Effect of cold plasma on proliferation rate of mammalian cells in vitro

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Abstract. Human keratinocytes HaCaT and human carcinoma cells A431 have been treated in vitro by a cold argon plasma jet with an average power value of 0.72 mW/cm$^2$. There were made estimations of proliferation rate and cell viability in a day after the exposition. In contrast to the cell viability of both cell lines there were revealed significant differences in proliferation rates of keratinocytes and cancer cells after plasma treatment.

1. Introduction

Cold atmospheric plasma is understood as a low-temperature nonequilibrium gas discharge plasma generated at atmospheric pressure with typical electron energies of 1-5 eV. Due to its low near room temperature it can be used for biological applications, for tissue treatment. It is known that cold atmospheric plasma can be used for the successful therapy of bacterial diseases [1]. There is also a lot of information about the selective effect of such plasma on cancer and normal cells of mammalians, and, in particular, humans [2]. The successful use of the plasma for these purposes is associated mainly with the generation of strong oxidant such as oxygen in plasma-chemical reactions. It is believed that reactive oxygen and nitrogen species, such as OH, H$_2$O$_2$, NO, molecules are most effective when exposed to cells [3] and may contribute to the destruction of deoxyribonucleic acid (DNA) in cancer cells, lipid peroxidation and enzyme activation [4]. However, the exact mechanism of the effect of cold plasma on human cells has not yet been clarified.

2. Experimental setup

In this work, the effect of the cold plasma on human skin cells, keratinocytes of the HaCaT line and human carcinoma cells A431 from the collection of cell cultures of the Koltzov Institute of Developmental Biology (IBR RAS) was studied. A plasma jet was used as a plasma source, which was formed in a plasma reactor by pumping argon at a rate of 3 L/min. The plasma power source was a high-voltage pulse generator developed at the Institute for Electrophysics and Electric Power (IEE RAS). Its operation is well described in the papers [5, 6].

Figure 1 shows an experimental setup. It can be conventionally divided into three parts: the plasma source 1, the treated object 2, and the diagnostic unit 3. The plasma reactor PR was a 1 mm thick quartz tube 5 mm in diameter. A metal rod with a diameter of 2 mm, recessed 10 mm from the end of
the tube, was used as a high-voltage electrode, and a metal ring mounted outside was used as a grounded electrode (figure 2).

As a power source, a rectangular pulse generator PG was used. It formed on the electrodes unipolar positive voltage pulses with an amplitude of 7 kV, a duration of 1.5 μs with nanosecond rise/fall time with a repetition rate of 3 kHz. When pumping, a plasma jet was formed at the tube nozzle.

The objects of the study were the above-described cell lines. The cells were grown in an incubator for several days and placed in the Gibco Dulbecco's Modified Eagle Medium (DMEM), which is widely used basal medium for supporting the growth of many different mammalian cells. The cell density in Petri dishes was 1500-2000 U/ml. The distance to the medium surface from the reactor's nozzle in all experiments was the same and equal to 40 mm. The Petri dishes were located on the grounded plate during the experiment. As it was shown in [7, 8] the grounded target favored to increase in the plasma jet current, and to increase in the OH-concentration, correspondingly [9]. The cells were treated for 1 minute. The control samples were not treated by the plasma. They were in Petri dishes just with the lids open for the same time.

In the course of the study, oscillograms of the voltage at the electrodes of the reactor were taken using a high-voltage probe HVP, and oscillograms of the jet current were taken using a current probe.
CP. According to the experimental data the power density of the jet was determined. Using the MDR-23 monochromator M, combined with a high-speed camera HSC and a personal computer PC, the optical spectrum of the jet radiation in the UV range was measured. Evaluation of the plasma effect on cells was carried out at the IBR RAS through the estimation of the cell viability and the rate of the cell proliferation 24 hours after the treatment. Cell viability was assessed by staining cells with a vital dye, propidium iodide. Proliferation rate was assessed by measuring the amount of the specific protein ki-67, which is produced during cell division and is a marker of tumor growth in oncology.

3. Results

Figure 3 (a) shows a typical oscillogram of the voltage at the electrodes of the plasma reactor and an oscillogram of the jet current. The corresponding $Q-V$ curve is presented in figure 3 (b).

![Figure 3](image)

Figure 3. Waveforms of the voltage at the plasma reactor and the jet current (a) and $Q-V$ of the plasma jet (b).

Based on these data and the jet diameter, the jet power was estimated. With a distance to the object of 40 mm and the diameter of the jet taken equal to the inner diameter of the plasma reactor tube, the power density was 0.72 mW/cm$^2$.

Preliminary studies of the jet spectrum depending on the parameters of the power pulses, the distance to the source, and the presence of the grounding plate under the target showed that the intensity of the OH and N$_2$ lines, which were responsible for the jet propagation and the main chemical reactions, increased significantly depending on the power supplied to the discharge, i.e. significantly depended on the amplitude and repetition rate of the pulses [9]. Figure 4 shows a typical jet spectrum at a pulse amplitude of 7 kV and a repetition rate of 3 kHz.

Despite the fact that the potential energy of metastable argon states $4^3P_2$ and $4^3P_0$, 11.55 eV and 11.72 eV, were less that the ionization energy of H$_2$O molecule (12.6 eV) [10], it was observed an emission from OH transition. It is likely to depend on the presence of the conductive grounded target. According to [11] OH radical can be obtained due to the local electric field near the liquid surface through the following reaction: $H_2O+e^- \rightarrow OH+H+e^-$. It was also proved by our previous works at different power dissipated in plasma at the presence of the targets with different conductivity [9].

Data on cell viability and proliferation rate one day after the plasma exposure are shown in figure 5. From the data obtained, it can be concluded that treatment of cell medium with plasma after a day does not cause significant apoptosis of both healthy and cancer cells. The number of dead cells in this case can change by 2%. But, as can be seen from the figure 5, the plasma strongly effects on the proliferation rate of cancer cells. The mechanism of this effect is unclear and it should be studied in detail in the future.
4. Conclusion
The cells of human skin were treated in vitro by the argon plasma jet with the pulse power supply, developed at IEE RAS. Due to the opportunity of smooth regulation of the input power there was chosen the optimum regime for generation of high OH concentration. The plasma treatment of cells for 1 minute did not cause essential changes in viability rate of the both lines. But there were revealed big differences in proliferation rate of human keratinocytes HaCaT and cancer cells A431 after the plasma treatment. The proliferation rate of cancer cells became almost two times less after the plasma exposure.

Thus, it can be concluded that by choosing the optimum plasma power density it is possible to achieve a noticeable decrease in the proliferative activity of cancer cells, while providing a minimal invasive effect on healthy keratinocytes.

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