

## In vitro antibacterial activity of porous TiO<sub>2</sub>–Ag composite layers against methicillin-resistant *Staphylococcus aureus*

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### Abstract

The aim of this study was the synthesis of a porous TiO<sub>2</sub>–Ag composite coating and assessment of its in vitro bactericidal activity against methicillin-resistant *Staphylococcus aureus*. The coating was produced by plasma electrolytic oxidation of Ti–6Al–7Nb medical alloy in a calcium acetate/calcium glycerophosphate electrolyte bearing Ag nanoparticles. Following oxidation, the surface of the titanium substrate was converted into the corresponding oxide (TiO<sub>2</sub>) bearing Ca and P species from the electrolyte. In addition, Ag was detected associated with particles present in the oxide layers. The coatings revealed a porous interconnected structure with pores up to 3 μm in size, a threefold increase in roughness and improved wettability relative to the non-oxidized specimens. The composite TiO<sub>2</sub>–Ag coating showed complete killing of methicillin-resistant *S. aureus* within 24 h in all culture conditions, whereas a 1000-fold increase in bacterial numbers was recorded with the ground titanium specimens and the samples oxidized in the absence of Ag nanoparticles.

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### 1. Introduction

Surgical implant procedures, such as partial or total joint arthroplasties, are commonly applied to restore joint function of affected patients. One of the limitations associated with total joint arthroplasties is represented by the implant associated infections. For instance, in the USA the annual infection rate for orthopaedic implants is 4.3% [1]. Such infections may lead to implant failure, revision surgery and even member amputation, all associated with extremely high medical costs in addition to the pain and suffering of the patients.

Approaches to prevent biomaterials associated infections rely first on prevention through the control of envi-

ronmental and personnel contamination. Additionally, strategies aimed at minimizing the incidence of infection through device design are explored. These include the modification of the chemistry and/or topography of the surface of implantable devices to make them resistant to bacterial adhesion, or to incorporate an antibacterial agent that will prevent bacterial colonization of the device upon implantation. Current strategies to inhibit adhesion of bacteria on the implantable devices involve the use of coatings bearing surfactants, proteins, hydrophilic negatively charged polysaccharides (e.g., hyaluronan and heparin) and specific polymers, like polyethylene glycol [2–5]. The addition of antibacterial agents on the surface of implantable devices is achieved by using several coating techniques, i.e., ion implantation (Ag<sup>+</sup>, Cu<sup>2+</sup>, F<sup>+</sup>) [6–8], electrodeposition [9], magnetron co-sputtering [10], anodic oxidation [11], plasma electrolytic oxidation (PEO) [12,13] and sol–gel

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coatings [14]. The primary advantage of these antibacterial coatings is the release of the bactericidal agent directly at the site of implantation, minimizing the risk of reaching concentrations that can cause harmful side reactions to the other parts of the body.

With the increase of microorganisms resistant to multiple antibiotics there is a need for alternative safe and cost-effective antibacterial agents. The antibacterial ability of silver and its compounds is well known. Metallic silver and silver salts have been used as bactericidal agents in silver-impregnated dressings for burn injuries, in polymers loaded with silver salts and as metallic silver coatings [15–17,10,18–21]. However, some limitations were found, like the interfering effect of silver salts and, in the case of bulk metallic silver, the impossibility to continuously release enough concentration of ions [22]. These limitations can be overcome by the use of Ag nanoparticles. Ag nanoparticles have the advantages of a high specific surface area, a high fraction of surface atoms and antibacterial efficacy [22–27].

Anodic oxidation is a technique that electrochemically enhances the native oxide layer present on the surface of several metals, including aluminium, magnesium and titanium [28,29]. The anodic oxidation process on titanium can be performed at voltages either below the dielectric breakdown of the oxide layer or above the breakdown limit, the latter being the PEO process. Most research has been reported on anodic oxides produced below the breakdown limit, when relatively thin amorphous TiO<sub>2</sub> films are formed, mainly for oral implants [30–32]. When the oxidation is performed at potentials above the breakdown limit, oxide layers with increased roughness, interconnected porosity and anatase and/or rutile phase composition may be formed [33]. Currently, the PEO process is applied for biofunctionalization of titanium to create bioactive porous oxide coatings containing Ca and P species, using calcium acetate and calcium glycerophosphate (Ca-GP/CA)-based electrolytes and specific oxidation conditions or post-treatment steps (hydrothermal treatment) [34,35].

The aim of this research was twofold (i) the synthesis of porous TiO<sub>2</sub>-Ag composite coatings on Ti-6Al-7Nb alloy by PEO; (ii) the assessment of in vitro bactericidal activity of the coatings against methicillin-resistant *Staphylococcus aureus*. Synthesis of a bactericidal coating following this approach has not previously been explored.

## 2. Materials and methods

### 2.1. Titanium specimens

The Ti-6Al-7Nb medical alloy supplied in the form of rods (ACNIS International, France) was machined into cylindrical discs with a thickness of 8 mm and a diameter of 20 mm. The discs were ground with 1200 grit paper (Struers, Denmark) using water as lubricating liquid. The samples were ultrasonically cleaned in ethanol, thoroughly

rinsed in deionized water and dried in a stream of compressed air prior to undergoing the PEO process.

### 2.2. Plasma electrolytic oxidation

The process was carried out in a double-wall glass electrolytic cell with a volume of 1000 ml. The electrolyte was prepared from 0.02 M Ca-GP and 0.15 M CA solutions, with and without the addition of 3.0 g l<sup>-1</sup> Ag nanoparticles (Sigma-Aldrich). The average size of Ag particles was measured using a Zetasizer NanoZS and was found to be 37 ± 6 nm. Titanium discs were screwed to an insulated metallic rod and suspended in the centre of the electrolytic cell as anode, surrounded by a cylindrical steel cathode. Cooling of the electrolyte during the oxidation process was performed by water circulation through the electrolytic cell jacket. The temperature of the electrolyte was thereby maintained at 25 ± 3 °C. The agitation of the electrolyte during the oxidation process was maintained at a speed of 500 rpm using a magnetic stirrer.

Oxidation was performed under galvanostatic conditions using a current density of 20 A dm<sup>-2</sup>. The current was applied by means of an AC (50 Hz) power supply type ACS 1500 (ET Power Systems Ltd., UK). The oxidation time was 5 min. The current and voltage transients were recorded during the process at 1 s intervals by a computer interfaced with the power supply through a National Instruments SCXI data acquisition system. After oxidation, the samples were thoroughly cleaned with deionized water and dried with blowing air.

Three different types of specimens were included for investigations, i.e., titanium discs ground with 1200 grit paper (TG), oxidized titanium in Ca-GP/CA electrolyte (TO) and oxidized titanium in Ca-GP/CA electrolyte bearing Ag nanoparticles (TO-Ag).

### 2.3. Surface morphology

The surface morphology was investigated by scanning electron microscopy (SEM) on a JEOL JSM-6500F microscope, using an electron beam energy of 5–20 keV and an emission current of 64 μA. Prior to investigation, the oxidized titanium samples were coated with a uniform carbon layer to ensure good electrical conductivity. Energy dispersive X-ray spectrometry (EDS) analyses were also performed on selected areas.

### 2.4. Chemical and phase composition

The X-ray fluorescence (XRF) analyses were performed with a Philips PW2400 X-ray fluorescence spectrometer. The data were processed by Uniquant v. 5.49 software.

The phase composition of titanium alloy and oxidized specimens was analyzed by X-ray diffraction (XRD). The measurements were carried out on a Bruker-AXS type DX Advance Series 2 diffractometer using Co K $\alpha$  radiation.

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