



Antimicrobial silver-hydroxyapatite composite coatings through two-stage electrochemical synthesis



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ABSTRACT

A two-stage electrochemical synthesis method was developed to prepare antimicrobial silver-hydroxyapatite (Ag-HA) composite coatings. In the first stage, a titanium substrate was coated with hydroxyapatite (HA) through electrochemical crystallization from aqueous solution. In the second stage, silver nanoparticles were deposited onto the HA layer through electrochemical reduction of aqueous Ag^+ to Ag^0 . Silver nanoparticles having a narrow size distribution were found to deposit uniformly on the HA layer. The quantity and size of silver nanoparticles were shown to be controlled by the electrochemical deposition time under constant applied electrical current density. Ag-HA composite coatings were found to be bioactive based upon the promotion of the deposition of additional calcium phosphate onto the Ag-HA coating surface from simulated body fluid. *Staphylococcus aureus* bacteria growth was inhibited by coatings of Ag-HA, while HA coatings without silver had little effect on growth of the bacteria. The novel composite Ag-HA coatings can potentially be used in orthopedic and dental implants to simultaneously improve osseointegration and prevent post-surgical infection.

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1. Introduction

Hydroxyapatite (HA) is a calcium phosphate compound that is found naturally as the mineral component of teeth and bone tissue. Orthopedic and dental implants are often coated with HA or related calcium phosphate compounds in order to speed osseointegration following surgery [1]. A number of in vivo and clinical studies have shown that HA promotes the osseointegration of load bearing implants [2–4]. As a result of its bioactivity, HA coatings have been used commercially for decades in dental and orthopedic applications. Commercial HA coatings are most commonly applied to implants using plasma spray [5], but a variety of other low temperature techniques can be used, including sol-gel synthesis [6], electrophoretic deposition [7], and electrochemical crystallization [8]. The electrochemical method allows crystalline HA to be deposited rapidly from aqueous solution under mild operating conditions. In addition, curved surfaces such as dental implant screws and orthopedic fixation pins may be evenly coated electrochemically in a single step. It was recently discovered that sub-micron thick HA coatings deposited electrochemically retain strong electrical polarization that is known to promote osseointegration [9]. The electrochemical technique therefore offers a low cost route for surface modification of implants with HA that has chemical and electrical properties that promote osseointegration. Strongly adherent,

sub-micron thick HA layers can be electrochemically synthesized on the surface within a few minutes.

One of the most serious complications following implant surgery is bone infection. To reduce the incidence of bacterial film formation on implant surfaces, local delivery of antibiotics is desirable [10]. In some procedures, surgeons use polymeric bone cement as a carrier for antibiotic release. Bone cements are typically based on non-biodegradable poly(methyl methacrylate), making the cement a non-ideal material for extended drug delivery. Moreover, there is evidence that local delivery of antibiotics significantly increases the likelihood of infection with antibiotic resistant bacteria following implant revision surgery [11]. One of the major concerns with the use of antibiotics is the relatively rapid rate that bacteria can become resistant to treatment [12,13]. Rapid clearance of antibiotic molecules from the body requires repeated doses for systemic delivery, and makes extended release through local delivery challenging.

Silver is well known for its broad spectrum antimicrobial activity, and has been gaining increasing attention as an alternative to traditional antibiotic molecules. It has high efficiency and low toxicity for long-term use [14–16]. Silver ions, silver compounds and silver particles have been used in pharmaceuticals and in medical devices to replace antibiotics or other antibacterial agents [17–21]. Bacteria may be killed upon surface contact with metallic silver nanoparticles, and through the extended release of low concentrations of silver ions through oxidative dissolution of the nanoparticles [22]. Silver nanoparticles have the advantages of long term antimicrobial activity from a single application,

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and a low probability of a microorganism developing resistance. A variety of chemical and physical methods have been developed to synthesize silver nanoparticles [23–26]. Among these approaches, the reduction of Ag^+ to Ag^0 is attractive for the potential to rapidly produce large quantities of product at low cost [27–30]. Silver ions can be reduced either chemically or electrochemically, with various synthesis methods allowing key nanoparticle properties such as particle size, size distribution, purity, crystallinity and shape to be controlled. Silver nanoparticles have been formed within thin ceramic coatings made through sol-gel synthesis by chemically reducing Ag^+ to Ag^0 [31–33].

Silver nanoparticles or silver atoms incorporated into HA coatings provide antimicrobial properties while maintaining the bioactivity of HA. Several methods have been used to create silver-containing HA coatings, including plasma spray [34], sputter coating [35], sol-gel synthesis [36], electrolytic synthesis followed by ion exchange [37], and electrophoretic deposition [38]. In the present study, a novel two-stage coating process is reported in which two electrochemical reactions are carried out in sequence to form a silver-hydroxyapatite (Ag-HA) composite thin film on a titanium substrate. The present study is motivated by the recent discovery that electrochemically synthesized sub-micron thick HA coatings retain strong quasi-permanent electrical polarization [9]. Electrical polarization is known to enhance biomineralization, cell adhesion, cell proliferation, and osseointegration [39–41]. The first stage of the synthesis is the electrochemical crystallization of HA from an aqueous solution onto a titanium substrate acting as a cathode. In the second stage, silver is electrochemically reduced from silver ions in aqueous solution onto the HA-coated titanium. The two stages are carried out under similar operating conditions, offering a rapid low cost implant coating method. The end result is a sub-micron thick HA coating decorated with silver nanoparticles. Separating the process into two stages allows the HA coating properties to be controlled independently of the silver loading. The silver loading can then be controlled in the second stage without altering the synthesis conditions known to produce polarized HA in the first stage. The resulting Ag-HA coating has the potential to act as an antimicrobial implant surface that inhibits bacterial growth while simultaneously enhancing osseointegration. Previous studies have shown that the loading of silver into HA must be optimized in order to provide an antibacterial effect without inducing cytotoxicity, as reviewed recently [42]. Future work will examine the cytotoxicity of the Ag-HA coatings made by two-stage electrochemical deposition in order to optimize the silver loading for potential in vivo applications.

2. Experimental procedure

2.1. Materials

Tris(hydroxymethyl)aminomethane (>99.8%), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (>99%), NaCl (>99.0%), and K_2HPO_4 (99.99%) were purchased from Sigma-Aldrich. Aqueous HCl solution (37%) was purchased from Mallinckrodt Chemicals. Titanium (Ti, 0.89 mm thick) and platinum (Pt, 0.127 mm thick) foils were obtained from Alfa Aesar. AgNO_3 (ACS grade) was obtained from Amresco.

2.2. Electrochemical crystallization of HA

A pre-cleaned Ti plate ($12.5 \times 12.5 \times 0.89$ mm) was used as the cathode while a Pt foil ($25 \times 25 \times 0.127$ mm) was used as the anode in the electrochemical crystallization. An electrolyte solution was prepared by dissolving 138 mM NaCl, 50 mM tris(hydroxymethyl)aminomethane, 1.3 mM CaCl_2 , 0.84 mM K_2HPO_4 in deionized water. The solution was buffered to pH = 7.2 with 37% HCl. The anode and cathode were immersed into the electrolyte solution. The electrolyte solution was heated in an oil bath to maintain constant temperature. When the temperature of the electrolyte solution arrived at -95°C , a constant current density of 12.5 mA/cm^2 (relative to

Pt electrode area) was applied for 2 min. The reaction results in selective crystallization of HA onto the titanium cathode.

2.3. Electrochemical reduction of silver

After the electrochemical deposition of HA, the electrodes were transferred to a 125 ml aqueous solution of NaCl. The solution was heated to 95°C under vigorous stirring. Then AgNO_3 having same concentration as the NaCl was added to the solution immediately before applying a DC electric field. Deposition time, was varied to investigate its effects on Ag-HA formation. The electrochemical reaction resulted in selective deposition of Ag nanoparticles onto the HA coated titanium cathode.

2.4. Characterization of the coatings

Morphology and composition of the samples were examined using a Zeiss-Leo DSM982 field emission scanning electron microscope (SEM) equipped with a Phoenix EDAX energy dispersive X-ray spectrometer (EDX). Surface composition was obtained with a Kratos AXIS Ultra DLD X-ray photoelectron spectrometer (XPS), equipped with a monochromatic Al anode X-ray gun ($K\alpha = 1486.6 \text{ eV}$). For crystal structure determination, four identically synthesized samples were scraped off the titanium substrate and combined to obtain enough material in the form of powder to analyze with X-ray diffraction (XRD). The powder was then analyzed with a Philips PW3020 XRD spectrometer using Cu $K\alpha$ radiation ($\lambda = 1.5418 \text{ \AA}$).

2.5. Mineralization from simulated body fluid

Solutions of $1.5\times$ simulated body fluid (SBF) were prepared following the literature [43]. Ag-HA composite coatings were placed into vials containing 5 ml of $1.5\times$ SBF at 37°C with pH of 7.25. After 24 h, the samples were taken out of the solution, rinsed with deionized water, and placed in a desiccator to dry. The mineralization was examined using SEM of the dried samples. Electrochemically synthesized HA coating without silver deposition was used as a control sample for comparison.

2.6. Antibacterial activity

A solution of *E. coli* (BL21(DE3)) bacteria in tryptic soy broth was grown overnight with shaking at 37°C . The bacteria concentration was then diluted with tryptic soy broth until the solution's absorbance value at 490 nm was 0.01. Bacteria growth curves were produced by placing 3 HA and 3 Ag-HA samples into wells of a 24 well plate. Each well was then filled with 2 ml of bacteria suspension, as well as three control wells with just bacteria suspension. The samples were then incubated at 37°C . Bacteria growth was measured at various times (0 h, 2 h, 4 h, and 8 h) using light scattering at 490 nm (PerkinElmer EnSpire Multimode Plate Reader) by placing 1200 μL of bacteria solution into 6 wells (200 μL in each well) of a 96 well microtitre plate. The 1200 μL solution was then placed back into the solution of their respective samples after each measurement. To measure a number of live bacteria in each treatment condition, bacteria solutions at the 0-h time point and 8-h time point were used to spread onto tryptic soy agar plates. The agar plates were then incubated at 37°C for 12 h. Numbers of the bacteria colonies from each plate were counted.

3. Results and discussion

3.1. Deposition of silver nanoparticles onto HA by electrochemical reduction

In the first electrochemical reaction stage, HA was crystallized onto a commercially pure titanium substrate using a current density of 12.5 mA/cm^2 at -95°C for 2 min, following the same procedure described in recent publications [9,44]. The electrochemical synthesis

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