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Using therapeutic ultrasound to promote irritated skin recovery after surfactant-induced barrier disruption

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ABSTRACT

Background: Surfactant-induced skin barrier disruption can enhance blood flow and water content in the superficial skin. The effect of therapeutic ultrasound on accelerating the recovery of superficial skin after skin barrier disruption has seldom been studied.

Objective: To understand the effects of therapeutic ultrasound on barrier recovery, we used the sodium lauryl sulfate irritation model and treatment with ultrasound intervention.

Methods: The study allocated 30 healthy subjects into an ultrasound group (n = 15) and a control group (n = 15), each divided into three subgroups (sodium lauryl sulfate at concentrations of 1.0%, 0.5%, and 0%). Pulsed ultrasound (1 MHz, 0.3 W/cm^2_{SATA}) was applied to ultrasound subgroups. The treatment effect was evaluated by the recovery rate of enhanced blood flow and water content.

Results: The results indicated a surfactant dose-dependent effect on blood flow, but not on water content. The recovery rates of enhanced blood flow were higher in the 0.5% and 1.0% ultrasound subgroups than in the control subgroups throughout the experiment. However, recovery rates of water content were higher in the ultrasound subgroups than in the control subgroups only on Day2.

Conclusions: Pulsed ultrasound accelerated the barrier recovery by reducing the enhanced blood flow and water content after skin barrier disruption.

1. Introduction

Ultrasound (US) is used widely in medicine as therapeutic tool for soft tissue lesions. Through both thermal and nonthermal mechanisms, US can promote healing in a variety of soft tissues, such as tendons, ligaments, joint capsules, and fascia [1,2].

Several studies have demonstrated that pulsed US promotes cutaneous tissue repair of diabetic wounds, skin flaps, and incisional wounds [3–7]. To sum up the aforementioned research regarding the early intervention on acute damage to skin tissue, US (pulsed, 0.75–3.0 MHz, 0.1–0.5 W/cm^2_{SATA}) can increase the collagen deposition and tissue tension of wounds, thus improving the survival rate of the skin flaps, as well as the blood vessel formation, epidermal cell proliferation, and vessel formation rates [3–7]. While the above interventions covered all the layers of cutaneous tissue, they did not focus on therapeutic US for superficial skin damage to the dermis and epidermis.

A dysfunctional barrier can be observed in various skin diseases, such as atopic dermatitis. When the skin barrier is disrupted, the disruption can simultaneously cause epidermal DNA synthesis and increase the release of cytokines [8,9], which facilitate the process of tissue repair. Choi et al. have demonstrated that the TGF-β of normal dermis and epidermis is upregulated after US treatment [10], suggesting that US intervention may accelerate activation in the inflammatory phase and promote the next proliferative stage of tissue [11]. Moreover, Mortimer & Dyson have reported that US can change the calcium permeability of the cell membrane in fibroblasts [12]. The increased concentration of intracellular calcium ions can activate the appropriate calcium-sensitive signal transduction pathways in the cell, thus promoting fibroblast proliferation [12]. Although no direct evidence suggests that the clinical advantages of ultrasound are due to altered membrane permeability and cytokines [13], Dyson has suggested that these changes could account for the promoted tissue repair

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after treatment with ultrasound [11].

Artificial disruption of the skin barrier is a type of damage to the skin barrier caused by a surfactant, an organic solvent [14]. According to the standard guidance published by the European Society of Contact Dermatitis (ESCD), sodium lauryl sulfate (SLS) is an anionic surfactant used extensively as a surfactant for skin barrier disruption [14]. It has been applied in dermatological research as a model of barrier disruption [15,16]. The surfactant destroys the lipids of the stratum corneum and thereby disrupts the barrier function of the skin, after which it permeates through the epidermal and dermal layers to cause a slight irritant reaction. In human stratum corneum cells, SLS increases the expression of cytokines, which leads to endothelial dysfunction and is characterized by vasodilation and increased capillary permeability [17,18]. The resulting syndrome from SLS irritation is clinically associated with increased blood flow and water content in human dermis [18,19]. Some in vivo studies have also found that the cytokine and skin blood flow in human forearm skin increased according to the SLS dosage, indicating an SLS dose-dependent effect on barrier damage [16,18,20]. Although it has been suggested that the irritation by topical administration of SLS can be used in clinical and experimental research [14,15,21], no previous studies have examined the application of US for barrier disruption in the irritation model.

To understand the effects of therapeutic US on barrier recovery, US was applied to the skin under the SLS-irritation model. This study aimed to test the hypothesis that US can promote barrier recovery by reducing the enhanced blood flow and water content after skin disruption. In this study, the model was used for studying the effect of US on the changes in superficial dermis, wherein one forearm of each participant in both groups was selected to receive SLS stimulation. Therapeutic US (1 MHz) was used as a stimulation method to deliver the sound waves to the irritated skin, and the skin blood flow and water content were quantitatively assessed by laser Doppler flowmeter and dielectric constant analyzer. Details of the current study are presented as follows.

2. Methods

2.1. Participants

The experimental protocol was approved by the institutional review board of Chung Shan Medical University Hospital, and informed consent for the study was obtained from all human subjects in accordance with the WORLD Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects; Trial Registration: ClinicalTrials.gov (NCT02257281). In total, 30 participants were recruited for this study with a poster approved by the Institutional Review Board. The 30 healthy subjects were randomly divided into two groups: an US group and a control group. All subjects were examined by doctors, and unsuitable subjects were excluded from the study. After the investigator explained the content to the subjects and they completely understood the test, they signed the informed consent. Healthy volunteers, males and females aged 20 to 45, were admitted. The exclusion criteria included vulnerable groups, metabolic disorders, malignancy, autoimmune diseases, allergies, irritant contact dermatitis, inflamed or infected skin, and persons meeting US treatment contraindications. The contraindications for US include infection, neoplasms, cancer, growing epiphyseal plate, metal pins, lack of sensation, and venous thrombosis [22]. Additional exclusion criteria included smoking or drinking, a recent blood extraction from the arm, aerobic exercise, medication (such as corticosteroid or adrenocorticotropic hormones), and excessive exposure to the sun near the experimental period.

2.2. Skin barrier disruption

Three circles of 2 cm in diameter were labeled along the midline of the volar forearm at 5, 10, and 15 cm distal to the antecubital crease. SLS (purity ≥ 99%, Sigma-Aldrich, Sigma Chemicals Co., St. Louis, MO, USA) and sterile water were mixed to prepare SLS solutions of three concentrations: 1%, 0.5%, and 0%. A pipette was used to drip the 60 µl SLS solution into 12 mm Finn chambers with paper filter discs (Epitest Ltd, Oy, Finland). The centers of the circular blocks were covered with Finn chambers with 1%, 0%, and 0.5% SLS solution, respectively, from the proximal to distal circles. Afterwards, the Finn chambers were affixed with non-occlusive dressing to the volar forearm and left for 24 h before removal. On removal of the SLS patches, the skin was gently washed with saline solution and allowed to dry. The day the chamber was removed was defined as Day0. The day following Day0 was defined as Day1, when the skin presented circular erythema (Fig. 1) [19]. Thus, disruption of the artificial skin barrier was completed. The homeostatic repair response began after skin barrier disruption [23]. Gradually, the barrier disruption in the dermal layer would recover [19,24].

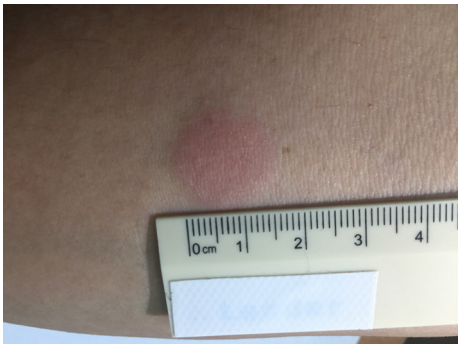

Fig. 1. Marked erythema induced by application of SLS. The erythema (1.0% SLS) was irritated on Day1.

2.3. Application of ultrasound

After the skin barrier disruption was completed, US intervention was applied twice daily to each erythema of the US group at fixed times (9:00 a.m. and 4:00p.m.) from Day1 to Day4, while the erythemas of the control group were free of US intervention. The US application was applied by a licensed physiotherapist. Each erythema was treated with 1.5 W/cm^2 (spatial average temporal peak, 20% duty cycle, 1 MHz) for 10 min. The machine model was a Sonopuls 692 with a 1.0-cm transducer head having a 0.5 cm^2 effective radiating area (Enraf Nonius BV, Rotterdam, the Netherlands). The operator moved the transducer head smoothly in circular motions. To ensure that the treatment was equal for all subjects, the probe was applied within the circular block. The jelly used for transmission was OptiLube Lubricating Jelly (Optimum Medical Solutions, UK). Additionally, the transducer head was sterilized by 75% alcohol before each US intervention.

2.4. Skin blood flow

Skin blood flow is one of the most important biophysical parameters for evaluating human skin barrier disruption [16,25,26]. To evaluate the increased blood flow caused by the skin irritation, a laser Doppler flowmeter (MoorVMS-LDF1; Moor Instruments Ltd., Devon, UK) was used according to the guidelines of the ESCD Standardization Group [25]. Doppler flowmetry is a noninvasive technique that measures the velocity of moving erythrocytes to a depth of approximately 1 mm. After the subject rested supine for about 15 min, the flow probe was applied with an adhesive holder to the center of the circular block, which had been drawn previously. The data during a period of 5 min were averaged for analysis. The measured data of normal skin before skin barrier disruption were defined as the baseline. The measurements were completed before nine o'clock in the morning from Day1 to Day5.

SLS-irritated barrier disruption is clinically associated with

increased blood flow and water content in human dermis [19,27]. The decline in the rate of the enhanced blood flow in recovery that presents after irritation was represented by the recovery rate of enhanced blood flow (ROBF). A value of ROBF close to 100% indicated recovery to nearly normal, meaning that the skin blood flow had been restored to the baseline. The following formula was used for calculation. The recovery rate of enhanced blood flow was given by % Recovery = $[(BF1 - BF_i)/(BF_1 - BF_0)] \times 100\%$, where BF_1 is 1st day blood flow before intervention, BF_0 is baseline blood flow before barrier disruption, and BF_i is blood flow after intervention at indicated time points.

2.5. Skin water content

The irritation after skin barrier disruption was combined with increased water content [19,27]. A dielectric constant analyzer (Moisture Meter D, Delfin Technologies Ltd., Kuopio, Finland) was used to detect the permittivity of the skin to represent the skin water content [27,28]. The reliability and validity of this approach are well established [28,29]. The principles of operation and physics have been described previously [30]. Briefly, a 300 MHz electromagnetic wave is produced by the analyzer and transmitted to the target tissue via a probe in contact with the skin. The amount of the incident wave that is reflected depends on the permittivity of the skin tissue, which itself depends on the amount of water in the tissue. Then the analyzer collects the reflected wave and displays the intensity of the wave as a dielectric constant [31]. The measured dielectric constant is therefore directly proportional to the water content of a tissue [28]. We selected a 10 mm diameter probe with an effective measuring depth of 1.5 mm, which is approximately the thickness of the dermis. The dielectric constant of each erythema was measured in triplicate and averaged for analysis. The measurement time of the water content was the same as the measurement time of the blood flow described above. The recovery rate of enhanced water content (ROWC) was calculated in the same way as the recovery rate of enhanced blood flow.

2.6. Statistical analysis

SPSS Version 14.0 was used for statistical analysis. Descriptive statistics were calculated for the demographic and dermatological variables. Continuous data are expressed as mean and standard deviation. Categorical data are expressed as frequencies and ratios. Independent *t*-test (continuous data) or chi-square test (categorical data) was used for comparison of the demographic variables and dermatological characteristics between the control and the US groups at the baseline. To examine the SLS dosage dependent effect, the dependent variables among the three subgroups (1.0%, 0.5% and 0%) were compared at the beginning of the study, for which one-way ANOVA with Tukey's HSD post-hoc analysis was used. To examine the changes in the dermatological variables over time, the blood flow and water content at different time points were compared (from baseline to Day5), for which repeated measures ANOVA with Tukey's HSD post hoc analysis was used. For testing the effect of US on the ROBF and ROWC, a comparison of the control and US subgroups was analyzed by Independent *t*-test. Statistical significance was set at $P < .05$.

3. Results

Table 1 summarizes the demographic and dermatological characteristics of the participants in the control and US groups at the beginning of this study. At the baseline, the blood flow (BF_0) and water content (WC_0), the dermatological variables, were measured before SLS irritation and US intervention. On the morning of Day1, the blood flow (BF_1) and water content (WC_1) were measured after SLS irritation but before US intervention. There were no statistically significant differences between the control and the US group in age, body height, body weight, right and left sides of forearm and dominant hand (Table 1, all $P > .05$), nor were there statistically significant differences in the dermatological characteristics of the two subgroups that were irritated with the same concentration of SLS solution (Table 1, all $P > .05$). These results demonstrated that there were no differences in demographic and dermatological characteristics between the control and US groups before the beginning of US intervention.

Table 1 shows that the BF_0 among the three subgroups had no statistical differences ($P > .05$). However, the WC_0 among the three subgroups had statistical differences ($P < .05$). Pairwise comparison showed that the WC_0 was higher in the 0.5% subgroup than in the 1.0% subgroup (Table 1, $P < .05$). There were also significant differences in BF_1 and WC_1 among the three subgroups ($P < .001$). BF_1 and WC_1 were significantly higher in the 1.0% ($P < .001$) and the 0.5% ($P < .05$) subgroups than in the 0% subgroup (Table 1), and BF_1 was higher in the 1.0% subgroup than in the 0.5% subgroup ($P < .05$).

Fig. 2 shows the changes of blood flow and water content irritated by SLS throughout the experiment. Regarding the changes in the dermatological variables over time, repeated measures ANOVA demonstrated significant differences among the dependent variables at different time points in the 1.0% and 0.5% control subgroups (Fig. 2a and b) (all $P < .001$). Blood flow was significantly increased over the baseline in the 1.0% and 0.5% control subgroups on Day1 (both $P < .001$), and it gradually returned to values close to the baseline on Day5 ($P < .05$ and $P < .001$, respectively) (Fig. 2a, left). The water content in the 1.0% and 0.5% control subgroups rapidly increased on Day1 (both $P < .001$) and gradually decreased to values below the baseline on Day5 ($P < .001$ and $P < .05$, respectively) (Fig. 2b, left). It is noteworthy that water content decreased to a level below the baseline by Day3 (1.0% Control) and Day4 (0.5% Control) ($P < .05$ and $P > .05$, respectively). Additionally, the time course of blood flow and water content changes held true for both ultrasound and non-ultrasound treatments (Fig. 2a and b).

As shown in Fig. 3, ROBF was higher in the 1.0% US subgroup than in the 1.0% control subgroup on Day2 (58.7 ± 20.0 vs. 39.6 ± 23.9, mean difference = 19.1, 95% CI 35.8 to 2.4, P = .027) and Day4 (84.5 ± 7.9 vs. 67.3 ± 22.4, mean difference = 17.2, 95% CI 29.8 to 4.6, P = .009), and a significant difference was also found on Day5 (84.5 ± 12.8 vs. 67.9 ± 23.9, mean difference = 16.5, 95% CI 30.8 to 2.2, P = .025). ROBF was higher in the 0.5% US subgroup than in the 0.5% control subgroup on each day of the experimental period. There were significant differences from Day2 (53.8 ± 26.2 vs. 24.5 ± 28.2, mean difference = 29.3, 95% CI 49.6 to 8.9, P = .006) to Day5 (87.6 ± 8.5 vs. 55.5 ± 20.6, mean difference = 32.0, 95% CI 43.8 to 20.3, $P < .001$) (Fig. 3b).

As shown in Fig. 4, ROWC was higher in the 1.0% US subgroup than in the 1.0% control subgroup only on Day2 (61.5 ± 23.5 vs. 13.13 ± 18.9, mean difference = 48.4, 95% CI 63.3 to 32.5, $P < .001$), but not significantly different on Day5 (179.6 ± 50.0 vs. 215.3 ± 46.2, mean difference = −35.7, 95% CI 0.26 to −71.7, P = .052). In addition, ROWC was higher in the 0.5% US subgroup than in the 0.5% control subgroup on Day2 (33.7 ± 30.4 vs. −24.2 ± 34.0, mean difference = 57.9, 95% CI 82 to 33.7, $P < .001$) and Day3 (82.47 ± 56.0 vs. 31.3 ± 44.5, mean difference = 51.1, 95% CI 89.0 to 13.3, $P < .001$) (Fig. 4). However, it was not significantly different on Day5 (147.1 ± 52.8 vs. 180.7 ± 50.0, mean difference = −33.7, 95% CI 4.8 to −72.1, P = .084).

4. Discussion

SLS destroys the skin barrier and increases the expression of the cytokines [17,32,33], which facilitate enhanced blood flow and water content in the superficial skin [14,18,19]. In this study, the time courses of blood flow and water content changes irritated by SLS were similar to those in studies reported previously [19,34]. The enhanced blood flow decreased gradually and finally returned to close to the baseline values

Table 1
Baseline and day 1 characteristics of the participants.

	Ultrasound group (N = 15)				Control group (N = 15)						
	1.0%	0.5%	0%	$P^{\dagger}$	1.0%	0.5%	0%	$P^{\dagger}$	P^{*} 1.0%	0.5%	0%
Age, years	28.4 ± 7.9				28.5 ± 8.5				.530		
Height, cm	164.6 ± 8.1				165.5 ± 7.5				.849		
Weight, kg	59.9 ± 10.9				61.9 ± 11.1				.872		
Gender, male/female	7/8				7/8				1.000		
Test forearm, right/left	8/7				7/8				.726		
Dominated/non-dominated	8/7				7/8				.726		
Blood flow of baseline (BF_0)	8.6 ± 3.7	7.7 ± 2.0	8.3 ± 0.8	.645	8.1 ± 1.3	7.4 ± 0.9	8.2 ± 1.3	.129	.534	.550	.865
Blood flow of day 1 (BF_1)	62.5 ± 32.0[a,c]	30.3 ± 5.7[b,c]	9.8 ± 1.9[a,b]	< .001	52.6 ± 19.7[a,c]	28.5 ± 7.6[b,c]	9.7 ± 2.0[a,b]	< .001	.329	.495	.954
Difference between BF_1 and BF_0	53.8 ± 32.9[a,c]	22.6 ± 5.1[b,c]	1.5 ± 2.2[a,b]	< .001	44.5 ± 19.9[a,c]	21.2 ± 7.7[b,c]	1.5 ± 2.8[a,b]	< .001	.372	.574	.976
Skin water content of baseline (SW_0)	29.0 ± 2.8[c]	32.3 ± 2.8[c]	30.7 ± 1.6	.007	28.1 ± 1.8[c]	31.4 ± 2.9[c]	30.0 ± 2.4	.001	.326	.442	.356
Skin water content of day 1 (SW_1)	38.1 ± 4.5[a]	38.6 ± 3.0[b]	32.1 ± 1.7[a,b]	< .001	36.5 ± 3.2[a]	37.0 ± 2.5[b]	30.7 ± 1.9[a,b]	< .001	.282	.505	.402
Difference between SW_1 and SW_0	9.1 ± 3.6[a,c]	6.3 ± 1.8[b,c]	1.4 ± 1.0[a,b]	< .001	8.3 ± 2.7[a,c]	5.6 ± 2.7[b,c]	0.7 ± 1.5[a,b]	< .001	.536	.911	.693

Abbreviation: BF_0, Blood flow of baseline; BF_1, Blood flow of day 1; SW_0, Skin water content of baseline; SW_1, Skin water content of day 1.
Data are expressed as ratios or mean ± standard deviation (n = 15).
1.0%, 0.5% and 0% subgroups: the three subgroups within the control (or ultrasound) group irritated with 1.0%, 0.5% and 0% SLS solution (n = 15, respectively).
* Between the control and ultrasound group analyzed by Independent *t*-test (continuous data) or chi-square test (categorical data).
† Among the three subgroups analyzed by one-way ANOVA.
[a] A significant difference between 1.0% SLS and 0% SLS subgroups (post hoc test with Tukey's HSD, P < .001).
[b] A significant difference between 0.5% SLS and 0% SLS subgroups (post hoc test with Tukey's HSD, P < .05).
[c] A significant difference between 1.0% SLS and 0.5% SLS subgroups (post hoc test with Tukey's HSD, P < .001).

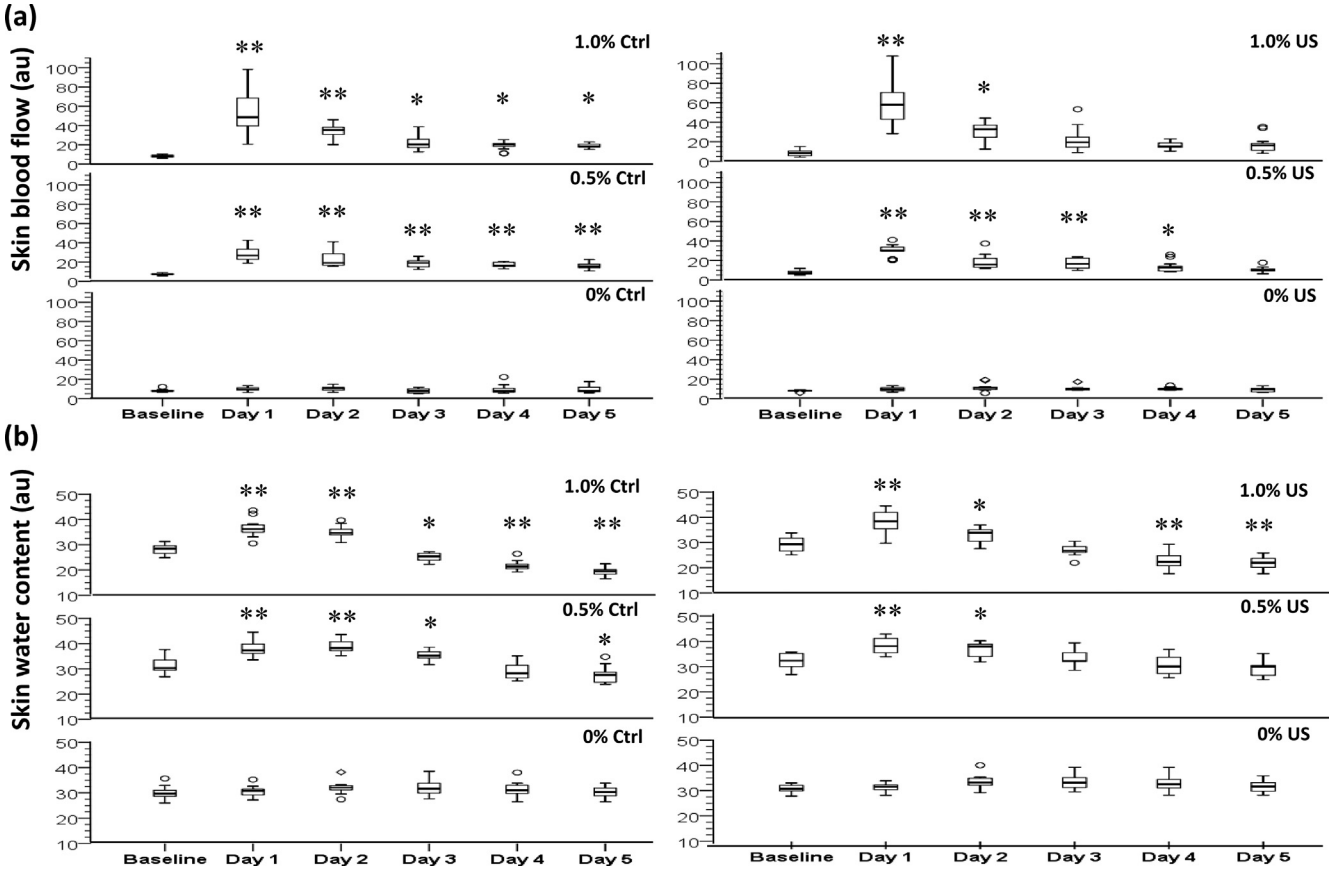

Fig. 2. Time-course for the dermatological variables in subgroups recorded throughout the experiment. The increased blood flow in the 1.0% and 0.5% control (Ctrl) subgroups on Day1 decreased gradually and was close to the baseline value on Day5 (Fig. 2a, left). Additionally, the increased water content in the 1.0% and 0.5% Ctrl subgroups on Day1 decreased gradually and was significantly below the baseline value on Day5 (**P < .001 and *P < .05, respectively) (Fig. 2b, left). The changes in the blood flow and water content in the ultrasound (US) subgroups are also presented in the course of time (Fig. 2, right). All results which are significantly different from the baseline are indicated by a double asterisk (**P < .001) or a single asterisk (*P < .05). Data are expressed as median and IQR (n = 15, each subgroup).

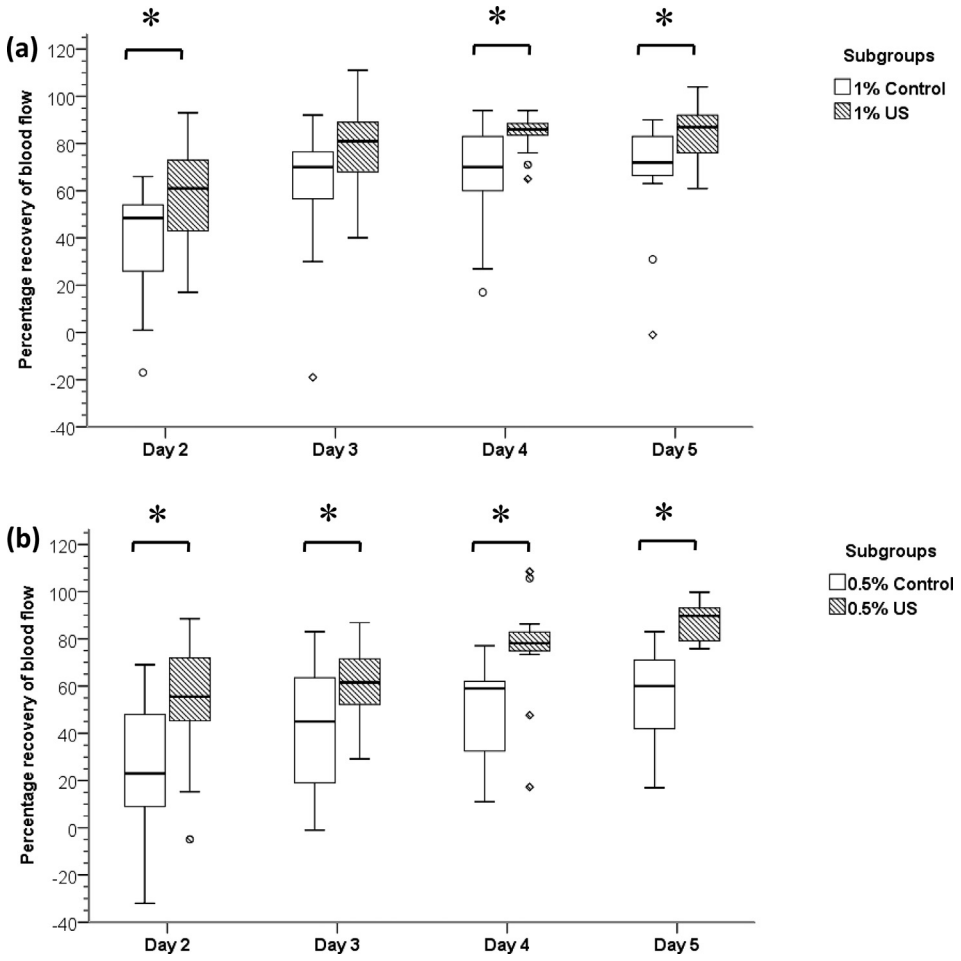

Fig. 3. Comparison of the recovery rate of enhanced blood flow (ROBF) between the control (Ctrl) and ultrasound (US) subgroups irritated with (a) 1.0% and (b) 0.5% SLS. Data are expressed as median and IQR (n = 15). Significant differences ($^{*}P < .05$) were found between the US and Ctrl subgroups. A value of ROBF close to 100% indicates good recovery, meaning that the blood flow was restored to the baseline.

(Fig. 2a), while the enhanced the water content decreased to baseline levels by Day3 (1.0% subgroups) and to values below the baseline on Day5 (Fig. 2b). At the beginning of the study, a dosage dependent effect on the enhanced blood flow (BF_1) was noted in both groups, but no such effect on the skin water content (WC_1) was noted (Table 1). As in previous studies, the blood flow changes were dependent on the dose of surfactant in irritated human skin [16,20]. The present study examined the application of US for barrier disruption in the irritation model. The results demonstrated that the recovery rates of the enhanced blood flow and water content induced by SLS were promoted by pulsed US (1 MHz, 1:4 $_{\text{duty cycle}}$, 0.3 W/cm^2_{SATA}), meaning that pulsed US may accelerate skin repair after barrier disruption (Figs. 3 and 4).

Little previous evidence has shown that therapeutic ultrasound is effective in treating superficial skin and barrier damage. Denda and Nakatani's research found that sound waves could accelerate barrier recovery [35]. They used audible sound (10 and 20 kHz) and US (30 kHz) to stimulate disrupted skin barriers on the flanks of hairless mice and indicated that the recovery of transepidermal water loss (TEWL) was accelerated. The authors postulated that sound could accelerate barrier recovery by influencing the homeostatic system [35]. Although the US frequencies used in that study and in the present study were different (30 kHz and 1 MHz), barrier recovery was promoted in both studies by the application of US. Especially, we have demonstrated the potential of therapeutic ultrasound for superficial skin repair in human subjects for the first time.

Previous studies have demonstrated that US has the potential to decrease blood flow in animal models [36–38] and human skin [39,40]. In one study on human skin, Ware et al. found an average decrease of 12% in blood flow in calf skin, despite an increase in skin temperature (3 MHz, continuous, 1.5 W/cm^2, 10 min) [39]. Recently, Shaik et al. have demonstrated the effects of US on decreasing skin blood flow in the forearm after applying two modes of US (3 MHz, pulsed $_{1:4}$, 0.25 W/cm^2_{SATP}, 5 min; 1 MHz, continuous, 0.8 W/cm^2, 3 min) [40]. In contrast, other studies with human subjects have demonstrated that US has the potential to increase blood flow [41,42]. Noble et al. demonstrated the real-time effects of US on increasing skin blood flow in the human forearm with the application of pulsed and continuous US (3 MHz, 1 W/cm^2, 6 min) [42]. However, the study designs of the previous studies and the present study had two important differences. First, the previous studies used healthy skin instead of irritated skin, and second, the numbers of US treatments in the previous and current studies were different (one treatment and eight treatments, respectively). While the results demonstrated that the application of US significantly promoted the recovery of irritated human skin, the underlying mechanisms of the changes in skin blood flow are still unclear. Shaik et al. presumed that the mechanism of the decrease in blood flow in human forearm skin in their study might have been due to the cooling effect of the transmission gel and the US transducer head, which would stimulate sympathetic vasoconstriction, or other underlying mechanisms [40]. The design of the parameters used in the present study was intended to avoid the thermal effect in principle due to the acutely irritated skin, which was treated with low dose pulsed US at 1 MHz. Hence, a possible explanation for the underlying mechanisms may be the cooling effect and non-thermal effect [13,39]. Unfortunately, we did not measure

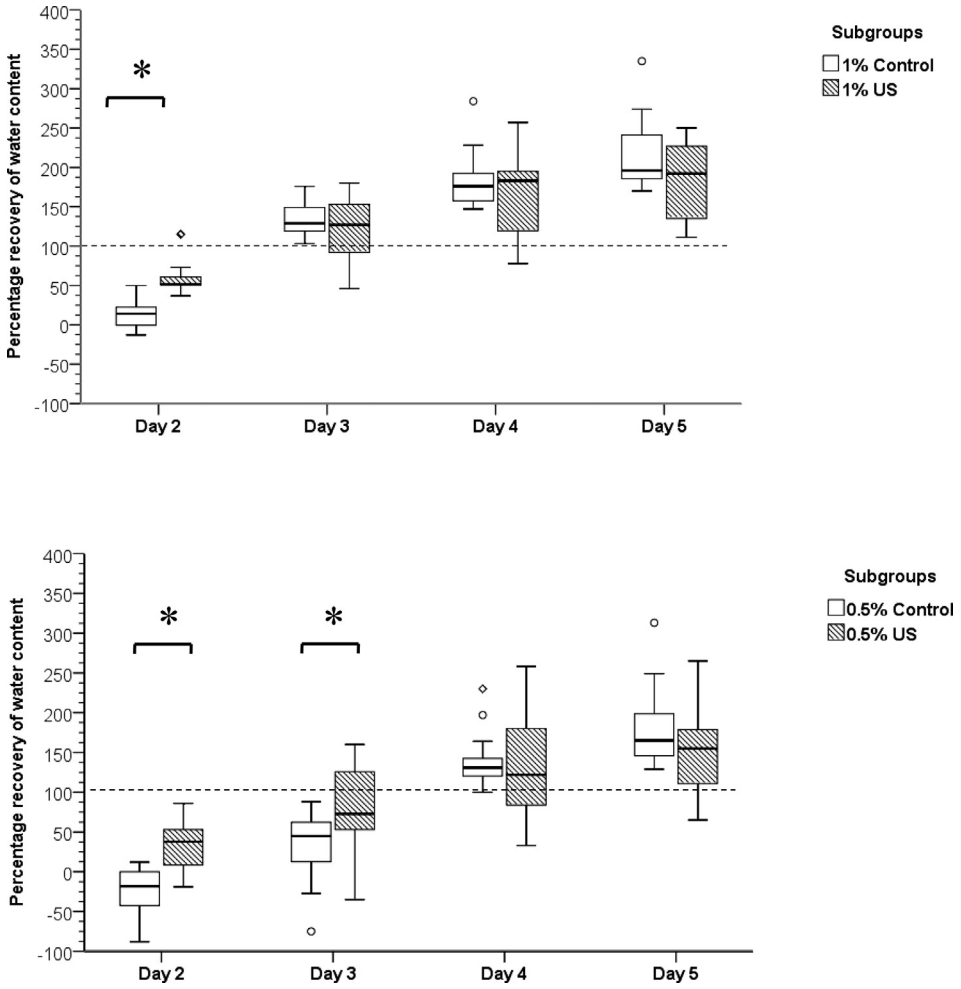

Fig. 4. Comparison of the recovery rate of enhanced water content (ROWC) between the control (Ctrl) and ultrasound (US) subgroups irritated with (a) 1.0% and (b) 0.5% SLS. Data are expressed in median and IQR (n = 15). Significant differences ($^{*}P < .05$) were found between the US and Ctrl subgroups only on Day2 and Day3. The dotted lines illustrate 100% of ROWC. A recovery rate above the dotted lines indicates it exceeded 100% of ROWC, which means that the skin water content was already lower than the baseline value during the recovery.

temperature changes throughout the experimental period, which may have further explained the observed decrease. Additionally, US at 1 MHz would have an effect on deep areas of the dermis besides the superficial layer. Investigation into the possible mechanism also should take into account this confounding factor in future research.

Clinically, US is expected to reduce the water content of injured tissue and thereby enhance the tissue repair capacity [43]. In the present study, the results demonstrated that application of US significantly promotes the recovery rate of the enhanced water content in human cutaneous tissue, thus supporting the work by Ferguson [44]. In contrast, a clinical note reported that the impedance of normal skin dropped after US (55 kHz, 15 W/cm2) was briefly applied on the volar forearm. The decrease in skin impedance represented increased water content after US [45]. In the present study, US was found to promote the recovery from enhanced water content only on Day2. The reason that therapeutic ultrasound was not effective in decreasing the water content on days 3, 4 and 5 (Fig. 4a) could be that the water content levels were already decreased to basal levels by Day3 (Fig. 2b). It is noteworthy that the ROWC exceeded 100% (dotted line) after Day3 and Day4 (Fig. 4a and b). An ROWC above the rate of 100% indicated that the decreased skin water content was already below the value of the baseline (Fig. 4). Above the 100% ROWC, a lower value indicated possibly better water retention in the dermis during recovery. Although ROWC was not different between the 1.0% subgroups on Day5 after exceeding the 100% ROWC, the p value was close to statistical significance (P = .052). This result implied that the US subgroup would have better water retention than the control subgroup if the experimental time were extended.

It is believed that the enhanced water content induced by SLS irritation will gradually return to a normal value close to the baseline due to spontaneous recovery [19]. However, the recovery from the enhanced water content is simultaneously accompanied by evaporation due to the ruptured stratum corneum caused by the barrier disruption [46]. Gradually, skin water is lost from the body surface to the point that the water content falls below the normal value (which is the baseline) [23]. The skin water content could be lower than the baseline in the last few days (Fig. 2b, 1.0%, and 0.5% control subgroups).

Pulsed US, as proposed in this study, may be effective in the treatment of human skin diseases related to barrier disruption, such as atopic dermatitis, psoriasis and irritant contact dermatitis. Clinical cases will be recruited in future research to explore the clinical feasibility. In addition, this experimental model could be applied in US research with different parameter sets for the dermatological variables. Further studies could explore different severities of barrier disruption under different US parameters in vivo, which would be required to establish therapeutic principles in the future. Since the mechanism that produces the changes of irritated skin recovery is unknown, further research is needed before any conclusion can be reached.

5. Conclusions

The present study found that the recovery from enhanced skin blood flow and skin water content after skin irritation was promoted by pulsed US. This implies that US intervention may accelerate skin recovery in cases of barrier disruption, and that US may have clinical applications for treating dermatological diseases in the future.

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Ethics approval

The experimental protocol was approved by the institutional review board of Chung Shan Medical University Hospital; Trial Registration: ClinicalTrials.gov (NCT02257281).

Declarations of interest

The authors completed the ICJME Form for Disclosure of Potential Conflicts of Interest and reported no conflicts of interest.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ultras.2018.08.007.

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