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Bactericidal activity and cytotoxicity of a zinc doped PEO titanium coating

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ABSTRACT

Metallic implants are susceptible to bacterial colonization even years after the implantation impairing the osseointegration process. The treatment of a colonized implant is highly demanding, and in most cases implant replacement is the only effective solution. To avoid the bacterial attachment and proliferation, bactericidal coatings are proposed as a long-term prevention tool. Those coatings must assure a bactericidal activity for a long period and cannot induce cytotoxic responses in eukaryotic cells. Among all the bactericidal agents, Zinc is one of the most investigated due to its broad bactericidal activity spectrum and its stimulatory effect on bone formation. The aim of this study is to obtain a titanium oxide coating containing Zinc and evaluate its bactericidal activity, cytotoxicity and ion release profile. The coating was obtained by Plasma Electrolytic Oxidation (PEO) on commercially pure titanium grade 4 at 350 V for 60 s. Samples were divided in two groups; the reference group was obtained in a base electrolyte containing calcium acetate and calcium glycerophosphate (called CaP group). The experimental group had Zinc acetate added as a Zinc source to the base electrolyte (called Zn-CaP group). The surface was characterized by Scanning Electron Microscopy (SEM) and X-ray Photoelectron Spectroscopy (XPS), while the ion dissolution was evaluated by Inductively Coupled Plasma - Atomic Emission Spectroscopy (ICP-AES). The bactericidal activity was determined against *Staphylococcus aureus* by fluorescence microscopy using a live/dead viability kit. The cytotoxicity against eukaryotic cells was evaluated using adipose derived stem cells (ADSC) using the lactate dehydrogenase (LDH) assay. Zinc, Calcium and Phosphorous were incorporated to the titanium oxide coating and no changes on the coating structure and morphology were observed by the addition of Zn to the electrolyte. ICP-AES results show the coatings released Ca, P and Z ions after 28 days of immersion in DI water. The ICP-AES profile suggests the ion release reach an equilibrium state after 7 days of immersion. The Zn-CaP coating presented bactericidal activity against *S. aureus*, showing a higher number of dead bacteria after 6 h of incubation and a lower number of living bacteria after 24 h compared to the CaP group. No cytotoxic effect was observed against ADSC by the presence of Zn on the coating, indicating the Zn-CaP coating has a potential to prevent bacterial colonization in metallic implants.

1. Introduction

Endosseous implants are susceptible to be colonized by bacteria even years after the implantation, when endogenous bacteria may migrate to the prosthesis surface [1]. This bacterial contamination impairs the osseointegration process leading to the implant failure, where the implant replacement is the only effective solution [2–4]. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are one of the most common colonizers strains on orthopedic and dental implants. Those bacteria form biofilms, an organized bacteria community involved by an extracellular matrix who protects the microorganisms from the host immune system and the antibiotic action, being the prosthesis removal the only effective treatment [5, 6]. Despite all the prophylactic measures bacterial infection in implants still have an expressive mortality rate, Lora-Tamayo et al. showed 7% of deaths among patients infected just with *S. aureus* in total knee arthroplasty [7]. To avoid the bacterial attachment and proliferation, bactericidal coatings that do not induce cytotoxic responses in eukaryotic cells are proposed as a long-term prevention tool.

Multifunctional coatings that prevent the bacterial adhesion and improve the bone growth by the presence of osteoinductor elements can be obtained by plasma electrolytic oxidation (PEO). This electrochemical technique allows the incorporation of bactericidal elements along with Ca and P on titanium surface. Ishizawa et al. showed for the first time the possibility to incorporate Ca and P on the TiO_2 structure by PEO; those coatings reduce the Ti^{4+} ion release, increase osteoblast

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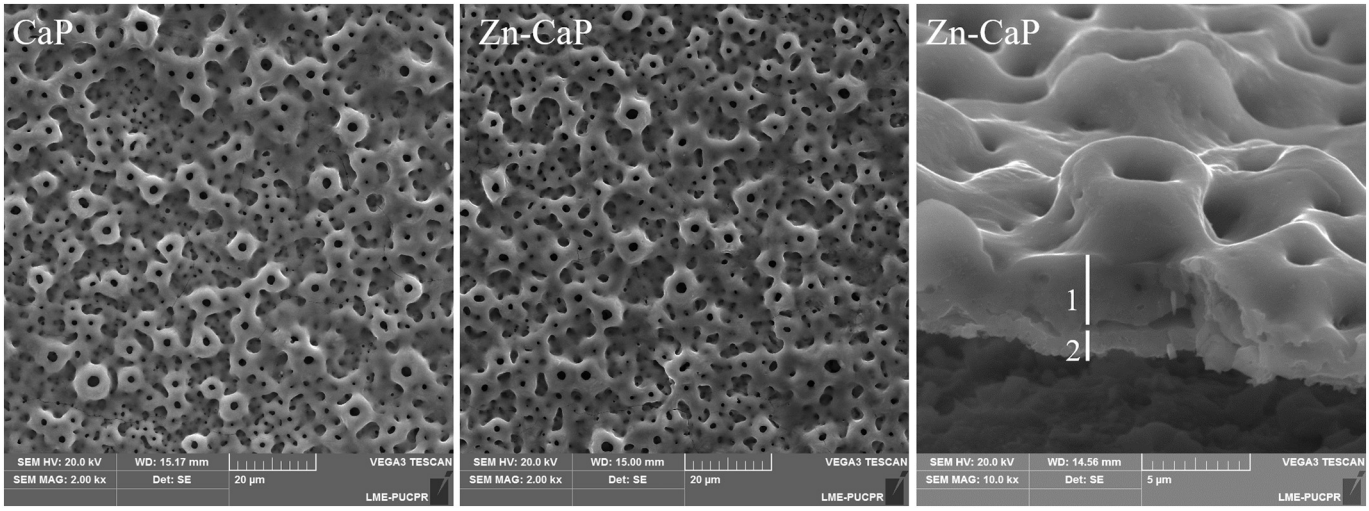

Fig. 1. Top view SEM images of the CaP and Zn-CaP coatings and cross-sectional image of the Zn-CaP coating. Number 1 outlines the outmost porous layer, while number 2 indicates the inner dense layer, both characteristic from coatings obtained by PEO.

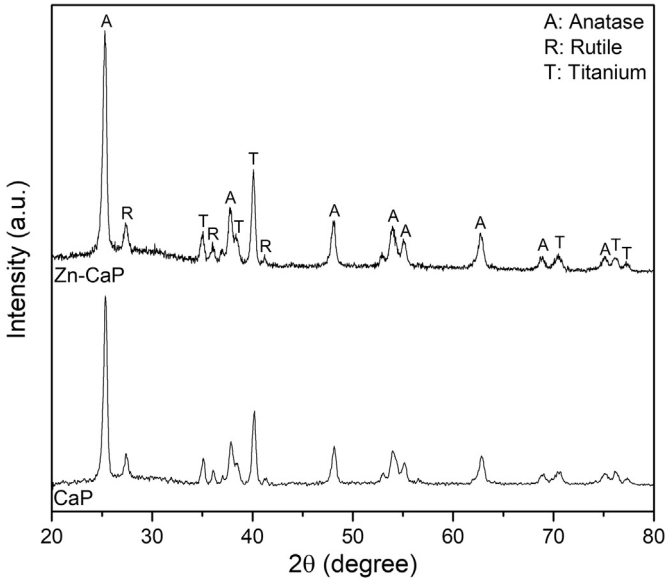

Fig. 2. XRD pattern of the CaP and Zn-CaP coatings. The XRD peaks were indexed by the following PDFs: Anatase [01-078-2486], Rutile [00-021-1276] and Titanium [01-089-3073].

Table 1
Elemental composition of the coatings determined by XPS.

	Element (% at)				
	Ti	O	Ca	P	Zn
CaP	6.3	67.4	12.7	13.6	–
Zn-CaP	3.9	66.7	8.4	11.3	9.7

adhesion and proliferation *in vitro*, and improve the osseointegration *in vivo* [8–11].

Zinc is an oligoelement present in abundance on the bone tissue [12]. Zinc influences the bone growth and mineralization and its deprivation impairs the bone metabolism [13]. Moonga et al. showed zinc is also involved in the control of bone resorption *in vitro*, by inhibiting the osteoclasts action [14]. Besides playing an important role on bone tissue, zinc is also essential for the function of > 300 enzymes, proteins, it regulates DNA synthesis, influences hormonal regulation and cell division [15–17].

Zinc has been extensively investigated as a broad spectrum bactericidal agent specially under the zinc oxide form [18]. Zinc oxide may act by the dissolution of zinc ions or the generation of reactive oxygen species (ROS), capable of disrupting the bacteria membrane [19]. Additionally, zinc oxide nanoparticles and structures may interact and attach to the cell wall, impairing the membrane function and possibly disrupting it [20]. Zinc ions are believed to compete with di-valent

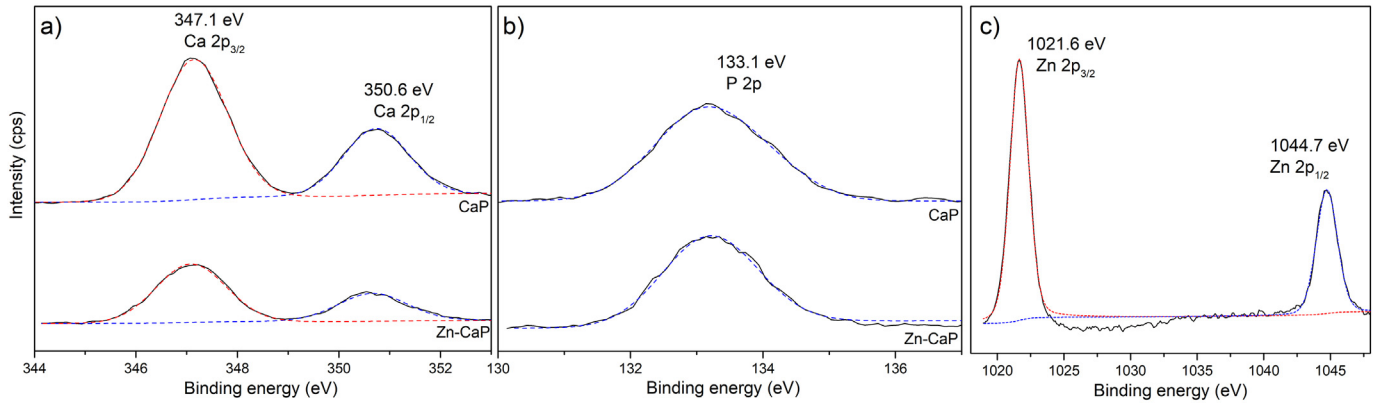

Fig. 3. Coatings high-resolution XPS spectra of a) Ca 2p, b) P 2p and c) Zn 2p.