The impact of air plasma jet on clinically significant consortiums of microorganisms

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Abstract: The inactivated effectiveness of the developed air plasma jet is demonstrated on epidemically significant microbiota from patients of medical institutions. Main bioactive components for air plasma jet are RONSs (e.t. NO, NO_2, HNO_2). The effect of the plasma jet was evaluated by inhibition zones on Petri dishes of surviving microorganisms.

Keywords: atmospheric pressure plasma jet, glow discharge, bactericidal components, inactivation, consortium of microorganisms

1. Introduction

To date, various promising sources of nonequilibrium plasma of atmospheric pressure for biomedical applications have been developed [1]. The main field of plasma medicine is the direct application of cold atmospheric plasma on/or in the human body for therapeutic purposes. A special place among them is occupied by plasma jets, which allow objects processing of various shapes and sizes outside the closed discharge volume. Plasma jets that are formed in a discharge in the air medium possess the greatest bactericidal effectiveness [2, 3].

In [4], we considered the effect of the air plasma jet on museum strains of microorganisms and proposed their consortiums as a more realistic model. It was shown that consortiums are more resistant to plasma treatment. In this article, we present the experimental results for inactivation of microbiota of medical institutions microorganisms by air plasma jet.

2. Experimental setup

The cold plasma jet is formed by direct current atmospheric pressure glow microdischarge in air [3]. The discharge chamber (Fig. 1, a)is composed of cylindrical 8 mm quartz tube, which has a rod copper cathode of 6 mm in diameter is coaxially disposed inside. Flat copper anode (4 mm in thickness) with a central hole (1.5 mm in diameter) is located at the tube tip. Interelectrode gap is fixed at 0.7 mm. Discharge is maintained by DC power supply with an output voltage up to 3 kV. A ballast resistor can be varied in the range of 1–300 kΩ. Air flow of 5 l/min into discharge chamber is provided through symmetrically arranged holes in cathode. Plasma generated in the discharge is blown out with the gas through anode hole into surrounding air. As a result, a glow of plasma jet of 2-3 mm in diameter and several centimeters in length is observed (Fig. 1, b).

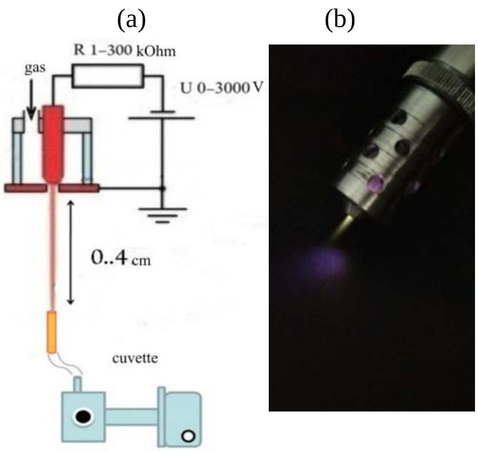

Fig.1. Scheme of the generation and diagnostics (a) and photo (b) of an air plasma jet.

3. Bactericidal components

Determination of the concentration of long-living chemically active species in the plasma jet is performed by IR absorption spectroscopy. The absorption spectra are registered using a Fourier IR spectrometer Nexus (Thermo-Nicolet) with a gas cell 186-0305 (Perkin-Elmer) with a controlled optical path from 11 cm up to 10 m and with germanium windows. A spectrum registration is carried out using a DTGS detector with a spectral range of 600-4000 cm^{-1} at a resolution of 2 cm^{-1} after 128 scans. The optical path gas cell equal to 135 cm has been selected. Gas was collected into the cell with a tube of 3.5 mm in diameter. The tube was placed along the jet axis and parallel to the gas flow direction.

The absorption spectrum of the bactericidal components bands of NO, NO_2, HNO_2 and N_2O are shown in Fig. 2 after removing the H_2O and CO_2 bands from the experimental spectrum.

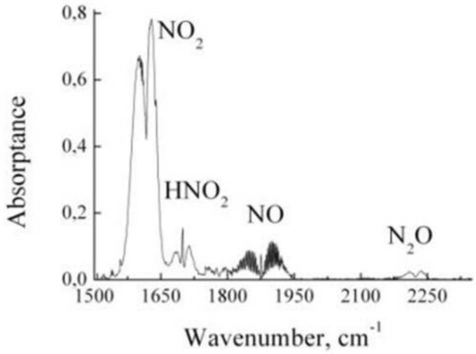

Fig. 2. Absorption spectrum of bactericidal component of air plasma jet.

Molar fractions of these active species are 400 ppm, 310 ppm, 85 ppm, 10 ppm near the anode and 40 ppm, 20 ppm, 10 ppm and 2 ppm at the zone of the jet impact on the bacteria (at 4 cm from anode).

Various bactericidal components are formed in microdischage and different zones of the jet [5].The main formation of nitrogen monoxide takes place in a nonequilibrium microdischage plasma where N, O, and OH are produced in plasma-chemical reactions involving electrons,. These processes are more efficient than thermal dissociation of nitrogen, oxygen and water molecules. As a consequence, the production of NO cannot be correctly described within the framework of the Zeldovich thermal mechanism and requires kinetic consideration with regard to plasma-chemical reactions. Since the conditions for thermal dissociation of nitrogen dioxide and nitrous acid molecules are provided in the active zone of the discharge, their concentration increases with a decrease in temperature downstream.

The absence of a noticeable concentration of ozone in the jet is apparently caused by the following reasons. Due to the low bond breaking energy, production of O_3 begins in the stream at a low temperature, i.e. after NO and NO_2. The fast three-body reaction of NO_2 formation "takes away" the oxygen atoms necessary to produce O_3. The nascent ozone molecules die in collisions with molecules of nitrogen monoxide and nitrogen dioxide, already having a significant concentration.

4. Use of an air plasma jet to inactivate consortiums

To establish informative criteria for quantifying the action of plasma jet on microorganisms, 7 strains of microorganisms isolated from patients of hospital with pneumonia, bronchitis and other respiratory system diseases were used as test models and type strains of *Escherichia coli ATCC 11229* and *Candida albicans ATCC 10231*. All studied strains differed in colony morphology and growth pattern.

The identification of the species of microorganisms isolated from the sputum of patients was carried out using a VITEK 2 compact analyser (bioMerieu, France).As a result of identification, it was found that isolates Nos. 374, 386, 171, 366 and 368 belonged to the species *Klebsiella pneumonia*, isolate No. 354 to the species *Escherichia coli*, and isolate No. 205 to the species *Klebsiella aerogenes*.

The following consortiums have been prepared for the study of the antibacterial effect of plasma jets on bacterial associations:

1) isolate No. 171 + isolate No. 205 (1: 1);
2) C. albicans ATCC 10231 + E. coli ATCC 11229 (1: 1);
3) isolate No. 368 + isolate No. 354 (1: 1);
4) isolate No. 368 + isolate No. 205 (1: 1);
5) isolate No. 386 + isolate No. 354 (1: 1);
6) C. albicans ATCC 10231 + No. 171 (1: 1);
7) isolate No. 368 + isolate No. 374 + E. coli ATCC 11229 (1: 1: 1).

To prepare consortiums, monocultures of microorganisms were sifted onto trypton soy agar (TSA) (Biolab, Hungary) and incubated in a thermostat at 37 ± 1 ° C for 18–24 hours. Prepared suspensions of microorganisms with a density of 10^8 CFU/ml in sterile saline using the turbidity standard. Suspensions of microorganisms were introduced in 0.1 ml in 100 ml of meat-trypton-soy broth (TSB) (Graso Biotech, Poland), and then kept at 37 ° C for 24 hours to form a stable community. At the end of the incubation time, the night cultures in TSB were centrifuged for 10 minutes at 2150 g, the supernatant was diluted with sterile saline to a concentration of 10^8 CFU/ml using the turbidity standard. The method of serial dilutions achieved the concentration of cells of microorganisms 10^6 CFU/ml. The suspension was applied in an amount of 0.1 ml on the surface of Petri dishes containing TSA and distributed with a sterile spatula.

Petri dishes coated with a consortium of microorganisms were treated with an air plasma jet at a distance of 4 cm between the anode and the microorganisms for 10 and 15 minutes. The mode and processing parameters did not change throughout all the experiments. Plasma-treated dishes with consortiums of microorganisms were placed in a thermostat and incubated at 37 ± 1 ° C for 18-24 hours.

Evaluation of the antimicrobial effectiveness of plasma treatment was carried out according to the size of the rounded zones of inhibition of the growth of microorganisms belonging to the consortiums on the surface of dense nutrient media in Petri dishes (Fig. 3, 4). The dishes were placed upside down on a dark matte surface and photographed. The assessment of growth inhibition zones was carried out in relative terms, in % of the total area of a Petri dish with a nutrient medium, by a decrease in visible growth of at least 90%. Very small single colonies detected within the inhibition zone only under special lighting conditions or magnification, as well as large atypical colonies were not taken into account. Free PC-Rect software [6] was used to determine the size of the zones of inhibition of growth of microorganisms on Petri dishes.

The results of the evaluation of growth inhibition zones are presented in Table 1. The sequence numbers of the consortiums in the table correspond to the numbers in Fig. 3 and 4.

As can be seen from the data presented in Table 1, the antimicrobial effect of plasma treatment was observed for all studied associations of microorganisms. The consortium consisting of isolates No. 368 and No. 205 (zone of growth inhibition 7.8% after 10 minutes treatment) was characterized by the highest resistance to the effects of an air plasma jet. The consortium of typical strains of *C. albicans* ATCC 10231 and *E. coli* ATCC 11229 turned out to be the most susceptible to treatment —

biodecontamination of microorganisms occurred at 10 minutes per 15.1% of the area of the Petri dish.

Table 1 - The results of the study of the antimicrobial efficacy of plasma irradiation against consortiums of microorganisms.

No.	Consortium	Inhibition zone (%)	
		10min	15 min
(1)	№ 368 + № 205	7.8	10.2
(2)	№ 368 + № 354	11.3	20.1
(3)	№ 386 + № 354	12.2	17.2
(4)	*C. albicans ATCC 10231 + E. coli ATCC 11229*	15.1	19.6
(5)	*C. albicansATCC 10231* + № 171	9.2	12.1
(6)	№ 171 +№ 205	11.6	13.2
(7)	№ 368 +№ 374 + *E. coliATCC 11229*	12.1	14.8

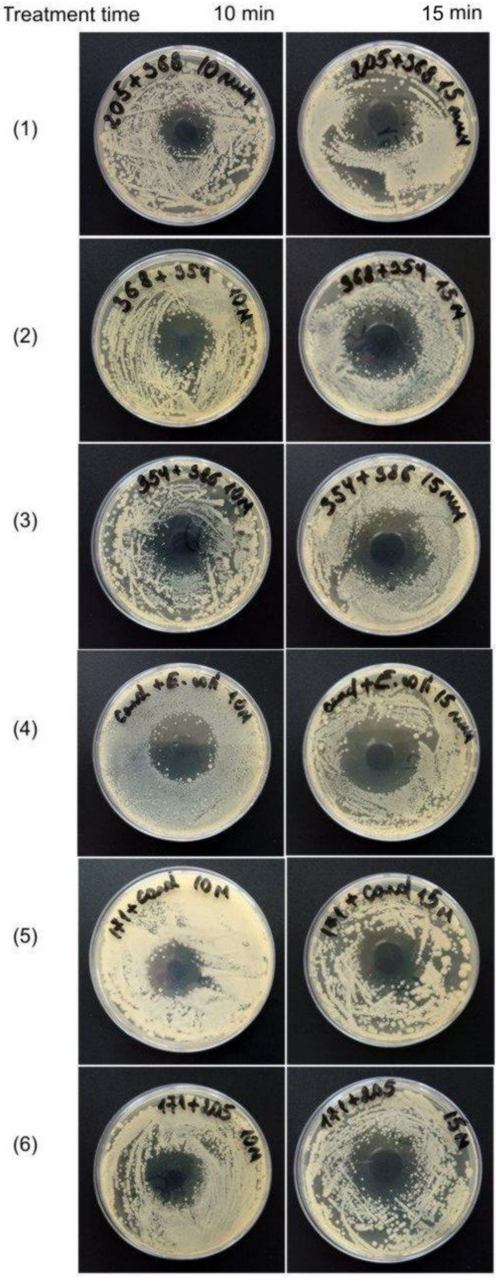

Fig 3. Inactivation of consortia of microorganisms consisting of two strains.

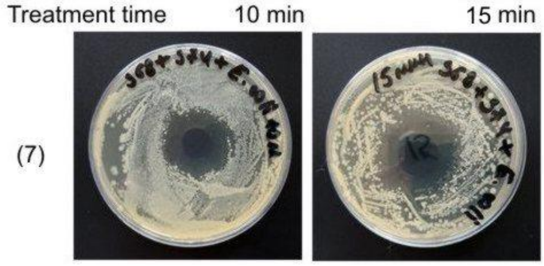

Fig.4. Inactivation of a consortium of microorganisms consisting of three strains

An increase in the processing time of Petri dishes to 15 minutes led to an increase in the area of the inhibition of growth of test strains on the surface of dense nutrient media. The largest increase in the size of the zone of inhibition was demonstrated by a consortium of typical strains of *C. albicans* ATCC 10231 and *E. coli* ATCC 11229 - the difference between 10 and 15 minutes was 4.51%, and also the consortiums comprising isolate No. 354 - growth inhibition zone increased by 4.5% for the consortium (isolate No. 386 + isolate No. 354) and 8.8% (isolate No. 368 + isolate No. 354).

Thus, consortiums of typical and natural strains of microorganisms used in this study provided reliable and illustrative results for evaluating the antimicrobial effectiveness of low-temperature atmospheric-pressure gas-discharge plasma, which indicates the feasibility of using such microbiological test models for studying the biological effects of human environmental factors physical and complex nature. Compared with the consortium of museum strains, which was considered in [4], these consortiums are more resistant to plasma action.

5. Acknowledgments

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6. References

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