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Synthesis and surface modification of polyurethanes with chitosan for antibacterial properties

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ABSTRACT

Surface modification and providing antibacterial properties to the materials or devices are getting great attention especially in the last decades. In this study, polyurethane (PU) films were prepared by synthesizing them in medical purity from toluene diisocyanate and polypropylene ethylene glycol without using any other ingredients and then the film surfaces were modified by covalent immobilization of chitosan (CH) which has antibacterial activity. CH immobilized PU films (PU-CH) were found to be more hydrophilic than control PU films. Electron Spectroscopy for Chemical Analysis (ESCA) and Atomic Force Microscopy (AFM) analyses showed higher nitrogen contents and rougher surface topography for PU-CH compared to PU films. Modification with CH significantly increased antibacterial activity against Gram positive (*Staphylococcus aureus*) and Gram negative (*Pseudomonas aeruginosa*) bacteria. It was observed that the number of bacteria colonies were less about 10^2–10^5 CFU/mL and number of attached viable bacteria decreased significantly after CH modification of PU films.

1. Introduction

Devices made of polyurethane (PU) are preferable in different biomedical applications because of their high biocompatibility, good physical and mechanical properties and availabilities (Hasirci & Aksoy, 2007; Wang & Wang, 2012; Zhou, Zhang, Guo, & Gu, 2014; Zhou, Hu, Li, & Li, 2014). Some of the applications of PUs can be given as artificial heart valves (Shi, 2013), injectable gels and micro/nano spheres (Guelcher et al., 2008), biodegradable scaffolds (Kiziltay et al., 2013; Parrag & Woodhouse, 2010), etc. Although they are biocompatible, intense research is still going on to increase their hemocompatibility and antibacterial activity (Aksoy, Hasirci, & Hasirci, 2008; Aksoy, Hasirci, Hasirci, Motta, et al., 2008; Zhou, Zhang, et al., 2014; Zhou, Hu, et al., 2014). One of the frequent reasons for the failure of medical devices and implants is the bacterial adhesion on the surface of the material which can lead to pathogenic biofilm formation and subsequent infectious complications (Bazaka, Jacob, Crawford, & Ivanova, 2012). There is quite a lot research focused on to prevent bacterial adhesion. One possible strategy is to modify the surface of the material with an antibacterial agent like silver (Tai, Ma, Liu, Yan, & Xue, 2012), chalcone (Sivakumar, Iyer, Natesan, & Doble, 2010), nitric oxide (Neidrauer et al., 2014), antibiotics (Kowalczuk, Ginalska, & Golus, 2010) or chitosan (Archana, Singh, Dutta, & Dutta, 2013; Liu, Hu, & Meng, 2009).

Chitosan (CH), the linear cationic (1–4)-2-amino-2-deoxy-β-D-glucan produced from chitin by partial deacetylation, is a prominent polysaccharide owing to its biocompatibility, antimicrobial activity, and absence of toxicity (Burke, Yilmaz, Hasirci, & Yilmaz, 2002; Du, Xu, Xu, & Fan, 2008; Isikli & Hasirci, 2012; Mengíbar et al., 2011; Muzzarelli et al., 1990; Muzzarelli, 2009, 2010). In literature it is reported that chitosan immobilized on acrylic acid grafted polypropylene fabric showed antibacterial assessment against *Pseudomonas aeruginosa* (*P. aeruginosa*) (Yang, Lin, Wu, & Chen, 2003). Similarly, chitosan anchored polyesters assessed biocide activity against several bacterial strains including *Escherichia coli* (*E.coli*) *Staphylococcus aureus* (*S. aureus*) (Hu, Jou, & Yang, 2003). Chitosan was also applied to food packages by immobilizing it on polypropylene films and strong antimicrobial activity against both *Bacillus subtilis* (*B. subtilis*) and *E. coli* was reported (Vartiainen, Rättö, Tapper, Paulussen, & Hurme, 2005). Tethering of chitosan onto cellulose membranes demonstrated

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higher antimicrobial activity for Gram positive than Gram negative bacteria (Nigmatullin et al., 2009) and chitosan grafted on acylated wool fabric showed antibacterial effect against both *E. coli* and *S. aureus* (Ranjbar-Mohammadi, Arami, Bahrami, Mazaheri, & Mahmoodi, 2010). Low-density polyethylene modified by chitosan and chitosan/pectin multilayer demonstrated antibacterial activitiy against *E. coli* and *S. aureus* (Popelka et al., 2012). Layer-by-layer (LBL) deposition of chitosan with pectin or phosvitin on electrospun cellulose acetate or cellulose mats also had antibacterial activity against *E. coli* and *S. aureus* (Xin et al., 2013; Zhou, Zhang, et al., 2014; Zhou, Hu, et al., 2014). The exact mechanism of antibacterial action of chitosan is still unknown although different mechanisms have been proposed. One explanation can be given as the strong interaction between positively charged chitosan molecules and negatively charged microbial cell membranes causes the leakage of proteinaceous fluid and other intracellular constituents out of cell membrane (Hashemi Doulabi, Mirzadeh, Imani, & Samadi, 2013; Kong et al., 2008; Zheng & Zhu, 2003).

In literature, there are some reports showing that modification with chitosan make polyurethanes antibacterial or help to release antibacterial agents (Lv, Luo, Deng, & Sun, 2013). Thin layers of chitosan/poly(vinyl alcohol) hydrogel coatings on polyurethane catheters had antibacterial activity against *E. coli*, *P. aeruginosa* and *S. aureus* (Yang, Lee, Lin, Yang, & Chen, 2007). Thermo-sensitive polyurethane membranes modified with chitosan were found antibacterial against *P. aeruginosa* and *S. aureus*, and in this study it was shown that CH coated PU samples were non-toxic, biocompatible and safe (Yang, Yang, Lin, Wu, & Chen, 2008). Similarly, modified fabrics (Liu et al., 2009) and thermoplastic polyurethane/chitosan laminated films (Chiu et al., 2008) demonstrated antibacterial activity against *S. aureus* and *E. coli*. It is also reported that layer-by-layer deposition of polysaccharide-based anionic lentinan sulfate and cationic polyelectrolyte chitosan onto polyurethanes showed antibacterial activity to *P. aeruginosa* (Wang, Hong, Chen, Lian, & Xiong, 2012).

In the present study, it is aimed to prepare antibacterial polyurethane (PU) films. For this purpose, PU films were synthesized by using toluene diisocyanate and polypropylene-ethylene glycol while no chain extender, catalyst or solvent was added. The surfaces of the films were modified by chitosan (CH) and antibacterial efficiencies of the coatings were examined against *S. aureus* and *P. aeruginosa*, the most common microbial pathogens encountered in biomaterial infections.

2. Experimental

2.1. Materials

Chitosan (low viscous <200 mPa s, 1% in acetic acid; 75–85% deacetylated), glutaraldehyde solution (GA, 25% aqueous solution), and diiodomethane were obtained from Sigma–Aldrich (Steinheim, Germany). Toluene diisocyanate (TDI, a mixture of 2,4- and 2,6-toluene diisocyanate in the ratio of 80:20) and polypropylene ethylene glycol (polyol) were obtained from Dow Chemical Company (Midland, Michigan, USA). Acrylamide (AAm), glacial acetic acid and sodium cacodylate trihydrate were obtained from Sigma (St. Louis, Missouri, USA). Nutrient Broth and Mueller-Hinton Agar were purchased from Merck (Darmstadt, Germany). American Type Culture Collection strains (Gram positive *Staphylococcus aureus* ATCC 25923 and Gram negative *Pseudomonas aeruginosa* ATCC 27853) were obtained from Biotechnology Laboratory of Gazi University.

2.2. Methods

2.2.1. Polyurethane synthesis

Polyurethane (PU) prepolymers were synthesized by condensation reaction of toluene diisocyanate (TDI) and polypropylene-ethyleneglycol (polyol) without adding any other ingredients (solvent, catalyst or activator) as described in earlier studies (Aksoy and Hasirci, 2012; Aksoy, Hasirci, & Hasirci, 2008; Aksoy, Akata, Bac, & Hasirci, 2007; Aksoy et al., 2007). Shortly, polyol was heated to 80 °C under vacuum and evacuated for at least 1 h. TDI (under nitrogen atmosphere) was added dropwise, the solution was stirred for 6 h, the viscous prepolymer was poured into glass molds, cured in vacuum oven about 15 days at 90 °C. Polymerized solid films were removed after immersing the molds in hot boiling distilled water.

2.2.2. Chitosan immobilization on polyurethane film

PU films were cut into pieces (7.5 mm × 7.5 mm), ultrasonically cleaned in isopropyl alcohol, dried at room temperature and then treated with oxygen plasma (100 W, 10 min; Advanced Plasma Systems Inc., St. Petersburg, USA, with Seren R300 13.56 MHz generator) to activate the surfaces of the films (Hasirci, 2011; Ozdemir, Hasirci, & Serbetci, 2002). Plasma treated films were coded as PU-plasma. Then the films were immersed in aqueous acrylamide solution (AAm; 50%, w/w) for 10 min, rinsed with water, dried in air and coded as PU-AAm (Parvin, Mirzadeh, & Khorasani, 2008). Then the films were immersed in glutaraldehyde solution (1%, v/v) for 3 h, rinsed with deionized water and immersed in chitosan solutions (5 mg/mL or 20 mg/mL chitosan in 1% glacial acetic acid) at 4 °C for 24 h (Fundueanu, Constantin, & Ascenzi, 2009). The chitosan (CH) immobilized films were rinsed with 1% glacial acetic acid, neutralized with NaOH and washed with deionized water. CH immobilized PU films were coded as PU-CH-0.5 and PU-CH-2.0 depending on the CH solution which they were treated (5 mg/mL or 20 mg/mL CH solutions, respectively). The steps of CH immobilization on PU films are shown schematically in Fig. 1. Meanwhile, stability of covalently immobilized CH was tested by applying Acid Orange7 (AO7) method. In this method, the samples were immersed in PBS for 1, 7 and 14 days, then the samples were removed and 1 mL AO7 (pH = 3; 0.1 mg/mL) was added into solutions. Absorbances were recorded at 485 nm with UV-microplate reader (Hu et al., 2003).

2.2.3. Surface characterization

Synthesized PU films and modified surfaces at each step were examined by using Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR; Perkin Elmer Spectrum 65, Waltham, Massachusetts, USA). The films were analyzed over a frequency range of 600–4000 cm^{-1} with the resolution of 4 cm^{-1}. All spectra were averaged over 32 scans. Radicals created after oxygen plasma treatment were determined with Electron Spin Resonance Spectrometer (ESR; Bruker-E580 Elexsys, Karlsruhe, Germany) in X-band continues wave (CW) mode. After plasma, the samples were immediately taken into quartz ESR tubes and kept at liquid nitrogen for ESR analysis. Atomic compositions of the films were analyzed by Electron Spectroscopy for Chemical Analysis system (ESCA; PHI 5000 VersaProbe, Chigasaki, Kanagawa, Japan) equipped with monochromatic Al Kα at 600 W power at the anode.

Surface hydrophilicity and surface free energy values of the films were evaluated after each step by contact angle measurements using deionized triple distilled water and diiodomethane. A drop of liquid (5 μL) was put on samples by a motor driving syringe at room temperature. For each material and for each liquid at least 6 measurements were done with a Goniometer equipped with high-performance image processing system (KSV-CAM200, Helsinki, Finland).

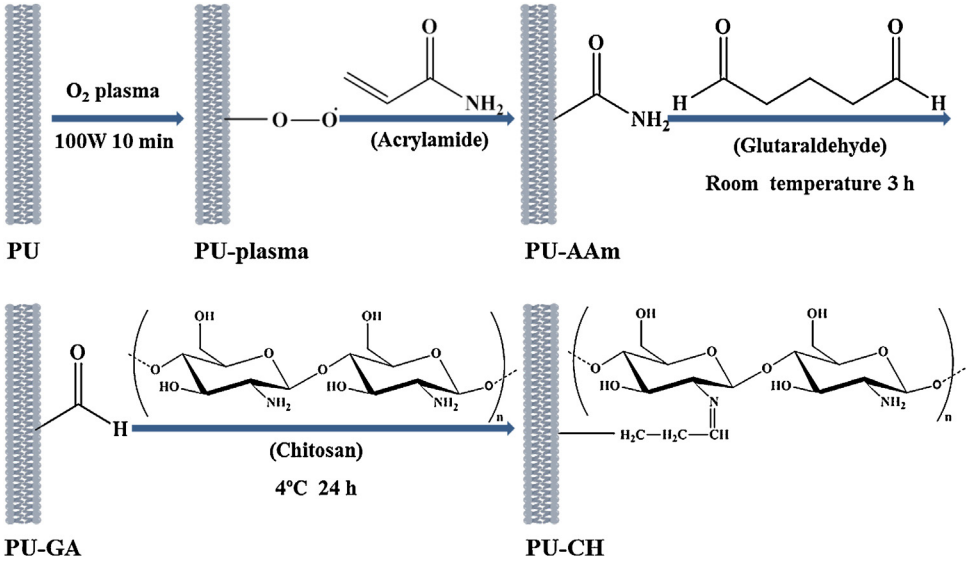

Fig. 1. Chitosan immobilization on PU film.

Total surface free energy (γ_S), and its polar component ($\gamma_S{}^p$) and dispersive component ($\gamma_S{}^d$) were calculated by using Owens–Wendt–Rabel–Kalble (OWRK) method (Eq. (1)).

$$\gamma_L(1+\cos\theta) = 2(\gamma_S{}^d\gamma_L{}^d)^{1/2} + 2(\gamma_S{}^p\gamma_L{}^p)^{1/2} \quad (1)$$

where θ stands for the contact angle between solid film and the liquid drop, γ_L is total surface free energy of the liquid and $\gamma_L{}^d$ and $\gamma_L{}^p$ are polar and dispersive components of the liquid, respectively.

Surface morphologies of all films were investigated by Atomic Force Microscopy (AFM; Veeco MultiMode V, Santa Barbara, California, USA) in tapping mode, with silicone tips.

2.2.4. Bacterial tests

Antibacterial activities of the prepared films were tested by using *Staphylococcus aureus* (Gram positive) and *Pseudomonas aeruginosa* (Gram negative), bacteria which are the most common microbial pathogens encountered in infections. For this purpose, first bacteria suspensions were obtained by the growth of each type of the bacteria in Nutrient broth at 37 °C for 16–18 h, aliquots of bacterial suspensions were taken out and incubated in broth for another 6–8 h to get the log phase of bacteria. The bacteria containing broths were then centrifuged at 3000 rpm for 10 min, and washed twice with phosphate-buffered saline (PBS; 0.01 M KH_2PO_4–Na_2HPO_4 containing 0.8% NaCl, pH 7.4) after the removal of the supernatants. Then bacteria were suspended in PBS to a McFarland turbidity of 0.5 (representing 10^7–10^8 colony forming units per milliliter; CFU/mL). No nutrients were added in the suspension in order to preserve the existing bacteria number constant. These suspensions were used in determining antibacterial activity and bacterial adhesion.

For determination of antibacterial activities, samples were placed in Petri dishes, bacterial suspension (100 μL) were placed on each sample, and incubated at 37 °C for 24 h. The films were then transferred into micro-centrifuge tubes containing 900 μL of sterilized PBS. Colony counting method was used to estimate the number of viable bacteria remained in the suspension. For this purpose, the bacteria solution was serially diluted and 100 μL of each dilution was placed onto Muller–Hinton agar plates. After incubation at 37 °C for 18 h, the number of viable bacteria was determined. Bacterial suspensions were tested under the same conditions served as controls.

Bacteria adhesions on the films were determined by Scanning Electron Microscopy (SEM; QUANTA 400F Field Emission, Hillsboro, Oregon, USA) and colony counting method. The samples were placed in 24-well plate, 1.0 mL of bacterial suspension was added on each sample and incubated at 37 °C for 4 h. Then, the suspension was removed and the films were washed three times with PBS. Bacteria attached on the surface of the films were fixed with GA (2.5% aqueous solution), washed three times with cacodylate buffer (0.1 M, pH 7.4), then with excess water and then dried at room temperature prior to SEM analysis. For the detection of the number of adhered bacteria, colony counting method was applied to the films after PBS washing. For this purpose, the films were placed in 1.0 mL PBS, ultrasonicated (Bandelin Sonorex, Berlin, Germany) for 1 min to remove the surface attached bacteria. Serial dilutions were performed to bacteria suspension, viable cells were counted and the results were expressed as CFU value per unit area (CFU/cm^2) (Chua, Neoh, Kang, & Wang, 2008). Bacteria experiments were carried our as triplicates. For statistical analysis, one-way analysis of variance with Student's t-Tests was used. A p-value of <0.05 was considered statistically significant.

3. Results and discussion

Polyurethane was synthesized by condensation reaction of TDI and polyol without adding any ingredient such as solvent, catalyst, initiator, preserver, or any other molecule. Transparent elastomeric films were obtained after the polymerization reaction and these films were modified with chitosan. A stable CH coat via covalent immobilization was obtained. Stability of CH on PU was checked by applying AO7 test. No color formation indicating no leakage of CH was observed in the PBS solutions which the samples were immersed.

3.1. Surface characterization

3.1.1. Chemical analyses

ATR-FTIR was used to examine surface chemistry of the films after each step (Fig. 2A). Untreated PU had two typical stretching vibration bands at 3300 cm^{-1} and 1726–1705 cm^{-1}. The band at 3300 cm^{-1} corresponds to NH stretching vibration in the presence of hydrogen bonding where strong C=O stretching bands were observed at 1720 cm^{-1}. Very sharp peaks at 1090 cm^{-1} and

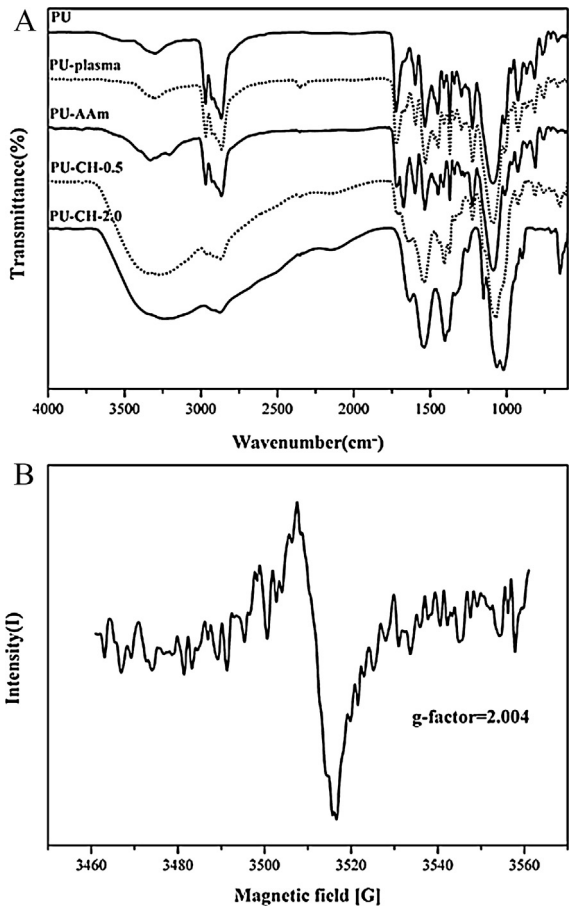

Fig. 2. (A) ATR-FTIR spectra of PU and surface modified PU films. (B) ESR spectrum of oxygen plasma treated PU film.

1247 cm^{-1}, represent C—O stretching and C—O—C vibration (Da Silva et al., 2008). PU and PU-plasma films had almost similar spectra. For PU-AAm films, the C=O (N—COO) band of PU at 1720 cm^{-1} decreased, while amide C=O (CO—NH_2) band at 1680 cm^{-1} increased. Also the stretching vibration of C—N at 1416 cm^{-1} and rocking of NH_2 at 1274 cm^{-1} were present. NH band of PU at 3300 cm^{-1}, shifted to 3331 and 3208 due to amide NH_2 stretching (Murugan, Mohan, & Bigotto, 1998). PU-CH-0.5 and PU-CH-2.0 films had a broad peak between 3200 and 3500 cm^{-1} corresponding to OH and NH stretching of chitosan. Also, CH has a typical series of bands between 1750 and 1500 cm^{-1}. The characteristic peak of N-acetylated chitin at 1640 cm^{-1} belongs to stretching of the carbonyl group of the amide I type. A band at 1545 cm^{-1} corresponds to NH_2 associated bond. The peaks observed at 1000–1200 cm^{-1} region are typical for saccharide structures (Vartiainen et al., 2005).

ESR analyses were achieved after oxygen plasma application to examine the existence of radicals on the films. Oxygen plasma creates mostly peroxide radicals depending on the plasma parameters (Ulubayram & Hasirci, 1993). Prior to AAm treatment and chitosan immobilization, existence of peroxide radicals were detected for PU-plasma samples (Fig. 2B). The *g*-value was obtained as 2.004 which is an evidence for peroxide radical (Dannoux, Esnouf, Amekraz, Dauvois, & Moulin, 2008).

ESCA is a sensitive surface characterization technique, in which the chemical structure near the surface of 0.5–8.0 nm can be analyzed with the quantification of surface atomic composition. Surface atomic compositions of all samples were analyzed by ESCA (Table 1). The core level spectra of carbon-1s atom (294–280 eV) and oxygen-1s atom (538–528 eV) are given in Fig. 3. Binding energies and the relative percentages (analyzed by Gaussian deconvolution algorithm of PeakFit v4.12) of C-1s and O-1s are given in Table 1. The surface atomic composition of samples changed after surface modification. Acrylamide grafting caused an increase in nitrogen content. The following step was chitosan immobilization and depending on the CH concentration, nitrogen content increased to 4.8% and 5.1% for PU-CH-0.5 and PU-CH-2.0 samples, respectively (Table 1). This increase in nitrogen is caused by the amine groups present on chitosan molecule.

Deconvoluted C-1s core-level spectrum of PU had three resolved peaks at 285.0, 286.6 and 289.2 eV (Fig. 3A). These peaks correspond to C—C (39.1%), C—O (55.9%), N—C=O (5.0%) groups (Table 1). Modification of PU films with AAm did not change the binding energies but the composition of the surface has altered. Conversion of C—O band (from 55.9 to 53.1%) to amide group (from 5.0 to 5.7%) has occurred indicating presence of AAm. For CH immobilized films, a fourth peak was observed at 288.1 eV which was attributed to O—C—O group of the saccharide unit of chitosan (Fig. 3A). Deconvoluted O-1s core-level spectrum of PU showed three resolved peaks at 531.1, 532.6 and 533.9 eV (Fig. 3B). These peaks correspond to N—C=O, C—O—C (or C—OH), and O—C=O groups with relative contents (Table 1). Presence of CH, enhanced hydroxyl content (from 80.7 to 83.3%) and decreased carboxyl groups (from 8.9 to 4.0%).

3.1.2. Wettability and surface free energy

Surface hydrophilicities of the prepared films were studied by goniometer after each step of modification. The measured water contact angles (θ) and surface free energy values are given in Table 2. After oxygen plasma treatment, θ values of PU decreased from 91° to 59°. This is expected due to increase in polarity by formation of peroxides. For PU-AAm, θ was found as 66° and it dropped to 57° for PU-CH-0.5 and to 41° for PU-CH-2.0 samples. Immobilization of chitosan introduced hydrophilic groups (such as hydroxyl and amino) and therefore increased hydrophilicity. There are similar reports in literature given for PU samples upon CH coating. For different polyurethanes and for various CH coatings, it was reported that water contact angle decreased from 99° to 91° (Alves et al., 2009), from 69° to 49° (Cai et al., 2002) and from 53° to 32° (Hu, Jou, & Yang, 2004). The decrease of the contact angles were in the range of about 8–21 degrees depending on the structures and functionalities of the polyurethanes and chitosans used in the processes. In our results, an enhanced hydrophilicity was observed and the decrease in contact angle was 34 and 50 degrees for PU-CH-0.5 and PU-CH-2.0, respectively.

Surface free energies (SFE) and their corresponding polar and dispersive components were found by OWRK method (Table 2). The total SFE value for pristine PU was 38.43 mJ m^{-2} having the highest dispersive component. Plasma application increased the polar component by creating peroxide radicals or hydroxide groups on the surface. An increase in total SFE was observed after chitosan immobilization. This is due to the increase of polar component values by introduction of additional polar groups (OH and NH_2) of molecules.

3.1.3. Morphological analyses

Modification steps altered the surface morphology of the film samples and these variations were examined by AFM analysis (Fig. 4). The root mean square roughness (Rq) of AFM is given in Table 2. Surface of pristine PU was very smooth compared to other samples (Rq = 2.8 nm). PU-plasma and PU-AAm films were highly rough (Rq = 19.8 nm and 12.8 nm, respectively). This can be explained by breaking of some bonds after plasma application, removal of leachables from the surface and creating roughness. AAm addition may fill some deep parts leading less Rq value. PU-CH showed a brush-like morphology with uniform distribution of

Table 1
ESCA results giving surface atomic compositions, binding energies and percent relative areas of C-1s and O-1s peaks.

Sample	Atomic %			Binding energy and relative area (%)						
				C-1s peaks				O-1s peaks		
	C-1s	O-1s	N-1s	C—C 285 eV	C—O 286.6 eV	O—C—O 288.1 eV	N—C=O 289.2 eV	N—C=O 531.1 eV	C—OC/C—O—H 532.6 eV	O—C=O 533.9 eV
PU	66.0	30.9	3.1	39.1	55.9	–	5.0	10.4	80.7	8.9
PU-AAm	67.8	28.5	3.7	41.2	53.1	–	5.7	12.4	80.6	7.0
PU-CH-0.5	57	38.2	4.8	24.3	56.0	16.4	3.3	10.2	82.4	7.4
PU-CH-2.0	62.7	32.1	5.1	32.0	49.4	16.9	1.7	12.7	83.3	4.0

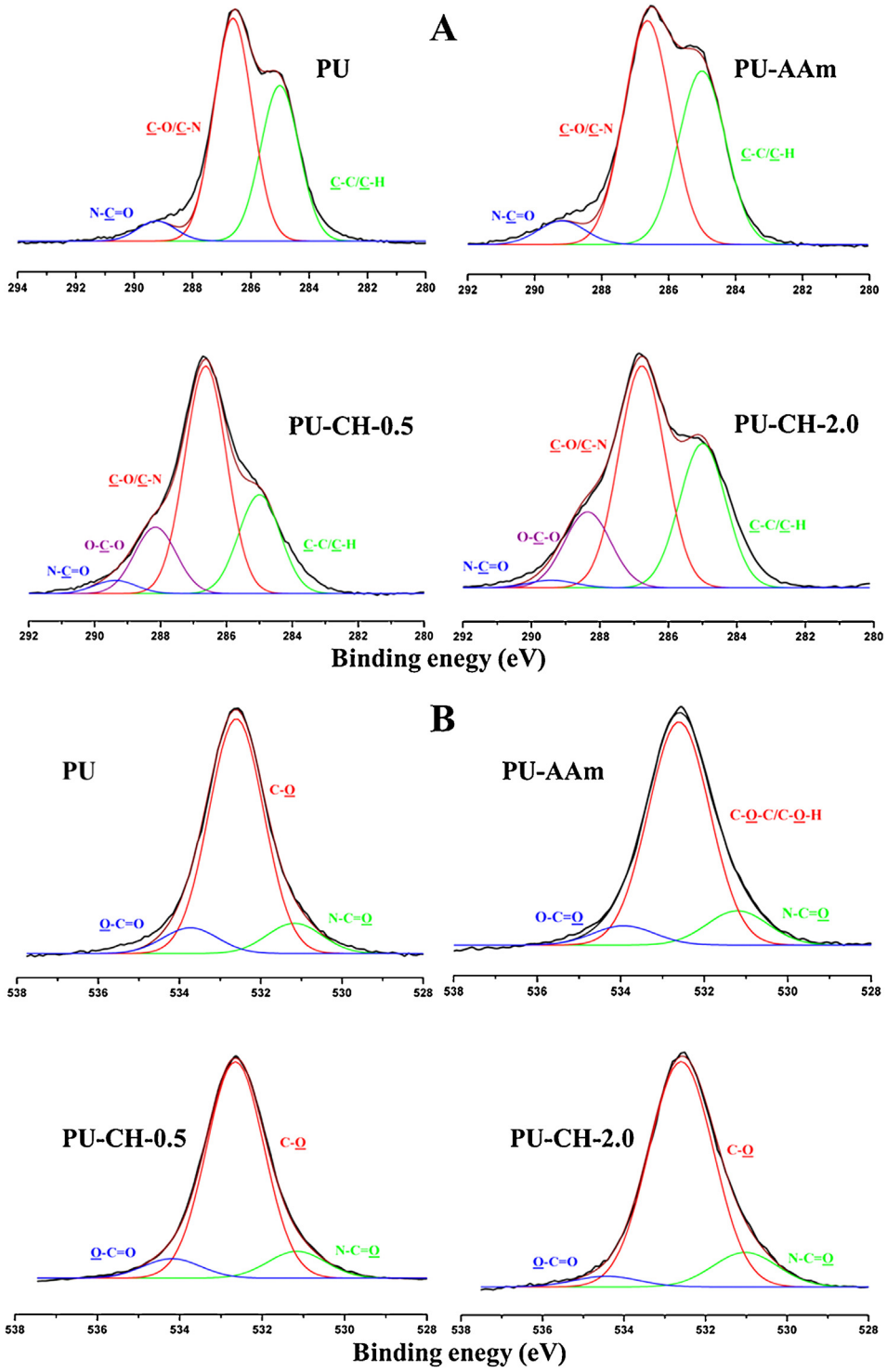

Fig. 3. ESCA spectra of PU and surface modified PU films: (A) Carbon-1s core level, (B) Oxygen-1s core level.

Table 2
Water contact angle, surface free energy and Rq values of PU and surface modified PU films.

Sample	Water contact angle (°)	Total SFE γ_s (mJ m^{-2})	Dispersive component SFE γ_s^d (mJ m^{-2})	Polar component SFE γ_s^p (mJ m^{-2})	Rq (nm)
PU	91 ± 3	38.43	37.54	0.89	2.8
PU-plasma	59 ± 2	49.79	37.04	12.75	19.8
PU-AAm	66 ± 2	41.42	32.06	9.36	12.8
PU-CH-0.5	57 ± 3	49.66	35.01	14.64	15.5
PU-CH-2.0	41 ± 4	54.68	29.81	24.87	19.6

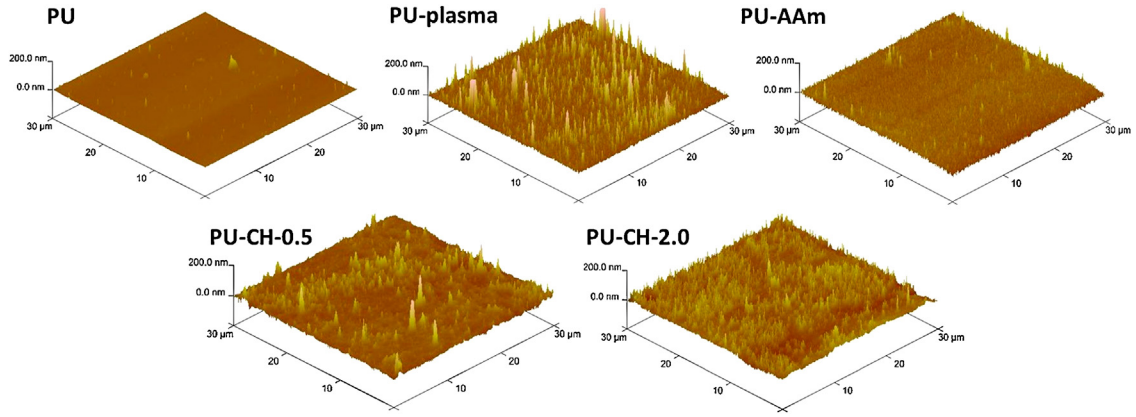

Fig. 4. AFM images of PU and surface modified PU films (30 × 30 µm2).

chitosan layer having Rq values as 15.5 nm and 19.6 nm for PU-CH-0.5 and PU-CH-2.0, respectively (Table 2). In literature, formation of globular structures on silicon matrix (Pastor, Matveeva, Valle-Gallego, Goycoolea, & Garcia-Fuentes, 2011), agglomerates on low density polyethylene (Popelka et al., 2012) and smoothening on polypropylene monofilament (Saxena, Ray, & Gupta, 2010) were reported up on chitosan coating on the materials. The variations of the topography depend on the chemistry of the substrate material as well as the coating process and type of chitosan used. In our study, brush formations are most probably result of the chitosan deposition on the top of the pins which were created after plasma. Although AAm treatment caused some filling, still there were some points which can accumulate chitosan macromolecules.

3.2. Bacterial evaluation

3.2.1. Antibacterial activity

The antibacterial effects of the prepared films were tested on two bacteria where one is Gram negative (*P. aeruginosa*) and the other is Gram positive (*S. aureus*). The results of antibacterial effects of PU and PUCH films are shown in Fig. 5. For *P. aeruginosa* control group (bacteria suspension), log concentration of bacteria was obtained as 7.2 log CFU/mL. After exposing the films of PU, PU-CH-0.5 and PU-CH-2.0 to the same bacteria suspension, these values reduced to 5.9 log CFU/mL, 4.0 log CFU/mL and 2.5 log CFU/mL, respectively. These differences were statistically significant ($p < 0.05$). When contacting with *S. aureus* bacterial suspension, PU did not demonstrate any significant reduction in bacteria colonies having 8.3 log CFU/mL. There was no significant difference between the control and PU films. On the other hand, for chitosan modified PU-CH-0.5 and PU-CH-2.0 films, this value reduced to 6.2 log CFU/mL and 4.2 log CFU/mL ($p < 0.05$), respectively. For both bacteria species (*P. aeruginosa* and *S. aureus*) chitosan immobilized films had strong antibacterial activities demonstrating higher efficiency parallel to the chitosan concentration.

The mechanism of antibacterial action of chitosan can be explained by the interaction between positively charged chitosan molecules and negatively charged microbial cell membranes which may prevent mass transfer across the cell wall, and may lead to leakage of intracellular constituents of the bacteria, thus accelerate dead of bacteria cells (Hu et al., 2004; Kong et al., 2008).

The antibacterial activity of chitosan immobilized films was found to be higher on *P. aeruginosa* than *S. aureus*. This effect may be due the bacteria surface polarity. It is known that the outer membrane of *P. aeruginosa* consists of essentially lipopolysaccharides containing phosphate and pyrophosphate groups which enhance negative charge density on the surface. This leads more effective attraction to the positive chitosan surface compared to *S. aureus* having peptidoglycan membrane. Similar effects on Gram negative bacteria are also given in literature (McCoy et al., 2012). As a result, very satisfactory antibacterial activity was obtained for both CH modified PU films for both bacteria types.

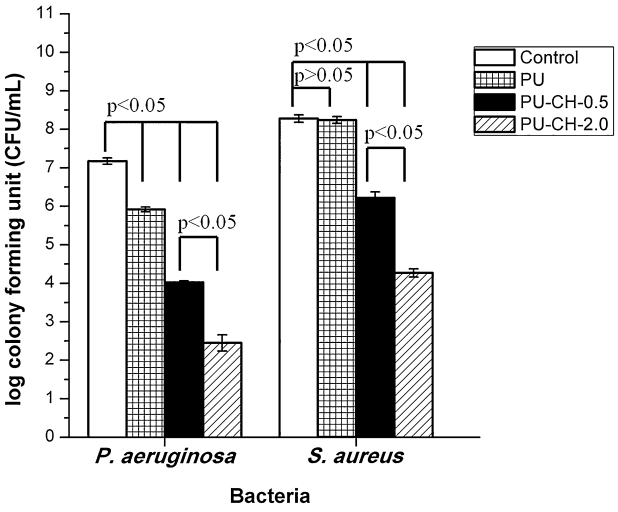

Fig. 5. Antibacterial effects of control, PU and chitosan immobilized PU films on survival of *P. aeruginosa* and *S. aureus*.

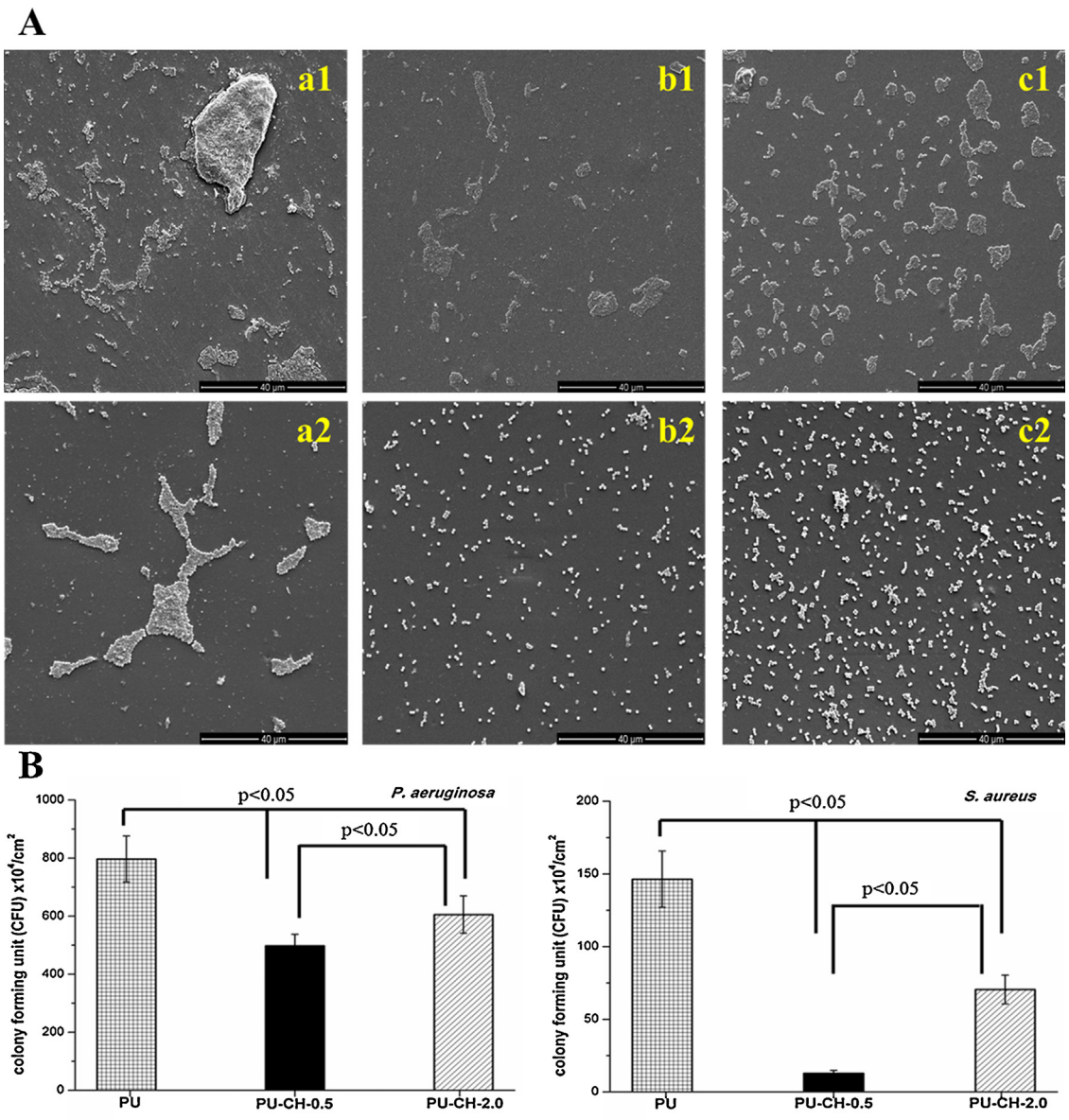

Fig. 6. (A) SEM images of *P. aeruginosa* bacterial adhesion for (a1) PU; (b1) PU-CH-0.5; (c1) PU-CH-2.0 and *S. aureus*. bacterial adhesion for (a2) PU; (b2) PU-CH-0.5; (c2) PU-CH-2.0 4 h incibation period. Scale bar = 40 μm. (B) Number of viable adherent *P. aeruginosa* and *S. aureus* cells on the PU and chitosan immobilized PU films.

3.2.2. Bacterial adhesion

Bacterial adhesion on medical devices is the initial and crucial step for biofilm formation which leads to the failure of the function of the device. Bacterial adhesion is a complex process affected by many parameters such as chemistry, porosity, crystallinity and charge of the surface as well as type of bacteria and environmental conditions (Katsikogianni & Missirlis, 2004). In this study, bacterial adhesion tests were conducted and then the surfaces were examined by SEM (Fig. 6A) and by colony counting methods (Fig. 6B). Adhesion of *P. aeruginosa* and *S. aureus* on both chitosan immobilized surfaces decreased compared to PU films. For PU, accumulation and colony formations were observed where for CH modified samples almost homogeneous distribution of bacteria occurred (Fig. 6A). Since SEM images can only provide qualitative information, colony counting method was used to detect the number of viable adhered bacterial cells. The number of viable adherent *P. aeruginosa* and *S. aureus* on both chitosan immobilized surfaces decreased significantly (in case of *P. aeruginosa* 37.5% for PU-CH-0.5 and 24% for PU-CH-2.0, and in case of *S. aureus* 91.2% for PU-CH-0.5 and 51.7% for PU-CH-2.0). These differences were statistically significant ($p < 0.05$) compared with PU films (Fig. 6B).

In literature it is given that there are various aspects of the surface of material which influence bacterial adhesion. Some of them are types of functional groups, electrical charge density, crystallinity, hydrophilicity, roughness, etc. In general, bacteria adhere more on hydrophobic surfaces than that of hydrophilic ones (Cerca, Pier, Vilanova, Oliveira, & Azeredo, 2005; Chen et al., 2012). Based on these results, it can be concluded that CH immobilization improved hydrophilicity by increasing the amount of polar groups and decreased the adhesion of bacteria. Significant reduction of adhesion for both bacteria was observed for the PU-CH-0.5 compared with PU-CH-2.0. Another reason can be the surface roughness which can promote bacterial adhesion and biofilm deposition (Chen et al., 2012). In this study it was observed that CH modification changed the chemistry as well as topography of the surface. Surface roughness significantly increased compared to pristine PU and

the effect was improved anti-adhesion performance of bacteria. The number of viable *P. aeruginosa* bacteria was higher than *S. aureus* on all samples.

4. Conclusions

Polyurethane films were synthesized from toluene diisocyanate and polypropylene-ethylene glycol without using any other ingredients. Films were modified by different concentrations of chitosan (CH). More hydrophilic and rough surfaces were obtained after immobilization of CH compared to pristine samples. The antibacterial tests showed that CH immobilized samples had strong antibacterial activity against *S. aureus* and *P. aeruginosa*. Bacterial adhesion tests indicated that the number of viable *P. aeruginosa* and *S. aureus* bacteria on modified surfaces decreased significantly. It can be concluded that, chitosan immobilized polyurethanes having antibacterial properties are potential candidates for the design and production of medical devices as urinary catheters. These kinds of materials can overcome pathogenic biofilm formation and subsequent infectious complications which are the frequent reasons for the failure of medical materials.

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