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# In vitro cytotoxicity evaluation of porous $TiO_2$–Ag antibacterial coatings for human fetal osteoblasts

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ABSTRACT

Implant-associated infections (IAIs) may be prevented by providing antibacterial properties to the implant surface prior to implantation. Using a plasma electrolytic oxidation (PEO) technique, we produced porous $TiO_2$ coatings bearing various concentrations of Ag nanoparticles (Ag NPs) (designated as 0 Ag, 0.3 Ag and 3.0 Ag) on a Ti–6Al–7Nb biomedical alloy. This study investigates the cytotoxicity of these coatings using a human osteoblastic cell line (SV-HFO) and evaluates their bactericidal activity against methicillin-resistant *Staphylococcus aureus* (MRSA). The release of Ag and the total amount of Ag in the coatings were determined using a graphite furnace atomic absorption spectrometry technique (GF-AAS) and flame-AAS, respectively. Cytotoxicity was evaluated using the AlamarBlue assay coupled with the scanning electron microscopy (SEM) observation of seeded cells and by fluorescence microscopy examination of the actin cytoskeleton and nuclei after 48 h of incubation. Antibacterial activity was assessed quantitatively using a direct contact assay. AlamarBlue viability assay, SEM and fluorescence microscopy observation of the SV-HFO cells showed no toxicity for 0 Ag and 0.3 Ag specimens, after 2, 5 and 7 days of culture, while 3.0 Ag surfaces appeared to be extremely cytotoxic. All Ag-bearing surfaces had good antibacterial activity, whereas Ag-free coatings showed an increase in bacterial numbers. Our results show that the 0.3 Ag coatings offer conditions for optimum cell growth next to antibacterial properties, which makes them extremely useful for the development of new antibacterial dental and orthopedic implants.



## 1. Introduction

Biomedical devices (e.g. artificial joints, dental implants and fracture fixation plates) are commonly used in total joint arthroplasties, dental implantation or bone trauma surgeries, improving the quality of life by relieving pain and restoring mobility and function. Despite advanced sterilization techniques, strict surgery rules and systemic antibiotic prophylaxis, implant-associated infections (IAIs) remain a great risk in such surgeries. IAI is a result of bacteria adhesion to the implant, colonization of its surface and subsequent biofilm formation at the implantation site. The biofilm has an extraordinary resistance to antibiotics and furthermore it can promote detaching of individual bacteria into the surrounding tissue and circulatory system, leading to further complications [1]. Once the IAI occurs, it is often impossible to heal without revision surgery, which most of the time requires the replacement of the implant. This devastating complication may lead to large skeletal defects, member amputation and even death. Besides patient trauma, the treatment of such infections incurs huge costs for the healthcare system [2–4].

Preventing bacterial adhesion on the biomedical devices or providing bactericidal activity to the biomedical device itself can be essential strategies to prevent IAI [5]. Therefore, research on surface modification of biomedical alloys to apply/form coatings/layers that kill any adherent and/or surrounding bacteria has garnered significant interest [6]. The unique advantage of these coatings/layers is the ability to provide locally, at the site of implantation, a controlled amount of the antibacterial agent that will prevent bacteria colonization. Furthermore, the local delivery of the antibacterial agents will reduce the risk of toxicity caused by conventional systemic delivery of antibiotics. Ideally, these coatings/layers should not change the structural integrity of the device and maintain the surface biocompatibility with the host tissue.

Previous studies demonstrated the potential of the plasma electrolytic oxidation (PEO) process to produce porous $TiO_2$–Ag antibacterial coatings on Mg and Ti biomedical alloys using electrolytes bearing Ag nanoparticles (Ag NPs) [7,8]. The coatings showed excellent in vitro antibacterial activity against methicillin-resistant

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