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Assessment the effect of non-thermal plasma on *Escherichia coli* and *Staphylococcus aureus* biofilm formtion *in vitro*

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Abstract

Biofilm formation represents one of the biggest problems facing scientists because of this phenomenon linkage with virulence of bacteria and other clinical environmental problems. In the present study, two clinical isolates, *Escherichia coli*, and *Staphylococcus aureus* were exposed to the non thermal plasma for different intervals of time (1, 2, 4, 8, and 16 min). The biofilm was measured post exposing. It was found that 2 min. exposing to non-thermal plasma reduced the biofilm formation by both clinical isolates significantly. It can be concluded that the ability of *S. aureus* to form biofilm higher than *E. coli* and exposing for 2 min to non-thermal plasma sufficient to reduce the biofilm formation by both isolates significantly.

Keywords: *Escherichia coli*, *Staphylococcus aureus*, *Biofilm*, *non-thermal plasma.*

تقييم تأثير البلازما غير الحرارية على تكوين الغشاء الحيوي لبكتريا *Escherichia coli* و *Staphylococcus aureus* في الزجاج

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الخلاصة

يعد تكوين الغشاء الحياتي واحد من اكبر المشاكل التي تواجه العلماء وذلك لان هذه الظاهرة ترتبط مع فوعة البكتريا وكذلك ترتبط بعدد من المشاكل السريرية والبيئية. في هذه الدراسة، عزلتين سريرية وهي الاشرشية القولونية والمكورات العنقودية عرضت الى البلازما غير الحرارية ولأوقات مختلفة (1، 2، 4، 8 و 16 دقيقة). وتم قياس تكون الغشاء الحياتي بعد التعرض. وقد وجد ان تعريض العزلتين للبلازما غير الحرارية ولمدة دقيقتين كافي لاختزال قابلية نوعي البكتريا على تكوين الغشاء الحياتي وبشكل معنوي. ويمكن الاستنتاج ان قابلية المكورات العنقودية اكبر من قابلية الأشرشيا القولونية على تكوين الغشاء الحياتي وان دقيقتين من التعرض للبلازما غير الحرارية كان كافي لأختزال قابليتهما على تكوين الغشاء الحياتي.

Introduction

Biofilms are a community of microorganisms attached to different surfaces by helping of different appendages and compound that inter in the structure of bacteria such as polysaccharides, proteins, and nucleic acids [1]. *Escherichia coli* biofilm development is a complex process that leads to form a

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biofilm, which plays an important role in causing several diseases related with by attaching of bacteria and resistance to wide spectrum of antibiotics. The biofilm formation is done by several steps, i. attachment of bacteria on the surfaces, ii developing the matrix from reversible to irreversible, iii developing biofilm, iv maturation of biofilm [2]. The appendages that may play an important role in adhesion of *E. coli* and form biofilm are flagella, pili and extracellular polysaccharides [3].

Staphylococcus aureus isolates are responsible for different problems associated with medical devices such as prostheses because of their ability to produce a biofilm on this surface [4]. In addition to *S. aureus* there are several species of produce biofilm such as *S. epidermidis, Pseudomonas aeruginosa* and *Bacillus* [3, 5]. The biofilm prevents antimicrobial agents from entering the bacterial cells and interfere the activities of antibiotics. As well as biofilms protect bacteria leading to continual infections [6].

There are several factors can control the biofilm formation such as chemical and physical factors. The temperature, pH and type of the surfaces control the biofilm formation [7]. A huge number of studies focused on the above mentioned factors and role of their roles on biofilm formation by Gram positive and negative bacteria [8]. Very scanty study focused on the role of non-thermal plasma on biofilm formation of Gram positive and negative bacteria. The present study aims to highlight the effect of non thermal plasma energy on the ability of *E. coli* and *S. aureus* to form biofilm.

Materials and methods

Clinical isolate

Number of clinical isolates of *E. coli* and *S. aureus* was used in this study the clinical sources, and the period of research is conducted. These isolates were procured from the Department of Biology, College of Science, University of Baghdad, Baghdad/ Iraq. The isolates were preserved on nutrient agar (Himedia, Mumbai, India) slants and stored at 4 ^{o}C until used.

Biofilm formation

Overnight cultures of *E. coli* and *S. aureus* in 5ml of Tryptose soy broth (TSB) (Himedia, India) were centrifuge at 3000 g for 15 min and the bacterial pellet was washed two times with sterile phosphate buffer saline (0.1 M, pH 7.2), and then two times with fresh TSB. The bacterial count was adjusted to 10^7 c.f.u/ml. 200 µl of inoculums were added to the wells of sterile flat-bottom polystyrene tissue culture plates, and incubated at 37°C for 24 h. The medium was then discarded, and the wells were washed three times with sterile PBS (0.1 M, pH 7.2) to remove non adherent bacterial cells. Biofilm was fixed by incubating the plates for 30 min at 61°C and then stained with crystal violet (0.4%, 5 min), After washing with PBS (0.1 M, pH 7.2) to remove the excess stain, the plates were left to dry at room temperature. The biofilm was determining by measure an absorbance of a stained biofilm when treating with acetone : ethanol (30 : 70) at a wavelength of 492 nm using ELISA reader [9].

Exposure of bacterial isolates to non-thermal plasma

S. aureus and *E. coli* isolates were grown into the nutrient broth for 18 h at 37 ^{o}C. The Bacterial suspensions were centrifuged and washed 3 times with PBS (0.1 M, pH 7.2). The pellets were re-suspended with PBS (0.1 M, pH 7.2), and divided into five tubes and exposing to non-thermal plasma for different time intervals (1, 2, 4, 8 and 16 min). The ability of each isolate to form biofilm was checked.

Statistical analysis

All values were calculated in mean value ± standard deviation (SD). Student's t-test was used to check the differences. $P<0.05$ was judged to be statistically significant.

Results

As mention earlier the ability of both clinical isolates to form biofilm was estimated. Figure-1 shows that it was found that the ability of *S. aureus* to form biofilm was significantly higher than that of *E. coli* to form biofilm ($P <0.05$).

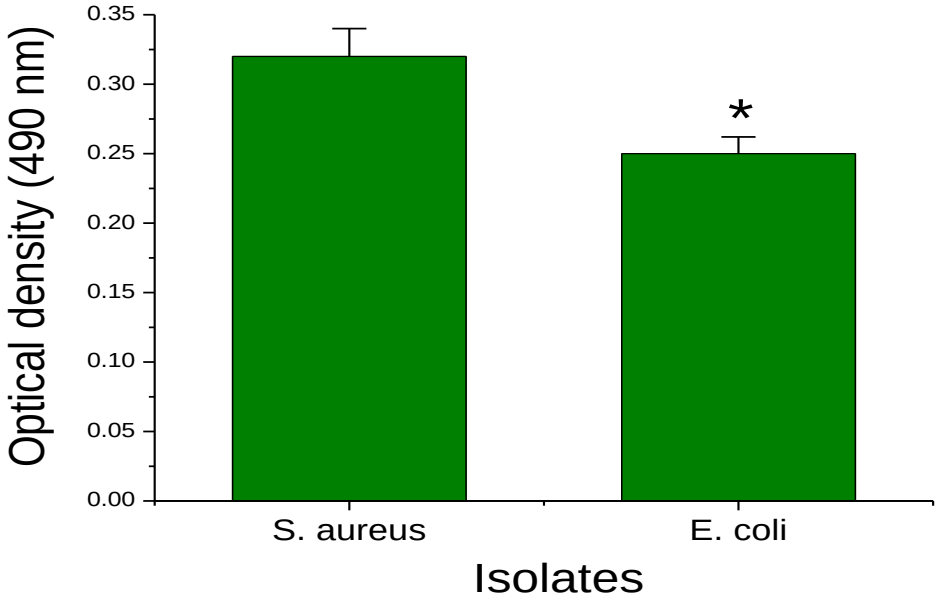

Figure 1-Biofilm formation of *S. aureus* and *E. coli* grown in Tryptose soy broth for 18 h. Asterisk indicates the significant difference.

Effect of exposing to non thermal plasma on biofilm formation

The previous section has shown that ability of both bacterial isolates to form a biofilm. Figure-2 shows effect of exposing to non thermal plasma at different time intervals on biofilm formation by *S. aureus*. The biofilm formation was decreased dramatically with time.

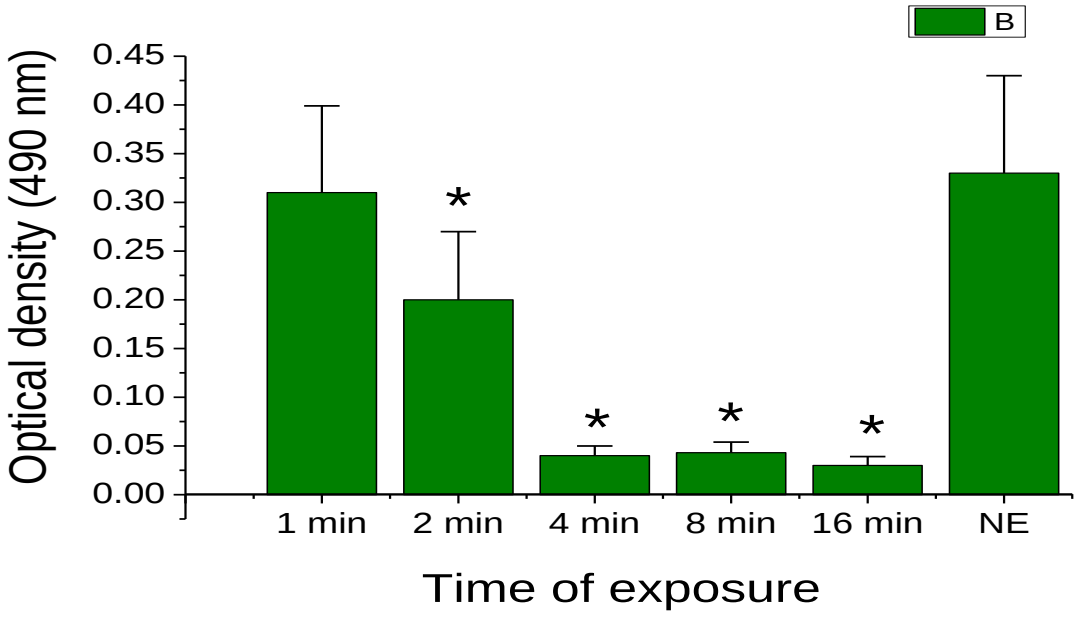

Figure 2- Effect of duration of exposure to non-thermal plasma energy on the biofilm formation by *S. aureus* in vitro. Asterisks indicate the significant difference in control. NE, non-exposed bacterial growth (control).

The significant decrease of biofilm was found as early as 2 min. post exposing to non-thermal plasma. The lowest level of biofilm was found at 4 min. There is no difference in terms of biofilm formation after this time point. A similar finding was observed when *E. coli* exposed to non-thermal plasma at different time intervals Figure-3.

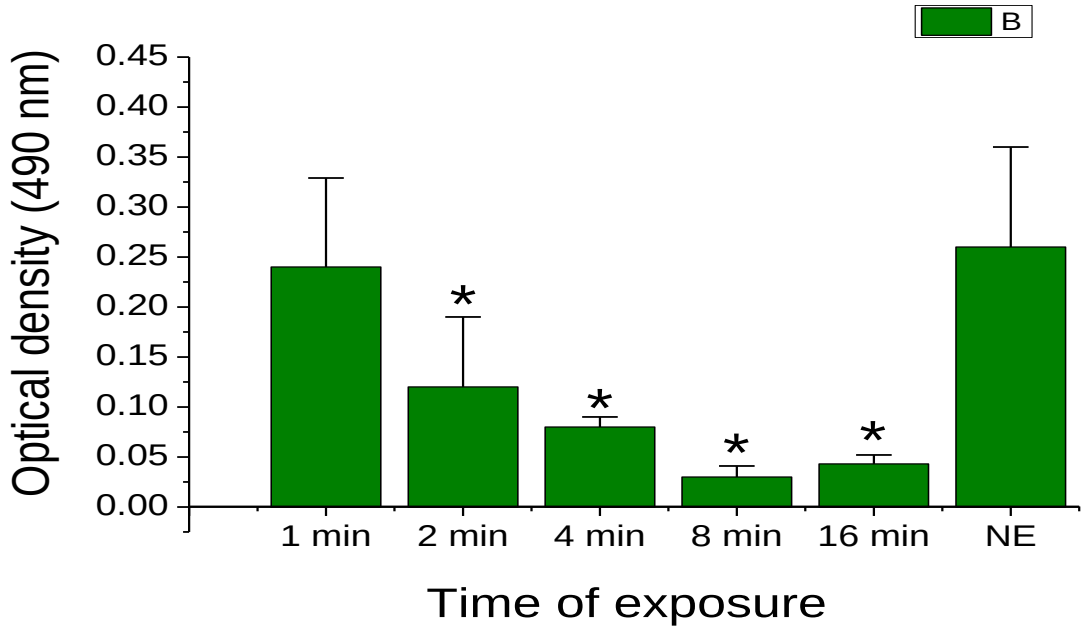

Figure 3- ffect of duration of exposure to non-thermal plasma energy on the biofilm formation by *E. coli* in vitro. Asterisks indicate the significant difference in control. NE, non-exposed bacterial growth (control).

Discussion

Several studies focused on effect of physical factors on formation of biofilm of clinical isolates [4]. In the present study, two clinical isolates, *E. coli*, and *S. aureus* were exposed to the non thermal plasma. The biofilm formation of both isolates was measured. It was found that two minutes of exposure of non-thermal plasma was sufficient to reduce the biofilm formation significantly in both isolates.

Biofilms are linked with the big challenge to control infection, even by the wide spectrum antibiotics [10]. The essential goal in the field of healthcare is to find a new strategy to limit the formation of biofilm by bacteria, which are difficult to get rid of them by traditional methods, especially the use of antibiotics. If scientific research can find such a technique that will occur a significant development in reducing the spread of pathogenic bacteria [10]. A previous study showed that the exposure of bacteria to the sublethal dose of plasma is leading to remove the activity of bacteria in vitro [11]. Thus the effect of non-thermal plasma against biofilm suggested being advisable in treating of biofilm formation by several species of bacteria in vitro. Effect of non-thermal plasma on different physiological activities of bacterial cells was reported by the previous study [12]. From current study, it can be concluded that the non-thermal plasma can reduce the biofilm and that may open wide range of applications that may be solve the biggest problem in the field of bioscience.

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