Effect of Atmospheric Non-Thermal Plasma on Adhesion Process of Pseudomonas Spp Bacteria

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Abstract

Non-thermal atmospheric pressure plasma has emerged as a new promising tool in medicine. The effectiveness of an Atmospheric Non Thermal Plasma (ANTP) for clinical and biological applications are studied. The effort achieved in this work includes a designing of plasma generating system and examine it on some medical and biological parameters. The dependencies of decreasing the adhesion of bacteria on the applied voltage and distance have been recorded. A series of experiments reveal that this effectiveness is due to the ability of direct discharge to bring charges to samples due to generation of so many mediators like reactive nitrogen species and free radicals. Increasing applied voltage and decreasing gap distance effect on decreasing Numbers of bacteria attached to epithelial cells to half.

Keywords: Atmospheric non- thermal plasma, Adhesion, *pseudomonas spp*.

Introduction

Plasma physics is a branch of physics science which appears as important science in the last centuries because its interest by the matter of the universe, plasma forms most of the universe matter. Also because, the plasma has unique properties and it can be generated in different laboratory methods. In other words, different plasmas with different properties can be obtained and then, can be used for wide range of applications.

According to its temperature, plasma can be classified to cold or hot plasma. The temperature of the cold one is approximately equal to the room temperature that is because, it is non- equilibrium plasma, i.e. its electrons have more large temperature greater than that of the neutral particles. For this reason, the cold plasma was used widely as a tool for different medical issues because it does not cause any thermal damage for the tissues [1].

Recently the development in cold plasma processes, which are working at atmospheric pressure by the growing requirements of new plasma technology such as a continuous plasma processing like plasma needle [2],the hair line plasma[3] and micro capillary plasma jet [4]. The dielectric barrier discharge (DBD) is most frequently used as a non-thermal plasma source that can be operated with different gasses at elevated pressures (up to atmospheric pressure) [5, 6].

Pseudomonas spp is opportunistic Gram negative pathogens. The ability of these pathogens to survive in multiple niches and to utilize many naturally occurring compounds as energy sources makes them one of the most ubiquitous bacteria in both the environment and the clinical setting, where they contaminate the floor, bed rails, and sinks, and the skin of patients and healthcare personnel. it is aerobic, motile, rod-shaped bacteria produce water –soluble pigments that diffuse through the medium [7]. On MacConkey media, the colonies are green or colorless, since lactose is not fermented [8]. They are opportunistic Gram negative pathogens. The ability of these pathogens to survive in multiple niches and to utilize many naturally occurring compounds as energy sources makes them one of the most ubiquitous bacteria in both the environment and the clinical setting, where they contaminate the floor, bed rails, and sinks, and the skin and urine tract infection of patients, also healthcare personnel [9].

Epithelial cells cover the whole surface of the body. It is made up of cells closely packed and ranged in one or more layers. This tissue is specialized to form the covering or lining of all internal and external body surfaces. An epithelial cell that occurs on surfaces on the interior of the body is known as endothelium. Epithelial cells are packed tightly together, with almost no intercellular spaces and only a small amount of intercellular substance. Epithelial cells, regardless of the type, is usually separated from the underlying tissue by a thin sheet of connective tissue; basement membrane. The basement membrane provides structural support for the epithelium and also binds it to neighbouring structures.

Adhesins are cell-surface components or appendages of bacteria that facilitate bacterial adhesion or adherence to other cells or to inanimate surfaces. Adhesins are a type of factor. Adherence is an essential step in bacterial pathogenesis or infection, required for colonizing a new host [10].

Most fimbriae of gram-negative bacteria function as adhesins, but in many cases it is a minor subunit protein at the tip of the fimbriae that is the actual adhesin. In gram-positive bacteria, a protein or polysaccharide surface layer serves as the specific adhesin. To effectively achieve adherence to host surfaces, many bacteria produce multiple adherence factors called adhesins [11].

Bacterial adhesins provide species and tissue tropism. Adhesins are expressed by both pathogenic bacteria and saprophytic bacteria. This prevalence marks them as key microbial virulence factors in addition to a bacterium's ability to produce toxins and resist the immune defenses of the host**.** Mature Fim H is displayed on the bacterial surface as a component of the type 1 fimbrial organelle [12].

The majority of bacterial pathogens exploit specific adhesion to host cells as their main virulence factor. "A large number of bacterial adhesins with individual receptor specificities have been identified." [11]. Many bacterial pathogens are able to express an array of different adhesins. Expression of these adhesins at different phases during infection play the most important role in adhesion based virulence.

DBD Circuit Modeling

The electrical gas discharge developed due to high voltage in the gap between the electrodes with at least one of them covered with a dielectric is called the dielectric barrier discharge [13]. At high pressures, the plasma is characterized with high electron density resulting in the formation of arcs. Such plasmas are termed as thermal plasmas. In order to reduce high current and the subsequent arc formation, one or both of the DBD electrodes are covered with a barrier material such as a dielectric, and hence the term dielectric barrier discharge [14,15]. The DBD is a highly transient, low-temperature non-equilibrium discharge.

DBD plasma can be explained with the help of a relatively simple model. Model the insulated electrode as a sphere of diameter D_{el}, while the object whose surface is being treated is modeled as a sphere of diameter $D_{ob.}$ In the absence of the object the electrode capacitance with respect to the far away (located at infinity) ground is given by the well-known formula $C_{el}= 2\pi\varepsilon_0 D_{el}$, where ε_0 is permittivity of free space. Now, if the object being treated has a relatively high dielectric constant (like that of water), it effectively expels most of the electric field from its interior when it is brought close to the electrode. From that point of view this object behaves like a good conductor and, therefore, its capacitance with respect to the far away ground can also be modeled by $C_{og}=2\pi\varepsilon_0 D_{ob}$. The region between the object and the electrode can be modeled roughly as a parallel plate capacitor with the value

$$C_{gab} = \frac{\pi\varepsilon_0 D^2{}_{el}}{2g} \quad \text{...} \ (1)$$

Where g is the gap distance. If the gap is significantly smaller than the electrode diameter. Note that

$$\frac{C_{gab}}{C_{og}} = \frac{D^2{}_{el}}{4gD_{og}} \quad \text{...} \ (2)$$

is significantly smaller than 1 for the typical choices of characteristic sizes. In the absence of any conduction current, the electrical models of the electrode by itself and the electrode near the treated object are well approximated by the circuits in Fig.(1 a, b). When the electrode is well removed from the ground, the magnitude of the applied voltage V is insufficient to create electric field strong enough to cause the breakdown and discharge.

However, when the object with a high dielectric constant is sufficiently close to the electrode, most of the applied voltage appears across the gap, this is because the capacitance of the object with respect to ground is much larger than the gap capacitance, and the voltage divides across these capacitors proportionally to the inverse of their size. This results in a strong electric field in the gap which can now lead to breakdown and discharge.

The electrical circuit model can be further refined by taking into account non-linear resistance and capacitance of the plasma created in the gap. The resulting circuit refinement is shown in Fig.(1-c). The refined circuit does not change the main conclusion that most of the applied voltage appears across the plasma gap.

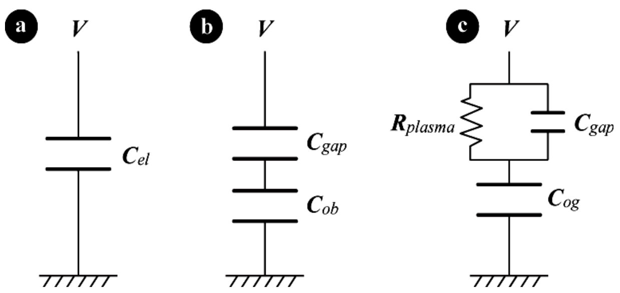

Fig.(1) Simplified electrical schematic of (a) electrode itself, (b) electrode near the treated object, and (c) plasma discharge on the treated object [13].

Dielectric barrier discharge (DBD) is significant among all types of non-thermal plasma, because they can provide none-equilibrium plasma conditions at bout atmospheric pressure in a much simple way. It can be operated in open air, outside a vessel .in addition to its flexibility with respect to geometrical configuration, operating medium and operating parameters are remarkable.

In many cases discharge conditions optimized in small laboratory experiments can be scaled up to large industrial installations, efficient low cost power supplies are available up to very high power levels [16, 17].

Experiment Setup

Dielectric barrier discharge DBD system is based on a conventional dielectric barrier discharge. It is basically a system driven by alternating current and high voltage applied between two conductors where one or both are covered with a dielectric. That is to limit the current and to prevent transition to an arc. A simplified schematic of the DBD system setup is shown in Fig.(2).

The system consists of power supply of high voltage (5-25 kV) related to a wire to the copper electrode is surrounded by Teflon; the end of the copper electrode is connected in contact with the glass thickness of 1 mm. Other part of the system is gradually moving catcher connected by a piece of mica to the copper electrode to prevent the transmission of discharge to the catcher moving. Plasma is generated by applying alternating polarity or pulsed high voltage between the insulated electrode and the sample which must be treated. The sample putted on an aluminum substrate. A 1 mm thick polished glass was used as an insulating dielectric barrier. The discharge occurs between the bottom surface of the glass and top surface of the sample. The distance where the discharge occurs was controlled to be (1-3 mm). To accomplish the control ability, the high voltage electrode was connected to a vertical catcher by a positioner. This positioner can be moved up and down easily. The diameter of the copper electrode employed was 2.5 cm. All the treatments are at room temperature and atmospheric pressure and were carried out according to the same procedure.

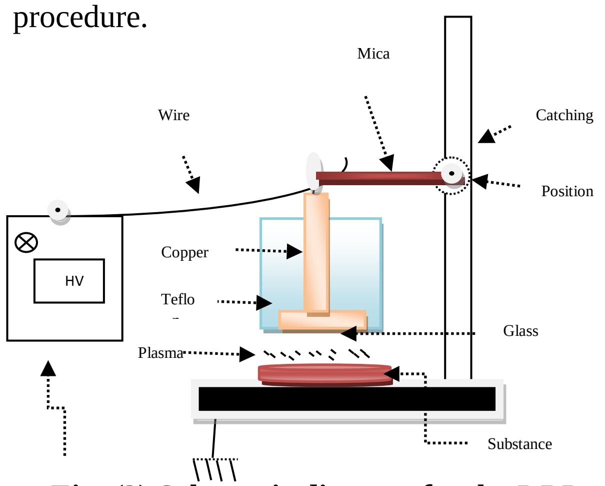

Fig. (3) Schematic diagram for the DBD system.

Method

Fifty patients complaining from different clinical signs and symptoms were admitted to Ramadi Teaching Hospital for seeking medical advise were included in the present study.

Midstream urine samples (MSU) were obtained from patient with urinary tract infections (UTI_s), were examined bacteriological. These samples were collected from patients visiting the out patients urology clinic and patients admitted in the urology department –Ramadi Teaching Hospital.

The results of this work were obtained from studying 50 urine samples. These samples were taken from patients complaining from signs and symptoms of urinary tract infections (UTI_s) they were 35 females and 15 males.

The technical detail were described in a methodological study, bacteria were transferred from deep agar subculture where they had kept after isolation to MacConkey agar plates. After growth for 16-48 hrs at 37 ^{0}C in Brain Heart Infusion (BHI) broth, the bacteria were centrifugated and resuspended in Phosphate Buffer Saline (PBS) pH (7.4). Uroepithelial cells (UEC_s) were obtained from the sediment of the midstream morning specimen of healthy females without bacteriuria. They were washed and resuspended in PBS [18]. 0.5 ml of an overnight culture of bacteria in B.H.I broth was gently added to 0.5 ml of UEC_s suspension. The mixture was mixed by inversion and then was incubated for 1 hr at 37^{0}C with shaking every 10 minutes .The UEC_S were then washed four times with PBS thus unattached bacteria were eliminated by repeated cycle of centrifugation and resuspension [18,19,20**]**. A drop of the final UEC_S suspension was deposited on a glass slide, air-dried, heat fixed and Gram stained. A control slide with no attached bacteria was stained at the same time. Examination of the slide was done by light microscopy under oil immersion to demonstrate the attachment bacteria to UEC_s [21,22]. Two tubes, one before DBD treatment, other after DBD treatment.

The adhesion for each bacterial isolate was estimated as the mean number of bacteria adhering to many epithelial cells. The results were compared with the results of control group.

Results and Discussion

Non-thermal atmospheric pressure dielectric barrier discharge DBD plasma was experimentally verified to significantly, the results presented in this article indicate that non-thermal dielectric barrier discharge treatment is capable of decreasing bacterial adhesion on epithelial cells.

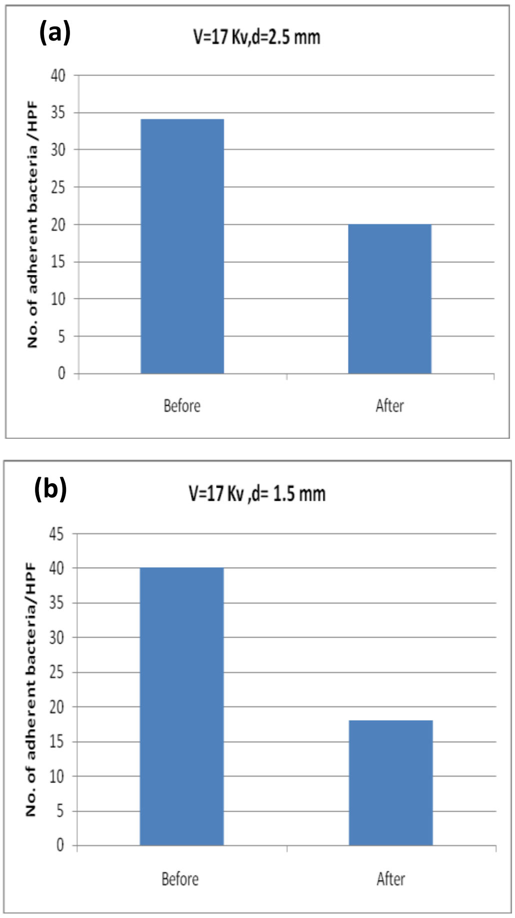

Fig.(4) Mean values of No. of adherent bacteria to epithelial cells before & after treatment with DBD at (V=17 kV) when gap distance. a) 2.5mm. b)1.5 mm.

Fig.(4) shows the mean values of No. of bacteria attached to epithelial cells before & after treatment with DBD, where it decreases after treatment at applied voltage 17 kV, gap distance 2.5 mm through period 1.30 minutes . While it decreases after treatment at applied voltage 17 kV, gap distance 1.5 mm through period 1.30 minutes.

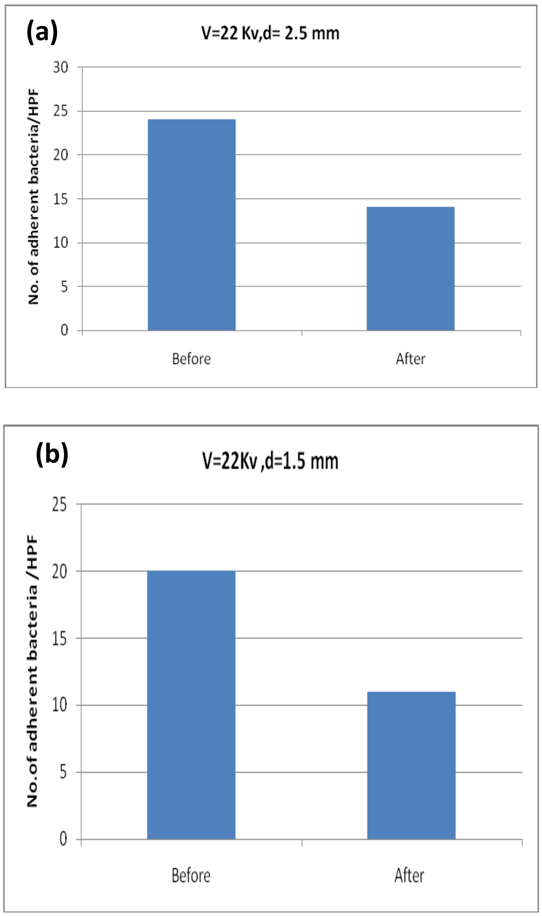

Fig. (5) Mean values of No. of adherent bacteria to epithelial cells before & after treatment with DBD at (V=22 kV) when gap distance. a) 2.5mm. b)1.5 mm.

Fig.(5) shows the mean values of No. of bacteria attached to epithelial cells before & after treatment with DBD, where it decreases after treatment at applied voltage 22 kV, gap distance 1.5 mm through period 1.30 minutes.

Comparing figures above, show increasing applied voltage and decreasing gap distance effect on decreasing No. of bacteria attached to epithelial cells .

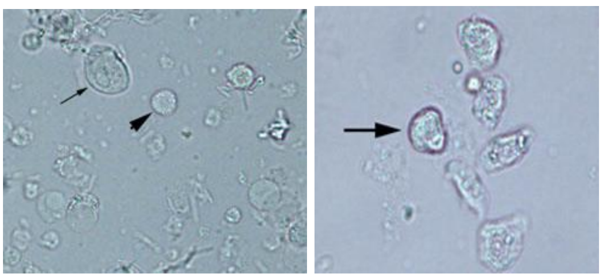

Fig. (6) Picture for bacterial adhesion on epithelial cells.

The specific mechanism for the plasma effect on epithelial cells is similarly unclear. Cold plasma produces long living (O_3,NO,HO_2,H_2O_2) and short lived (OH,O electronically excited) neutral particles and charged particles(ions and electrons). All of these could be toxic to cells, induce low levels of cell membrane damage and potentially change intercellular signaling pathways. Specific plasmas can be created to produce either neutrals or charged particles in order to elucidate the critical mechanism [22,5].

The properties of the cold plasma can most likely be assigned electrical stresses that rupture the bacterial cell wall. Also, breaks chemical bonds, and interactions with short living plasma species gradually enhance the bacterial death rate. Prolongation of treatment time and elevated applied voltage significantly improved the bacteria destruction efficiency at DBD plasma.

Conclusions

Plasma device inactivates bacteria. The dependencies of the degree of the exposure time, applied voltage and distance have been recorded. A series of experiments reveal that this effectiveness is due to the ability of direct discharge to bring charges to samples due to generation of so many mediators like reactive nitrogen species and free radicals. This DBD air plasma could be a promising technique in applications for biological and medical materials contaminated with microorganisms. Results presented in this paper indicate that there are several possible ejects produced by plasma components on microorganisms.

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الخلاصة

تعد البلازما غير الحرارية العاملة بضغط جوي وسيلة ترويجية حديثة في الطب. تم الاعتماد على البلازما الباردة بضغط جوي للتطبيقات البايلوجية والسريرية. يتضمن هذا البحث تصميم لنظام توليد البلازما غير الحرارية في الضغط الجوي الاعتيادي واختباره على التطبيقات الطبية والبايلوجية. الاعتمادات لانخفاض الالتصاق البكتيري على الفولتية المطبقة، المسافة التي تكون مسجلة. وقد بينت سلسلة من الاختبارات بان هذه التأثيرات الناتجة من التفريغ المباشر لجلب الشحنات الى العينات الناتجة من توليد عدد من العوامل مثل أصناف النتروجين المتفاعلة والجذور الحرة. الزيادة بالفولطية المطبقة وتناقص مسافة الفجوة تاثير على انخفاض عدد البكتريا الملتصقة بالخلايا الطلائية الى النصف.