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Long-term antibiotic delivery by chitosan-based composite coatings with bone regenerative potential

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ABSTRACT

Composite coatings with bone-bioactivity and drug-eluting capacity are considered as promising materials for titanium bone implants. In this work, drug-eluting chitosan-bioactive glass coatings were fabricated by a single-step electrophoretic deposition technique. Drug-loading and -releasing capacity of the composite coatings were carried out using the vancomycin antibiotic. Uniform coatings with a thickness of ~55 μm containing 23.7 wt% bioactive glass particles and various amounts of the antibiotic (380–630 μg/cm^2) were produced. The coatings were bioactive in terms of apatite-forming ability in simulated body fluid and showed favorable cell adhesion and growth. *In vitro* biological tests also indicated that the composite coatings had better cellular affinity than pristine chitosan coatings. The *in vitro* elution kinetics of the composite coating revealed an initial burst release of around 40% of the drug within the first elution step of 1 h and following by a continuous eluting over 4 weeks, revealing long-term drug-delivering potential. Antibacterial tests using survival assay against Gram-positive *Staphylococcus aureus* bacteria determined the effect of vancomycin release on reduction of infection risk. Almost no bacteria were survived on the coatings prepared from the EPD suspension containing ≥0.5 g/l vancomycin. The developed chitosan-based composite coatings with bone bioactivity and long-term drug-delivery ability may be potentially useful for metallic implants to reduce infection risk.

1. Introduction

Titanium and titanium-based alloys are extensively used as suitable materials for orthopedic and dental implants [1]. This is primarily due to their excellent mechanical properties, chemical stability, biocompatibility and their capacity bond with bone and other tissues [2]. However, aseptic loosening and implant-associated infections are the most serious complications about titanium implants and major causes of failures [3]. Aseptic loosening is the predominant factor limiting the longevity of the prosthesis, accounting for over 75% of joint replacement failure [4]. Orthopedic implant-associated infections occur less frequently than aseptic failures but represent the most devastating complication, with high morbidity and substantial cost [5]. The biocompatibility and antibacterial performance of titanium implants can be improved by the surface modifications, either by controlling over roughness and topography [6,7] or the coating with bioactive and antibacterial compositions [8,9]. Ideally, implant's surfaces should promote osteoblast functions and concomitantly inhibit bacterial colonization [3]. Various composite coatings have been developed which offer the opportunity to add biocompatibility [10], osteointegration [11] and antimicrobial functionality [8] to titanium implants.

Natural biopolymers are promising candidates for tissue engineering applications. This is primarily due to their similarities with the extracellular matrix, good biological performance, biodegradability, biocompatibility and low manufacture and disposal costs [12,13]. Among various natural biopolymers, chitosan (CS) has gained much attention because of its biocompatibility [14], biodegradability [15], non-toxicity [16] and antibacterial and antimicrobial activities [17].

In order to promote osteointegration and bioactivity of CS, bioactive glass (BG) particles are commonly utilized. BG particles provide bioactivity and introduce a surface topography to the polymer matrix, which is favorable for cellular adhesion and stimulates bone repair [18]. On the other hand, local delivery of antibiotics from CS coatings to prevent risk of implant infections has gained much attention [19,20]. We have recently shown that

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incorporation of antibiotics in CS coatings provided drug-eluting potential without degrading the cell response [21].

Among various available methods for the preparation composite coatings on metallic implants [22–24], electrophoretic deposition is very promising and effective process, mainly owing to its simplicity, low equipment cost, the possibility of deposition on substrates of complex shape, high purity and microstructural homogeneity of deposits [25,26]. Recent studies have demonstrated the feasibility of cationic EPD of CS [27,28] and CS-BG composite coatings [29,30] on metallic surfaces. Effect of processing parameters such as voltage, time, pH and concentration on the deposition kinetics was studied [29,30]. Apatite-forming ability and corrosion resistance of the coatings were also shown [9,31]. Meanwhile, a few investigations have been performed to incorporate antibiotic drugs into CS-based composite coatings to gain long-term drug-delivering potential. Sustained release time for antibiotic drug is a crucial factor for bone infection. The scaffold for treating bone infection from bacteria should be one which would give a sustained release drug over 4–6-week period [32]. It is notable that the burst release may help to maintain the patient therapeutic regime, while the sustained release will maintain the plasma concentration level [33]. Therefore, fabrication of CS-BG composite coatings with bone regenerative and sustained drug-eluting potential is potentially useful for metallic implants. Patel *et al.* [34] examined the cell response and bactericidal capacity of CS-BG-ampicillin composite coatings prepared by EPD. A continuous and sustained release of ampicillin was reported, while the drug release provided bactericidal capacity against *Streptococcus mutans.* In continuation of this work, we have developed novel composite coatings with long-term drug-delivering potential of vancomycin to discourage bacterial adhesion to metallic implants while favoring the activity of osteoblast cells. The advancement of this work compared to that of Patel *et al.* [34] is related to the composition of the coatings, which affect the drug-releasing behavior and antimicrobial response. First, vancomycin drug was utilized because the most common pathogens causing bone infection are the Gram-positive *Staphylococcus aureus* and *S. epidermidis* [35]. This antibiotic is commonly used as an effective therapy for treating serious implant infections [32,36] with no negative influence on osteoblast cells *in vitro* [32] and bone growth *in vivo* [37]. Second, higher amount of BG particles was incorporated in the coating. The amino groups of CS react with PO_4^{3-} and hydroxyl groups of BG particles causing CS-BG coatings structurally more stable and physically robust such that the CS macromolecular chains are closely packed. Additionally, BG particles could alter the degradation of the CS matrix through hydrolysis in aqueous solutions; hence, the drug release was more controlled through composition modification. Besides cytotoxicity assessment, *in vitro* biocoactivity by evaluating apatite formation in simulated body fluid as well as proliferation of by employing alkaline phosphatase (ALP) activity were examined. Therefore, through detailed analyses of physicochemical and biological properties of the prepared films, more information on the potential of the electrodeposited coatings for surface modification of titanium implants has been obtained.

2. Materials and methods

2.1. Electrophoretic deposition

An aqueous chitosan solution with a concentration of 0.5 g/l was prepared by dissolving chitosan flakes (85% deacetylated, 190–310 kDa; Sigma-Aldrich, USA) in 1% (v/v) glacial acetic acid (Merck, Germany). The pH of 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 residuals (Whatman® quantitative filter paper, Grade 42, pore size 2.5 μm). A melt-drive BG powder with a nominal composition of (wt%) 45 SiO_2, 24.5 Na_2O, 24.5 CaO, 6 P_2O_5 with a mean particle size of ~100 nm (Nik Ceram Razi Biomedical Engineering Company, Iran) was added into the CS solution to prepare a composite suspension with a concentration of 0.5 g/l BG. Magnetic stirring and sonication (WiseClean WUC-D10H) were afforded for 30 min for homogenization. Then vancomycin antibiotic was supplied by Dana Tabriz Company (Iran) and used as a model drug. Suspensions containing various amounts of drug (0.25, 0.5, 1, 1.5 and 2 g/l) were prepared by dispersing vancomycin in the CS-BG suspension using magnetic stirring for 30 min.

Biomedical-grade titanium foils (ASTM B265, ATI Allegheny Ludlum, USA) with dimensions of 10 mm × 20 mm × 0.45 mm were used as both anode and cathode. An electronic image and schematic of the EPD setup are shown in Electronic Supplementary Document S. The EPD process was carried out at 10 and 15 V/cm for the CS and CS-BG composite coatings, respectively. After 10 min of deposition, samples were dried and stored in a desiccator for further characterization. To prepare samples for drug release and cell culture studies, 2 g/l vancomycin was added to the CS-BG suspension and the EPD process was afforded as explained above.

2.2. Material characterization

The characteristics of the prepared coatings were evaluated by various analytical techniques. Scanning electron microscopy (SEM, VEGA TESCAN, Czech Republic) was used to observe topography, morphology, microstructure and thickness of the coating. Atomic force microscopy (AFM, DME DualScope™ C-26 Controller, scanning probe and optical microscope) was utilized to measure the surface roughness in a non-contact mode. The patterns were scribed by using a $200 \times 200\ \mu m^2$ piezoelectric scanner which can digitize the data into 1024×1024 pixels. Surface hydrophilicity was determined by a 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 composite components, 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. Thermogravimetric analysis (TGA; Mettler Toledo, Switzerland) was performed to estimate the amount of BG particles in the coatings. The composite coatings were removed from the substrates and were analyzed in air at a heating rate of 10 K/min.

2.3. Bone bioactivity

In vitro bone bioactivity of the coatings was evaluated by the apatite-forming ability in simulated body fluid (SBF) solution [38] at 37 °C for 28 days. The following reagents from Sigma–Aldrich (USA) were used to prepare SBF solution: NaCl, $NaHCO_3$, KCl, $K_2HPO_4 \cdot 3H_2O$, $MgCl_2 \cdot 6H_2O$, $CaCl_2$, Na_2SO_4, Tris–hydroxymethyl aminomethane and HCl (1.0 M). The surface morphology and mineralized apatite particles were analyzed by SEM and energy-dispersive spectroscopy (EDS) measurements.

2.4. Drug-release studies

The drug-release studies were conducted in an autoclaved phosphate-buffered saline solution (PBS) with controlled pH and temperature of 7.4 and 37 °C. The specimens were immersed in PBS for various times and the solution was analyzed by