Study of plasma parameters of non-thermal plasma jet for antimicrobial treatment

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This study examined the effectiveness of inhibiting bacteria that cause skin diseases using a homemade plasma system known as a microwave-induced plasma jet (MIPJ) operating under atmospheric pressure (APPJ). The system utilized argon gas and a voltage source of up to 2.4 GHz to generate a non-thermal plasma. The inhibition efficiency of thermal plasma was tested against gram-positive (Staphylococcus aureus) and gram-negative (Pseudomonas aeruginosa) bacteria. These bacteria were exposed to the plasma column at various voltages (175-195 V), with a gas flow rate of 5 L/min, a 60-second exposure time, and a 5 cm distance between the plasma and the bacteria samples. The plasma system inhibited Gram-negative bacteria (Pseudomonas) by changing voltages during exposure. The rate of bacterial inhibition at 175, 180, 185, 190 and 195 volts was of 100%, 85%, 75%, 80% and 99%, respectively. When exposed to the plasma, gram-positive bacteria (Staphylococcus aureus) were completely inhibited for all voltages.

The MIPJ system proved to be an effective tool for treating different types of bacteria. The study also highlighted the impact of voltage change on bacteria inhibition, emphasizing that increasing the voltage generates highspeed particles able to penetrate the external structure of bacteria playing a crucial role in bacteria inactivation by the plasma jet.

Keywords: Staphylococcus aureus; pseudomonas; plasma jet (MIPJ); inhibition; treatment; voltages.

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1. Introduction

The term plasma was used to describe an ionized gas in 1927 - Langmuir was reminded of the way blood plasma carries red and white cells and the way an electrified liquid carries electrons and ions. Langmuir, along with his colleague Lewi Tonks, was researching the physics and chemistry of tungsten lamps, with the aim of finding a way to greatly extend the life of the filament (a goal which he eventually achieved). In the process, he developed the theory of plasma envelopes - the layered boundaries that form between ionized plasma and solid surfaces. He also discovered that certain regions of a plasma discharge tube exhibit periodic variations in electron density, which we now call Langmuir waves. This was the genesis of plasma physics. Interestingly, Langmuir's research nowadays forms the theoretical basis for most plasma processing techniques for manufacturing integrated circuits [1].

Plasma is defined as an assembly of charged particles, called electrons and ions, that collectively interact with forces exerted by electric and magnetic fields [2].

For example, the substance in stars or nebulae is plasma. There is also man-made plasma in our planet, used daily in industrial and medical applications [3].

Plasma has been used in medical investigations and has received a great deal of interest, especially in the fields of biological research, as it has shown a noticeable effect on the samples used [4].

In recent years, plasma has been widely studied and used in medicine. Non-thermal plasma is plasma generated by partial ionization of gases, allowing its use with low thermal damage under certain conditions. Typically, non-thermal plasma generators produce a mixture of reactive species, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), in addition to charged particles and ultraviolet radiation. These reactive species have shown various biological effects, including antimicrobial, anticancer, wound healing, tissue regeneration, plant surface treatment, and anti-inflammatory activities [5-6]. As a result, plasma medicine has emerged as a new field for the use of non-thermal plasma in medical treatments [7]. Non-thermal plasma treatments are relatively painless, non-surgical, and do not require the use of chemicals or harmful radiation. Given the wide range of studies on non-thermal plasma medicine, organizing and summarizing publications can help develop this field and provide guidance for future researchers. Understanding the developmental trends and new advancements in plasma medicine is of utmost importance. However, keeping pace with the rapidly changing landscape of biomedical research poses a challenge.

1.1. Bacteria

Bacteria are microscopic, usually unicellular, prokaryotic organisms that can be found everywhere. Bacteria can live inside the soil, in the ocean, and inside the human intestine. Bacteria do not have a membrane-bound, well-defined nucleus or other cellular organelles like eukaryotic cells. Bacteria can be beneficial, such as in the fermentation process, or dangerous, such as when they cause infection. Most bacteria are one micron in size. The cell membrane can be found in

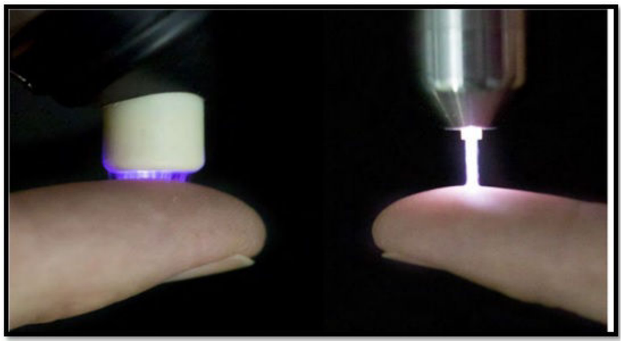

FIGURE 1. Photograph of DBD direct exposure (left) and Plasma jet indirect exposure (right) [13].

all bacterial cells in the form of a lipid bilayer. The membrane is an internal barrier between cells called the cytoplasm [8].

1.2. Staphylococcus aureus

Staphylococcus aureus is a gram-positive bacteria that appears under a microscope to be about 1 μm in diameter. Its cells form clusters like grapes, as in Fig. 1, as cell division occurs at more than one level. These bacteria are often found commensal with the skin, skin glands, and mucous membranes, especially in the nose of healthy individuals. Estimates indicate that 20-30% of the population carries these bacteria [9]. It is also called Staphylococcus aureus. There are some infections caused by Staphylococcus aureus, which include: skin infections (boils and abscesses) and impetigo. Staphylococcus aureus can also cause more serious infections, including meningitis (inflammation of the membranes lining the brain), osteomyelitis (infection of the bones and bone marrow), pneumonia (infection of one or both lungs), and septic phlebitis (infection of a vein) [10,11].

1.3. Pseudomonas aeruginosa

Pseudomonas aeruginosa is a gram-negative bacteria that appears rod-shaped under the microscope. Pseudomonas aeruginosa can be found in soil, water, animals, humans, and plants. Some infections caused by Pseudomonas aeruginosa include bloodstream (bacteria), eye (bacterial keratitis), heart (endocarditis), respiratory tract (pneumonia), and urinary tract [8].

1.4. Types of exposure

Samples can be exposed to plasma in two ways: directly or indirectly. Direct exposure to the tissue causes it to act as an electrode, enabling electricity to flow through it. Most direct exposure systems use ambient air as the gas, leading to exposure to various active processes such as heat, UV light, charged particles, and reactive species formed in plasma. In the case of indirect exposure which is used in this research, plasma is generated between two electrodes and transmitted to the target area through a gas flow, with noble gases like argon or helium serving as the working gas. This method may also subject the samples to certain active processes [12].

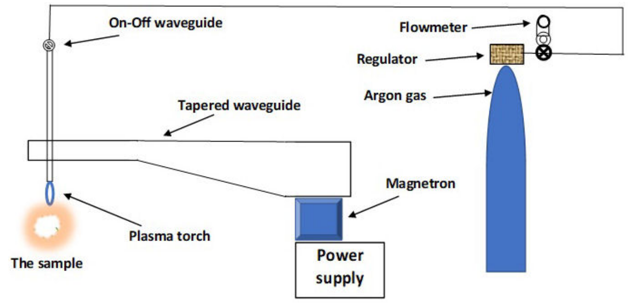

FIGURE 2. Microwave induced plasma jet.

2. Experimental section

2.1. Plasma jet system

The plasma jet device which is used in this research consists of a hollow tube with an outer diameter of 8 mm that is inserted into a tube connected to a high-voltage power source. Under certain conditions, an argon plasma jet can be extracted from the end of the nozzle tube due to the absence of discharge inside the plastic tube. The plasma jet obtained in this way is cool enough to be in direct contact with human skin without electric shock and can be used for medical treatment and pollution removal. The high-voltage power source generates a high-voltage pulsed waveform of 250 volts and a frequency of 2.45 Hz. The microwave induced plasma jet (MIPJ) system consists of five main parts:

1- Microwave source.

2- Waveguide.

3- Plasma discharge tube.

4- Ignition system.

5- Flow meter.

The applied voltage was changed to obtain the best inhibition in of bacteria.

2.2. Plasma treatment sample

Bacteria were treated with plasma jetting using an argon gas flow rate of 5 L/min. The bacterial sample was treated for a fixed time of 60 seconds and variable voltages (175, 180, 185, 190, 195, 200 volts) for each group. After the treatment was completed, the plates were incubated at 37°C for 24 hours, and bacterial colonies were counted. Bacterial suspensions of Staphylococcus aureus and Pseudomonas aeruginosa at certain concentrations were used. The streak plate method was adopted as stated in Noelle *et al*., (2016) and Nussbaum *et al*., (2002) [14].

1- Preparing the culture medium for bacterial growth.

2- Prepare the bacterial inoculum for both models at a concentration of 1.5×108 bacterial cells per ml by mixing the bacteria with the physiological solution under sterile conditions.

3- Exposing bacterial models to plasma positives at different dimensions and voltages.

4- After the bacterial samples were exposed to plasma, they were cultured on petri dishes using the method of plotting on the plate.

5- It was incubated for 24-48 hours and then the results were read.

2.3. Control

The culture medium without any bacteria was used as a positive control. Bacteria not exposed to pathogens were grown on separate plates as a negative control.

3. Results and discussion

The results were analyzed by testing the optimal conditions for two types of bacteria: (Pseudomonas aeruginosa) and (Staphylococcus bacteria) with a time (60 seconds), gas flow (5 liters/minute), distance (5 cm), and variable voltages as shown in the following states. The inhibition rate depends strongly on the characteristics of the device used (applied voltage, gas flow rate, treatment time, and frequency). When the natural frequency of a system is equal to the frequency of the force of the incident wave, it causes vibrations at the same frequency with huge amplitude, so the resonant frequency occurs. The condition for the frequency to occur is that

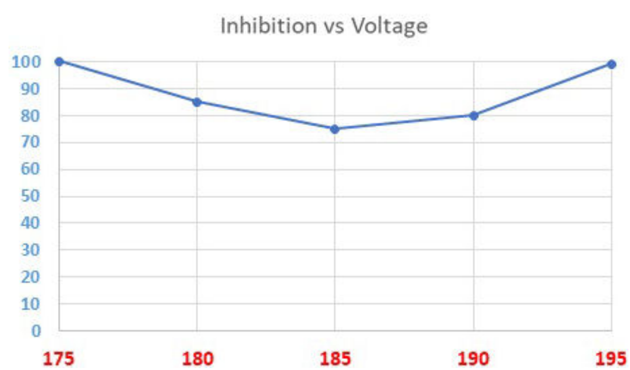

FIGURE 3. Rate of inactivation of Pseudomonas aeruginosa bacteria with changing voltages.

TABLE I. Percentage of inhibition of Pseudomonas aeruginosa bacteria when folate is changed while the above parameters remain constant.

Voltage (v)	175	180	185	190	195
Inhibition %	100	85	75	80	99

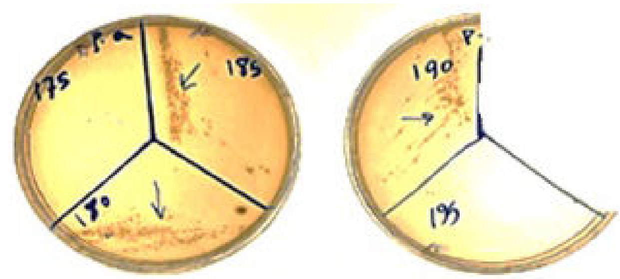

FIGURE 4. Pesudomonas bacteria exposed to a plasma jet system at a distance of 5 cm and at different voltage 175, 180, 185, 190, 195 V.

TABLE II. Rate of inactivation of Staphylococcus bacteria when changing folates with the above parameters constant.

Voltage (v)	175	180	185	190	195
Inhibition %	100	100	100	100	100

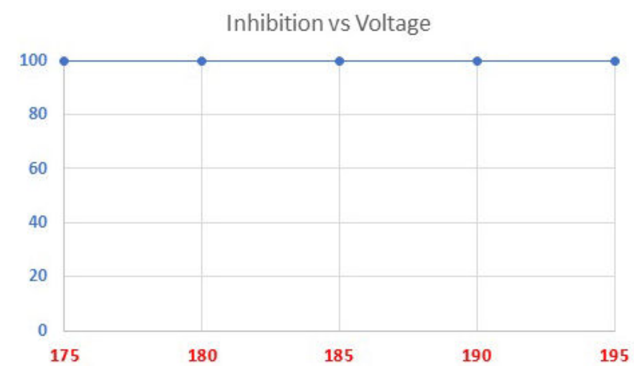

FIGURE 5. Rate of inactivation of Staphylococus bacteria with changing voltages.

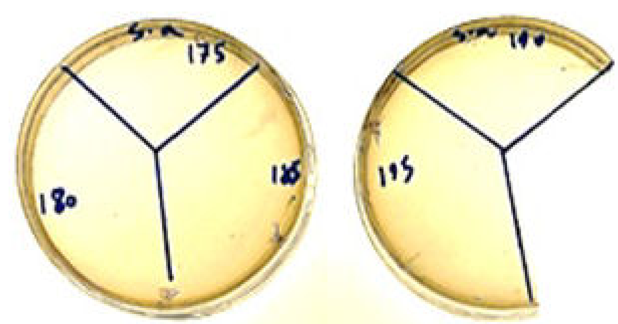

FIGURE 6. Staphylococus bacteria exposed to a plasma jet system at a distance of 5 cm and at different voltage 175, 180, 185, 190, 195 V.

the frequency of the external force is equal to the natural frequency of the vibrating body through which the collapse of the outer membrane occurs. This study is compatible with [15] due to its focus on inhibiting bacteria, the plasma frequency and wavelength used, and the external disruption of the bacteria by charged particles Bacteria can be inactivated when exposed to an atmospheric pressure plasma jet of several voltages. It was found that Pseudomonas aeruginosa

FIGURE 7. Staphylococcus bacteria and Pesudomonas bacteria without plasma exposure.

bacteria are less sensitive to treatment with plasma jetting than Staphylococcus bacteria. Table I, II Figs. 3-6 and 7 show the rate of inactivation of Pseudomonas aeruginosa bacteria with changing voltages and the result is consistent with [8].

4. Conclusions

The atmospheric plasma jet system is an effective tool for inactivating certain types of bacteria. The rate of voltage change has been shown to affect the gas flow, distance, and time, while high-speed particle discharge that penetrates the external structure of the bacteria plays a dominant role in the inactivation process caused by the plasma jet. It has been found that Pseudomonas bacteria are less sensitive to plasma jet treatment than Staphylococcus bacteria, as inhibition increases with increasing voltage, and this depends on the nature of the structural structure of the bacteria.

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