Low Temperature Plasma Treatment of Living Human Cells

Stephanie Tümmel, Nina Mertens, Jiejun Wang, Wolfgang Viöl*

The purpose of this article is to provide a sustainable dielectric barrier discharge for the treatment of living biological tissue. The investigation aims at the innocuousness of plasma treatment by DBD with biological tissue as counterelectrode on biomaterial. Instead of human tissue, pork and in vitro living epidermal human cells are used for the plasma treatment. Potential detrimental effects like current conduction, temperature, ozone and UV-irradiation are investigated. All the levels are below the danger threshold. After plasma treatment of human cells, no cell damage could be detected. Hence applications on human beings with a DBD plasma source seems to be possible.

Introduction

The dielectric barrier discharge is accomplished in air and under atmospheric pressure. Dissertations and educational books from the first 20 years of the 20th century already gave a detailed account of applications for high frequency treatment, which has been offered in several fields of skin care since then. Up till now the real therapeutic advantage of this treatment, which is a plasma treatment (plasma = gas discharge), is only insufficiently researched as far as the effect-mechanism, efficiency and compatibility are concerned.

While it is very easy to create low pressure plasma, the development of a plasma source for the application under atmospheric conditions on living biological tissue like skin is more complex. Among several options to create low temperature plasma at atmospheric pressure, only two forms of gas discharges can be considered for the plasma treatment of skin:

- potential-free plasma jet (plasma needle, plasma pencil),
- dielectric barrier discharge (DBD),

because only these gas discharges include the necessary homogeneity. The development of the plasma needle is persecuted in the Netherlands, while the plasma pencil is developed in the USA[1–3]

With a new design of the electrode the application of a DBD seems to be possible while the tissue itself acts as counterelectrode.[4–9]

The potential detrimental effects to be considered are electrical current conduction, ozone and UV-irradiation. Because of the human body's resistance to high voltage, the electrical current conduction should be beyond the danger threshold. Ozone occurs under the presence of atmospheric oxygen directly above the treated region of the skin. The high level of energy on inner particles and the existence of numerous insignificant levels of energy are causing the plasma to emit electromagnetic radiation of several frequencies for example UV-irradiation. It can be expected that the plasma treatment causes an increase in skin temperature. Due to this the discharge temperature and the temperature of the skin are measured.[10]

The purpose of this paper is to provide evidence that the treatment is harmless despite the previously mentioned possible risk factors (temperature, ozone, UV-irradiation and current conduction). The experiments are performed using pork, an accepted alternative to human tissue.[11,12]

To gain insight into the influences exerted on cellular level, living epidermal cells of the HaCaT cell line are treated with plasma.[13] Potential cyto-reply could be necrosis or apotheosis as the negative reaction, or increased cell division, which is the positive reaction.[14]
Experimental Part

Figure 1 shows the test arrangement of the DBD on pork as the counter-electrode. The dielectric barrier electrode sparks pulses with an amplitude amounted to $\mu_{\text{sto}} = 5\text{–}6\text{kV}$ with a pulse length of $t_p = 200\text{–}300\text{ns}$ and a pulse repetition rate of $f_p = 518\text{Hz}$. With a micrometer screw the discharge gap $d$ between electrode and skin is exactly adjusted.

The temperature of the gas discharge is measured with an optical sensor (Universal Fiber Optic Sensor FTI 10 from Soliton Laser and Messtechnik) within the discharge. The heating of the treated region of the pork is acquired contactless by an infrared-thermometer (IR 1001 A from Voltcraft). Due to the similarity of pork and human skin, the same emission coefficient is assumed.

The level of ozone concentration is measured by ozone switch from Eco Sensors. The measurement spans 20 s and takes place right beside the gas discharge in a distance of 5 mm.

Voltage and current through the tissue are measured with a needle, which is inserted through the back of the pork. The needle is movable, so current, voltage and electrical power can be measured not only against the gap $d$ between skin and electrode but also against the distance $e$ between needle point and skin as well, as seen in Figure 1. The applied current in this project is because of the pulse length $t_p = 200\text{–}300\text{ns}$, an alternating current of high frequency. Only the peaks of current and voltage are measured, because only these have a therapeutic use at all the stimulation currents. However, the compatibility of current and human beings depends on the maximum electrical power. Hence, the electrical energy and then the electrical power are calculated from the values. The comparable body-resistance for alternating current is about $R_{\text{hand}} = 600\ \Omega$, therefore the measurement operates with this resistance ($R_{\text{M}} = 600\ \Omega$).

The UV-irradiation-spectrum is recorded with the Flame Star II — camera system from La Vision. It records the emission spectrum in the range of $\lambda = 186\text{–}413\text{nm}$. This is sufficient because the share of atomic oxygen is about $10^{-3}$ to $10^{-4}\%$ in one filament. The smallest possible gap $d$ is $d_{\text{min}} = 0.5\text{mm}$. Below this value there will be no discharge. So there is enough O$_2$ under atmospheric pressure that all UV-C-irradiation ($\lambda = 100\text{–}280\text{nm}$) is absorbed.

The treated material is pork, which is most similar to human tissue. For the experiments, it is heated to $T_{\text{body}} = 37\ ^\circ\text{C}$ while the skin has a temperature of $T_{\text{surface}} = 30\ ^\circ\text{C}$. Via the heating method, which is shown in Figure 2, both temperatures are kept constant. During the measurements this is checked by three temperature sensors, as depicted in Figure 2. Upon the skin but not in the gas discharge with the universal fibre optic sensor FTI 10 from Soliton laser technology and measurement, contactless with the infrared-thermometer IR 1001 A and in the nearby ambient air with an ambient thermometer EM-913R from Oregon Scientific.

As an evidence of the harmlessness at cellular level a cytotoxicity assay is established. The effect of the plasma treatment is tested depending on the duration of the treatment on living human cell cultures.

The HaCaT-cells are disseminated in two 24-multiwell-plates in 3 ml Dulbecco’s modified Eagle’s Medium (DMEM) and 7% fetale calfserum (FCS). The incubation is performed at $T_l = 38\ ^\circ\text{C}$ and a CO$_2$-level of about 5%. Under these conditions the cells will keep on dividing continuously. After 2 days the treatment is tested depending on the duration of the treatment.
Results and Discussion

After the data assessment, the degree of exposure of the plasma treatment can be evaluated.

Temperature

The dielectric barrier discharge on pork is shown in Figure 3. During the plasma treatment in air for $t_B = 20 \text{ s}$ no rise of the skin temperature is noticed, which drops out of the zone of measurement error ($\Delta T_{\text{surface}} = 0.1 \text{ K}$). The cell-destruction starts at $T_{\text{limit}} = 42 \text{ °C}$. At a normal skin temperature about $T_{\text{surface}} \approx 30 \text{ °C}$, the measured minor change is far away from the dangerous threshold. The gas temperature of the discharge is measured at a temperature of $T_{\text{discharge}} = 33 \text{ °C}$.

Ozone Level

The German MAK of ozone concentration amounts to 0.1 ppm, which means the maximum allowable concentration at work environment. As an exceptional case for a period of 5 min 0.2 ppm are allowed.$^{[15,25]}$

In a distance of 5 mm to the gas discharge and during a treatment time of $t_B = 20 \text{ s}$ the level of 0.01 ppm of ozone concentration is measured, so the threshold of 0.1 ppm is not reached. The plasma generator would have to generate a plasma for a time span of more than 3 min to achieve the threshold, but this is definitely out of the question. Furthermore, because of its reactivity with materials of the ambience the ozone concentration degrades very fast. For indoors a half-life period of 2–6 min is published.$^{[15]}$

Electrical Measurement

The gas discharge acts with a high voltage pulse of about $u_{HF} = 5–6 \text{ kV}$. The measured maximum voltage in the pork reaches $u_{\text{max}} = 102 \text{ V}$ for a short time of about $t_P = 200–300 \text{ ns}$. The maximum displacement currents are about $i_{\text{max}} = 483 \text{ mA}$ as depicted in Figure 4. The values depend on the distance $e$ between needle point and skin and the gap $d$ between electrode and skin. The maximum peak voltage (see Figure 5) is nearly the same in all the tested distances $d$ and $e$. It shows that the resistance of the skin and the subjacent tissue pale in comparison to $R_M = 600 \Omega$.

Figure 6 shows the electrical energy depending on distance $d$. The variation of gap $e$ is not depicted, because the measured values do not alter. The electrical energy is used to calculate the electrical effective power $P_{\text{electr}}$ which is needed to appraise the compatibility of the current.

The real electrical energy is determined over the full period $t_P$ with

$$E_{\text{electr}} = \int_0^{t_P} i(t)u(t)dt$$

(1)
The maximum electrical power from about $P_{\text{electr.}} \approx 2 \text{ mW}$ can be calculated by

$$E_{\text{electr.}} \cdot f_p = P_{\text{electr.}}$$

with $E_{\text{electr.}} = 3.7 \text{ mJ}$ and $f_p = 518 \text{ Hz}$.

This is the effective power of the discharge, which is deposited into the biological tissue.

Attention must be paid to the very short pulse length. Independent of the duration of the plasma treatment the single discharges have durabilities of $t_p = 200–300 \text{ ns}$. It is published that a pulse length below $t_p = 10 \mu \text{s}$ is harmless, because nerves are not stimulated, regardless of the current conduction and the voltage.\(^{[14,16,17,26]}\)

Furthermore, it should be noted that the risk of ventricular fibrillation depends on the frequency. The recommended value about $i_{\text{max\,50\,Hz}} = 80 \text{ mA}$ applies for $f_p = 50 \text{ Hz}$. The allowable current for other frequencies is published as

$$i_{\text{max}} = i_{\text{max\,50\,Hz}} \cdot F$$

$F$ is called frequency-factor. With $F_{518\text{ Hz}} = 10$ the maximum allowable current conduction is calculated as $i_{\text{max\,518\,Hz}} = 800 \text{ mA}$.\(^{[16,18]}\)

This leads to a guideline of the electrical power concerning the human body of $P_{\text{max\,518\,Hz}} \approx 2 \text{ kW}$.\(^{[18,23]}\)

In spite of the high current and voltage the real power ($P_{\text{electr.}} = 2 \text{ mW}$) is well below the guideline. Standard used transcutaneous electrical nerve stimulation (TENS) apparatus in the human medicine works with a considerably higher electrical power.\(^{[27]}\)

The results of current and voltage measurement reveal that plasma treatment is a harmless discharge for the human body.

**UV-Irradiation**

The spectrum of the UV-irradiation is recorded with the Flame Star II—camera system from La Vision. UV-C-rays ($\lambda = 100–280 \text{ nm}$) are particularly dangerous. This short-wavy irradiation badly damages living cells. UV-B-irradiation ($\lambda = 280–315 \text{ nm}$) is responsible for sun burn and at last also skin cancer. The UV-A-rays ($\lambda = 315–400 \text{ nm}$) go further into the skin than UV-B or -C, but they are relatively harmless.\(^{[34,17,21]}\)

The spectrograph measures within the range of $\lambda = 186–413 \text{ nm}$. The peaks are represented relatively to each other. The spectrum is shown in Figure 7 and contains only excited nitrogen lines of the second positive system.\(^{[28]}\) This leads to the conclusion that mainly the emitted UV-irradiation is the UV-A-irradiation. Only one peak is in the region of the UV-B-irradiation, UV-C is completely missing. This is because of the absorption of UV-C-irradiation by $O_2$.\(^{[19,20]}\)

The intensity is measured with a detector in the range of 250–400 nm. The average intensity amounts to 0.035–0.04 mW⋅m\(^{-2}\) for a time of $t_B = 20 \text{ s}$. The threshold is approximately $E_{\text{lim\,it}} = 0.1 \text{ W} \cdot \text{m}^{-2}$, this means that the relative UV emission is about an order of magnitude smaller.\(^{[29]}\)

**Plasma Treatment of HaCaT Cells**

Two hours after treatment the results will show if there is immediate cell damage. The ratio of dead plasma treated cells is about 8.3%. This is very near to the result of the untreated control cells (7.2%). In consideration of the
standard deviation, no cell damage can be detected in this case.

Figure 8 shows the ratio of dead cells to the basic cell population calculated in percent. The lower ratio of dead cells after 24 h can be explained by the continuously ongoing cell division. In the meantime more cells are generated than cells dying off. So the death ratio of the untreated HaCaT as well as the plasma treated HaCaT cells decreases. So the dead/common cell ratios for the 2 and 24 h time frame are very similar. In regard of the standard deviation a prolonged cell damage caused by plasma treatment of this HaCaT cells cannot be noticed.

Looking at the results of the enumeration of the cells (see Figure 8) it becomes apparent that the plasma treatment in this case does not cause a cell damage, which would prohibit an application.

**Conclusion**

The results of the measurements allow the following conclusions: There is no risk of a burn. The level of the ozone concentration is well below the German MAK. The electrical power does not stress the human body. The UV emissions are negligible and neither necrosis nor exceptional apoptosis are noticed. The previously noted cytotoxicity assay showed no negative consequences arising out of plasma treatment on living HaCaT cells.

After long term testing is performed and the harmlessness of this procedure is determined it can be used for several different medical applications.

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