Effect of low temperature plasma of atmospheric pressure on single-cell model organisms of ciliate *Paramecium caudatum*

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Abstract. We studied low-temperature plasma effects on *Paramecium caudatum* using the original electric arc plasmatron. The decrease in the number of experimental cells has been shown, which may indicate membrane destructive pathology due to oxidative stress induced by the plasma impact. It is concluded that reactions at the population level of *Paramecium caudatum* are effective indices of primary information about plasma radiation cytotoxicity.

1. Introduction

The modern state of medicine indicates the need for radical changes in the medical process, because many traditional medicines leave in the body toxic substances, pharmacological preparations have a number of side effects (toxic and allergic reactions, change immunobiological properties of the body, etc.), and then, viruses, pathogenic biological entities exhibit greater adaptability to recently highly effective antibiotics [1, 2]. In the process of chemotherapeutic and antibiotic therapy it is necessary to know well antimicrobial activity of applied medicines, in addition there is a danger of secondary resistance to the drug used, resulting from spontaneous mutations of viruses and pathogenic microorganisms.

Recently, developing rapidly an extensive interdisciplinary area of plasma medicine, free of the aforementioned shortcomings, because of the simultaneous presence of plasma many medical factors leading to a synergistic effect of exposure [3, 4]. Naturally, consideration of this problem it is important to lead on three levels: cell, tissue and organism. Choice of the object of study of this work is due precisely to these considerations.

Choice *Paramecium caudatum* as on object of this study is due to the following criteria. Thanks to a combination in *Paramecium* signs of the cell and organism it can be studied as cellular and organismic form of reactions to external influence. Compared to others protozoa groups these infusoria have the most complex structure and are distinguished by the variety and complexity of functions.

It is of great importance of type of plasma source used for creating a technological environment characterized by many parameters distributions of which in importance to a large extent depends on the specific biomedical impact object.

The source of plasma in this work was electric arc plasmatron of direct current a new type with a high service life and a plasma jet practically free from metal particles of electrodes detailed in the work [5] and references cited therein.
2. Materials and Methods

The basic design components of the plasmatron are water-cooled rod cathode and nozzle anode. To protect the cathode from oxidation into the gap between the cathode and the anode an inert gas is supplied (argon) in the atmosphere of which an arc discharge is excited. Copper anode structure provides distributed nature anodic arc binding by filing in the gas channel of the anode process gas (oxygen, air, freons, etc.). This gas arrives in a circular slot moving to the axis of the plasmatron in radial direction. In the zone of its mixing with the shielding gas of the cathode there is a vortex zone in which is formed fixing arc length distributed anode binding surrounding anode from the outside anode nozzle channel. This feature leads to a sharp decrease in the current density of the anode and reduce the rate of its erosion.

To get information on characteristics technological plasma jet in the functional zone diagnostic methods were used: optical emission spectroscopy, thermal imager and chemical barbater. The following data were obtained [5]: plasma radiation spectrum in the range 200 – 700 nm, presence of chemical radicals NO\textsuperscript{-}, OH\textsuperscript{-}, N\textsubscript{2}\textsuperscript{+} and their density, gas temperature distribution in the functional zone of the plasmatron.

The study of the low-temperature plasma effect was carried out on the cell culture of *Paramecium caudatum*, which showed effectiveness in determining the role of oxidative stress [6]. In the experiment, test -tubes with ciliates were placed under the plasma jet at a distance of 15 cm. Time of exposure of plasma radiation on the cells was 0.5 min, 1 min, 3 min and 5 min. After the action of the plasma jet, we implemented a microscopy with a video recording of morphological and functional changes in cells from the test samples. 2.5 h and 24 h after irradiation, we determined the number of living cells.

Cells that have been outside the plasma impact zone during the equivalent time and non-irradiated intact cells from the vial with stock culture served as a control. Ph measurements were carried out at all stages of the experiment. Selection of culture from one vial and filling of experimental vessel with sample with cells was carried out up to 5 times.

The data was processed statistically with the calculation of mean for the whole sample, mean error, variance using the functions included in the Excel 2013 software package. After checking the distribution for normality, the significance of the differences between the groups was assessed by t-criteria using Statistica 10.0. The differences were considered statistically significant at p < 0.05.

3. Results and Discussion

It is established that the action of the plasma jet decreases the number of living cells, the severity of the cytotoxic effect depends on the time of exposure to the plasma flow. The effect of low-temperature plasma for 0.5 and 1 min did not cause a statistically significant decrease in the number of living cells (Fig. 1).

Immediately after irradiation for 3 minutes, the number of living cells decreased by 2 times (p<0.01), and after 5 minutes – by 14.5 times compared with intact cells (p< 0.005). After 2.5 hours of experience in the tubes under the action of atmospheric plasma 3 min and 5 min, the cell survival was 0 %. In addition, changes in cell shape have been recorded in all experimental samples. To the greatest extent, the number of atypical cells appeared at 3 and 5 minutes exposure of the plasma jet. Apparently, charged plasma particles being in direct contact with the cell can accumulate on its surface and cause electrostatic potential. This can lead to changes in morphology, changing strength and stretching the cell membrane [7, 8]. In addition to the morphological changes of cells, after exposure to low-temperature plasma, there were disturbance in the mobility of *Paramecium caudatum*. Infusoria were moving slowly, rotational movement differed from the normal. It is known that the motor activity of Paramecia is largely formed on the basis of the ion channels built into the membrane.
of the motion organs (cilia), and reflects the functional state of the cell. It is known that *Paramecium caudatum* functions to maintain the membrane potential. As a result of reduction of membrane potential, cells move more slowly or rotate in one place around one end.

Fig. 1. The effect of plasma on the number of Paramecium caudatum cells through 2.5 h and 24 h (experimental samples exposure time is 0.5 min, 1 min, 3 min, 5 min; the distance of exposure to cells - 15cm from the plasmatron outlet)

The number reduction of *Paramecium caudatum* and morphological changes in cells indicate a destructive membrane pathology, in the occurrence of which the processes of free radical oxidation also play an important role. Free radicals can attack directly polyunsaturated fatty acids in membranes and initiate lipid peroxidation. The main effect of lipid peroxidation is a decrease in membrane fluidity, which significantly changes the functional properties of the cell membrane. It is possible that bioactive radicals NO·, OH·, N2· trigger a cascade of chain reactions, which lead to the development of oxidative stress, and as a consequence to the death of cells *Paramecium caudatum*.

To date, the direct mechanisms of action of low-temperature plasma have not been studied in detail, however, the works of Laroussi M. et al., 2003; Mogul R. Et al., 2003; Kalghatgi, et al., 2011 [8-10] indicate changes in the structure of membranes, DNA and enzymatic activity of cells.

### 4. Conclusions

Identification and analysis of biological effects, which are determined by physical effects at the cellular level and the body as a whole are of great interest. Our studies have shown that the reactions at the population level of *Paramecium caudatum* are informative enough to obtain primary data on the cytotoxicity of plasma radiation, which can be used in the future.
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