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Electrodeposition of alginate/chitosan layer-by-layer composite coatings on titanium substrates

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ABSTRACT

In this study, alginate/chitosan layer-by-layer composite coatings were prepared on titanium substrates via electrodeposition. The mechanism of anodic deposition of anionic alginate based on the pH decrease at the anode surface, while the pH increase at the cathode surface enabled the deposition of cationic chitosan coatings. The surface of coatings was characterized by using attenuated total reflection–Fourier transform infrared spectroscopy (ATR–FTIR), X-ray photoelectron spectroscopy (XPS), and scanning electron microscopy (SEM). The properties of coatings were characterized by X-ray diffraction (XRD) and differential thermal analysis (DTA). Indirect in vitro cytotoxicity test showed that the extracts of coating had no significant effects on cell viability. Moreover, in vitro cytocompatibility test exhibited cell population and spreading tendency, suggesting that the coatings were non-toxic to L929 cells. However, the results revealed that alginate coating was more benefit for cells growing than chitosan coating. The results indicated that the proposed method could be used to fabricate alginate/chitosan layer-by-layer composite coatings on the titanium surface at room temperature and such composite coatings might have potential applications in tissue engineering scaffolds field.

1. Introduction

Electrodeposition, a versatile and efficient approach for fabricating thin and thick films, coatings and free-standing bodies, is an area of increasing interest (Boccaccini, Keim, Ma, Li, & Zhitomirsky, 2010). It is a process in which an imposed electric field is employed to direct charged particles dispersed in a liquid towards an electrode for the assembly of thin films. Compared with other methods of preparing coatings, such as solution casting (López-Lacomba et al., 2006), silane reactions (Bumgardner et al., 2003), and layer-by-layer technique (Cai, Rechtenbach, Hao, Bossert, & Jandt, 2005), electrodeposition has advantages of short processing time, the possibility of room temperature processing, and no require for cross-link agents. The coatings or films prepared with the method of electrodeposition have been widely used in the biomedical and biotechnology fields. Roether et al. applied, for the first time, electrodeposition to coat three-dimensional porous biodegradable polymer (polylactic acid) substates with bioglass particles for bone tissue engineering (Roether et al., 2002). Zhitomirsky, Roether, Boccaccini, and Zhitomirsky (2009) have developed bioactive glass/polymer composite coatings with or without HA nanoparticle inclusions for biomedical applications via electrodeposition. Related work has been investigated electrodeposition of chitosan with paired sidewall electrodes (Cheng et al., 2010). More recently, Gray et al. (2012) reported an anodic method to deposit hydrogel films of the aminopolysaccharide chitosan. The mechanisms of electrodeposition have been investigated and there were only a few known mechanisms for polymer electrodeposition, including electro-polymerization mechanism (Glenis, Horowitz, Tourillon, & Garnier,1984), neutralization mechanism and Ca^{2+}-alginate deposition mechanism (Cheng et al., 2011). For example, neutralization mechanisms (Fernandes et al., 2003; Luo, Xu, Du, & Chen, 2004) could be used to explain electrodeposition of pH-responsive film-forming biopolymers. These mechanisms for biopolymer eletrodeposition relied on the use of an electrical signal to trigger a reversible sol–gel transition.

Chitosan is a cationic natural polymer that carries positive charges with a molecular structure of (1 and 4)-linked 2-amino-2- deoxy-β-D-glucan. Aminopolysaccharide chitosan has been electrodeposited by a cathodic neutralization mechanism (Redepenning, Venkataraman, Chen, & Stafford, 2003). In the process of electrodeposition, the pH of solution near the cathode would increase, which were connected with the electrochemically generated concentration gradient of reactant OH^- ions. When

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the pH was above its p*K*a value (~6.3), chitosan solution could, under given condition, undergo a sol–gel transition. The subsequent neutralization of the NH_3^+ groups of chitosan chains in solution near the cathode impeled chitosan to deposite on the electrode.

Alginate is a kind of unbranched polysaccharides consisting of (1 → 4) linked β-D-ManA(M) and α-L-GulA(G) residues at different proportions and with different sequential occurrence. To our knowledge, an alginate solution could form a homogeneous gel at pH below p*K*a. These gels were presumably stabilized by intermolecular hydrogen bonds. The anodic deposition of the acidic polysaccharide alginic acid also was attracting attention because it offered broad opportunities for a diverse range of application. Also the neutralization mechanism allowed for electrodeposition of alginate. Sodium alginate with carboxylate groups ($-COO^-$) "recognized" the local low pH at the anode and "responded" by undergoing protonation (to form –COOH) and precipitation (or gelation) at the anode surface.

In this study, we hypothesized that chitosan and alginate could be deposited onto the titanium electrodes layer-by-layer via electrodeposition. Scheme 1 showed that the electrodes were first contacted with neutral sodium alginate and then acidic chitosan solution, respectively. As expected, two thin layers were deposited on the surface of the titanium electrode. The material deposited was chitosan and alginate multilayer composite films, which were tested by SEM, XRD, XPS and ATR–FTIR.

2. Experimental

2.1. Materials.

Chitosan (Mw 100,000, degree of deacetylation (DDA) 87%) and sodium alginate (SA, 1.28 Pa s for a 2 wt% aqueous solution at 30 °C) were purchased from Zhejiang Golden-Shell Biochemical Co., Ltd. (Zhejiang, China) and Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), respectively. Commercial pure titanium was supplied by Shanxi baoji new metal materials co., LTD. Other reagents and solvents were of analytic grade and purchased from Beijing Chemical Reagent Company. Ultrapure water with specific resistance around 18.25 MΩ cm was used to prepare the solution.

2.2. Titanium substrates preparation

Titanium pieces were cut into rectangular plates (6 × 2 × 0.25 cm) as electrode. All the plates were polished with raw emery paper and fine sandpaper in sequence. Then, the polished plates were chemical polished with mixed acid solution (nitric acid: hydrofluoric acid: water volume ratio 4:1:5). After that, they were ultrasonically cleaned with acetone, ethanol, and ultrapure water for 30 min each then rinsed with ultrapure water.

2.3. Eletrodeposited process

The thickness of the deposited layer was observed to be dependent upon the deposition time, the applied voltage, and the concentration of chitosan and sodium alginate. Chitosan and sodium alginate solution were obtained by dissolving 75 mg of each material in 100 mL of deionized water and 100 mL of 1% (V/V) acetic acid, respectively. All the stock solutions were stirred with magnet for about 30 min. The pH of sodium alginate and chitosan solution was 2.8 and 7.4, respectively. During electrodeposited process, Ti plate was used as anode to deposite first layer alginate and parallel platinum plate as counterelectrode. After dried, depositing of second layer chitosan, the above Ti plate was used as cathode and parallel platinum plate as counterelectrode, then repeated for several times. The electrolyzer cell was cuboid-shaped (5.5 × 3 × 2 cm). Deposition was performed by connecting both the cathode and the anode to a direct current power supply (Model 1719A-5, Dahua electronic) with a constant voltage of 20 V for 20 min. After deposition, the electrodes were rinsed with ultrapure water, and finally dried at 50 °C in the oven overnight.

2.4. Characterization

The surface chemistry of the coatings was investigated by attenuated total reflection Fourier transform infrared spectroscopy (ATR–FTIR; Nicolet Spectra 5700 spectrometer, Nicolet Instrument, Thermo Company, Madison, USA). X-ray diffraction pattern (XRD) of the coatings was identified by using a Rigaku D/Max2500VB2+/Pc diffractometer (Rigaku Company, Tokyo, Japan) with 40 kV and 50 mA with Cu *K*α radiation (λ = 0.154 nm). The scanning scope of 2θ was 5–50° and the scanning rate was 5°/min. Surface morphology of the coatings was observed by scanning electron microscopy by using a Hitachi S-4700 microscope with sputter coated of gold before observation. Thermogravimetric analysis was performed on TGA Q500 (TA Instruments). Each membrane sample in an aluminum copple with the same weight (about 6 mg) was run from room temperature to 800 °C at a scanning rate of 10 °C/min under a nitrogen atmosphere. X-ray photoelectron spectroscopy (XPS) spectra were obtained by using a VG ESCALAB MKII X-ray photoelectron spectrometer (VG Scientific Ltd., UK) with Al *K*α radiation. Survey spectra were recorded for 0–1350 eV binding energy range.

2.5. Swelling properties study

Swelling behavior of the electrodeposited chitosan coating, alginate coating and alginate/chitosan coatings (1 mm × 1 mm) were determined by equilibrium swelling studies, according to a recently procedure (Yang et al., 2010). At room temperature, each coating was immersed in 100 mL PBS buffer solution, respectively. The swollen coatings were processed with filter paper to remove adsorbed water on the surface of coatings. The swelling percentage was calculated from the following equation:

$$E_{sw} = \left[\frac{(w_t - w_o)}{w_o}\right] \times 100$$

where w_t is the weight of the sample at the time t, and w_o is the initial weight of the sample. In order to make the experiments more accurate, experiments had been repeated for three times.

2.6. In vitro cell cytotoxicity

In vitro cytotoxicity of the extracts and cytocompatibility of the alginate/chitosan coatings and alginate/chitosan/alginate coatings were assessed by using mouse L929 fibroblasts cells line.

2.6.1. Indirect cytotoxicity assay

Mouse L929 fibroblast cells were cultured in 200 μL DMEM (Dullecco's modified Eagle's medium; Sigma–Aldrich, USA) medium with 10% FBS (fetal bovine serum), together with 1.0% penicillin-streptomycin and 1.2% glutamine at 37 °C under a wet atmosphere containing 5% CO_2. When cells reached 80–90% confluence, they were trypsinized with 0.25% trypsin containing 1 mL Ethylene Diamine Tetraacetie Acid (EDTA) and suspended in the culture medium. For the MTT assay, the coatings were previously sterilized by high temperature in a hermetically-sealed instrument and then placed in 24-well tissue culture plates under aseptic condition. The samples were then incubated in 1 mL of DMEM at 37 °C for 24 h. After that, the coatings were removed and the extracts were obtained and further diluted to get extraction medium samples. Mouse fibroblasts cells were seeded in wells of a 96-well plate at a density of 4000 cells per well. After incubation for