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Ti surface modification with a natural antioxidant and antimicrobial agent

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ABSTRACT

Ti surface was modified with a natural antioxidant and antimicrobial agent, torularhodin, by its incorporation onto a Ti/TiO_2 substrate via anodization process. This natural antioxidant and antimicrobial agent can be produced from waste materials, as an alternative to synthetic antimicrobial compounds.
Scanning electron microscopy (SEM), Structural Fourier Transform Infrared (FTIR) spectroscopy, contact angle (CA) measurements, and atomic force microscopy (AFM) are employed to assess the surface properties of resulting coatings.
Surface and electrochemical analyses highlighted that torularhodin was embedded in the TiO_2 shielding layer, improving the antibacterial activity with about 65% compared to Ti/TiO_2. Several species of microorganisms: *Staphylococcus aureus*, *Candida albicans* and *Aspergillus fumigatus* were utilized to test the antimicrobial effect. Hemolysis and cytotoxicity tests were additionally performed, showing good results.
This nanostructured Ti surface based on a natural antioxidant and antibacterial agent is a promising biomaterial for medical device purposes.

1. Introduction

Titanium (Ti) based materials are the most common and successful biomaterials used for implantable medical devices (MD), due to their biocompatibility and because they do not cause in vivo inflammatory response [1,2]. For implants, rapid and stable bone tissue integration is very important. For this purpose, the surface properties became key factors. Considering that the cell surface is structured at the nanoscale level (receptors and filopodia) and in vivo cells live inside an extracellular matrix with nanoscale collagen fibrils, they are able to respond to nanostructures [3]. Since the first level of bone structural hierarchy is also in the nanometer domain, researchers have become more interested in creating nano-modified biomaterials [4]. These provide surface and/or chemical properties closer to native bone [2]. The growth of titanium oxide (TiO_2) nanotubes with many —OH groups on the surface (high surface energy), represents a way of surface nano-structuring. This may lead to improved biomimetic apatite precipitation, osteoblast adhesion, cytoskeleton organization, proliferation and phenotypic expression [4,5]. TiO_2 nanotubes can also determine bladder stent urothelialization and skin growth, reduce inflammation and decrease bacterial functions [5].

The surface of biomaterials used for MD is important not only for the surrounding tissue cells. Tissue cells have to compete with pathogens [3]. If initial attachment and permanent adherence of pathogens on MD occur first, there is a danger of infection [6]. Treating these infections is more difficult once pathogens are already adhered to the implant surface and a biofilm is formed [5]. The predominant nosocomial fungal pathogens include *Aspergillus* spp., *Candida* spp., *Fusarium* spp., and other molds, including *Mucorales* spp. [7]. *Candida albicans* represents a major threat to vulnerable patients. In hospital and clinics, *Candida* species may cause invasive infections of immune compromised patients. *Candida* cells may enter the blood by direct penetration from epithelial tissues, during or after the surgery and polytrauma, or may spread from biofilms produced on MD [7–9]. Also, infections with mold pathogens have emerged as an increasing risk faced by patients; the *Aspergillus* family accounts for most of these infections and in particular, *Aspergillus fumigatus* is the most airborne-pathogenic fungus and is capable of living under extensive environmental stress. The improvement in transplant medicine is often complicated by the threat of invasive aspergillosis [10–12]. On the other hand, bacteria cause many forms of infection. *Staphylococcus aureus* is responsible for hospital acquired infection of surgical wounds and, together with *Staphylococcus epidermidis*, causes infections associated with indwelling medical devices [13,14].

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Taking into account the above description is important to find alternative ways to treat MD related infections [5]. One way is to use synthetic compounds. Polyethylene glycol (PEG) was found to be a compound with antibacterial properties. Results have shown a synergistic antibacterial effect of PEG and TiO_2 nanotubes. The antibacterial effect of the Ti substrate was found to increase by nanotube formation and polymer incorporation in the shielding layer during anodization [15]. Another way to create surfaces resistant to microbial adhesion and colonization is incorporation of natural compounds on MD [16, 17]. Natural antimicrobial agents have attracted the attention of many researchers, as an alternative to synthetic antimicrobial compounds. The antimicrobial effect of natural agents is due to the ability of these compounds to interfere with the metabolism of pathogens similar with those of synthetic antimicrobial agents [18–21]. Cells of *Rhodotorula rubra* and *Rhodotorula mucilaginosa* yeasts produce pigments like carotenoids to protect themselves from the visible light effect and near ultraviolet light [22–25]. Some of these natural pigments have a great antioxidant and antimicrobial potential, torularhodin (3′,4′-didehydro-β-ψ-caroten-16-oic acid) being one of them [22–24]. An advantage of using those natural antioxidant and antimicrobial agents is that they can be produced from waste material, reducing environmental pollution. Their production is independent of season and geographical conditions, and leads with controllable yield [26].

The carotenoid biosynthesis pathway includes increasing oxidation levels from ψ-carotene to torulene. In the final step, torulene is hydroxylated and oxidized to torularhodin by mixed function oxidase [23]. Thus, torularhodin is the carotenoid at the highest oxidation level in biosynthesis. Moreover, it was discovered that during exposure to air or in other situations, carotenoids can spontaneously add oxygen. By oxygen addition, they form potentially bioactive, oxygen-rich, carotenoid–oxygen copolymers. In vitro results of a Polymerase Chain Reaction (PCR) gene expression test and in vivo experiments showed that also these spontaneously formed copolymers are biologically active [27].

Taking into account the last tendencies to develop new antibacterial surfaces using natural compounds, the aim of this paper was to create and to evaluate a nano-structured Ti surface modified with a natural antioxidant and antimicrobial compound for MD purposes. Such a surface was created using one-step anodization, a simple and costless method.

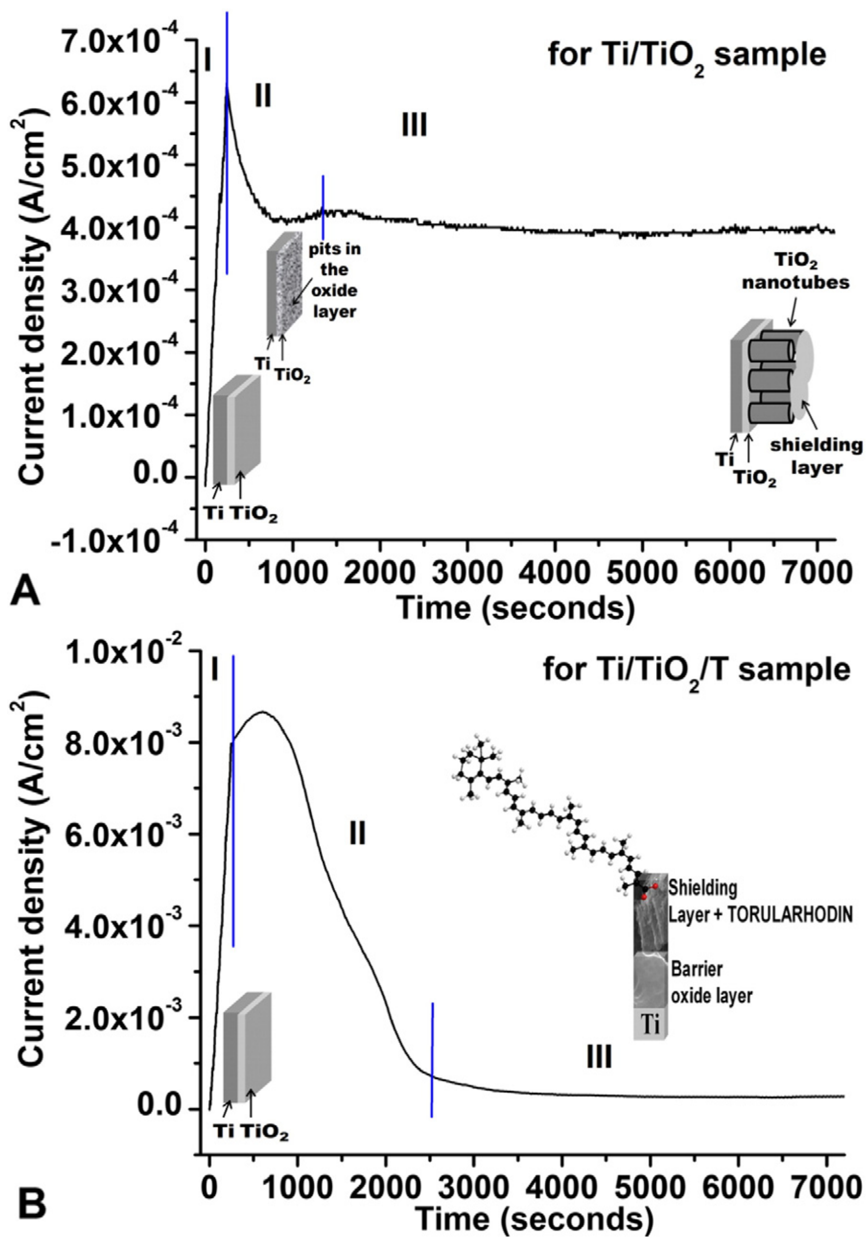

Fig. 1. Evolution of current density in time during anodization for Ti/TiO_2 and $Ti/TiO_2/T$.