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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).