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Plasma Modified Foley Catheters Against to Catheter Associated Urinary Tract Infection (CAUTI)

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Abstract

In this study; the foley catheters were modified by plasma polymerization technique and coated with alginate gels. This modification processes were applied to prevent bacterial adhesion onto the foley catheter surfaces. Here the most effective parameters were evaluated as the plasma polymerization system power and plasma exposure time for the effective catheter surface modification. The obtained results showed that the plasma power affected hidrophilicity of the surface and wettability of the foley catheters was increased. The plasma exposure time increased the amount of EDA deposition over the catheter surfaces and then caused more hydrophilicity for the foley catheter surfaces also. In this study, the bacterial strain of *E. Coli* had a hydrophobic nature and therefore the increase in hydrophilicity of the surface of foley catheters decreased the adhesion risk for the *E. Coli* onto the catheter surfaces. As the numerical values of the obtained data; while the amount of bacterial colony formation unit was at 108 level initially, it was decreased down to 10^4-10^3 level by changing the surface hydrophilicities as expected.

Key Words: Foley catheter, plasma modification, alginate, urinary tract infection.

INTRODUCTION

Catheters, urethral or ureteral stents, nephrostomy tubes and other urological implants are frequently affected by bacterial adhesion and encrustation due to their permanent contact with urine [1,2]. This bacterial adhesion and encrustation increase the risk of blockage of the device lumen and infection of the urinary tract [3-5]. Bacterial adhesion and encrustation can be due to the previous formation of an organic layer on the polymer surface mainly constituted by proteins able to bind ions, aggregate crystals and support microbial attachment [6]. Therefore, the related research were focused on the characterization and modification of the mentioned devices (ie., catheters and stents in urinary system). In last decade many different types of new attempts have been examined such as hydrophilic outer layers in that kinds of devices, antimicrobial agent impregnated or coated surfaces, low surface energy and carbon-rich materials, highly biocompatible substances, biodegradable material and cell or protein grafted surfaces [7,8]. Unfortunately, these problems (i.e., encrustation and infection) associated with diverse materials used in urinary

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systems is as yet an unresolved problem and still requires an urgent solution because hundreds of millions of these devices are used in all over the world on an annual basis [9]. In the related literature, polymeric coatings (with or without any active agents especially antibiotics) of these devices are seem to be most promising for the modification of these devices. In this approach, due to the nature of the soft and slippery surfaces when hydrated, hydrogels may be beneficial if used as a coating material for uriary catheter [10].

In this presented study; alginate coated foley catheters were modified by plasma polymerization and examined to prevent the bacterial adhesion by changing the surface hydrophilicity against to the bacterial strain of *E. Coli* as in vitro studies. Here, alginate was used as the coating material due to the hydrogel structure, non-toxic and biocompatible character of this biopolymer [11-15].

EXPERIMENTAL

Sodium alginate (SA), calcium chloride and ethylenediamine (EDA) were purchased from Fluka (Switzerland). Acetic acid was obtained from Carlo Erba (Italy).

Modification of Foley Catheters

Foley catheters were first modified with ethylene diamine (EDA) plasma. In this part of the study, EDA was applied as the coating material and the effects of plasma exposure time and plasma power were investigated over the bacterial adherence. The samples were prepared by cutting the Foley catheters 10 mm length and placed into the plasma reactor. First of all the reactor was evacuated and then EDA was applied into the reactor. Plasma exposure time was changed as 10-20-30 min and the plasma power was also changed as 15-25-35 W to obtain modified Foley catheters with different surface properties.

After the plasma modification with EDA the catheter samples were immersed into the alginate gels which was prepared by dissolution of sodium alginate with 1% (by mass) of concentration. The catheters were kept in alginate gel for 10 min and then gel coated catheters were immersed into the $CaCl_2$ (5% by mass) solution and they kept there for 30 min. Alginate coated catheters were dried at 35°C for more than one day. This procedure was repeated with EDA plasma modified Foley catheters which were modified at different modification conditions (i.e., 10-20-30 min plasma exposure time and the 15-25-35 W plasma power).

Characterization of The Catheters

Morphological Evaluations

Morphological evaluations of the modified and unmodified catheters were made by a scanning electron microscope (SEM, JEOL, Japan). In these studies, a small piece of catheter was put onto the sample holder, coated with gold and then the SEM micrographs were taken.

Contact Angle Measurements

In the general aspect, physicochemical surface properties of the biomedical devices and the bacterial surface are the most effective parameter for the device associated infections. Some of these properties are surface potential, functional groups at the surface, surface receptor sites, surface energy, roughness, textures, molecular mobility and the hydrophobicity or hydrophilicity of the surfaces. The hydrophobicity or hydrophilicity of the surfaces can be defined by water contact angle measurements easily. On the other hand it is well known that the infection is begun with bacterial biofilm following the bacterial adherence to the

biomedical surfaces. At this point, the similarities of the bacteria and biomedical device surfaces based upon their hydrophilities is very important for the mentioned biofilm formation and infections. In this respect, in most of the related researches the authors have been reported that the hydrophobic bacteria are preffered more hydrophobic biomedical surfaces while the hydrophilic bacteria are preferred more hydrophilic biomedical surfaces.

In the presented study, relatively hydrophobic bacteria of *Escherichia Coli* was evaluated as the bacterial strain and the obtained results showed that the highest bacterial adherence was obtained with native (or unmodified) catheter. The bacterial adherence was lower for the EDA and HEMA plasma coated catheters and alginate attached EDA plasma modified catheters. On the other hand for the comparison of bacterial adherence and catheter hydrophilicities, the water contact angle values of the catheters were determined using a sessile drop technique. In this part of the study, the contact angle values of the native and modified catheters were used and about 5 µl of distilled water was individually dropped at more than five different locations on the catheter surfaces. The shape of the drop was captured with a CCD video camera (Hitachi D.S.P, VK-C220 E, Japan) by lighting from the backside of the drop using a tungsten lamp. The contact angle values were calculated by using the following equation.

$$\tan(\theta/2) = 2h/d \quad (1)$$

Here; (θ) is the contact angle, h is the height of the droplet from the catheter surface, and d is the diameter of the circle of the drop contacting the catheter.

Antibacterial Efficiency Tests

E. coli strain used in the experiment was obtained from a clinical case. Bacterial suspensions (1 mL) in brain-heart infusion broth (BHI, Oxoid ltd, UK) with 20% glycerol were stored -70^{o}C. A fresh aliquot was taken and used to inoculate 5 mL of BHI broth for each experiment. Cultures were grown overnight at 36ºC and 1 mL was centrifuged for 3 minutes and re-suspended in 1 mL NaCl 0.9%. Bacterial density was measured at 600 nm and diluted to a defined final concentration in BHI broth.

Urinary catheters were used in the experiments (Bıçakcılar®, Foley Catheter, Turkey). Segments were cut into 10 mm length with 5 mm in diameter. Approximate surface are of the catheters were 500 mm^2 were incubated in physiologic saline buffered, 0.5 McFarland *E. coli* (2x10^8) for 24 h at 37ºC. At the end of 24 h grafts were washed 3 times with saline and vortexed for two minutes in 2 ml of physiologic saline. 100 micro liters of samples of vortexed material were incubated in blood agar. Colony numbers were assessed 24 h later .

RESULTS AND DISCUSSION

Modification and Characterization of Catheters

Catheter associated urinary tract infections (CAUTI) and encrustations (or calcifications) are the most important problems in the treatment of urinary system diseases. These problems occur due to the unsuitable nature of the catheter. Most practical and effective solution is the modification of these catheters via chemically or biochemically. On the other hand bacterial adhesion facilitates the encrustation (and calcification or stone formation).

In this study, the urinary catheters were modified by plasma polymerization technique and chemical modification against to bacterial adherance onto the catheters and catheter associated urinary tract infections. In the first group of experiments, the catheters were modified with ethylenediamine

(EDA) plasma and ionic complexation of EDA with alginate polymer. The reason for this selection of these modifiers is about lowering the surface tension and increase the repellency power of the catheters against to stone crystal (especially calcium compounds) formation.

Morphological Evaluations

Unmodified and modified catheters were characterized morphologically with a scanning electron microscope (JEOL, JSM 5600 SEM, Japan). The surface structure micrographs of the unmodified and modified catheters were given in

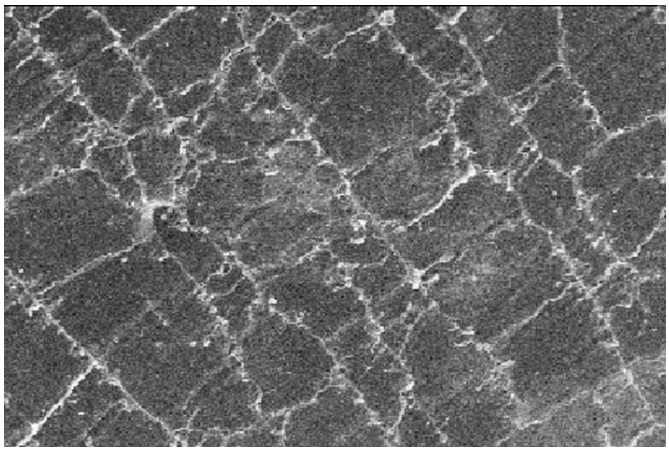

Figure 1.A. SEM micrograph of unmodified Foley catheter surface.

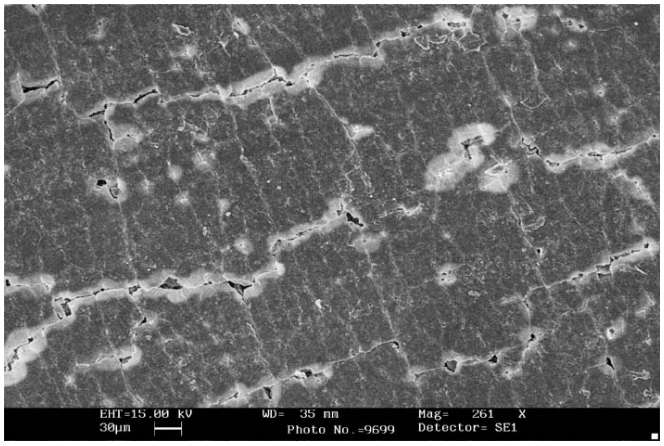

Figure 1.B. SEM micrograph of EDA plasma modified Foley catheter surface.

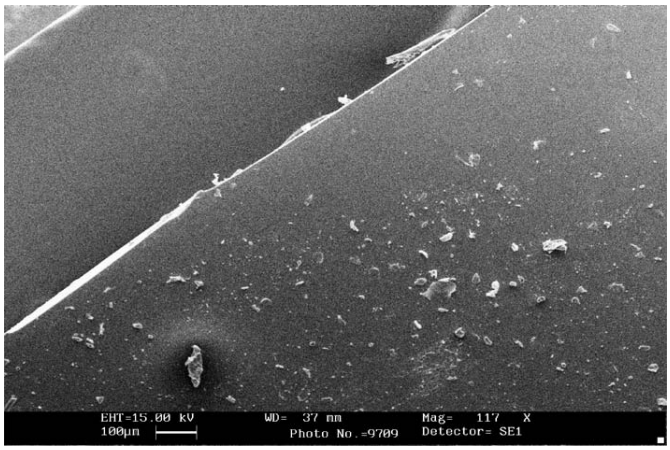

Figure 1.C. SEM micrograph of EDA plasma modified-alginate coated Foley catheter surface

Figure 1. In the case of unmodified catheters, there are some regular cracks over the catheter surface while there is thin and continuous layer in EDA plasma modified catheter and also more dense layer in alginate coated (following EDA plasma modification) catheter as seen in Figure 1.A, B and C respectively.

Bacterial Adherance Studies

In the general aspect, physicochemical surface properties of the biomedical devices surface and the bacterial surface are the most effective parameter for the device associated infections. Some of these properties are surface potential, functional groups at the surface, surface receptor sites, surface energy, roughness, texturity, molecular mobility and the hydrophobicity or hydrophilicity of the surfaces. The hydrophobicity or hydrophilicity of the surfaces can be defined by water contact angle measurements easily. On the other hand it is well known that the infection is begun with bacterial biofilm following the bacterial adherance to the biomedical surfaces. At this point, the similarities of the bacteria and biomedical device surfaces based upon their hydrophilicities is very important for the mentioned biofilm formation and infections. In this respect, in most of the related researches the

authors have been reported that the hydrophobic bacteria prefere more hydrophobic biomedical surfaces while the hydrophilic bacteria prefer more hydrophilic biomedical surfaces [16,10,3].

In the bacterial adherance studies, initially different types of modified and unmodified catheters were evaluated to get the highest resistancy against to bacterial adhesion. During these studies the most common and problematic bacterial line of *Escherichia Coli (E.Coli)* obtained from the urinary system was used. This type of pathogenic *E. Coli* has a relatively hydrophobic surface structure as given in related literature [17]. Therefore, the modification procedures were orientated to get more hydrophilic catheter surfaces at initial stages of this study. EDA and hydroxyethylmethacrylate (HEMA) were used as the modification materials in the plasma modification studies to get more hydrophilic surfaces. On the other hand, alginate coated catheters were used as the modified form to correlate the bacterial adherance and encrustation behavior of these catheters.

For the comparison of bacterial adherance and catheter hydrophilicities the water contact angle values of the catheters were determined using a sessile drop technique. In this part of the study, the contact angle values of the unmodified and all modified catheters were used and about 5 µl of distilled water was individually dropped at more than five different locations on the catheter surfaces. The shape of the drop was captured with a CCD video camera (Hitachi D.S.P, VK-C220 E, Japan) with lighting from the backside of the drop using a tungsten lamp.

In the plasma modification studies, it is very well known that the plasma power and plasma exposure time (or monomer flow rate) are the most important parameters for plasma modifications [18]. Therefore, the plasma power and plasma exposure time were selected as the effective parameters to obtain more hydrophilic surfaces by using EDA.

Effects of Plasma Power

Unmodified catheters were exposed to EDA plasma for 10 minutes at different plasma power (i.e., 15, 25 and 35 W). Furthermore the EDA plasma modified catheters were interacted with alginate solutions to coat these catheters with alginate. The unmodified and EDA plasma modified catheters were incubated with *E. Coli* suspension for bacterial adherance studies. The obtained results were summarized in Table 1, 2. and 3. for EDA plasma modified and alginate coated catheters, respectively.

In the EDA plasma modified catheters the bacterial adherance was not changed significantly (and also the surface hydrophilicity was not changed similarly). On the other hand even slightly decrease in bacterial adherance was obtained than modified form, especially bacterial adhesion was low in the case of higher plasma power (Table 1).

Table 1. Effects of plasma power on bacterial adherance and contact angle values (EDA plasma modified forms).

Sample	Bacterial Adherance (CFU)	Contact Angle (°) (θ)
Unmodified Catheter	$6x10^6$	77±1
(10 min/15 W EDA Plasma)	10^5	67±2
(10 min/25 W EDA Plasma)	10^5	66±3
(10 min/35 W EDA Plasma)	10^4-10^5	70±1
Initial Bacterial Population	$2x10^8$	

In the last part of the plasma power differences

evaluations, EDA plasma modified (with different plasma powers) catheters were reacted with alginate solutions and then these alginate coated catheters were incubated in bacterial suspension to investigate the bacterial adherance resistancy. The obtained results were given in Table 2. The bacterial adherance values were significantly low according to the other modifications and unmodified forms. Furthermore, the hydrophilicity values were also decreased by increasing the plasma power. This can be speculated that the EDA deposition was higher in the case of higher plasma power values and therefore higher amount of alginate could be reacted with EDA in this case.

Table 2. Effects of plasma power on bacterial adherance and contact angle values (Alginate coated following EDA plasma modified forms).

Sample	Bacterial Adherance (CFU)	Contact Angle (°) (θ)
Unmodified Catheter	$6x10^6$	77±1
(10 min/15 W EDA Plasma+Alginate)	$9x10^3$	76±2
(10 min/25 W EDA Plasma+Alginate)	$6x10^3$	75±2
(10 min/35 W EDA Plasma+Alginate)	$5x10^3$	67±1
Initial Bacterial Population	$2x10^8$	

Effects of Plasma Exposure Time

In this part of the study, the plasma modifications were achieved for different plasma exposure time at constant plasma power (i.e., 25 w). During the studies both EDA plasma modified and alginate coated catheters were evaluated for their bacterial adherance resistancies. In the first group EDA plasma modification was performed for different plasma exposure time (i.e.,10, 20, 25 min.) at 25 W of plasma power. The obtained results were given in Table 3.

Table 3. Bacterial Adherance and Contact Angle values for EDA plasma Modified Catheters (different plasma exposure time).

Sample	Bacterial Adherance (CFU)	Contact Angle (°) (θ)
Unmodified Catheter	$6x10^6$	77±1
(10 min/25 W EDA Plasma)	10^5	66±3
(20 min/25 W EDA Plasma)	10^5	67±1
(30 min/35 W EDA Plasma)	$5x10^3$-$7x10^3$	65±3
Initial Bacterial Population	$2x10^8$	

Table 4. Bacterial Adherance and Contact Angle values for EDA plasma Alginate coated catheters (different plasma exposure time).

Sample	Bacterial Adherance (CFU)	Contact Angle (°) (θ)
Unmodified Catheter	$6x10^6$	77±1
(10 min/15 W EDA Plasma+Alginate)	$6x10^3$	75±2
(20 min/25 W EDA Plasma+Alginate)	$3x10^3$	73±2
(30 min/35 W EDA Plasma+Alginate)	$2x10^3$	74±1
Initial Bacterial Population	$2x10^8$	

Both bacterial adherance and contact angle values were decreased by increasing the plasma exposure

times. This can be expressed that the bacterial adherance was decreased due to the increase in hydrophilic character of the catheters and the bacterial line (i.e.,*E.coli*) was relatively hydrophobic and it preffered more hydrophobic surfaces.

In the last part, EDA plasma modified catheters were coated with alginate and they were incubated in bacterial suspension to evaluate the alginate coating against to bacterial adherance.

The obtained results were given in Table 4. Overall bacterial adherance values were very low according to the unmodified forms even if the contact angle values were not changed significantly.This result was most probably due to the natural structure of alginate and more plasma exposure time caused more EDA and than alginate deposition over the catheters.

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