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Plasma can reduce *Staphylococcus epidermidis* biofilm formation on medical polymers

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ABSTRACT

Medical polymers, such as urinary catheters are widely used biomaterials. One of the main problem for using the urinary catheters is biofilm formation on their surface, when they are used in a long time in the body. Virulence and pathogenicity of *Staphylococcus epidermidis* is often enhanced when growing as a biofilm. Many techniques have been presented to reduce the biofilm formation by surface modification. One of the most revolutionary techniques allowing such surface modifications is the plasma surface modification. In this work, plasma effects on *S. epidermidis* biofilm formation on urinary catheter surface have been investigated. Plasma was produced in a Pyrex glass tube containing nitrogen with pressure 1.6×10^{-1} Torr for plasma treatment of a catheter surface. Discharge voltage was about 1.2 kV and current was 150 mA. Each set of plasma treated catheter samples was inoculated by cultivation of *S. epidermidis* on 50 ml of Tryptic soy broth medium in the shaking incubator for 48 h at 37°C and 100 rpm. Then, amount of biofilm formation on the surface of polymer were assessed by crystal violet binding assay and sonication method. The results of these experiments indicated reduced biofilm formation on the modified surface around 50-60% compared to non-modified surface. This study shows that plasma surface modification can be used to reduce biofilm formation on medical polymers such as urinary catheter.

Keywords: Urinary catheters, Biofilm, Surface modification, Medical polymer

Introduction

Medical polymers such as urinary catheters have played an important role in disease management and the advancement of health care (1-3). Urinary catheters are used for introduction into the bladder through the urethrea for the withdrawal of urine. When urinary catheters are implantedinside bladder for a long time, they can become places for bacteria to biofilm formation and infection frequently results and is by far one of the major clinical complications (4).

Microbial biofilm is accumulation of micro-organisms embedded in an extracellular matrix that is composed primarily of exopolysaccharides (EPS) and extracellular DNA (eDNA) (5). Biofilms display a complex three-dimensional structure, increased resistance to antibiotics, environmental stresses and the host immune response and responsible for over 80% of microbial infections in the body (6).

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Staphylococcus epidermidis is very likely to contaminate patient-care equipment such as urinary catheters in the hospital setting. *Staphylococcus epidermidis* produces slime layers, which forms a hydrophobic biofilm. This film is adhesive to hydrophobic biopolymers of prosthetics such as urinary catheters, creating diseases such as urinary tract infection. Virulence and pathogenicity of *S. epidermidis*is often enhanced when growing as a biofilm (7, 8). The adhesion of bacteria to a surface of medical polymer appertains on surface characteristics of polymer. Therefore one of the ways to tackle this problem is to control the physicochemical interactions between the bacteria and medical polymer surface due to reduce the biofilm formation (9). Many techniques have been presented to reduce the biofilm formation by surface modification. One of the most revolutionary techniques allowing such surface modifications is plasma treatment.

Plasma is defined as a partially or completely ionized gas with approximately equal amount of positively and negatively charged particles. Plasma treatments can be used to modify the surface properties of polymers and improve their performance in various applications (10). One of the plasma treatments is Glow discharge plasma (GDP) which is a frequently used method for cleaning, preparation, and modification of biomaterial and implant surfaces (11, 12).

We propose here to use Glow discharge plasma treatment to conduct surface modification of urinary catheters. Therefore, we investigated GDP effects on *S. epidermidis* biofilm formation on urinary catheter surface.

Materials and Methods

Materials

Urinary catheter was purchased from Well Lead Medical Co., Ltd. All microbial media and chemicals were obtained from Merck Co., Ltd., Germany, and *Staphylococcus epidermidis* strain was previously isolated from a urinary infection sample.

Plasma treatment

Plasma was generated in a Pyrex glass tube containing pressure 1.6×10^{-1} Torr of nitrogen for plasma treatment of a catheter surface by Glow discharge plasma was made in Iran, Discharge voltage was about 1.2 kV and current was 150 mA. Urinary catheter samples (1×0.5×0.5 cm) were placed inside the glass tube in the positive Coulomb region of discharge for 20 min (Fig. 1).

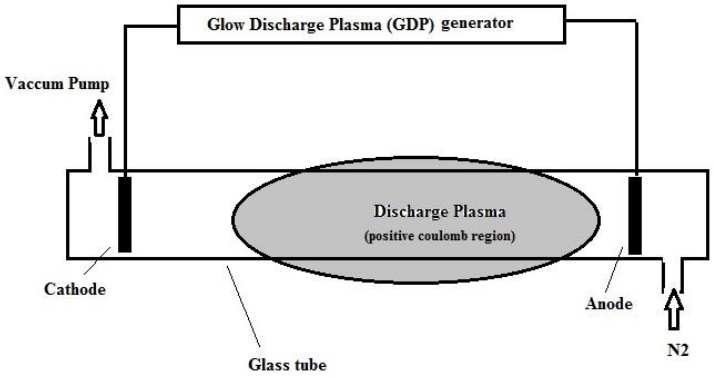

Figure 1. The Glow discharge plasma (GDP) apparatus and the glass tube that the samples were placed in the positive coulomb region of discharge used for plasma treatment.

Biofilm formation

Evaluation of biofilm formation ability of *Staphylococcus epidermidis* strain on plasma treated catheter was performed in tryptic soy broth (TSB) medium. All set of catheter samples inoculated by an overnight cultivation of *S. epidermidis* (1 ml of *S. epidermidis* culture with OD_{600}=1) in the shaking incubator for 48h at 37°C and 100 rpm. At the end of

incubation period, each sample was aseptically removed from the broth culture for biofilm quantification using both of the crystal violet binding assay and sonicate method.

Crystal violet binding assay

Biofilms were produced on the modified and non-modified polymer surface as described above. Each sample was washed 6 times with 3 ml of sterile distilled water. Then the samples were stained with 2.5 ml crystal violet (Fishers scientific, USA) for 15 minutes and the excess stain washed off under running tap water. After the samples were air dried, were immersed in 2.5 ml of 33% glacial acetic acid (Fishers scientific, USA). The re-solubilized liquid for each sample was poured into a cuvette. The absorbance (optical density) of each re-solubilized liquid was measured against the optical density of blank reading without inoculation at wavelength of 600 nm for *Staphylococcus* strains the using a spectroscope. The absorbance of negative control was subtracted from the absorbance values to determine the actual values (13).

Sonicate method

Biofilms were produced on the modified and non-modified polymer surface as described above. Each sample was washed 6 times with 3 ml of sterile distilled water. Then, the samples were transferred into sterile flasks containing 5 ml saline. The flask was sealed and immersed in an ultrasonic bath. Sonication at 30 kHz with a power output of 400 W, as specified by the manufacturer, was performed at 37°C for 10 seconds and vortexed for 10 seconds. Then a tenfold serial dilution of the sonicate samples prepared and 100 µL of each dilution was cultured on 20 ml TSA at 35ºC for 24 h. The cultured bacteria were enumerated by colony counting. The number of CFU/ml after final rinsing was recorded as a quantitative baseline, facilitating evaluation of the different detachment methods. Finally, the number of CFU/ml of samples was compared with the number of CFU/ml of negative control (non-modified polymer) (14).

AFM image

Biofilms were produced on the modified and non-modified polymer surface as described above. Each sample was washed 6 times with 3 ml of sterile distilled water. Then, photographs were taken from their surface by atomic force microscopy (AFM).

Statistical analyses

Analyses were performed by SPSS software version 16. All the experiments were done in triplicates and data are mean of three independent measurements with significance of $p \leq 0.05$.

Results

Effects of plasma treatment on the surface hydrophobicity of polymer

The surface of most medical polymers is hydrophobic. Results of Fourier Transform Infrared Spectrometery (ATR-FTIR), indicated that after nitrogen plasma treatment, the surface hydrophobicity was changed due to formation the C=O and O–H groups on the surface of polymer (Fig. 2). The intensity of peaks at 3448 cm^{-1} and 1632 cm^{-1} corresponding to OH and C=O polar groups bonds are increased on the surface of polymer.

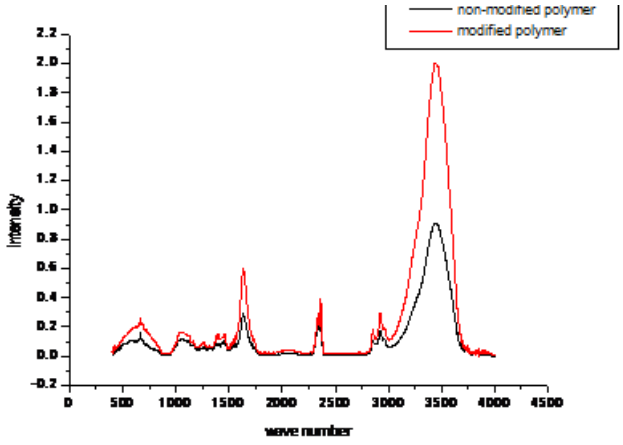

Figure 2. FTIR for modified and non-modified polymer surfaces.

Effects of plasma treatment on the surface inequality and roughness of polymer

AFM images of polymer surface before the plasma treatment and after that indicated while nitrogen plasma treatment decreased the surface inequality, it increased the surface roughness (Figs. 3, 4).

Biofilm formation on the surface of modified polymer

As shown in Figure 5, the amount of crystal violet adsorption by modified sample is about 60% less than the pristine sample. It indicates reduce of biofilm formation on the modified surface compared to non-modified surface. On the other hand, the result of colony count also confirmed this finding (about 54%).

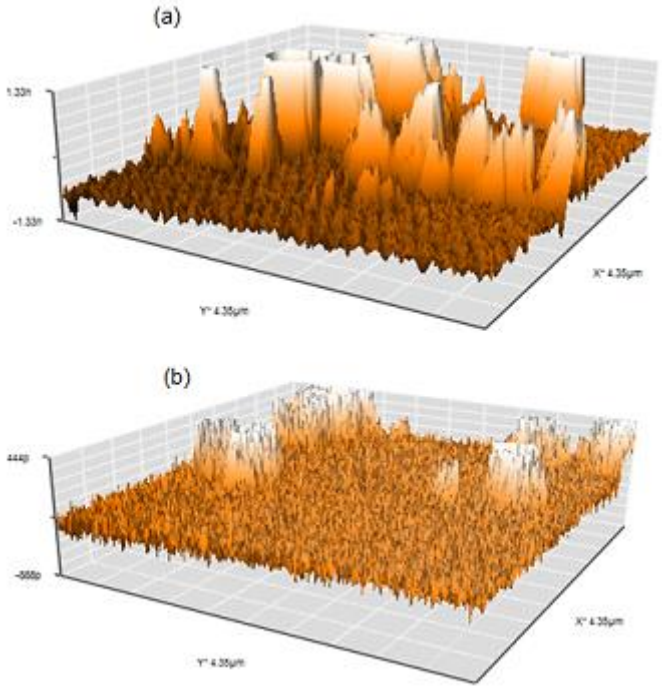

Figure 3. AFM micrographs of non-modified (a), and modified (b) polymer surfaces.

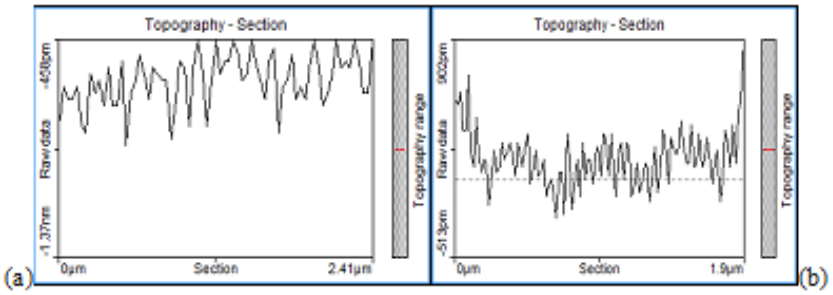

Figure4. AFM micrographs analyses of non-modified (a), and modified (b) polymer surfaces.

Discussion

Similar to the surface of medical polymers such as urinary catheter, the surface of microorganisms is usually hydrophobic too. Therefore, surface of these polymers is an appropriate place for attachment of microorganisms and subsequently biofilm formation (15). In the present study, we used the plasma treatment for surface modification of polymer for making it hydrophilic in order to reduce the attachment of bacteria. During plasma surface modification, the substrate is exposed to a reactive

environment of a partially ionized gas comprising large concentrations of excited atomic, molecular, ionic, and free radical species. The nature of the interactions between the excited species and the solid surface will determine the type and degree of the chemical and physical modifications that will take place (10).

Our results as shown in Figure 5 indicated that biofilm formation was significantly decreased after plasma treatment. According to Figure 2, it seems that the hydrophilic nature of polymer surface (due to increase of OH groups on the surface of polymer after plasma treatment) was responsible for this phenomenon. Similarly, a d.c. oxygen treatment of medical grade poly(vinyl chloride) resulted in a 70% reduction in bacterial adhesion for the four strains of *P. aeruginosa* (16). On the other hand, the analysis of AFM micrographs before and after the plasma treatment (Fig. 4) was indicative of increased surface roughness and reduced surface inequality. Indeed plasma decreased the size of the accessible pores and made them unavailable for colonization. In an earlier investigation argon plasma treatment was used to reduce levels of *S. epidermidis* adhesion to Ar-treated polyethylene (17). The results of both treatments confirm that two factors, namely polymer hydrophilic surface and reduction of contact area between bacterium and substrate, might reduce the ability of attachment and subsequently biofilm formation by some microorganisms such as *S. epidermidis*.

We conclude that plasma surface modification can be used to reduce *S. epidermidis* biofilm formation on medical polymers such as urinary catheter. So, it can reduce the rate of infection in patients with long-term use of these catheters.

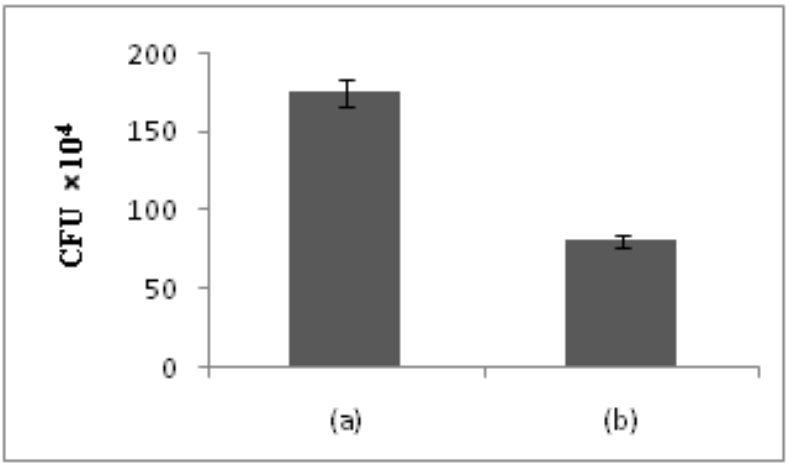

Figure 5. Comparison of *Staphylococcus epidermidis* biofilm on non-modified surfaces (a), and modified surfaces (b) by crystal violet binding assay.

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