

Plasma surface modification as a new approach to protect urinary catheter against *Escherichia coli* biofilm formation

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ABSTRACT

Background and Objectives: Biomaterials are widely used in medical devices such as urinary catheters. One of the main problems associated with long term using of the urinary catheters is biofilm formation on their surfaces. Many techniques have been presented to reduce the biofilm formation. One of the most revolutionary techniques allowing such surface fiction-alization is plasma surface modification.

Materials and Methods: In this study, a glow discharge plasma (GDP) effect on *Escherichia coli* biofilm formation on the surface of urinary catheter in the pressure of 1.6×10^{-1} Torr of nitrogen, discharge voltage about 1.2 kV and current of 150 mA for 20 minutes has been investigated. Crystal violet binding assay and sonication method were performed in order to evaluate the amount of biofilm formation on tested biomaterials.

Results: Characterization of modified surfaces by Attenuated Total Reflectance Fourier Transform Infrared Spectrometry (ATR-FTIR) and atomic force microscopy (AFM) revealed a noticeable change in hydrophobicity and roughness of catheter surfaces achieved by nitrogen plasma. The results of crystal violet binding assay and sonication method showed that the amount of biofilm formation on modified surface was about 86% less than the pristine sample.

Conclusion: Plasma surface modification can reduce the risk of infections in patients with long-term use of urinary catheters.

Keywords: Plasma treatment, Glow discharge plasma, Surface modification, Biofilm, Catheter

INTRODUCTION

The interaction of biological systems and engineered biomaterials is one of the key element in biotechnology (1). Biomaterials are widely used in medical devices, such as the urinary catheters (2). Urinary catheters are used for introduction into the bladder

through the urethra for the withdrawal of urine. One of the main problems for using the urinary catheters is biofilm formation on their surface specially when they are used in the body for a long time (3).

Biofilms are slime-enclosed aggregations of microbes that grow and attach to surfaces by extracellular polymers. Biofilm can form on any surfaces especially once it has been conditioned by proteins and other molecules present in the environment. Biofilm formation on medical devices causes serious failures (4) and responsible for over 80% of microbial infections in the body (5, 6). In the biofilm, microorganisms are more resistant to therapeutic agents such as antibiotics.

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Escherichia coli is the most common cause of urinary tract infection. The virulence of *E. coli* which is responsible for urinary infection is often enhanced when growing as a biofilm (7-9). The adhesion of bacteria to a surface of medical polymer appertains on surface characteristics of polymer (10). Many techniques such as new types of biomaterial production and surface modification methods have been presented to reduce the biofilm formation (11-18). One of the most revolutionary techniques allowing such surface fictionalization is the plasma surface modification (10, 15, 19).

Plasma is defined as a partially or completely ionized gas with approximately equal amount of positively and negatively charged particles (10). Plasma can be classified into 2 categories, equilibrium plasma and non-equilibrium plasma according to the temperature conditions. Non-equilibrium plasmas can be initiated at substantially lower temperatures, enabling their application for surface cleaning and fictionalization of polymer surfaces (10, 20-23) which one of them is Glow discharge plasma (GDP) (23, 24).

In the present study, a GDP effect on *E. coli* biofilm formation on urinary catheter surface has been investigated.

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 *E. coli* 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) placed in the positive Coulomb region of discharge for 20 minutes.

Biofilm formation. Evaluation of biofilm formation ability of *E. coli* strain on plasma treated catheter was performed in tryptic soy broth (TSB) medium. All set of catheter samples inoculated by an overnight cultivation of *E. coli* (1 ml of *E. coli* cul-

ture with $OD_{600}=1$) in the shaking incubator for 48 h 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 sonication 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. The samples were air dried and then 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 *E. coli* strains using a spectroscope. The absorbance of negative control was subtracted from the absorbance values to determine the actual values (25).

Sonication 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 ten fold serial dilution of the sonicated samples prepared and 100 µL of each dilution was cultured on 20 ml TSA and incubated at 35 °C for 24 h. The cultured bacteria were enumerated by colony counting. The number of CFU after final rinsing was recorded as a quantitative baseline, facilitating evaluation of the different detachment methods. Finally, the number of CFU compared with the number of CFU of negative control (non-modified polymer) (26).

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, photos were taken from their surfaces by atomic force microscopy

(AFM).

RESULTS

Effects of plasma treatment on the surface hydrophobicity of polymer. The surface of most medical polymers is hydrophobic. Results of Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) indicated that nitrogen plasma treatment could modify the surface hydrophobicity, that is because of formation of the C=O and O-H groups on the surface of polymer (Fig. 1).

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.

Effects of plasma treatment on the surface inequality and roughness of polymer. AFM images of polymer surface before and after the plasma treatment indicated while nitrogen plasma treatment decreased the surface inequality; it increased the surface roughness (Figs. 2 and 3).

Biofilm formation on the surface of modified polymer. As shown in Fig. 4, the amount of crystal violet adsorption by modified sample is about 86% less than the pristine sample. It indicates reduction of biofilm formation on the modified surface compared to non-modified surface. On the other hand, the number of CFU also confirmed this result (Fig. 5). AFM image indicated that the number of bacteria on the polymer surface after the plasma treatment decreased (Fig. 6).

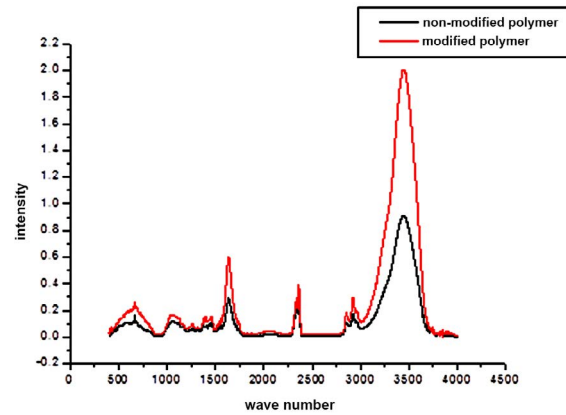


Fig. 1. FTIR for modified and non-modified polymer surfaces.

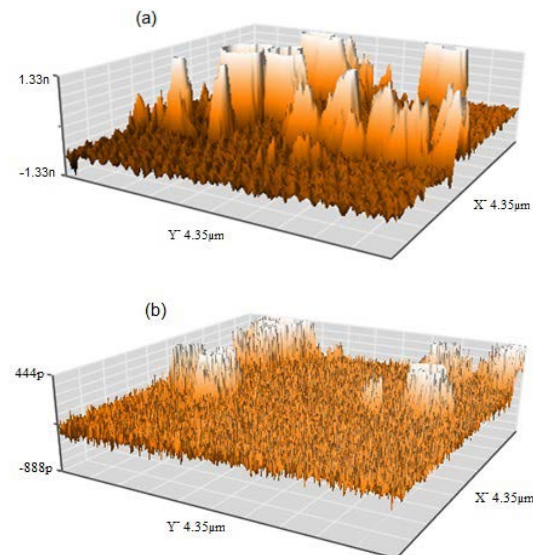


Fig. 2. AFM micrographs of non-modified (a) and modified (b) polymer surfaces in challenge with *E. coli* biofilms.

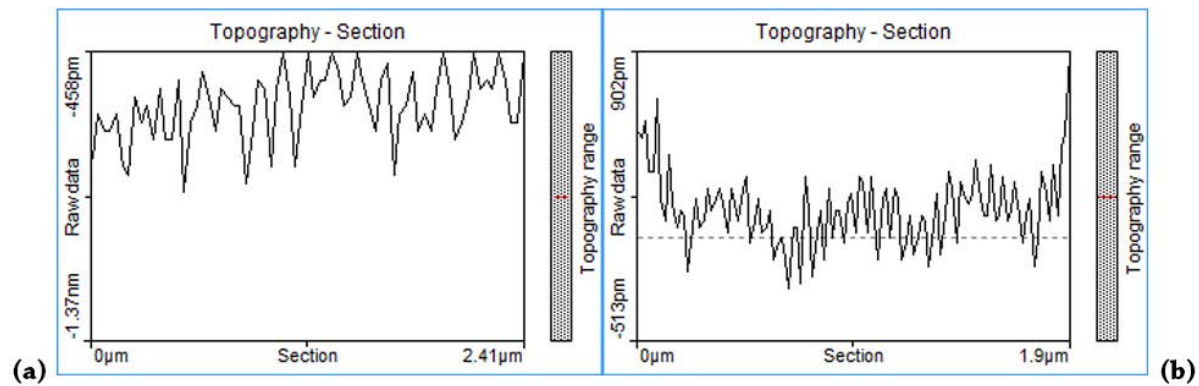


Fig. 3. AFM micrographs analysis of non-modified (a) and modified (b) polymer surfaces.

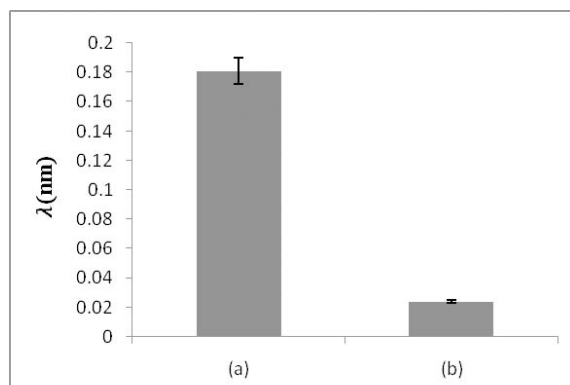


Fig. 4. Comparison of *E. coli* biofilms on non-modified surfaces (a) and modified surfaces (b) by crystal violet binding assay.

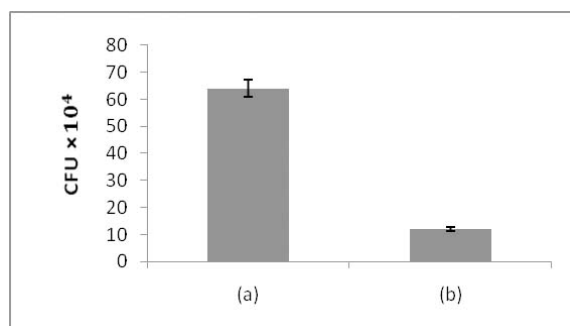


Fig. 5. Comparison of *E. coli* biofilms on non-modified surfaces (a) and modified surfaces (b) by sonication method.

DISCUSSION

Surfaces of microorganisms usually are hydrophobic. In the other hand, surface of medical polymers such as urinary catheter usually is hydrophobic too. Therefore, surface of these polymers is an appropriate place for attachment of microorganisms and subsequently biofilm formation. In this 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).

Results in Figs 4- 5 and AFM images of polymer surface (Fig. 6) indicated that biofilm formation af-

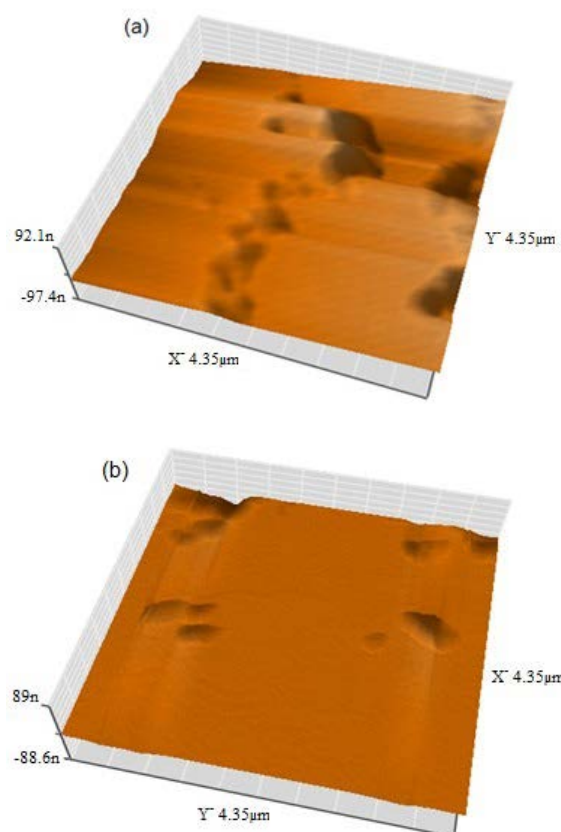


Fig. 6. AFM micrographs for the amount of biofilm formation on non-modified (a) and modified (b) polymer surfaces.

ter plasma treatment was significantly decreased. According to Fig. 1, 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. 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 *Pseudomonas aeruginosa* (27). On the other hand, the analysis of AFM micrographs before and after the plasma treatment (Fig. 3) 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. Wang et al. used argon plasma treatment to reduce levels of *Staphylococcus epidermidis* adhesion to Ar-treated polyethylene (28). Therefore, the resultant of these two factors, namely polymer hydrophilic surface and reduction of contact area between bacterium and substrate, could reduce the ability of attachment and subsequently biofilm formation by some microorganisms

such as *E. coli*.

In conclusion, Plasma surface modification can be used to reduce *E. coli* biofilm formation on medical polymers such as urinary catheter. So it can reduce the rate of infections in patients with long-term use of these catheters.

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