Sterilization of *Staphylococcus aureus* by an Atmospheric Non-Thermal Plasma needle

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Abstract
The current study is concerned about the production of plasma needle at atmospheric pressure air for sterilization by killing Staphylococcus aureus using an Atmospheric Non-Thermal Plasma needle. The discharge was produced by applying high voltage AC source of frequency (10) kHz and potential difference of (0-8) kV across the electrodes with flow rate (1-5l/min.) of argon gas. The discharge was characterized by measuring current and voltage with a high frequency. The results showed the best time to kill Staphylococcus aureus was 6 minutes, where the voltage was 8 kV and the distance between the sample of the bacteria and the head of the plasma needle is 2cm and the gas flow is 5L/min.
Keywords: plasma needle, high voltage, Staphylococcus aureus, atmospheric pressure

1. Introduction

Plasma is considered as the fourth state of matter, it is the most abundant state in the universe. It is not a human invention and it present in nature as a fire in the sun, stars, in the tails of comets and as flashes of lightning [1]. The number of applications of plasma technology in many fields including microelectronics, metallurgy, polymer engineering and biomedical engineering is growing rapidly. One of the advantages of this technology is that surface properties such as hardness, corrosion resistance also other chemical and physical properties can be selectively modified without affecting the bulk characteristics of the materials.[2]
In comparison with other methods for surface modification (layer by layer deposition, dipping, etc.) plasma surface modification offers a shorter and more economical method for the covalent attachment of bioactive molecules to the substrate without obstructing the bulk properties [2,3]. Thus plasma technology has great important in the development of new biomaterials.
Plasma consists of a mixture of positively and negatively charged ions, electrons and neutral species (atoms, molecules). It can be divided into two main categories; hot plasma (near-equilibrium plasma) and cold plasma (non-equilibrium plasma). Hot plasma consists of very high temperature particles and they are close to the maximal degree of ionization. Cold plasma is composed of low temperature particles and relatively high temperature electrons and they have a low degree of ionization [4], so cold plasma can be further subdivided into low pressure and atmospheric pressure cold plasma. Atmospheric pressure cold plasma is the basis of one of the most promising methods of achieving a more flexible, reliable, less expensive and continuous method of surface modification [5] .
Different forms of energy (thermal, electric current, electromagnetic radiations, light from a laser, etc.) are used to create the plasma regardless of the nature of the energy source. Depending on the type of energy supplied and the amount of energy transferred to the plasma, the properties of the plasma change in terms of electron density or temperature [6]. In common, man-made plasma, electrical energy is usually injected into a system in a continuous manner in order to avoid stoppage of the plasma discharge, plasma is most commonly produced by passing an electric current through the gas.[1]
Different frequencies of power sources direct current, alternating current, low frequency, radio frequency, microwave and etc. are used for the generation of discharges such as atmospheric and low pressure glow discharge, corona, magnetron and dielectric barrier discharge (DBD) [1,7]. Plasma parameters must be designed specifically for a given application plasma sources have their own peculiarities, advantages, and disadvantages. The selection of a plasma source and design for the production of novel material is a great challenge for scientists and industry. [7]

2. Theory

Non-equilibrium plasmas under atmospheric pressure have been developed as an effective means for surface modification of polymers [8]. The biological applications of the atmosphere plasma have attracted a great interest from both plasma as

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well as biological research communities. Plasma sterilization and plasma interaction with microorganisms are two of the most interesting and ongoing research areas in the biological application of atmosphere plasma .

Staphylococci are Gram-positive bacteria, with diameters of 0.5 – 1.5 μm and characterized by individual cocci, which divide in more than one plane to form grape-like clusters. To date, there are 32 species and eight sub-species in the genus Staphylococcus, many of which preferentially colonize the human body [7]. Staphylococcus aureus is not always pathogenic, it is a common cause of skin infections such as skin abscess, respiratory infections such as sinusitis, and food poisoning. Pathogenic strains often promote infections by producing virulence factors such as potent protein toxins, and the expression of cell-surface proteins that bind and inactivate antibodies. The cell wall of S. aureus is a tough protective coat, which is relatively amorphous in appearance, about 20- 40 nm thick, underneath the cell wall is the cytoplasm that is enclosed by the cytoplasmic membrane. Peptidoglycan is the basic component of the cell wall, and makes up 50% of the cell wall mass [8,9].These bacteria are the most common pathogenic bacteria isolated from wounds and burns infected were chosen in this study therefore, the aim of this study is to demonstrate the role and effect of non-thermal plasma on these bacteria.

3. Materials and Methods

A high voltage power supply of frequency 10 kHz was used to generate the discharge in the air gap between two electrodes covered by dielectric medium. The current and voltage of the discharge were measured by high frequency digital oscilloscope (Tektronix TDS 2002). The applied voltage and discharge current were measured and analyzed. Plasma jet system is based on a conventional plasma discharge which is basically a system driven by alternating current. High voltage is applied between two conductors where one or both are covered with a dielectric to limit the current and to prevent transition to an arc.

Non-thermal plasma was generated by applying alternating voltage (0 to 3 kV) (peak-to-peak) between an insulated high-voltage electrode and the grounded base holding the sample. A variable voltage and current power supply was used for treating samples. The power supply was connected to a stainless steel tube. The dielectric prevented current flow between electrodes, creating plasma with high reactive species concentrations, but minimal gas heating. The discharge distance between the dielectric and the sample was varying from (1 to 3cm).

In frequency (10 kHz), that is to limit the current and to prevent transition to an arc. The system consists of power supply of high voltage varying from (0 to 15 kV) related to a wire to the stainless steel tube. Other part of the system is gradually connected by a piece of mica to stainless steel to prevent the transmission of discharge to the catcher. The discharge occurs between the bottom surface of the tube and top surface of the sample. The distance where the discharge occurs was controlled to be from 1 to 3 cm. The diameter of the glass tube was 2.5 cm. All the treatments are at room temperature and atmospheric pressure and were carried out according to the same procedure. Figure 1 shows the schematic diagram of the experimental set-up used in this study.

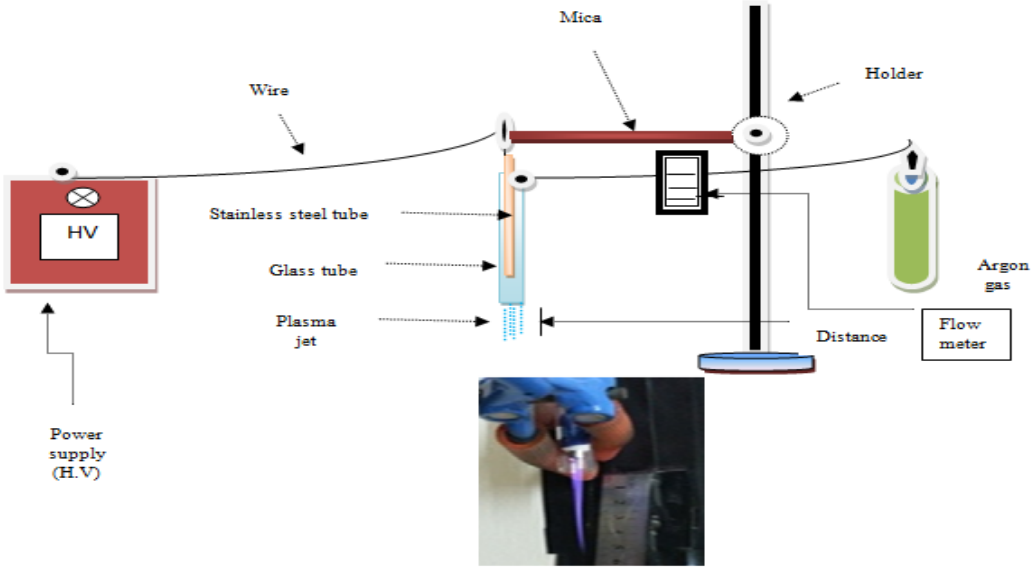

Figure (1): Production system non-thermal plasma needle and image it.

4. Bacterial strains

The non-thermal plasma needle generated from this system were applied on Staphylococcus aureus, these bacteria samples were collected from Al-Yarmouk Teaching Hospital / Department of wounds and burns - microbiology laboratories.

Culture Medium

MacConkey agar and mannitol salt agar, as well as nutrient broth were used in this study, which prepared as recommended by the manufacturing company, and then sterilized by an autoclave at 121 ◦C for 15 min.

Preparation of Samples and Bacterial Inoculation

Culturing the swab on MacConkey agar and after incubated for 24 hr at 37 0C a one colony was taking from a fresh solid medium and suspension with a distilled water to have 108 (CFU/ml) as determined by 0.5 Mcfarland standard. The resuspended culture was serially diluted to 1:10, 1:100 , 1:1000 , 1: 10000 , 1: 100000, of the original . The third suspension was used in this experiment to Staphylococcus aureus, to evaluate the effect of non-thermal plasma needle system on S. aureus.

Samples Treatment by Non-Thermal Plasma Needle

In this experiment 1mL of prepared bacterial suspension was placed on agar surface. This volume was selected as it spread to ≈ 1 cm2 over the agar surface; thus, the bacterial samples were placed in a petri dish is exposed to the non-thermal plasma needle. Samples were exposed to non-thermal plasma needle at different times starting from (1-6) minutes with the change in the distance, flow and voltages. A petri dish containing a bacterial suspension was placed down plasma needle. The distance between the non-thermal plasma needle and the bacterial petri dish was change at (2-3) cm. The operational conditions of the system at the exposure were fixed at voltage (4.9 - 8 kV) and frequency (10kHz). In addition to exposed samples, control samples were inoculated without exposed to plasma. After the treatment, the exposed bacteria selective samples to non-thermal plasma needle were plated in petri dishes containing the media for S. aureus and then placed in the incubator for 24 hours at 37◦C . After incubation , the colony forming units (CFU) were counted in order to check the efficiency of bacterial inactivation using non thermal plasma needle system .

5. Results and Discussions

Role of applied Voltage On *Staphylococcus aureus*:

Staphylococcus aureus were exposed to non-thermal plasma needle at two different values of voltages (4.9-8) kV and constant distances 2cm. The killing rate for bacteria was different at these two values of voltages and at different flow of gas (1-5) L/min. Results showed killing all bacteria when increase the voltage to 8 kV and at a distance 2cm at a gas flow of 5 L/min and different time. The first voltages did not result in a complete killing rate, but a lower kill rate was obtained, this indicates that the increase in voltages has an effect on the bacterial killing rate. Figure (2) showed relationship between killing rate and time of exposure on bacterial samples to non-thermal plasma of bacteria.

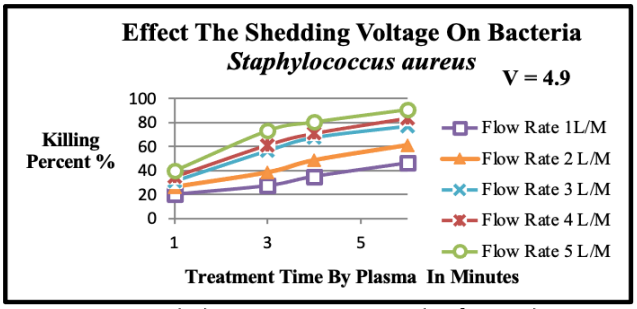

Figure (2): Staphylococcus aureus at the first voltage 4.9 kV and distance 2cm.

The figure (3) showed the relationship between killing rate and time of exposure on bacterial samples to non-thermal plasma when the voltages were increased to 8 kV.

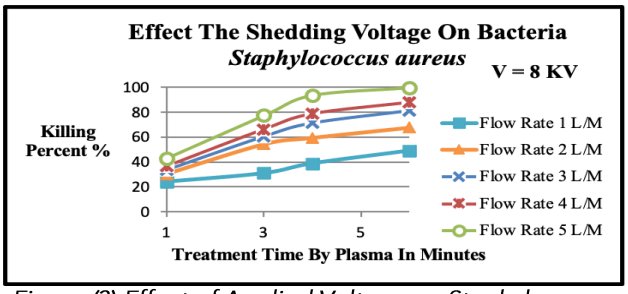

Figure (3) Effect of Applied Voltage on Staphylococcus aureus at voltage 8 kV and distance 2cm.

Figure (4) showed Staphylococcus aureus before and after treatment by non-thermal plasma needle at different times.

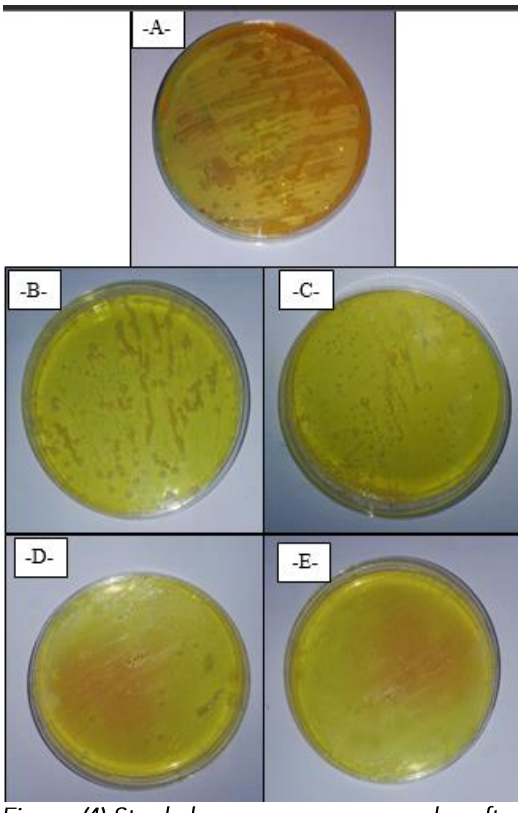

Figure (4) Staphylococcus aureus samples after exposure to non-thermal plasma needle system.

A: control, B: 1 minutes, C: 3 minutes, D: 4 minutes , E: 6 minutes.

Role of Distance on Staphylococcus aureus

The effect of distance on the rate of killing bacteria, results showed when exposed Staphylococcus aureus to non-thermal plasma needle after increasing the distance to 3cm between the needle and bacteria and to different voltages, where the rate of killing bacteria are decreased. These results showed the relative proportion between the rate of killing bacteria and distance, when the distance between bacteria and plasma needle increases, the rate of killing decreases and vice versa, relationship between the kill rate and the treatment time of Staphylococcus aureus at 3 cm and 4.9 kV(see figure 5).

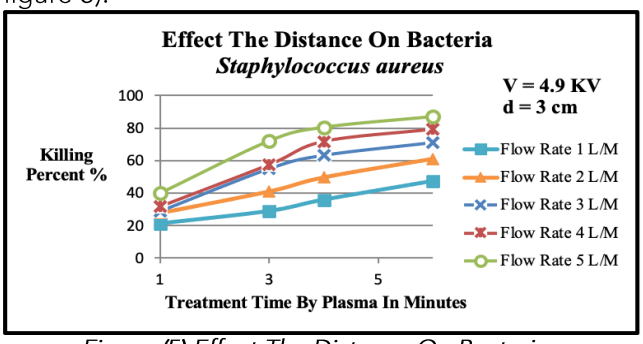

Figure (5) Effect The Distance On Bacteria Staphylococcus aureus at distance 3cm and voltage 4.9 kV and different flow rate.

Furthermore, when the voltages are increased to 8 kV and the distance is constant at 3 cm, the results were decreased even when the voltages increased, this shows the clear effect of the distance on the killing rate of bacteria, figure (6) showed effect the distance On Staphylococcus aureus at 3cm when increasing voltage to 8 kV.

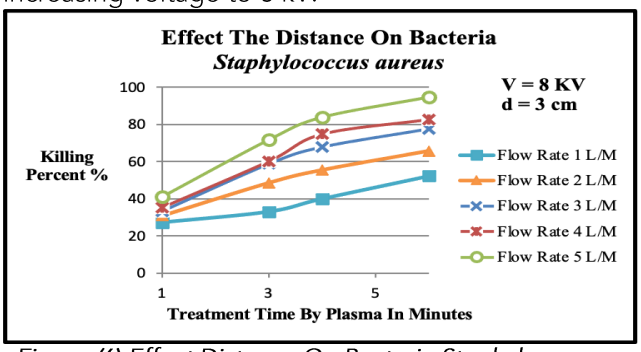

Figure (6) Effect Distance On Bacteria Staphylococcus aureus at distance 3cm and voltage 8 kV and different flow rate.

S. aureus was exposed to non-thermal plasma to evaluate the inhibitory effect of non-thermal plasma needle. The previous results showed that S. aureus is more resistant to non-thermal plasma needle. This is related to the difference in the structure of the cell wall for Gram positive bacteria, so the cell wall of Gram positive contains peptidoglycan, which is responsible for protecting the cell, thus providing these bacteria with higher strength and rigidity, making then harder to be sterilize [10,7,11].

The effect of non-thermal plasma on bacteria was linked to the charged particles found in plasma, as these particles play a large role in tearing the outer membrane of bacterial cells, the electrostatic force caused by the buildup of charges on the outer surface of the cell membrane can overcome the tensile strength of the membrane and thus cause damage and tearing in cell membrane [10,12].

Reactive species produced in electron-impact excitation and dissociation in non-thermal plasma a significant contribution to the plasma sterilization process. Air plasmas, for example, are excellent sources of reactive oxygen-based and nitrogen-based species (ROS and RNS), such as O, O∗ 2, O3, OH, NO, NO2, etc. These species have direct chemical interactions with the cell membrane, where these species diffuse through the bacterial cell wall causing the local damage possible by oxidation of cytoplasmic membrane, also cause the leakage of the intracellular components outside the cell and act with these intracellular components [13]. The presence of (ROS) is enhanced by the presence of some level of humidity, which leads to the generation of hydroxyl1(OH) and oxygen radicals that play an important role in killing the targeted microorganism [7,13]. These results are in agreement with the previous study of M.U. Hussein (2017) [14].

6. Conclusions

This study can be conclude the best time to kill *Staphylococcus aureus* was 6 minutes, where the voltages were 8 kV and the distance between the sample of the bacteria and the head of the plasma needle is 2cm and the gas flow is 5L/min.

7. References

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