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Advanced biopolymer-coated drug-releasing titania nanotubes (TNTs) implants with simultaneously enhanced osteoblast adhesion and antibacterial properties

Tushar Kumeria[a,1], Htwe Mon[b,1], Moom Sinn Aw[a,1], Karan Gulati[a], Abel Santos[a], Hans J. Griesser[c,**], Dusan Losic[a,*]

[a] School of Chemical Engineering, The University of Adelaide, Adelaide, SA 5005, Australia
[b] Sansom Institute for Health Research, School of Pharmacy and Medical Sciences, University of South Australia, Frome Road, Adelaide, SA 5000, Australia
[c] Mawson Institute, University of South Australia, Mawson Lakes, SA 5095, Australia

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ABSTRACT

Here, we report on the development of advanced biopolymer-coated drug-releasing implants based on titanium (Ti) featuring titania nanotubes (TNTs) on its surface. These TNT arrays were fabricated on the Ti surface by electrochemical anodization, followed by the loading and release of a model antibiotic drug, gentamicin. The osteoblastic adhesion and antibacterial properties of these TNT–Ti samples are significantly improved by loading antibacterial payloads inside the nanotubes and modifying their surface with two biopolymer coatings (PLGA and chitosan). The improved osteoblast adhesion and antibacterial properties of these drug-releasing TNT–Ti samples are confirmed by the adhesion and proliferation studies of osteoblasts and model Gram-positive bacteria (*Staphylococcus epidermidis*). The adhesion of these cells on TNT–Ti samples is monitored by fluorescence and scanning electron microscopies. Results reveal the ability of these biopolymer-coated drug-releasing TNT–Ti substrates to promote osteoblast adhesion and proliferation, while effectively preventing bacterial colonization by impeding their proliferation and biofilm formation. The proposed approach could overcome inherent problems associated with bacterial infections on Ti-based implants, simultaneously enabling the development of orthopedic implants with enhanced and synergistic antibacterial functionalities and bone cell promotion.

1. Introduction

A wide variety of orthopedic devices are implanted every year in millions of patients suffering from bone-related injuries or diseases, including fracture fixations, artificial joints, and prostheses [1,2]. The benefits obtained from implants can be numerous, although they are susceptible to multiple problems such as infections, lack of integration, inflammations, and total rejection by the host body. Bacterial infections are the main cause of implant failures (ca. 10%) [1,2]. These are typically caused by adhesion, colonization, and biofilm formation by bacteria colonies on the implant surface after implantation [3]. Bacterial infections require complicated and costly clinical treatments (i.e. up to US $18,000 each) and can lead to morbidity and mortality in patients [4–8]. Therefore, considering the large number of patients requiring orthopedic implants, alternative approaches are urgently needed to prevent implants from bacterial infections. Current therapies to treat bacterial infections are based on prolonged and repetitive systemic administration of therapeutics. Intravenous delivery of antibiotics is the standard treatment, which is usually prescribed for 6–8 weeks upon surgical implantation [9]. However, these therapies are not always effective due to bacterial colonies forming biofilms on the implant surface. These biofilms provide bacteria with protection against the host immune system and also reduce the efficacy of systemic therapy since they act as natural barriers that hinder the diffusion of administered antibiotics [3,10,11].

Therefore, the use of biofilm-disrupting agents and localized administration of antibiotics have become one of the most promising alternatives to address this problem. In this regard, a number of strategies have been developed so far, including bioactive sol–gel glass, injectable polymers, peptides, or protein-based surface

* Corresponding author. Tel.: +61 8 8313 1535; fax: +61 8 8303 4373.
** Corresponding author. Tel.: +61 8 830 23703; fax: +61 8 830 25639.
E-mail addresses: hans.griesser@unisa.edu.au (H.J. Griesser), dusan.losic@adelaide.edu.au (D. Losic).
[1] These authors contributed equally to this work.

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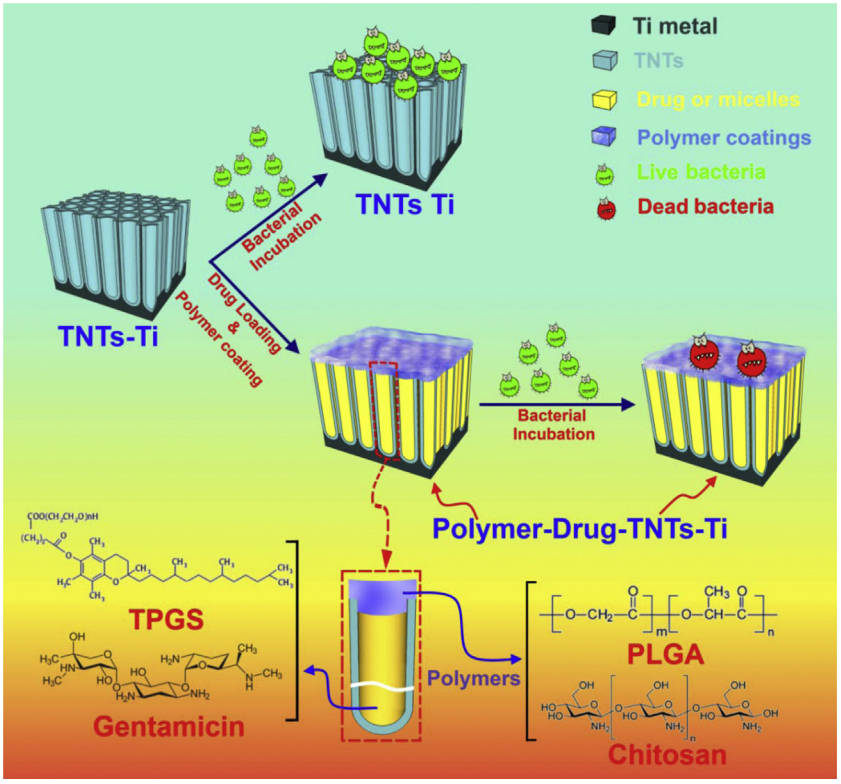
coatings, poly(methyl methacrylate) (PMMA) beads, and bone cements [12–18]. Nevertheless, these alternatives present some inherent shortcomings, such as toxicity, removal complications due to cemented revisions, exothermic polymerization leading to thermal injury to tissue, protein denaturation that inhibits growth factors, insufficient antibiotics dosage for direct exchange, and low fracture toughness, low mechanical strength, and load bearing capacity [19–23]. In the case of orthopedic implants based on titanium (Ti), one of the most promising approaches to overcome these limitations is to generate a layer of titania nanotubes (TNTs) on the implant surface via electrochemical anodization [24,25]. This electrochemical approach is based on a simple, scalable, and cost-effective industrial process that can be applied on currently used medical implants based on Ti and Ti alloys of a variety of forms and shapes (including screws, plates, nails, and wires) [26]. TNT layers have a high surface area and loading capacity, excellent chemical inertness, mechanical robustness, good biocompatibility, tunable nanotube dimensions, and surface chemistry. Furthermore, the duration and kinetics of drug released from TNT structures can be controlled ad-lib by either engineering the nanotubes' dimensions or modifying their surface chemistry, and alternatively, by incorporating polymeric coatings on the TNT–Ti implant surface through plasma polymerization or dip-coating [27–31]. For these reasons, drug-eluting TNT–Ti implants for localized delivery of therapeutics (e.g. anti-inflammatory, antibiotics, anticancer drugs, and proteins) have attracted huge attention during recent years [9,24,25,27–29,31]. However, their potential applicability as an active nanostructured surface for antibacterial proliferation and biofilm formation has not been adequately explored yet. So far, only a few studies have reported on the antibacterial properties of TNTs loaded with antibacterial agents (i.e. antibiotics and silver) [32–38]. Therefore, given the widespread use of Ti-based implants in orthopedics and their susceptibility to clinical complications associated with bacterial biofilms, more in-depth studies should be carried out in order to fully exploit the potential applicability of TNTs in orthopedics. In particular, studies showing combined effect of antibacterial and osteogenic properties of drug-releasing TNT-based Ti implants are scarce. This is a fundamental aspect to translate TNT–Ti implants into real clinical therapies, where antibacterial properties and bone cell promotion/integration are recognized as the most critical factors.

Thus, our study is aimed at enhancing the therapeutic performance of drug-releasing TNT–Ti implants in terms of osteoblast adhesion and antibacterial properties. To this end, we have developed Ti implants featuring two active layers, namely: (i) TNT layers loaded with an antibacterial drug and (ii) a coating based on specific biopolymers with improved osteoblast adhesion and antibacterial properties. This concept is graphically summarized in Scheme 1. First, TNTs were synthesized on flat Ti foils by means of electrochemical anodization and loaded with a model antibacterial drug. The top surface of the Ti plates was subsequently coated with a biopolymer film. This coating enables a controlled and extended release of the drug from the nanotubes and, at the same time, improves bone cell adhesion, promotes implant integration, and prevents the formation of bacterial biofilms. Gentamicin sulfate, an aminoglycoside antibiotic commonly prescribed against implant infections, was selected as a model drug due to its wide bactericidal spectrum [39]. To increase their bioavailability and extend their release in a physiological environment, gentamicin molecules were encapsulated in a micelle polymer nanocarrier, namely d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) prior to their loading into TNTs. Two bioactive polymers, i.e. polylactic-co-glycolic acid (PLGA) and chitosan were investigated as functional coatings on gentamicin-loaded TNT–Ti samples. The osteoblast adhesion and antibacterial properties of the resulting TNT–Ti samples were assessed through a



Scheme 1. Schematic diagram summarizing the ability to impart effective antibacterial properties to the proposed TNT–Ti drug-releasing implants combining drug/micelle (drug/micelle model: gentamicin/TPGS) payloads and biopolymer coatings (PLGA and chitosan). The lower part of the scheme shows the chemical structure of the drug/micelle payloads and the two biopolymers used for coating the surface of TNT–Ti samples. PLGA: polylactic-*co*-glycolic acid.