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Synthesis of new antibacterial composite coating for titanium based on highly ordered nanoporous silica and silver nanoparticles

Miguel A. Massa [a], Cristian Covarrubias [a,*], Mauricio Bittner [b,c], Ignacio Andrés Fuentevilla [b], Pavel Capetillo [a], Alfredo Von Marttens [a], Juan Carlos Carvajal [a]

[a] Laboratory of Nanobiomaterials, Institute for Research in Dental Sciences, Faculty of Dentistry, University of Chile, Independencia, Santiago, Chile
[b] Laboratory of Oral Microbiology and Biotechnology, Faculty of Biological Sciences, Andrés Bello University, Santiago, Chile
[c] Laboratory of Oral Microbiology and Biotechnology, Faculty of Dentistry, Andrés Bello University, Santiago, Chile

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ABSTRACT

Infection is the most common factor that leads to dental titanium implant failure. Antibacterial implant surfaces based on nano-scale modifications of the titanium appear as an attractive strategy for control of peri-implantitis. In the present work, the preparation and antibacterial properties of a novel composite coating for titanium based on nanoporous silica and silver nanoparticles are presented. Starch-capped silver nanoparticles (AgNPs) were synthesized and then incorporated into sol–gel based solution system. The AgNP-doped nanoporous silica coatings were prepared on titanium surface using a combined sol–gel and evaporation-induced self-assembly (EISA) method. The coating nanostructure was characterized by XRD, SEM–EDX, and HR-TEM. Antibacterial activity was evaluated against *Aggregatibacter actinomycetemcomitans*, a representative pathogen of dental peri-implantitis. Colony-forming units (CFUs) were counted within the biofilm and at the planktonic state. Biofilm development was quantified using crystal violet staining and viability of adherent bacteria was confirmed with the Live/Dead fluorescence assay.

Silica-based composite coating containing AgNPs (AgNP/NSC) was prepared on titanium surface by direct incorporation of AgNP suspension into the sol–gel system. The self-assembly technique enabled the spontaneous formation of a highly ordered nanoporosity in the coating structure, which is a desired property for osseointegration aspects of titanium implant surface. AgNP/NSC coating produces a strong antibacterial effect on titanium surface by not only killing the adherent bacteria but also reducing the extent of biofilm formation. Biofilm survival is reduced by more than 70% on the AgNP/NSC-modified titanium surface, compared to the control. This antibacterial effect was verified for up to 7 days of incubation. The long-term antibacterial activity exhibited by the nanostructured AgNP/NSC-titanium surface against *A. actinomycetemcomitans* suggests that this type of nano-scale surface modification is a promissory strategy to control infections associated with dental implant rehabilitation.

1. Introduction

Multiple factors affect the clinical success of dental implants, including osseointegration of bone implants and the degree of bacterial colonization surrounding the implants [1]. Peri-implantitis is the inflammatory disease marked by bacterial infection and the destructive process affecting the soft and hard tissues around osseointegrated implants, leading to the loss of supporting bone [2,3]. Although the treatment of the infected implants is a challenge in clinical practice, its consequent loosening is still most common in implanted devices. In recent years, strategies based on the modification of the geometry and physicochemical properties of the titanium implants are being investigated to prevent or reduce their bacterial colonization [4,5]. One of these approaches consists in the fabrication of antibacterial coatings on the titanium implant surface. Antibacterial polymeric coatings loaded with antibiotics such as vancomycin and gentamicin have been prepared on titanium surfaces [6,7]. Coatings loaded with non-antibiotic organic antimicrobials (NOA) such as chlorhexidine, chloroxylenol, and polyhexamethylene biguanide have also been studied [8–10]. However, antibiotics and NOA cannot be incorporated into the traditional hydroxyapatite coatings during their formation because of the extremely high processing temperature. On the other hand, physical adsorption of these antimicrobial agents onto the surface of coatings limits the loaded amount and release characteristics [11]. Antibiotics and NOA also raise the risks by new multidrug-resistant strains (MRS). Precisely, the multidrug-resistance of microorganisms has increased [12,13], and several reports indicate that some antibiotics and NOA agents are harmful to cell functions [14,15].

Recently, studies have introduced silver nanoparticles (AgNPs) as a new antimicrobial generation for biomedical applications with many

* Corresponding author at: Sergio Livingstone 943, Independencia, Santiago, Chile. Tel.: +56 2 9781719; fax: +56 2 978 1754.
E-mail address: ccovarru@u.uchile.cl (C. Covarrubias).

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advantages over antibiotics and NOA, such as good antibacterial activity, excellent biocompatibility, and satisfactory stability [16–18]. The study of bactericidal metallic nanoparticles is particularly timely considering the recent increase of MRS. Silver nanoparticles present unique physical, chemical and biological properties, better than micrometric size particles. Nanometric silver has a large total surface area available for bacterial interaction and exhibits a stronger antibacterial effect, which affects cellular functions as well as structures [19,20]. Current research is focused on improving the antibacterial properties of biomaterials by using silver nanoparticles. Several antimicrobial dental products based on nanosilver have been studied, such as silver-doped hydroxyapatite [21], dental adhesives [22], primers [23], and mouthwash loaded with AgNPs [24], pointing to increasing interest in the potential applications of nanosilver in the dental area. Regarding the use of silver nanoparticles to produce antimicrobial surfaces on titanium implants, Juan et al. [25] reported the preparation of nanosilver-modified titanium surfaces through a simple coating method. A silane-modified titanium surface was immersed in an aqueous suspension of silver nanoparticles. The titanium surfaces loaded with 4.26% of AgNP killed over 94% of *Staphylococcus aureus* and *Escherichia coli* in bacterial suspensions. Although bacterial inhibition of the material surface was not quantified, SEM examination revealed that an important decrease in the bacteria adhesion occurred on the AgNP-modified titanium surface. Despite the promising antibacterial activity, silver nanoparticles were sparsely deposited on the titanium surface, forming aggregates, and probably weakly bonded to the silane-modified titanium surface.

In a previous work, we reported the synthesis and bioactive properties of a nanostructured porous silica coating on titanium surfaces by a combined sol–gel and evaporation-induced self-assembly process [26]. This silica coating with highly ordered sub-10 nm porosity improves titanium osseointegration, speeding up the osteoblasts' adhesive response and promoting the osteogenic differentiation of stem cells; probably due to mechanical stimulus from the nanostructured topography. We hypothesize that an adequate incorporation of AgNPs into this nanoporous silica coating could generate a surface with antimicrobial properties oriented at preventing peri-implant infection. Sol–gel technique offers finer control of the nanostructure [27], as compared to previously reported methods for incorporating AgNPs on titanium surface. Sol–gel coatings enable a more homogeneous distribution and better attachment of AgNPs on the titanium surface. In addition, future studies could lead to the development of a bifunctional coating for dental titanium implants combining both osseointegration and antimicrobial properties.

We have now produced a nanostructured silica coating loaded with AgNPs on titanium surfaces by incorporating the metallic nanoparticles during a combined sol–gel and evaporation-induced self-assembly process. The antibacterial properties of titanium surface modified with the nanostructured coating are evaluated against a representative pathogen of dental peri-implantitis.

2. Materials and methods

2.1. Synthesis of silver nanoparticles

In order to obtain a more biocompatible nanomaterial, silver nanoparticles were synthesized using a green chemistry approach. In this case soluble starch was used as a biocompatible reducing and stabilizing agent. In a typical synthesis, 1 g of soluble starch was added to 100 mL of distiller water and heated in a microwave oven. After complete dissolution, 1 mL of a 2 M aq solution of silver nitrate was added with stirring (2000 ppm AgNP suspension). The reaction mixture was stirred for 2 min and then heated at 70 °C for 55 min in an oil bath.

2.2. Synthesis of silica coatings on a titanium surface

Silica coatings were prepared on sheets of Ti6Al4V titanium alloy (Zimmer Dental Inc.) using the evaporation induced self-assembly (EISA) sol–gel technique [28]. Titanium sheets (15 × 15 × 1 mm) were sanded with silicon carbide paper (800 grit) and cleaned ultrasonically with acetone and ethanol before use. The sol–gel precursor solutions were prepared using both the amphiphilic triblock copolymer Pluronic P123 (P123) (EO20PO70EO20, MW = 5800, Aldrich) and poly(ethylene glycol) (MW = 600, PEG) as pore structure directing agents (SDA). Briefly, 3.7 g of tetraethyl orthosilicate (TEOS 98%, Aldrich) was prehydrolyzed in a solution containing 20 mL of ethanol (95%) acidified with 0.5 mL of 0.5 N HCl (pH 2.0) with vigorous stirring at room temperature for 20 min. After that, 5 mL or 2.5 mL of 2000 ppm AgNP suspension was added to the TEOS solution to obtain silica films with 5 wt.% or 2.5 wt.% AgNP content, respectively. This prehydrolyzed silica/AgNP solution was added to a solution containing 2 g of the SDA dissolved in 20 mL of ethanol. The resulting solution was then submitted to an aging period at room temperature for 24 h with stirring, and films were prepared by slip-coating on the titanium sheets. Pure nanoporous silica coatings (NSC) were prepared as control, using the same procedure described above, but without adding the AgNP suspension to the TEOS solution.

For the slip-coating procedure [26], the titanium sheet was suspended in an inverted position from tweezers attached to a clamp fixed loosely enough to a stand to allow rotation of the tweezers. The polished side was brought in contact with the silica sol. The titanium sheet was kept in this half-immersed position for 20 s, slipped away horizontally by rotating the tweezers, and then dried in a vertical position for 40 s. The silica coatings were kept for 24 h at 35 °C, and then calcined by heating at a rate of 0.5 °C/min to 400 °C, holding that temperature for 4 h to remove the SDA.

2.3. Material characterization

AgNPs were examined by transmission electron microscopy (TEM) in a Philips Tecnai 12 Bio Twin microscope. Specimens were prepared by transferring a small drop of synthesized suspension to carbon-film-coated copper grids. Particle size distribution of the silver nanoparticles was obtained using dynamic light scattering (DLS) with a ZetaPALS instrument (Brookhaven Instruments).

The unmodified and coated titanium surfaces were examined by scanning electron microscopy (SEM) in a Jeol JSM 5410 microscope equipped with energy-dispersive X-ray spectroscopy (EDX). The structural order of the porous silica coatings was analyzed by low angle X-ray diffraction (XRD) within a 2θ range of 0.5–5°. XRD patterns were collected on a Siemens D 5000 diffractometer using CuKα radiation at a scanning speed of 0.2°/min. The porous nanostructure was examined by high resolution transmission electron microscopy (HRTEM) on a FEI-Tecnai G2 F20 S-Twin HRTEM microscope equipped with a Field Emission Gun (FEG) operating at an accelerating voltage of 120 kV. Plan-view film specimens were prepared by removing the silica films from a titanium sheet and suspending them in ethanol. This suspension was then dispersed on a holey carbon film supported by a copper grid.

2.4. Antibacterial activity

The antimicrobial activity of the AgNP/NSC-modified titanium surfaces was tested against *Aggregatibacter actinomycetemcomitans* (serotype b). The strain was grown in BHI broth or agar (Brain Heart Infusion, Oxoid, Wesel, Germany) and incubated in a 5% CO_2 atmosphere at 37 °C for 48 h. From a grown plate, bacteria were transferred to fresh BHI medium to a density equivalent to McFarland 2 standard. Each sterilized titanium disk was placed in 12-well plates, with the coated titanium side facing up. Then, 990 μL of fresh BHI medium and 10 μL of the inoculum were added to each well, and incubated for 1, 2, 4 and 6 days in a 5% CO_2 atmosphere at 37 °C. After the incubation period, antibacterial activity was evaluated by total viable counts of each well. To remove bacteria from the disks we used the protocol reported elsewhere [29] with some modifications. Briefly, after the incubation period