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Chitosan/poly (vinyl pyrollidone) coatings improve the antibacterial properties of poly(ethylene terephthalate)

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ABSTRACT

Chitosan/poly (vinyl pyrollidone) (CHI/PVP) coatings were prepared to improve the antibacterial properties of poly (ethylene terephthalate) (PET) by a simple dip-coating method. The binding capability of CHI/PVP coatings was enhanced by successively pretreatment of PET by polyetherimide and polyacrylic acid and crosslinking. Measurements of water contact angle and atomic force microscope revealed that the coatings created a highly hydrophilic surface with low roughness. Adherences of *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) on PET with CHI/PVP coating were significantly reduced. Bactericidal activity of CHI/PVP coatings was good against *E. coli* and *S. aureus* and the adding of PVP obviously increased its antiadhesion property. In vitro cytotoxicity tests, cell morphology and activity evaluation of human umbilical vein endothelial cells showed that CHI/PVP coatings had good biocompatibility.

1. Introduction

With the rapid developments of material science, medical and biological, studies on biomedical materials have achieved a great success, but still face various challenges, among which infection is a main obstacle [1]. In the world, about 64% of the hospital acquired infections are caused by the adherence of pathogens on medical implant and devices [2]. Only in the United States, more than 100,000 people die of device-related infections annually [3]. In Europe, patients spend about 50 million euros on the treatment of catheter-related infection and there are 5000 infection death cases per year. The general process of infection is the adhering of bacteria, colonizing and multiplication into colonies, secreting extracellular matrix and finally forming biofilm which induce infection through releasing planktonic cells and toxins. With the transmitting passages of nutrients and wastes, bacteria inside the biofilm change its original phenotype by crossed propagation and gene transmission among bacteria and only the bacteria in the surface layer can reproduce. Just owning to the formation of multifunctional films, antibacterial agents can hardly play its role and also generate drug resistant bacteria which can resist 1000-folds dose antibiotics in comparison with planktonic bacteria [4–6].

Poly (ethylene terephthalate) (PET) has been widely used as implant material in cardiovascular due to its favorable mechanical properties and moderate biocompatibility. However once PET implanted in human body as medical material, bacteria could easily colonize on the surface and further lead to infection, which directly call forth high morbidity and mortality [7,8]. Due to the chemical inertia, high crystallinity and hydrophobic specialties of PET, it is difficult to carry out surface modification on PET [9]. Although surface modification based on different chemical reactions have been reported [10–12], surface treatments based on physical approach are more widely applied due to its facility, versatile and low cost [13].

Several kind of hydrophilic polymer, such as polyethylene glycol [14], polyvinyl alcohol [15] and poly(vinyl pyrollidone) (PVP) [16] have been explored as adhesion resistant surfaces due to the molecular high motility of chains and hydrated film forming ability. Contact killing surface [17] can be prepared through conjugating base material with antibacterial peptides, quaternary ammonium salts, phosphonium salts or chitosan (CHI). However, neither antiadhesion surface nor contact killing surface possesses ideal antibacterial properties. Therefore, designing a multifunctional surface by composition of antiadhesion and contact killing polymers in a simple way to modify the medical devices is a better access [12]. PVP has been widely concerned due to its hydrophilicity, lubricity, antiadhesion property and favorable biocompatibility. CHI, as an effective bactericidal polycation natural macromolecule, has been widely used in packaging materials, textiles, surface antibacterial modification and so on [18,19]. Moreover, PVP and CHI can form into a homogeneous phase due to the strong hydrogen bonding forces between two kinds of molecules [20,21]. The goal of this study is to develop a stable multifunctional coating that combines the antiadhesion property of PVP and contact killing

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ability of CHI by blending method. PET substrate (or silicon wafer) was successively pretreated by polyetherimide (PEI) and polyacrylic acid (PAA) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)/N-hydroxysulfosuccin-imide (sulfo-NHS) with the objective to enhance the binding capability of CHI/PVP coatings.

2. Materials and methods

2.1. Material

Chitosan (K2210, viscosity: 55 cps, degree of deacetylation is 92%) used in this work was obtained from Sanland-chem international Inc. PVP (*K* value: 29–32), PEI (M_w: 25 kDa) and PAA (35 wt%, M_w: 10 kDa) were purchased from Sigma–Aldrich chemical. PET sheets were purchased from Hangzhou Magnetic Tape Corporation of China. Trypicase soy agar (TSA) and Trypicase soy broth (TSB) were purchased from Hangzhou Baisi Corporation of China. *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) were kindly provided by Prof. Jian Xu (Zhejiang University, Hangzhou, China). Ultrapure distilled water was obtained after purification using a Millipore Milli-Q system (USA).

2.2. Clean and pretreatment of PET

After cutting into certain shape (3 cm × 2 cm), PET was successively cleaned in ethanol, acetone and water for 10 min respectively and dried with N_2. Then PET was successively pretreated by PEI (5 mg/ml, 30 min) and PAA (5 mg/ml, 30 min) and sulfo-NHS solution (EDC: 10 mmol, sulfo-NHS: 20 mmol, 12 h) at 25 °C.

2.3. Preparation of CHI/PVP dipping solutions and formation of coatings on PET

In the process of CHI/PVP coatings preparation, PVP (wt% = 0 wt%, 15 wt%, 25 wt%, 35 wt%, 50 wt% in CHI and PVP total amount) and CHI (1 wt%) were successively added into HAc/NaAc buffer (0.1 M, pH 4.0) with magnetic stirring and this process continued 2 h. A simple dip-coating method was used to prepare CHI/PVP coatings on the pre-treated PET substrates. The coating coated PET sheets dried at room temperature for 24 h, then at 30 °C for 12 h under vacuum prior to further characterizations.

2.4. Characterization

In PET pretreatment process, the changes of thickness, surface wettability and morphology were measured by spectroscopic ellipsometry (M-2000DI™, J.A. Woollam), drop shape analysis (KRŰSS, DSA10-MK2) and atomic force microscope (AFM, SPA 400, Seiko instrument Inc.) respectively. Spectroscopic ellipsometry was carried out to measure the film thickness on silicon wafer instead of PET sheet. The sessile dropping method was used to detect the PET surfaces within 15 s after the water droplet contacted the coating. AFM images were performed in the tapping mode under ambient conditions using a commercial scanning probe microscope, equipped with a silicon cantilever, nanosensors, typical spring constant 40 N m^{-1}. The morphology and thickness of the coating were examined by field emission scanning electron microscopy (FE-SEM, FEI, SiRion 100) and AFM. The surface wettability of coating was examined by contact angle measurement.

2.5. In vitro antibacterial test

Antimicrobial tests of CHI/PVP coatings were conducted qualitatively and quantitatively by the shake-flask culture method, waterborne test and zone inhibition method respectively with *E. coli* and *S. aureus* as model bacteria.

For the shake-flask culture method, the CHI/PVP coated PET sheets were placed in test tubes with 10 ml 1.1×10^5 cells/ml of initial *E. coli* (or *S. aureus*) suspension in PBS. These tubes were shaken at 200 rpm in incubators at a constant temperature of 37 °C. At predetermined time, cells were pipetted out from the tubes and consecutive dilutions were prepared by taking 0.1 ml of the previous solution and mixed with 9.9 ml of PBS. From the solution, 0.2 ml was plated onto the triplicate solid agar using the spread plate method. After incubating for 24 h, the number of viable bacteria was counted and the results after multiplying with the dilution factor were expressed as mean colony forming units (CFU) per ml. The survival ratio of bacteria was defined as the percentage of viable bacteria in the suspension relative to the total number of the initial bacteria in the suspension. Results represent mean ±SD of triplicates from three separate experiments ($P < 0.05$).

For the waterborne assay, the CHI/PVP coatings coated PET sheets were immersed into sterile plastic tubes with 10 ml 1.1×10^7 cells/ml initial bacteria suspension in PBS, and these tubes were shaken at 200 rpm at 37 °C for 4 h. The sheets were then removed and washed gently three times with sterile PBS and immersed into 3 vol% glutaraldehyde solution in PBS at 4 °C for 4 h. The glutaraldehyde solution was then removed and the substrates were washed with PBS, followed by step dehydration with 25, 50, 70, 95 and 100 vol% ethanol for 10 min each. The sheets were then dried and sputter-coated with a thin film of gold for imaging purposes. The substrates after waterborne test were characterized by SEM.

Zone inhibition test was carried out with a modified agar diffusion assay. CHI/PVP coatings coated PET sheets were placed on nutrient agar in petri dishes which had been seeded with 20 μl of bacterial cell suspensions. The petri dishes were examined for zone of inhibition after 24 h incubation at 37 °C. The area of the whole zone was calculated and then subtracted from the film disc area, and the difference in area was reported as the zone of inhibition.

2.6. In vitro cytotoxicity test

Human umbilical vein endothelial cells (HUVECs) were used to evaluate cell toxicity by cell morphological evaluation and cell activity test. HUVECs were maintained in RPMI.1640 supplemented with 10% heat-inactivated (30 min, 56 °C) fetal bovine serum (Sijiqin Biotech, China, lot no.020613.2), 80 units/ml penicillin, 100 μg/ml streptomycin 50 μg/mL gentamicin (Gibco) and kept at 37 °C in a humidified 5% CO_2 atmosphere. The CHI/PVP coatings coated PET sheets, sterilized in 75% ethanol and swollen in PBS, were placed into 96-well tissue culturing polystyrene (TCPS) plates (NUCLONTM, Cat. No.167008). The HUVECs were then seeded in culture media to give a final density of 1×10^4 cells/well and incubated for 24 h at 37 °C in an atmosphere of 5% CO_2 in air. Cell activity was determined by 3-(4, 5-cimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay. The absorbance values were measured by using microplate reader (Bio-Rad, model 550) at wavelength 570 nm, blanked with DMSO solution. Five replicates were read for each sample, the mean value of the five was used as the final result.

The cell monolayers on different substrates were also stained with fluorescein diacetate (FDA, Sigma) for fluorescence microscope investigation (Olympus DP71 microscope at 20× magnification in fluorescein filter, 488 nm excitation). FDA is an indicator of membrane integrity and cytoplasmic esterase activity. Stock solutions were prepared by dissolving 5.0 mg/ml FDA in acetone. The working solution was freshly prepared by adding 5.0 μl of FDA stock solution into 5.0 ml of PBS. FDA solution (20 μl) was added into each well and incubated for 5 min. The sheets were then washed twice with PBS and placed on a glass slide for fluorescence microscope examination. The 488 nm wavelength of the