Influence of low-temperature atmospheric pressure plasma on the vital functions of microorganisms

V P Savinov¹,², V G Yakunin¹, O V Karpukhina²,⁶, A N Inozemtsev², V Y Timoshenko¹,³ and V A Riaby⁴

¹ Lomonosov Moscow State University, Faculty of Physics, 119991, Moscow, Russia
² Lomonosov Moscow State University, Faculty of Biology, 11991, Moscow, Russia
³ Semenov Institute of Chemical Physics, Russian Academy of Sciences, 115409, Moscow, Russia
⁴ Moscow Aviation Institute (National Research University), 125993, Moscow, Russia

E-mail: savinov1983@yahoo.com
E-mail: karpukhina.msu@yandex.ru

Abstract. Experimental research on inactivating various types of microorganisms with low-temperature plasma at atmospheric pressure demonstrates great advantages of this method. It is shown that when treating the cells with cold plasma for 3 minutes by using a compact plasma source (plasmatron) a partial or complete disruption of the functional activity of Paramecium caudatum cells occurs. We have found out that plasma treatment for 5 minutes suppresses completely the vital functions of pathogenic microorganisms such as Mucor, Fusarium, Aspergillus and Bacillus mesentericus cells.

1. Introduction

The prospects of research on the impact of low-temperature plasma on biological models of different levels of organization are undeniable. Developing effective methods of influence on pathogenic microorganisms is a current trend of applying low-temperature plasma in the field of biological and ecological safety [1]. One of the ways to use low-temperature plasma in biology, ecology and medicine is to inactivate bacteria and viruses and to destroy the cell structures of cancer cells/tumors, etc. [2-4].

To further advance this technology, it is necessary to study in detail the most complex physical, chemical and biological mechanisms of plasma interaction with cells, microorganisms and other biological objects. Of great significance is the type of plasma source used, which creates a technological environment characterized by many parameters, the importance of which largely depends on the specific biological object of exposure. The crucial active factors of low-temperature plasma include the influence of electromagnetic fields and irradiations as well as the impact of chemical agents. This is not only ultraviolet and infrared radiation, but also the flows of free electrons, ions, neutral atoms and molecules; this allows us to evaluate the impact of many simultaneously acting factors of low-temperature plasma [5, 6]. It is obvious that the impact of chemical agents of low-temperature plasma can activate the mechanisms of oxidative metabolism in the cells. At low intensity free radical oxidation is one of the types of normal metabolic processes [7]. Under normal conditions the reaction of formation and consumption of peroxides are balanced, and the peroxidation of cell
lipids occurs at a certain stationary level. Under various influences such balance can change and cause serious violations of biological membranes [7-8].

Currently special attention is paid to the biological effects of reactive oxygen and nitrogen (and some other) forms formed in low-temperature plasma in the main biological processes, such as cellular metabolism, proliferation, survival, etc. (see for example Refs. [10-11]).

In our paper we investigate an effect of low-temperature plasma on inactivation of various types of microorganisms.

2. Experimental technique
The source of low-temperature plasma at atmospheric pressure was an arc-discharge plasmatron with extended operational life and plasma jet practically free from metal contaminations (see Ref. [6] and references cited therein). The basic design components of the plasma source are water-cooled rod cathode and nozzle anode (see Figure 1). To protect the cathode from oxidation in the gap between the cathode and the anode an inert gas is supplied (Ar) in the atmosphere where an arc discharge is excited. The copper anode structure provides distributed anodic arc-discharge binding by filing in the gas channel 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 mixing with the shielding gas of the cathode there is a vortex zone. This feature leads to a sharp decrease in the current density of the anode and reduces the rate of its erosion.

2.1. Study of unicellular organism Paramecium caudatum survival
We studied the effect of low-temperature plasma on the cell culture of Paramecium caudatum. This unicell is a promising model for assessing the viability and metabolic changes in living systems in vitro not only under the action of chemicals, but also of physical (electromagnetic fields of different frequency and intensity) factors.

In the present work the exposure time of low-temperature plasma on experimental samples containing approximately the same number of cells of Paramecium caudatum (N=64) was 0.5 min, 1 min, 3 min and 5 min, respectively. The control was a sample with intact cells from a vial of uterine culture that were not exposed to the plasma jet impact. The experiments were conducted 5 times and the obtained experimental data were processed statistically using Excel, Statistica 6.0 application packages. The statistical significance of main differences was determined by the Mann–Whitney criterion.

2.2. Study of pathogenic microorganisms survival
In addition, we investigated the effect of low-temperature plasma on the development of fungi of Mucor, Fusarium, Aspergillus genera, and bacteria Bacillus mesentericus. The pathogenic microorganisms were grown on the natural nutrient medium.
Five samples were prepared for plasma treatment for 1, 2, 3, 4 and 5 min, as well as an intact sample. The samples were treated with the plasma flow torch at a distance of 15 cm. Then the samples were incubated in a thermostat for 15 days at 18 °C. The absence of growth of pathogenic organism colonies was confirmed by microscopic methods.

3. Results and Discussion

We have defined the viability and functional state of Paramecium caudatum cells in 2.5, 24, 48, 72 and 96 hours after exposure to factors that determine the biological effects of plasma. During the experiments, it was found that the action of the plasma jet decreases the number of viable cells, while the severity of the cytotoxic effect depends on the time of exposure to the plasma flow (Figure 2).

![Figure 2](image)

**Figure 2.** Number of Paramecium caudatum cells in 2.5 and 96 hours after exposure for 0.5, 1, 3 and 5 min and the intact group. The distance of plasma jet to the cells was about 15 cm (* statistically significant difference of values compared with the intact group, p <0.05).  

Oxidative stress is an imbalance between the formation of radical oxidants and their reduction, which affects the level of quasi-stationary concentration of short-lived components and the level of oxidation products. The normal functioning of cells and the body as a whole depends on the level of free radicals, the rate of oxidation and the content of oxidation products. Low-temperature plasma generates a large number of physically and chemically active factors that, when interacting with the cell, can cause its oxidative damage and, as a consequence, death [9]. Thus, after 24 hours in a sample exposed to plasma for 5 minutes, 100% cell death was recorded. The effect of low-temperature plasma for 1 and 3 minutes on Paramecium caudatum cells significantly reduced their number after 4 days.

In addition, we have established the modes of low-temperature plasma action which has a bactericidal effect on pathogens. A study of the bacteria Bacillus mesentericus survival in low-temperature plasma of atmospheric pressure showed that after the treatment of samples for 3 and 5 minutes the bacteria died completely (Figure 3). There was no growth of microorganisms after treatment in cold argon plasma for 10 days of cultivation in the natural nutrient medium.

The survival rate of mold fungi in the mode of exposure to plasma of atmospheric pressure showed that the complete inactivation of the microorganisms Mucor, Fusarium, Aspergillus is observed after processing the samples for 5 minutes (Figure 3).
4. Conclusion
The developed arc-discharge plasma source is used to generate cold plasma jets at atmospheric pressure, which can result in partial or complete disruption of the functional activity of *Paramecium caudatum* cells. The plasma treatment for a few minutes can be used as non-specific bactericidal action against such pathogenic bacteria as *Bacillus mesentericus* and fungi of the genus *Mucor, Fusarium, Aspergillus*. While the proposed plasma treatment seems to be promising to solve actual problems in various spheres of biosafety, further studies of the effect of low-temperature plasma on various biological targets are required to determine the role of different plasma components and the synergy between them in these effects.

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