



Antibacterial and cytocompatible nanotextured Ti surface incorporating silver via single step hydrothermal processing



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ABSTRACT

Nanosurface modification of Titanium (Ti) implants and prosthesis is proved to enhance osseointegration at the tissue–implant interface. However, many of these products lack adequate antibacterial capability, which leads to implant loosening. As a curative strategy, in this study, nanotextured Ti substrates embedded with silver nanoparticles were developed through a single step hydrothermal processing in an alkaline medium containing silver nitrate at different concentrations (15, 30 and 75 μM). Scanning electron micrographs revealed a non-periodically oriented nanoleafy structure on Ti (TNL) decorated with Ag nanoparticles (nanoAg), which was verified by XPS, XRD and EDS analysis. This TNLAg substrate proved to be mechanically stable upon nanoindentation and nanoscratch tests. Silver ions at detectable levels were released for a period of ~28 days only from substrates incorporating higher nanoAg content. The samples demonstrated antibacterial activity towards both *Escherichia coli* and *Staphylococcus aureus*, with a more favorable response to the former. Simultaneously, Ti substrates incorporating nanoAg at all concentrations supported the viability, proliferation and osteogenic differentiation of mesenchymal stem cells. Overall, nanoAg incorporation into surface modified Ti via a simple one-step thermochemical method is a favorable strategy for producing implants with dual characteristics of antibacterial activity and cell compatibility.

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1. Introduction

Titanium (Ti) implants are widely used for orthopedic applications because of its superior mechanical properties, resistance to corrosion, and biocompatibility [1,2]. However, bacterial infection is one of the major challenges associated with Ti implants [3]. *Staphylococcus aureus* is the leading etiologic agent responsible for orthopedic implant infections, and account together for two out of three infection isolates [3,4]. These bacteria form biofilm, which are resistant to host defense mechanisms or antibiotics [5,6]. All these can lead to implant failure, thus necessitating revision surgery or even amputation [6]. To address this limitation, researchers have rendered the metallic surface resistant to bacteria through the use of various antibacterial agents such as silver and zinc oxide nanoparticles [7,8]. Amongst them, silver has a broad antimicrobial spectrum against Gram positive and Gram negative bacteria, including resistant strains [9,10] and is also known for their potent antibiofilm properties [11]. Nevertheless, high concentrations of silver may generate potential toxicity to mammalian cells [12,13]. Hence, to provide an implant surface that promotes mammalian cell adhesion,

while concurrently reduce bacterial colonization, appropriate strategies have to be adopted for incorporating optimal silver concentration on the material surface.

Recent reports have established that nanoscale modifications of Ti surface using physicochemical, morphological and biochemical approaches result in higher bone to implant contact ratio and improved osseointegration [14,15]. These nanotopographical cues typically mimic the native bone architecture at the nanoscale [16], and provide enhanced adsorption of RGD (arginine-glycine-aspartate) rich extracellular matrix proteins (ECM), thereby promoting favorable cellular response [15]. To impart the added functionality of antibacterial effects, various methods have been proposed to incorporate silver nanoparticles on to the nanostructured implants. These include techniques such as UV light-irradiation in silver nitrate solution [17], magnetron sputtering of Ag [18], silver plasma immersion ion implantation [19], and heating pre-heated Ti metal in silver nitrate solution [20]. Majority of these studies thus far have utilized a protracted two-step process of silver deposition on to titanium substrates. However, it is always desirable to adopt simplistic protocols for developing surface modified implants, enabling an easy clinical translation.

In this study, we propose a simple, single-step and cost-effective approach of hydrothermal processing of metallic Ti in alkaline pH containing silver salt to generate nanotextured titania embedded with silver nanoparticles. Our group had earlier demonstrated that a specific non-periodic nanoleafy topography generated through hydrothermal

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treatment of Ti exhibited good osteogenic differentiation and osseointegration *in vitro* [21,15] and *in vivo* [15]. Herein, this nanoleafy Ti surface is rendered antibacterial and cell compatible by using different concentrations (15 μM , 30 μM and 75 μM) of silver salt added to the alkaline medium during hydrothermal processing. The modified surfaces were tested against both Gram positive (*S. aureus*) and Gram negative (*E. coli*) strains as well as osteogenic induced mesenchymal stem cells (MSCs) *in vitro*.

2. Materials and methods

2.1. Fabrication of nanotextured Ti surface incorporating Ag

Commercially pure Ti substrates ($14 \times 14 \times 1 \text{ mm}^3$) (98%, Grade 2, Jayon Surgicals, India) were used for the study. These plates were manually polished with silicon carbide papers of grit sizes ranging from 120 to 4000 (Buehler Carbimet, USA) and finishing was done using diamond polish (Metadi fluid, Buehler, USA). Further, the samples were ultrasonically cleaned with acetone, ethanol, and deionised water sequentially (5 min each) and dried.

Nanoleafy topography on Ti substrates (abbreviated as TNL hereafter) was generated via a hydrothermal processing technique as reported earlier by our group [22,23]. Briefly, the polished and cleaned Ti substrates were immersed in 40 mL sodium hydroxide (NaOH) (1 M) in a Teflon container housed within a stainless steel autoclave and positioned in a furnace whose temperature set at 200 °C for 4 h represented in Fig. 1. After the treatment, samples were subjected to ultrasonic cleaning in distilled water for 10 min to remove any residual byproducts and dried. For silver incorporation, NaOH solution was additionally supplemented with varied amounts of silver nitrate (15 μM , 30 μM , 75 μM). Hence, a total of four substrates were taken for the study (A) TNL – titania nanoleaf (B) TNLAg15 – TNL with 15 μM silver nitrate (C) TNLAg30 – TNL with 30 μM silver nitrate and (D) TNLAg75 – TNL with 75 μM silver nitrate.

2.2. Characterization of Ti substrates

2.2.1. Morphological and elemental analysis

The surface morphologies of the substrates were studied using scanning electron microscope (SEM) (JEOL, JSM6490LA) at an accelerating voltage of 15 kV. Elemental analysis was carried out using energy dispersive spectrum (EDS) (JEOL, JSM6490LA) detector attached to the SEM. Different fields were analyzed to get an average distribution of Ag nanoparticles over Ti surfaces.

2.2.2. Crystallinity

The crystallinity of TNL surfaces with and without Ag was characterized by X-ray diffractometer (XRD) using PANalytical X'Pert PRO X-ray diffractometer fitted with CuK α source at a wavelength of $\lambda = 1.541 \text{ \AA}$. The spectrum was recorded in the range from 2° to 80° at a step size of 0.02°. Phase identification was carried out with the help of standard JCPDS database.

2.2.3. Compositional analysis

The chemical composition of TNL and TNLAg substrates were determined by X-ray photoelectron spectroscopy (XPS) (Model ESCALAB220IXL) equipped with an Axis Ultra, Kratos (1486.7 eV) monochromatic source for excitation. XPS spectra over a binding energy (BE) range of 0–1200 eV were obtained using analyzer pass energy of 117.4 eV.

2.2.4. Quantification of silver release

Silver ion release from TNLAg substrates was quantified by immersing the substrates in phosphate buffer saline (PBS) at 37 °C and pH 7.4. The sample was collected during intermittent time intervals (Days 1, 14 and 28) and analyzed by inductively-coupled plasma atomic emission spectrometry (ICP-AES; Thermo Electron IRIS INTREPID II XSP DUO) to determine the amount of silver ions being released from the substrates. Fresh PBS was used as the blank for the analysis.

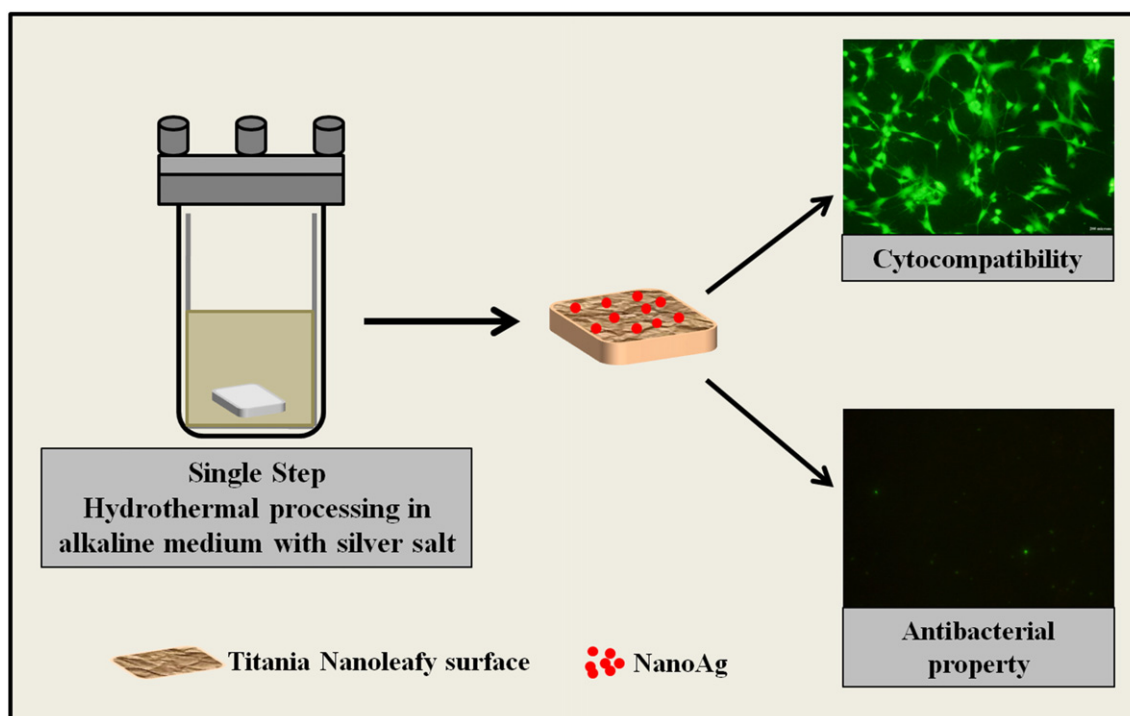


Fig. 1. Schematic diagram of the development of cytocompatible and antibacterial nanotextured Ti surface incorporating silver by single step hydrothermal processing.

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