A novel application of low discharge voltage atmospheric microplasma for transdermal drug delivery

K. Shimizu[1, 2], K. Hayashida [1], M. Blajan[2,] K. Kusamori[3], H. Katsumi[3], and A. Yamamoto[3]

[1]*Shizuoka University, Graduate School of Engineering, Hamamatsu, 432-8561, Japan.*
[2]*. Shizuoka University, Organization for Innovation and Social Collaboration, Hamamatsu, 432-8561, Japan.*
[3] *Kyoto Pharmaceutical University, Department of Biopharmaceutics, Kyoto, 607-8414, Japan*

Atmospheric plasma for medical application has been gathered attention such as sterilization, treatment of cancer cells and blood coagulation etc. Dermatology is one of the application fields of the atmospheric plasma, which has the potential to be a novel tool for wound healing, skin rejuvenation and treatment of wrinkles. In this study, we have investigated the enhancement of percutaneous absorption of dye as alternatives agents of transdermal drugs. We have been exploring the feasibility of the atmospheric microplasma irradiation on the enhancement a percutaneous absorption of drug for the alternative method to the conventional hypodermic needle. Hypodermic needles are very often the only way to deliver large molecular drugs into dermis, although transdermal delivery has attractive possibility, since the safety drug delivery method without any injection needle could be desired. Pig skin was used as a biological sample, which was exposed to the atmospheric microplasma, and was analyzed by ATR-FTIR (Attenuated total reflection-Fourier transform infrared) spectroscopy. A tape stripping test, a representative evaluation method for skin barrier performance was also conducted to compare with the atmospheric microplasma irradiation. TEWL (Transepidermal water loss) was also measured and compared with and without irradiation of atmospheric microplasma, since TEWL is defined as an amount of water evaporation from inner body through skin and indicates the barrier function of the skin. According to the experimental results, the thickness of a SC (stratum corneum), the outermost layer of a skin could be decreased by the atmospheric microplasma irradiation. This result suggests that the atmospheric microplasma could have a potential to enhance the percutaneous absorption.

1. Introduction

Recently, atmospheric plasma applications have been studied, especially in a wide range of medical applications [1] such as sterilization [2] decomposition of harmful substances [3] and surface treatment [4]. In addition, Blood coagulation [5], wound healing [6] and treatment of cancer cells [7] have been studied as a biomedical applications. These processes are possible due to the generation of ions, active species or other chemical species. As described above, interactions with the atmospheric plasma and the biological matter have been gathered attention.

Dermatology is one of the medical fields where is expected to apply the atmospheric plasma. Various devices for the dermatological application have been developed and evaluated the effects on a skin by irradiating with atmospheric plasma [8]. These applications indicate that medical applications using the atmospheric plasma have a potential to become an innovative method to enhance the drug delivery.

We have conducted the percutaneous absorbance by the irradiation of the atmospheric microplasma application.

This dosing method has advantages such as a stable blood concentration of the drug and avoidance of a drug metabolism. On the other hand, there are disadvantages such as the limitation of drug molecular weight [9] and need of moderate lipid solubility [10]. The SC (stratum corneum) layer inhibits the drug penetration, since it acts as a barrier to protect inner skin [11], and the skin barrier performance was depended by the thickness of the SC) [12].

In this study, pig skin was used as a biological sample, which was exposed to atmospheric microplasma. Exposed sample was analyzed by ATR-FTIR spectroscopy and comparing with the result of tape stripping test. TEWL (transepidermal water loss) which related to the barrier function of skin was also measured. In addition, percutaneous absorption test was conducted to estimate the effect of the atmospheric microplasma irradiation.

2. Experimental setup

2.1. Preparation of skin sample

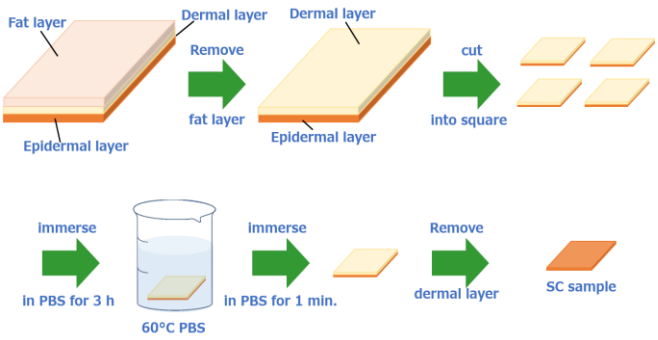

Fig. 1 Preparation of pig skin sample for microplasma irradiation.

Yucatan Micropig skin (female, 6 months) removed from the back was used as the skin sample. A fat layer of the pig skin was removed with a scissor and sand paper. Then it was immersed in PBS for 3 hours. Subsequently, the pig skin was immersed in PBS heated at 60 °C for 1 min. Finally, the stratum corneum layer was peeled off with tweezers, which was used as a biological sample. Thickness of the SC sample was about 500 µm. Preparation of the skin sample is shown in figure 1.

2.2. Procedure of atmospheric microplasma irradiation

Figure 2 shows the experimental setup for the atmospheric microplasma irradiation procedure. Discharge voltage was measured using a high-voltage probe (Tektronix P6105A) and an oscilloscope (Tektronix TDS 2014). Discharge current was measured using a current probe (Tektronix P6021) and an oscilloscope.

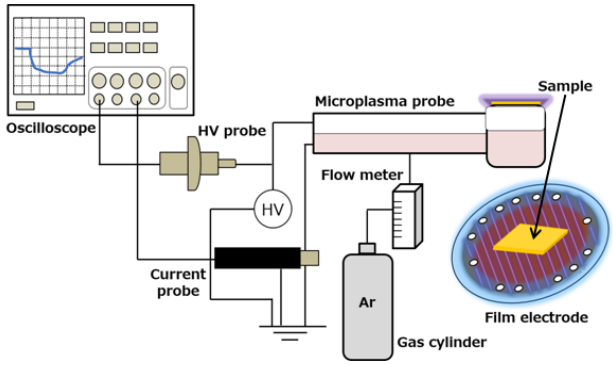

Fig. 2 The experimental setup for microplasma irradiation.

A film electrode was utilized to generate the microplasma. The film electrode has two conductive parts and dielectric layer. The dielectric layer is sandwiched between the conductive parts [13]. The atmospheric microplasma was generated on the gap made by the conductive part and dielectric layer. The film electrode was energized by a negative pulse voltage and generated with flowing argon gas (5 L/min.). Fig. 3 shows the film type microplasma electrode utilized in series of experiments and generated atmospheric microplasma.

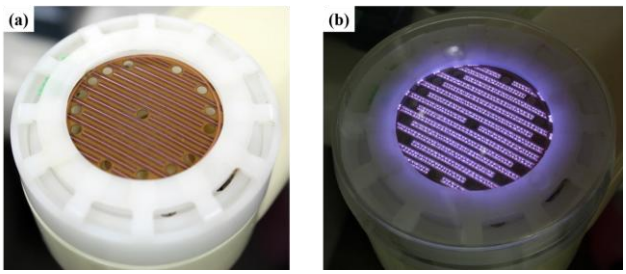

Fig. 3 (a): The film type microplasma electrode, (b): Generated microplasma on the film electrode (1 kV, Ar).

Ground was connected to the surface of the film electrode, and high voltage was connected to the back side of the film. Thus there was no electric shock, even when the living thing was touched to the surface of the film electrode. Discharge voltage, frequency and pulse width were set at about 1 kV, 10 kHz and 5.0 µs respectively as shown in Fig. 4. The pig skin sample was put on the electrode and exposed to the atmospheric microplasma as shown in fig. 2. Exposure time was set at 1 min and 5 min.

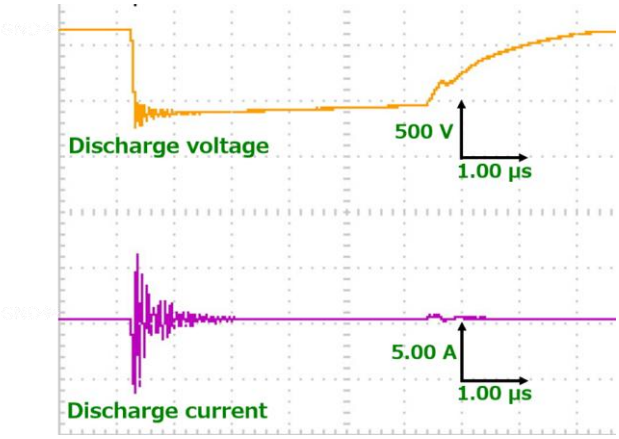

Fig. 4 Waveforms of the negative pulse voltage and corresponding discharge current.

2.2. *In Vivo* transdermal absorption

The pig skin sample was set on the Franz-type diffusion cell (TP-8S, Biocomsystems) which effective area was 1.8 cm^2. In this study, phenol red solution was used to estimate the effect of atmospheric microplasma irradiation for the transdermal drug delivery. 400 µl of phenol red (0.04 v/w %) was applied on the donor part of the Franz-type diffusion cell. Receptor part was filled by 7 ml of PBS (Phosphate buffered saline). The PBS in the receptor part was sampled 4 ml and filled same amount of sampled PBS. Phenol red concentration was estimated by spectrophotometer (SP-300, Optima). The measurement wavelength was set at 560 nm. Temperature of the receptor component was maintained using a water bass at 32 °C [14].

2.3. Tape stripping test

A tape stripping test is a representative method for estimating a barrier performance of the skin or the other properties of a stratum corneum [15]. In this study, a tape stripping test was carried out to evaluate the atmospheric microplasma irradiation to the pig skin. Scotch tape (Sumitomo, 3M) was stuck to a surface of the pig skin sample and peeled off. The tape striping was conducted 10 and 20 times. After the tape stripping test, the pig skin samples were analyzed by ATR-FTIR spectroscopy to compare the atmospheric microplasma irradiation.

2.4. ATR-FTIR spectroscopy

There is a wide variety of analytical methods for skin structure or other properties such as Raman spectroscopy [16], X-ray diffraction [17], electron diffraction [18] and TEM [19]. ATR (attenuated total reflection) method combined with FTIR is one of the useful methods, which is also utilized for analyzing the skin [20]. The exposed pig skin sample was analyzed with ATR-FTIR spectroscopy. Resolution and cumulated number were set at 1.0 cm^{-1} and

64 times, respectively. Several peaks associated with chemical functional group were found. In this study, the measuring wave number was set at between 2800 cm^{-1} and 3100 cm^{-1}, which indicate CH_2 symmetric and asymmetric stretching mode, respectively [21]. The absorbance of the spectrum was decreased as the thickness of the skin was decreased [21]. In addition, the measuring wave number range indicates the change of a chain conformation, especially, the change of the peak shape [22].

2.4. TEWL measurement

Barrier properties of the stratum corneum can be confirmed by the TEWL (transepidermal water loss), a water evaporation from inner body through skin [12]. In this study, pig skin samples which treated by the atmospheric microplasma irradiation were also evaluated by using this method. Pig skin samples (LWD, 6 months, male) were exposed to the atmospheric plasma for 1 and 5 minutes. After that, TEWL was measured with evaporimeter (H4500, Nikkiso Thermo) to compare with the control samples.

3. Results and discussion

3. 1. Phenolred absorption of pig skin

Fig. 5 Photograph of the Franz-type diffusion cell for the transepidermal absorption test using phenol red (left:control sample, right: after 5min. microplasma irradiation).

Fig. 5 shows the Flanz-type diffusion cells before atmospheric microplasma irradiation and after microplasma irradiation (5 min.). Phenol red penetration through the pig skin was observed with spectrometer (see Fig. 5 right). In the light of series of experiments, the atmospheric microplasma irradiation could have the potential to enhance the transdermal absorption for the drug delivery through the SC.

3. 2. Tape stripping test on CH_2 spectra

Fig. 6 shows CH_2 spectra of the pig skin sample before and after the tape stripping test. Absorbance of the CH_2 peaks was decreased, when the number of the tape stripping was increased. This result shows that the barrier performance of the stratum corneum (SC) was decreased as the increase of the tape stripping, since SC layer was removed by applying and peeling the scotch tape off [19].

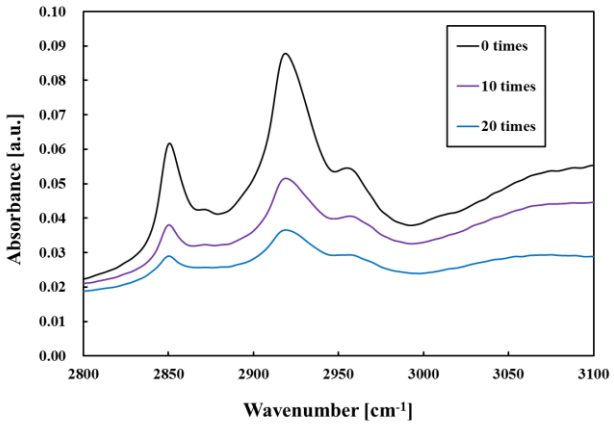

Fig. 6 CH_2 spectra of the pig skin sample conducted the tape stripping test.

3. 3. Effect of microplasma irradiation on CH_2 spectra

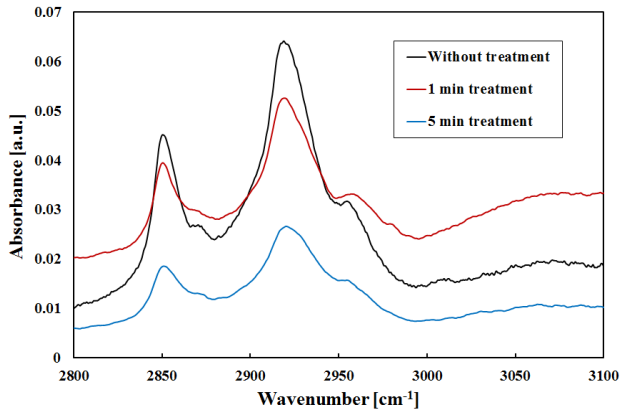

Fig. 7 The CH_2 spectra of the pig skin sample exposed by the atmospheric microplasma.

Fig. 7 shows the CH_2 spectra of the pig skin sample exposed to the atmospheric microplasma. The absorbance of the skin sample was decreased as the exposure time of the atmospheric microplasma was increased. This result could be occured due to the SC layer was shaved by the atmospheric microplasma irradiation, and suggested that the atmospheric microplasma has a potential to enhance the drug penetration through the skin since the SC layer performs as a barrier.

3. 4. TEWL measurement for estimating barrier function of skin

Fig. 8 shows the TEWL measurements of the control sample, microplasma irradiation sample, and tape stripping test sample, each measurements were repeated 4 times. TEWL of the atmospheric microplasma irradiation sample (exposure time; 5min) were increased compared with the control sample (only Ar gas flow). After microplasma irradiation on the pig skin sample, TEWL increased to almost twice, and this result suggests the barrier properties were decreased by irradiating the atmospheric

microplasma. While TEWL of tape stripping sample skin increased more than 3 times.

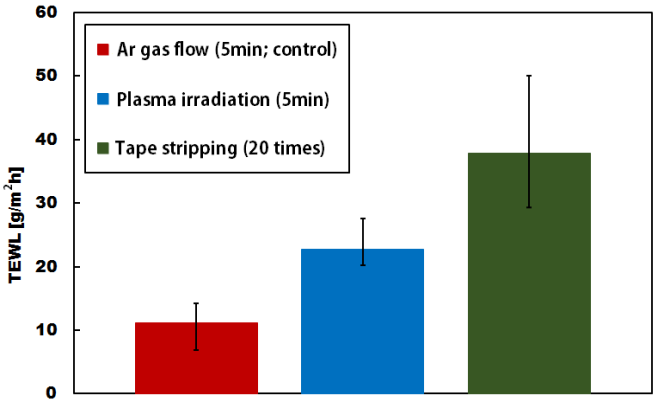

Fig. 8 TEWL measurements of the control sample of the pig skin, and 5 min. microplasma irradiation, and 20 times tape stripping test.

This result suggests the barrier properties were decreased by irradiating the atmospheric microplasma, since TEWL is defined as an amount of water evaporation from inner body through skin and indicates the barrier function of the skin.

4. Conclusion

The atmospheric microplasma was irradiated to the pig skin samples to investigate the feasibility of enhancement the drug penetration through the SC. The following results were obtained through the series of experimental study.

1. With the atmospheric microplasma irradiation, phenol red absorption could be enhanced through the pig skin compared with the control sample.
2. Surface analysis by the ATR-FTIR, the absorbance of the CH_2 peak was decreased as the increased of the exposure time. Compared with the tape stripping test, stratum corneum layer could be decreased by the atmospheric microplasma irradiation. This result suggested the enhancement the drug penetration through the skin.
3. As the result of TEWL measurement, barrier properties of the pig skin sample was decreased by the atmospheric microplasma irradiation.

It could be difficult to cure the various kinds of symptoms such as Alzheimer's disease by only applying the atmospheric plasma. We need to combine together with the safety transdermal drug delivery method and transdermal agent such as peptide vaccine without any injection needle. Further investigation is necessary to improve the atmospheric microplasma irradiation for future clinical test. Safety of patients is the most important to make this novel technology come true.

5. References

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