Materials Science and Engineering C 41 (2014) 240–248

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Characterization and antibacterial performance of electrodeposited chitosan–vancomycin composite coatings for prevention of implant-associated infections

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ARTICLE INFO

Article history:
Received 3 November 2013
Received in revised form 10 March 2014
Accepted 17 April 2014
Available online 26 April 2014

Keywords:
Biodegradable coating
Electrophoretic deposition
Antibacterial
Chitosan
Drug release
Cytotoxicity

ABSTRACT

Orthopaedic implant-associated infections are one of the most serious complications in orthopaedic surgery and a major cause of implant failure. In the present work, drug-eluting coatings based on chitosan containing various amounts of vancomycin were prepared by a cathodic electrophoretic deposition process on titanium foils. A three-step release mechanism of the antibiotic from the films in a phosphate-buffered saline solution was noticed. At the early stage, physical encapsulation of the drug in the hydrogel network controlled the release rate. At the late stage, however, *in vitro* degradation/deattachment of chitosan was responsible for the controlled release. Cytotoxicity evaluation of the drug-eluting coatings *via* culturing in human osteosarcoma cells (MG-63 osteoblast-like cell line) showed no adverse effect on the biocompatibility. Antibacterial tests against Gram-positive *Staphylococcus aureus* also demonstrated that the infection risk of titanium foils was significantly reduced due to the antibiotic release. Additionally, i*n vitro* electrochemical corrosion studies by polarization technique revealed that the corrosion current density was significantly lower for the titanium foils with drug-eluting coatings compared to that of uncoated titanium.

1. Introduction

Implant-associated infections are one of the most serious complications in orthopaedic and trauma surgery as it may result in poor functional outcome, implant failure, chronic osteomyelitis or even death [1]. Bacterial colonization and biofilm formation are particularly problematic because sessile bacteria can withstand host immune responses and are significantly more resistant to antibiotics, biocides and hydrodynamic shear forces than their planktonic counterparts [2]. The presence of biofilms and the poor vascularization of the bone/implant interface make the infections extremely difficult to treat [3]. Annually, ~750,000 surgical site infections occur in the US that cost more than $1.6 billion in excess hospital charges [4]. With the increasing use of orthopaedic devices, the number of infected implants will continue to rise [5]. Therefore, great concerns have been taken to reduce these infections through progressing in operating standards, minimizing the possibility of contamination during surgery, reducing the establishment of infection by peri-operative antibiotic prophylaxis, and confining of pathogenic strains by patient isolation [6]. An ideal strategy to combat implant-associated infections would be the prevention of infection at the site of the implant. Physiochemical modifications of implant surfaces [7], anti-adhesive and antimicrobial coatings [3,8], and drug-eluting composite coatings [9] are some ways to inhibit the bacterial adhesion [10]. Among these techniques, local and controlled delivery of antibiotics [9] and antimicrobial peptides [3,11] through implant surfaces has received much attention. Due to the potential and low systemic side effects of this approach [12], incorporating the drug macromolecules in organic [13,14], inorganic [15,16] and composite coatings [17,18] have been explored. Vester et al. [1] have utilized poly (D,L-lactide) as the biodegradable carrier and gentamicin as the antibiotic to modify the surface of orthopaedic implants and examined bacterial growth *in vitro*. They showed that the drug was released rapidly with an initial burst in aqueous solution which was followed by a slow release. Bacterial adhesion was successfully prevented and osteoblasts were not negatively affected by the gentamicin released from the coating. Gentamicin–hydroxyapatite coatings have also revealed good biocompatibility and bony integration in a rabbit model [19].

In the present work, we prepared biodegradable and drug-eluting chitosan-based coatings on titanium substrates by cathodic electrophoretic deposition. Chitosan is a linear, semi-crystalline polysaccharide composed of (1 → 4)-2-acetamido-2-deoxy-β-D-glucan (*N*-acetyl D-glucosamine) and (1 → 4)-2-amino-2-deoxy-β-D-glucan (D-glucosamine) units [20]. Biocompatibility [21], non-toxicity [22],

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http://dx.doi.org/10.1016/j.msec.2014.04.036
0928-4931/

biodegradability [23], antibacterial and antimicrobial activity [24,25] of chitosan have made this polysaccharide as a promising candidate for drug delivery [26] and tissue engineering [20,27]. The most common pathogens causing infection are the Gram-positive *Staphylococcus aureus* and *Staphylococcus epidermidis*, which are found at the site of approximately 90% of all implants [28]. Vancomycin is a glycopeptide antibiotic indicated for the treatment of serious, life-threatening infections by Gram-positive bacteria and has been widely utilized to treat and prevent osteomyelitis and deep infections [18,29]. Chitosan–vancomycin composite coatings have been explored as a drug delivery system to prevent bone infections [9,13]. Various techniques including solvent casting [30], dip coating [31], layer by layer deposition [32], electrochemical method [13] and electrophoretic deposition (EPD) [33,34] have been used to prepare composite coatings on orthopaedic implants. Recently, composite orthopaedic coatings with antibacterial capability containing chitosan, bioglass particles and silver nanoparticles have been fabricated by employing a single-step EPD process [33]. Studying of the structural and preliminary *in vitro* bactericidal and cellular properties showed that composite coatings containing 342 μg of Ag nanoparticles were cytotoxic on MG-63 osteoblast-like cells. Patel et al. [17] have developed composite coatings of chitosan-bioglass nanoparticles *via* cathodic EPD. Uniform coating with thicknesses of a few to tens of micrometers were produced through controlling the EPD parameters. EPD is a facile and straightforward procedure to prepare biocompatible coatings for medical applications [35]. EPD is a simple and inexpensive technique that enables uniform deposition of organic [36], inorganic [37], and composite coatings [33,34] on implants of complex shape with high purity and microstructural homogeneity. Recently, cationic EPD of chitosan [36,38] and chitosan-mediated electro-synthesis of various composites [34,35] have been reported.

Herein, we developed antibacterial drug-eluting coatings composed of chitosan and a glycopeptide antibiotic (vancomycin) through the cathodic EPD technique. Although, the deposition of vancomycin–chitosan coatings on titanium alloys has been investigated by electrochemical method [13], based on our knowledge, it is the first report to use a single step EPD to incorporating vancomycin into the chitosan matrix. The EPD process of chitosan–vancomycin coatings and the interactions between drug and chitosan macromolecules were investigated. The physicochemical and biological properties of coatings in terms of degradation, drug release, antibactericidal capacity and osteoblast like-cell responses were also studied. Although for studying the bactericidal capacity of the coatings *S. aurues* assay was utilized, the findings can be rationalized to other Gram-positive bacteria such as *S. epidermidis*.

2. Materials and methods

2.1. Electrophoretic deposition

A chitosan solution in distilled water (0.5 g/l) was prepared by dissolving chitosan flakes (85% deacetylated, 190–310 kDa; Sigma-Aldrich, USA) in glacial acetic acid (Merck, Germany). The pH of the solution was adjusted to a value of 3 ± 0.05 by a Metrohm® 827 pH Lab Meter (USA Inc.). The solution was stirred overnight and then filtered to remove any residuals. Vancomycin (Dana Tabriz Company, Iran) was added to the chitosan solution to prepare macromolecule suspensions for EPD. The pH of the suspensions was controlled to be in the range of 3 ± 0.1. The weight ratio of drug to polymer in suspension was changed in the range of 0.5–4. Constant voltage EPD at an electric field of 10 V/cm was performed to prepare the coatings. Biomedical grade titanium foils (Alfa Aesar, USA) with dimensions of 10 mm × 15 mm × 0.25 mm were used as both anode and cathode. The titanium substrates were grounded and polished before treating by EPD to facilitate cross-sectional SEM studies. Deposition experiments were carried out at room temperature for 10 min.

2.2. Material characterization

The characteristics of the prepared coatings were evaluated by various analytical techniques. Scanning electron microscopy (SEM, VEGA TESCAN, Czech) was used to observe topography, morphology, microstructure, and thickness of the coatings. The operating voltage was 5 kV and no preparation techniques were utilized before imaging. Atomic force microscopy (AFM, Auto Probe CP-Research-Veeco Instruments Inc., USA) was utilized to measure the surface roughness in a non-contact mode. The patterns were scribed by using a 5 × 5 μm^2 piezoelectric scanner which can digitize the data into 1024 × 1024 pixels. The AFM tip was a pyramidal Si tip (NT-MDT NSG-10) with a tip radius of about 10 nm and an aspect ratio of about 1.2. The speed of the tip was 1 μm/s. The wettability of the films was determined by an OCA15 plus video-based optical contact angle meter (Dataphysics Instruments GmbH, Filderstadt, Germany). The images of a water droplet (4 μl) spreading on the sample surface were recorded by a camera and then analyzed using the software supplied by the manufacturer. Zeta potential measurement was carried out by a Malvern zeta sizer (Model HS C1330-3000, UK). In order to identify the chemical structure of coatings and possible interaction between the polysaccharide and glycopeptides, Fourier transform infrared spectroscopy (FTIR, ABB Bomem MB100, USA) in the range of 400–4000 cm^{-1} with a resolution of 4 cm^{-1} was utilized. To perform differential scanning calorimetry (DSC Q100, TA instrument, USA), the coatings were gently removed from the substrate and about 5 mg of the each material was collected in aluminum pans with cover sealed. The measurements were performed under nitrogen purge over the temperature range 25–280 °C at a heating rate of 10 °C/min.

2.3. In vitro drug release studies

Drug release studies were conducted in a phosphate-buffered saline solution (PBS) in a shaken incubator with controlled pH and temperature of 7.4 and 37 °C, respectively. At various time intervals, the solutions were collected and analyzed by ultraviolet–visible spectroscopy (6705 UV–Vis. Spectrophotometer, JENWAY, UK) at a wavelength of 280 nm. It should be mentioned that each test was repeated three times to ensure reproducibility of the results. Calibration curve with a root mean square (R^2) of >0.997 was constructed by using vancomycin solutions in PBS with concentrations of 5–100 μg/ml (Fig. 1). The intra-day precision on the assay was 0.54%, 1.36% and 0.67% (RSD, $n = 3$), determined by preparing, processing and analyzing three individual vancomycin standards at the concentrations of 40, 50 and 60 μg/ml, respectively (Table 1). The value of RSD was less than 2%, showing the

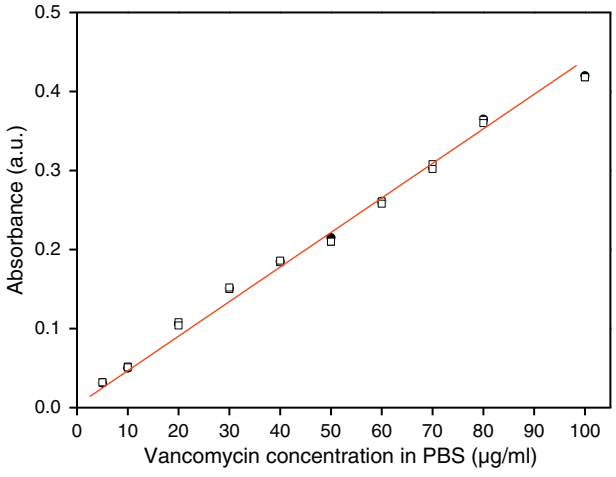

Fig. 1. Relationship between optical density and concentration of vancomycin in the PBS solution.