Chapter 6

Enhancement of Percutaneous Absorption on Skin by Plasma Drug Delivery Method

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Additional information is available at the end of the chapter

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Abstract

Transdermal drug delivery (TDD) is a painless method of low-dose drug delivery. The advantages and disadvantages of transdermal drug delivery methods are named and basic methods such as using chemical enhancers, iontophoresis and electrophoresis are introduced. One of the promising methods make use of plasma which is generated in atmospheric pressure mostly in volume or on surface dielectric barrier discharge (DBD) or in plasma jet. As the plasma produces various particles according to the used gas, UV radiation and heat, their effects on skin and barrier function are described. Improvement of transdermal drug delivery of hydrophilic drug galantamine hydrobromide (GaHBr) using microplasma electrode is introduced.

Keywords: microplasma, plasma jet, plasma drug delivery, skin, stratum corneum

1. Introduction of atmospheric plasma for medical application

Medical applications of atmospheric plasma have been intensively studied [1]. These studies have reported numerous applications, such as for sterilization [2, 3], decomposition of harmful substances [4] and surface treatments [5–7]. In recent years, this area of research has been focused on the practical utilization of atmospheric plasma in the medical filed, such as for blood coagulation [8, 9], wound healing [10–12] and cancer treatment [13–15]. On the other hand, dermatology is one area of medicine where atmospheric plasma applications are currently used in practice [16], such as in a device to treat acne or wrinkles [17] and to treat stretch marks in combination with ultrasonic waves [18, 19]. As explained above, various research and development efforts have been conducted in the application of plasma medicine; however, we do not yet know whether plasma irradiation can be used to treat various symptoms. Though, the basic

mechanisms of cell response [13–15] and the bacterial sterilization process [2, 3] have been studied intensively, effectiveness of plasma medicine in treating depression, which is a social problem in contemporary countries such as Japan, and Alzheimer's disease, which is an increasing problem in aging societies, has not still been investigated. The use of plasma medicine to treat various other diseases, such as cerebral infarction and high blood pressure, also requires additional investigation. Reactive oxygen species (ROSs) and reactive nitrogen species (RNSs) could play important roles in the beneficial effects of plasma medicine. Mechanisms underlying these effects could be quite complicated because of the physiological activation in the body by ROS signalling [20]. Fundamental research to elucidate the cellular response is currently under investigation. In contrast, reactive species generated in plasma or exposed liquid medium have demonstrated efficacy for treating various cancers, but not for the previously mentioned mental disorders, other associated symptoms or diseases such as diabetes, cerebral infarction and high blood pressure [21]. Fortunately, mechanisms of depression and Alzheimer's disease have been revealed in detail. The inhibited production of neurotransmitters (serotonin, noradrenalin and dopamine) causes depression, and Alzheimer's disease occurs because of the deposition of a peptide called amyloid-ß on the cerebral cortex. Research and development of effective drugs for treating mental disorders are proceeding [22]. These drugs are administrated orally; however, the effects of many of these drugs are delayed because of absorption in the small intestine. Moreover, as is the case with oral administration, absorption of drugs in additional locations is different for each person. Thus, administration by injection is used if a precise or high dose of drugs is needed [23, 24]. Administration of poorly absorbed drugs is also the same. However, frequent injections are not desirable in the view of a patient's quality of life (QOL). We can point out the following questions to guide the future of plasma medicine and develop it into a strong tool for the treatment of various symptoms:

1. Is plasma medicine an effective method for treating various symptoms?

2. If not, what combination of drugs in addition to plasma irradiation will be required?

3. Can plasma irradiation be an effective method for drug administration?

As mentioned in many of the references, recent studies of plasma medicine are mostly focused on direct or indirect actions of the active species. Hence, clinical cases, such as Alzheimer's disease, diabetes, cerebral infarction and high blood pressure, have not yet been investigated to determine whether reactive species and plasma medicine could be an effective treatment. Applying plasma medicine for treatment of various symptoms, we have been considering plasma irradiation as a method to deliver appropriate drugs [25]. One instance of this use is the enhancement of percutaneous absorption by atmospheric microplasma irradiation. Percutaneous absorption is a drug administration method in which drugs are absorbed into the skin or body. In this sense, this method is similar to an injection; however, there is no need for a needle in plasma irradiation. Administering drugs in ways that do not require needles could increase patient QOL and reduce the rate of infections. In addition, percutaneous absorption has several advantages, such as preventing metabolism of the drug [24] and stabilizing the concentration of drug in the blood [26]. However, it has disadvantages as well, such as the limitation of the molecular weight of the drug [27] and the need for moderate

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solubility of the drug in lipids [28]. The stratum corneum layer inhibits drug penetration because the skin acts as a barrier to guard our body from foreign substances.

2. Advantages and disadvantages of TDD

Transdermal drug delivery (TDD) is an alternative to oral and parenteral routes of administration. It can offer some advantages over other methods of drug delivery [29, 30]:

1. Reduction of dosing frequency, due to longer duration of delivery.
2. Lower dosing of drugs.
3. Circumvention of first-pass inactivation by liver that can metabolize drugs.
4. Reduction of gastrointestinal irritation.
5. Lower probability of over or under dosing.
6. No pain.
7. No risk of infection by a contaminated needle or via wounds caused by a needle.
8. Suitable for patients with problems of swallowing.

Disadvantages of TTDS:

1. Only some drugs are permeable through the skin.
2. Limited to drugs with a daily dose 10 mg and less.
3. Slow drug delivery.
4. Possible skin irritation.

3. Stratum corneum structure

Stratum corneum, an upper layer of the skin, is a lipid-rich matrix with embedded corneocyte cells, which are dead keratinocytes. Corneocytes are tied together by a protein called corneodesmosome (**Figure 1**). Renewal of stratum corneum occurs every 14 days [31]. This layer is the first barrier for transdermal drug delivery. Lipid-rich matrix is used for transdermal drug delivery (TDD)—intercellular pathway. The lipid-rich matrix is composed of hydrophilic domain—head of ceramides and lipophilic domain—tails (**Figure 1**). The lipids in stratum corneum include mostly ceramides (41%), cholesterol (27%), cholesteryl esters (10%) and fatty acids (9%) with a small fraction of cholesterol sulphate (2%) [32]. The presence of ceramides indicates that lipids will be structured [31].

The permeability of stratum corneum lipid membranes depends strongly on the ceramide composition of these membranes. Molecular dynamics simulations show that ceramides with shorter (four- to eight-carbon acyl chains) fatty acid chains increase skin permeability, whereas

further shortening of the chain leads to increased resistance to penetration almost as good as that of ceramides from healthy skin (24 carbons long on average) [33]. In order to enhance skin permeability, mechanisms like alternation of lipids of stratum corneum and its fluidity or creation of the disordering effect between alkyl chains of lipids of stratum corneum have been proposed [34].

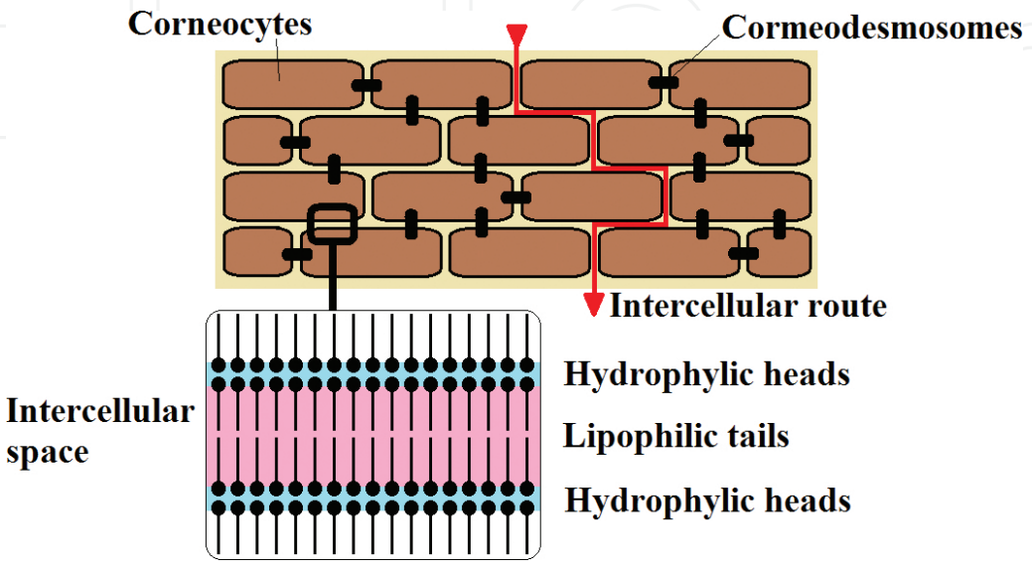

Figure 1. Stratum corneum layer with intercellular space [31].

4. Diagnostics of barrier properties of the skin—TEWL (transepidermal water loss), TST (tape stripping test), ATR-FTIR (attenuated total reflection-Fourier transform infrared spectroscopy)

There are wide varieties of methods available for analysing skin structure and other properties including its barrier function, such as Raman spectroscopy [35], X-ray diffraction [36], electron diffraction [37] and transmission electron microscopy [38]. Barrier properties of the stratum corneum can be confirmed by the transepidermal water loss (TEWL) test, which indicates water evaporation from the inner body through skin [21]. This test expresses the amount of water in grams evaporated per square metre in 1 h. Stratum corneum is a barrier against water diffusion and some other chemicals. The better the barrier function of the skin, the higher the water content and the lower the TEWL value. The tape striping test was described for the first time by Pinkus [39] in 1951. This method is based on removing of stratum corneum layers of the skin. The amount of removed stratum corneum is not constant and it depends on many parameters such as cohesion between cells, hydration or body sites [40]. This technique has been used for evaluation of the barrier function of skin, i.e. for investigating the depth of penetration of drugs [41], the influence of drug enhancers on stratum corneum [42], pH profiles [43] and many others. The tape stripping test (TST) is a representative method for estimating the barrier performance of skin and other properties of the stratum corneum [44]. When the

Scotch tape is placed on skin and peeled off, stratum corneum layer is stuck to the surface of the tape. Therefore, the barrier performance is decreased. Attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR) is an effective method when a material which is not well transmissive is measured, and the method can be used for liquid or solid samples. The information tells us about several micrometres of surface; thus, it is also a useful method for analysing skin [45, 46]. ATR-FTIR spectra can give us information about hydration of skin [47], structure of proteins [48] and lipids [49] and about their changes during skin treatment by chemical or other drug enhancers [50, 51]. There are several vibrational bands associated with chemical functional groups. Vibrational bands at 2850 and 2920 cm^{-1} belong to CH_2 symmetric and asymmetric stretching modes, respectively [52]. The absorbance of the spectrum decreases as the thickness of the skin decreases and the wavenumber indicates a change in chain conformation, especially a change in peak shape [49].

5. Introduction to chemical and physical transdermal drug delivery methods

The stratum corneum provides a barrier to any chemical entering the body and only small molecules having a molecular weight of less than 500 Da (Daltons) can passively diffuse through the skin at rates resulting in therapeutic effects. There are several methods for overcoming this barrier such as using chemical enhancers or physical ways using electric current or plasma discharge.

5.1. Chemical enhancers in transdermal drug delivery

Chemical enhancers are substances that change properties of skin for better penetration of drugs. The most well-known chemical enhancers are alcohols, fatty acids, terpenes and azone. An increase in permeability is very often caused by fluidising the lipids in stratum corneum. However, it is not valid for high concentration of short chain alcohols (like ethanol), where there was observed only a decrease of the absorbance of CH_2 stretching bands and no shift of their position; thus, there is only the extraction of lipids [53]. Many enhancers have long hydrocarbon chains, and it was found out that for fatty acids and fatty alcohols, enhancement is related to the length of the hydrophobic group chain [32]. Enhancer can interact with polar head or with hydrophobic tails of lipid bilayer or with proteins. Water increases fluidity of stratum corneum by insertion of water molecules between polar head groups. Dimethylsulfoxide (DMSO) interacts with intercellular lipids and keratin. Azone disrupts lipid structure, and oleic acid increases fluidity of intercellular lipids. The most effective enhancers are those which interact with lipids and also with proteins of stratum corneum [54]. Combinations of chemical enhancers have been used to maximize the effect of the permeability of drugs, and chemical enhancers are also combined with physical enhancing methods such as iontophoresis [55].

5.2. Iontophoresis in transdermal drug delivery

Iontophoresis (**Figure 2**) is a class of noninvasive methods to increase penetrations of ions through the skin by applying electric current using low voltages of up to 10 V (**Figure 2**).

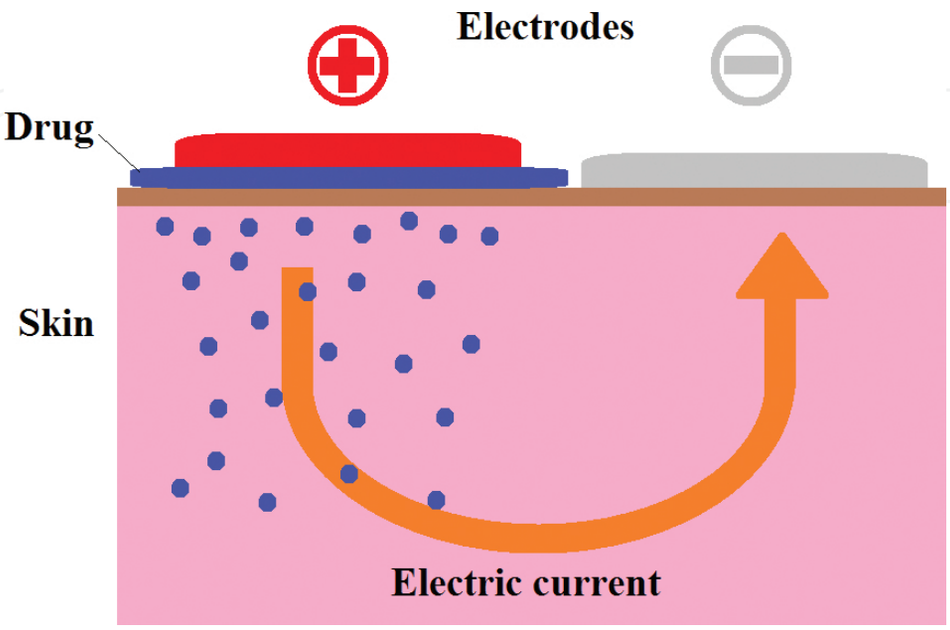

Figure 2. Application of iontophoresis to skin.

Various waveforms of applied current have been investigated [56], and it has been shown that various waveforms have various effectivities for skin permeability [57]. Iontophoresis enhances transdermal drug delivery by three mechanisms:

1. Ion-electric field interaction causing moving of drug (ions) away from the electrode through the skin.
2. Flow of electric current increases skin permeability.
3. Electro-osmosis caused by solvent flow from the anode to cathode because of negatively charged skin due to amino acids in cell membranes.

Iontophoresis is used for ionisable drugs, and it is most effective for molecules with weight of up to 7 kDa [58] or 10–15 kDa according to Kalluri and Banga [59]. The disadvantages of iontophoresis are: difficulties with stabilizing the therapeutic agent in the application vehicle, complexity of the drug release system and prolonged skin exposure to an electric current [60]. The main changes on skin after iontophoresis are an increase of the hydration of stratum corneum and a decrease of electrical resistance of the skin [61]. Analysis of asymmetric CH_2 peak, in ATR-FTIR spectra, did not show lipid alkyl chain disorders characterised by band shift or band broadening even at high current densities in some previous works [61–64]. On the other hand, in the recent work of Prasad et al. [65], they observed a shift of asymmetric CH_2 band with increasing of current density that achieved 8 cm^{-1} at 0.2 mA/cm^2. Also decreasing of lipid and protein bands intensity indicates lipid and protein extraction. The spectra also

demonstrated a split in amide II band into 1553 and 1541 cm^{-1}. The split could be due to the disruption in hydrogen bonding associated with the head of ceramides, breaking interlamellar hydrogen bonding of the lipid bilayer and disrupting the barrier property of stratum corneum, resulting in loosening of lipid-protein domains, thus allowing higher flux as compared to the passive treatment [65]. Reversibility studies were conducted *in vivo* after 24 and 48 h of the application of iontophoresis. It was observed that the recovery process had started in 24 h and almost total recovery of epidermal as well as dermal changes was found in 48 h with low current density DC iontophoresis. However, with iontophoresis using 0.5 mA/cm^2 current density, edema along with focal disruption of the epidermis persisted [65].

5.3. Electroporation in transdermal drug delivery

Unlike iontophoresis, electroporation (**Figure 3**) uses high voltage (HV) (over 100 V) pulses for short time (in range of microseconds to milliseconds). Cells exposed to an electrical pulse open pores in the cell membrane and allow macromolecules to enter. It was confirmed that delivery of drugs of at least 40 kDa can be achieved [66]. The disadvantages of electroporation are as follows: (1) if the pulses have not adequate length and intensity, pores can be too large or cause cell damage, non-specific amount of material can be released to the cell [67]. (2) The created pores can persist for several hours, which allow a higher amount of drug to be delivered [30].

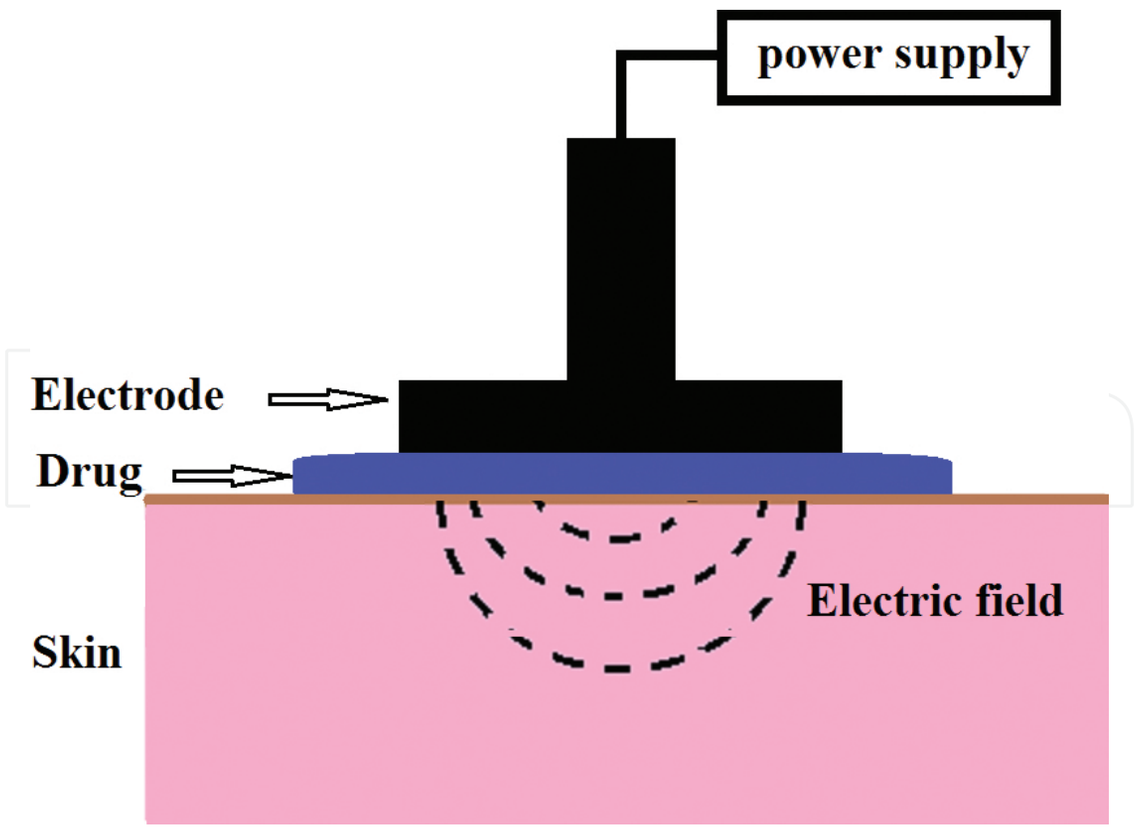

Figure 3. Application of electroporation application.

6. Plasma sources used for skin treatment

Several kinds of plasma sources were used for investigation of transdermal drug delivery so far, such as volume dielectric barrier discharge (DBD), surface DBD and plasma jet.

6.1. Volume DBD

Volume DBD (**Figure 4**) with one isolated electrode has been used in the study of Kalghatgi et al. [68]. Plasma can be with direct contact with a treated object (placed between electrodes) but with increasing distance of electrodes, ignition voltage also increases, too. This means that very high voltages need to be used in real applications. Kalghatgi et al. used pulsed discharge with a pulse duration of 1–10 μs. They applied 10 kV in the frequency range of 50–3.5 kHz to HV electrode isolated by quartz glass. The skin was treated from 15 to 120 s.

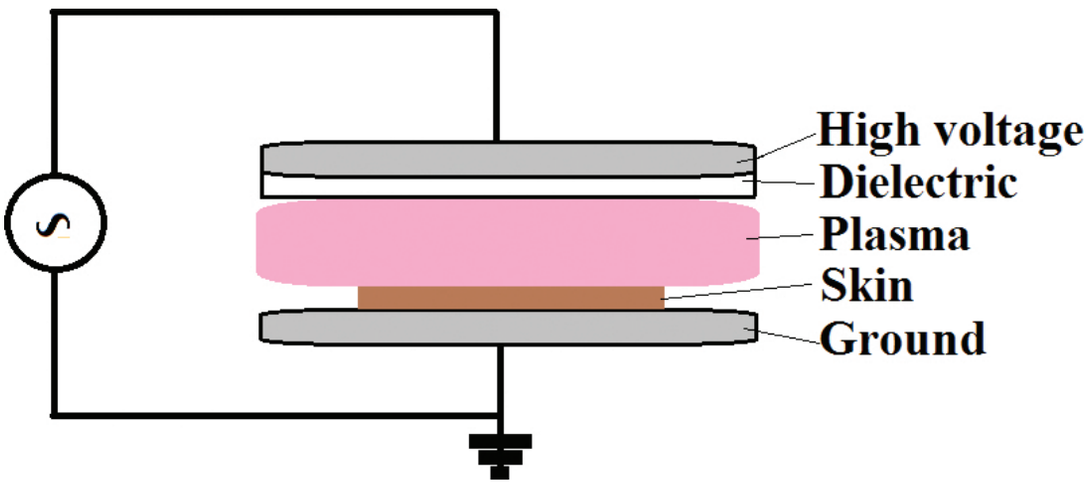

Figure 4. Volume dielectric barrier discharge.

6.2. Surface DBD

Another version of DBD is surface DBD used in Shimizu et al. [23, 69]. Both electrodes were in contact with isolator layer. Each electrode had a thickness in micrometre dimensions. These low dimensions allowed the decrease of ignition voltage to hundreds of volts. During treatment of skin, electrodes were powered by 600 V with an AC frequency of 27 kHz. Electrodes are created by two copper grids each with a thickness of 18 μm with insulation layer between them with thickness of 100 μm. The high-voltage electrode was covered with an insulation layer and the grounded electrode faced the skin samples at a distance of approximately 1.5 mm (**Figure 5**). Thus, there was no risk of electric shock to users, even when touching the surface of the film electrode. The waveform of the applied voltage and corresponding discharge current are shown in **Figure 5**. Holes between electrode lines allowed gas to pass towards the sample (**Figures 5** and **6**). Argon gas was supplied to the microplasma electrode systems with a flow rate of 5 L/min through the tube that was connected to the electrode. The exposure time was usually between 1 and 5 min (**Figure 7**).

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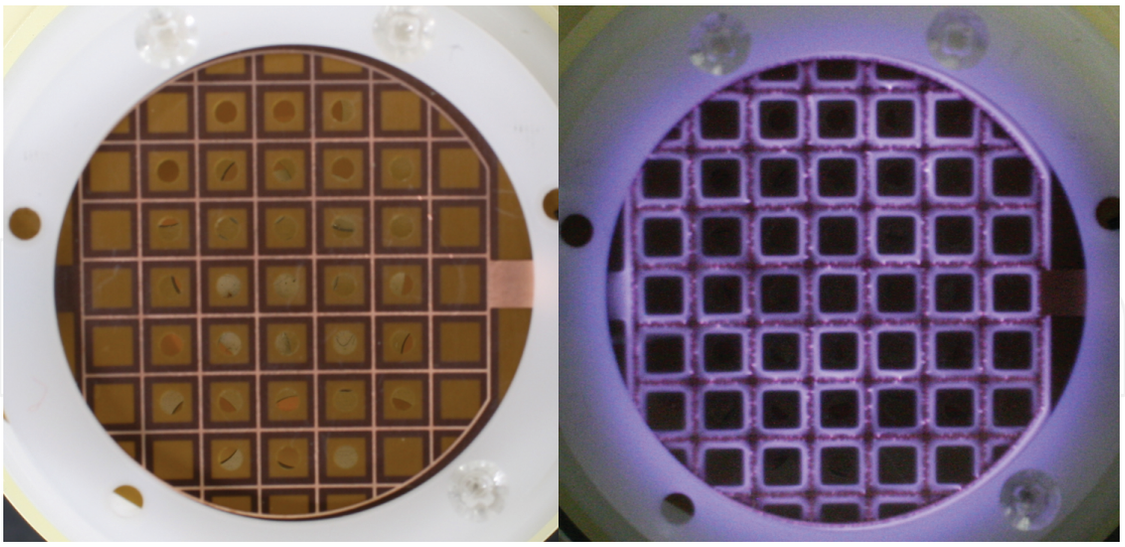

Figure 5. Top view of microplasma electrode, before discharge (left), discharge with Ar gas – 600 V (right) [69].

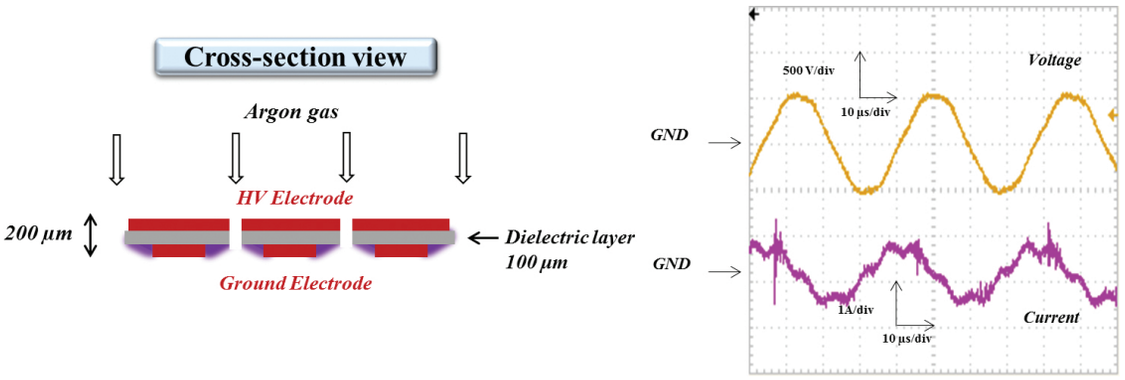

Figure 6. Cross section of microplasma electrode (left); waveforms of microplasma discharge (right) [69].

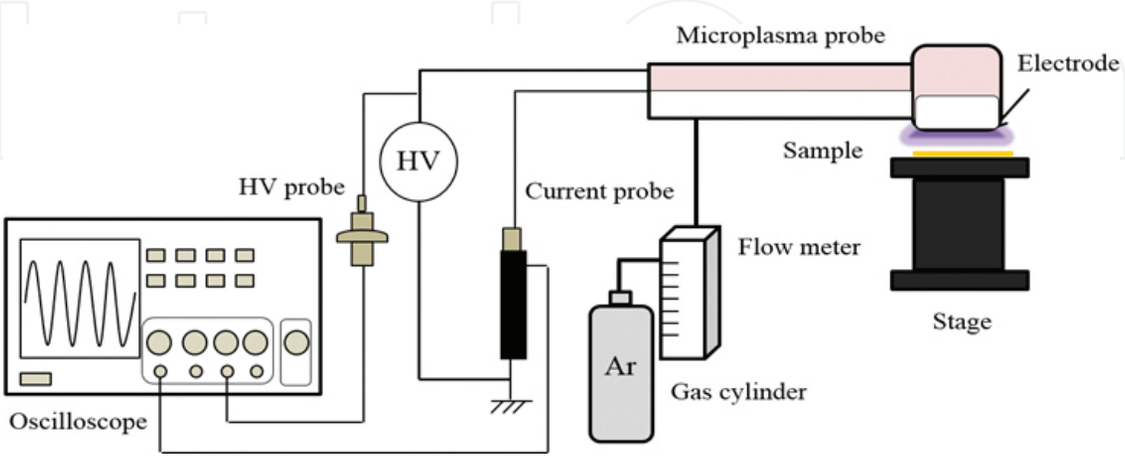

Figure 7. Experimental set-up with microplasma electrode [23].

6.3. Plasma jet

Plasma jets consist usually from a gas nozzle with one, two or three electrodes [70, 71]. The plasma jet can be realised by two ways—active plasma jets (expanding plasma contains free and high energetic electrons) and remote plasma jets (plasma is potential free and consists of relaxing and recombining active species from inside the nozzle) [70]. The plasma jet in **Figure 8** consists of a Pyrex tube and a central tungsten high voltage (HV) electrode. The grounded electrode is an aluminium ring located at the end of the outer surface of the Pyrex tube. The distance between the skin sample and the outlet of the plasma jet was set to 2 mm. The sample was isolated from the holder by a 30-mm thick PVC isolator or without isolator to compare the effect of the conductive layer under the skin surface as the human body is not isolator. The treatment time of the sample was set from several seconds to 1 min. Argon, nitrogen or argon-water vapour gases were introduced into the plasma jet. The waveform of the voltage and current of argon plasma jet is depicted in **Figure 8** [72].

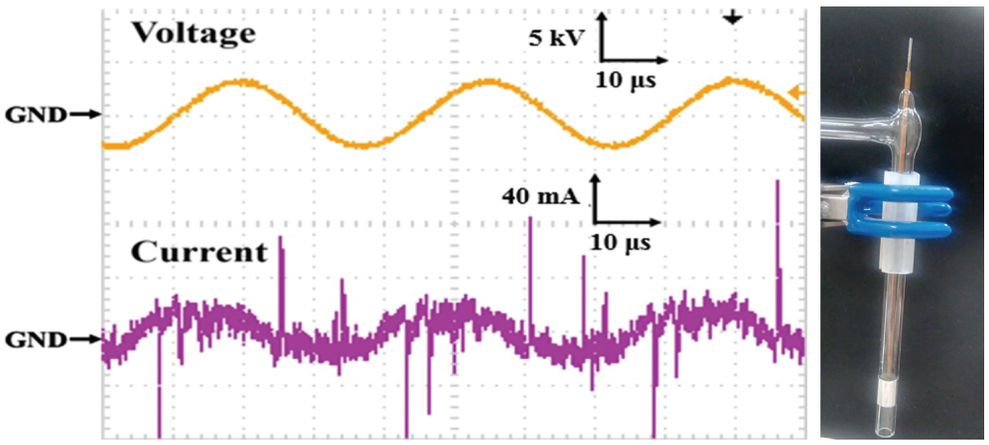

Figure 8. Waveform of plasma jet discharge (left) [72] and plasma jet electrode (right).

7. Plasma effects on skin barrier and transdermal drug delivery

7.1. Chemical reactions plasma particles with the skin

If argon plasma is used for skin treatment, the plasma produces electrons, argon ions, metastable and excited states. These particles can react with the skin directly. But, if argon or nitrogen plasma is working in atmospheric air, it makes the situation more complex because molecules of air (O_2, N_2, CO_2 and H_2O) can enter the volume of the plasma jet [73, 74]. In this case, argon can react with skin also indirectly through the excitation or dissociation or ionization process with air molecules. The reaction results in the creation of a number of species. It is difficult to find the molecule causing changes in skin. Pig skins treated by argon, nitrogen and argon/water plasma were compared by measuring ATR-FTIR spectra and monitoring of the shift of asymmetric stretching CH_2 band near 2920 cm^{-1}. A comparison of argon and nitrogen plasma jets has shown that argon can play a role in reactions with molecules

of stratum corneum, but a similar effect can be achieved by nitrogen plasma itself as similar shift can be observed. A significantly higher value of shift was observed when argon with water vapours was used. The asymmetric stretching band was shifted 3.5 cm^{-1} (**Figure 9**). This result indicates that the stratum corneum became the most permeable after treatment of Ar plasma with water vapours. On the other hand, we observed low wavenumber shifts of the maxima of amide I and amide II (**Figure 9**).

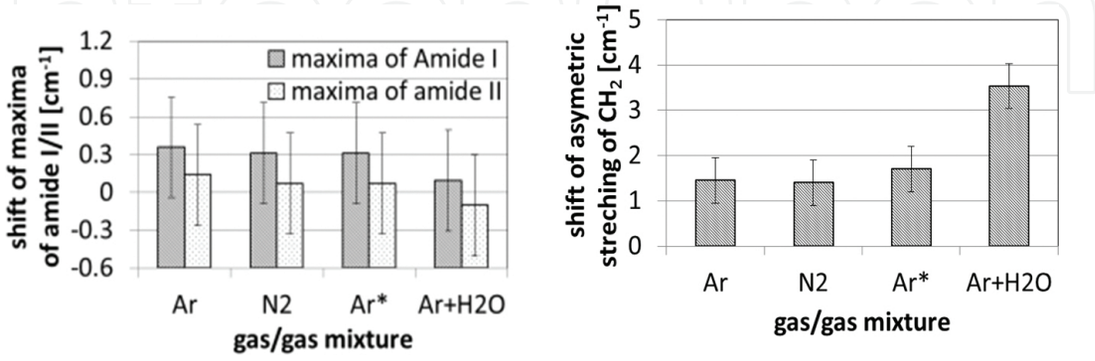

Figure 9. Shift of asymmetric stretching band of CH_2 to higher wavenumbers for the gases used in plasma jet discharge. Flow 10 L/min = (Ar*; N_2), 3 L/min = (Ar; Ar+H_2O).

Pig skin treated by argon microplasma shows shift of asymmetric stretching CH_2 band near 2920 cm^{-1} after 1, 3 and 5 min of irradiation comparable with the plasma jet (**Figure 10**).

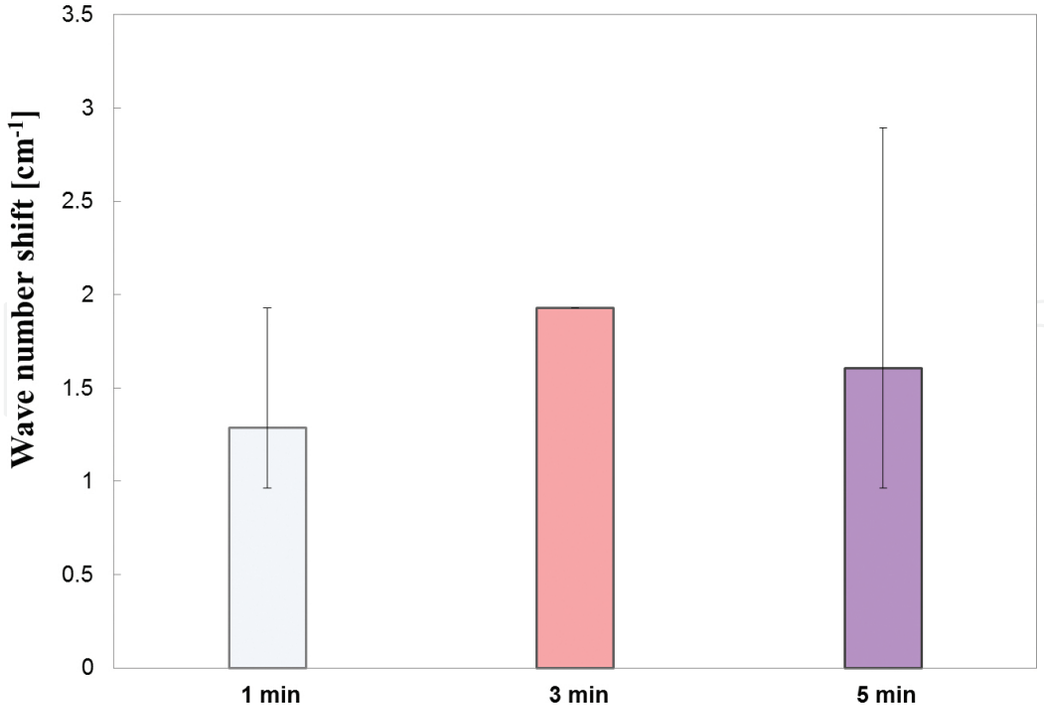

Figure 10. Shift of asymmetric stretching band of CH_2 to higher wavenumbers for Ar gas after microplasma irradiation [69].

When argon flew through the water reservoir, the argon ensured a higher concentration of water vapours in the discharge. The high shift of the asymmetric stretching band of CH_2 indicates that H_2O and the created OH molecules can play important roles in increasing the shift of the asymmetric stretching band of CH_2 in stratum corneum. OH could be created mainly by two channels [75]:

$$Ar_m + H_2O \rightarrow OH + H + Ar \quad (1)$$

$$H_2O + e \rightarrow OH + H + e \quad (2)$$

Simulation of the interaction of O and OH radicals with α-linolenic acid as a representative of fatty acid [76] showed that OH radicals most typically abstract an H atom from the fatty acids, which can lead to the creation of a double bond and also to the incorporation of alcohols or aldehyde groups, increasing hydrophilic properties of fatty acids and changing the lipid composition of the skin, causing an increase of skin permeability. Creation of these groups increases the hydrophilic character of the lipid layer. Incorporation of oxygen to stratum corneum lipids was also confirmed by increasing of C–O and C=O bonds after treatment of skin layer by atmospheric plasma [77]. Also, micropores (10 nm–1 μm in size) were observed in an artificial cell membrane system consisting of supported lipid bilayers after DBD plasma irradiation [78]. Later, it was found out that these micropores are induced transient species such as OH and OOH formed by plasma and they caused lipid peroxidation leading to truncated lipid chains, which induced pore formation [79]. These temporal pores were observed in skin after DBD plasma treatment and it was allowed to penetrate large molecules through the skin in several minutes. These pores had lifetimes of less than 5 min after treatment [68].

7.2. Effect of heating on skin barrier

Heating of the skin can increase permeability of stratum corneum [80]. Reasons of improving of transdermal delivery through the skin by increasing temperature are structural changes of stratum corneum lipids. Plasma can raise skin temperature from 30°C to more than 100°C which depends on the irradiated time, power of plasma source or used gas. Structural changes of stratum corneum lipids occur between 20 and 40°C, from orthorhombic to hexagonal ordered lipids. These changes can be indicated by CH_2 symmetric stretching frequency with an increase of 0.5 cm^{-1} in ATR-FTIR spectra or it is possible to observe by CH_2 scissoring mode where transition is revealed by splitting of the scissoring modes to produce a doublet with components at 1473 and 1463 cm^{-1}. Ordered lipid chains change to disordered chains at around 80–90°C. But some scientific groups identified more transitions in lipid structure [52]. For example, four phase transitions in temperature ranges 35–42°C, 65–75°C, 78–86°C and 90–115°C, respectively. Transitions below 75°C are reversible [81]. Between 35°C and 42°C two transitions can occur belonging to solid fluid transition and disruption of lipids covalently

linked to corneocites at 37 and at 40°C to 'orthorhombic to hexagonal' change in structure. But too high a temperature can cause damage to the skin. Thermal conditions that cause burns of the skin are functions of the time and method of how the skin is exposed to the heat. Longer exposure of skin to a temperature higher than 43°C can lead to the formation of blisters [82]. It was found out that fast heating of skin can increase skin permeability without damaging deeper tissues. Investigation of high temperatures of up to 315°C applied to skin for 100 ms, 1 s and 5 s showed small variations between drug deliveries of calcein [82]. Exposure for 1 s or 5 s should be sufficient to equalize temperature in the full thickness of skin, but the 100 ms exposure should have influence only on stratum corneum. Between 100 and 150°C, permeability of skin was increased a few fold. This increase was attributed to lipid melting in the stratum corneum. In the range of 150–250°C, transdermal flux increased by two orders, attributable to disruption of stratum corneum keratin network structure. Above 300°C, transdermal flux increased by three orders, attributable to decomposition and vaporization of the stratum corneum [83].

7.3. Effect of UV radiation on skin barrier

The wavelength range of 100–400 nm is called UV light and is usually divided into three ranges: UVA (320–400 nm), UVB (280–320 nm) and UVC (100–280 nm). UV light can cause damage to skin. It is well-known that atmospheric plasma can generate wide range of UV light dependent on the used gas [84, 85]. UVC radiation can reach only stratum corneum and it consists of emission of excited NO molecule (200–280 nm—NO-gama system) or nitrogen molecule (120–200 nm—LBH system) in plasma discharges. The source of UVB radiation is emission of OH and nitrogen (second positive system of nitrogen). UVA is composed mostly of second positive system of nitrogen. UVA and UVB can reach deeper layers of skin like epidermis and UVA also dermis. UV radiation can cause formation of hydroxides and epoxides, hydrogenation of double bonds and breaking of carbon chains. The effect of UV on lipids depends on their structure, and only double bonds of fatty acids are sensitive to the formation of oxygenated molecules by oxygen in the air. These changes can be amplified or weakened by the surrounding atmosphere around the treated skin. When lipids of stratum corneum like cholesterol, cholesterol sulphate, ceramide III, linolenic acid and dipalmitoylphosphatidylcholine were irradiated by UV light up to 240 min, peroxidative changes occurred only in lipids with double bonds such as cholesterol and linolenic acid [86]. But the changes are not permanent and the order of stratum corneum lipids and water-loss protection can recover after 3 days and it returns to its initial state, thanks to repair processes in the skin [87]. Reactive oxygen species like superoxide anion radicals, singlet oxygen, hydrogen peroxide and hydroxyl radical can be created by UV light. For example, superoxide anion radicals are precursors to other oxygen reactive species, and it was found that there exists a correlation between the superoxide anion radical's concentration and degree of oxidation of lipids [88]. UV light is also used for treatment of some skin diseases but delivered doses have to be controlled and maintained in safety level to not penetrate to deeper layers of skin [89].

7.4. Acidifying effect of plasma on skin

Acidifying effect was observed after applying plasma to stripped lipids from stratum corneum. A higher decrease of pH was observed in discharges, which produced a higher amount of NO_x species. The same effect was observed on human skin, but after 30 min after plasma treatment, the pH of skin return to initial value [90]. The decrease of pH depends on parameters of the plasma discharge and the treatment time. Previous work has attributed the pH shift of water on plasma-treated lipids to the interaction of reactive species with the surface. Nitrates formed in water droplets could form nitric acid. NOx species could adhere on lipid surface or deposited nitric acid on the film surfaces by gaseous HNO_3 [91]. The recovery of pH in the post-plasma phase was attributed to the decrease of acidifying agents on the substrate surfaces by both diffusion and desorption processes.

7.5. Skin etching and skin damage by plasma

The etching effect of plasma is demonstrated in **Figure 11** by a cross section of the skin. The stratum corneum layer (the white part in (a) control sample) was removed after 20 tape stripping cycles, as shown in (b). After 30 s of atmospheric plasma jet irradiation, most of the stratum corneum layer was removed, as shown in (c). In the microplasma case, even after 5 min irradiation (d), the stratum corneum layer remained similar to the control sample, as shown in (a) [23]. Little difference in physical appearance was observed between the control pig skin sample and after microplasma irradiation. For the tape stripping test, surface asperity was decreased and after 10 s of atmospheric plasma jet irradiation, small pores (ranging from 40 to 100 μm) were observed. Physical damages on skin could be considered to arise from the electric field or an etching effect from bombardment with charged particles [92].

Surface potential can lead to a strong electric field across the skin and finally penetrate the skin to form holes. Holes were confirmed after longer operation (3–5 min) of atmospheric plasma irradiation. Pores and holes are shown in **Figure 12** after 10 and 30 s of operation of the plasma jet.

This physical damage could affect the barrier function of skin samples. When the effect of plasma jet was tested on PEN film, after 10 s of plasma jet irradiation, the surface potential increased to about 10% of the driven voltage. After long-term operation, it reached almost the same value as the driven voltage [23]. A similar problem could happen in electroporation but it uses very short operation times in the range of microseconds or milliseconds. High surface potential can induce current flow through the skin and it will increase the skin temperature and might affect cells of skin and cause thermal damage of skin [93]. On the contrary, the surface potential remained low in the microplasma case. The thickness of stratum corneum after 30 s of treatment of plasma jet is equivalent to 20 times of striping by tape. This is also confirmed by TEWL (**Figure 13**), which also shows the same values. On the other hand, TEWL after 5 min of microplasma irradiation of the pig skin sample, TEWL increased to almost double its original value, suggesting that the barrier properties were decreased through atmospheric microplasma irradiation but it is less effective than plasma jet [23].

http://dx.doi.org/10.5772/65116

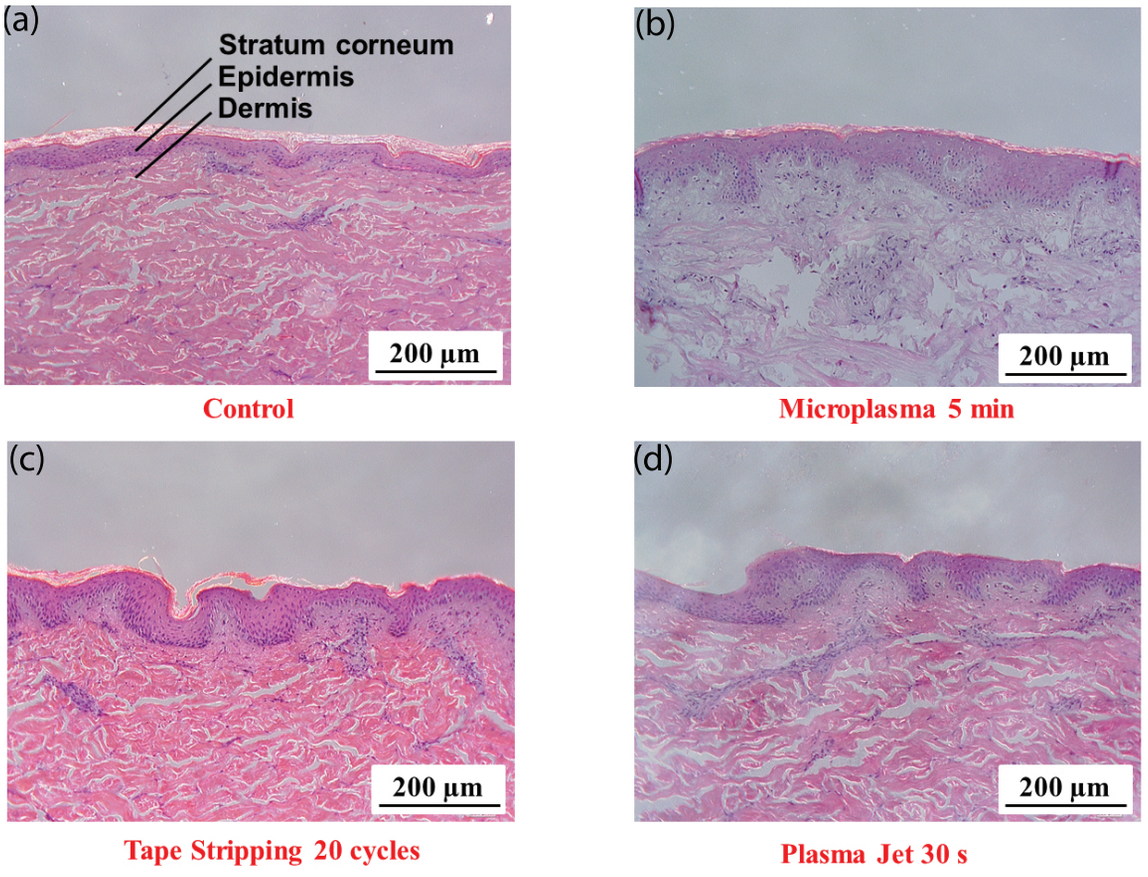

Figure 11. Cross section of pig skin after atmospheric plasma irradiation, and tape stripping test. (a) Control sample, stratum corneum thickness: 18.09 ± 1.64 µm; (b) tape stripping test — 20 times, stratum corneum thickness: 5.99 ± 1.24; (c) plasma jet — 30 s, stratum corneum thickness: 3.49 ± 0.61; (d) microplasma — 5 min, stratum corneum thickness: 13.40 ± 1.46 [72].

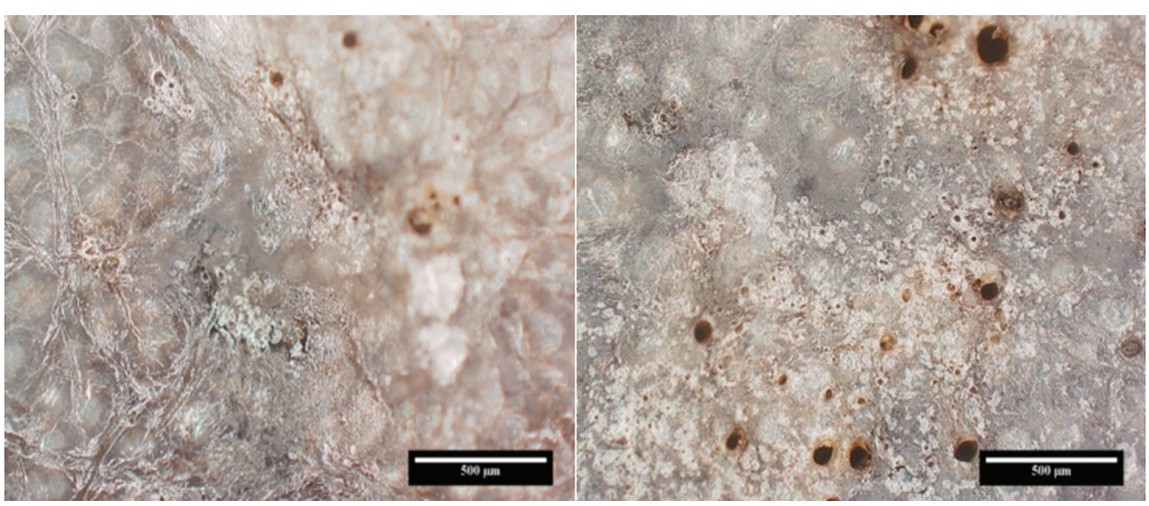

Figure 12. Left: 10 s of treatment by plasma jet; Right: 30 s of treatment by plasma jet [72].

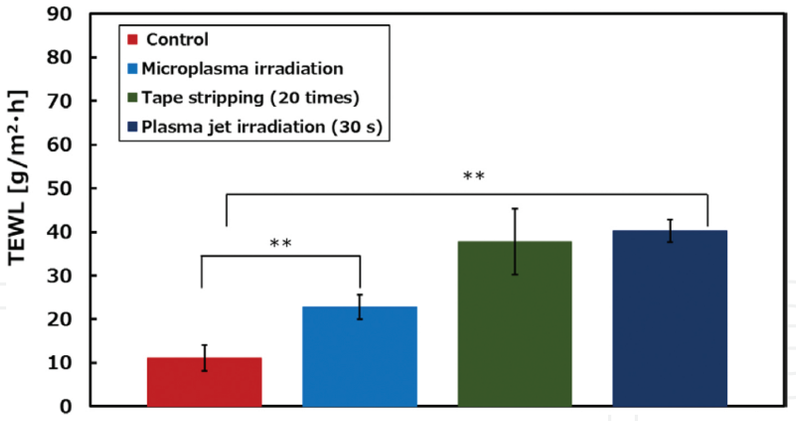

Figure 13. Variations of TEWL values before and after microplasma irradiation, tape striping test and plasma jet irradiation [72].

8. Improvement of galantamine hydrobromide (GaHBr) permeation by plasma irradiation

Galantamine hydrobromide (GaHBr) (**Figure 14**) is an alkaloid, isolated from plant species such as Narcissus and Lycoris species [93]. Treatment of Alzheimer's disease occurs by inhibition of acetylcholinesterase enzyme. The chemical structure of galantamine hydrobromide is shown in **Figure 14**.

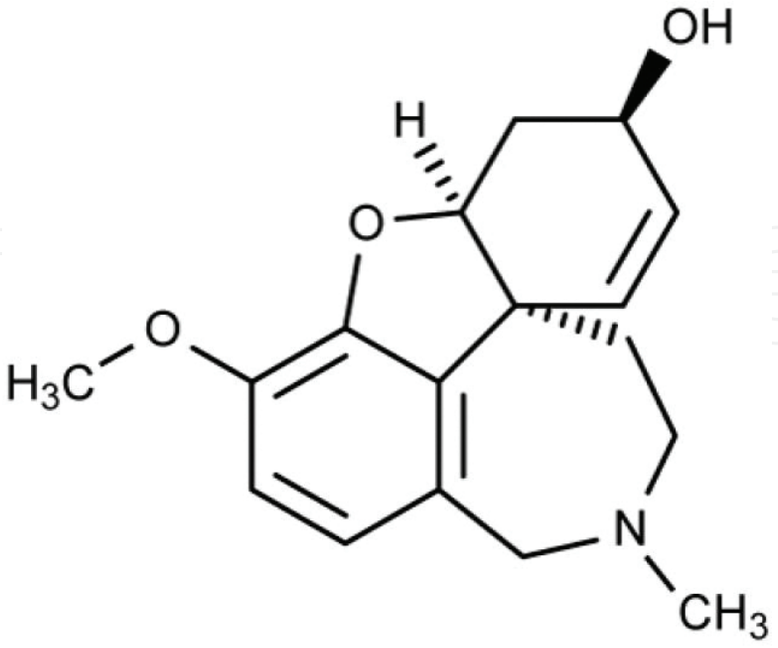

Figure 14. Chemical structure of galantamine hydrobromide.

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As GaHBr is a hydrophilic molecule with a low molecular weight (368 Da), usually transdermal patches with chemical enhancers are used for transdermal delivery [94]. Using microplasma for the first time for enhancement of transdermal delivery of GaHBr, a slight improvement was observed with comparison of passive diffusion (**Figure 15**). Pig skin was irradiated for 3 min and then a water solution with GaHBr was applied on the skin. The permeation profiles of GaHBr through the pig skin delivered 5.35 ± 2.34 and 11.53 ± 2.89 μg/cm^2 for the control and the plasma-irradiated sample 24 h post-experiment, respectively [69]. These results lead to the expectation that microplasma discharge can be used to enhance the skin delivery of hydrophilic drugs and larger molecular drugs in the future.

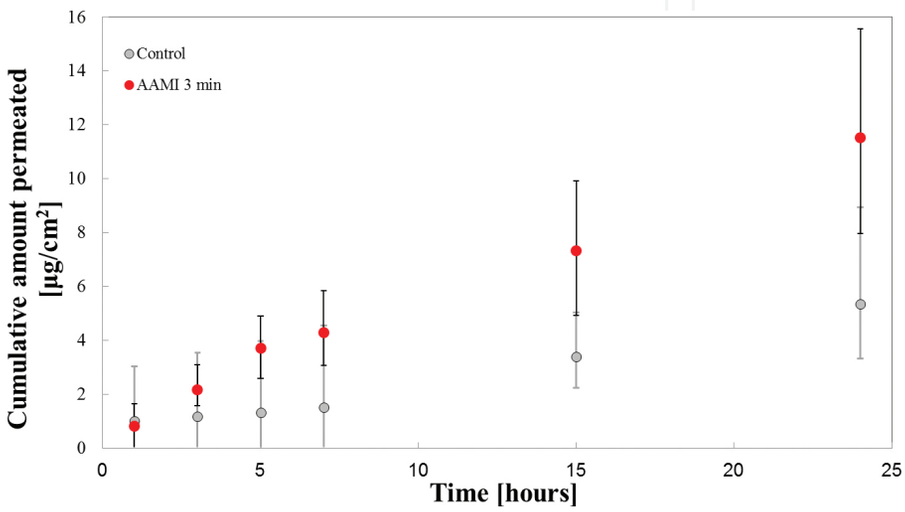

Figure 15. Amount of penetrated GaHBr—passive diffusion and after microplasma irradiation (red circles) [69].

9. Outlook of plasma drug delivery

Plasma as a source of UV light, heat, ions, reactive radicals and metastable states could be a successful tool for transdermal drug delivery. Each of these parameters can be used for improvement of skin permeability. Stratum corneum can be altered by chemical reaction of reactive oxygen species or by UV causing peroxidation of intercellular lipids. Undesirable heating effects can be tuned by the length of the plasma pulse and then a change of the conformation of lipids can be achieved also by increasing gas temperature in plasma. Improvement of permeability of skin by plasma was proved by ATR-FTIR and TEWL methods. A plasma jet is effective in etching of stratum corneum but can cause damage to skin because of the presence of a high electric field. We believe that plasma jet could be considered as a trade-off relationship, i.e. accompanied by physical damage or pain on the skin, similar to needles and creation of pores can cause risk of infection. On the other hand, microplasma seems to be more suitable for future drug delivery because it caused lower damage of skin. Transdermal drug delivery of galantamine hydrobromide by microplasma showed slight improvement in comparison with passive diffusion but further investigation is needed for drug testing

or improvement in plasma properties in the future. We suggest that the use of a transdermal drug delivery with plasma irradiation is feasible, and thus, could be combined with appropriate drugs to target various symptoms such as Alzheimer's disease, diabetes and other symptoms that cannot be cured by applying plasma irradiation alone. This novel method will reveal new directions for the future of plasma medicine. Our aim is to combine a safe transdermal drug delivery method with a transdermal agent such as a peptide vaccine without requiring an injection needle and causing physical damage to the skin. Safety of patients, without causing harmful damage or physical damage to the skin, is critical for enabling this novel technology.

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