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Inactivation of Consortiums of Microorganisms by Air Plasma Jet at Atmospheric Pressure

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ABSTRACT: We realize an atmospheric pressure glow discharge air plasma jet at direct current and detect the bactericidal components in plasma jet using optical emission and infrared-absorption spectroscopy. We investigate degree of inactivation of planktonic microorganisms and their consortium after treatment with air plasma jet. We then test the efficiency of plasma inactivation on the natural consortium and find a decreased ability of the surviving microorganisms to form biofilms.

KEY WORDS: atmospheric pressure glow discharge, plasma jet, IR-absorption spectroscopy, inactivation of microorganisms, plasma medicine

I. INTRODUCTION

Among the variety of low-temperature plasma, sources that may be promising for biomedical application are being developed.[1–3] A basic requirement for nondestructive medical applications is a cold plasma source at atmospheric pressure, wherein the gas temperature does not exceed 45°C. Special attention has focused on the development and application of plasma jets[4] to treat objects of complex shapes and sizes outside a closed discharge volume. However, despite extensive research, the mechanisms of plasma jet action on microorganisms remain poorly understood. In almost all articles about plasma medicine, the accent is on the bactericidal action of reactive oxygen and nitrogen species (RONSs; e.g., NO, O_3, OH, H_2O_2, etc.) that are generated by plasma jets.[5] Plasma jets at the discharge current of 30 mA were comparatively investigated for different working gas mixtures, including 95% He + 5% O_2, 95% Ar + 5% O_2, N_2, and air,[6] and it was established that an air plasma jet has more effective inactivating ability.

Microbiology has transitioned from traditional notions of microorganisms as single-celled organisms to the concept of microbial communities as integral structures regulating vital functions, depending on changes in environmental conditions. These factors must be considered when developing microbial test objects to assess the impact of plasma exposure.[7] Typically, consortia are natural associations, but strains with predetermined properties can also be selected.[8] The consortium of microorganisms CG/N-1,

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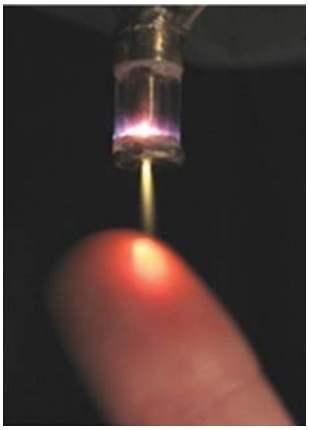

FIG. 1: Chamber and atmospheric pressure plasma jet in air

consisting of the strains *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, was selected as the test consortium for our study, which focuses on features of inactivation using atmospheric pressure air plasma jet.

II. EXPERIMENTAL SETUP AND DIAGNOSTIC METHODS

We formed a cold plasma jet (Fig. 1) using direct current (DC) glow discharge[9,10] in air at atmospheric pressure. The discharge chamber had a cylindrical 8-mm quartz tube, inside of which a copper rod cathode (6 mm in diameter) was coaxially disposed. A flat copper anode (4-mm thick) with a central hole (1.5-mm diameter) was located at the tube tip. An interelectrode gap remained fixed at 0.7 mm. Discharge was maintained by DC or ripple current power supply, with an output voltage of up to 3 kV. A ballast resistor could be varied within the range of 1–300 kΩ. Symmetrically arranged holes in the cathode provided an airflow into the discharge chamber of 5 L/min. Plasma generated in the discharge blew out with the gas into the surrounding air through the anode hole. This resulted in a glow of the plasma jet that was 2–3 mm in diameter and several centimeters long.[11]

The bactericidal components in the plasma jet were detected using optical emission and infrared (IR)-absorption spectroscopy. We used a high-resolution scanning monochromator MDD-500x2 (Solar, USSR) (with two diffraction gratings) that provided a photoelectric registration of the emission spectra of plasma jet. The inverse linear dispersion of the monochromator measured 0.5 nm/mm. Plasma jet spectra were registered in two directions: along the jet axis (end on) and side on. The vibration–rotation bands of NO, OH (A–*X*) and N_2^+ were present in the end-on spectrum of the jet. The side-on jet emission spectrum was radically different, with its intensity several orders of magnitude lower. In bactericidal range, we observed NO and OH bands as well as a broad NO_2 band, probably due to a chemiluminescent three-body reaction.[12]

We determined the concentration of long-living chemically active species in the plasma jet using IR-absorption spectroscopy.[13] In the absorption spectra of air jet, we observed NO, NO_2, HNO_2, and N_2O bands. The molar fractions of these active species at the zone of the jet impact on the bacteria were 40, 20, 10, and 2 ppm, correspondingly.

We could not detect hydroxyl using IR-absorption spectroscopy in our experiments. The reasons for this may be connected to the high values of reaction rate constants for OH, products of the plasma chemical reactions that were present in the jets,[14] and the components mentioned above. Therefore, we expected the concentration of OH radicals to drastically decrease as soon as forming stopped, that is, outside of the interelectrode gap. Thus, in our experiment, radicals were practically absent in IR spectra registered at a distance of 4 cm. Moreover, a measurement procedure using IR spectroscopy in our case has a longer duration than the life of OH radicals.

A suspension of microorganisms with an initial concentration of 10^5 colony-forming units (CFU)/mL was deposited uniformly onto the surface of a dense, undifferentiated, nutrient medium to measure inactivation of bacteria in Petri dishes. The temperature of the plasma jet in the bacteria exposure zone did not exceed 30°C. Inactivation ability was determined using a colony counting method. The identity of bacterial growth on untreated and pretreated agar plates by plasma jet allowed us to infer that inactivation occurred due to plasma impact on the cells and not to changes in nutrient medium properties.

The bacterial consortium and stains of consortium microbe components were seeded in the undifferentiated nutrient agar and in three differential diagnostic mediums (Endo agar, Federal Budget Institution of Science, State Research Center for Applied Microbiology and Biotechnology, Russia; Baird–Parker agar, Biolab Zrt., Hungary; and King B medium, Carl Roth GmbH + Co. KG, Germany). In addition to the quantitative accounting of microorganisms, a control of morphological properties of the surviving microorganisms was carried out as well.

III. RESULTS AND DISCUSSION

The efficiency of plasma exposure is estimated to be the percentage of surviving cells in strains of the planktonic microorganisms and their consortia (Fig. 2a). It was found that the characteristic *D* times, defined as the time interval during which the number of surviving microorganisms is reduced by 10, were rather different. For monocultures of *S. aureus*, *E. coli*, and *P. aeruginosa*, the characteristic *D* times were all ~3 min, but *D* time for the consortium of all three of the strains of microorganisms exceeded 5 min.

For an active area determination of plasma jet impact on microorganisms, each monoculture was fitted into separate Petri dishes. Treatments by plasma jet were carried out at a distance of 4 cm from the anode, with the same exposure time of 10 min. The areas of microorganism sterilization were ~27%, 30%, and 29% of the total area of control Petri dishes with *E. coli, P. aeruginosa*, and *S. aureus*, respectively.

In the experiments, the consortium CG/N-1 contained microorganisms in the ratio of 1:1:1, and a general concentration of 10^5 CFU/mL was used. Plasma jet exposure was performed under the same conditions as in the previous cases (4 cm from the anode and

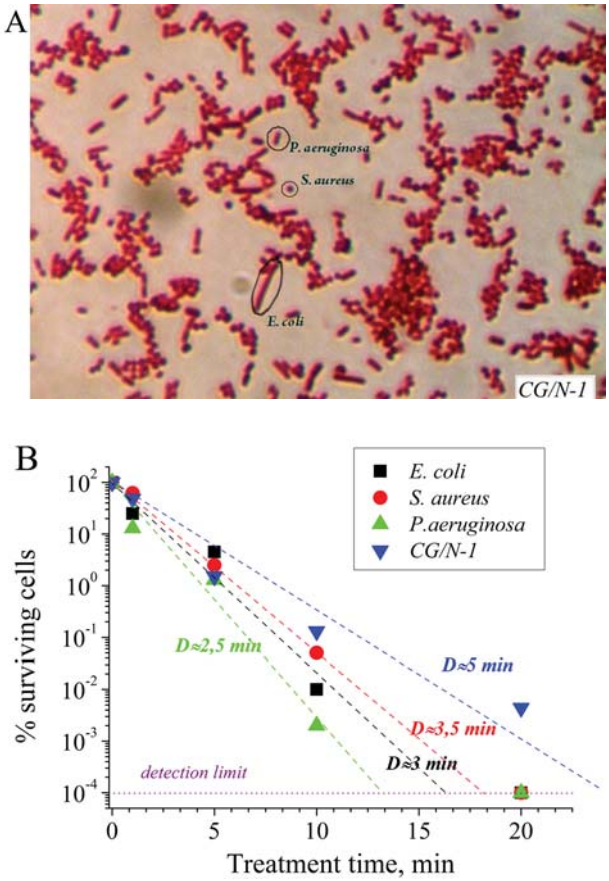

FIG. 2: (a) Consortium through a microscope; (b) inactivation curves of individual microorganisms and their consortium as a result of plasma jet exposure

for 10 min). The consortium was placed on the common nutrient medium and differentiated nutrient media, corresponding to a specific microorganism. The areas of sterilization measured ~20% on the general nutrient medium and ~10%, 15%, and 10% on the differentiated nutrient media for *E. coli*, *P. aeruginosa*, and *S. aureus*, respectively.

It should be noted that the influence area of plasma jet for the consortium on the general nutrient medium was 1.5–2 times larger than that on the selective nutrient media. This may be associated with a higher viability of consortium on the differentiated nutrient medium. Figure 3 demonstrates the inactivation difference of consortium and monoculture: the inhibition zones of monocultures of microorganisms and their consortium differ by approximately twofold, confirming a greater stability of consortium of microorganisms to plasma influence. Thus, we established that the consortium of microorganisms, consisting of strains *S. aureus, E. coli*, and *P. aeruginosa* in a ratio of 1:1:1, was steadier against plasma influence than the monobacterial population. Efficiency of

FIG. 3: (a) Control, (b) *Pseudomonas* monoculture, and (c) consortium CG/N-1 at identical media after treatment by plasma jet

plasma influence depends on the structure of consortium and the initial concentration of microorganisms on the substrate.

As an example of a treatment of natural consortium, several toothbrushes were tested. One individual used the toothbrushes daily, for 3 mo, and in parallel. We identified the bacterial consortium on the toothbrushes to consist of *Staphylococcus*, *Enterococcus*, and *Enterobacter* (Fig. 4). The general concentration of microorganisms was ~$4.5 \cdot 10^4$ CFU/sample. These strains are characterized by their stability to the external environment and their ability to form biofilms. In this experiment, we used a discharge current of 100 mA when the plasma jet temperature was 60°C. Concentrations of some of bactericidal components included NO, 1100 ppm; NO_2, 240 ppm; and HNO_2, 150 ppm. After samples were treated with plasma for 20 min, the concentration of microorganisms decreased to $1.3 \cdot 10^2$ CFU/sample. At 20 min, *Enterobacter* was not revealed at all, only a few bacteria of *Staphylococcus* were present, and the concentration of

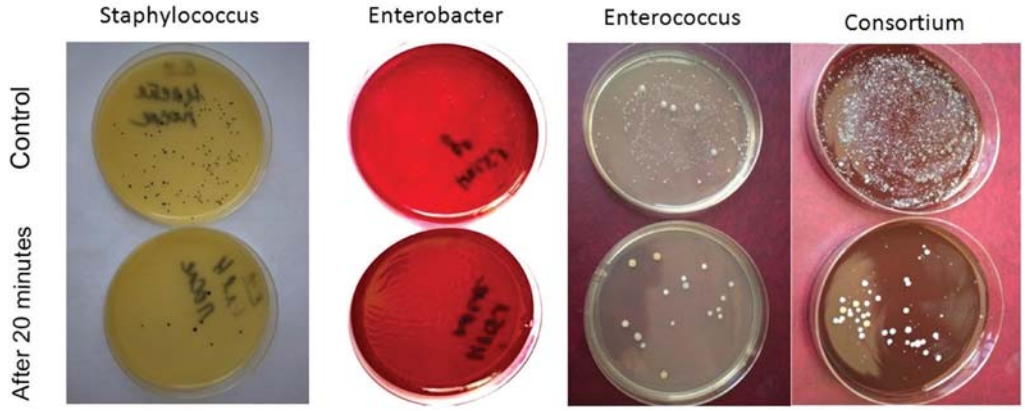

FIG. 4: Control and treatment Petri dishes for bacteria consortium. Several toothbrushes were used by one person, daily, for 3 months, and in parallel.

Enterococcus cells decreased by 97%. Thus, the ability of the survived microorganisms to form biofilms decreased.

IV. CONCLUSIONS

We realized the atmospheric pressure glow discharge air plasma jet at DC. Bactericidal components in plasma jet were detected using optical emission and IR-absorption spectroscopy. The main bactericidal components of plasma jet in air were NO, NO_2, HNO_2, and N_2O, and we determined axial distributions of the active components along the plasma jet.

We also investigated the inactivation effect that occurred during air plasma jet treatment of planktonic microorganisms and their consortium. We demonstrated that the consortium CG/N-1 is more resistant to plasma exposure than certain strains of microorganisms. For monocultures of *S. aureus*, *E. coli*, and *P. aeruginosa*, the characteristic *D* times were practically the same at ~3 min. However, *D* time exceeded 5 min for the consortium of the three microorganism strains.

Treatment of real samples (toothbrushes) showed good results for inactivation of microorganisms (consortium of bacteria: *Staphylococcus*, *Enterococcus*, *Enterobacter*) located on the brushes. The concentration of bacterial cells after plasma treatment decreased by 97%, and the ability of the surviving microorganisms to reduce biofilm formation occurred as well.

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