baseline.
Abbreviations: H10, 10-shot HIFU treatment; H20, 20-shot HIFU treatment; SEM, standard errors of mean; SD, standard deviation.

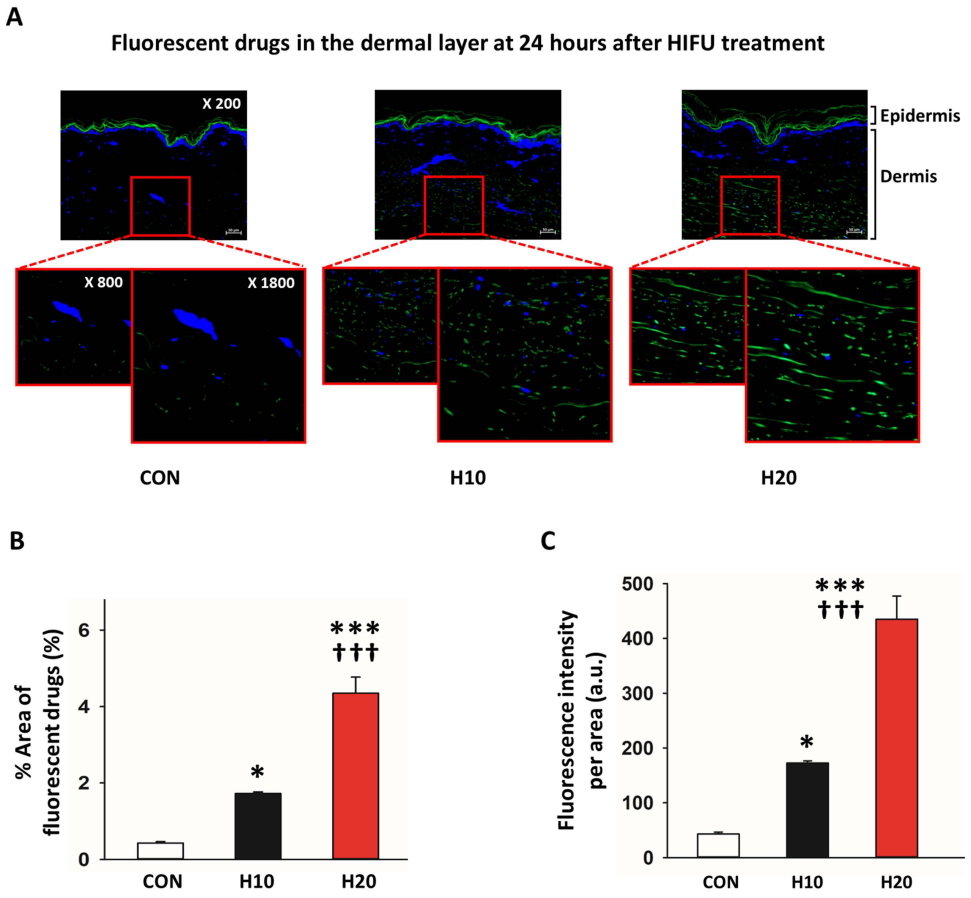

Figure 2 More fluorescent drugs permeated into the skin after HIFU treatment. (**A**) Each skin tissue was collected from the Franz diffusion cell at 24 hours after drug application and the permeated fluorescent drugs and their areas were observed from the epidermis to the dermis under a fluorescence microscope. A greater amount of fluorescent drug was found in the H10 and H20 groups. As a result of quantitative analysis, (**B**) the % area of fluorescent drug and (**C**) the fluorescence intensity per area were all significantly higher in the H10 and H20 groups compared to the control group ($p < 0.05$, $p < 0.001$). Notably, this event was shown in an energy-dependent manner ($p < 0.001$) (**B** and **C**).
Note: The symbols, *And †Indicate statistical significance between control group and H10 or H20, and H10 and H10, respectively (*$p < 0.05$; ***,†††$p < 0.001$).
Abbreviations: CON, control; H10, HIFU 10 shots; H20, HIFU 20 shots.

(Figure 3B) (Table 4). For the penetration depth, the control group was markedly different from the H10 group ($p < 0.01$), as well as from the H20 group ($p < 0.05$). But there was no difference between the H10 and H20 groups. Collectively, our results indicate that HIFU has a critical role in delivering drugs into the deeper layer of the dermis, thereby facilitating transdermal drug penetration.

Discussion

The present study showed that HIFU treatment markedly enhanced topical drug penetration into the human skin. In the Franz diffusion cell, the fluorescent intensity of the FITC-labelled drug was higher in the HIFU treatment groups at all

Table 2 % Area of Fluorescent Drug in Each Group

Group	HIFU	% Area of Fluorescent Drug			Rate of Change (%) (vs Control)	*p*-Value (vs Control)
		Mean	SEM	SD		
Control	No treat.	0.43	0.03	0.06	–	–
H10	10 shots	1.72	0.04	0.07	3	0.010
H20	20 shots	4.35	0.42	0.73	9.12	< 0.001

Abbreviations: H10, 10-shot HIFU treatment; H20, 20-shot HIFU treatment; SEM, standard errors of mean; SD, standard deviation.

Table 3 Fluorescent Intensity per Area in Each Group

Group	HIFU	Fluorescent Intensity Per Area			Rate of Change (%) (vs Control)	*p*-Value (vs Control)
		Mean	SEM	SD		
Control	No treat.	43.07	3.54	6.13	–	–
H10	10 shots	172.51	4.06	7.04	300.53	0.010
H20	20 shots	435.02	42.24	73.16	910.03	< 0.001

Abbreviations: H10, 10-shot HIFU treatment; H20, 20-shot HIFU treatment; SEM, standard errors of mean; SD, standard deviation.

the time points compared to the control group. Notably, there was a significant difference in fluorescence intensity between the H10 and control groups at 24 hours after HIFU treatment. The tissue slice analysis revealed that a greater amount of the drug was permeated into the skin in the HIFU treatment groups. Furthermore, HIFU promoted drug penetration into the deeper layer of the skin. All the events occurred in an energy-dependent manner. Accordingly, these results demonstrate that HIFU has a direct impact on permeating drugs into the skin and ultimately facilitates transdermal drug delivery.

It is well established that the ultrasound-induced mechanical and thermal effects have a crucial role in augmenting the skin permeability of drugs.[21,25] The previous studies reported that ultrasonic waves vibrate and disturb skin tissue and its layers, which in turn open a new pathway in the skin that can allow drugs to easily penetrate.[18,35] Low-to-high frequencies at the range of 20 kHz–16 MHz are used to make a drug passage in the skin and can disrupt a key barrier, SC, thus facilitating skin permeability.[18,36] Specifically, pioneering studies have already been conducted for high-frequency ultrasound-dependent sonophoresis a few decades ago. They showed that the transdermal transport of salicylic acid was much elevated at the frequency of 10 MHz and 16 MHz compared to at 2 MHz, further finding that the diffusion lag time was reduced at the higher frequency groups.[27,28] Notably, high frequency sonophoresis was found to lower skin barrier function since higher ultrasound frequencies are more likely to concentrate ultrasound energy into the SC due to their short wavelength, which in turn can create aqueous passages in the SC and ultimately promoting skin permeability.[27–30] These results indicate that SC lipids (cutaneous barrier function) can be more effectively disrupted at higher ultrasound frequencies, promoting skin permeability.

In addition, it was reported that micro jets and shock waves generated by ultrasound-driven cavitation induce a structural alteration in SC lipids and disrupt them to make diffusion channels for transdermal drug delivery.[25,30,37] In effect, a scanning electron microscopy investigation found that after sonophoresis, pores, whose size is approximately 1–

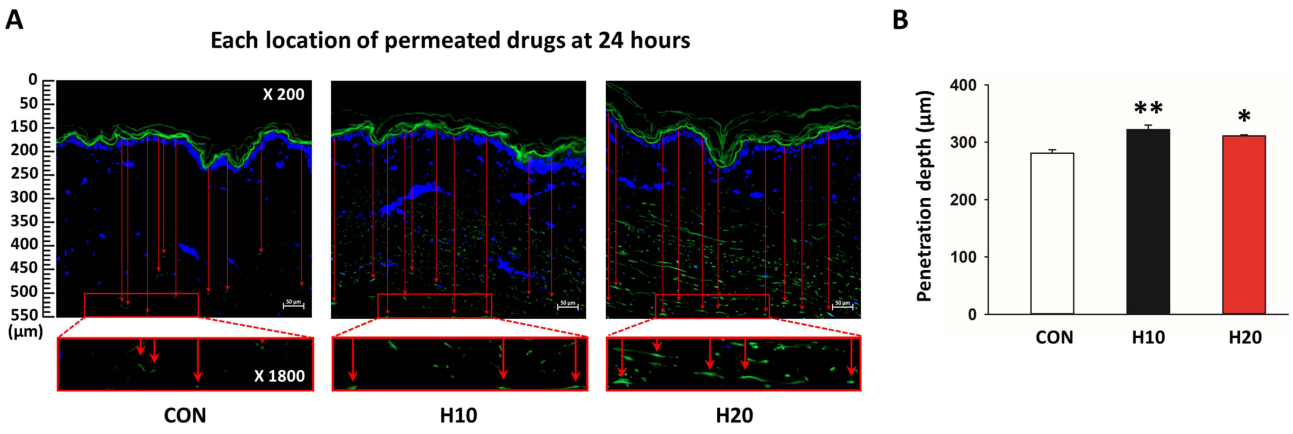

Figure 3 Fluorescent drugs found in the deeper layer of the skin after HIFU treatment. (**A**) The penetration depth of fluorescent drug was explored in each tissue slice. The fluorescent particles were found in the dermal layer in all groups. (**B**) However, it was found that the penetration depth was much deeper in the H10 and H20 groups compared to the control group ($p<0.01$, $p<0.05$).
Note: The symbol, *Indicates statistical significance between control group and H10 or H20 group (*$p<0.05$; **$p<0.01$).
Abbreviations: CON, control; H10, HIFU 10 shots; H20, HIFU 20 shots.

Table 4 Drug Penetration Depth in Each Group

Group	HIFU	Drug Penetration Depth			Rate of Change (%) (vs Control)	*p*-value (vs Control)
		Mean	SEM	SD		
Control	No treat.	281.09	5.93	10.28	–	–
H10	10 shots	322.05	7.79	13.49	14.57	0.006
H20	20 shots	311.02	2.10	3.64	10.65	0.025

Abbreviations: H10, 10-shot HIFU treatment; H20, 20-shot HIFU treatment; SEM, standard errors of mean; SD, standard deviation.

2 μm, were formed on the SC surface.[38] Furthermore, high-intensity ultrasound is more advantageous for sonoporation, greatly contributing to enhancing cellular drug uptake.[25,30] It was reported that high-intensity ultrasound markedly increased the cumulative amounts of permeated drugs compared to the medium-intensity ones.[19] Collectively, these results suggest that HIFU can promote skin and cellular permeability by increasing its mechanical forces on the surrounding tissue and cells.

With regard to thermal effects, HIFU can create focused and precise heating zones in the tissue and induce more rapidly localized and controlled thermal effects compared to the low-frequency one.[21,39] It is well known that HIFU can induce a mild hyperthermia, approximately 40–45°C, inside the skin, resulting in the non-invasive local augmentation of temperature. More importantly, this hyperthermia increases tissue plasticity and vascular permeability to improve nutrient transport, blood flow, and cellular drug uptake, thereby greatly contributing to transdermal drug delivery.[21,24] In our study, we used a cartridge (1.5-mm depth, 7 MHz, Energy 0.1 J/cm^2) designed to irradiate HIFU to a small and limited area in a circular manner and thus enhance cavitation and thermal effects. This cartridge vibrates surrounding tissue 70,000 times per second and creates a mild hyperthermia inside the skin, suggesting that these features enable our HIFU system to facilitate transdermal drug penetration.

Our study showed that HIFU treatment significantly enhanced drug penetration into the skin at each time point (Figure 1C and D). There was a gradual rise in the amount of the permeated drug in the non-treatment group. In contrast, after HIFU treatment, this increase was markedly promoted in an energy-dependent manner ($p < 0.05$) (Figure 1C–E). Moreover, consistent with this result, a larger amount of the drug was observed in the dermis at 24 hours after drug application (Figure 2A). The % area of fluorescent drug and the fluorescence intensity per area were all much higher in the HIFU treatment groups compared to the control group ($p < 0.05$, $p < 0.001$) (Figure 2B and C). It has not yet been fully elucidated how HIFU promotes transdermal drug penetration, but it can be speculated that HIFU-mediated mechanical and thermal effects increase preferentially skin permeability by vibrating surrounding tissues and making passages in the SC through which drugs can easily penetrate. And in turn they improve vascular and cellular permeability inside the skin, ultimately facilitating transdermal drug penetration.

In addition, our study found that the drug was permeated into the deeper part of the skin at 24 hours after drug application ($p < 0.05$, $p < 0.01$) (Figure 3A and B). In the H10 and H20 groups, the penetration depth was 322.05 ± 7.79 μm and 311.02 ± 2.10 μm, respectively. Skin thickness varies greatly depending on the area. These differences also appear depending on gender and ethnicity.[40,41] Kılınç et al showed that in the facial skin (forehead, nose, upper lip, ear, scalp, and cheek), epidermal thicknesses ranged from 65.91 ± 14.44 μm to 120.91 ± 44.74 μm, dermal thicknesses from 1150 ± 217.43 μm to 1498.33 ± 388.56 μm. For the body skin, its epidermal and dermal thickness was found to be thicker than those of the facial one.[40] Given these previous data, our results (total thickness, approximately, 311–322 μm) can be considered to correspond to the upper dermis. Meanwhile, it was observed that the drug was permeated approximately up to 281.09 ± 5.93 μm even in the control group. But the fluorescent drugs in the control group were much dimmer and smaller than those in the HIFU treatment groups (Figure 2A). Furthermore, the % area of the fluorescent drug and the fluorescence intensity per area were all significantly smaller in the control group compared to the HIFU treatment groups (Figure 2B and C). Accordingly, several fluorescent particles were observed in the dermal area in the control group, but given their permeation area and fluorescence intensity, the penetration effect of the control group is considered insignificant.

Our study showed that larger amounts of the drug were permeated into the deeper part of the skin post-HIFU treatment compared to the non-treatment. The difference between HIFU treatment and non-treatment groups was found to be significant, and the strength of this effect was observed in an energy-dependent manner. Collectively, these results indicate that HIFU directly facilitates transdermal drug penetration.

In the present study, the sample size was "n = 3" in each group, which was a relatively small number of samples. In this respect, there is a limitation of generalization caused by the sample size. However, despite the small number of tissue samples, the significance of the results of this study is that the transdermal drug penetration effect of HIFU was consistently observed in all samples. In addition, this study provided the mechanism of HIFU-mediated drug delivery from a physical perspective such as cavitation and thermal effects, while its molecular mechanism was not investigated in this study. As the importance of transdermal drug delivery systems (TDDS) in the clinical environment increases, mechanism studies must also be conducted at a molecular level. Comparative studies on the effectiveness of TDDS between HIFU and low-to-moderate frequency ultrasound are an interesting research topic. These studies will further elucidate the role and position of HIFU in TDDS. Lastly, the safety assessment of HIFU was not conducted in this study. HIFU can induce skin erythema, swelling, pigmentation, or even pain. However, this study applied HIFU to the skin tissue at a very low energy level (0.1 J). Considering the safety results of another HIFU study that showed mild and transient adverse events such as pain and erythema at 0.3 J and 1.2 J,[42] it is expected that there will be little safety problem. Thus, further studies are warranted to examine the efficacy and safety of HIFU in a larger sample size with various parameters, to investigate the molecular mechanism of HIFU-mediated transdermal drug delivery, and to compare the effectiveness of TDDS between HIFU and low-to-moderate frequency ultrasound.

Conclusion

The present study reported HIFU-assisted transdermal drug delivery in the human skin tissue. After HIFU treatment, topical drug penetration was markedly promoted in an energy-dependent manner. Notably, we measured cumulative changes in the fluorescence intensity of the drug over time, further, performed an in-depth analysis in the tissue slice. Interestingly, the HIFU treatment promoted drugs to penetrate into the skin, as well as allowed them to be more deeply permeated to the dermis. These results demonstrate that HIFU directly drives transdermal drug penetration. Importantly, it indicates that we can deliver drugs into the skin while performing HIFU treatment in dermatological settings.

Low-to-moderate frequency ultrasound has been widely used for transdermal drug delivery, but HIFU has not received much attention in this field. In this study, we offer reliable outcomes concerning HIFU-driven transdermal drug penetration for the first time, strongly suggesting that HIFU can be an effective modality for transdermal drug delivery.

Ethical Statement

This study was exempt from review by the Institutional Review Board (IRB). In accordance with the National Bioethics Law, IRB review is exempted only in cases where the risk to research subjects and the public is minimal. In this study, the researcher of this study cannot identify the personal information of human specimen donors, the researcher did not collect this specimen directly and purchased it from a bio company (see also Materials and Methods section), and this human specimen is a research material that has been separated and processed for use by the general public.

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Disclosure

The authors report no conflicts of interest in this work.

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