Incorporation of silver nanoparticles into magnetron-sputtered calcium phosphate layers on titanium as an antibacterial coating

Maria A. Surmeneva [a], Anna A. Sharonova [a], Svitlana Chernousova [c], Oleg Prymak [c], Kateryna Loza [c], Mikhail S. Tkachev [a], Ivan A. Shulepov [b], Matthias Epple [c,*], Roman A. Surmenev [a,*]

[a] Centre of Technology, Department of Experimental Physics, Lenin Avenue 43, National Research Tomsk Polytechnic University, 634050 Tomsk, Russia
[b] Material Properties Measurements Centre, National Research Tomsk Polytechnic University, 634050 Tomsk, Russia
[c] Inorganic Chemistry and Center for Nanointegration Duisburg-Essen (CeNIDE), Universitaetsstr, 5-7, University of Duisburg-Essen, 45117 Essen, Germany

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ABSTRACT

A three-layer system of nanocrystalline hydroxyapatite (first layer; 1000 nm thick), silver nanoparticles (second layer; 1.5 μg Ag cm^{-2}) and calcium phosphate (third layer, either 150 or 1000 nm thick) on titanium was prepared by a combination of electrophoretic deposition of silver nanoparticles and the deposition of calcium phosphate by radio frequency magnetron sputtering. Scanning electron microscopy showed that the silver nanoparticles were evenly distributed over the surface. The adhesion of multilayered coating on the substrate was evaluated using the scratch test method. The resistance to cracking and delamination indicated that the multilayered coating has good resistance to contact damage. The release of silver ions from the hydroxyapatite/silver nanoparticle/calcium phosphate system into the phosphate-buffered saline (PBS) solution was measured by atomic absorption spectroscopy (AAS). Approximately one-third of the incorporated silver was released after 3 days immersion into PBS, indicating a total release time of the order of weeks. There were no signs of cracks on the surface of the coating after immersion after various periods, indicating the excellent mechanical stability of the multilayered coating in the physiological environment. An antimicrobial effect against *Escherichia coli* was found for a 150 nm thick outer layer of the calcium phosphate using a semi-quantitative turbidity test.

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1. Introduction

The introduction of silver nanoparticles has had a pronounced impact on applications of silver in consumer products and medical devices [1,2]. Silver nanoparticles (AgNPs) show efficient antimicrobial properties due to their large specific surface area, which enhances the release of silver ions [3–7]. However, *in vitro* studies have demonstrated that high concentrations of silver nanoparticles can lead to cell death (see Refs. [1,5,8,9] for overviews on the biological effects of silver).

Typically, antibacterial surfaces are created by introducing bactericidal compounds, such as antibiotics or metals, using different methods [10]. For instance, the incorporation of silver nanoparticles into polymer matrices is a common strategy to prepare composite coatings [11–14]. However, an even distribution of silver nanoparticles into polymer matrices is challenging because of the strong tendency for particle aggregation to reduce the surface energy of the nanoparticles.

An alternative strategy involves the fabrication of uniformly dispersed silver nanoparticles embedded into a calcium phosphate coating (CaP). CaP coatings (mainly as hydroxyapatite) are widely used for orthopaedic implants to stimulate osteointegration and to accelerate bone formation. They are typically applied with plasma spraying, sol-gel processing, or plasma sputtering [15]. However, a hydroxyapatite (HA) coating on a metallic implant is also susceptible to bacterial infection because its microscale topography, which is beneficial for osteointegration and also favourable for bacterial colonization [16,17].

The homogeneous incorporation of silver nanoparticles into a calcium phosphate coating by plasma spraying is a challenging task because the nanoparticles sinter at the high temperature of

the plasma flame. Our strategy is therefore based on the sequential preparation of calcium phosphate surface coatings that contain internal silver nanoparticles as an antibacterial agent. The silver nanoparticles can then be used as a source of silver ions when they are released from the coating, *e.g.,* in the case of a pH drop during inflammation [18]. This extends our earlier approaches where a layer of electrophoretically deposited silver nanoparticles was sputter-coated with a layer of calcium phosphate, [19] and the successful incorporation of silver ions into the magnetron-sputtered coatings of the calcium phosphate [20]. The hydroxyapatite coating above and below the silver nanoparticles will slowly degrade and cause a release of silver ions directly at the surface-tissue interface. Moreover, the durability of the antimicrobial surface is enhanced because the silver nanoparticles are not washed away since they are firmly incorporated between two layers of calcium phosphate. Finally, the amount of silver in the coating can be varied through the synthesis process, *i.e.,* by varying the thickness of the three layers.

2. Materials and methods

2.1. Deposition of the bottom and top calcium phosphate layers by RF magnetron sputtering

A hydroxyapatite powder, $Ca_{10}(PO_4)_6(OH)_2$, was prepared by mechanochemical activation and used as a precursor to prepare a target for sputtering. The powder was pressed and then sintered in air at 1100 °C for 1 h. The chemical composition of the powder and the target was confirmed by X-ray powder diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and energy dispersive X-ray spectroscopy, as previously reported [21]. The target material had a molar calcium to phosphate ratio of $Ca/P = 1.67 \pm 0.02$, consistent with stoichiometric hydroxyapatite [22]. Plates of technically pure titanium were used (1×1 cm^{-2}) as the substrate. The titanium samples were acid-etched for 5–10 s in a mixture of HF and HNO_3. The volume ratio of the aqueous HF (48 wt%): aqueous HNO_3 (65 wt%): H_2O was 1: 4: 5.

A commercially available apparatus with an RF (13.56 MHz, COMDEL) magnetron source was used to deposit the calcium phosphate coatings [23]. The bottom hydroxyapatite layer was deposited at an RF power level of 500 W in an argon atmosphere (0.4 Pa) for 8 h onto a substrate mounted in a grounded substrate holder. The top calcium phosphate layer above the surface of silver nanoparticles was deposited at 500 W for either 8 or 2 h, which resulted in a layer thickness of 900 ± 90 or 150 ± 20 nm, respectively.

The bottom calcium phosphate layer obtained by RF magnetron sputtering was annealed for 2 h at a temperature of 600 °C before the electrophoretic deposition of the silver nanoparticles. The heat treatment was performed in air with a conventional furnace. Each layer design was prepared at least ten times to validate the reproducibility.

2.2. Silver nanoparticles

Poly(vinylpyrrolidone) (PVP)-stabilized silver nanoparticles were synthesized by reduction with glucose in the presence of PVP following Refs. [8,24,25]. In brief, 2 g of glucose and 1 g of PVP were dissolved in 40 g of water and heated to 90 °C. Then, 0.5 g of $AgNO_3$ dissolved in 1 mL of water was quickly added. The dispersion was kept at 90 °C for 1 h and then allowed to cool to room temperature. The particles were collected by ultracentrifugation (29,400g, 20,000 rpm, 20 min), redispersed in pure water and collected again by ultracentrifugation. This procedure was repeated three times. Thereby, nitrate, excess glucose and its oxidation products, excess

PVP, and excess Ag$^+$ were all removed. Ultrapure water was prepared with an ELGA Purelab ultra instrument.

The hydrodynamic diameter of the silver nanoparticles was measured by dynamic light scattering (DLS) using a Malvern Zetasizer Nano ZS instrument and nanoparticle tracking analysis (NTA) using a NanoSight LN 10 instrument. The zeta (ζ) potential of the silver nanoparticles was measured with a Malvern Zetasizer Nano ZS instrument. The polydispersity index (PDI) was below 0.3 in all cases. The concentration of silver was determined by AAS (Thermo Electron Corporation, M-Series) with a detection limit of 1 μg L^{-1}.

Prior to electrophoretic deposition, the silver nanoparticles were dispersed in ethanol by ultrasonication. Electrophoretic deposition was performed at a constant voltage of 30 V for 30 min. The distance between the two electrodes was 3 mm and the silver concentration in the dispersion was 60 mg L^{-1}.

2.3. Characterization methods

The morphology and composition of the coatings after each preparation stage were determined using an ESEM Quanta 400 FEG scanning electron microscope (SEM), equipped with energy-dispersive X-ray spectroscopy (EDX; Genesis 4000, SUTW-Si(Li) detector) operating in a high vacuum with gold/palladium-sputtered samples. To confirm the formation of a layered structure, cross sections were prepared with a cross section polisher (Jeol IB-09010CP). To determine the internal structure and phase composition of the studied samples, a Panalytical Empyrean X-ray powder diffractometer with Cu Kα radiation source ($\lambda = 1.54$ Å; 40 kV and 40 mA) was used. The coatings were investigated using grazing incidence X-ray diffraction (GIXRD) with incident beam angles of $\Phi = 1.0$ and 2.0° (with respect to the sample surface) and at 2θ from 5 to 90° with a step size of 0.05°. Rietveld refinement with the programme package TOPAS 4.2 from Bruker was performed to determine the average crystallite size, the microstrain, and the crystallographic density as well as the lattice parameters and the individual phase content. For each Rietveld refinement, the instrumental correction as determined with a standard powder sample LaB$_6$ from NIST (National Institute of Standards and Technology) as a standard reference material (SRM 660b; a(LaB$_6$) = 4.15689 Å) was taken into account. Silver (#4-0783), hydroxyapatite (#4-0432) and titanium (#44-1294) patterns from the ICDD database were used as References.

The adhesion of the multilayer coatings to the underlying titanium alloy was investigated with a micro-scratch test method using a CSEM Micro Scratch Tester. The micro-scratch tests were performed using a Rockwell C indenter with a diamond tip radius of 200 μm, a scratch length of 10 mm, a load range of 0.01–7 N, and a normal pressure increase rate of 7 N min^{-1}. Six independent scratches were applied to each sample. The critical load was determined at the point when the delamination of the coating from the substrate began, using the friction coefficient. Images of the scratches were recorded with optical microscopy and SEM.

2.4. Ion release kinetics and antibacterial assay

Dissolution studies were conducted in a phosphate buffered saline (PBS) solution, followed by analysis of the immersion medium by AAS. The samples were incubated in PBS (5 mL) for 3 days at 37 ± 2 °C to simulate physiological conditions (pH 7.4). The total amount of silver in the coatings was determined by AAS after the complete dissolution of all layers in concentrated aqueous HNO_3 (65 wt%).

The antibacterial activity of the three-layer coatings was evaluated with the Gram-negative strain of *Escherichia coli DH5α* (*E. coli*) with a semi-quantitative turbidity test. The samples containing silver nanoparticles were incubated in PBS (6 samples with