Interaction of cold atmospheric plasma jet with bio targets: different sensitivities of human cell lines

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Abstract. Cold atmospheric plasma (CAP) jet device is developed and CAP jet was applied for the treatment of normal and cancer cells. The characteristics of plasma jet consisting of a sequence of cathode directed streamers are studied in the experiment and numerical fluid model simulations. A content of mixture of active radicals and ions due to plasma enhanced chemical reactions is calculated with 0D chemical model near the surface of biotarget. The evolution of spatial distribution of the streamer parameters (electric field, electron and ion density, electron energy) delivered by the streamer to the biotarget is calculated. The results of cell viability analysis of normal cells, HEK 293 (kidney embryonic stem cells) and malignant cells, A431 (skin carcinoma) demonstrate the different sensitivity to the indirect CAP jet treatment.

1. Introduction
Recently the plasma devices generating the streamer type of breakdown in a mixture of noble gas and air are widely used in medicine [1]. The sinusoidal voltage with an amplitude of 2.5–10 kV applied to the electrode inside of dielectric tube initiates the periodic streamer formation near the powered electrode and propagation outside of tube. Usually the noble gas (helium, argon) is pumping through the dielectric tube since the critical voltage of the breakdown in the noble gas is essentially lower that in the atmospheric air. The streamers propagate over a laminar jet of noble gas and induces multiple chemical reactions in the mixture of nitrogen, oxygen, water vapor and noble gas some distance from the dielectric tube inlet. The biotarget treated with the plasma jet is exposed to this chemical cocktail of different radicals, ions and the large electric field delivered by the streamer head. In [2], the plasma treatment was found to act differently on healthy and malignant cells in some cases, killing the latter more efficiently. The selectivity of cold atmospheric plasma (CAP) jet treatment of normal and cancer...
cells is still an open question widely discussed recently. In this work, in experimental and theoretical study we analyse the streamer formation, propagation and interaction with bio target. The plasma enhanced chemical reactions and evolution of active species are simulated with 0D chemical model. The effects of CAP exposure time on the viability of healthy and malignant cells are presented.

2. Experimental study of CAP jet
The photo of the plasma device is shown in figure 1. A tip of the powered electrode is seen in figure 1 as a bright spot. The radius of dielectric tube is 0.4 cm. The capillary with radius of 0.2 cm is inserted inside the dielectric tube closer to the inlet. The gas (argon or helium) is pumped through the dielectric tube with a rate of 3.5–8 l/min. The frequency of applied voltage is 25 kHz and the voltage amplitude is 3–5 kV. The grounded electrode is a metal ring near the tube inlet. The biotarget or culture media is placed 2–3 cm apart from the tube inlet.

![Figure 1. Plasma device generating streamers in helium.](image)

![Figure 2. Calculation domain for simulation of dynamics of streamer formation and propagation.](image)

3. Theoretical study of CAP jet dynamics and plasma chemistry
The plasma dynamics is simulated in the framework of the fluid model approach (see [3] for calculation details). The calculation domain with the cylindrical symmetry is shown from figure 2. In the experiment and simulations, the streamer forms near the tip of powered electrode during a positive cycle of the applied alternating voltage. The streamer propagates first inside of the dielectric tube and then over noble gas jet, approaching the target surface. The spatial electric field distribution over an area of plasma jet-water contact is shown in figure 3. A water layer (the dielectric constant $\varepsilon_w = 80$) with $H = 1.6$ mm covers the cells colony which is mimicked by a dielectric object with $r = 2$ mm, $h = 0.85$ mm and $\varepsilon_s = 15$. 


Figure 3. Spatial distribution of electric field (in kV\cdot cm^{-1}), U = 5 kV.

As seen from figure 3, the electric field strength reaches 5 kV\cdot cm^{-1} over cells colony.

The evolution of some species including hydrogen during plasma device operation is shown from figure 4. The results were obtained on the basis of 0D chemistry model including 872 reactions and 98 components. The electron temperature and concentration were taken from 2D plasma dynamics calculations and approximated by periodic functions.

Figure 4. Evolution of components of hydrogen group and electron temperature.

4. Experimental study of sensitivity of bio targets to the CAP treatment

The human skin carcinoma cells A431 and normal embryonic kidney cells HEK 293 were seeded in 96-well flat-bottom plates at a density of 5000–10000 cells per well in 100 \mu L of DMEM F12 media.
The same DMEM F12 media in 12-well plates was exposure to the CAP jet (with buffer gas argon) during 5, 10 and 15 min. After that, 100 μL of treated media was added to the cells of each well of 96-well flat-bottom plates with cells.

![Graph](image)

**Figure 5.** Live cells (%) as a function of exposure time: a) – A431 human skin carcinoma; b) – HEK293 human embryonic cells. Indirect CAP treatment. Control – cells treated with non-irradiated media. Data are presented as mean of three independent experiments ± SD

To analyse the effect of the CAP jet on the cells, MMT assay of cell viability was performed after the treatment. The viability of A431 cancer cells and HEK293 normal cells shown in figure 5 was quantified at 24 hours after CAP exposure at 4.9 kV. As seen in figure 5a, a greater than 90% reduction in A431 (skin carcinoma) cell viability was registered for the case of 5 min indirect treatment. For the cases of 10 and 15 min, A431 cells’ viability is almost the same. In contrast to A431 cells, HEK293 normal cells were more resistant to CAP treatment (see figure 5b). Indeed, the data showed that 80% stayed alive after the indirect treatment with the CAP-exposed media (5 min).
For the experimental conditions of 10 and 15 min of CAP exposure, the viability of HEK293 cells decreases to 40% and 38%, respectively, and these values are also lower than for A549 cells at the same treatment conditions.

5. Conclusion
The operation characteristics of plasma device generating cold atmospheric plasma jet are studied in the experiment and in numerical simulations. The spatial distribution of the plasma parameters (electric field, electron and ion density, electron energy) delivered by the streamers to the biotarget and the production of active radicals and ions in plasma enhanced chemical reactions were calculated. The different sensitivity of normal and cancer cells on CAP jet treatment has been demonstrated. The results of cell viability obtained with MTT assay showed that the indirect CAP jet treatment acts stronger on malignant cells, A431 (skin carcinoma) than on normal cells, HEK293 (kidney embryonic stem cells). In this type of treatment, first, the media was exposed to the CAP jet during a given time and then this media was added to the cells. We found, that for the malignant A431 cells, the indirect treatment with the media irradiated for 5 min is sufficient to reduce the number of survivals to 10% compared to the control case.

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