Noninvasive Transdermal Delivery of Biomolecules Using Arc-poration

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Abstract: The transdermal drug delivery system is the most suitable method for increasing the bioavailability of drugs, minimizing side effects, and improving patient compliance. However, due to the stratum corneum skin barrier, transdermal drug delivery remains limited. To disrupt the stratum corneum, we developed an arc-poration device, which is an arc discharge-based device that creates micropores on the stratum corneum while minimizing skin damage. Optical images and histological analysis using reconstituted human skin and porcine skin show that micropores with an average diameter of approximately 100 micrometers are created only to the depth of the stratum corneum, not viable epidermis, by the treatment of arc-poration. In addition, the Franz diffusion cell experiment using reconstituted human skin showed a remarkable increase in permeability following pretreatment with arc-poration. Clinical results indicate that the skin improvement effect of cosmetics is enhanced by pretreatment of arc-poration is statistically significant, and there are no abnormal skin responses. Taken together, our results indicate that arc-poration can increase skin permeability by creating stratum corneum-specific micropores. Here we suggest arc-poration as a novel technique that can overcome the limitations of transdermal drug delivery.

Keywords: transdermal drug delivery; arc discharge; arc-poration; skin permeability; stratum corneum; cosmetics

1. Introduction

The transdermal drug delivery system (TDDS) is emerging as an attractive alternative to drug delivery via oral administration or subcutaneous injection [1]. The TDDS enables controlled and long-term release of drugs by avoiding first-pass effects, enzymatic digestion, and exposure to extreme changes in pH that occur during oral administration [2,3]. Drug administration is even possible for patients who are unconscious or have difficulty swallowing via the TDDS [4]. In addition, the TDDS can minimize pain and injection risks, thereby improving patient compliance [5]. In terms of skin diseases, especially, TDDS is an effective administration route that can be applied directly to skin lesions, and it avoids the side effects of conventional administration routes [6].

The most critical step in TDDS is that the drug must pass through the two epidermal skin barriers: the stratum corneum and the tight junctions on the stratum granulosum. The stratum corneum, the primary skin barrier, very strictly prevents the penetration of external substances and pathogens [7]. Nevertheless, small molecules with molecular weights of less than 500 Da and moderate lipophilicity can penetrate the stratum corneum to some extent, but not macromolecules and hydrophilic molecules [1,8]. The tight junctions on the stratum granulosum obstruct molecules that pass through the stratum corneum from penetrating deep into the skin [9]. However, the tight junction is less strict than the stratum corneum because it can selectively allow ions, uncharged molecules, and macromolecules to pass through the charge-selective pore and leak pathways [10]. This suggests that the most important challenge for the TDDS is to disrupt the stratum corneum.

FDA-approved drugs for transdermal delivery are small molecules with a molecular weight of less than 500 Da capable of passive delivery [11]. Recently, a number of innovative biologics, consisting of antibodies, peptides, or nucleic acids for various diseases, have been developed [12]. However, they cannot penetrate the skin with passive delivery methods because most of them are low lipophilic macromolecules. To increase the permeability of biologics, various active delivery techniques have been developed, including iontophoresis, sonophoresis, electroporation, thermal ablation, and microneedle [13]. Of those techniques, only the microneedle and thermal ablation techniques create micropores on the skin. The microneedle procedure is in the spotlight as a technique in which drugs can effectively penetrate the skin with a controlled release, but the occurrence of abnormal skin responses cannot be ruled out because it is an invasive method [14,15]. In the case of thermal ablation using radio frequency, the generated micropores are formed in a certain diameter to the depth of epidermis [16]. In contrast, in the case of thermal ablation using the lasers, the depth of the micropores can be adjusted by controlling the thermal energy applied to the skin, but a masking process is additionally required for a certain diameter [17,18]. Given the importance of stratum corneum-specific disruption and of minimizing damage, it is essential to develop a technique that can control both the depth and diameter of micropores [19].

Dielectric barrier discharge is a nonthermal plasma that occurs between the high voltage cathode and anode electrodes. In the TDDS, dielectric barrier discharge enhances the penetration of molecules by etching the stratum corneum [20,21]. However, dielectric barrier discharge damages a large area of the stratum corneum and produces harmful substances, such as ozone and nitrogen oxides, when plasma is generated, so there is a possibility for abnormal skin responses and respiratory disorders [14,22]. Unlike dielectric barrier discharge, arc discharge transmits thermal energy generated by high current. When dielectric breakdown occurs for the ignition of arc discharge, thermionic emission is induced in the cathode area by high current density and thermal electrons transmit to anode area [23,24]. In addition, the erosion of electrodes generated by arc discharge shows in areas sized between tens and hundreds of micrometers [25,26]. Arc discharge has been used for various purposes, including welding and lamps, but has not yet been used in the TDDS. We considered the

possibility that arc discharge could be applied to TDDS in a noninvasive manner similar to the thermal ablation technique. Previously, we reported that arc discharge causes sporadic damage to the surface of a porcine skin, although this finding has not been analyzed intensively [27]. In this study, we used reconstituted human skin and porcine skin as alternatives to animal experiments to investigate the characteristics of micropores created by an arc discharge-based device and the penetration of biomolecules through those micropores. Furthermore, we evaluated the effectiveness of arc discharge in cosmetics clinical trials and the safety of an arc discharge-based device.

2. Materials and methods

2.1. Device

Skin micropores were generated using the arc-poration device (MEDICUBE AGE-R ATS AIR SHOT, APR Co., Ltd.) manufactured by Easytem Inc. The device consists of five outputs depending on duty cycle (10%–90%). Level 5, which has a 90% duty cycle, was used in this study. To efficiently generate the skin micropores, the device was kept in contact with the skin by tapping, sweeping, or brushing.

2.2. Cosmetics ingredients

The ingredients in the MEDICUBE Deep Vitac Ampoule (APR Co., Ltd.) are as follows:

water, ascorbic acid, butylene glycol, dipropylene glycol, propanediol, dicaprylyl carbonate, propylene glycol dicaprylate/dicaprate, diisopropyl sebacate, polysorbate 60, glutathione, glycerin, (-)-alpha-bisabolol, glyceryl stearate, panthenol, citric acid, chitosan, pullulan, sodium gluconate, sodium hyaluronate, helianthus annuus (sunflower) seed oil, dimethicone, disodium EDTA, ethylhexyl methoxycinnamate, guar hydroxypropyltrimonium chloride, sodium metabisulfite, tris (tetramethylhydroxypiperidinol) citrate, xanthan gum, cyclopentasiloxane, dimethicone/vinyl dimethicone crosspolymer, Phaseolus Radiatus seed extract, Citrus Paradisi (grapefruit) fruit extract, Opuntia Ficus-Indica fruit extract, Myrciaria Dubia fruit extract, beta-carotene, Daucus Carota Sativa (carrot) seed oil, Curcuma Longa (turmeric) root extract, Terminalia Ferdinandiana fruit extract, beta-glucan, Betula Platyphylla Japonica Bark extract, ethylhexylglycerin, Rumex Crispus root extract, sodium hydroxide, fragrance, linalool, limonene, and 1,2-hexanediol.

Whereas the MEDICUBE Super Cica Cream (APR Co., Ltd.) is composed of the following ingredients:

water, butylene glycol, glycerin, caprylic/capric triglyceride, cyclohexasiloxane, pentylene glycol, 1,2-hexanediol, dipropylene glycol, Limnanthes Alba (meadowfoam) seed oil, Theobroma Grandiflorum seed butter, Melia Azadirachta leaf extract, Melia Azadirachta flower extract, Ocimum Sanctum leaf extract, hydrolyzed hyaluronic acid, Centella Asiatica leaf extract, Centella Asiatica root extract, Centella Asiatica extract, Curcuma Longa (turmeric) root extract, Theobroma Cacao (cocoa) seed extract, Corallina Officinalis extract, Glycyrrhiza Uralensis (Licorice) extract, ethylhexyl olivate, panthenol, ectoin, hydrogenated polydecene, sodium acrylates copolymer, cetearyl olivate, sorbitan olivate, polyglyceryl-4 oleate, hydroxyethyl acrylate/sodium acryloyldimethyl taurate copolymer, synthetic beeswax, dimethicone/vinyl dimethicone crosspolymer, hydroxyacetophenone, salicylic acid, ethylhexylglycerin, dipotassium glycyrrhizate, sodium phytate, sorbitan isostearate, polyglyceryl-10 oleate, hydrogenated lecithin, silica, dextrin, allantoin, polyglyceryl-10 stearate, madecassoside, tocopherol, ceramide NP, asiatic acid, asiaticoside, madecassic acid, and xanthan gum.

2.3. Skin micropore formation using arc-poration device

For the micropore formation in the porcine skin, a porcine skin was kept at room temperature until the surface was dry. Prior to the arc-poration treatment, the porcine skin was marked with stamp ink so the micropores coud be easily detected. After the arc-poration treatment, the micropores were observed under a microscope, and their diameters were calculated. To determine the depth of the micropores, a reconstituted human skin (Neoderm, Tegoscience Inc.) was used. After the arc-poration treatment, a reconstituted human skin was fixed with 4% formaldehyde and then embedded with paraffin. The paraffin block was sliced to a thickness of 5 um. These sections were deparaffinized with xylene and then dehydrated with alcohol. The sections were stained with hematoxylin and washed in water. Next, the sections were stained with eosin and rehydrated with EtOH-Xylene steps. After mounting with Canada balsam solution, stained sections were observed under a microscope.

2.4. Permeation test using Franz diffusion cell

The Franz diffusion cell system was used to determine the penetrance of caffeine after the arc-poration treatment. Phosphate-buffered solution and a magnetic stirrer were placed in the receiving compartment of the Franz diffusion cell. Reconstituted human skin that had or had not received arc-poration treatment were placed between a donor chamber and a receptor chamber, followed by the addition of a 2.2% caffeine solution on the surface of the reconstituted human skin. At each time point, samples were collected from the receiving compartment and analyzed to determine the concentration of caffeine using HPLC. The experiments were duplicated.

2.5. Clinical evaluation of arc-poration

This split-face clinical trial evaluated the effectiveness of arc-poration and two other cosmetic products in improving skin gloss, dermal hydration, skin flakiness, skin tone, skin tone evenness, skin pigmentation, skin pore tightening, and skin texture.

This study was appropriately conducted based on the World Medical Association Declaration of Helsinki, and in accordance with following applicable regulatory requirements: “Bioethics and Safety Act”; “Cosmetics Act” of the Republic of Korea; public announcement from Ministry of Food and Drug Safety; Regulation for the designation of testing institution for drugs, etc., cosmetics, and medical devices; Korea Good Clinical Practice for Drugs; Guideline for cosmetic testing in human volunteers and In Vitro tests; Guideline for test methods for substantiation of labeling and advertisements of cosmetics; Guideline for Effectiveness Assessment of Functional Cosmetics; and Standard Operating Procedure of Global Institute of Dermatological Sciences.

In total, 22 Korean adult women aged 20–60 years who met the inclusion criteria and were not included in the exclusion criteria were enrolled for this study. The average age of the participants was 48.762 ± 6.715 years, and all individuals provided written informed consent. Out of 22 participants, 21 completed this study.

After cleansing their face, all participants were treated with the arc-poration device for 2 min to the left side of the face four times a week (every Tuesday, Thursday, Saturday, and Sunday) for the 4 week study period. Additionally, the MEDICUBE Deep Vitac Ampoule and MEDICUBE Super Cica Cream were applied to the whole face twice each day (every morning and night) after cleansing the face during the study period. Prior to the skin evaluation, all individuals washed the study area with the same detergent in an indoor space under constant temperature (22°C ± 2°C) and humidity conditions (50% ± 10% relative humidity) without direct sunlight and waited for at least 30 min.

Skin gloss, dermal hydration, skin flakiness, or skin tone were evaluated using Mark-Vu (PSI Plus Co., Ltd.)/SkinGlossMeter (Delfin Technologies Ltd.), MoistureMeterD Compact (Delfin Technologies Ltd.), Visioscan VC 20plus (Courage+Khazaka Electronic GmbH), and

Mark-Vu/Chromameter CR-400 (Konica Minolta Sensing, Inc.), respectively. Skin tone evenness, pigmentation, pore tightening, and texture were evaluated using Antera 3D.

For the statistical analysis of this study, IBM SPSS Statistics 27 software (IBM Co., USA) was used. To analyze the significance of the evaluation results, the paired t-test and Wilcoxon signed-rank test were used for preuse and postuse comparisons according to the human body application test and normality test results of each item. In the case of group comparison, the independent t-test and Mann–Whitney U-test were used. Statistical significance was defined by a p-value of <0.05 in the 95% confidence interval.

For clinical evaluation immediately after one-time treatment, 22 Korean adult women aged 20–60 years (average age of 49.091 ± 7.151 years) who met the inclusion criteria, provided written informed consent, and were not included in the exclusion criteria were enrolled for this study. All 22 participants completed this study. All participants were treated with the arc-poration device once for 1 min to the left side of their cleansed face. Then, the MEDICUBE Deep Vitac Ampoule and the MEDICUBE Super Cica Cream were applied to the whole face once. Skin gloss, dermal hydration, skin flakiness, skin pore tightening, and skin texture were evaluated without cleansing the face.

2.6. Measurement of ozone, nitrogen oxide, and nitrogen dioxide

To measure the concentration of ozone and nitrogen oxides, the arc-poration device was placed on a jig and operated at level 5 for 5 min. Then, a sampling tube was installed within 5 mm of the device's application region, and the maximum concentration was measured during the device's operation time. The concentrations of ozone and nitrogen oxides were measured using the ozone concentration tester (2B Technologies) and NOx analyzer (rbr Messtechnik GmbH), respectively.

3. Results

3.1. Development of arc-poration device

Figure 1A represents a conceptual diagram of arc-poration. Briefly, it shows that the penetration of biomolecules into the skin can be promoted through micropores created by an arc discharge that is ignited by high voltage-induced dielectric breakdown. The breakdown voltage of air is known to be approximately 3 kV/mm [28]. This suggests that a high voltage in kilovolt units would be required for the arc discharge generated between the electrode and the skin surface. However, the applied voltage generally used in handheld electronic devices is very low. To generate high voltage from low applied voltage, we used transformer in which the voltage applied to the primary coil was elevated to the winding ratio of the secondary coil via electromagnetic induction (Fig. 1a). As a result, 3.3 Vdc of the applied voltage was transformed to 1.91 ± 0.4 kVpp of alternating current (AC) voltage (Fig. 1b). To prevent severe burning of the skin caused by thermal energy of continued arc discharge, we also generated pulsed arc discharge using 20 Hz of burst frequency, applying duty cycle in the range of 10%–90%, regulating the burning period (Fig. 1c).

Although the output voltage was elevated to 1.91 ± 0.4 kVpp, it was not sufficient to ignite an arc discharge between the electrode and the skin surface. Dielectric breakdown strength reportedly decreases as the frequency increases [29,30]. In other words, the high frequency may lower the breakdown voltage depending on the distance between the electrode and the skin surface. To ignite the arc discharge efficiently, we applied 90 kHz of frequency as a carrier frequency into the burst frequency (Fig. 1c). As a result, the output carrier frequency was measured at 74.17 ± 0.36 kHz under the no load condition (Fig. 1b). Although it differs from the applied frequency due to the complexity of the circuit, there is no significant difference in functionality.

Here Fig.1

3.2. Process of arc-poration

Figure 2 describes the process of arc-poration once the device contacts the skin. Initially, one of electrodes is in contact with the skin surface as a ground electrode. Once the other electrode enters within the distance where dielectric breakdown can occur, an arc discharge is generated, and micropores are created on the skin surface by the thermal energy. When both electrodes contact the skin surface, the AC current drops due to high skin resistance and flows between the two electrodes through the skin (Fig. 1b). From the moment one electrode is detached from the skin, an arc discharge occurs and micropores are created on the skin until that electrode is outside the range where dielectric breakdown can occur. Through this mechanism, micropores can be easily created by sweeping, brushing, or tapping the skin with the arc-poration device.

Here Fig. 2

3.3. Skin micropore generation using arc-poration device

A porcine skin was used to verify the ability of arc-poration to create micropores. Since a porcine skin has many pores, stamp ink was applied before the arc-poration treatment to distinguish newly created micropores (Fig. 3a). We found multiple newly created micropores on the porcine skin treated with arc-poration (Fig. 3b). To determine the pores' diameter, the longest length of the pore shape was calculated. The pores had an average diameter of 97.45 ± 19.33 micrometers (Fig. 3c), which is similar to the diameter of the pores generated by microporation [16] or microneedle [31].

Here Fig. 3

Invasive procedures may cause skin infection, and damage deep in the skin can cause inflammatory reactions, such as pain, erythema, and bruising [14]. Thus, the arc-poration was not only designed to be noninvasive, but also to cause minimal damage to the skin through depth control. A reconstituted human skin was used as an alternative to human and animal to determine the depth of micropores created by arc-poration. A reconstituted human skin consists of stratum corneum, viable epidermis, and dermis (Fig. 4a). Histological analysis shows the depth of micropores created by arc-poration on a reconstituted human skin. Interestingly, the micropores were formed only on the stratum corneum (Fig. 4b). The presence of stratum corneum-specific micropores indicate that arc-poration can break through the skin barrier that prevents substance penetration, making a channel for transdermal delivery.

Here Fig. 4

3.4. Increased permeability of caffeine by arc-poration treatment

Given the stratum corneum's function as a skin barrier, it can be assumed that the stratum corneum-specific micropores created by the arc-poration facilitate the penetration of biomolecules into the skin. To test this, we performed a Franz diffusion cell experiment with both arc-poration-treated and nonarc-poration-treated reconstituted human skin (Fig. 5a). Caffeine, which is widely used in dermatological applications, was used as a test substance to measure permeability [32]. The concentration of caffeine penetrated through the stratum corneum to the dermal layer was measured using HPLC. The concentration of permeated caffeine gradually increased over time. At 2, 4, and 8 h after caffeine treatment, the average

concentrations of permeated caffeine are 5.9, 4.8, and 4.5-fold higher in arc-poration-treated sample than those in the nontreated sample, respectively (Fig. 5b). This result indicates the enhancement of transdermal delivery through stratum corneum-specific micropores.

Here Fig. 5

3.5. Clinical evaluation of arc-poration treatment

Next, we investigated whether increased skin permeation through the stratum corneum-specific micropores increased the clinical effect of active ingredients. Two types of cosmetics containing antioxidants and anti-inflammatory, moisturizing, and soothing ingredients were applied after pretreatment or nontreatment of arc-poration. Skin gloss, dermal hydration, skin flakiness, skin tone, skin tone evenness, skin pigmentation, skin pore tightening, and skin texture were evaluated.

After 4 weeks of application of the study products, significant improvements in skin gloss, dermal hydration, skin flakiness, skin tone, skin tone evenness, skin pigmentation, and skin texture were observed only in the application area (cosmetics only area) in comparison to the findings at baseline, confirming the skin improvement effects of cosmetics. These were further improved when arc-poration device was used together with cosmetics (AP area); these significant improvements in the AP area were statistically higher than those in the cosmetic area ($p < 0.05$) (Fig. 6a–f, h). The evaluation of skin pore tightening after 4 weeks showed that the pore volume in the cosmetics only area decreased compared to that at baseline, but the difference was not statistically significant. In contrast, the AP area showed significant improvement in skin pore tightening ($p = 0.002$), which was statistically significantly different from that of the cosmetics only area ($p < 0.05$) (Fig. 6g).

Here Fig. 6

After 2 weeks, the skin area that received only cosmetics showed significant improvements in skin gloss, dermal hydration, skin flakiness, skin tone evenness, and skin pigmentation compared to those at baseline. However, 4 weeks later, there were no significant improvements in skin tone or texture in the cosmetics only area, although there was a tendency for the skin tone and texture to improve compared to that at baseline. Even in the evaluation of skin pore tightening after 2 weeks, the pore volume in the cosmetics only area did not improve compared to that at baseline. In contrast, significant improvements in skin gloss, dermal hydration, skin flakiness, skin tone, skin tone evenness, skin pigmentation, skin pore tightening, and skin texture were observed in the AP area compared to those at baselines; the AP area showed a statistically significant difference compared to that in the cosmetics only area only in dermal hydration, skin tone evenness, and skin texture ($p < 0.05$) (Fig. 7a).

Reportedly, long-term exposure to a particular electrical stimulation or low-frequency massage can induce skin rejuvenation through collagen synthesis [33,34]. To exclude these possibilities, changes in skin condition were evaluated after one-time treatment of the study products. Clinical evaluations were performed without cleansing the face after study product application (Fig. 7b). Significant improvements in skin gloss, dermal hydration (0.5/1.5 mm), skin flakiness, skin pore tightening, and skin texture were observed in the cosmetics only area when compared to those at baselines. These results were assumed to be due to the temporary effect immediately after applying cosmetics. Interestingly, these improvements in the cosmetics only area, except those of skin flakiness, were significantly enhanced in the AP area; the enhancements in the AP area were statistically significantly different from those in the cosmetics only area ($p < 0.05$). Skin gloss and skin flakiness may appear to be improved

by cosmetics on the surface of the skin, whereas dermal hydration and skin texture can only be improved if the active ingredients contained in cosmetics penetrate the skin. Therefore, these results indicate that the improvement in skin condition by cosmetics is enhanced by skin penetration of active ingredients through arc-poration. Taken together, arc-poration makes skin improvement effects by cosmetics more effective and faster due to the absorption promotion of active ingredients by arc-poration.

Here Fig. 7

3.6. Safety evaluation

The safety of devices used on the human body should be considered prior to their effectiveness. Therefore, we considered the safety of the arc-poration device from its early stages of development. First, when the device contacts the skin, the voltage drops so that a microcurrent that is below the standard allowable current for the human body flows. Second, the pain that may occur due to low-frequency electrical stimulation was excluded by using high frequencies [35]. Third, skin damage caused by the thermal energy of arc discharge was minimized by applying high frequency to shorten the duration [36]. Last, arc-poration device was insulated through an isolation transformer to prevent electrical shocks caused by unexpected events (Table 1 and Fig. 1).

Here Table 1

Ozone and nitrogen oxides have been known to occur through the dielectric breakdown of air, such as lightening, pulsed discharge, and spark discharge [37-39]. Ozone and nitrogen oxides irritate the eyes and skin on contact, and the nose and throat when inhaled. To check this concern in the actual environment the arc-poration device will be used in, the concentrations of ozone and nitrogen oxides were measured within 5 mm of the application region of the device (Table 1). The concentration of ozone generated by the use of the device was 0.004 ppm, which is less than 0.1 ppm, which is the minimum value among the occupational short-term exposure limit values determined by each country. It is even less than 0.05 ppm, the minimum long-term exposure limit value [40]. The concentrations of nitrogen oxide and nitrogen dioxide produced by the use of the device were both less than 1 ppm. According to the Occupational Safety and Health Administration, the exposure limit values of nitrogen monoxide and nitrogen dioxide are 25 ppm and 5 ppm, respectively. These results indicate that the concentrations of ozone and nitrogen oxides produced by arc-poration are far less than those of the international exposure limit standards.

To evaluate the safety of arc-poration on the human skin, all clinical participants were monitored for abnormal skin responses, including erythema, edema, scaling, itching, stinging, burning, tightness, and prickling. We observed no abnormal skin responses in the application area before or after the arc-poration treatment during the study period (Table 1). Taken together, these data indicate that the arc-poration device is a safe device, which minimizes damage and generates harmful substances below the allowable level.

4. Discussion

Based on the thermal effect of arc discharge, we developed a handheld device that can create micropores on the skin surface using a modified arc discharge. We named this technique arc-poration, which means creating micropores via arc discharge.

The stratum corneum serves as a barrier to prevent penetration of external substances and water loss in the skin [7]. In other words, it is essential to disturb the stratum corneum to deliver drugs or cosmetics transdermally. The selective formation of microchannels on the

stratum corneum is important for minimizing skin damage [19]. However, except for the physical method, thermal ablation is the only other method with the reported ability to selectively create micropores on the stratum corneum under particular conditions. In thermal ablation, the strength of the heat applied to the skin must be tightly regulated for depth control, and a masking step is required to adjust the diameter of the micropore [17,18]. From this perspective, we investigated transdermal delivery using novel technology and then focused on arc discharge. Previously, we reported that arc discharge generated by the application of high voltage causes skin damage and may function to support transdermal delivery along with electroporation [27]. Although the previous study did not evaluate arc discharge itself in terms of the micropore depth, skin permeability, and clinical effect, it suggested the possibility of creating stratum corneum-specific micropores using arc discharge. In this paper, we describe a noninvasive device applied with arc-poration, which can create micropores using a modified arc discharge. Experiments with a reconstituted human skin and a porcine skin demonstrated that the micropores formed by arc-poration were limited on the stratum corneum. Furthermore, these experiments showed that the treatment of arc-poration creates micropores with the diameter of 97.45 ± 19.33 mm, which is similar to the diameter of micropores formed by thermal ablation or microneedle. Interestingly, these were achieved without the masking steps required to adjust the diameter of the micropores during thermal ablation, indicating that arc-poration is a unique tool for creating stratum corneum-specific micropores of a particular size.

When dielectric breakdown occurs, the voltage drops, whereas the current elevates dramatically [41]. The heat generated by the high current causes damage to the skin, suggesting the importance of the device safety. To avoid continuous burning to the skin, we adjusted the burning period by applying burst frequency and a high carrier frequency to the arc-poration device. As a result, micropores could be created only on the stratum corneum without severe damage to the skin. Another concern is the electrical injury caused by the current exceeding the allowable value for passing through the human body. When the current reaches a maximum during the occurrence of arc discharge, the resistance represents a minimum value, resulting in the voltage drop [42]. That is, just before the electrode touches the skin, the voltage is dropped by arc discharge, and then the current intensity is lowered due to the high resistance of the skin. Here we show that the current intensity represents 21 ± 4 mA at 354.98 ± 4.29 kHz of frequency when a 500-ohm load is applied, which is similar to the situation where the device contacts the skin. This current intensity could be considered safe for the human body because it does not exceed the allowable current limit for humans of 100 mA of current when exceeding 1.5 kHz of frequency according to IEC 60601, a series of technical standards for the safety of medical electrical equipment.

One of the important considerations of facial devices is the safety of eyes. With the use of most devices that use electric stimulation or laser, it is critical to avoid patient's eye are or have them wear protective equipment on the eyes. In arc discharge, the conditions of dielectric breakdown vary depending on the humidity. The breakdown voltage increases as the humidity increases [43]. Since human eyes are always wet, arc discharge does not occur in the eyes when using the arc-poration device, suggesting the device's safety for the eyes.

Ascorbic acid is involved in collagen synthesis promotion, melanogenesis inhibition, and antioxidation [44]. However, since ascorbic acid is hydrophilic and has a negative charge when ionized, it is extremely difficult for ascorbic acid to pass through the hydrophobic and negatively charged stratum corneum. We clinically evaluated arc-poration using cosmetics containing ascorbic acid as the main active ingredient. Our clinical results show that the skin improvement effect of cosmetics is enhanced by the pretreatment of arc-poration in all aspects, including melanogenesis and collagen synthesis. Especially, the pretreatment of arc-poration was remarkably effective for skin pore tightening. Enlarged skin pores are caused by

decreased skin elasticity due to loose collagen around the pores [45]. These findings suggest that arc-poration may facilitate the penetration of ascorbic acid deep into the skin for its skin improvement effects. Although it may be the penetration effects of other ingredients into the skin, there is no doubt that arc-poration promotes penetration of biomolecules into the skin.

To date, tens of drugs have been FDA-approved for transdermal delivery, but most of them have a molecular weight of less than 500 Daltons, which is essential for a passive delivery method [11]. A variety of innovative biologics, including antibodies, peptides, and nucleic acids, have been developed to treat skin cancer and atopic dermatitis [46-48]. However, biologic drugs cannot pass through the stratum corneum by passive transdermal delivery methods due to their high molecular weights of 1 kDa to 1000 kDa, suggesting the requirement for active delivery methods [49]. Although we evaluated the penetration-promoting effects and clinical effects of arc-poration with caffeine and cosmetics, respectively, the biomolecules for arc-poration are not limited to them. For example, among the biologic modalities, IgM monoclonal antibodies have a molecular weight of more than 900 kDa and a diameter of 29 nm at extended conformation [50]. Since the diameter of the micropores created by arc-poration is about 100 mm, IgM monoclonal antibodies can be expected to pass through the stratum corneum via the micropores. In other words, most biologics are smaller than IgM monoclonal antibodies; thus, there is no size limit for biologics to pass through the micropores created by arc-poration. The biologics may not be able to pass through the tight junctions on the stratum granulosum, a second skin barrier, as arc-poration only disrupts the stratum corneum. However, tight junctions are dysregulated in atopy dermatitis and skin cancers, allowing the biologics to deeply penetrate the skin [51,52]. This suggests that stratum corneum-specific micropores created by arc-poration allow biologics to be concentrated in the lesion.

5. Conclusions

Passage through the stratum corneum remains the biggest challenge for transdermal drug delivery. In this study, we demonstrated that arc-poration, an arc discharge-based technique, can selectively create micropores on the stratum corneum, thereby increasing skin permeability. Clinical results showed that the skin improvement effect of cosmetics was enhanced by pretreatment of arc-poration, and even one-time use of arc-poration showed superior immediate results compared to those following the application of cosmetics alone. These results suggest that the arc-poration device can be a highly effective beauty device for transdermal delivery of cosmetics. Although clinical trials were conducted with cosmetics, considering the diameter size of the micropores created by arc-poration, it would be also possible to promote skin penetration of macromolecules by pretreatment of arc-poration. To demonstrate the possibility of an arc-poration device as a therapeutic use in the future, we will evaluate skin permeability and efficacy of various biologics after arc-poration pretreatment.

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Conflicts of Interest: The authors declare no conflict of interest. All authors have approved the final article.

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Figures

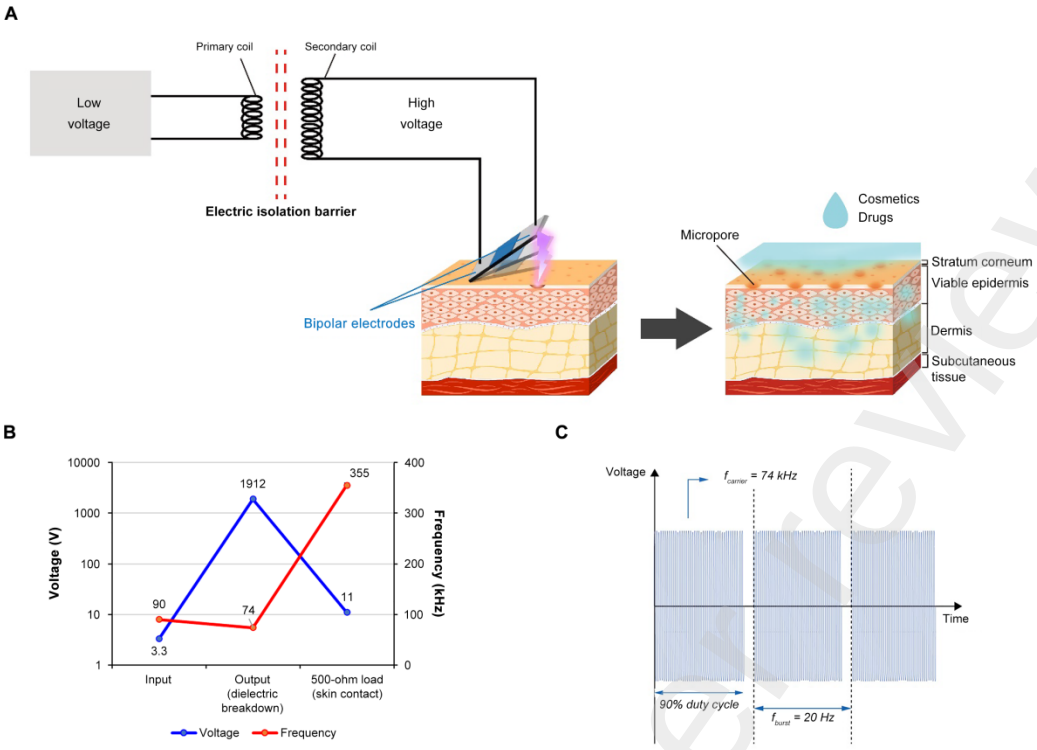

Figure 1

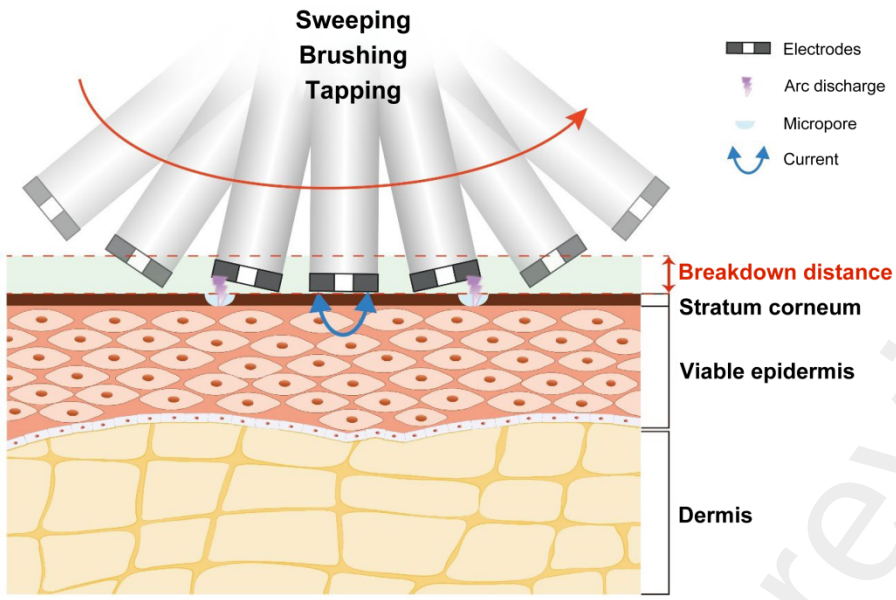

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Figure 2

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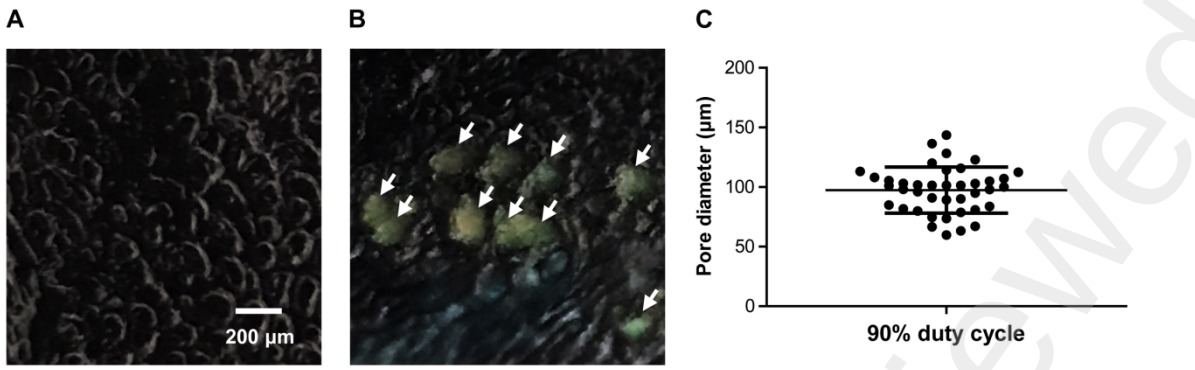

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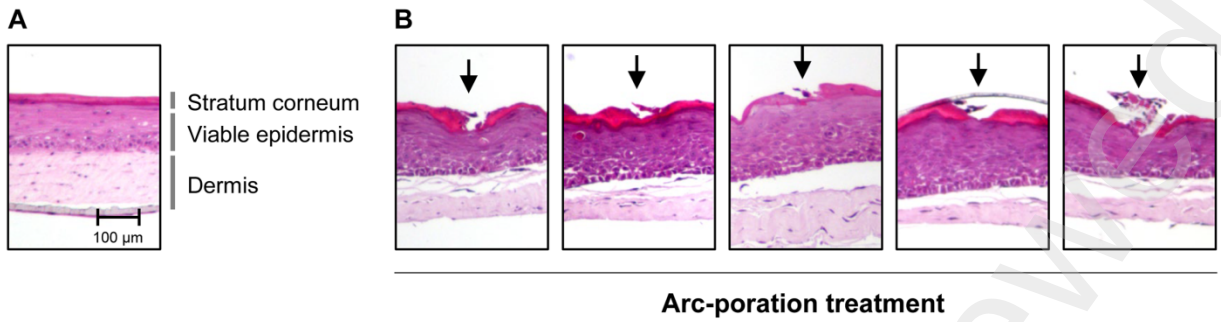

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Figure 4

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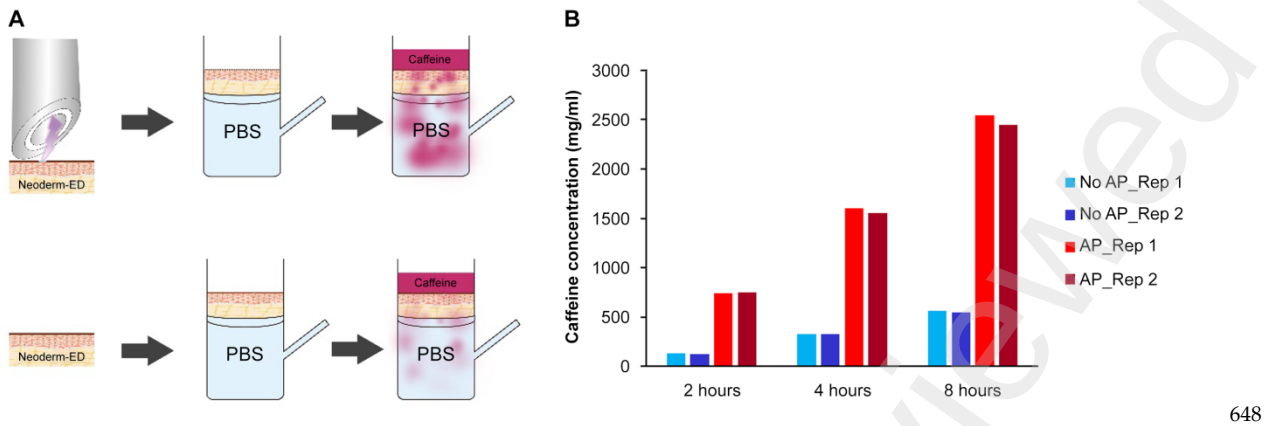

Figure 5

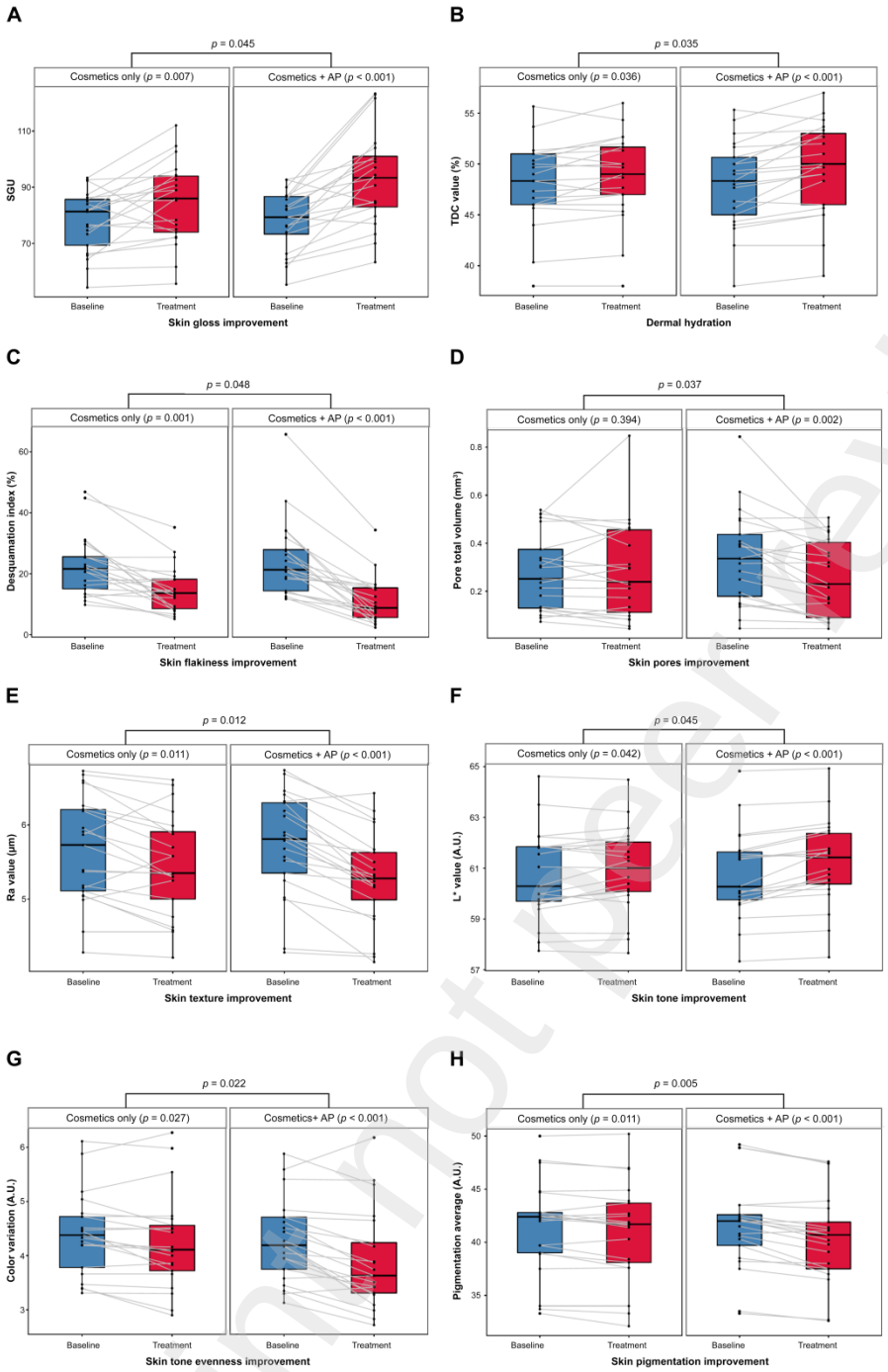

Figure 6

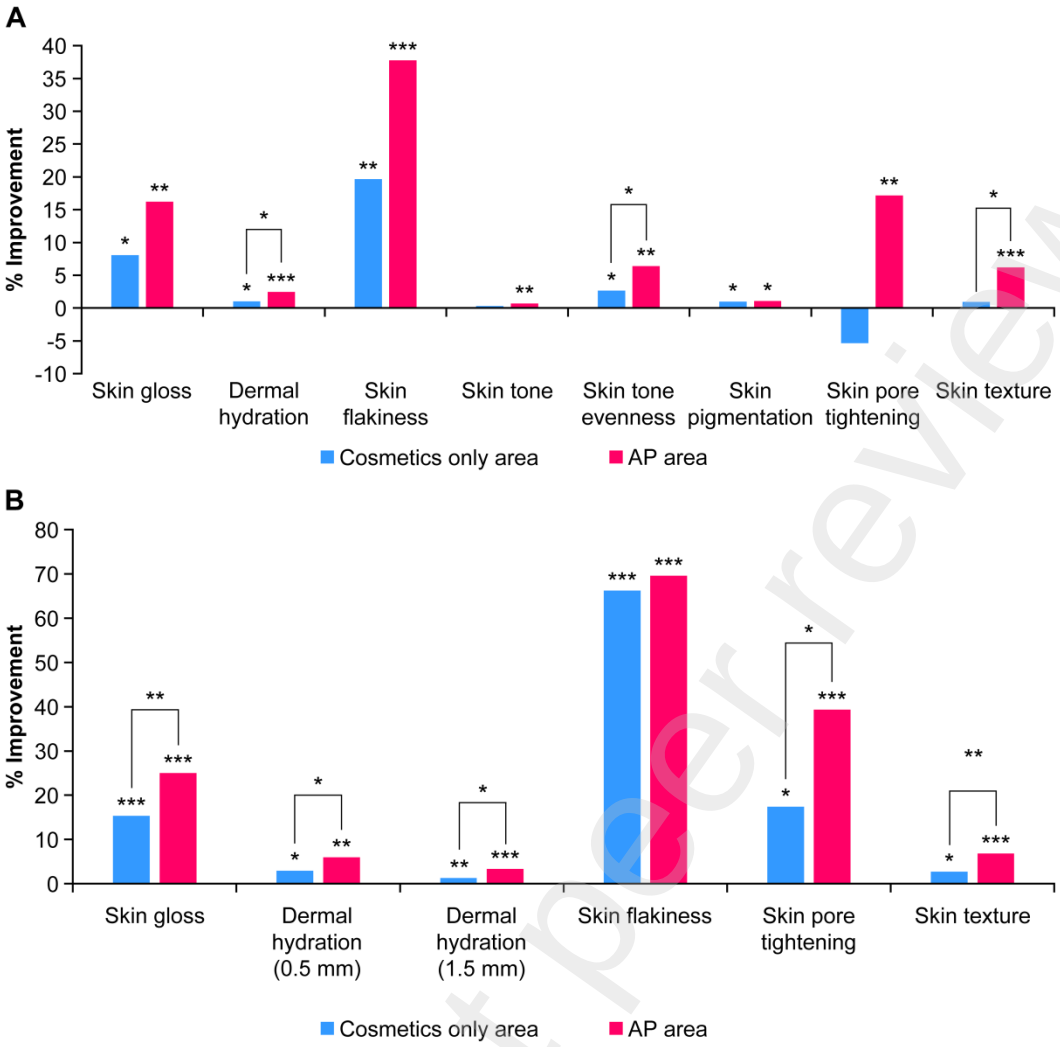

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Figure 7

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Figure captions

Figure 1. (A) Schematic diagram of transdermal delivery system using arc-poration. (B) Changes in voltage and frequency at the indicated step. Data are expressed as mean ± SD (n = 5). (C) Schematic diagram of carrier frequency in burst frequency used in this study.

Figure 2. Schematic diagram for a process of arc-poration on the skin surface.

Figure 3. Micropore formation on a porcine skin using arc-poration. (A) Normal porcine skin. (B) Arc-poration-treated porcine skin. Arrows indicate the micropores. (C) The micropores' diameter. Error bars represent standard deviation (n = 40).

Figure 4. Micropore formation on reconstituted human skin model using arc-poration. (A) Structure of reconstituted human skin. (B) Arc-poration-treated reconstituted human skin. Arrows indicate the micropores.

Figure 5. The permeation of caffeine using reconstituted human skin model. (A) Schematic diagram for the procedure of the Franz diffusion cell experiment. (B) Measurement of caffeine concentration harvested from the Franz diffusion cell with (AP) or without (No AP) arc-poration. The experiment was duplicated. AP: arc-poration.

Figure 6. Clinical evaluation of skin after 4 weeks of arc-poration device usage with cosmetics. (A) Skin gloss improvement, (B) dermal hydration, (C) skin flakiness improvement, (D) skin pores improvement, (E) skin texture improvement, (f) skin tone improvement, (G) skin tone evenness improvement, and (H) skin pigmentation improvement was measured. Data are expressed as mean ± SD (n = 21).

Figure 7. Clinical evaluation of arc-poration device with cosmetics after 2 weeks of usage (A) and immediately after one-time use (B). The improvement rate was obtained compared to baseline. Asterisks indicate *p*-values (*$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Table

Table 1. Evaluation of safety after arc-poration treatment

Risk factor	Control subject	Required value (preferred value)	Observed value
Electric stimulations[1]	Contact current	<100 mA	22.04 ± 0.54 mA
	Pain	>20 kHz	354.98 ± 4.29 kHz
	Thermal damage	50–500 kHz	74.17 ± 0.36 kHz
	Electric shock	Insulation	Isolation transformer
Hazardous substances	Ozone	<0.05 ppm	0.004 ppm
	Nitrogen oxide	<25 ppm	<1 ppm
	Nitrogen dioxide	<5 ppm	<1 ppm
Abnormal skin responses	Erythema	(Not observed)	Not observed
	Edema	(Not observed)	Not observed
	Scaling	(Not observed)	Not observed
	Itching	(Not observed)	Not observed
	Stinging	(Not observed)	Not observed
	Burning	(Not observed)	Not observed
	Tightness	(Not observed)	Not observed
	Prickling	(Not observed)	Not observed

[1] These data were obtained from Figure 1.