



Single-step electrochemical deposition of antimicrobial orthopaedic coatings based on a bioactive glass/chitosan/nano-silver composite system



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ABSTRACT

Composite orthopaedic coatings with antibacterial capability containing chitosan, Bioglass[®] particles (9.8 μm) and silver nanoparticles (Ag-np) were fabricated using a single-step electrophoretic deposition (EPD) technique, and their structural and preliminary in vitro bactericidal and cellular properties were investigated. Stainless steel 316 was used as a standard metallic orthopaedic substrate. The coatings were compared with EPD coatings of chitosan and chitosan/Bioglass[®]. The ability of chitosan as both a complexing and stabilizing agent was utilized to form uniformly deposited Ag-np. Due to the presence of Bioglass[®] particles, the coatings were bioactive in terms of forming carbonated hydroxyapatite in simulated body fluid (SBF). Less than 7 wt.% of the incorporated silver was released over the course of 28 days in SBF and the possibility of manipulating the release rate by varying the deposition order of coating layers was shown. The low released concentration of Ag ions (<2.5 ppm) was efficiently antibacterial against *Staphylococcus aureus* up to 10 days. Although chitosan and chitosan/Bioglass[®] coating supported proliferation of MG-63 osteoblast-like cells up to 7 days of culture, chitosan/Bioglass[®]/Ag-np coatings containing 342 μg of Ag-np showed cytotoxic effects. This was attributed to the relatively high concentration of Ag-np incorporated in the coatings.

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1. Introduction

In spite of notable advances in clinical methods, implanting a metallic orthopaedic device with long-term survivability still represents a major challenge for orthopaedic surgeons [1]. In terms of improving the surface functionality of metallic implants [2], bioactive glass coatings have demonstrated promising functionality [3]. These coatings can be further improved by combining bioactive glass with an organic polymer. Addition of a polymer phase to an inorganic coating may mechanically strengthen the glass or

ceramic coating structure [4], eliminate high-temperature processing [5], control the ionic release rate of the bioactive ceramic [6], and perhaps more importantly, can provide a platform for incorporation and release of biomolecules and drugs which often require room-temperature processing [7].

Chitosan, a natural polysaccharide, is a well-known candidate for such a composite system. It is a linear, cationic polymer consisting of β (1 → 4)-glucosamine and N-acetyl-D-glucosamine and is obtained from N-deacetylation of chitin. Notable features of this biopolymer are susceptibility to enzymatic degradation, accelerated angiogenesis, little fibrous encapsulation, ability to link to and deliver growth factors, and improved cellular adhesion [8,9]. Moreover, chitosan has excellent film-forming property and has been the focus of many coating systems for biofunctionalization of metallic implants [10–13].

Implantation of an orthopaedic device in the body is always associated with a risk of microbial infection which is more severe

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in open-fractured bones and joint revision surgeries [14]. More importantly, formation of bacterial biofilms on the implant presents additional complications, as these are extremely resistant to both the immune system and antibiotics [15]. Unfortunately systemic drug administration in such clinical condition is not sufficiently effective due to impaired blood circulation at the injury site and low local concentration of drug. Therefore local delivery of drugs and biomolecules can be an effective approach to treat infections with high local concentrations of drug, with long-term controlled release and without the risk of systemic toxicity or formation of biofilms [16]. Various organic and inorganic coating systems with therapeutic capability have been explored [17].

As increased antibiotic resistance is a major medical concern, silver—an agent which has not developed bacterial resistance—is emerging as an antibacterial component with high potential. It works against a broad range of gram-positive (e.g. *Staphylococcus aureus* and *Staphylococcus epidermidis*) and gram-negative (e.g. *Escherichia coli*, *Pseudomonas aeruginosa*) bacteria [2]. Silver exhibits a bactericidal effect at very low concentrations without toxicity to human cells [18]. It has been incorporated in a variety of organic [19,20] and inorganic [21–24] coatings. More interestingly, it has been shown that silver nanoparticles have larger surface area contacting bacterial cells and hence have a higher level of interaction and faster release rate [25]. Films of chitosan-nano-silver/heparin produced via layer-by-layer deposition have shown antibacterial effects to *E. coli* without cytotoxicity to osteoblast cells [26]. Ag-doped HA/PEEK composite coatings cold-sprayed on glass slides have inhibited the growth of bacteria [27]. In another study by Roy et al. [28], significant reduction in *S. aureus* and *P. aeruginosa* colonies were observed on Ag-tricalcium phosphate-coated Ti surfaces.

Among different techniques exploited for composite orthopaedic coatings, electrophoretic deposition (EPD) is particularly attractive because it can be utilized to produce uniform coatings with controlled properties on complex-shaped and porous structures, at ambient temperature and without the need for expensive processing equipment [29]. Co-deposition of polymers and ceramics is also another interesting feature of EPD [5,29,30]. In EPD, surface-charged particles in suspension move toward an oppositely charged electrode (i.e. the substrate) under an applied electrical field and form a coating. Many different composite biomaterial coatings have been fabricated by EPD [30]. Recently we have investigated the influence of EPD parameters on co-deposition of Bioglass® particles and chitosan from aqueous medium [5]. Pang et al. [31] have applied EPD to co-deposit multilayers of composite chitosan/silver/hydroxyapatite (HAP) nanoparticles. They have reported formation of amorphous Ag–chitosan complexes in their coatings and have controlled the release kinetics of ionic silver from these coatings by application of an additional chitosan film.

We have previously studied in detail the effect of EPD parameters on deposition of chitosan coatings [10], Bioglass® coatings [32] and chitosan/Bioglass® composite coatings [5]. The next step is addition of an antibacterial function to the chitosan/Bioglass® composite coatings and we are keen to demonstrate the potential of EPD as a single-step platform for obtaining such a coating. Hence, the goal of this research is the single-step fabrication and characterization of multifunctional resorbable chitosan/bioactive glass composite coatings containing silver in the form of Ag nanoparticles (Ag-np). In this work, co-deposition of the chitosan/bioactive glass composite coating in addition to simultaneous *in situ* formation and deposition of Ag-np as antibacterial agent has been demonstrated for the first time. More importantly, considering the environmental concerns associated with free nanoparticles, the benefit of the developed technique is the absence of loose nanoparticles in all steps. The microstructural aspects of the coatings have been examined by different techniques. A preliminary *in vitro*

cellular and antibacterial study to characterize the behaviour of these films was conducted. This investigation can contribute to a better understanding of the interaction between the biological environment and these novel materials intended for orthopaedic and bone engineering applications.

2. Experimental procedures

2.1. Materials

45S5 Bioglass® powder with nominal composition 45SiO₂–24.5Na₂O–24.5CaO–6P₂O₅ (wt.%) was kindly supplied by Dr. I. Thompson (Kings College London, UK). The particle size was in the range 1.6 and 26.7 μm with a median particle size of 9.8 μm. Medium molecular weight chitosan (MW = 80 kDa) with a degree of deacetylation of about 85% and acetic acid (>98%) were purchased from Sigma–Aldrich and 0.05 M silver nitrate solution (AVS TITRINORM) from VWR was used. The following reagents from Sigma–Aldrich were used to prepare simulated body fluid (SBF) solution [33]: NaCl, NaHCO₃, KCl, K₂HPO₄·3H₂O, MgCl₂·6H₂O, CaCl₂, Na₂SO₄, Tris-hydroxymethyl aminomethane and HCl (1.0 M).

2.2. Electrophoretic deposition

Dilute solutions of chitosan (0.5 mg ml⁻¹) in 1 vol.% acetic acid in deionized water were prepared by magnetic stirring at room temperature for 24 h. Suspensions containing Bioglass® were prepared by dispersing the particles in the chitosan solutions via magnetic stirring and sonication (USC 300 sonicator, VWR International, Malaysia) for periods of 600 s each. For coatings containing Ag-np, silver nitrate solution was added to the prepared suspensions with a final concentration of 1 mM. The pH of the suspensions was measured by using Jenway 3510 pH meter (Essex, UK).

AISI 316L stainless steel is among the most commonly used metals for orthopaedic implant applications [34]. Thus, for electrophoretic deposition, AISI 316L (Advent Research Materials Ltd., Oxford, UK) foils (20 mm × 10 mm × 0.2 mm) were utilized as deposition substrate. It is pertinent to point out that for similar substrate surface conditions, as long as the substrate is electrically conductive, the EPD rate is independent of the substrate material [29]. Therefore, the methodology applied here is extendable to other conductive implant substrate materials such as Ti alloys. Substrates were washed with acetone and dried prior to deposition. A gold counter-electrode was used in the EPD cell. Experiments were performed at 21 ± 2 °C. The distance between the electrodes was kept constant at 1.5 cm and the suspensions were gently stirred during deposition by a magnetic stirrer. The constant electric voltage was applied by a Thurlby Thandar Instruments (TTi) EL561 power supply (Huntingdon, UK). Four different coatings were deposited. The EPD experimental condition for each coating is outlined in Table 1. After deposition, the cathodic films were gently rinsed with deionized water, dried and stored in a desiccator for further characterization.

2.3. Characterization of coatings

2.3.1. Microstructural characterization

High-resolution scanning electron microscopy (SEM; LEO Gemini 1525) was used to study the microstructural features. The samples were coated with chromium using an Emitech K575X sputter coater (Emitech Ltd., UK) beforehand to avoid any charging artefacts during imaging. The LEO Gemini 1525 microscope was equipped for energy-dispersive X-ray spectrometry

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