The acidification of lipid film surfaces by non-thermal DBD at atmospheric pressure in air

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Abstract. We studied the acidifying efficiency of a cold atmospheric pressure plasma treatment and ambient air as a working gas on lipid films. Acidification of a thin water film could be observed on plasma-treated surfaces of wool wax, pork sebum and human lipids. This pH shift was partly attributable to NO\textsubscript{x} species and to the formation of nitric acid in the upper layers of the substrates. The acidic compounds on the lipid surfaces resulted in pH shifts for up to 2 h after plasma exposure, which might be beneficial for pH-targeted therapies in dermatology.

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1. Introduction

Plasma sources for the biomedical field have been developed using diverse technical approaches to meet the claims of plasma medicine. These sources have been applied for sterilization and wound healing during the last decade with increasing insights into the dynamics of plasma interactions with biological components [1]–[6]. Direct treatment with air plasmas at atmospheric pressure is known to have physical and chemical bactericidal effects. A local reduction of bacterial colonization within the time frame of seconds to minutes has been observed in several studies [7]–[10]. However, how plasmas interact with biomaterials is not fully resolved and there are still uncertainties regarding cooperation and synergies between particular plasma agents. In terms of therapeutical applications in plasma medicine, the biocidal effects of plasmas on microorganisms within the active plasma phase are important, but alterations of the tissue surface caused by interactions with plasma species are also of relevance.

In the case of human skin surfaces exposed to plasmas, the outermost layer to interact directly with the chemical compounds produced is the hydrolipid film covering the epidermis. This continuously renewing emulsion is generated by transepidermal water, lipids, amino acids, lactic acids and free fatty acids [11]. It can be considered as the substratum for a complex microbial ecosystem consisting of numerous bacteria of various genera [12]. Ecologic shifts in microbial inhabitants can result in skin diseases: in contrast it is well known that the microbial composition of wounds is shifted because of the altered conditions compared with healthy skin [13, 14]. These shifts correlate with changes of the environmental pH values, which can therefore be considered as an important determinant of metabolism. Hence, pH-targeted approaches are of growing interest for therapeutic strategies in wound healing [15]. The hydrolipid film of healthy skin is balanced at a pH value of 5.4–5.9 [16]. Studies on affected skin areas from patients with diseases such as ichthyosis and atopic dermatitis, as well as from wounds from chronic venous leg ulcers and pressure sores, have revealed elevated cutaneous pH [17]–[19]. Thus, these areas undergo enhanced susceptibility to pathogen growth, as most relevant pathogenic bacteria on human skin possess optimum growth at pH values above 6, while their growth is inhibited at a lower pH [20].
This background forms the basis of studies on the efficacy of acidification by plasma treatment of lipid films. Furthermore, acidification by plasma exposure might also contribute to our understanding of plasma processing of other biomaterials such as wood [21].

2. Experimental part

2.1. Dielectric barrier discharge (DBD) source

The experimental setup is illustrated in figure 1. By applying high voltage pulses to an electrode covered by a dielectric platen, a discharge in the air gap between the dielectric surface and the glass substrate on the counter electrode is ignited.

The dielectric is made of alumina with a diameter of 10 mm, an insulation thickness of 1 mm and a relative permittivity of $\epsilon_r = 9.6$. The plasma propagates in the multi-filamentary mode, consisting of stochastically distributed streamers. The power supply is identical to the apparatus used in the work of Kuchenbecker et al [22]. It generates high voltage-pulsed packages of some 10 $\mu$s (see figure 3(a)) with a maximum amplitude of the alternating voltage up to 13 kV at repetition rates between 200 and 300 Hz. The electrical parameters were measured by applying two high voltage probes 1000:1 Tektronix P6015A and oscilloscope Yokokawa DL1740EL Dual 500 MHz. Measured voltage traces were digitized and transmitted to a personal computer for processing using MATLAB software.

The $Q$–$U$ plot method was applied for measuring energy dissipated into the discharge. Typically, these plots have a parallelogram shape, as shown in figure 2.

From the enclosed area of the Lissajous curve in 1943 Manley first derived expressions for the energy dissipated into the discharge [23]. According to Manley, the total charge $dQ$ transferred by the microdischarges per half cycle of an applied sinusoidal voltage is given by

$$dQ = 2C_{\text{die}}u_{\text{peak}} - [(C_{\text{die}} + C_{\text{gap}})/C_{\text{die}}]u_b,$$

whereby $C_{\text{die}}$ is the capacitance of the dielectric, $C_{\text{gap}}$ is the capacitance of the gas gap, $u_{\text{peak}}$ is the peak value of the applied voltage and $u_b$ is the effective gas breakdown voltage. Because...
there are both a forward and a reverse discharge in each cycle, the energy $W$ coupled into the discharge amounts to

$$W = 2dQ u_b.$$  (2)

Inserting equation (1) into (2) leads to

$$W = 4u_b C_{\text{die}}[u_{\text{peak}} - [(C_{\text{die}} + C_{\text{gas}})/C_{\text{die}}]u_b].$$  (3)

The mean power dissipated in the discharge can be calculated by multiplying the mean energy $W$ per cycle with the repetition frequency $f$ of the applied voltage pulses [24]:

$$P = W f.$$  (4)

2.2. Methodology and diagnostics

For the experiments, wool wax (adeps lanae anhydricum) as well as sebum stripped from pork skin and lipid layers stripped from a human forehead were plasma-treated on microscope slides measuring 76 mm $\times$ 26 mm $\times$ 1 mm. For the purpose of defined layers, 5-mm-wide Scotch Magic Tape No. 810 was stuck along both 76 mm sides of the glass surfaces. The biomaterial was then deposited onto the glass surface and spare material was removed by scraping the tape lengthwise, resulting in layers of 76 mm $\times$ 16 mm $\times$ 63 $\mu$m.

As soon as the layers were established, the samples were plasma treated for different durations at a constant distance of $d_{\text{gap}} = 0.5$ mm (see figure 1). Immediately after exposure, a droplet of 20 $\mu$l deionized water was pipetted onto the treated area of each specimen. The pH of the droplet was determined potentiometrically by applying a 12 mm diameter Derma Unit SSC 3 glass electrode (Courage and Khazaka Electronics, Cologne, Germany). This method requires the complete coverage of the treated area with water. The electrode was brought into contact with the water drop. The water spread over the complete area of the glass membrane and a thin film approximately 0.2 mm thick formed between the glass electrode and the lipid surface.

In another series of tests, to analyze the water content 20 $\mu$l droplets were pipetted onto plasma-treated surfaces. After 20 s, the droplets were extracted from each surface by a pipette and dropped onto substance specific test strips. In two test series, the concentrations of nitrate ($\text{NO}_3^-$, Reflectoquant 1.16971.0001, Merck) and nitrite ($\text{NO}_2^-$, Reflectoquant 1.16973.0001, Merck) were determined using a RQFlex reflectometer (Merck, Darmstadt, Germany). The
reflectometer is based on the principle of remission photometry and the concentrations of specific analytes can be derived from the difference in intensity of emitted and reflected light from substance specific test strips [25]. Calibration was achieved by preprogrammed bar codes resulting in a measurement accuracy of ±10% in the mean effective range.

3. Results and discussion

3.1. Power measurements

Each pulse package is a damped sine wave with a frequency of about 100 kHz. For the first three periods of the sine wave, breakdown conditions in the gas gap are fulfilled and microdischarges are ignited. This corresponds to the findings of Kuchenbecker et al. At a constant gap, the average power and the total transferred charge are proportional to the supplied voltage. Therefore, dissipated energy into the discharge was determined for the first three periods separately (see figures 3(b)–(d)) and summed up to

\[ P = (W_b + W_c + W_d) f. \]  

(5)

The mean discharge power was determined for two different voltage amplitudes and repetition rates of the power supply. For an amplitude of 6.3 kV at a repetition rate of 271 Hz and 11.5 kV at 206 Hz mean discharge powers of \( P_{\text{low}} = 18 \text{ mW} \) and \( P_{\text{high}} = 66 \text{ mW} \) were obtained, respectively.

3.2. Plasma treatment of lipid films

The application of a dielectric barrier discharge to lipid films of wool wax and pork sebum led to significant decreases in pH values, as depicted in figures 4 and 5. The results for wool wax and pork sebum clearly indicated the inversely proportional scaling of pH with increasing plasma energy. After 5 s of plasma treatment, the pH was reduced by one unit, while after 480 s both surfaces led to pH values in the water droplet of 2.4 at a plasma power of \( P_{\text{low}} = 18 \text{ mW} \) and 2.8 at \( P_{\text{high}} = 66 \text{ mW} \).

The pH characteristics of plasma-treated human lipid films are presented in figure 6. Because of the acidic constituents in the lipid film, the initial pH value was considerably lower than in the former experiments. In our experiments, the pH varied between 4.6 and 6.2 prior to treatment, which is in good agreement with previous measurements of Zlotogorski [26]. After 60 s of exposure, the plasma treatment caused a pH shift down to a mean value of 3.7.

To evaluate the duration of acidification, wool wax surfaces were plasma-treated and then stored untreated at ambient conditions. The corresponding pH values, determined at different periods after plasma treatment, are depicted in figure 7.

Even at 2 h after plasma treatment, the wool wax surfaces did not recover to the initial pH value of 7.3 but remained about 1.3 units lower. We attribute the recovery of pH in the post-plasma phase to the decrease of acidifying agents on the substrate surfaces by both diffusion and desorption processes.

3.3. Sample diagnostics

In general, the dominant species produced by the gas discharge at this experimental conditions is ozone, while the amount of NO\(_x\) species is rather small [27]. Still, NO\(_x\) species are known
to produce strong acids in the presence of water and thereby may contribute noticeably to pH shifts \cite{28, 29}. Consequently, to identify possible agents causing acidification in our experiments, amounts of nitrates, nitrites and oxonium ions were measured. Using the approximation of equation (6) below, the concentrations of oxonium ions were calculated from measured pH values. As the water volume was kept constant, the amount of oxonium ions in the droplet could be calculated.

\[
\text{pH} \approx -\log(c_{\text{H}_3\text{O}^+}).
\]  \hspace{1cm} (6)

The amounts of nitrates and nitrites could be determined from the measured concentrations in the water droplet. Because of cross reaction of the nitrate test strips with nitrite content in the solution and assuming linear failure, the nitrate amount curve had to be corrected by

\[
\frac{dn_{\text{NO}_3^-}}{dE} = \frac{dn_{\text{measure}}}{dE} - \frac{dn_{\text{NO}_2^-}}{dE}.
\]  \hspace{1cm} (7)

Figure 8 clearly shows the correlation between surface acidification and nitrate content on the lipid surface caused by formation of NO\textsubscript{x} species in the discharge volume. From that we
conclude that the formation of nitric acid may play an important role for surface acidification. There are multiple pathways for the formation of nitric acid in the gas discharge. A prominent educt for formation of nitric and nitrous acids is nitrogen dioxide NO₂, which is produced in non-equilibrium discharges by various reactions [30].

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Figure 6. pH values of plasma-treated human lipid layer at constant power. Plasma treatment times varied between 1 and 60 s.

Figure 7. Mean values and standard deviation of pH up to 2 h after plasma treatment at $P_{\text{low}} = 18$ mW for 30 and 60 s of wool wax and ambient air as the working gas.
Figure 8. Amount of nitrate $\text{NO}_3^-$ (from reflectometric measurements), oxonium ions $\text{H}_3\text{O}^+$ (from pH measurements) and nitrite $\text{NO}_2^-$ (from reflectometric measurements) in droplets of 20 µl deionized water on plasma-treated wool wax. Plasma power was $P_{\text{low}}$. The working gas was ambient air.

Gaseous HNO$_3$ can be produced by the reaction mechanism (8), below, in the discharge volume, whereby OH is generated by dissociation of H$_2$O molecules present in the discharge under atmospheric conditions. Gaseous HNO$_3$ can then dissolve into the aqueous phase

$$\text{NO}_2 + \text{OH} \rightarrow \text{HNO}_3.$$  

(8)

In the aqueous phase (see reaction mechanism 9, below), the dissolution of gaseous NO$_2$ produces nitrous as well as nitric acid

$$2 \text{NO}_2 + \text{H}_2\text{O} \rightarrow \text{HNO}_2 + \text{HNO}_3.$$  

(9)

4. Conclusion

From the results, we attribute the pH shift of water on plasma-treated lipid films to acidic constituents on the lipid film surfaces. These are most likely generated by the interaction of chemically reactive species with the surface. The amounts of nitrates in the water droplets and most likely the formation of nitric acid could be sufficient to explain the pH shifts, but the complete kinetics of acidification, especially in the presence of ozone, are to be investigated in more detail.

We attribute the nitrate content in the water droplets either to adhesion of NO$_x$ species on the lipid surfaces, or to the deposition of nitric acid on the film surfaces by gaseous HNO$_3$.

The sustainable mechanism of surface acidification was active in our experiments even 2 h after plasma exposure. These preliminary observations need further investigations as they might offer a first step into pH targeted plasma therapies in dermatology.
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