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The Effect of the Non-Thermal Plasma Needle on *Pseudomonas Aeruginosa* Bacteria

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Abstract

The development in the field of medical physics has led to the use of devices that are manufactured under normal conditions to make tremendous progress in the world of development in medical treatment by using these devices with modern techniques by reducing the use of antibiotics and relying on these tools and devices that link between physics and modern therapeutic medicine. In this research, a non-thermal plasma system for argon gas operated at normal atmospheric pressure was designed, this system was applied on *Pseudomonas Aeruginosa* bacteria isolated from burn patients from Yarmouk Teaching Hospital. These bacteria were exposed to this system, the results showed that these bacteria were killed at time (5 min) and (8kV) where the argon gas flow rate was (5*l/min*).

Keywords: Non Thermal Plasma, Plasma Needle, *Pseudomonas Aeruginosa* Bacteria.

تأثير البلازما غير الحرارية على بكتيريا سيدوموناس ايروجينوسا

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الخلاصة

أدى التطور في مجال الفيزياء الطبية الى استخدام الاجهزة التي يتم تصنيعها وتعمل تحت ظروف طبيعية الى احداث طفرة في العلاج الطبي وذلك بتقليل استخدام المضادات الحيوية والاعتماد على هذه الادوات والاجهزة التي تربط ما بين الفيزياء والطب العلاجي الحديث ، في هذه البحث تم تصميم منظومة بلازما غير حرارية لغاز الارجون تعمل عند الضغط الجوي الاعتيادي ، حيث تم تطبيق هذه المنظومة على بكتيريا (*Pseudomonas Aeruginosa*) التي عزلت من المرضى المصابين بالحروق من مستشفى اليرموك التعليمي حيث تم تعريض هذه البكتريا لهذه المنظومة حيث تم الحصول على نسبة القتل التام لهذه البكتريا عند الزمن (5دقيقة) وعند فولتية (8 كيلو فولت) وتدفق غاز (5 لتر/دقيقة).

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Introduction

The term "plasma" is used to describe a partially or completely ionized gas containing electrons, ions, and neutrals. Although there is always a small degree of ionization in any gas, a stricter definition of plasma is "a quasi-neutral gas of charged and neutral particles which exhibits collective behavior" [1, 2]. Non-thermal plasma (NTP) is a weakly ionized gas far from thermodynamic equilibrium. While electron temperature is 1-10 eV, electrons are not able to transfer their entire kinetic energy gained from an externally applied electric field onto bigger particles and thus the gas remains non-thermal ($T_e >> T_g$; $T_g \approx 300$ - 1000 K [3]).

Because the ions and the neutrals remain relatively cold, this characteristic provides the possibility of using cold plasmas for the treatment of heat sensitive materials including polymers and biological tissues [4].

Plasma needle is a type of non-thermal glow discharge which operates under normal atmospheric pressure and is composed of one pole or two poles, one called the cathode and the other is named the anode and run on the noble gases. one of the important characteristics of this type of plasma approach it to room temperature and operating under normal atmospheric pressure as it allows for processing of sensitive surfaces, as well as can penetrate small depths [5, 6].

These characteristics give great potential for the use of plasma needle in the field of bio-medicine, as it is able to remove bacterial contamination topical cells without causing any necrosis in neighboring cells during treatment [7].

Plasma needle has a very simple design as it consists of a metal or syringe needle, is used as High-voltage electrode. Gas flows through the electrode at different flow rate measured by unit (*l/min*). Flow velocity can be controlled by a flow meter [8].

As it is characterized by being small, careful in their work, flexible, and non-heat. This small size distinguishes the plasma needle from other plasma atmospheric pressure that deal with large areas, and this small size is desirable in dental treatment in medical use, which requires high accuracy [9].

Pseudomonas aeruginosa

Is a common-negative, rod-shaped bacterium that can cause disease in plants and animals, including humans [10]. found in soil, water, and most man-made environments throughout the world. Is found not only in normal atmospheres, but also with little oxygen, and has thus colonized in many natural and artificial environments. Because it thrives on moist surfaces, this bacterium is also found on and in medical equipment's including catheters, causing cross infections in hospitals and clinics. It uses a wide range of organic material for food; in animals the versatility enables the organism to infect damaged tissues or people with reduced immunity [11, 12].

Experiment Setup

A non-thermoplastic plasma needle was designed using argon gas and at normal atmospheric pressure. The plasma needle was manufactured from PYREX glass. The inner electrode from metal stainless its diameter is (3 mm) represents the cathode, The external electrode made from a copper represents anode thick (0.8 mm) placed outside the tube at the front of the needle and was isolated by silicon to prevent discharge at the tip of the needle. Figure-1 shows the non-thermal argon plasma needle.

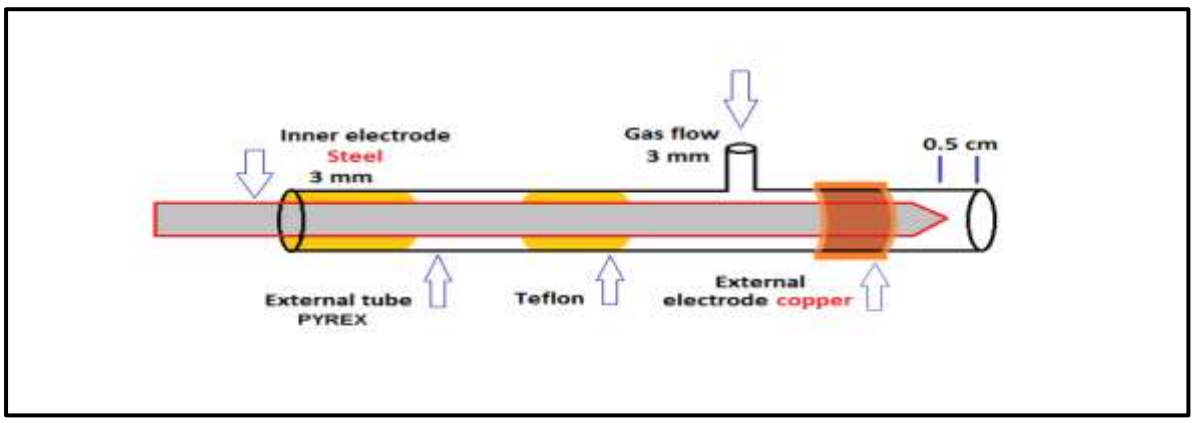

Figure1- Schematic for design non thermal argon plasma needle.

The non-thermal plasma needle system linked to power supply (AC) range voltages (0-30) kV, the inner electrode of the needle connected to the cathode source of power supply, whereas external electrode of the needle connected to the anode source of power supply. The flow rate of the argon gas adjusted by a flow rotameter (1-5 *l/min*). Figure -2 illustrate the non-thermal plasma needle system used to kill bacteria.

Gram-negative (*Pseudomonas aeruginosa*) bacteria isolated and taken from Al-Yarmouk Teaching Hospital / Department of wounds and burns - microbiology laboratories. This bacteria is the most common types of bacteria for patients which infected by wounds.

These bacteria have been selected to demonstrate the effect of non-thermal plasma on these bacteria, by exposing the bacteria isolate to the non-thermal plasma at a certain time.

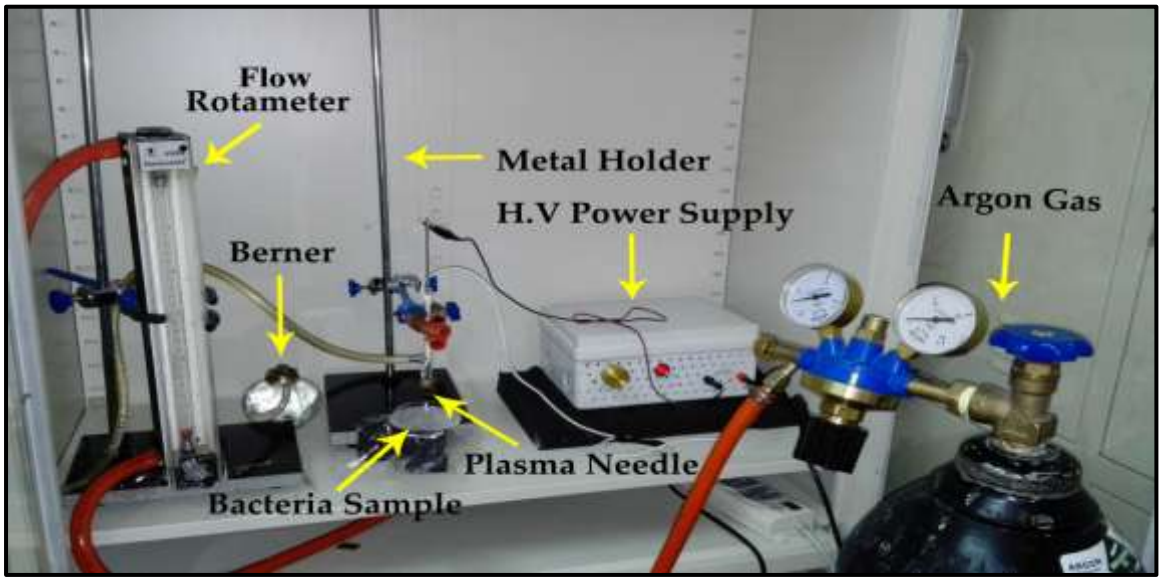

Figure 2- the non-thermal plasma needle system.

Method

The medium was inoculated with bacteria after taking a colony from a fresh solid medium at 37 °C and incubated for overnight, from this suspension, suspension with a number of bacteria about 1.5×10^{8} (CFU/ml) were made as determined by 0.5Mcfarland standard. 1: 100000 suspension used in this experiment to (*Pseudomonas aeruginosa)* bacteria to evaluate the effect of non-thermal plasma needle system on Gram negative bacteria.

A petri dish containing (1ml) of bacterial suspension was placed down plasma needle. The distance between the non-thermal plasma needle and the bacterial petri dish constant at (2 cm). The operational conditions of the system at the exposure were fixed at voltage (4.9, 8 kV).

control isolates inoculated but not exposed to plasma . After the treatment, the exposed bacteria isolates for non-thermal plasma needle, planted in petri dishes containing on the media which special for each bacteria and then placed in the incubator for 24 hours at 37°C. After incubation, the colony forming units (CFU) counted in order to check the efficiency of bacterial inactivation using non thermal plasma needle system.

Results and Discussion:

Bacteria isolates of Gram negative (*Pseudomonas aeruginosa)* were exposed to non-thermal plasma needle at two different values of voltages (4.9,8) kV and constant distances (2cm). The killing percentage for bacteria was different at these two values of voltages and at the different flow of gas (1-5 *l/min)*. The results obtained for the bacteria showed when increase The voltage to (8 kV) and at a distance (2cm) obtained on complete killing at gas flow (5 *l/min)*, the Gram negative (*Pseudomonas aeruginosa)* was killing in (5 min).

While the first voltages did not obtain a complete killing percentage, but a lower killing percentage was obtained, this indicates that the increase in voltages has an effect on bacterial killing percentage,

The following Figure-3 show the relationship between killing percentage and time of exposure bacterial isolates to non-thermal argon plasma needle.

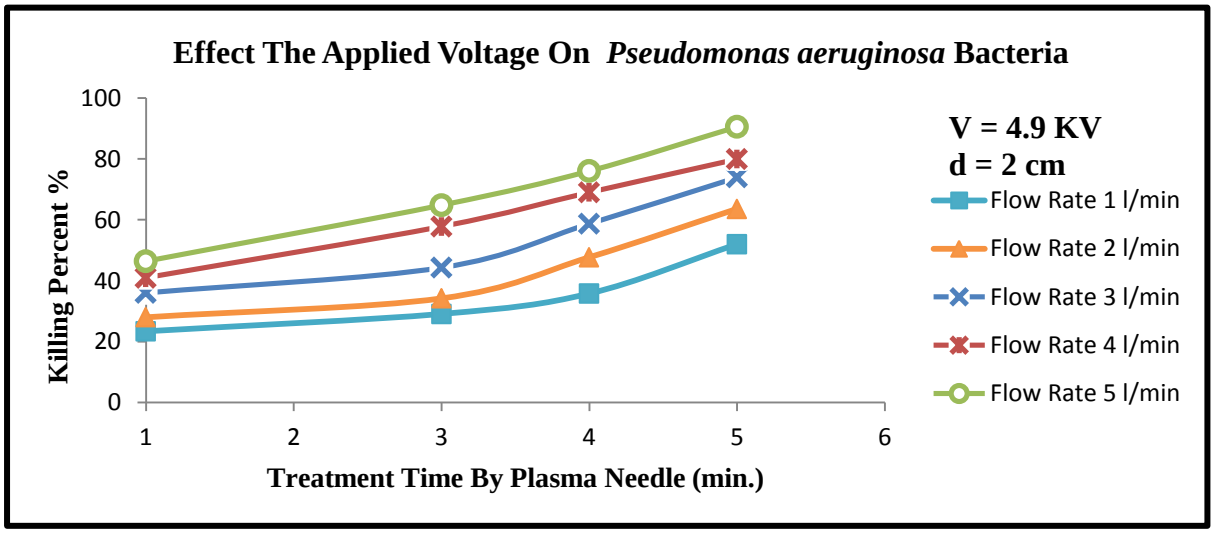

Figure 3- The effect the non-thermal argon plasma needle on bacteria *Pseudomonas aeruginosa* at 4.9 kV and different flow rate in distance 2cm.

The results showed that the killing percentage of (*Pseudomonas aeruginosa)* bacteria increases by increasing the gas flow when the voltages are (4.9kV), where the killing percentage is more than (51%) at the gas flow (1 *l/min*) and at the (5min). While the killing percentage (*Pseudomonas aeruginosa)* bacteria more than (90%) when the flow of gas (5 *l/min*) in (5min). This shows the obvious effect of the increasing gas flow on bacteria killing percentage, Where the number of passing gas particles increases during the plasma tube, leading to direct effect on the cell wall. When increasing the voltage to (8kV) get on complete killing percentage of the bacteria, this shows the effect of increasing the value of the voltages on the killing percentage of bacteria as in the Figure-4, where the results showed that the killing percentage of (*Pseudomonas aeruginosa)* bacteria when exposed to plasma directly proportional with the height applied voltage.

The results showed that the rate of killing of (*Pseudomonas aeruginosa)* bacteria increases by increasing the gas flow when the voltages (8kV), the killing percentage is more than (65%) at the gas flow (1 *l/min*) in (5min). While the killing percentage (*Pseudomonas aeruginosa)* bacteria were killed completely (100%) when the flow of gas (5 *l/min*) in (5min). Figure -4 shows the effect of non-thermal plasma needle at (8 kV) on (*Pseudomonas aeruginosa)* bacteria.

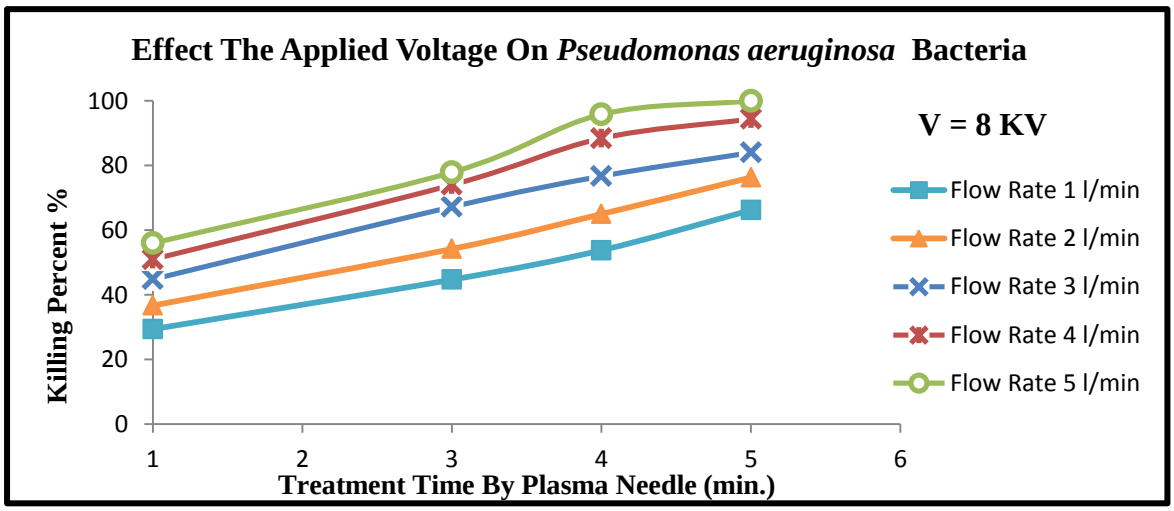

Figure 4-The effect of the non-thermal argon plasma needle on bacteria *Pseudomonas aeruginosa* at 8 kV and distance 2cm at different flow rate.

When the voltages used are increased to (8 kV) with a constant distance of (2cm) between the isolate of the bacteria and the tip of the plasma needle, there is an increase in the killing percentage of the bacteria. This indicates that the proportion between the voltages and the killing percentage of the bacteria is proportional.

The special effects of the high voltage and high velocity particle discharge penetrating through the external structure of the bacterium is also play a dominant role throughout the inactivation of the bacterium caused by plasma. If bacterium area unit processing with a high voltage, the cell membrane's structure and charge distribution consequence of the high velocity particle discharge the outer structure of bacterium, namely cell wall and cell membrane of culture from, coating of the spore, could be destroyed and cytoplasm would be released, which would cause the death of the bacteria[13, 2].

Reactive species produced in electron-impact excitation and dissociation in non-thermal plasma can make 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}, O_3, OH, NO, NO_2, etc. These species have direct chemical interactions with the membrane of bacteria , where these species diffuse through the bacterial cell wall causing the local damage possible by the oxidation of cytoplasmic membrane [14]. The effect of non-thermal plasma on bacteria is linked to the charged particles found in plasma, as these particles play a large role in tearing the outer membrane of bacterial cells[15, 11]. Figure-5 shows the bacteria isolates before exposure to plasma and after exposure.

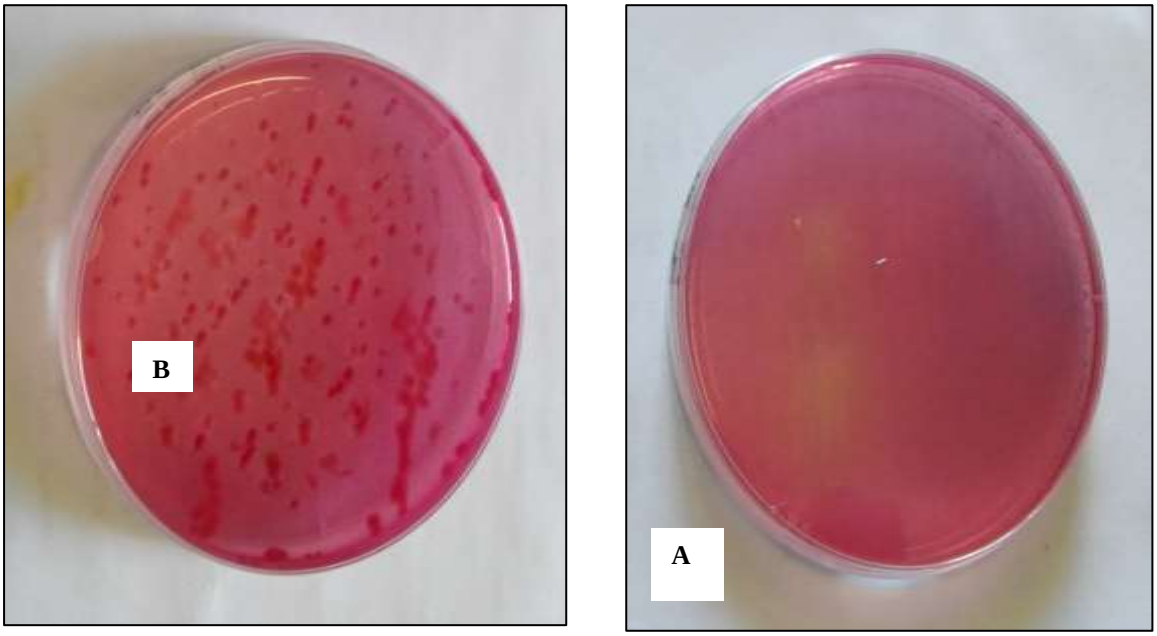

Figure 5- A (*Pseudomonas aeruginosa)* isolates before and after exposure to non-thermal argon plasma needle.

Conclusions

From the above results, one can conclude that:

1- A non-thermal plasma needle was designed to operate at atmospheric Pressure.

2- The killing percentage depends on the plasma system operating conditions such as applied voltage on bacteria, gas flow and distance between tip needle and isolates bacteria, the perfect and partial killing percentage increase with the applied voltage increasing.

3- Increasing gas flow lead to increases the killing percentage in (*Pseudomonas aeruginosa)* bacteria, as gas molecules during increased flow velocity play a large role in increasing the killing percentage.

4- Increasing the voltages means an increase in the processed energy to non-thermal plasma needle, thus increasing these voltages leads to complete killing, there was a complete killing of bacteria at (8 kV).

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