Chapter 6

Microplasma Drug Delivery

Kazuo Shimizu and Jaroslav Krištof

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.73498

Abstract

There are several techniques to perform drug delivery. One of the newest methods of drug delivery through the skin is the application of plasma. Reactive species generated by plasma can change the chemical composition of the skin, change the structure and extract lipids of the lipid barrier, create pores in or etch the surface of the skin. These changes have an influence on the barrier function of the skin which can be decreased. The main barrier of the skin is called stratum corneum. The structure and composition of the stratum corneum and function of the components is described. Possible interaction of plasma particles with skin is presented and compared with interaction of plasma species with carbon or hydrocarbon surfaces. Active species which can effectively interact with lipid molecules is introduced. Hydrophilic drugs and drugs with high molecular weight can penetrate very difficult through the skin or cannot penetrate the skin at all. As a model, a drug, Cyclosporine A, was studied. Cyclosporine A is a lipophilic drug with a molecular weight of 1203 Da which is used during and after organ transplantation to prevent rejection. The Hairless Yucatan micropig was used to simulate human skin. A film electrode was used to generate plasma that was used for skin treatment. An AC voltage ($V_{0\text{-}p}$ = 0.6–1.5 kV, 25 kHz) was applied with flowing gas (5 L/min). The barrier function of the skin was evaluated by a Franz cell experiment and high performance liquid chromatography (HPLC) for a particular drug. An effective amount of drug in human body was determined by pharmacokinetic model.

Keywords: microplasma, plasma drug delivery, stratum corneum, transdermal drug delivery

1. Introduction of atmospheric plasma for medical application

Plasma applications are used in many applications from etching of surfaces [1], deposition of superconducting materials [2], and improvement of adhesion properties of polymers [3] to sterilization [4] and various medical treatments. Plasma in biomedical field is intensively

studied these days. Research is mainly oriented to wound healing [5], cancer treatment [6], dermatology [7] and drug delivery to the cells [8]. Drug delivery through the skin is also studied very intensively and for a very long time [9, 10] but using plasma is a relatively new approach and only some studies exist [11–15]. For successful penetration of the drug through the skin, it is necessary to know the composition and structure of the stratum corneum and its lipid barrier. The stratum corneum is the main barrier of the skin, so knowledge of the function(s) of the molecules which participate in this barrier is crucial. Plasma sources can produce different kinds of particles with lifetimes from several nanoseconds to several seconds [16–18]. The configuration of using a specific gas or mixture of gases can prefer certain type of particles. Each particle can have different effects on the skin such as penetration depth, reactivity, and the ability to convert to more reactive particles. The effects of the particles to carbon and hydrocarbon surfaces will be described. The success of using of plasma for transdermal delivery of certain molecules and also feasibility of using model drug, Cyclosporine A, will be presented.

2. Plasma discharges

These days many types of plasma discharges exist. If we want to use plasma in medical applications (plasma drug delivery in our case), mostly atmospheric discharges are required. Atmospheric discharges can be generated from DC, through low frequency AC, radiofrequency to microwave frequencies. Generally, we can divide plasma sources into three categories:

1. Discharges where plasma is directly in contact with the sample (skin). In the case of direct plasma treatment of the tissues or skin, the human body serves as one of the electrodes and partial current flows through the tissue. The plasma has a low temperature (up to 45°C). The sample is several millimeters from plasma source and active species directly treat sample [19].
2. Plasma is blown out by gas flow to the sample (skin) from the place where the plasma was created. The distance between the plasma nozzle and the sample can be set from several millimeters to centimeters [20]. The diameter of the plasma plume can reach the diameter of the needle [21].
3. Plasma is not in contact with the sample (skin). The sample is treated by long living particles such as certain radicals, ions, metastable species or particles with very long lifetimes (for example, ozone). Plasma usually has a very high temperature up to several thousand degrees or very small dimensions. A typical example of high temperature plasma source for medical treatment is "PLASON," which is used for the production of NO radicals [22], or microwave plasma torches [23]. On the other hand, a high temperature is sometimes necessary for the production of certain type of species. Sources with low dimensions are surface plasma discharges. Microplasma DBD (**Figure 1**) is a plasma source used in Shimizu et al. [11], where electrodes had a thickness in micrometer dimensions that allowed a decrease of ignition voltage to hundreds of Volts. Advantages of surface DBD sources is that they can be very large; and the electrode can be very thin and placed on polymer which can copy the surface of treated sample [24]. A disadvantage is that the distance between the electrode and the sample must be very short, approximately 1–2 mm.

http://dx.doi.org/10.5772/intechopen.73498

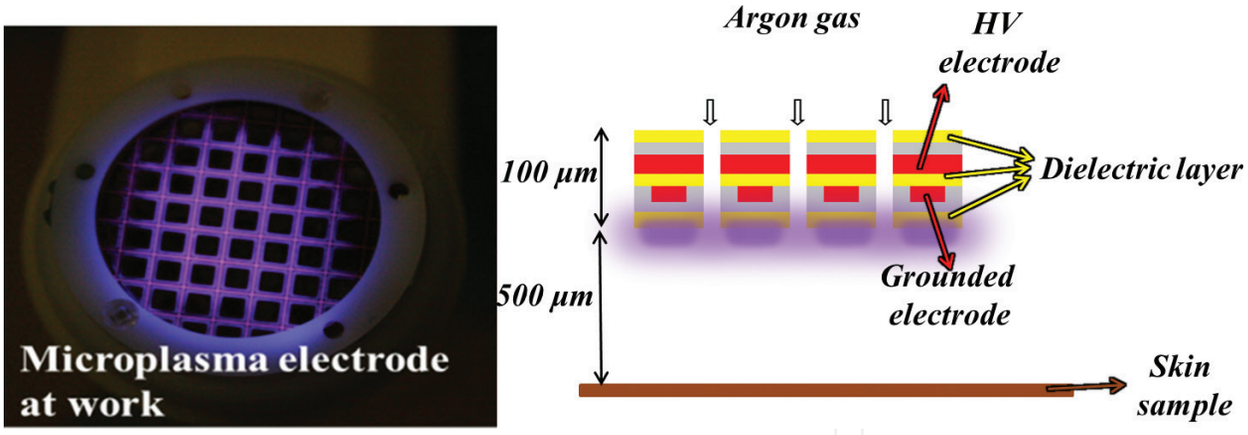

Figure 1. Microplasma discharge at work (left). Schematic of electrode and skin treatment (right) (reproduced from Ref. [13], with the permission of the American Vacuum Society).

3. Stratum corneum structure

The stratum corneum is an upper layer of the skin. This layer is the main barrier of the skin which protects the body against loss of water and ensures that molecules from outside will not enter the body. The stratum corneum is composed of corneocyte cells placed in a lipid-rich matrix. Human stratum corneum (thickness of 10–20 μm) contains from 10 to 25 layers of corneocytes. Corneocytes are composed of fibrillary keratin and water inside a cornified envelope. The cornified envelope is composed of crosslinked fillagrin, loricrin and involucrin [25]. Lipids in the lipid matrix are organized in lamellar layers following a tri-layer broad-narrow-broad arrangement [26]. The width of the tri-layer structures can be 6 or 13 nm. These lamellae can be packed in dense orthorhombic, less dense hexagonal or disordered liquid phase [25]. The lipid matrix consists of ceramides (41%), cholesterol (27%), cholesterol esters (10%), fatty acids (9%) with a small fraction of cholesterol sulfate (2%) [27] and glucosylceramides. Lipid chains are usually in a solid crystalline or gel state. At higher temperatures, lipids change their state to a liquid crystalline and they are more permeable (**Figure 2**).

After disruption of the stratum corneum, cholesterol synthesis leads to repairing of the barrier and it starts 90 min after barrier disruption [28]. The renewal of the stratum corneum occurs every 14 days [29]. Lipid-rich matrix is used for the transdermal delivery (TDD)—intercellular pathway, and it is composed of hydrophilic domain—head of ceramides and lipophilic domain—tail of cermaides. The stratum corneum can be divided into three main layers with different compositions and barrier functions [30]. The layers can be characterized by the concentration of K, Na, ceramides and fillagrin (**Figure 3**).

The concentration of Na is high in the whole stratum corneum and the concentration of K is high in the upper layer but low in the rest of the stratum corneum. The concentration of ceramides decreases from the upper layer to the lower layer. The concentration of arginine, which is the product of fillagrin, is the highest in the middle layer and lowest in the rest of the stratum corneum. The upper layer of the stratum corneum with dimension of 2/5 of the whole thickness (~ 8 μm) appears as a layer which allows passive flow of ions inside and

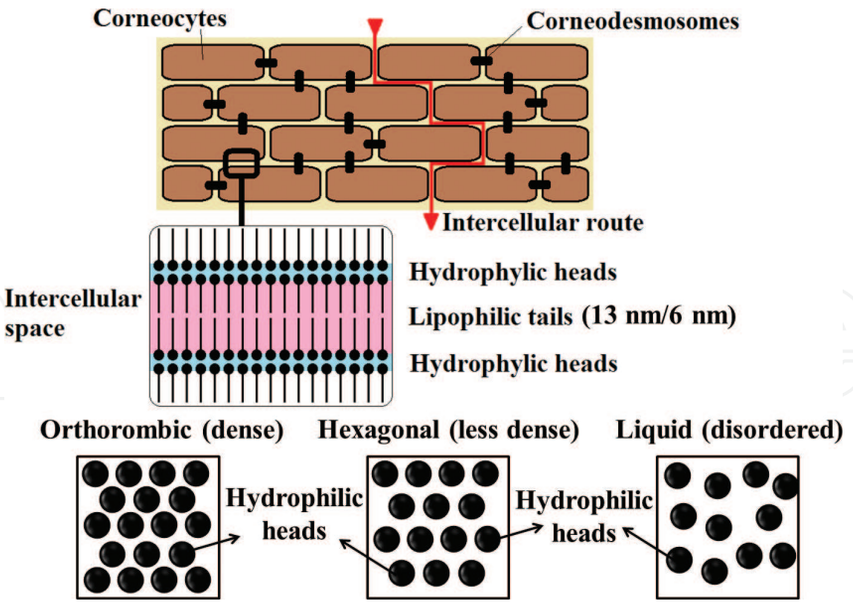

Figure 2. Model of the stratum corneum and with lateral and lamellar organization of lipids in the lipid matrix [26].

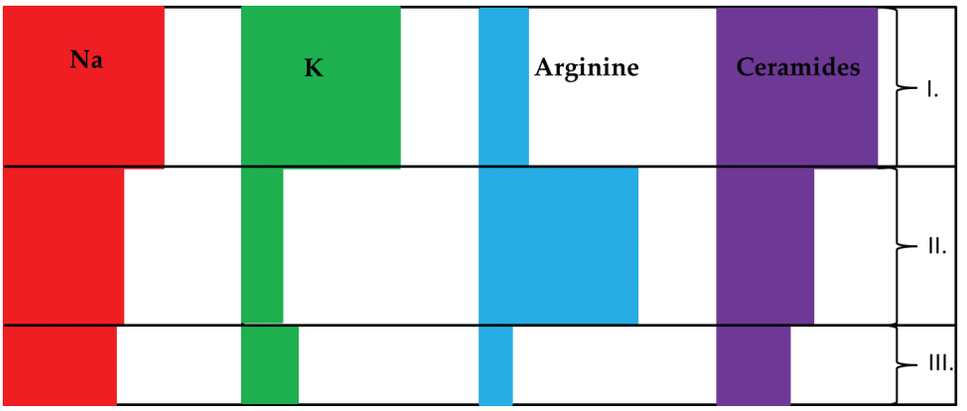

Figure 3. Three layers of the stratum corneum characterized by normalized amount of Na, K, arginine and ceramides [30]. The thickness of red, green blue and violet rectangular indicates relative concentration. I. Denotes the upper layer, II. Denotes the middle layer, and III. Denotes the lower layer.

outside. The amount of Na and K can be changed by the influence of the outer environment. Liquids and ions can easily penetrate the corneocytes in this layer and intracellular Na can be flushed out when corneocytes absorb water or other liquids. The middle layer of the stratum corneum appears as a first real barrier. The thickness is approximately 2/5 of the whole stratum cornea as in the previous case. Ions such as K^+ and Cr^{6+} cannot penetrate into this layer. Hydration of the skin is a function of the second layer because of the high concentration of arginine. It was observed that Cr^{3+} was able to penetrate into the middle layer of the stratum corneum, but not into the third layer, which is the reason why the third layer can be called a second barrier. The third layer has a thickness of 1/5 of the stratum corneum (~ 4 μm) and the barrier function of the stratum corneum increases toward the viable epidermis.

http://dx.doi.org/10.5772/intechopen.73498

3.1. Corneocytes

Corneocytes contain a lipid envelope bounded to the exterior protein envelope. Corneocytes of the lipid envelope function as a semipermeable membrane that allows water molecules to penetrate but not the larger hygroscopic molecules [31]. Intercellular lipid lamellae and lipids of the lipid envelope of corneocytes are connected through an ester bond (R—CO—O—R1) [32].

3.2. Role of ceramides

The percentages of some ceramides in the lipid matrix are 29.9% of ceramide 6, 21.7% of ceramide 2, 14.8% of ceramide 4, 13.9% of ceramide 5, 11.9% of ceramide 3 and 7.8% of ceramide 1 [33] (**Figure 4**).

The permeability of this membrane can be increased by decreasing the concentration of cholesterol and increasing the amount of short-tailed fatty acids and unsaturated fatty acids. A decrease of ceramides also decreases the barrier function of the skin. Ceramides form a multilayer lamellar structure with other lipids. Ceramides also act as a water modulator and their decrease also decreases the water-holding capacity [34]. The majority of ceramides of the stratum corneum has an even number of carbons and the most abundant has 44 and 46 carbons. Ceramides with number of carbons higher than 60 were also observed in a non-negligible concentration [35]. The permeability of the synthetic lipid membrane was not changed with the length of the ceramides. The changes in head groups of ceramides also did not change the permeability [36]. Other research showed that the chain length of ceramides has a huge influence on the barrier permeability. It was also observed that a synthetic barrier composed of a limited amount of ceramides (3 in this case) has some effect on barrier properties. A pig skin containing lamellae with a wider distribution of ceramides has higher permeability than synthetic lamellae with three ceramides. This can be explained by mismatches between various lengths in the lipid lamellae. The wider distribution of ceramide chains affects lamellar

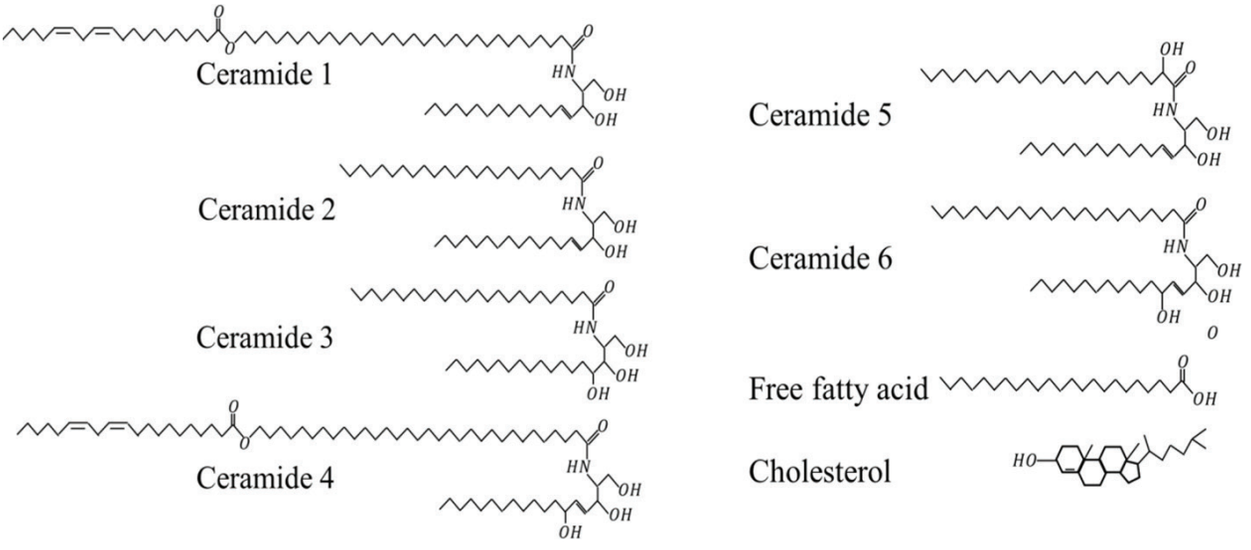

Figure 4. Structure of some ceramides fatty acid and cholesterol in the lipid matrix [33].

and also lateral organization. The wider distribution leads to a hexagonal organization, and synthetic ceramides with a low ceramide chain distribution leads to an orthorhombic organization [37]. A long-periodic phase is formed in the presence of a certain level of unsaturated ceramides, and a long-periodic phase is associated with fluid domain inside. A saturated ceramide EOS-S is important for the stabilization of the orthorhombic phase [38].

3.3. Role of fatty acids

The reduction of free fatty acid (FFA) chains was observed in skin diseases which are known by impaired skin barrier function. The reduction of the fatty acid chain can be caused by an increase in the amount of shorter FFA chains such as with 16 or 18 carbons. A shorter chain allows an increase in vibrations followed by conformational disordering. An increase in the number of unsaturated FFA reduces the packing density of the lipid organization [36]. An analysis of the composition of free fatty acids of human stratum corneum showed a dominant presence of saturated free fatty acids with carbon chain lengths from 16 to 30. The most abundant were FFAs with 24 and 26 carbons (50% of all FFAs). The content of unsaturated FFAs appeared to be around 2% of all FFAs; mostly with 18 carbons and with traces of FFAs with 16, 17 and 20 carbons; 1% of di-unsaturated FFAs (chain length of 18 carbons) [35].

3.4. Role of cholesterol

Cholesterol is situated near the ester bond of ceramides and this position allows the formation of a hydrogen bond between the cholesterol ─OH group and the carbonyl group C═O. The presence of cholesterol has an influence on the formation of the long periodicity phase of ceramides (13 nm). There is a minimal amount of required cholesterol for the formation of the long periodicity phase of ceramides (without cholesterol, long periodicity phase is not formed). Cholesterol does not influence only the presence of long periodicity phase but also the packing density in the long periodicity phase [39]. Cholesterol is very important for correct skin barrier function [40].

4. Plasma treatment

4.1. Sputtering/etching

Sputtering is very often the result of an interaction of the target surface or molecule with ion fluency. When the energy of an ion exceeds a certain value during collision, an atom absorbing this energy can leave its position in the molecule and a vacancy is created (free bond). If the atom that has left still has enough energy, other atoms can be released by following collisions and other vacancies can be created. Some of the atoms can be released from the target material which leads to a process called "physical sputtering."

4.1.1. Physical sputtering

The key parameters of physical sputtering are the binding energy in the target molecule and also the energy and mass of the impinging ion. The dependency on temperature is weak. The

threshold energy is relatively high (in range 10–100 eV) depending on the target/impinging ion combination. The sputtered yield changes with the angle of incident, the substrate material or the roughness of surface. Eroded species consist of atoms or small clusters of target material. Investigation of metallic targets with impinging metallic ions showed that the mechanism of sputtering depends on the ratio of impinging ion mass (M1) and the mass of the atom in the sample (M2) [41]. In the case of M1 < M2, the threshold energy does not depend on the mass M1 or M2. As the mass M2 increases, the probability of reflection of ion increases. The mechanism of sputtering follows the next steps:

1. Ion penetrates through the surface layer.
2. Ion is implanted in sublayer and deform structure of material.
3. Atom of the first layer is sputtered by impinging ion.

If M2 ≥ M1, the threshold energy for sputtering is higher than in the first case. The threshold energy also does not depend on the mass of M1 and M2, and sputtering follows the mechanism:

1. Ion penetrates through the surface layer and creates vacancy, releasing atom.
2. Impinging ion and also released atom deform structure of material.
3. Atom of the first layer is sputtered by released atom.

The threshold is higher in the second case because atoms of the target are sputtered by secondary atoms which were released by ion bombardment. Pure physical sputtering can be achieved mostly in discharges with inert gases. Bombardment of lipids inside the stratum corneum will follow mostly the second mechanism when argon is used and the first one in the case of helium. Physical sputtering is also present in nitrogen, oxygen or air plasma, but chemical sputtering can be much more dominant.

4.1.1.1. Argon bombardment/argon plasma

Argon is one of the simplest media for treatment of biological material. Excited states of Ar (Ar*), metastable states (Ar_m) and Ar ions (Ar^+, Ar_2^+) [42] can be produced in any electrical discharges. Ionization energy of Ar^+ and Ar_2^+ is 15.8 and 15.5 eV, respectively. Argon metastable states have a relatively long lifetime equal to 38 s [43] in vacuum. However, this time is reduced in atmospheric pressure. The energy difference between the ground state and the metastable state of Ar is 11.55 eV, which can be released by collision. A molecular dynamics simulation demonstrated the sputtering of a lipid-like material by argon ions (**Figure 5A** and **B**). The sputtering threshold energy was between 10 and 20 eV. The yield of sputtered particles increases to four carbon atoms per ion impacting the surface at an energy of 50 eV and increases more slowly up to 10 at 100 eV [44]. If we suppose a similar behavior of lipids and polymers during bombardment, argon ion bombardment of the polymers can lead to decreasing of side chains of the polymer, volatile CH_4, CO, CO_2, $HCOOCH_3$ and H_2, and disordered polymer structure [45]. The sputtering of polymer surface by Ar ions leads to graphite-like

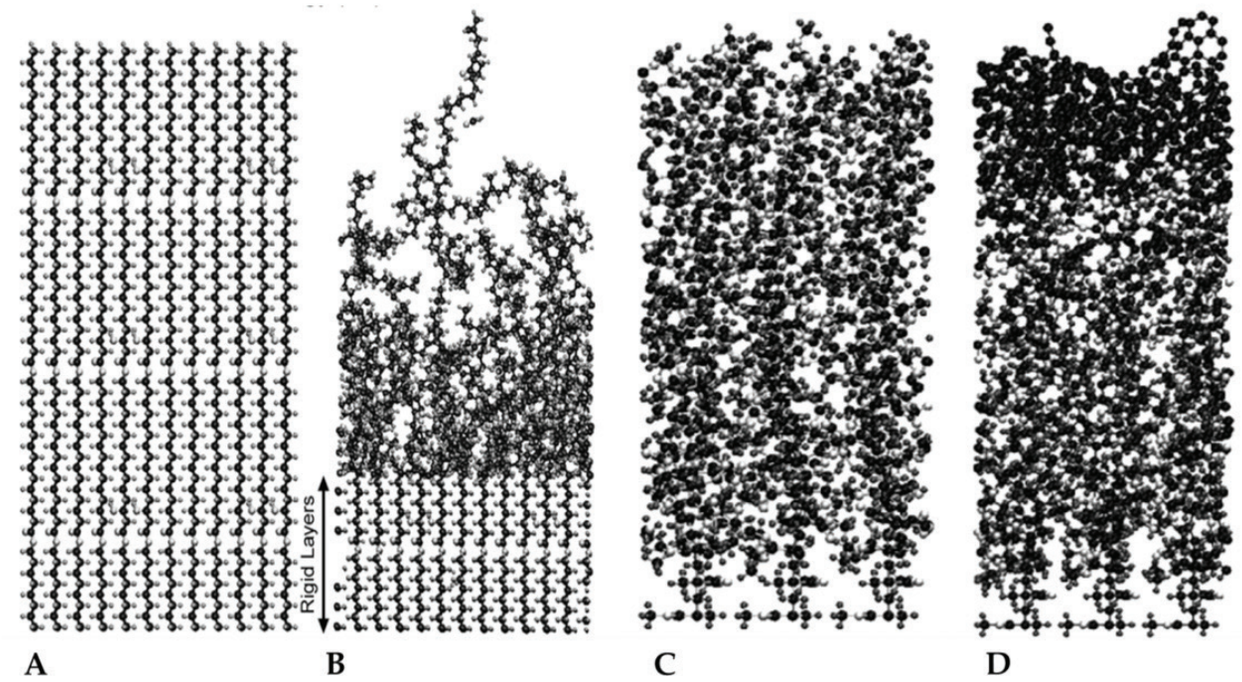

Figure 5. Molecular dynamic simulation. A. Lipid-like surface (black—C, gray—H, white—O). B. Lipid-like surface after bombardment by argon ions. [44] C. Virgin polymethylmethacrylate (composed of C—black, H—gray and O—White) D. Polymethylmethacrylate after bombardment by argon ions (damaged, carbon rich layer is formed on surface) [48].

structure, decreasing the rate of sputtering [46]. If the surface consists of too much of O and H, a thin C-rich layer is not created because released O and H atoms can cooperate in surface sputtering and react with free carbon bonds [44]. Comparison of the lipid-like surface and polymethylmethacrylate in **Figure 5A** and **B** shows forming of carbon-like surface in the case of polymer, but this surface is not formed in the case of the lipid-like surface. Etching of skin surface was confirmed by measuring the thickness of the stratum corneum in the skin cross section (**Figure 6**) [11]. The first layer of the stratum corneum was etched after 5 min of irradiation. If argon plasma treatment is realized in atmospheric air, air particles can participate in treatment with processes other than physical sputtering. Chemical sputtering or surface functionalizing can start to be effective. In this case, plasma treatment causes wettability and hydrophilicity of surfaces by increasing the number of functional groups such as oxygen or nitrogen. Argon plasma treatment is able to increase oxygen functional groups on polymer surface if argon plasma discharge is working on atmospheric air [47]. Molecular dynamics (MD) simulation of bombardment of polymers showed that the main products of etching are CO or H_2, polymer units and C_xH_y fragments during transient sputtering. After the formation of a damaged layer, the products of sputtering are H, H_2, CO, and C_n. Oxygen leaves polymers more difficult than hydrogen because oxygen is larger and less mobile, and that is the reason why oxygen is concentrated under a damaged layer with strong C—O—C bonds and the surface of polymer is created in the layer of amorphous carbon. This process is not valid for all polymers. If polymers compose of many H or O atoms, amorphous layer of carbon is not formed because released H and O atom can etch carbon atoms [48].

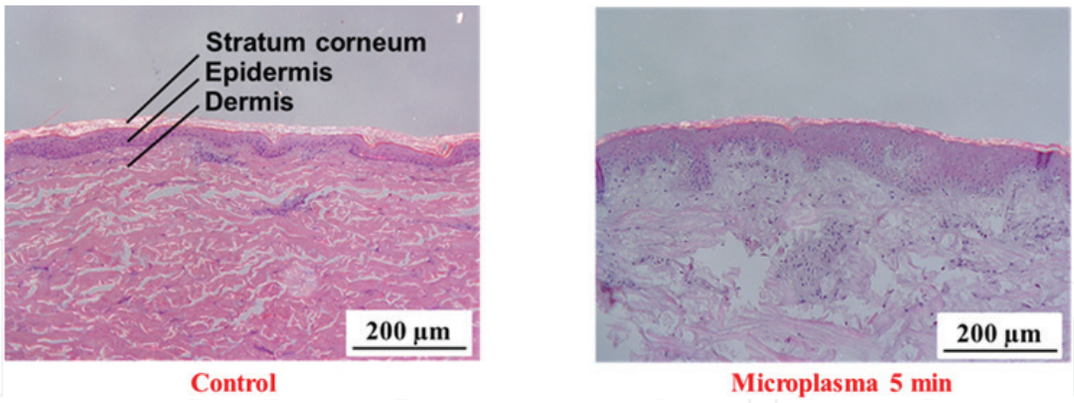

Figure 6. Cross section of pig skin before atmospheric plasma irradiation, the stratum corneum thickness: 18.09 ± 1.64 µm (left—control), and after atmospheric plasma irradiation (right), the stratum corneum thickness: 13.40 ± 1.46 [11, 49].

4.1.1.2. Helium bombardment/helium plasma

Helium is an inert gas with light atomic mass, which means that the sputtering of any surface is lower in comparison with argon. All atoms in the lipid matrix of the stratum corneum are heavier than helium, so atoms are sputtered mostly by the first mechanism described in Section 4.1.1. Investigation of sputtering of polymers by high energy (1 keV) He and Ar ions demonstrated that light atoms such as hydrogen are sputtered at first, followed by heavier carbon and oxygen [50]. On the other hand, the penetration depth increases with decreasing atomic mass, which means that an atomic mass of helium allows penetration deeper inside the surface than argon [51]. However, helium plasma treatment of crystalline carbon with low energy ions (ion temperature ~0.1 eV) demonstrated mass loss and disorder in carbon structure [52]. Helium plasma-treated polycarbonate containing C, O, H atoms did not change the number of C and O atoms, but a change of structure by breaking carbonate groups was observed [53]. Helium is effective in increasing oxygen and also nitrogen functional groups coming from surrounding atmospheric air [47].

4.1.2. Chemical sputtering

Unlike physical sputtering, chemical sputtering (sometimes known as reactive etching) has no or a very low threshold. Chemical sputtering varies strongly with surface temperature and it is highly selective depending on the target/impinging particle combination. The impinging particle also involves neutral reactive species which help to increase the number of sputtered atoms. Eroded species consist of molecules involving impinging atoms and target. The number of sputtered atoms increases by decreasing the energy of bonds and also by decreasing the molecular weights of the atoms in the molecule. The rate of reactions on the surface depends on its structure. If the surface is damaged by sputtering, the reaction rate can increase by orders of magnitude. Atoms react with free bonds after ion bombardment. Chemical sputtering is much more effective, sometimes 1 or 2 orders.

4.1.2.1. Reactive etching/plasma treatment by gases containing oxygen

The dominant mechanism of sputtering by oxygen plasma discharge is the bombardment of the sample by ions creating vacancies. Oxygen molecules, ozone or radicals react with these defects and form H_2O, CO or CO_2. When an unsaturated bond is created in argon plasma, this bond cannot react with any chemically active species to form a volatile by-product. The only way to form those carbon-carbons unsaturated bonds is to react with others carbons and form a highly crosslinked material. Physical sputtering of hydrocarbon film by argon ions can be increased by a beam of molecular oxygen, which leads to chemical sputtering. Molecular oxygen alone cannot sputter the surface but can be adsorbed. Bombardment induces reactions between adsorbed molecular oxygen and bonds created by Ar^+ bombardment on hydrocarbon film. The sputtered amount of atoms and molecules is proportional to the flux of molecular oxygen and the ratio of O_2/Ar^+. An increase of molecular flux increases the number of adsorbed molecules and the number of chemical reactions on the surface [54]. A similar effect can be achieved in Ar/O_2 plasma discharge where except molecular oxygen, other active species such as O_3, radicals O and metastable states $O_2(a)$ and Ar_m are also present. Unsaturated bonds created by Ar/O_2 plasma treatment lead to crosslinking and oxidation [55]. Pure oxygen post-discharge treatment of hexatriacontane resulted in a mass decrease, which corresponds to etching, but no chemical modifications were observed [56]. In this case, only long living particles can interact with hexatriacontane. Wertheimer et al. [57] showed that oxygen radicals $O(^3P)$ and metastable $O(^1D)$ alone are not very effective in interacting with a polymer surface with saturated aliphatic carbons. When hexatriacontane was treated in N_2-O_2 discharge, grafting with etching compete to each other. The mass of hexatriacontane and the amount of oxygen increases followed by etching, and decreases the amount of oxygen [56]. Joubert et al. showed that molecular oxygen does not participate in plasma surface reaction when they compared discharges of N_2O plasma and N_2-O_2 plasma with the same concentration of atomic oxygen. They concluded that O_2 only provides oxygen radicals [58].

4.1.2.2. Reactive etching/plasma treatment by gases containing nitrogen

Nitrogen is a very stable molecule and its concentration of atomic radicals is low in comparison with oxygen. On the other hand, nitrogen creates a lot of metastable states. It was observed that even so nitrogen states coming from nitrogen discharge can cause chemical sputtering [59]. Bombardment of carbon film by N_2^+ ions with an energy under threshold of physical sputtering demonstrated the formation of CN radicals. If these radicals are formed on the surface, it creates HCN and OHCN after reaction with hydrogen and water. If the radical is formed in sublayers, C_2N_2 molecule is created [60]. In the case of nitrogen plasma of hydrocarbon film, we can also observe erosion of the surface and formation CN radicals. However, an admixture of methane can cause deposition of C_xH_y groups. Erosion will compete with deposition and CN, CNH and C_2N_2 groups can be incorporated into the film [61, 62].

4.1.2.3. Reactive etching/plasma treatment by gases containing hydrogen

Hydrogen is the lightest atom, so similar to helium, the sputtering effect of hydrogen ions H^+, H_2^+ and H_3^+ is much lower than by heavy ions. Hydrogen atomic radicals can be formed in discharge which leads to more effective reactive etching. Etching by hydrogen plasma causes incorporation of H and reduction of dangling bonds, unlike oxygen plasma which increases the number of dangling bonds [63]. It was observed that sputtering of the surface with a combination of hydrogen atoms and argon ion enhances sputtering more than their sum. A hydrogen-rich surface is easier to etch than a surface with a deficiency of hydrogen or a more crosslinked carbon network, as the hydrogen atoms can react with free carbon bonds after bombardment by Ar^+ ions. The ratio of the flux of hydrogen atoms and Ar^+ ions is crucial for effective etching. It is the most effective at a ratio 400:1. It means that the atomic flux should be several times higher than the ion flux. If the flux ratio is too low, most of the ion-induced defects recombine before they can be passivated by incident H [64, 65]. Another way to increase sputtering is using an N_2-H_2 mixture. Ions N^+, NH_2^+, NH_3^+, NH_4^+, N_2H^+ and H_3^+ increase the etching rate to a higher value than N_2 or H_2 plasma alone [66].

4.2. Lipid peroxidation

Lipids in the lipid membrane of the stratum corneum do not have to be etched by ions but radicals created in plasma or secondary created in the stratum corneum can degrade lipids by the process called lipid peroxidation. Lipid peroxidation is an oxidative degradation of lipids. The reaction occurs on unsaturated fatty acids. As molecular oxygen is not reactive enough to start oxidation, it must be changed to a more reactive state such as hydroxyl radical (OH), superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroperoxyl radical (HO_2•), lipid peroxyl radical (LOO•), alkoxyl radical (LO•), metastable singlet oxygen (O_2(a)), or iron-oxygen complexes (ferryl-, perferryl radical) [67]. Reactive oxygen species abstract hydrogen atoms from the methylene group and create a lipid radical. The rate of lipid peroxidation exponentially increases with the number of bis-allylic carbons, because this bond is the weakest in the molecule with no relation to chain length. After abstraction of hydrogen, the lipid radical can react with molecular atmospheric oxygen and form a lipid peroxyl radical. At a low concentration of atmospheric oxygen, lipids can react with each other in the lipid membrane [67]. The lipid peroxyl radical can abstract hydrogen from another lipid to form lipid radical (L•) which can react with atmospheric oxygen and again to form lipid peroxyl radical (LOO•). Oxidized lipids form more rigid domains. LOOH is more polar than fatty acids; it can disrupt the structure of the lipid membrane.

Superoxide anion radical (O_2-•) reactivity is low in aqueous environments, but it increases in hydrophobic environment.

Hydroperoxyl radical (HO_2•) is much more reactive than superoxide anion radicals. Deprotonation can lead to formation of O_2-• at a pH of 4.8. Weak reactivity of O_2-• allows penetrating deeper in the lipid bilayer than HO_2•. The poor reactivity and relatively long

half-life of $O_2^{-\bullet}$ allows it to diffuse more effectively from its generation site to targets such as membrane lipid bilayers than $HO_2\bullet$ or other reactive species.

Hydrogen peroxide (H_2O_2) has limited reactivity and can diffuse to target longer time and has potential to create other reactive short-living species near the lipid membrane. Creation of lipid radical can be realized in the presence of iron as a catalyst.

Hydroxyl radical (OH•) reactivity is very high and its lifetime is very short; it can react with any molecule in tissue. It can be created from H_2O_2 by catalysis of iron or ultrasound in water [67]:

$$\mathrm{Fe(II)-complex} + \mathrm{H_2O_2} \rightarrow \mathrm{Fe(III)-complex} + \mathrm{OH^-} + \mathrm{OH} \quad (1)$$

5. Application of plasma discharges in transdermal drug delivery

DBD plasma discharge in direct contact with skin was used for transdermal delivery in the air [15]. The time of plasma application was set up to 2 min in pulsed mode 1–10 μs and frequency ranged from 50 Hz to 3.5 kHz. The delivery of large molecules such as dextran molecules with a molecular weight 10 kDa can penetrate to a depth of 600 μm within 1 h and larger molecules such as albumin (66 kDa), IgG human immunoglobulin (115 kDa) and SiO_2 nanoparticles with a diameter of 50 nm can penetrate to a depth of 200 μm within 1 h. Even liposomes with a diameter of 100 nm were delivered to a depth of 100 μm after 1 h. Remote microplasma or plasma jet in argon gas were investigated for transdermal delivery of galantamine hydrobromide (368 Da) [14] and Cyclosporine A (1200 Da) [13]. After 3 min of microplasma treatment, the permeability of galantamine hydrobromide increased by a factor 2 after 24 h in comparison with non-treated skin. The permeability of Cyclosporine A was not able to penetrate through the stratum corneum without plasma treatment. Argon plasma and microplasma showed

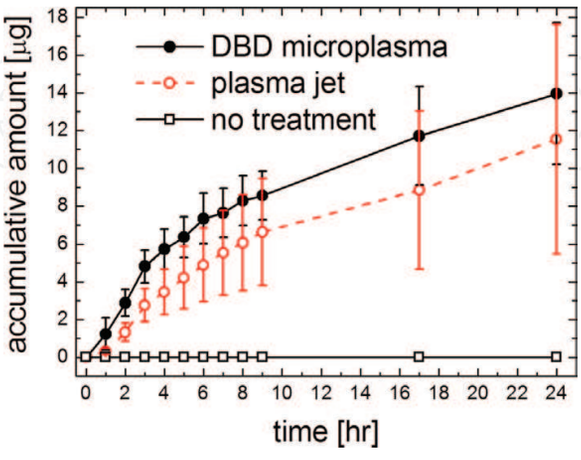

Figure 7. Accumulative amount of penetrated Cyclosporine A through the epidermal layer of the pig skin (four samples were used for microplasma dielectric barrier discharge treatment and five samples for plasma jet treatment) [13] (reproduced from Ref. [13], with the permission of the American Vacuum Society).

http://dx.doi.org/10.5772/intechopen.73498

similar results. About 9 and 8 μg/cm^2 of Cyclosporine A penetrated through the epidermal layer using microplasma or plasma jet, respectively (**Figure 7**). The maximum delivery rate was reached after 3 h of delivery and decreased to its minimal value after 9 h (**Figure 8**)

Although, it was shown that microplasma can enhance the permeability of Cyclosporine A, it is only half of the problem which must be solved before clinical use. The second important question is, whether this penetrated amount is sufficient. This problem can be solved with pharmacokinetics, and it was shown that the treated area of the skin must be equal to 225 cm^2 and the concentration of Cyclosporine A should be 400 mg/ml in propylene glycol. If this condition is fulfilled, a therapeutic concentration in blood can be achieved (**Figure 9**). However, the therapeutic concentration is achieved only for up to 10 h and then another treatment of the skin is needed.

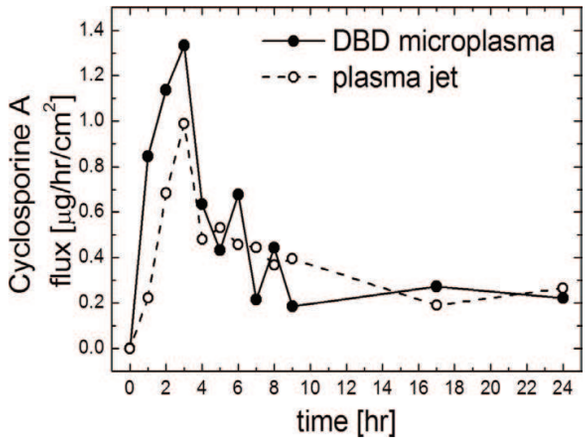

Figure 8. Evolution of the rate of drug delivery through the epidermal layer of the pig skin (four samples were used for microplasma dielectric barrier discharge treatment and five samples for plasma jet treatment) [13] (reproduced from Ref. [13], with the permission of the American Vacuum Society).

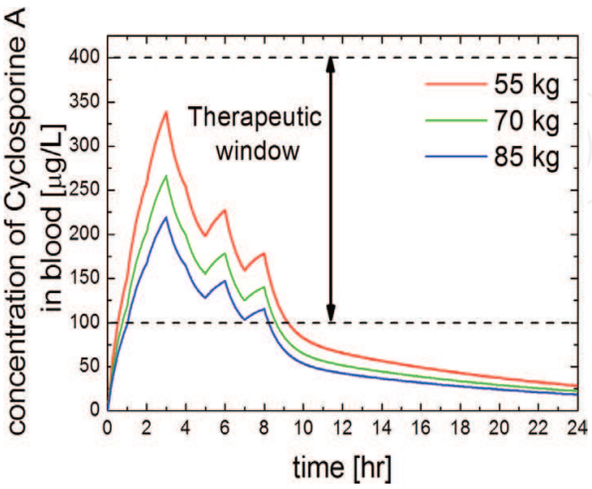

Figure 9. Concentration of Cyclosporine A in blood of adults 55, 70 and 85 kg in weight (400 mg/ml of Cycloporine A in propylene glycol). The two peaks at 6 and 8 h come from experimental data of drug flux containing some errors [13] (reproduced from Ref. [13], with the permission of the American Vacuum Society).

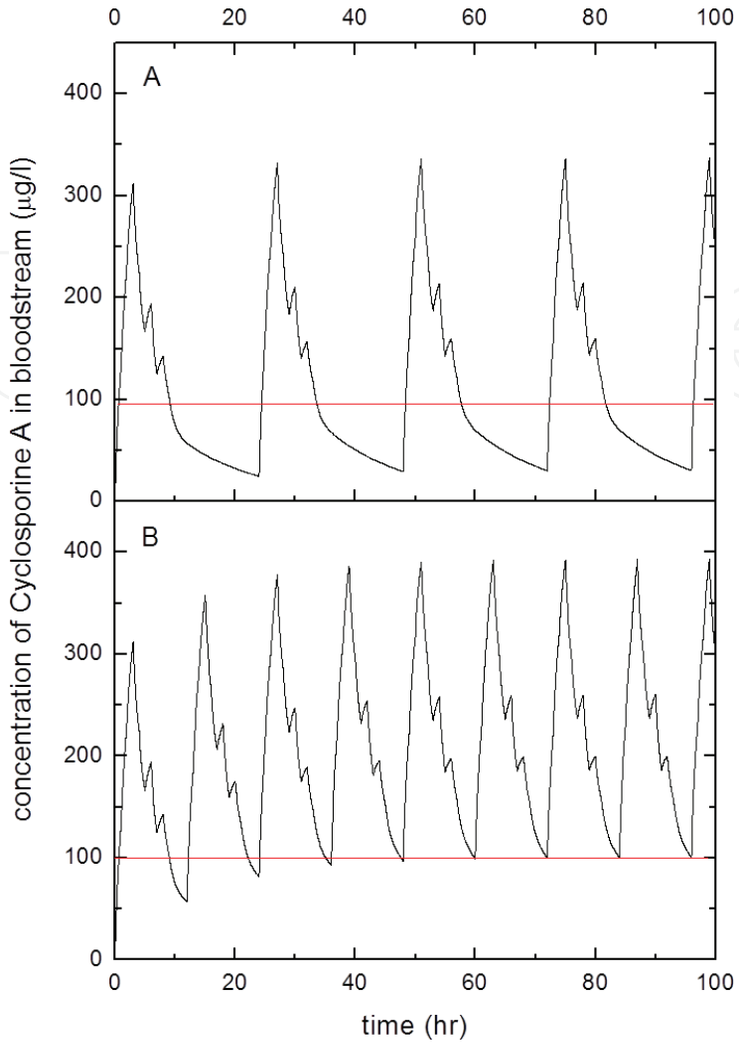

Figure 10. (A) Transdermal drug delivery of Cyclosporine A every 24 h. (B) Transdermal drug delivery of Cyclosporine A every 12 h. The weight of the patient was 55 kg in the model. The red line indicates the lowest therapeutic concentration [68].

Repeated treatment by microplasma and application of Cyclosporine A every 12 h/24 h, to a human of 55 kg is shown in **Figure 10**. As it is seen, treatment every 24 h is not sufficient, and it must be realized at least every 12 h [68]

6. Conclusion

The stratum corneum is the main barrier of the skin. The strength of this barrier is not homogeneous through the whole thickness, but it is stronger in the direction of the living tissues. The lipid matrix is mainly used for drug delivery through the skin. This matrix is composed of lipid molecules which are crucial for barrier properties. If these molecules are peroxided or shortened by breaking bonds, the permeability of the skin can be changed. Lipid molecules are hydrocarbons composed mainly of carbon, hydrogen, oxygen and nitrogen. If plasma is applied on the skin, we suppose that this surface treatment can be compared to

treatment of the other materials, such as polymers. The thickness of the stratum corneum will be decreased. How effective the interaction of plasma can be, depends on the use of a gas or gas mixture. The presence of ions can be very effective to start the interaction with the skin. Usually, oxygen containing plasma can strongly etch the surface with the help of reactivity of active species with free bonds created by ions. If the result is a fluidic or heavy crosslinked structure, the structure depends on the amount of species which is able to react with the free bonds. On the other hand, helium plasma is not so strong of an etching medium and the result of the interaction can be less destructive. However, plasma treatment can also bring some reactive species close to the lipid membrane which can react with lipids without the help of ions and cause peroxidation of lipids and decrease their barrier function. It was shown that plasma sources can influence the skin and help the penetration of various kinds of molecules. Microplasma treatment of the skin and delivery of the Cyclosporine A showed that delivery of a therapeutic amount of drug is possible.

Acknowledgements

We would like to thank to Professor Damon M. Chandler, Mr. Hideto Miyamoto and Dr. Marius G. Blajan of Shizuoka University, for fruitful discussion. This work was supported by JSPS KAKENHI Grant Number JP16H04085.

Author details

Kazuo Shimizu* and Jaroslav Krištof

*Address all correspondence to: shimizu@cjr.shizuoka.ac.jp

Shizuoka University, Hamamatsu, Japan

References

[1] Donnelly VM, Kornblit A. Plasma etching: Yesterday, today, and tomorrow. Journal of Vacuum Science and Technology A. 2013;**31**(5):050825. DOI: 10.1116/1.4819316

[2] Proslier T, Klug JA, Becker NC, Elam JW, Pellin MJ. Atomic layer deposition of superconductors. ECS Transactions. 2011;**41**(2):237. DOI: 10.1149/1.3633673

[3] Hegemann D, Brunner H, Oehr Ch. Plasma treatment of polymers for surface and adhesion improvement. Nuclear Instruments and Methods in Physics Research Section B. 2003;**208**:281. DOI: 10.1016/S0168-583X(03)00644-X

[4] Shintani H, Sakudo A, Burke P, McDonnell G. Gas plasma sterilization of microorganisms and mechanisms of action (review). Experimental and Therapeutic Medicine. 2010; **1**:731. DOI: 10.3892/etm.2010.136

[5] Bekeschus S, Schmidt A, Weltmann K-D, von Woedtke T. The plasma jet kINPen—A powerful tool for wound healing. Clinical Plasma Medicine. 2016;**4**(1):19. DOI: 10.1016/j.cpme.2016.01.001

[6] Yan D, Sherman JH, Keidar M. Cold atmospheric plasma, a novel promising anti-cancer treatment modality. Oncotarget. 2017;**8**(9):15977. DOI: 10.18632/oncotarget.13304

[7] Park H, Kim E, Kim J, Ro Y, Ko J. High-intensity focused ultrasound for the treatment of wrinkles and skin laxity in seven different facial areas. Annals of Dermatology. 2015; **27**(6):688. DOI: 10.5021/ad.2015.27.6.688

[8] Vijayarangan V, Delalande A, Dozias S, Pouvesle J-M, Pichon C, Robert E. Cold atmospheric plasma parameters investigation for efficient drug delivery in HeLa cells. IEEE Transactions on Radiation and Plasma Medical Sciences. 2017;**PP**(99):1. DOI: 10.1109/TRPMS.2017.2759322

[9] Langer R. Transdermal drug delivery: Past progress, current status, and future prospects. Advanced Drug Delivery Reviews. 2004;**56**:557. DOI: 10.1016/j.addr.2003.10.021

[10] Prausnitz MR, Langer R. Transdermal drug delivery. Nature Biotechnology. 2008;**26**(11): 1261. DOI: 10.1038/nbt.1504

[11] Shimizu K, Hayashida K, Blajan M. Novel method to improve transdermal drug delivery by atmospheric microplasma irradiation. Biointerphases. 2015;**10**(2):029517. DOI: 10.1116/1.4919708

[12] Shimizu K, Tran AN, Blajan M. Effect of microplasma irradiation on skin barrier function. Japanese Journal of Applied Physics. 2016;**55**(7S2):07LG01. DOI: 10.7567/JJAP.55.07LG01

[13] Kristof J, Miyamoto H, Tran AN, Blajan M, Shimizu K. Feasibility of transdermal delivery of cyclosporine A using plasma discharges. Biointerphases. 2017;**12**(2):02B40. DOI: 10.1116/1.4982826

[14] Shimizu K, Tran AN, Kristof J, Blajan M. Investigation of atmospheric microplasma for improving skin permeability. In: Proceedings of the 2016 Electrostatics joint conference; 13-18 June; Lafayette, USA; 2016. p. I4

[15] Kalghatgi S, Tsai C, Gray R, Pappas D. Transdermal drug delivery using cold plasmas. In: 22nd Int'l Symposium on Plasma Chemistry; 5-10 July; Antwerp, Belgium. 2015. p. 0-22-6

[16] Phelps AV, Molnar JP. Lifetimes of metastable states of Noble gases. Physics Review. 1953;**89**:1202. DOI: 10.1103/PhysRev.89.1202

[17] Das MB, Karmakar S. Lifetime measurement of excited atomic and ionicstates of some noble gases using the high-frequency deflection technique. Pramana—Journal de Physique. 2005;**65**(6):1061. DOI: 10.1007/BF02705281

[18] Klopovskii KS, Kovalev AS, Lopaev DV, Popov NA, Rakhimov AT, Rakhimova TV. New mechanism of singlet-oxygen production in processes with participation of electronically and vibrationally excited ozone molecules. JETP. 1995;**80**(4):603

[19] Bibinov N, Rajasekaran P, Mertmann P, Wandke D, Viöl W, Awakowicz P. Basics and biomedical applications of dielectric barrier discharge (DBD). In: Laskovski AN, editor. Biomedical Engineering, Trends in Materials Science. Rijeka, Croatia: InTech; 2011. p. 123. DOI: 10.5772/13192

[20] Emmert S, Brehmer F, Hanßle H, Helmke A, Mertens N, Raees Ahmed R, Simon D, Wandke D, Maus-Friedrichs W, Daschlein G, Schon M-P, Viol W. Atmospheric pressure plasma in dermatology: Ulcus treatment and much more. Clinical Plasma Medicine. 2013;**1**:24. DOI: 10.1016/j.cpme.2012.11.002

[21] Bora B, Jain J, Inestrosa-Izurieta MJ, Avaria G, Moreno J, Pavez C, Marcelain K, Armisen R, Soto L. Development of plasma needle to be used for biomedical applications. Journal of Physics: Conference Series. 2016;**720**:012038. DOI: 10.1088/1742-6596/720/1/012038

[22] Pekshev AV, Shekhter AB, Vagapov AB, Sharapov NA, Vanin AF. Study of plasma-chemical NO-containing gasflow for treatment of wounds and inflammatory processes. Nitric Oxide. Forthcoming. DOI: 10.1016/j.niox.2017.06.002

[23] Ferreira CM, Gordiets B, Tatarova E, Henriques J, Dias FM. Air-water microwave plasma torch as a NO source for biomedical applications. Chemical Physics. 2012;**398**:248. DOI: 10.1016/j.chemphys.2011.05.024

[24] Boekema BKHL, Vlig M, Guijt D, Hijnen K, Hofmann S, Smits P, Sobota A, van Veldhuizen EM, Bruggeman P, Middelkoop E. A new flexible DBD device for treating infected wounds: In vitro and ex vivo evaluation and comparison with a RF argon plasma jet. Journal of Physics D: Applied Physics. 2016;**49**:044001. DOI: 10.1088/0022-3727/49/4/044001

[25] Van Smeden J, Janssens M, Gooris GS, Bouwstra JA. The important role of stratum corneum lipids for the cutaneous barrier function. Biochimica et Biophysica Acta. 2014;**1841**:295. DOI: 10.1016/j.bbalip.2013.11.006

[26] Hill JR, Wertz PW. Molecular models of the intercellular lipid lamellae from epidermal stratum corneum. Biochimica et Biophysica Acta. 2003;**1616**:121. DOI: 10.1016/S0005-2736(03)00238-4

[27] Suhonen TM, Bouwstra JA, Urtti A. Chemical enhancement of percutaneous absorption in relation to stratum corneum structural alterations. Journal of Controlled Release. 1999;**59**:149. DOI: 10.1016/S0168-3659(98)00187-4

[28] Dayan N. Stratum corneum: The role of lipids and ceramides. Cosmetics & Toiletries Magazine. 2006;**121**(1):37

[29] Hadgraft J, Lane ME. Skin: The ultimate interface. Physical Chemistry Chemical Physics. 2011;**13**:5215. DOI: 10.1039/c0cp02943b

[30] Kubo A, Ishizaki I, Kubo A, Kawasaki H, Nagao K, Ohashi Y, Amagai M. The stratum corneum comprises three layers with distinct metal-ion barrier properties. Scientific Reports. 2013;**3**:1731. DOI: 10.1038/srep01731

[31] Elias PM, Gruber R, Crumrin D, Menon G, Williams ML, Wakefield JS, Holleran WM, Uchida Y. Formation and functions of the corneocyte lipid envelope (CLE). Biochimica

et Biophysica Acta (BBA)—Molecular and Cell Biology of Lipids. 2014;**1841**(3):314. DOI: 10.1016/j.bbalip.2013.09.011

[32] Downing DT. Lipid and protein structures in the permeability barrier of mammalian epidermis. Journal of Lipid Research. 1992;**33**:301. PMID: 1569381

[33] Wertz PW. The nature of the epidermal barrier: Biochemical aspects. Advanced Drug Delivery Reviews. 1996;**18**:283. DOI: 10.1016/0169-409X (95)00077-K

[34] Imokawa G, Koichi I. Role of ceramide in the barrier function of the stratum corneum, implications for the pathogenesis of atopic dermatitis. Journal of Clinical & Experimental Dermatology Research. 2014;**5**(1):1000206. DOI: 10.4172/2155-9554.1000206

[35] Van Smeden J, Boiten WA, Hankemeier T, Rissmann R, Bouwstra JA, Vreeken RJ. Combined LC/MS-platform for analysis of all major stratum corneum lipids, and the profiling of skin substitutes. Biochimica et Biophysica Acta. 2014;**1841**:70. DOI: 10.1016/j.bbalip.2013.10.002

[36] Uchiyama M, Oguri M, Mojumdar EH, Gooris GS, Bouwstra JA. Free fatty acids chain length distribution affects the permeability of skin. Biochimica et Biophysica Acta. 2016;**1858**:2050. DOI: 10.1016/j.bbamem.2016.06.001

[37] Mojumdar EH, Kariman Z, van Kerckhove L, Gooris GS, Bouwstra JA. The role of ceramide chain length distribution on the barrier properties of the skin lipid membranes. Biochimica et Biophysica Acta. 2014;**1838**:2473. DOI: 10.1016/j.bbamem.2014.05.023

[38] De Sousa Neto D, Gooris G, Bouwstra J. Effect of the omega-acylceramides on the lipid organization of stratum corneum model membranes evaluated by X-ray diffraction and FTIR studies (part I). Chemistry and Physics of Lipids. 2011;**164**:184. DOI: 10.1016/j.chemphyslip.2010.12.007

[39] Mojumdar EH, Gooris GS, Groen D, Barlow DJ, Lawrence MJ, Demé B, Bouwstra JA. Stratum corneum lipid matrix: Location of acyl ceramide and cholesterol in the unit cell of the long periodicity phase. Biochimica et Biophysica Acta (BBA)—Biomembranes. 2016;**1858**(8):1926. DOI: 10.1016/j.bbamem.2016.05.006

[40] Vávrová K, Kováčik A, Opálka L. Ceramides in the skin barrier. European Pharmaceutical Journal. 2017;**64**(1):1. DOI: 10.1515/afpuc-2017-0004

[41] Yan C, Zhang QY. Rare event molecular dynamics simulations of plasma induced surface ablation. AIP Advances. 2012;**2**:032107. DOI: 10.1063/1.4738951

[42] Bashir M, Rees JM, Bashir S, Zimmerman WB. Characterization of atmospheric pressure microplasma produced from argon and a mixture of argon–ethylenediamine. Physics Letters A. 2014;**378**(32-33):2395. DOI: 10.1016/j.physleta.2014.05.049

[43] Zinner M, Spoden P, Kraemer T, Birkl G, Ertmer W. Precision measurement of the metastable 3P2 lifetime of neon. Physical Review A. 2003;**67**:010501. DOI: 10.1103/PhysRevA.67.010501

[44] Babaeva NY, Ning N, Graves DB, Kushner MJ. Ion activation energy delivered to wounds by atmospheric pressure dielectric-barrier discharges: Sputtering of lipid-like surfaces. Journal of Physics D: Applied Physics. 2012;**45**:115203. DOI: 10.1088/0022-3727/45/11/115203

[45] Pignataro B, Fragal ME, Puglisi O. AFM and XPS study of ion bombarded poly (methyl methacrylate). Nuclear Instruments and Methods in Physics Research Section B. 1997; **131**:141. DOI: 10.1016/S0168-583X (97)00297-8

[46] Bachman BJ, Vasile MJ. Ion bombardment of polyimide films. Journal of Vacuum Science and Technology A. 1989;**7**(4):2709. DOI: 10.1116/1.575779

[47] Van Deynse A, Morent R, De Geyter N. Surface modification of polymers using atmospheric pressure cold plasma technology. In: Méndez-Vilas A, Solano A, editors. Polymer Science: Research Advances, Practical Applications and Educational Aspects. Formatex Research Center; 2016. pp. 506-516

[48] Choudhary GK, Vegh JJ, Graves DB. Molecular dynamics simulations of oxygen-containing polymer sputtering and the Ohnishi parameter. Journal of Physics D: Applied Physics. 2009;**42**:242001. DOI: 10.1088/0022-3727/42/24/242001

[49] Shimizu K, Kristof J. Enhancement of percutaneous absorption on skin by plasma drug delivery method. In: Maiti S, Sen KK, editors. Advanced Technology for Delivering Therapeutics. InTech; 2017. p. 111. DOI: 10.5772/65116

[50] Rzeznik L, Fleming Y, Tom Wirtz T, Philipp P. Experimental and simulation-based investigation of He, Ne and Ar irradiation of polymers for ion microscopy. Beilstein Journal of Nanotechnology. 2016;**7**:1113. DOI: 10.3762/bjnano.7.104

[51] Livengood R, Tan S, Greenzweig Y, Notte J, McVey S. Subsurface damage from helium ions as a function of dose, beam energy, and dose. Journal of Vacuum Science and Technology B. 2009;**27**(6):3244. DOI: 10.1116/1.3237101

[52] Kim HS, Noh SJ, Kweon JJ, Lee CE. Influence of irradiation with low-energy helium ions on graphite and tungsten for fusion applications. Journal of the Korean Physical Society. 2013;**63**(7):1422. DOI: 10.3938/jkps.63.1422

[53] Bergeron A, Klemberg-Sapieha JE, Martinu L. Structure of the interfacial region between polycarbonate and plasma-deposited $SiN_{1.3}$ and SiO_2 optical coatings studied by ellipsometry. Journal of Vacuum Science and Technology A. 1998;**16**(6):3227. DOI: 10.1116/1.581527

[54] Hopf C, Schluter M, Jacob W. Chemical sputtering of carbon films by argon ions and molecular oxygen at cryogenic temperatures. Applied Physics Letters. 2007;**90**:224106. DOI: 10.1063/1.2745267

[55] Murillo R, Poncin-Epaillard F, Segui Y. Plasma etching of organic material: Combined effects of charged and neutral species. European Physical Journal Applied Physics. 2007; **37**:299. DOI: 10.1051/epjap:2007031

[56] Hody V, Belmonte T, Czerwiec T, Henrion G, Thiebaut JM. Oxygen grafting and etching of hexatriacontane in late N_2–O_2 post-discharges. Thin Solid Films. 2006;**506-507**:212. DOI: 10.1016/j.tsf.2005.08.016

[57] Wertheimer MR, Fozza AC, Hollander A. Industrial processing of polymers by low-pressure plasmas: The role of VUV radiation. Nuclear Instruments and Methods in Physics Research Section B. 1999;**151**:65. DOI: 10.1016/S0168-583X (99)00073-7

[58] Joubert O, Pelletier J, Arnal Y. The etching of polymers in oxygen-based plasmas: A parametric study. Journal of Applied Physics. 1989;**65**(12):5096. DOI: 10.1063/1.343186

[59] Vázquez L, Buijnsters JG. Chemical and physical sputtering effects on the surface morphology of carbon films grown by plasma chemical vapor deposition. Journal of Applied Physics. 2009;**106**(3):033504. DOI: 10.1063/1.3184349

[60] Hammer P, Gissler W. Chemical sputtering of carbon films by low energy N_2+ ion bombardment. Diamond and Related Materials. 1996;**5**(10):1152. DOI: 10.1016/0925-9635 (96)00527-4

[61] Hong J, Granier A, Goullet A, Turban G. In situ deposition and etching process of a-C:H:N films in a dual electron cyclotron resonance–radio frequency plasma. Diamond and Related Materials. 2000;**9**(3-6):573. DOI: 10.1016/S0925-9635(99)00263-0

[62] Hong J, Turban G. Etching process of hydrogenated amorphous carbon (a-C:H) thin films in a dual ECR–r.f. nitrogen plasma. Diamond and Related Materials. 1999;**8**(2-5): 572. DOI: 10.1016/S0925-9635(98)00337-9

[63] Yun DY, Choi WS, Park YS, Hong B. Effect of H_2 and O_2 plasma etching treatment on the surface of diamond-like carbon thin film. Applied Surface Science. 2008;**254**:7925. DOI: 10.1016/j.apsusc.2008.03.170

[64] Hopf C, von Keudell A, Jacob W. Chemical sputtering of hydrocarbon films by low-energy Ar+ ion and H atom impact. Nuclear Fusion. 2002;**42**:L27. DOI: 10.1088/0029-5515/42/12/101

[65] Hopf C, von Keudell A, Jacob W. Chemical sputtering of hydrocarbon films. Journal of Applied Physics. 2003;**94**:2373. DOI: 10.1063/1.1594273

[66] Voitsenya VS, Masuzaki S, Motojima O, Sagara A, Jacob W. Impact of N_2+H_2 mixture plasma on carbon-containing film. Problems of Atomic Science and Technology. Series: Plasma Physics. 2006;**6**:141

[67] Min B, Ahn DU. Mechanism of lipid peroxidation in meat and meat products—A review. Food Science and Biotechnology. 2005;**14**(1):152

[68] Kristof J, Miyamoto H, Blajan M, Shimizu K. Pharmacokinetics of cyclosporine A of transdermal delivery using microplasma and oral administration. In: Luca D, Sirghi L, Costin C, editors. Recent Advances in Technology Research and Education; 25-28 September. Romania: Alexandru Ioan Cuza University of Iași; 2017. p. 161. DOI: 10.1007/978-3-319-67459-9_21