Investigation of atmospheric micro-plasma for improving skin permeability

K. Shimizu, An N. Tran, Kristof Jaroslav, and Marius G. Blajan
Dept. of Electrical and Electronic Engineering
Shizuoka University, Japan
phone: (81) 54-478-1443
e-mail: shimizu@cir.shizuoka.ac.jp

***Abstract*—This paper accessed a new application of argon atmospheric microplasma irradiation (AAMI) in improving skin permeability. All of ex vivo investigations were performed on Yucatan hairless micro-pig skin. A percutaneous absorption of Galantamine hydrobromide (GaHBr) – an Alzheimer drug was also carried out in order to clarify the enhancing effect of AAMI. After 3 min of AAMI, the cumulative amount of GaHbr permeated through skins was increased by approximately 2 times at 24 h-post experiment (from 5.35 ± 2.34 μg/cm^2 to 11.53 ± 2.89 μg/cm^2). These obtained results pointed out a positive effect of AAMI on facilitating the osmosis of hydrophilic drug and also provided a proposed mechanism for this interaction.**

I. INTRODUCTION

Transdermal administration of medication has its unique advantages, for instance, avoiding the first-pass metabolism [1], [2], preventing gastrointestinal drug degradation [3] and easy controlling of allergic reaction, etc. However, because of the obstruction of the outer stratum corneum (SC) layer, the substances which have either molecular weight greater than 500 Da [3], [4] or hydrophilic characteristic [5] encounter the difficulty in absorption through skin.

Recently, atmospheric plasma applications have attracted attention due to their many potential advantages, such as the presence of highly reactive species [e.g., reactive oxygen species (ROSs) and reactive nitrogen species (RNSs)] [6] - [8], and no requirement for costly vacuum enclosures. Particularly in the medical and biological fields, several recent studies have demonstrated that plasma is highly efficient for skin treatment [9], [10], including skin disinfection [11] - [13] and rejuvenation therapy [8].

Therefore, the goal of our study is (1) To investigate the possibility of atmospheric-pressure argon plasma irradiation (AAMI) for improving skin permeability as an appropriate tool for skin treatment; and (2) To assess drug delivery through the skin without injection needles [14] and without any damages to the skin [15]. In conclusion, this study indicates that AAMI is expected to be a promising alternative method that can promote drug delivery through the skin and can simultaneously minimize the pain from other skin penetration enhancement manipulations.

II. Materials and methods

A. Chemicals

GaHBr was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). It has a high purity of 98% and can be obtained commercially. Galantamine hydrobromide (GaHBr) has an effect on inhibiting the production of acetylcholinesterase, which is considered to cause memory loss or confusion in Alzheimer disease (AD) patients. Also, it can promote the release of acetylcholine, which is a neurotransmitter transmitting signals between nerve cells in the brain and plays an important role in the perception. This drug does not cure AD, but it works to reduce behavioral symptoms of the disease in patients. GaHBr is currently introduced into the body by oral route, this may cause nausea, gastrointestinal and vomiting [16], [17].

GaHBr is relatively a hydrophilic drug with low octanol-water partition coefficient (log P = 1.75) and has a molecular weight of 368.265 g/mol. GaHBr was dissolved in distilled water with concentration of 20 mg/ml for the upcoming experiments.

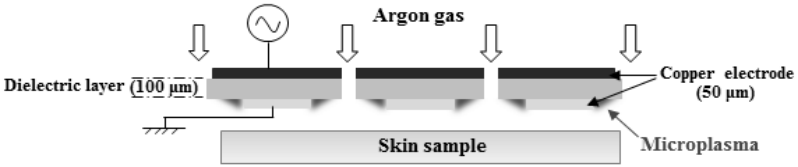
B. Skin sample preparation

Pig skin has been commonly used in dermatological studies, because of its form and the permeation rate of drugs are most similar to those of a human skin [18], [19]. The Yucatan micropig (YMP) has less hair upon the surface of the body and the hair density is comparable to the human [20]. Therefore, the YMP skin was selected for this ex vivo study.

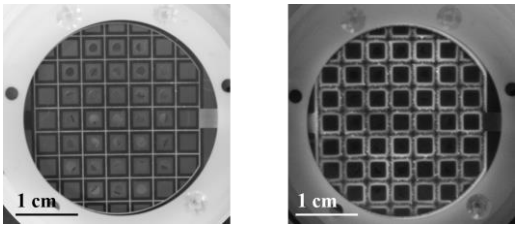
Frozen YMP dorsal skin sheets (female pigs, 5 months old) were purchased from Charles River Japan, Inc. (Yokohama, Japan). The skin sheets were stored at -80 ºC in a freezer until the series of experiments. Skin samples were equilibrated in a chamber at approximately 27 ºC for 30 min before starting each experiment. The subcutaneous fat maximally removed skin was soaked in 4 ºC phosphate buffered saline (PBS) for 3 hours, then was put in 60 ºC PBS for 1 min [21]. Thereafter, the epidermal layer of the skin was carefully peeled off to a thickness of roughly 200 μm using a tweezer.

C. Microplasma irradiation procedure

A thin-film type electrode was utilized for the microplasma generation as shown in Fig. 1. This electrode consists of a dielectric layer of 100 μm thickness, sandwiched by two copper electrode layers of 50 μm thickness. The high-voltage applied electrode was covered with an insulation layer and the grounded electrode faced the skin samples at a distance of approximately 1.5 mm. Due to this electrode structure, the starting discharge voltage was comparatively low with the amplitude of 600 V (zero-to-peak). The dielectric barrier discharge (DBD) microplasma was generated using a neon-sign transformer (frequency of 27 kHz; LECIP αNEON M-1H). The waveform of the applied voltage and corresponding discharge current are shown in Fig. 2. Argon gas was supplied to the microplasma electrodes systems with a flow rate of 5 L/min, via the tube that was connected to the electrode. During the irradiation time which was less than 5 min, the plasma temperature was measured to be below 40 ºC (Fig. 3).



(a) Schematic image of cross-sectional view.



(b) Top view of electrodes without plasma and with plasma discharge on the surface.
Fig. 1. Images of microplasma electrodes.

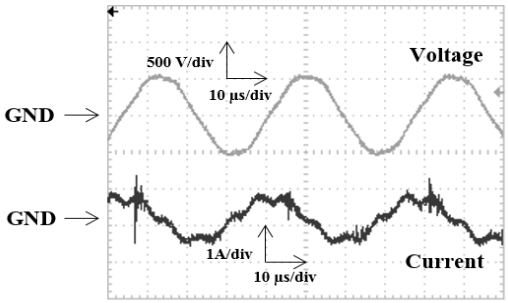


Fig. 2. Applied AC high voltage and corresponding discharge current of AAMI.

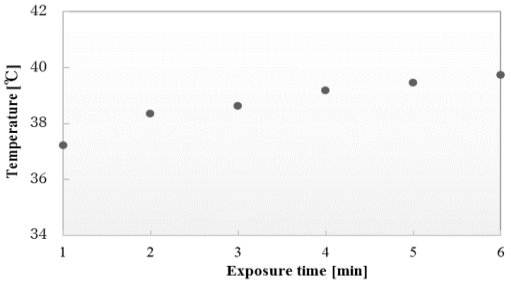


Fig. 3. Plasma temperature at the electrode with increasing exposure time.

D. Assessment of skin barrier integrity

The changes in lipid composition in the pig SC were investigated using attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy (JASCO FT/IR 6300) [22], [23]. As shown in Fig. 4, the principle of the ATR method is based on the property of total internal reflection resulting in an evanescent wave, which is formed by the reflection of

the infrared light off the internal surface, extending into the sample with a few micrometers in depth (0.5 – 5 μm). FTIR spectra were obtained by the measurement of this evanescent wave-induced attenuated energy.

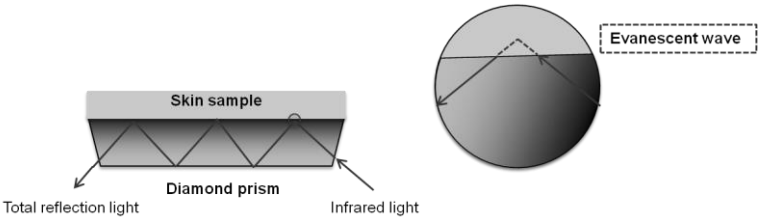


Fig. 4. A basic principle of ATR method.

In this study, ATR-FTIR analysis was conducted with a diamond prism. It has a higher refractive index (2.4) than that of the pig skin (1.55), which is required for the formation of total internal reflection at the interface [24]. For each analysis, 64 scans of infrared spectra were taken with a resolution of 4 cm^{-1}.

In addition, the barrier function alteration of the skin, which is correlated closely with the permeation behavior, was monitored by transepidermal water loss (TEWL) measurement with an evaporimeter (Nikkiso Thermo H4500) [25].

E. Assessment of skin barrier integrity

The permeation of GaHBr through the previously described epidermal layer of YMP skin was accessed ex vivo using a Franz diffusion cell (TP-8S, Biocomsystems). The sample was mounted in the cell which has a diffusion area of 1.8 cm^2, with the SC facing the donor compartment and the dermis facing the receiver compartment. The donor compartment was filled with 0.5 ml of 20 mg/ml GaHBr solvent, and the receptor compartment was filled with 7 ml of distilled water. During the experiment, the system was kept at 32 ºC by a water bath under constant stirring [26]. 2 ml of receptor solution was sampled at 1, 3, 5, 7, 15 and 24 h post-application. The same volume of distilled water was refilled back to the receptor solution each time after sampling.

Concentration of GaHBr contained in the sampled solution was quantified by HPLC systems which consists an Aligent (Palo Alto, CA) chromatograph quipped with an isocratic pump (1100 series). The analysis was performed under the conditions shown in table 1.

TABLE 1: CONDITIONS FOR HPLC ANALYSIS

Column	Inert-ODS-S1	
Flow rate	0.4 ml/min	
Volume of injection	5 μl	
Column temperature	50 ºC	
Mobile phase	A:B = 20:80	A: 10mM Ammonium acetate solvent
		B: Acetonitrite
Wavelength of UV-VIS detector	289 nm	

The peak of GaHBr was obtained at a retention time of 8.22 min. As shown in Fig.5, the standard calibration curve plotted within the range of 1 to 10 μg/ml, shows the good linearity (r^2 = 0.9996).

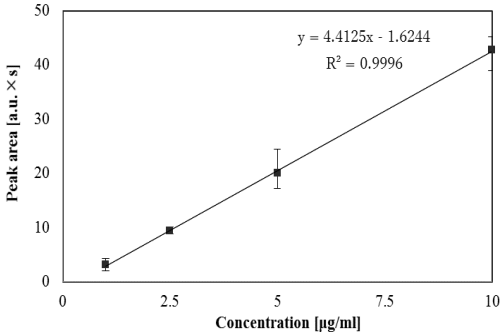


Fig. 5. A calibration curve for the concentrations of GaHBr (n = 3).

III. RESULTS AND DICUSSION

A. ATR-FTIR Analysis

Figure 6 shows the obtained ATR spectra of the pig SC before and after 3 min of AAMI. The modes at roughly 1544 and 1644 cm^{-1} were assigned to the keratin, which is the major protein of the corneocytes [23]. The absorbance at 2850 and 2918 cm^{-1} were assigned to the symmetric and asymmetric methylene (CH_2) stretching modes, which are sensitive elements to access the alterations or phase transitions in SC lipid and packing of acyl chains [27].

The reduction in these CH_2 stretching absorbance over exposure time was shown in Fig.7. In addition, it was revealed that these CH_2 peaks decreased as the thickness of the skin decreased [23]. Therefore, this result suggests that there was a physical effect of AAMI depending on the exposure time on the SC surface. That could lead to a lower number of SC layer, resulting in the reduction in the absorbance of keratin modes.

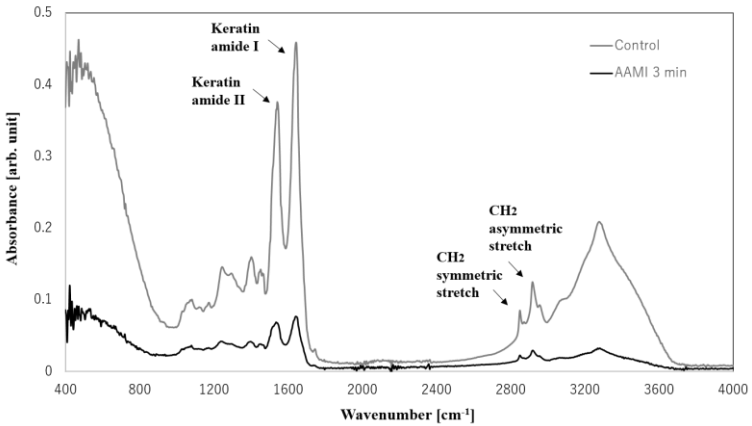


Fig. 6. ATR spectra of the SC layer of YMP skin.

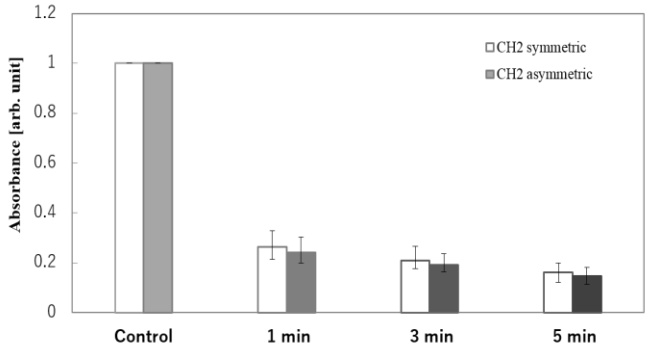


Fig. 7. Reduction in absorbance of methylene groups with increasing exposure time of AAMI (n=3).

In addition, wavenumber of asymmetric CH_2 shifted to higher wave number when increasing the exposure time (Fig. 8). This spectral shift had a maximum value of 1.928 cm^{-1} after 3 min of AAMI. Lin *et al*. utilized the skin of a nude mice and the pig SC to investigate in vitro the structural changes in the lipid after treating the samples with enhancers of vitamin C, DMSO. They obtained the upward spectral shift of CH_2 peaks as a results of the lipid-disorder [28]. Furthermore, Tero *et al*. performed DBD plasma irradiation to an artificial cell membrane and revealed that the irradiation induced pore formation (10 nm to 1 μm in size) in the cell membrane comprising supported lipid bilayers, which have a similar composition to intercellular lipid bilayers [29].

Accordingly, we also considered that the active species, charged species, or photons that were generated by plasma could play a role to enhance drug penetration by the disturbance of the intercellular lipid bilayers.

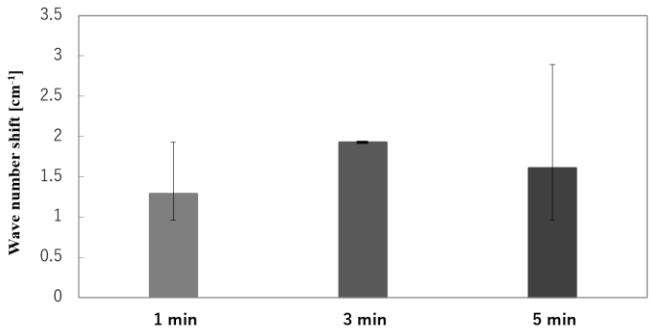


Fig. 8. The upward wavenumber shifts of asymmetric CH_2 stretching mode after AAMI (n=3).

Figure 9 shows the alteration in TEWL values before and after AAMI. The longer the skin sample was irradiated by microplasma, the greater TEWL value was obtained, that was in agreement with the plasma-induced skin barrier defects mentioned above. After 3

min of the irradiation, the TEWL value (13.82 ± 2.97 g/m^2/h) was increased nearly 2 times compared to that of the control (7.68 ± 1.15 g/m^2/h).

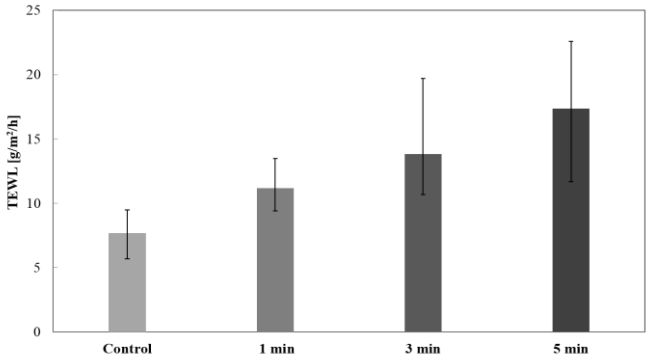


Fig. 9. Variations in TEWL values before and after AAMI (n=6).

B. Improvement of GaHBr permeation.

We carried out the previously described permeation experiment of GaHBr through a YMP. 3 skin samples were target of only Argon gas flow as the control samples and 3 skin samples were irradiated by Argon microplasma for 3 min as the irradiated samples. The permeation profiles of GaHBr through these samples are shown in Fig.10. As expected, the cumulative amount of GaHBr permeated through skin demonstrated an approximately 2 times increase after plasma application ($p < 0.05$–degree of reliability in T-test). These cumulative amount at 24 h post-experiment were 5.35 ± 2.34 μg/cm^2 and 11.53 ± 2.89 μg/cm^2 for the control and the plasma-irradiated sample, respectively. This results leads to the expectation that AAMI can be used to enhance the skin delivery of hydrophilic drugs and larger molecular drugs in the future.

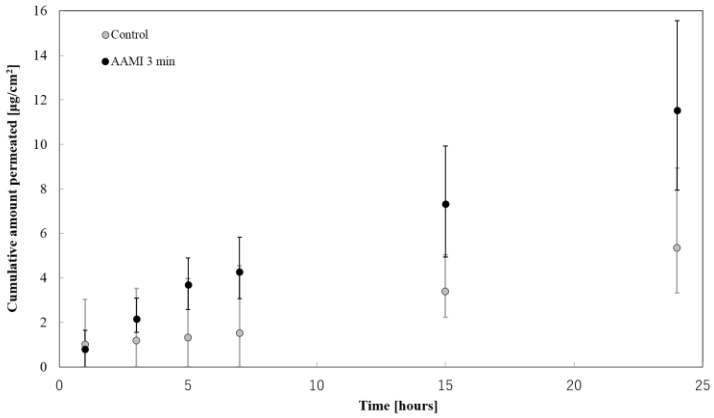


Fig. 10. Time course of the cumulative amount of GaHBr through the skin sample (n=3).

IV. Conclusion

Owing to the presence of many highly reactive species (ROS, RNS, etc.), atmospheric-pressure argon microplasma irradiation is an efficient method to chemically modify a material substance. In this study, the enhanced transdermal activity of 3min AAMI on Galantamine hydrobromide, which has slightly hydrophilic characteristics and small molecular weight, has been confirmed as described below:

i. Asymmetric CH_2 peak arising from the SC lipid, was shifted from 2918.25 ± 0.48 cm^{-1} to 2920.34 ± 0.45 cm^{-1}.
ii. The absorbance of symmetric CH_2 peak was reduced from 0.086 ± 0.001 to 0.018 ± 0.003. Also, the absorbance of asymmetric CH_2 peak was reduced from 0.124 ± 0.001 to 0.024 ± 0.004.
iii. TEWL value was decreased from 7.68 ± 1.15 $g/m^2/h$ to 13.82 ± 2.97 $g/m^2/h$.
iv. The cumulative amount of GaHbr at 24 h-post experiment was increased from 5.35 ± 2.34 $\mu g/cm^2$ to 11.53 ± 2.89 $\mu g/cm^2$.

The mechanism was considered to be that the AAMI caused the SC lipid to be chemically modified due to the active species, ions and photons that change the bonds and had also a physical effect on SC. However, microlasma is a surface DBD and the electric field is closed between the electrodes [30]. Therefore, this method could not give any harmful physical damages to the target samples [31].

This may lead to a feasible effect of improving skin permeability and facilitating the transdermal absorption of drugs. Moreover, AAMI has the ability to avoid undesirable damage to the skin and can be expected to minimize physical pain. These findings have suggested some practical application of AAMI in medical fields and have provided a basis for further researching in the future.

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