



Full length article

Strontium (Sr) and silver (Ag) loaded nanotubular structures with combined osteoinductive and antimicrobial activities



Hao Cheng^{a,b,c}, Wei Xiong^a, Zhong Fang^a, Hanfeng Guan^a, Wei Wu^a, Yong Li^a, Yong Zhang^a, Mario Moisés Alvarez^{b,c,d,e}, Biao Gao^f, Kaifu Huo^f, Jiangwen Xu^f, Na Xu^f, Chengcheng Zhang^f, Jijiang Fu^{f,*}, Ali Khademhosseini^{b,c,e,g,h,i,*}, Feng Li^{a,*}

^aOrthopaedic Department, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1095 Jiefang Avenue, Wuhan 430030, China

^bBiomaterials Innovation Research Center, Division of Biomedical Engineering, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston 02139, MA, USA

^cHarvard-Massachusetts Institute of Technology Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge 02139, MA, USA

^dCentro de Biotecnología-FEMSA, Tecnológico de Monterrey, Ave. Eugenio Garza Sada 2501 Sur Col. Tecnológico, CP 64849 Monterrey, Nuevo León, Mexico

^eMicrosystems Technologies Laboratories, Massachusetts Institute of Technology, Cambridge 02139, MA, USA

^fThe State Key Laboratory of Refractories and Metallurgy, School of Materials and Metallurgy, Wuhan University of Science and Technology, Wuhan 430081, China

^gWyss Institute for Biologically Inspired Engineering, Harvard University, Boston 02115, MA, USA

^hDepartment of Bioindustrial Technologies, College of Animal Bioscience and Technology, Konkuk University, Hwayang-dong, Gwangjin-gu, Seoul 143-701, Republic of Korea

ⁱDepartment of Physics, King Abdulaziz University, Jeddah 21569, Saudi Arabia

ARTICLE INFO

Article history:

Received 12 July 2015

Received in revised form 16 November 2015

Accepted 19 November 2015

Available online 2 December 2015

Keywords:

Strontium

Silver

Titanium

Nanotubular structures

Antibacterial

Osteogenic

ABSTRACT

Two frequent problems are associated with the titanium surfaces of bone/dental implants: lack of native tissue integration and associated infection. These problems have prompted a significant body of research regarding the modification of these surfaces. The present study describes a hydrothermal treatment for the fabrication of strontium (Sr) and silver (Ag) loaded nanotubular structures with different tube diameters on titanium surfaces. The Sr loading from a Sr(OH)₂ solution was regulated by the size of the inner diameter of the titanium nanotubes (NT) (30 nm or 80 nm, formed at 10 V or 40 V, respectively). The quantity of Ag was adjusted by immersing the samples in 1.5 or 2.0 M AgNO₃ solutions. Sr and Ag were released in a controllable and prolonged manner from the NT-Ag,Sr samples, with negligible cytotoxicity. Prominent antibacterial activity was observed due to the release of Ag. Sr incorporation enhanced the initial cell adhesion, migration, and proliferation of preosteoblast MC3T3-E1 cells. Sr release also up-regulated the expression of osteogenic genes and induced mineralization, as suggested by the presence of more mineralized calcium nodules in cells cultured on NT-Ag,Sr surfaces. *In vivo* experiments showed that the Sr-loaded samples accelerated the formation of new bone in both osteoporosis and bone defect models, as confirmed by X-ray, Micro-CT evaluation, and histomorphometric analysis of rats implanted with NT-Ag,Sr samples. The antibacterial activity and outstanding osteogenic properties of NT-Ag,Sr samples highlight their excellent potential for use in clinical applications.

Statement of Significance

Two frequent problems associated with Ti surfaces, widely used in orthopedic and dental arenas, are their lack of native tissue integration and risk of infection.

We describe a novel approach for the fabrication of strontium (Sr) and silver (Ag) loaded nanotubular structures on titanium surfaces. A relevant aspect of this work is the demonstration of long-lasting and controllable Ag release, leading to excellent antibacterial and anti-adherent properties against methicillin-resistant *Staphylococcus aureus* (MRSA), and Gram-negative bacteria such as *Escherichia coli*.

* Corresponding authors at: Biomaterials Innovation Research Center, Division of Biomedical Engineering, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston 02139, MA, USA (A. Khademhosseini). The State Key Laboratory of Refractories and Metallurgy, School of Materials and Metallurgy, Wuhan University of Science and Technology, Wuhan 430081, China (J. Fu). Orthopaedic Department, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1095 Jiefang Avenue, Wuhan Avenue, Wuhan 430030, China (F. Li).

E-mail addresses: fujijiang@wust.edu.cn (J. Fu), alikh@rics.bwh.harvard.edu (A. Khademhosseini), lifengmd@hust.edu.cn (F. Li).

The extended release of Sr accelerates the filling of bone defects by improving the repair of damaged cortical bone and increasing trabecular bone microarchitecture. Our results highlight the potential of Sr and Ag loaded nanotubular structures for use in clinical applications.

© 2015 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.

1. Introduction

Titanium (Ti) and its alloys are frequently the materials of choice for orthopedic and dental implants due to their biocompatibility, chemical stability, and mechanical properties [1,2]. However, the use of Ti implants still presents two important challenges: lack of native tissue integration and high incidence of associated infection [3]. Deficient bonding of Ti implants to bone tissues often results in the loosening or failure of the implant [3]. Postoperative infection, in turn, is a frequent and severe complication in orthopedic surgery—especially infections caused by antibiotic-resistant or gram-negative bacteria [4,5]. Severe postoperative infection often necessitates repeated surgeries and/or implant removal with the consequent extended hospitalization and cost [4]. These problems are exacerbated in patients suffering from bone healing problems associated with diabetes, aging, or osteoporosis [6,7].

Different strategies have been attempted to alleviate these issues. The Ti surfaces have been treated with antibacterial or anti-adhesive materials to inhibit bacterial attachment and proliferation [5–9]. In addition, molecules such as bone morphogenetic protein-2 (BMP-2), vascular endothelial growth factor (VEGF), and RGD have also been incorporated into Ti implant surfaces to impart osteogenesis and enhance osteointegration [10–13]. Nonetheless, most coatings tested thus far lack the desired stability, and their fabrication process is often elaborated, and time consuming. To our knowledge, antibacterial and osteogenic activity—two key properties for implants—have not been integrated together in any of the existing Ti implants. A simple strategy for Ti surface engineering that would impart both stable osteogenesis and sustained antibacterial activity, for example by devising a controlled delivery/release system, would have great scientific and clinical significance.

Modification of the surface nanotopography by inclusion of a TiO₂ nanotube (NTs) coating could result in an excellent delivery platform. TiO₂-NTs could be used as a scaffold to allow for the slow release of the therapeutic agents [4,14,15], particularly inorganic bioactive elements such as Ag, Sr, and other trace elements. Recently, TiO₂ NTs have been shown to improve cell adhesion, elongation, differentiation, and migration due to their structural similarity to bone collagen fibrils [16–20].

In this contribution, we explore the loading and controlled delivery of Ag and Sr. Ag is a well-recognized and efficient antimicrobial agent that inhibits or kills a vast spectrum of microorganisms, including antibiotic-resistant and gram-negative bacteria. Ag has additional advantageous properties, such as stability under physiological conditions and low cytotoxicity [21–28]. The biofilm preventing properties of different Ag-containing materials have been investigated; in particular, Ag ions exhibit the highest antibacterial activity among different Ag forms [29–32]. However, controlling Ag dosage/delivery to obtain consistent therapeutic scores continues to be a challenge. Sr stimulates bone formation and inhibits osteoclastic activity. This element has been introduced into clinical practice in the form of oral strontium ranelate as a widely used prescription against osteoporosis [17,18]. Oral administration of Sr enhances bone to implant fixation in naive and osteoporotic rats [33,34]. However, a constant *in situ* Sr release directly at the implant-tissue interface is more effective, as it simultaneously stimulates bone formation minimizing the side effects potentially related to high Sr oral doses [15]. The incorporation of Sr into

osteogenic materials, including bioactive glasses and calcium phosphates, has been reported [35–39], but the inclusion of increasing amounts of Sr appreciably changed the solubility of calcium phosphates and bioactivity of the bio glasses [40]. The development of a more efficient delivery platform for Sr—one that achieves a long-lasting and controlled release at a reasonably constant rate—would be clinically useful. Sr and Ag have been incorporated individually into TiO₂ nanotubes. The resulting coatings demonstrated remarkable antibacterial properties and osteogenesis activity in our previous studies [4,41]. Here, a nanotubular structure loaded with both Ag and Sr (NT-Ag.Sr) was fabricated on Ti implants by treating the NTs successively with Sr(OH)₂ and AgNO₃ solutions in a controlled manner. We prepared a set of NT and NT-Ag.Sr implants with different Sr and Ag loadings in order to determine whether an optimal quantity of Sr and Ag could be incorporated into an NT of the desired diameter to provide reliable controlled release of Ag and Sr. This controlled release from the NT-Ag.Sr coatings would be expected to expedite osteointegration and new bone formation both in an osteoporosis condition and a bone defect model, while simultaneously showing long-term antibacterial properties.

2. Materials and methods

2.1. Fabrication of nanotubular arrays loaded with Ag and Sr

Titanium foils (average size of 10.0 mm × 10.0 mm × 1.0 mm) and titanium rods (diameter: 1.0 mm; length: 12 mm) with a purity of 99.7% (Aldrich, USA) were polished using SiC (silicon carbide) sandpapers. The foils were ultrasonically cleaned for 10 min at a frequency of 100 Hz (successively with acetone, ethanol (>99.7%), and deionized water (DI)) and anodized. The Ti foil was used as the anode and graphite foil as the cathode. Electrodes were placed 1 cm apart. The electrolyte consisted in an ethylene glycol solution that contained 5 vol. % DI water and 0.5 wt. % ammonium fluoride (NH₄F). Anodizing was conducted under direct current (DC) at two different voltage/time conditions (10 V for 1 h) and (40 V for 40 min) using a power supply (IT6834, ITECH, China). Samples obtained using these protocols are subsequently referred to as NT₁₀ and NT₄₀, respectively. The anodized samples were rinsed with DI water, air dried, and hydrothermally treated for 3 h at 200 °C in 40 ml of 0.02 M Sr(OH)₂ solution (pH = 12.2) in a 60 mL-Teflon-lined autoclave to fabricate the Sr-incorporated NTs (denoted as NT₁₀-Sr₃ and NT₄₀-Sr₃). The samples were then rinsed with 1 M HCl to eliminate the residual Sr(OH)₂. The Sr-incorporated NTs samples (NT₁₀-Sr₃ and NT₄₀-Sr₃) were then soaked in 1.5 M or 2.0 M AgNO₃ solution (pH = 5.8) at room temperature for 10 min, rinsed with DI water, and UV irradiated for 10 min using a high-pressure Hg lamp. The Ag-loading was repeated twice. The resulting materials were denoted as NT₁₀-Ag_{1.5}Sr₃, NT₁₀-Ag_{2.0}Sr₃, NT₄₀-Ag_{1.5}Sr₃, and NT₄₀-Ag_{2.0}Sr₃. Samples of these materials were generically referred to as NT-Ag.Sr.

We characterized the surface topography of the NT-Ag.Sr specimens using field-emission scanning electron microscopy (FE-SEM) (FEI Nova 400 Nano) and analyzed their crystalline structure by X-ray diffraction (XRD) (Philips X0 Pert Pro); X-ray photoelectron spectroscopy (XPS) (ESCALABMK-II) and transmission electron microscopy (HRTEM, TECNAI) were used to analyze the surface chemical composition of the samples.

ID	Title	Pages
161	Strontium (Sr) and silver (Ag) loaded nanotubular structures with combined osteoinductive and antimicrobial activities	13

Download Full-Text Now



<http://fulltext.study/article/161>



-  Categorized Journals
Thousands of scientific journals broken down into different categories to simplify your search
-  Full-Text Access
The full-text version of all the articles are available for you to purchase at the lowest price
-  Free Downloadable Articles
In each journal some of the articles are available to download for free
-  Free PDF Preview
A preview of the first 2 pages of each article is available for you to download for free

<http://FullText.Study>