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THE EFFECT OF COLD PLASMA ON THE INHIBITION OF THE GENUS STAPHYLOCOCCUS BACTERIA

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Annotation. Experimentally investigated the effect of cold plasma of dielectric barrier discharge on inhibition of bacteria of the staphylococcus family. It has been determined that when cleaning the coaxial discharge system in a fazzle with a gas flow rate of 180 l/h, a capacity of 40 W and a distance of 15 mm, full inactivation of the staphylococcus was reached within 10 minutes. For surface discharge, a similar effect was observed at 50 W, 10 mm distance and 15 minutes processing time.

Keywords: atmospheric plasma, coaxial discharge system, surface discharge, staphylococcus, inhibition

Introduction. Given the high pathogenicity and potential danger of staphylococci (Staphylococcus aureus, Staphylococcus saprophyticus) that can cause a wide range of infectious diseases, there is an urgent need to develop and implement new effective methods of disinfection, providing reliable inactivation of micro-organism data on different types of surfaces and objects in the external environment.

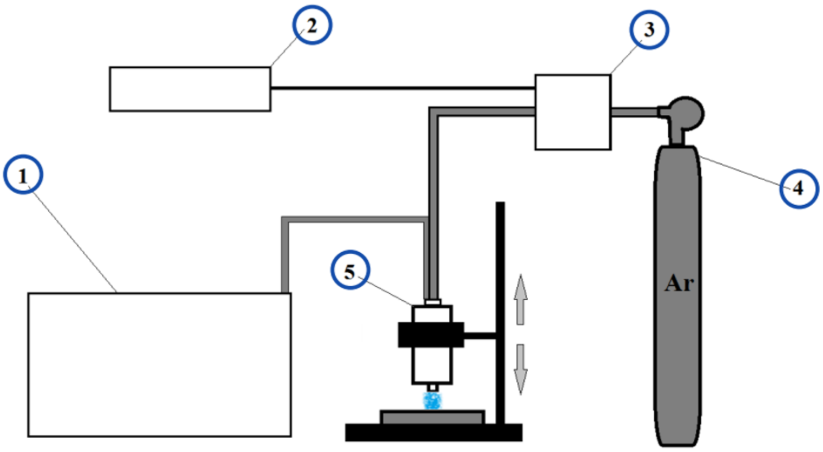
Bacteria of the genus Staphylococcus, including Staphylococcus aureus and coagulation-negative species (Staphylococcus saprophyticus), are among the most common human pathogens. They cause a wide range of diseases, from superficial pyroderma to severe invasive infections (pneumonia, endocarditis, osteomyelitis), as well as toxicosis. The threat is the spread of methicillin-resistant Staphylococcus aureus (MRSA) strains that are resistant to most β-lactam antibiotics [1]. The ability of staphylococci to form biofilms on the surface of medical implants and catheters further complicates therapy and requires new, nonscientific approaches to antimicrobial treatment.

Cold plasma is a partially ionized gas containing an unbalanced mixture of electrons, ions, neutral atoms, excited molecules and photons with temperatures close to the room. Plasma-generated active forms of oxygen and nitrogen, UV radiation and charged particles have a powerful bactericide effect, damaging the walls, membranes, proteins and DNA of microorganisms [2,3]. At the same time, the low plasma temperature allows biological tissues to be treated without risk of thermal damage.

It has been generally established that cold plasma can be an effective way to affect biological tissues in order to achieve various effects: antibacterial effect and inactivation of viruses, biocatalyst (aid in the decomposition of substances alien to the body – xenobiotics), for the treatment of diseases, associated with chronic inflammation and dangerous changes in the structure of proteins, which cause them to lose their function. Separately and actively discussed is the role of other plasma products – ozone and peroxynitrite – in bactericidal activity, that is, in direct destruction of microbes, which makes it a promising antiseptic. Thus, the interest in cold plasma applications in medicine and the need to develop effective and safe clinical protocols require deciphering key mechanisms of plasma influence on cells and tissue.

In this article, the author shows that a key role in the mechanism of inactivation is played by active forms of oxygen and nitrogen (in particular, atomic oxygen), generated by plasma and causing irreversible damage to cellular structures. The results confirm the prospects of using developed plasma sources to create effective and safe disinfection systems.

Basic Concepts. A system of atmospheric discharge plasma generation was used to clean the surface of the substrates. An schematic of the atmospheric plasma generation system is shown in Figure 1.



1 – power supply; 2 – control and power unit WP-12; 3 – gas flow regulator; 4 – gas cylinder (Ar); 5 – discharge system

Figure 1 – Diagram of an experimental coaxial type discharge system with dielectric barrier spread

Basic characteristics of the plasma generation system:
– type of discharge – dielectric barrier;
– discharge power >40 W
– working gas flow rate 70-350 l/min
– gas type – Ar

Among the many ways of creating a diffused dielectric barrier discharge, the simplest technical implementation is the coaxial configuration of the discharge system. When the gas passes through a tube in the area between the electrodes, atmospheric discharge is formed.

Two types of discharge systems were used to generate dielectric barrier plasma (DRB) at atmospheric pressure:
– coaxial discharge system;
– a planar discharge system.

The coaxial discharge system is a dielectric tube with metal electrodes inside and outside it (Figure 2). The quartz tube has an external diameter of 8 mm. The internal electrode is a hollow metal rod with a diameter of 1 mm. The outer electrode consists of a copper foil closely attached to a quartz tube, 10 mm wide.

The gas is continuously pumped through the discharge system, thereby removing heat from the discharge zone, excited particles and products of various plasma chemical reactions that typically impair the stability and performance of the discharge.

This discharge system provides a temperature in the processing area below 40 °C, as well as high stability by inhibiting streamer through a dielectric barrier between electrodes.

The planar discharge system is designed for handling large areas and consists of two flat electrodes, one of which is covered by a dielectric barrier.

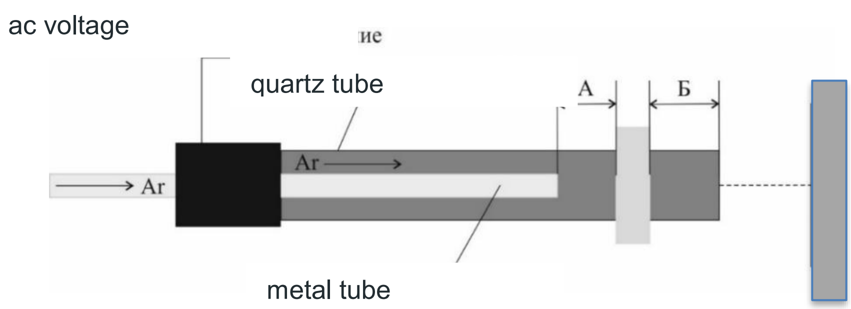


Figure 2 – Diagram of an experimental coaxial type discharge system with dielectric barrier spread

Growth of culture. Two species of bacteria of the genus Staphylococcus have been used as test cultures:

1 Staphylococcus aureus is a gram-positive cockle with a wide range of pathogenicity and clinical significance.

2 Staphylococcus saprophyticus – a causative agent of extracorporeal infections, also used as a model object.

Cultures are provided by the microbiology laboratory of the biological faculty of BSU.

The planting of microorganisms was carried out in a continuous layer on the surface of the dense nutrient medium in petri dishes using a bacteriological loop in the area of the burner flame. The flame heats the surrounding air, causing a local upward flow of air from the working surface, which reduces the likelihood of aerosols, dust and unwanted microorganisms that do not participate in the experiment. Continuous planting is a method of cultivating microorganisms in a solid nutrient medium, where the surface of the agar-agar is evenly covered by a microbial suspension to produce uniform monotone growth throughout the entire area of the petri dish.

The cultivation of staphylococcus requires a specially prepared nutritional environment, which is associated with control of growth conditions, reproducibility of results, safety and diagnostic informational. For the preparation of a nutritious medium, chicken fillet and agar-agar are needed as a source of proteins and amino acids, while agar-agar serves as a solid gel-forming component. The chicken fillet needs to be cooked, and the resulting broth needs to be filtered. In the hot solution, the agar-agar is dissolved so that when cooled it will freeze and form a floorboard surface suitable for growing colonies. The mixture is sterilized in alcohol flames to exclude foreign microorganisms, then the solution is distributed over sterile petri dishes where it is fixed and ready for stapling of the staph. Cultivated Staphylococcus aureus and Staphylococcus saprophyticus are shown in figures 3 and 4.

Petri cups with a seeded surface were treated with a plasma torch at a distance of 10–15 mm from the slit of the discharge system nozzle to the surface of the medium. The processing time (from 1 to 15 minutes) and discharge power (40 and 60 W) varied. The plasma gas flow rate (argon) was 130–180 L/h.

After processing, petri dishes were placed in a thermostat for incubation at the optimal temperature. The effectiveness of disinfection was assessed by a visual method that took into account the number of colonizing units grown on the surface of the nutrient medium. The degree of inhibition was defined as the percentage ratio of the number of colonies that grew in treated and control (untreated) cups.

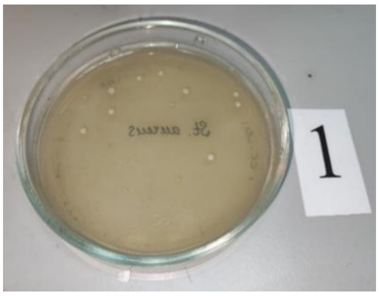


Figure 3 – Cultivated Staphylococcus aureus

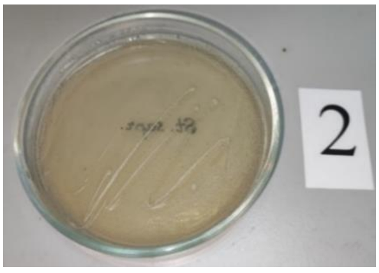


Figure 4 – Cultivated Staphylococcus saprophyticus

Results. Experiments on the disinfection of a staphylococcus-populated surface of a nutrient medium have demonstrated the high efficiency of the plasma sources developed. The static analysis did not reveal significant differences in plasma sensitivity between gold and saprophyte staphylococcus, allowing the data for both species to be considered together.

Figure 5 shows the dependency of the bacterial inhibition percentage on the processing time for coaxial system. It can be seen that the process of inactivation has a pronounced time dependency. At 40 W and 15 mm, full inhibition (100% bacterial death) was achieved after 10 minutes of processing. The increase of capacity to 60 W allowed a reduction in the time for full disinfection to 10 minutes, with inhibition of more than 90% of colonies observed after 5 minutes of treatment.

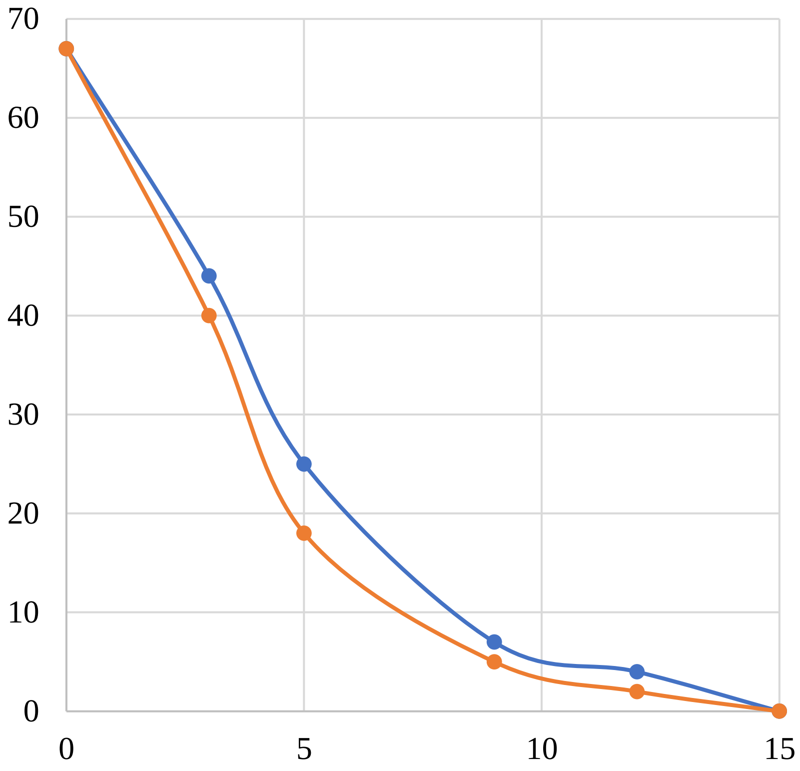


Figure 5 – Percentage of inhibition per surface treatment time

Conclusion. During the work, it was determined that for a coaxial discharge system with a gas flow rate of 180 l/h, a capacity of 40 W and a distance of 15 mm, full inactivation of the staphylococcus could be achieved in 10 minutes. For the surface discharge, a similar effect was observed at 50 W, 10 mm distance and 15 minutes processing time.

Thus, the numerical results confirm the energy efficiency and high antimicrobial activity of the dielectric discharge barrier plasma: optimal modes allow to form a stable flame up to 24 mm long, reduce energy consumption by 50%, and ensure complete disinfection of bacterial cultures in 10–15 minutes.

References

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