XPS analysis of the skin treated by microplasma

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Abstract

A microplasma electrode was used for skin treatment using argon, oxygen, nitrogen or ambient air. The presence of various particles is important for the interpretation of the microplasma effect on the skin. The production of long living particles was detected by an FTIR spectrometer and the presence of air between the sample and the electrode was monitored by the O_2 monitor. In the case of gases other than air, we concluded that the concentration of processing gas is at least 99.5 %. The epidermal layer of pig skin was used for observing changes caused by microplasma treatment. The XPS spectra of carbon and oxygen were analysed. Copyright © 2018 VBRI Press.

Keywords: Skin, XPS, microplasma discharge, transdermal drug delivery.

Introduction

The skin is the largest organ of the body. The main role of the skin is to prevent the loss of water from the body and to prevent the penetration of outer molecules into the body. This barrier function is fulfilled by the uppermost part called stratum corneum, a lipid-rich matrix with embedded corneocyte cells. The need for the understanding of skin diseases, skin aging and creation of wrinkles, hydration and transdermal drug delivery process led to the investigation of the skin composition, structure, and their changes. Today, several methods exist for the treatment of wrinkles by chemical solutions or creams[1], ultrasound[2], laser treatment[3] or plasma treatment[4]. These techniques can affect the skin and for this reason, they are also investigated as tools for improving transdermal delivery of drugs. The use of chemical enhancers[5], lasers[6], ultrasound[7], electroporation[8] are well-known and longtime investigated methods for transdermal delivery but the use of plasma is relatively new in this field with only some studies[9-13]. The pathway for drug delivery through the stratum corneum is realized by lipid bi-layer situated between corneocyte cells. Structure, chemical composition, extraction of lipids or whole parts of stratum corneum layer was studied by several experimental methods such as ATR-FTIR (Attenuated Total Reflection – Furrier Transform InfraRed) spectrometry, XRD (X-Ray Diffraction) and others[14]. XPS (X-ray Photoelectron Spectroscopy) is a suitable method for analyzing the skin or lipid composition. Marschewski *et al.*[15] used XPS to study the effect of their plasma discharge on skin lipids.

The argon microplasma discharge was successfully used for the study of transdermal delivery of Cyclosporine A in our previous study[13].

As a next step, we investigated changes to the skin caused by microplasma discharge using argon, nitrogen, oxygen or ambient air as a working gas in this study. First, we confirmed the presence of gases which could interact with skin sample. After that, XPS was used for the analysis of the treated skin sample in each of the mentioned gases. The results were discussed and compared with other studies.

Experimental

Sample preparation

Pig skin of Yucatan micropig from Charles River Japan, Inc. (Yokohama, Japan) was used for investigating the influence of the plasma treatment. The fat layer of the skin was removed and then the skin was soaked at 4 ºC in phosphate buffered saline (PBS) for 3 hours. The epidermal layer of a thickness of 200 μm was peeled after bath in 60 ºC PBS for 1 min. Prepared skin was treated by microplasma for 5 min, then dried and analyzed by XPS.

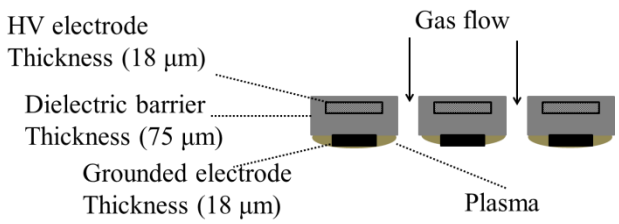

Fig. 1. Microplasma electrode used for skin treatment.

Skin treatment

The electrode used in the experiment is shown in **Fig.1**. The electrodes have been coupled by using a Neon transformer (ALPHA Neon M-5) with an AC frequency of 27 kHz. The voltage and current were monitored with a (Tektronics P60015A) high-voltage probe and a (Tektronics P6021) current probe. The distance between the skin sample and the outlet of the plasma jet was set to 1 mm. The sample was isolated from the holder by a 30-mm thick PVC isolator. The treatment time of the sample was set to 5 min, and the sample was not in contact with the plasma during this time. The gas was introduced into the microplasma device through a (Yamato) flow meter. Argon (purity 99.999%), nitrogen (purity 99.9995%), oxygen (purity 99.9%) and ambient air were used for the plasma generation.

We investigated the influence of the used gas using the following settings:

(a) Argon as the working gas, electrode coupled by 760 V, gas flow rate of 5 l/min.
(b) Nitrogen as the working gas, electrode coupled by 1.3 kV, gas flow rate of 5 l/min.
(c) Oxygen as the working gas, electrode coupled by 1.5 kV, gas flow rate of 10 l/min.
(d) Ambient air working gas, electrode coupled by 1.3 kV, argon gas flow rate of 10 l/min.

Gas composition analysis between skin and microplasma electrode

Analysis of the composition of gases which are in contact with the pig skin was implemented by the set-up in the **Fig. 2**. The distance of 1 mm from the surface of the electrode is the tube with a diameter of 3 cm and with a length of 10 cm. The tube is covered with a plastic shield with 9 holes to allow gas to enter. The other part of the tube was connected to the chamber with the O_2 monitor (OXY-1). At the end of the chamber was a pressure gauge followed by a 509 cm long plastic tube (diameter 0.4 cm) and another pressure gauge. The last pressure gauge was connected to an FTIR (Furrier Transform InfraRed) spectrometer pumped by the oil rotary vacuum pump. The gas flow to the FTIR spectrometer was set to a low value of F = 0.5 – 1 l/min depending on the used gas according to the following formula (1)

$$F = \frac{\pi(P1-P2)r^4}{8\eta l} \quad (1)$$

where F is the gas flow, $P1$ ($P1$ = atmospheric) is the pressure at the chamber with the O_2 monitor, $P2$ ($P2$ = 99-99.5 kPa) is the pressure at the side of the FTIR spectrometer, l is the length of the tube connecting 2 pressure gauges, r is the radius of the tube and η is the viscosity of the gas. The low gas flow was chosen to have minimal influence on the flow of gases between the electrode and the plastic shield mentioned above.

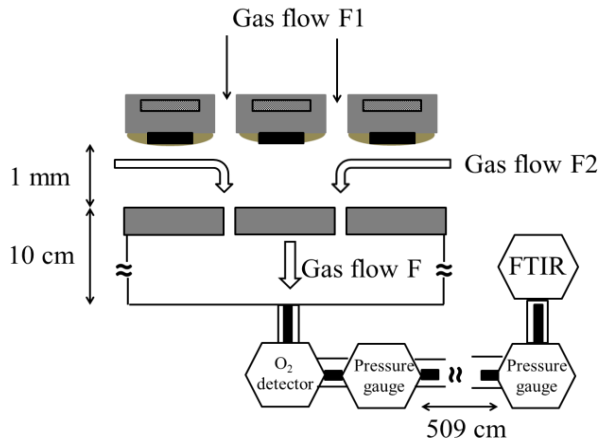

Fig. 2. Experimental set-up for the measuring of the production of long living molecules in microplasma.

Results and discussion

Plasma particles production

The O_2 monitor detected an increasing percentage of O_2 with the gas flow $F1$ through the electrode. The higher was gas flow $F1$ the lower could be flow $F2$ from air surrounding electrode. 100 % of the O_2 was detected at the gas flowrate of 5 l/min (**Fig. 3**). The FTIR spectra showed only the presence of ozone produced by plasma. In the case of argon and nitrogen, percentage of oxygen was decreasing to 0% at 5 l/min (**Fig. 3**). In this case we did not observe a spectrum of any molecule except traces of CO_2. These results indicated that during skin treatment by the discharge gas, mostly only this gas is present without any admixture of air. The oxygen monitor cannot detect oxygen with concentration lower than 0.1%. The ratio of N_2:O_2 in the atmosphere is 3.73. We concluded that the concentration of air during the treatment of skin in the case of argon, nitrogen or oxygen is less than 0.5%. On the other hand, the air microplasma discharge produced several molecules and we could detect ozone, NO_2, N_2O, CO, CO_2.

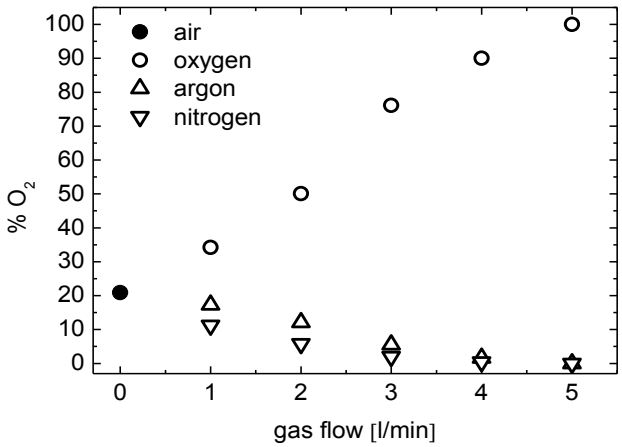

Fig. 3. Evolution of the percentage of oxygen between the sample and the electrode with increasing gas flow F1through the microplasma electrode.

Argon

Argon plasma is the simplest medium that we used for the skin treatment. Argon is the major gas which fills the space between the sample and electrode. We suppose that the presence of other gases is negligible. The surface DBD microplama produced excited states of Ar (Ar*), metastable states (Ar_m) and Ar ions (Ar^+, Ar_2^+)[16]. However, outside of the plasma, where electrons were not present, only metastable states and ions can survive and only these particles can participate in the skin treatment. Argon metastable states have relatively long lifetime equal to 38 s[17] in vacuum. However, this time is reduced in atmospheric pressure.

Nitrogen

Nitrogen discharge, similar to argon, produces ions N_4^+, N_2^+ and a metastable state $N_2(A)$ with a lifetime of 1 s[18]. The treatment by nitrogen should be similar except the case when nitrogen can be dissociated and create reactive ion or radical.

Oxygen

Oxygen discharge produces O_2^+ ions, ozone and metastable $O_2(a)$ with a lifetime of 4700 s[19]. Singlet oxygen $O_2(a)$ because of a very long lifetime can participate in the interaction with skin. Watabe *et. al.*[20] demonstrated that unsaturated bonds of lipids decreased with the time of irradiation of $O_2(a)$.Another long living molecule is ozone and the exposure of the skin to ozone can lead to lipid peroxidation[21].

Air

As it was mentioned above, air microplasma produces a wide range of particles which are composed from nitrogen and oxygen such as $O_2(a)$, O_3, $N_2(A)$, HNO_2, HNO_3, N_2O_5, H_2O_2, NO and ions O_2^+, N_2^+[22].

Air plasma could probably combine the effects of oxygen and nitrogen.

XPS analysis

The lipid bilayer of the stratum corneum is the main route for drugs in transdermal delivery. These lipids are composed of hydrophilic heads and lipophilic tails. More specifically, according to Wertz[23] lipids are formed of 50% of ceramides, 25% of cholesterol, 10 – 15% of free fatty acids. In the case of ceramides, it was 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[23] (**Fig.4**). The permeability of this membrane can be increased by decreasing concentration of cholesterol, increasing amount of short tailed fatty acids and unsaturated fatty acids.

XPS spectra were fitted by a Gaussian function with FWHM 1.5 eV in the case of carbon lines and 1.8 eV in the case of oxygen lines.

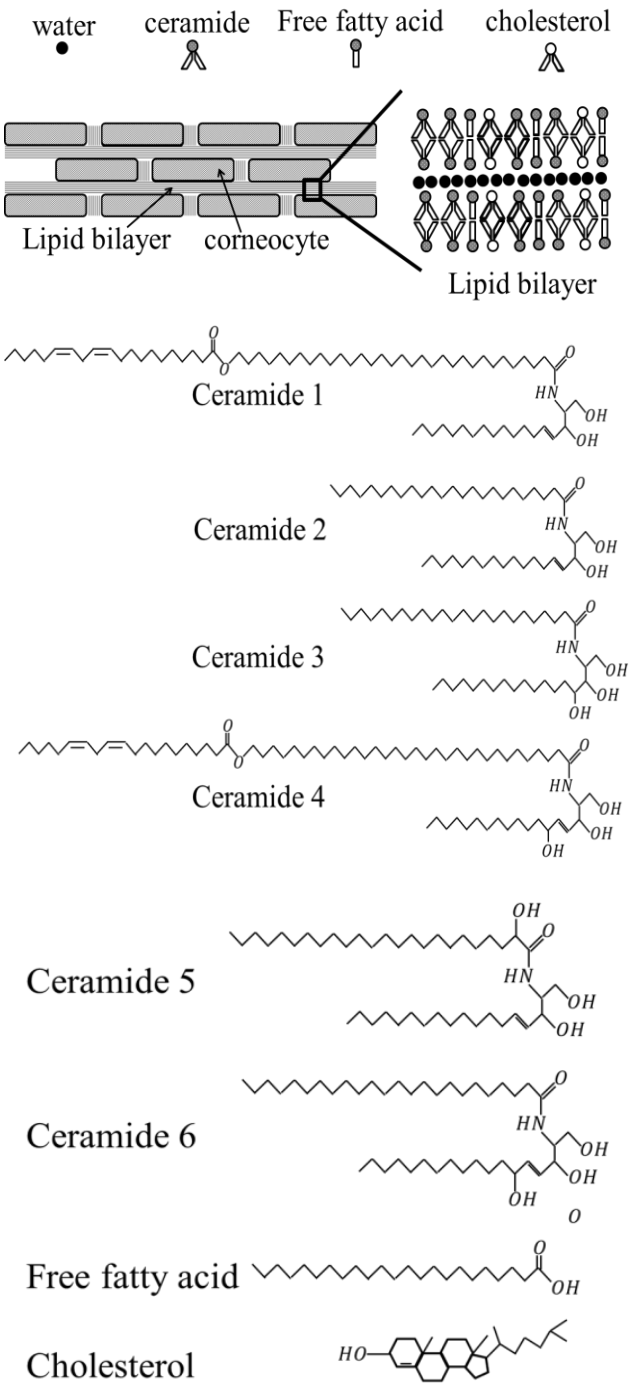

Fig. 4. Stratum corneum with bilayer lipid membrane and main components of membrane such as ceramides, free fatty acids and cholesterol.

Treatment of the pig skin by argon microplasma indicate a slight decrease of C-C and C-H bonds, reduced amount of O-C=O and N-C=O bonds in the carbon spectra (**Fig. 5**) and a reduced amount of O=C-OH bonds in the oxygen spectra (**Fig. 6**). The most effective in the decrease of all bonds C-C, C-H and O-C=O and N-C=O in the carbon spectra and also in the oxygen spectra (C-O, C=O and O=C-OH) is air microplasma.

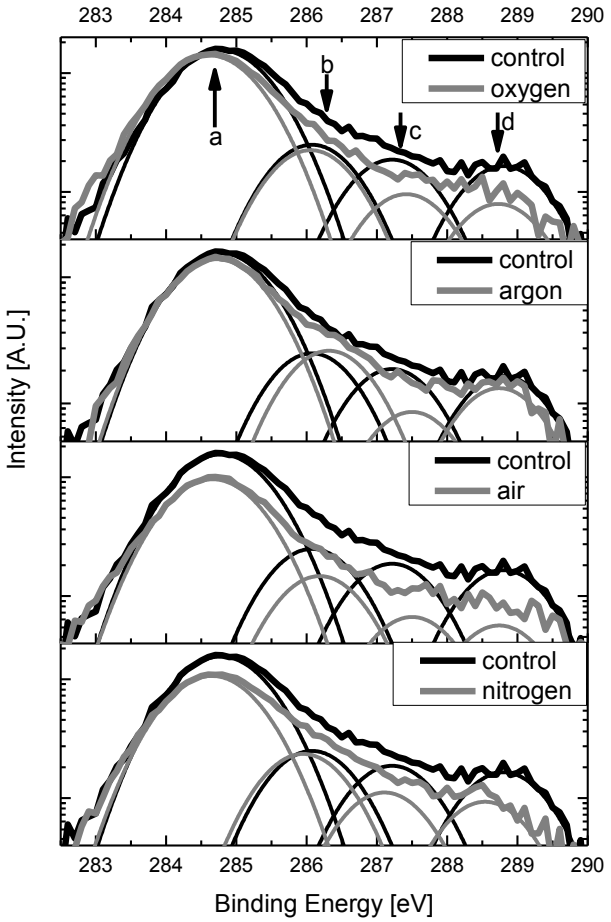

Fig. 5. Carbon XPS spectra of pig skin. Positions a, b, c, d represents peaks of carbon bonds:
a: C-C, C-H, b: C-O, C-N c: O=C-N d: O=C-O. (Because peak at 284.6 is too high, we chose logarithmic scale)

In the oxygen microplasma C-O, C-N bonds stayed unchanged and C-C, C-H slightly decreased (carbon spectra in **Fig. 5**) and O=C-OH is slightly increased (oxygen spectra in **Fig. 6**). Nitrogen microplasma reduced C-C, C-H bonds (**Fig. 5**) and increased O=C-OH bonds (**Fig. 6**). Results indicate that argon and nitrogen react mainly with sidechains of hydrocarbons which contained oxygen. Air and oxygen can significantly decrease the C-C and C-H bonds of the main chain, too. Mostly microplasma caused the decreasing of all kinds of bonds, sometimes more or less effective depending on the used gas. We can conclude that the interaction of microplasma with the skin or lipid bilayer is based on breaking bonds of molecules. This can lead to a more "liquid" phase of lipid membrane and probably to an increase in its permeability. In the case of argon it was confirmed in Kristof *et al.*[13]. But for other gases, it has to be proved if the effect is the same. Slight etching of the skin surface by microplasma was confirmed before in the skin section measurements after the argon microplasma treatment in Shimizu *et al.*[11]. Argon ion bombardment of the PMMA also confirmed decreasing of side chains of the polymer and led to volatile CH_4, CO, CO_2, $HCOOCH_3$ and H_2 and polymer was changed to a sort of disordered polyethylene[24]. Also argon ion bombardment in the study of Bachman *et al.*[25] confirms that after argon bombardment of polymer, surface is changing to graphite-like if energy or concentration of Ar ions is sufficient.

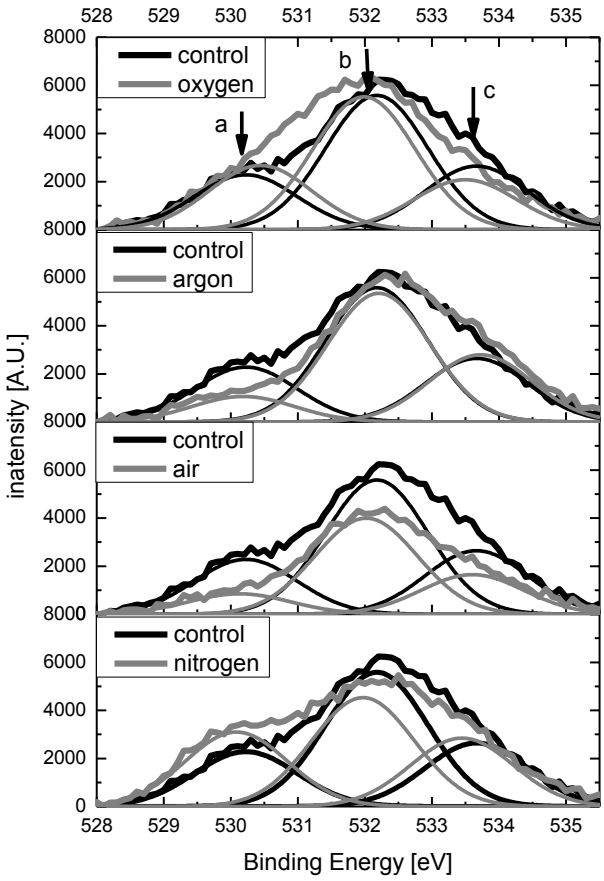

Fig. 6. Oxygen XPS spectra of pig skin. Positions a, b, c represents peaks of oxygen bonds:
a: O=C-OH, b: C-O c: C=O

Hirschberg *et al.*[26] investigated the influence of plasma sources working in the air on human lipid composition. They used plasma sources which were in direct contact with lipids by microdischarges. They observed a decrease of C-C bonds and an increase of C-N, C-O, O=C-O and O=C-N bonds. Emmert *et al.*[27] confirmed this behavior by changing the concentration of carbon, oxygen and nitrogen. Plasma in last the two studies was in contact with the skin and it means that the concentration of reactive species such as radicals can be much higher than in our study. We concluded that the main active species in the case of microplasma are ions and it confirms also the comparison of ion bombardment of polymers which demonstrated similar trends.

Conclusion

Skin treatment by microplasma discharge was investigated by the XPS analysis of the epidermal layer. We confirmed by oxygen monitor that during the treatment only gas used in microplasma discharge with the concentration of at least 99.5% interacted with skin

and impurities from the air could be at a maximum of 0.5%. Oxygen, argon, nitrogen and ambient air were used as processing gases. The amount of most of the bonds decreased after 5 minutes of treatment, and the most effective was when ambient air was used. A comparison with the results in literature when skin was treated by plasma or polymers were bombarded by ions, we conluded that most probably, the changes on the skin are caused by ions generated by microplasma.

Acknowledgements

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