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Research Article

Enhanced Transdermal Drug Delivery by Sonophoresis and Simultaneous Application of Sonophoresis and Iontophoresis

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Abstract. Transdermal drug delivery has advantages of topical drug administration compared to the other conventional administration methods. However, the skin penetration of drugs is limited by the barrier properties of stratum corneum. The combinational strategy has been investigated to improve the skin permeability of the drug. For this study, we devised an improved device that can perform not only the single application of sonophoresis or iontophoresis but also the simultaneous application. The enhancement effect of sonophoresis was evaluated for various cosmeceutical drugs using a Franz diffusion cell. The enhancement ratio of niacinamide and retinol with sonophoresis was increased to 402% and 292%, respectively. The relationship was found between the enhancement effect of sonophoresis and the physicochemical properties of drugs. In particular, the simultaneous treatment of sonophoresis and iontophoresis enhanced skin penetration of glutamic acid to 240% using the fabricated device. The simultaneous application showed significantly higher enhancement ratio than application of sonophoresis or iontophoresis alone. Moreover, the improved device achieved skin penetration enhancement of various cosmeceutical drugs with lower intensity and a short application time. This combined strategy of transdermal physical enhancement methods is advantageous in terms of decline in energy density, thereby reducing the skin irritation. The miniaturized device with sonophoresis and iontophoresis is a promising approach due to enhanced transdermal drug delivery and feasibility of self-administration in cosmetic and therapeutic fields.

KEY WORDS: sonophoresis; ultrasound; iontophoresis; transdermal drug delivery; skin penetration enhancer; physical enhancer.

INTRODUCTION

Transdermal drug delivery has many advantages over the conventional drug delivery routes. Topical drug administration through skin is a non-invasive and convenient method compared to typical drug administration methods such as intravenous and oral administration (1,2). However, we should overcome the skin barrier property for effective delivery into the skin. The stratum corneum is the outmost layer of skin consisting of flattened dead keratinocytes surrounded by lipid matrix, which is the main barrier to transcutaneous delivery (3,4). To enhance the permeability of skin, various methods have been investigated for the past several decades (5–7). There are two ways to enhance skin permeability: chemical enhancement methods including azone derivatives, fatty acids, alcohols, esters, sulfoxides, pyrrolidones, glycols, surfactants, and terpenes and physical enhancement methods including electroporation, ultrasound, iontophoresis, and microneedles (8–10). In this study, we focused on two physical methods: sonophoresis and iontophoresis.

Sonophoresis increases the permeability of the skin and transfer drugs effectively. This method uses ultrasound waves at various frequencies. In general, it is believed that low-frequency ultrasound (~ kHz) induces a greater transdermal enhancement than high-frequency ultrasound (3–16 MHz) (11–14). Ahmet *et al*. have reported that skin permeability of drug linearly depends on the ultrasound intensity and exposure time, and the enhancement is directly proportional to the total ultrasound energy density (15). Although sonophoresis has been studied for a long time, the fundamental mechanism behind sonophoresis has not been identified. Several mechanisms have been proposed such as thermal effects by absorbing ultrasound energy and cavitation effects caused by the collapse and oscillation of cavitation bubbles in the ultrasound field (16).

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Iontophoresis is one of the physical permeation enhancers using an electric field (17). The repulsive forces between the same charge push the molecule into the skin; thus, iontophoresis promotes drug delivery with a low current density (18). Two mechanisms are possible to explain iontophoretic drug delivery: electro-osmosis and electrophoresis. In electro-osmosis, drug is delivered by solvent flow occurred electrically. The electro-phoretic mechanism involves the skin penetration is induced by a direct interaction between electric field and a charged molecule (8). Iontophoresis offers the fast drug release as well as delivery of both charged and uncharged species. In particular, this method has the significant advantage of being easily coupled with other transdermal enhancement methods (19,20). Therefore, iontophoretic devices recently gained considerable attention in cosmetic and therapeutic fields.

Sonophoretic and iontophoretic devices have been widely applied in cosmetic and therapeutic fields. Several models of sonophoretic device are designed for treatment of diabetes, cancer, asthma, cardiovascular diseases, and skin wounds (21). The sonophoretic devices have been available on the market for therapeutic applications. Various iontophoretic systems were approved by the Food and Drug Administration (FDA) and also commercialized. These iontophoretic devices are feasible to deliver anesthetics, anti-oxidant, and anti-cancer agents on the skin (22).

Researchers have investigated combinational strategies to improve transdermal drug delivery. Shirouzu *et al.* (23) reported that the combination of sonophoresis and iontophoresis synergistically enhances the skin penetration of vitamin B_{12}. Long Le *et al.* (24) investigated the enhancement of heparin transdermal penetration by incorporating both sonophoresis and iontophoresis. Only lower voltage/current is required to achieve the desired transdermal enhancement when the skin is treated with a combination of these two methods, thereby reducing the skin irritation (24). One of the proposed mechanisms is that enhancement effect is mainly caused by the stratum corneum diffusivity of molecules increased by sonophoresis and the electro-osmotic water flow by iontophoresis application (25).

In this work, we designed the miniaturized devices which can perform both sonophoresis and iontophoresis simultaneously to enhance skin permeability of the cosmeceutical drugs (Fig. 1). This simultaneous application is differentiated from previous studies in which researchers first applied sonophoresis followed by iontophoresis (25,26). We investigated the effect of ultrasound at two frequencies (280 and 350 kHz) and different intensities (levels 1, 2, and 3) on the porcine skin. The enhancement ratio by sonophoresis was evaluated on hydrophilic and hydrophobic drugs. Simultaneous treatment with sonophoresis and iontophoresis exhibited favorable delivery efficiency of glutamic acid into the skin.

MATERIALS AND METHODS

Materials

Phosphate-buffered saline (PBS), Tween 80, niacinamide, arbutin, glutamic acid, retinol, and alpha-bisabolol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Caffeine and minoxidil were purchased from Tokyo Chemical Industry (Chuo-ku, Tokyo, Japan). Other chemicals were purchased from Daejung Chemicals (Siheung, South Korea). Tegaderm Film 1622W used as a protective film was purchased from the 3M Co. (Maplewood, MN, USA).

Instruments of Sonophoresis and Iontophoresis

The devices for this experiment were designed in two versions to operate at 280 kHz and 350 kHz, respectively. Table I shows the operating conditions of two devices. Low frequency ultrasound was applied at 280 kHz and 350 kHz. The intensity of sonophoresis was set to 500 $mW/cm^2 \pm 10\%$ and divided into three levels by the different duty cycles such as 30%, 60%, and 90%. Current density of iontophoresis is also divided into three levels: 100, 150, and 200 $\mu A/cm^2$, respectively.

The block diagram of the device is shown in Figure S1. There is a micro controller unit (MCU) to support operation such as sonophoresis, iontophoresis, and load sensing for efficient device operation. The iontophoresis block is composed of constant current circuits in order to maintain a constant current of 100, 150, and 200 $\mu A/cm^2$ even if the load changes. Also, controlled ultrasound signals are emitted through the ultrasound transducer and these pulsed signals are shown in Figure S2. The Bluetooth module is applied to managing the device operation and use history. The transducer module consists of the head, transducer, and insulation film. The role of the insulation film prevents the current flow between the head and transducer. The transducer module is designed to support single mode and simultaneous mode of sonophoresis and iontophoresis.

The device was applied to the porcine dorsal skin with sonophoresis and iontophoresis. The skin was treated by the device for 4 min. The time was determined to set up the condition similar to actual device application in cosmetic field. The operating condition of the simultaneous mode was the same as each condition for the single mode. Using the Bluetooth function, the operating system was continually checked by a smartphone application during this process.

Skin Preparation

The dorsal skin of mini-pig was supplied by Cronex (South Korea) without drying and stored at −80°C and thawed at room temperature prior to the experiment. The skin has been verified through various virus, bacteria, and parasite tests by Cronex. Additionally, the integrity of the skin was verified by applying fluorescein. The porcine skin was mounted on the Franz diffusion cell and then 0.3 ml of 1 mM fluorescein (Sigma-Aldrich) solution (PBS buffer based) was applied into donor chamber. After 20 min, we measured the fluorescence of PBS solution collected from the receiver chamber with a spectrofluorophotometer (Dong-il Shimadzu, South Korea). The porcine skin without fluorescence signal was considered as having integrity, and the skin showing strong fluorescence signal was discarded. The verified skins through this method were used for experiments.

The mini-pig is promising model animal for percutaneous drug absorption because mini-pig and human skin are similar in consideration of cellular composition, morphology, and epidermal thickness (27,28). From previous research, there is

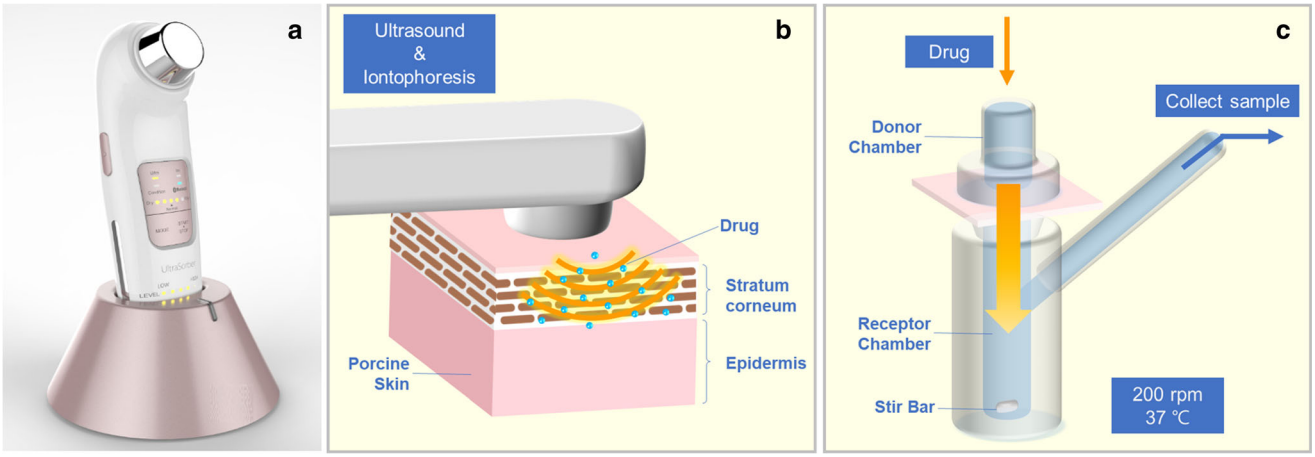

Fig. 1. Schematic illustration of the treatment of the sonophoresis and iontophoresis by the device and skin penetration experiment. **a** The image of fabricated device for sonophoresis and iontophoresis. **b** Application of ultrasound and iontophoresis. **c** Skin penetration experiment using Franz diffusion cell

not a quantitative, but a qualitative similarity between percutaneous drug absorption across porcine and human skin.

Skin Penetration Experiment

Various cosmetic ingredients were prepared for the skin penetration experiments. Table II presents the concentrations and formulations of each ingredients experimental materials. All hydrophilic drugs were dissolved in PBS for the skin penetration experiment. For the hydrophobic drugs, the formulations were prepared differently from hydrophilic drugs because hydrophobic drugs are insoluble in PBS. Retinol and alpha-bisabolol were used in emulsion form. The formulation of minoxidil was prepared in proportions of 5:2:3 for propylene glycol (PG), distilled water (DW), and ethanol (EtOH), respectively. The concentrations of these drugs were determined by the concentration of the actual cosmetic.

Porcine skin was used in the size of 3 cm in width, 3 cm in length, and 1 mm in thickness. A hole on the protective film (Tegaderm, 3M) was 2.5 cm in diameter in order to specify the area treated by the device. This film was attached on the porcine skin and then the porcine skin was placed on aluminum foil. The formulations were applied on the porcine skin at a volume of 100 μl and then treated by each condition of device (Fig. 1). Control group was treated with the device without power setup under the same conditions. After the treatment, the remaining formulation on the skin was removed by Pasteur pipette. The treated skin was mounted on the Franz diffusion cell. The receiver chamber was filled with phosphate buffer saline (PBS, pH 7.4), and the donor chamber was filled with 300 μl of the drug formulation. Franz diffusion cells were maintained at 37°C. Samples were taken at 3, 5, and 24 h by withdrawing and refilling the receiver chamber with fresh PBS. For the hydrophobic drugs, 0.5% (*v*/*v*) Tween 80-PBS solution was used.

HPLC and Analytical Method

All quantitative analyses of the samples were conducted with a High-Performance Liquid Chromatography system (HPLC, Younglin YL 9100 HPLC system, South Korea) equipped with a vacuum degasser, a quaternary pump, a column oven, and a UV/Vis detector. The C18 reverse-phase column (Zorbax SB-C18 type, 150 mm × 3.0 mm, 3.5 μm) was kept at 40°C by isocratic elution with the flow rate of 0.5 ml/min. The mobile phase composition was 0.2% (*v*/*v*%) trifluoroacetic acid (TFA) or 80% (*v*/*v*%) methanol in distilled water (DW). Samples were detected at each specific wavelength as shown in Table II using UV/Vis detector.

Data Analysis

The enhancement ratio was quantified by the peak area of the sample treated with each application condition divided by that of the control. We defined the enhancement ratio of each sample as follows:

$$\text{Enhancement ratio} = \left(\frac{\text{Peak area of the treated sample}}{\text{Peak area of the control}}\right) \times 100(\%) \quad (1)$$

Equation (1) was used to convert the accumulative peak area of each drug after 3, 5, and 24 h to the

Table I. Various Levels of Energy Flux and Application Time for Ultrasound and Iontophoresis

Frequency	Mode		Level 1	Level 2	Level 3	Time
280 kHz or 350 kHz	Ultrasound	Intensity (mW/cm^2)		500 ± 10%		4 min
		Duty ratio (clk = 10 Hz)	30%	60%	98%	
	Iontophoresis	Current density (μA/cm^2)	100	150	200	

Table II. The Concentrations and HPLC Conditions of the Hydrophilic and Hydrophobic Drugs used in the Experiments

	Active ingredient	Effect	Concentration (w/v%)	Mobile phase (v/v%)	Wavelength (nm)
Hydrophilic	Niacinamide	Whitening	5	0.2% TFA	254
	Arbutin	Whitening	5	0.2% TFA	222
	Caffeine	Reducing cellulite	2	80% Methanol	272
	Glutamic acid	Skin conditioning	1	0.2% TFA	215
Hydrophobic	Retinol	Anti-wrinkle, whitening	0.3	80% Methanol	325
	Alpha-bisabolol	Whitening	0.5	80% Methanol	200
	Minoxidil	Hair growth	1	80% Methanol	250

enhancement ratio. At each 3.5 and 24 h, the relative enhancement ratio of the treatment group was calculated by dividing the value of the treatment group by the value of control group. A total accumulated value of 300% of control group was converted to 100%.

Statistical Analysis

The number of samples in all the tests was at least three. All results in this study are shown as the mean ± SD. Statistical analysis was performed by two-tailed Student's *t* test. The differences were considered statistical significant based on the *p* value whether it is less than 0.05.

RESULTS AND DISCUSSION

Effect of Sonophoresis on Hydrophilic Drugs

The aim of skin penetration experiment was to evaluate the enhancement effect of sonophoresis for various drugs. Two versions of the deviceswith different frequencies (280 and 350 kHz) were designed, and ultrasound was treated to porcine skin for 4 min with three levels of the intensity (levels 1, 2, and 3). As shown in Fig. 2, all the hydrophilic drugs showed significant skin penetration enhancement with the ultrasound treatment compared to the control group. Niacinamide and arbutin, which are widely used in cosmetics as a whitening agents, had the highest enhancement ratio of 402% and 319% at 350 kHz and intensity level 2, respectively. The enhancement ratio of caffeine was more than doubled to 217% by applying ultrasound at 350 kHz and intensity level 3 (Table S1).

In general, application of ultrasound to the skin makes it possible to increase the fluidity of lipids and drug permeation through the intracellular pathway. A molecule with a lower molecular weight is able to more easily penetrate the skin through the pores created by ultrasound. Morimoto *et. al.* (29) have suggested that ultrasound treatment improves the transdermal transport of hydrophilic drugs by convective solvent flow. From these results, the relationship was found between enhancement effect by sonophoresis and the physicochemical properties of hydrophilic drugs. The molecular weights of hydrophilic drugs used in the experiment are 122.12, 272.25, and 194.19 Da for niacinamide, arbutin, and caffeine, respectively. Niacinamide with the lowest molecular weight showed the most enhanced skin permeability than the other hydrophilic drugs. The hydrophilic drugs have a major physicochemical difference: p*K*a values of 3.35, 9.82, and 14 for niacinamide, arbutin, and caffeine, respectively. The drug with lower p*K*a value showed a greater the enhancement ratio (Fig. 2). The p*K*a value indicated the dissociation of the drug, which implies that niacinamide was more soluble in water than arbutin and caffeine. This correlation is mainly because more dissociated molecules are easily transferred by convective solvent flow caused by sonophoresis.

Effect of Sonophoresis on Hydrophobic Drugs

We evaluated the effect of sonophoresis on hydrophobic drugs (Table II). For all treated groups, the significant enhancement was caused by the application of ultrasound (*t* test, $P < 0.05$). Retinol, known as vitamin A, showed the highest enhancement ratio of 292% at 350 kHz and intensity level 2 (Fig. 3). The enhancement ratios of alpha-bisabolol and minoxidil were 240% and 232% at 350 kHz and intensity level 3 (Table S1).

In transdermal drug delivery, the intercellular pathway *via* intercellular lipids predominates over the intracellular pathway. Especially, hydrophobic drugs prefer to penetrate skin through the intercellular pathway because stratum corneum has lipophilic properties (30). The oil–water partition coefficient (Log P) is a measure of hydrophilicity or hydrophobicity of drug. The higher Log *P* value indicates the more hydrophobic drug. The Log *P* values of drugs were 5.68, 3.91, and 1.24 for retinol, alpha-bisabolol, and minoxidil, respectively. In Fig. 3, more lipophilic drug showed the higher enhancement ratio. In all group, it was also observed that the enhancement ratio from 0 to 5 h was significantly higher than that from 5 h to 24 h. This result indicated that sonophoresis could induced the fast transport of hydrophobic drugs through an intracellular pathway. The skin penetration of drug after 5 h was also significantly enhanced compared to the control group. It is assumed that the cavitation bubbles induced the disorder in stratum corneum, resulting in hydrophobic drugs to easily permeate across the stratum corneum. So, the simultaneous activation of the intracellular pathway by sonophoresis and the intercellular pathway might have led to the skin penetration enhancement.

However, the enhancement ratio was not affected by the intensity levels in most of the samples in this study. This issue was also observed in previous studies. Ahmet *et al.* reported on a non-linearity between the enhancement and intensity at a high intensity levels (15). One possible explanation is that skin penetration enhancement is not proportional to the intensity above a certain level. Terahara *et al.* demonstrated

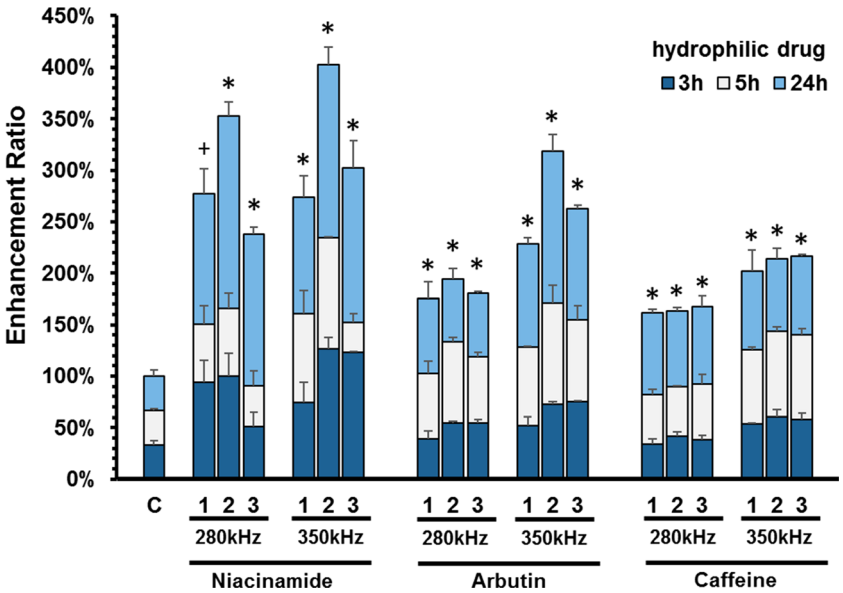

Fig. 2. Enhancement ratio of sonophoresis on the hydrophilic drugs at different frequencies (280and 350 kHz) and intensities (levels 1, 2, and 3). Each data point represents the mean ± SD ($n = 3$; $^{+}P < 0.05$; $^{*}P < 0.01$, two-tailed t test)

that as the intensity increased, skin conductivity was increased up to a threshold limit and then declined afterwards (31). Similarly, the most enhancement ratios were achieved at intensity level 2 and sudden decline on the enhancement ratios were observed at intensity level 3 for niacinamide, arbutin, and retinol (Figs. 2 and 3).

All the treated groups showed better enhancement ratio at higher frequency (350 kHz), but the enhancement ratio varied among different intensity levels. Unlike previous studies, higher frequency resulted in better skin permeability. The reason for the inconsistent result is that the difference between two frequency values (280 kHz and 350 kHz) was not enough to correlate the enhancement ratio with the frequency level.

Enhanced Transdermal Delivery of Glutamic Acid by Simultaneous Application of Sonophoresis and Iontophoresis

The experiment was carried out at different frequencies (280 and 350 kHz) and intensities (levels 1, 2, and 3) with the simultaneous application as well as single application of ultrasound and iontophoresis to evaluate the skin permeation enhancement effect of sonophoresis and iontophoresis. Glutamic acid, a negative charged molecule, was employed as the model drug. Therefore, a cathode with the same charge was applied on the skin. Iontophoresis was also applied to three level of current density of 100, 150, and 200 μA/cm^2. After the application of the device, no change in appearance of the skin was observed.

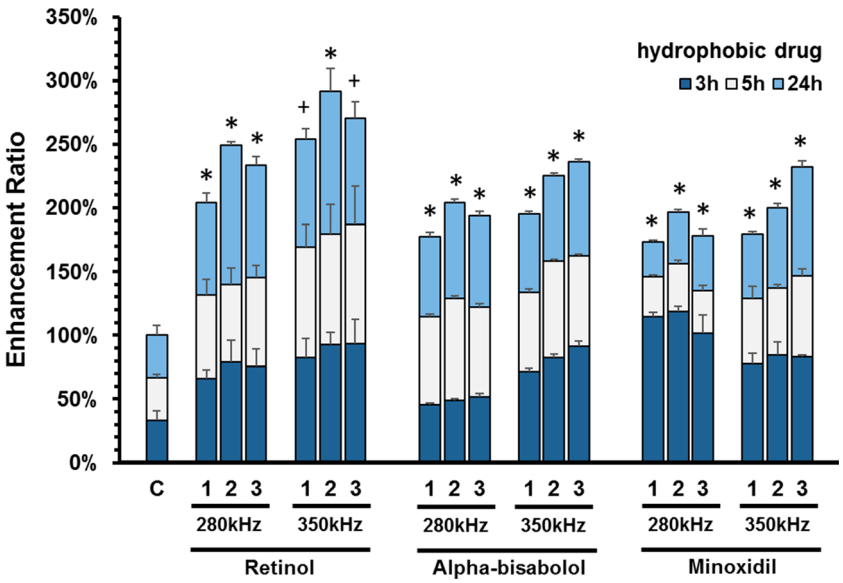

Fig. 3. Enhancement ratio of sonophoresis on the hydrophobic drugs at different frequencies (280 and 350 kHz) and intensities (levels 1, 2, and 3). Each data point represents the mean ± SD ($n = 3$; $^{+}P < 0.05$; $^{*}P < 0.01$, two-tailed t test)

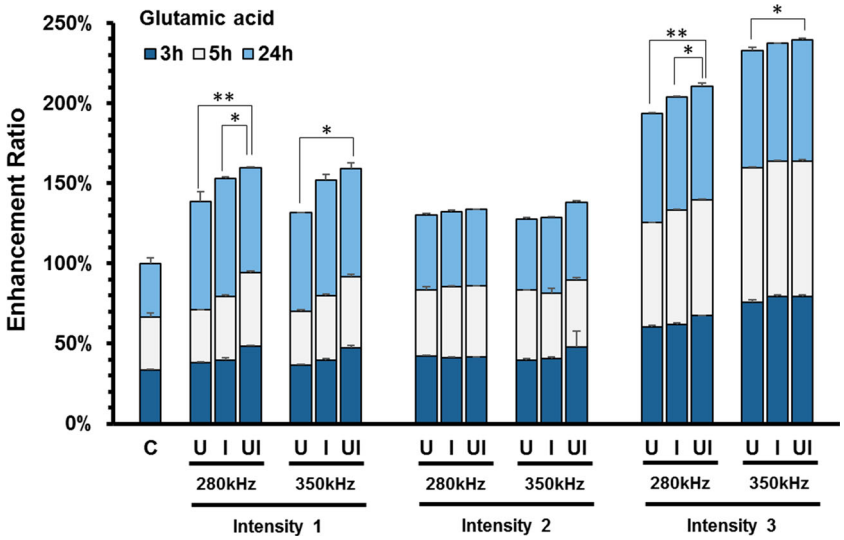

Fig. 4. Enhancement ratio of the glutamic acid on skin at different frequencies (280 and 350 kHz) and intensities (levels 1, 2, and 3). U: only ultrasound treatment, I: only iontophoresis treatment, UI: the simultaneous treatment of ultrasound and iontophoresis. All treated groups have a statistically significant difference compared to the control ($P < 0.01$, two-tailed t test). Each data point represents the mean ± SD ($n = 3$; $^{*}P < 0.05$; $^{**}P < 0.005$, two-tailed t test)

In this experiment, the enhancement of all treated groups showed the significant difference compared to that of the control group (t test, $P < 0.01$). It was also observed that iontophoresis has a stronger effect on enhancing skin permeation compared with sonophoresis shown in Fig. 4. The reason for this result is that glutamic acid has a negative charge and ionic property. The enhancement ratio was more than doubled to 240% by applying sonophoresis and iontophoresis simultaneously at 350 kHz and intensity level 3 (Table S2). More importantly, simultaneous application of sonophoresis and iontophoresis induced significant enhancement effect compared to ultrasound or iontophoresis individual application. The combined treatment of these physical enhancement methods increased the skin permeability of glutamic acid without losing their enhancement effect.

The combined effect is primarily due to the respective mechanisms of ultrasound and iontophoresis. The removal of lipids from the stratum corneum through the ultrasound treatment led to the fluidization of the stratum corneum and ultimately enhanced the drug permeation (32). Iontophoresis usually delivers the drugs through the electrically induced solvent flow (electro-osmosis) and a direct interaction with the electric field (electro-phoretic) (8). From these mechanisms, it is reasonable to infer that this combined strategy of two physical enhancers induced a structural change in the stratum corneum and promoted a solvent flow at the same time.

CONCLUSION

In summary, we have designed the miniaturized device for this study, which can operate not only the single application but also the simultaneous application of sonophoresis and iontophoresis. In spite of a short application time and lower intensity level than previous studies, the improved device achieved significant skin penetration enhancement of various hydrophilic and hydrophobic drugs. In particular, the enhancement ratio of niacinamide and retinol increased up to 402% and 292%, respectively. It was also found that the physicochemical properties of the drugs influence the enhancement effect by sonophoresis. We believe that these results can be used as a reference for the effects of physical enhancers on various drugs. Furthermore, the combination of sonophoresis and iontophoresis induces the combined effect without losing their own function. The simultaneous application is a promising approach because this strategy achieves transdermal enhancement at the low-energy density. Considering safety, this simultaneous application has advantage of reduced the skin irritation because this strategy enhances transdermal drug delivery at the low-energy density. Thus, this study suggests the miniaturized device is able to be practically used for self-administration in cosmetic and therapeutic fields. This simultaneous application of sonophoresis and iontophoresis can be extended for therapeutic fields as well as cosmetic fields.

FUNDING INFORMATION

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