Investigation of the role of chemically active radicals in the antibacterial properties of a low-temperature plasma jet at ambient pressure mixed with argon and air

N A Ashurbekov¹, Z M Isaeva¹, K M Rabadanov¹, G S Shakhsinov¹, A A Murtazaeva¹² and E K Israpov²

¹Dagestan State University, 43a M. Gadjiev St., Makhachkala, 367001, Russia
²Institute of Physics, DFRC of RAS, 94 M. Yaragsky St., Makhachkala, 367003, Russia

E-mail: nashurb@mail.ru

Abstract. The work is devoted to the study of a low-temperature plasma jet of atmospheric pressure in a mixture of air with argon, used in plasma medicine. The intensities of the spectral emission bands of OH (309 nm), N₂ (337 nm), and N₂ (356 nm) in different parts of the plasma jet were investigated depending on the argon flow rate and the amplitude of voltage pulses at the electrodes of the dielectric barrier discharge. The antibacterial effect of such a plasma jet on the surface of biological tissues has been experimentally investigated. Numerical simulation of ionization processes in the studied plasma source has been performed in the COMSOL Multiphysics software using. The dynamics of the distribution of excited molecules in the plasma torch as well as the kinetic plasma processes inside the plasma torch affecting the spatial distribution of its main characteristics have been analyzed.

1. Introduction

Cold atmospheric-pressure plasma (CAPP) generates a large number of chemically and biologically active molecular complexes [1-9], without significantly increasing the temperature of the surrounding gas. The presence of such particles makes CAPP applicable in various fields. Currently, the main areas of CAPP use are biomedical applications and materials science. The former include, for example, sterilization [1, 6], cancer treatment [9], acceleration of biological tissue regeneration and wound healing [10]. Thus, the possibilities of using a plasma jet in medicine are quite wide [11].

To create a plasma jet, an inert gas (helium or argon) is usually pumped through the air at atmospheric pressure in the discharge gap. An important feature of such a plasma jet is the generation of chemically active nitrogen- and oxygen-containing molecular complexes. A few percent of gas impurity may be added to the flowing gas in order to change the concentration of chemically active substances formed in the discharge. The effect of the impurity on the discharge characteristics has been investigated in a number of studies. For example, in [12-14], the dependence of the propagation velocity of the plasma front was investigated depending on the composition and velocity of the gas flow when a percentage of another gas was added to the flowing gas.

The investigation of various chemically active particles behavior in a plasma jet is of significant interest. Such studies were performed using various methods, including laser-induced fluorescence.
and mass spectrometry [17]. Oxygenated radicals and nitrogen-containing radicals are reactive species of greatest interest for medical applications [18, 19]. Oxygenated radicals affect cell metabolism, cell membranes, oxidation of lipids, proteins and DNA. The action of nitrogen-containing radicals is reduced to the effect on cells, and on cellular signaling. In addition, the effect on biological tissues of positive and negative ions, for example, $\text{N}_2^+$ and $\text{O}_3^-$, has been studied in [17, 20]. Basically, the role of ions is reduced to the catalysis of oxidative processes in the presence of oxygen-containing radicals.

One of the many neutral particles that have received a lot of attention is the OH molecule because of its importance in biomedical applications [16, 21, 22]. It was shown that OH is mainly formed inside the discharge capillary with water vapor impurities. The main chemical processes of OH generation were studied in [23].

Also, many works were devoted to the study of atomic oxygen [24-26]. For example, in [24] it was found that the maximum density of O is obtained at the exit from the capillary, while moving away from the exit of the capillary, the density of O decays rapidly.

The generation of ozone is also of considerable interest to researchers [27-30].

The aim of this work is to experimentally study the plasma jet of a barrier capillary pulse-periodic discharge of atmospheric pressure in a mixture of air with argon from the point of view of its use for antibacterial purposes in plasma medicine.

The study was carried out using spectroscopic methods in combination with numerical simulation methods and is a continuation of work [31], where the study of the effect of a plasma jet on the optical parameters of biological tissues was carried out.

2. Materials and methods
The experimental model of an atmospheric pressure plasma generator in a mixture of Ar and air is described in detail in our works [31, 32]. The barrier discharge was formed in a quartz tube with an outer diameter of 7 mm and an inner diameter of 1 mm. As one of the electrodes, we used a metal rod 0.5 mm in diameter installed inside a quartz tube. At a distance of 60 mm from it, a second electrode was installed outside the quartz tube. It was a grounded metal ring 10 mm wide and with a diameter that coincided with the outer diameter of the quartz tube. The grounded electrode was located at a distance of 10 mm from the exit of the quartz tube. To form a dielectric barrier discharge, a high-voltage transformer-type pulse generator was used, operating in a frequency-periodic mode with a repetition rate of aperiodic voltage pulses of 100 Hz. The amplitude of the positive half-wave of the voltage pulse had an adjustable value in the range of 8-15 kV. The voltage pulse rise time was approximately 50 ns. Argon was pumped through a discharge tube filled with air at atmospheric pressure in the direction of the grounded electrode at a controlled gas flow rate.

To record the optical characteristics along the plasma jet, the radiation was transmitted to the input of the spectrograph using a quartz fiber. Further, the optical radiation of the plasma torch was projected through the lens onto the entrance slit of the MS3504i spectrograph (SOL-Instruments, Belarus). The spectral composition of the radiation at the output of the spectrograph was digitally recorded with an HS103H CCD camera (Hamamatsu, Japan) mounted at one of the outlet ports of the spectrograph.

To study the antibacterial properties of the plasma jet, the effect of plasma on biological tissues with pathologies was studied. Biotissue samples were positioned perpendicular to the torch at a distance of up to 15 mm from the exit from the plasma jet source. The argon flow rate was fixed at 0.32 lpm, at which the plasma torch reached its maximum length. Measurements of the plasma torch temperature showed that the gas temperature did not rise above 2°C relative to room temperature, which made it possible to exclude the thermal effect of plasma on biological tissues.

3. Research results
Figure 1 shows typical optical patterns of a plasma jet at different velocities of argon flow through air.
Figure 2 demonstrates the spectral composition of the plasma torch radiation. Analysis of this spectrum shows that it contains mainly the spectral lines of argon and molecular bands of nitrogen, as well as the hydroxyl group. The most intensive groups of molecular bands corresponded to N$_2$ (337 nm), Ar (763.5 nm), OH (309 nm). The molecular band with a wavelength of 309 nm indicates the presence of water vapor in the gaseous medium, where OH radicals are formed as a result of the dissociation of water molecules. The radiation intensities of molecular bands OH (309 nm), N$_2$ (337 nm), N$_2$ (356 nm) were experimentally investigated depending on the distance along the plasma jet (figure 3). Analysis shows a strong dependence of the intensities of the spectral bands of the radiation of the plasma jet not only on the distance from the outlet of the discharge tube, but also on the consumption of Ar and the amplitude of the voltage pulses across the discharge tube. This dependence makes it possible to choose such conditions in the discharge, under which certain radicals compounds necessary for a targeted effect on biological tissues are most efficiently generated.

Figure 1. Photographs of a plasma jet of atmospheric pressure in a mixture of air with argon at different flow rate of argon.

Figure 2. Characteristic spectral distribution of the plasma torch radiation intensity.

A number of works, in particular [33], also indicate that the emission spectra of low-temperature jets in a mixture of argon with air contain the lines of water vapor, including the OH group (309 nm) and nitrogen (314 nm) [34].
Figure 3. The intensity of OH spectral bands (309 nm) (a), N$_2$ (337 nm) (b) and N$_2$ (356 nm) (c) depending on Ar consumption at a distance of 2 – (a) and 5 – (b) mm from the outlet of the plasma jet source.
Our studies show that with distance from the outlet nozzle of the discharge tube, the ratio of the two indicated maxima changes. These measurements were carried out at an Ar flow rate of 0.32 lpm, at which the length of the plasma jet turned out to be maximum under the conditions of our experiment.

Figure 4 demonstrates that, with distance from the outlet nozzle of the discharge tube, the first maximum of 309 nm virtually disappears, while the second maximum at a wavelength of 314 nm increases. The appearance of additional maxima at 353 and 375 nm radiation wavelengths of molecular nitrogen is also observed. Studies have shown a pronounced decrease in the intensity of spectral lines of argon in the long-wavelength area of the spectrum along the plasma jet, while the intensities of spectral lines in the UV region of the spectrum increase.

Figure 4. Spectral distribution of the radiation intensity, taken orthogonal to the gas flow, at a distance from the nozzle (2 – a, 5 – b, 10 – c, 15 – d, in mm) at an Ar flow rate of 0.32 slm.

4. Investigation of the bactericidal effect

Studies of the effectiveness of low-temperature plasma flows application in the treatment of trophic ulcers and purulent wounds were carried out together with specialists from Dagestan State Medical University of the Ministry of Health of Russia on bioassays obtained from patients with purulent-inflammatory diseases of soft tissues. The investigated materials were fragments of biological tissues with dimensions of $10 \times 10 \times 5$ mm. After plasma probing, a bacteriological study of biological tissues for the resistance of microorganisms to antibiotics was carried out on the basis of Dagestan Antiplague Station Federal Bacteriological Center of Rospotrebnadzor (Russian Federal State Agency for Health and Consumer Rights). For each sample of biological tissues, three series of measurements were carried out. The final result for the test samples was determined by averaging serial measurements.
These studies have shown that when biological tissues are irradiated for more than 15 minutes, a number of bacteria, after plasma treatment, pass from the antibiotic-resistant to penicillin form to an intermediate form, which makes it possible to combine antibiotic treatment with plasma treatment. In addition, the maximum antibacterial effect was achieved when the biological tissue was located at a distance of about 10 mm from the end of the discharge tube issuing the plasma jet.

A more detailed description of the results of the analysis of the bactericidal properties of the investigated type of plasma jet will be the subject of a separate publication.

5. Numerical simulation
The numerical model consisted of two coupled sub-models: a flow dynamics model and a gas-discharge model. The flow dynamics model calculates the pressure, velocity field, and the density of the background gas components. The discharge model is solved for electron density, electron energy density, electric potential, and the densities of active particles formed during the discharge. This model was implemented using COMSOL Multiphysics software [35].

The input parameters of the flow dynamics model are the gas flow velocity in the capillary, its chemical composition and air humidity (if humid air is taken). The output parameters are the pressure of the mixture, the field of velocities, the densities of the constituents of the background gas, represented by the mass fraction of each component, and the gas temperature in cases where the temperature is of interest.

The model consists of \( N \) equations solved under the assumption of a laminar flow. In the model, the Navier-Stokes equation is solved together with the continuity equation, which in general form written as the following system of differential equations:

\[
\frac{\partial \rho}{\partial t} - \nabla \cdot \left( \rho \mathbf{u} \right) = 0 ,
\]

\[
\frac{\partial \rho}{\partial t} + \nabla \cdot \left( \rho \mathbf{u} \right) = 0 ,
\]

(1)

(2)

where \( \mu \) is the dynamic viscosity of the flow, \( \mathbf{u} \) is the velocity vector (m/s), \( p \) is the pressure (Pa), and \( I \) is the unit matrix.

Equation (1) is the equation of motion for the laminar flow in this case (equation of momentum balance), which is solved for the velocity of gas mixture \( \mathbf{u} \). Equation (2) is the continuity equation (the equation of gas mixture mass conservation), which is solved for the density of gas mixture \( \rho \) (kg/m\(^3\)).

It is also essential that the mass conservation equation for each \( j \)-th particle is added:

\[
\frac{\partial \rho}{\partial t} + \nabla \cdot ( \rho \omega_j \mathbf{u} ) = -\nabla \cdot \mathbf{j}_i + R_i ,
\]

(3)

where \( \omega_j \) is the mass fraction (dimensionless quantity), \( \mathbf{j}_i \) is the mass flow relative to the mass averaged velocity (kg/m\(^2\)·s), and \( R_i \) is the rate of appearance or loss of particles.

The plasma model is a hydrodynamic model based on the particle continuity equation with the drift-diffusion approximation, the drift-diffusion equation for the mean electron energy, and the Poisson equation.

Unlike electrons for heavy particles (Ar, Ar\( s \), Ar\(+ \)), the continuity equation is formulated using mass fraction instead of particle density.

The model equations are supplemented with initial and boundary conditions.

In the simulation, the following ratio of the plasma components was assumed: N\(_2\)/O\(_2\)/Ar = 78/20/2.

Figure5 shows the calculated characteristics of the spatial distribution of the argon flow velocities near the end of the discharge tube and the change in the argon flow velocity in the axial direction.

Calculations were made of the voltage of excited particles along the plasma jet as a function of the argon flow velocity and pulse amplitude. The simulation results are in qualitative agreement with the experimental results of the spectroscopic characteristics of the plasma jet.
6. Conclusion

Thus, a plasma jet of atmospheric pressure in a mixture of air with argon, created by a pulse-periodic barrier discharge in a capillary tube, predominantly contains excited particles of argon, nitrogen molecules, and also the particles of a hydroxyl group. The most intensive groups of molecular bands correspond to N₂ (337 nm), Ar (763.5 nm) and OH (309 nm). The molecular band with a wavelength of 309 nm is associated with the presence of water vapor in a gaseous medium, where OH radicals are formed as a result of the dissociation of water molecules.

The intensities of the spectral bands of the plasma jet radiation strongly depend not only on the distance from the outlet of the discharge tube, but also on the consumption of Ar and the amplitude of voltage pulses across the discharge tube. This fact makes it possible to choose the most effective conditions for the formation of certain radical compounds necessary for a targeted effect on biological tissues.

With a growing distance from the end of the discharge tube, the first OH spectral band (309 nm) practically disappears, but the second maximum at a wavelength of 314 nm increases. Additional maxima also grow at the wavelengths of molecular nitrogen emission with wavelengths of 353 and 375 nm.

When biological samples of trophic ulcers and purulent wounds are irradiated for more than 15 minutes, a number of bacteria, after plasma treatment, move from the antibiotic-resistant to penicillin group to an intermediate form. The maximum antibacterial effect depends not only on the characteristics of the barrier discharge, but also on the distance between the biological tissue and the end of the discharge tube.

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