DEACTIVATION OF STAPHYLOCOCCUS AUREUS AND ESCHERICHIA COLI USING PLASMA NEEDLE AT ATMOSPHERIC PRESSURE

[1]Hamad Raheem H., [2]Maha Adel Mahmood

[1]Department of Physics, College of Science, Baghdad University, Baghdad, Iraq
[2]Department of Basic Science, College of Dentistry, Baghdad University, Baghdad, Iraq
E-mail: dr.hammad6000@yahoo.com

Abstract

Non-thermal (low-temperature) plasma may act as an alternative approach to control superficial wounds and skin infections when the effectiveness of chemical agents is weak due to natural pathogen or biofilm resistance. In this paper an atmospheric pressure plasma needle jet device local made which generates a cold plasma jet 1mm diameter and about 30 mm in length sustained by low-frequency excitation was used to study the deactivation of two bacterial isolates Escherichia coli and Staphylococcus aureus. Escherichia coli and Staphylococcus aureus were used as a model system to optimize the conditions for bacterial destruction. Plasma working gas flow rate, treatment time and needle to sample distance are varied. Plasma treatment of Escherichia coli results in formation of inhibition zone of 25 mm and Staphylococcus aureus in 29 mm inhibition zone respectively. Prolongation of treatment time improves the destruction efficiency. Sample treatment during plasma treatment has been monitored. The temperature can reach up to 32ºC at shortest needle-to- sample distances and 80 s treated time where the ambient temperature was 29ºC.

Keywords: *Plasma needle, plasma bacteria deactivation, non-thermal plasma*

1. INTRODUCTION

Some major drawbacks of the conventional sterilization techniques are the high processing temperatures (ovens and autoclaves) which makes it impossible to sterilize heat-sensitive materials like polymers, the use of toxic chemicals and the long sterilization times needed approximately 12 hours in the case of ethylene oxide exposure[1]. Another interesting sterilization method

is the use of gamma irradiation, but this is an expensive technique and may cause the material to undergo undesirable changes during sterilization.

It has been known for a long time that ionized gases have biotical effects, but only in 1996 successful killing of bacteria with plasma was reported [2].

One of the attractive features of non-equilibrium plasmas is the ability to achieve enhanced gas phase chemistry without the need for elevated gas temperatures. This is because these plasmas exhibit electron energies much higher than that of the ions and the neutral species. The energetic electrons enter into collision with the background gas, causing enhanced level of dissociation, excitation, and ionization. Because the ions and the neutrals remain relatively cold, the plasma does not cause any thermal damage to articles it comes in contact with.

In physics plasma is considered the fourth state of matter next to solids, liquids and gases. On the basis of their high bactericidal effectiveness plasmas are also used to sterilize medical devices and in packaging of food stuffs. The development of diverse, usually non-thermal atmospheric-pressure plasma sources makes its use also in the (bio) medical field possible. While in the past only the thermal properties of plasmas (> 80 °C) were utilized – cauterization, sterilization of heat-resistant instruments or for cosmetic, reconstructive procedures – current research is directed primarily at the non-thermal effects of plasma [3].

These plasma-generated active species are useful for several bio-medical applications such as sterilization of implants and surgical instruments. Sensitive applications of plasma, like subjecting human body or internal organs to plasma treatment for medical purposes, are also possible.

Biomedical applications of plasmas have recently been summarized as “Plasma medicine. “Within this context, there is an increasing interest on the development and characterization of flexible plasma sources which can be used under atmospheric pressure conditions. Plasma jets, barrier dischar-

ges, and other micro plasma devices are therefore under intensive investigation [4,5].

The aim of this work was to use *Escherichia coli* and *Staphylococcus aureus* as a model system to optimize the conditions, Plasma working gas flow rate, treatment time and needle to sample distance for bacterial destruction.

2. EXPERIMENTAL WORKS

2.1. Plasma Needle Apparatus

Non-equilibrium plasma generated by a plasma needle was used for the treatment of bacteria. The plasma torch shown in Fig.1 local made is consisting from 10cc glass syringe adapted to a hallow stainless steel needle with inner diameter 1.04mm from one side and the working gas (Ar) into other side through Teflon gas fitting. The needle is powered by high voltage of sinusoidal shape of 7.5kV and frequency of 28 kHz peak to peak generated by high voltage transformer. The Ar gas is directed to the needle through the syringe and mixes with ambient air around the tip of the needle. The Ar gas was flowing at 1slm (standard liters per minute) flow rate through the needle. The jet length depends on argon gas flow rate and it can reach 3.5 cm in air at gas flow rate 1slm.

Figure 1. Plasma needle torch [6]

2.2. Sample Preparation

Two bacterial isolates (S. aureus and E. coli) were obtained from Al-Yarmook Teaching Hospital. They were activated by cultivating S. aureus on blood agar and E. coli on MacConkey agar for 20 h at 37ºC. Sub-cultures on solid nutritional media were incubated under the same conditions.

Bacterial suspension was prepared according to no.5 McFarland standards tube whose density corresponds to an approximate cell density of 1×10^8 colony forming unit per milliliter (cfu ml^{-1}), from this initial suspension, other suspensions in cfu /ml were made with cell density 1:10, 1:100 and 1:1000 of the original. Plastic Petri dishes were filled to a depth of 4 mm with agar, which is an aqueous growth medium with a jelly-like consistency. After cooling and solidification, the bacterial inoculum was uniformly spread using sterile cotton swab on a sterile Petri dish of nutrient agar forming a bacterial lawn. (from antibiotic original)

2.3. Plasma treatment

There were three adjustable parameters for our plasma treatment against the studied isolates. The exposure time was varied in a range from 20 to 80 s, the needle-to-sample separation d from 2 to 6 mm, and the gas flow from 1 to 5 slpm.

At first, the plasma torch was raised, installed a Petri dish with its center immediately below the needle, and then lowered the plasma torch to the desired separation d. Next, plasma treatment was start by positioning the Petri dish so that the plasma impinged on a desired spot in the inoculated portion of the dish. At the end of the exposure time, the dish was moved to treat another spot. This step was repeated to treat a total of three spots on the dish, as shown in Fig. 2. The fourth spot was the control for the experiment; it was exposed to the gas flow without plasma glow. The control spot indicates the effects of argon flow without any plasma-generated species.

2.4. Incubation and imaging

To visually observe the effect of plasma treatment, the treated samples were incubated so that visible colonies formed. Petri dishes were covered to prevent atmospheric contamination. All plates were then incubated in an incubator at 37°C for 48 hours so that bacteria multiplied. And the inhibition diameter zones were observed and measured in mm. (from antibiotic original). After incubation we imaged the dishes with a digital color camera using white photographer's lights for illumination.

2.5 Temperature tests

For the purpose of measuring temperature increase during the plasma treatment, the temperature was monitor by infrared thermometer.

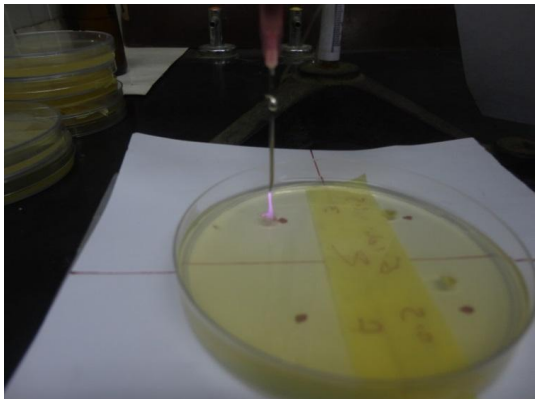

Figure 2. Plasma samples treatment

3. RESULTS AND DISCUSSION

Susceptibility to the argon plasma treatment was determined by applying plasma to planktonic bacterial cells plated on nutritive agar plates. To check the effect of non-ionized argon gas, it was applied to parallel samples. As a control, bacterial plates were left untreated. The bactericidal effect was determined as the diameter of inhibition zones (Diz) in millimeter after treatment with plasma or non-ionized argon compared with the untreated control . figure 3 showed the (Diz) in millimeter for S. aureus cultivated on agar plate verse plasma exposed time using three different distances between the needle end and agar surface (2, 4, 6 mm), three different gas flow rat (1, 2.5, 5 slpm) and exposer time ranged between 20- 80s. figure 4 showed the (Diz) in millimeter for E. coli at the seam condition. Fig 3 and 4 shows that an increase in inhibition zones when the distances between needle end and agar surface was increased from 2mm to 6mm for all argon gas flow rate. Also show increase in inhibition zones with increasing plasma exposer time for all flow rat and for all distances. The maximum increase in inhibition zone for S. aureus was seen at 4 mm distance, 2.5 slpm, 80 s. or at 6mm distance, 2.5 slpm, 80 s (29 mm) respectively.

The exposure of S. aureus to plasma work at gas flow rate of 2.5 slpm for 80 seconds at distance of 4 or 6 mm was the best killing condition where the inhibition zone of 29 mm. From this result we can conclude that the gas flow doesn't affect the inhibition zones rather what the role will due to the time which play a great role in this case. The most favorable conditions in E.coli inactivation was the exposure of E.coli to either 5 slpm at 2mm distance for 80 s (25mm) or 2.5 slpm at 4 and 6mm distance for 80 s and this is corresponds with the results obtained for S. aureus.

The diameter of inhibition zone of both bacteria (S. aureus and E.coli) was increased as the time increased from 20 to 80 s for each gas flow and for each distance used in this investigation. The inhibition zone of both bacteria (S. aureus and E.coli) was increased with an increase of distances at gas flow 1 or 2.5 slpm but this is not the case with 5 slpm. Under most conditions, in our temperature tests, the agar temperature remained below 32°C. The agar temperature did not exceed 32°C for the all experiment conditions even increased the exposure time to 80 s. From this result we conclude that it is possible to operate a plasma needle so that there is no killing due to heat [7,8].

To summarize, all conditions are most attractive for possible clinical applications, and these are achieved at short exposure time but large separation between plasma needle and Petri dish. The 80 s treatment proves to be efficient even at a low flow rate whereas higher flow rate appear to be less efficient for shorter treatment times.

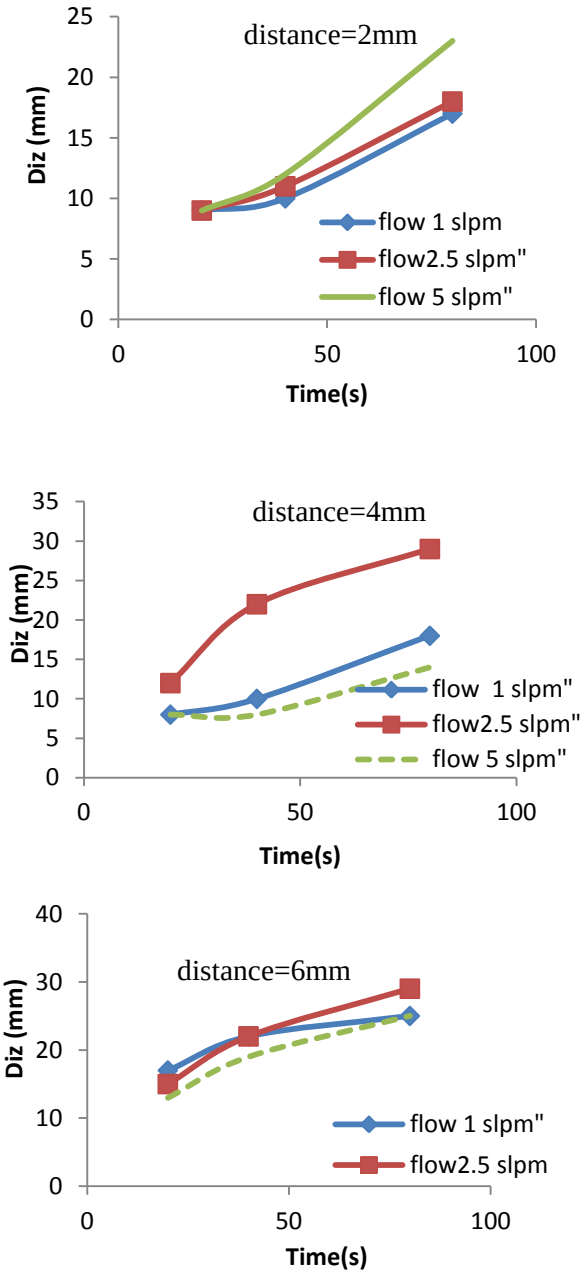

Figure 3. The diameter of inhibition zones (Diz) in millimeter for S. aureus cultivated on agar plate verse plasma exposed time

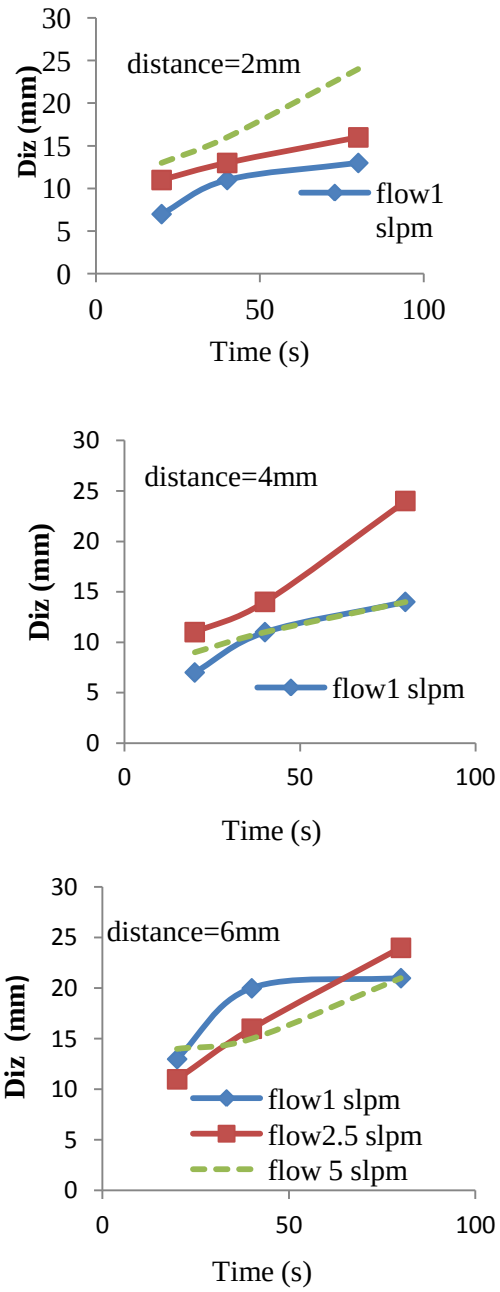

Figure 4. The diameter of inhibition zones (Diz) in millimeter for E. coli cultivated on agar plate verse plasma exposed time

The flow of the Argon gas plays an important role in the resulting concentrations of bacteria. This is presumably due to different resulting concentrations of reactive particles (radicals) where the flow of the inert Argon gas pushes atmospheric gases further away from the plasma core, and while the densities of UV photons could remain unperturbed, the densities of chemically active radicals would be reduced because of the increasing gas flow. This is, on the other hand, a simplified explanation as operating conditions may be quite different depending on the percentage of atmospheric gases. E. coli is a Gram-negative bacteria, which means that it has an additional outer membrane made of lipopolysaccharides and protein. If mechanical erosion of the bacterial membrane is one of the factors responsible for its inactivation, we can assume that E. coli will suffer less damage in the same treatment conditions, as compared to S. aureus[7]. The damage induced by plasma in the case of E. coli consisted of punctured, eroded and morphologically transformed bacteria, while for S. aureus, the bacteria were ruptured with their cellular contents released onto the substrate surface.

Another very strong disinfectant is UV radiation but its role in atmospheric plasma sterilization goes from modest to highly effective depending on the type and concentration of bacteria or spores, the amount and composition[10,11].

4. CONCLUSIONS

The plasma needle is capable of treatment under conditions that are attractive to clinical uses as it is capable of killing S. aureus and E.coli with a treatment time of seconds, and without an elevated temperature. It found that the plasma needle can be operated under conditions where the bactericidal effect is attributable to atmospheric chemical species produced by the plasma and not due to heat. Most importantly, the treatment time proved to be more important than merely the gas flow rate and the distance between plasma needle and patria dishes within the plasma jet length.

References

[1] B. J Park, D. H. Lee, J. C. Park, I. S. Lee, K. Y. Lee, S. O. Hyun, M. S. Chun, and K. H. Chung,. "Sterilization using a microwave-induced argon plasma system at atmospheric pressure", Physics of Plasmas, 10, 2003,pp. 4539-4544.

[2] M.Laroussi, "Sterilization of contaminated matter with an atmospheric pressure plasma", IEEE Trans Plasma, 24, 1966,pp.1188-91.

[3] M. G.Kong, G.Kroesen, G. Morfill, T.Nosenko, T. Shimizu, J. Van Dijk, and J. L. Zimmermann, "Plasma Medicine: an Introductory Review", New J Phys, 11, 2009, pp. 115012-14.

[4] M. Moreau, N.Orange, and M. G. J. Feuilloley, "Nonthermal plasma technologies: new tools for bio-decontamination", Biotechnol Adv 26, 2008, pp.610–617.

[5] Hammad R. Humud, Ahmad S. Wasfi and Wafaa Abd Al-Razaq, "A low temperature atmospheric pressure plasma jet", International Review of Physics (I.RE.PHY), 7,1, 2013, pp.40-43.

[6] Hammad R. Humud, Ahmad S. Wasfi, Wafaa Abd Al-Razaq, and Mazin S. El-Ansary, "Argon plasma needle source", Iraqi Journal of Physics, 10,17,2012, pp.53-57.

[7] J. Goree, B. Liu, D. Drake, and E. Stoffels, "Killing of S. mutans bacteria using a plasma needle at atmospheric pressure", IEEE Trans. Plasma Sci., 34, 4, Aug. 2006, pp.1317–1324,.

[8] Hammad R. Humud, Maha Adel Mahmood, and Wafaa Abd Al-Razaq, "Strain specifity in antimicrobial activity of non-thermal plasma", Iraqi Journal of Physics, 11,20, 2013, pp.110-115.

[9] K. Y. Lee, B. Joo Park, D. Hee Lee, I. S. Lee, O. S. Hyun, K. H. Chung and J. C. Park, "Sterilization of Escherichia coli and MRSA using microwave - induced argon plasma at atmospheric pressure" Surf. Coat. Technol, 193, 2005, pp.35–38.

[10] F. Iza, G. J. Kim, S. M. Lee, J. K. Lee, J. L. Walsh, Y. T. Zhang, and M. G. Kong, "Microplasmas: sources, particle kinetics, and biomedical applications," Plasma Process. Polym., 5, 4, Jun. 2008, pp.322–344.

[11] T. Nosenko, T. Shimizu and G. E. Morfill "Designing plasmas for chronic wound disinfection", New J. Phys. 11, 2009, pp.115013–32.