Polyhydroxyalkanoate Coating on Silver Loaded Titania Nanotubes: Osteogenic and Antibacterial Applications

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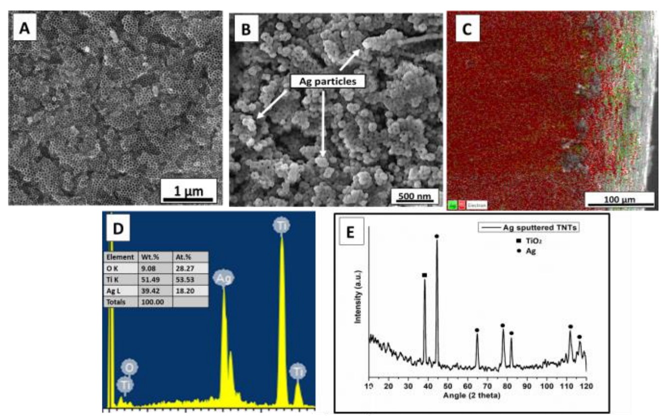
Statement of Purpose: Commonly used metallic materials for load-bearing implants and internal fixation devices are prone to corrosion and bacterial infections in the presence of body minerals. Pure Titanium (Ti) is biocompatible and corrosion resistant owing to the formation of passive oxide layer on surface. The electrochemically anodized (EA) self-ordering array of titania nanotubes (TNTs) have space for drug loading for medicinal purposes. In this study, we plan to deposit few nanometers layer of Ag on TNTs via sputtering. Ag coated TNTs will be further subjected to electrophoretic deposition (EPD) of medium chain length polyhydroxyalkanoates (MCL-PHAs) for osteogenic effect. MCL-PHAs are biocompatible, biodegradable and promote bone regeneration[1]. MCL-PHAs layer is expected to degrade and the dissociation products (ions) will enhance osteoblasts adhesion and proliferation. Ag release from TNTs will inhibit bacterial growth. The proposed research is focused on the translation of the multifunctional, i.e. antibacterial and osteogenic coatings on 3D Ti implants and conducting *in-vivo* testing leading towards clinical trials.

Methods: The experimental procedure consists of three main steps: 1) Synthesis of TNTs; 2) Ag sputtering on TNTs; and 3) PHA coating on Ag-sputtered TNTs. TNTs were synthesized in an electrolyte comprising of ammonium fluoride (NH_4F), ethylene glycol and de-ionized water at 20 V for 2 hours at room temperature. Annealed samples (at 500°C for 4 hours) were subjected to Ag sputtering in vacuum. To prevent the toxic effect of Ag, only a few nanometers (25 and 50 nm) thick layer was deposited over TNTs. In the next step, EPD of MCL-PHAs was performed on Ag-sputtered TNTs. The process parameters (voltage, time, inter-electrode spacing, concentration of polymer suspension, etc.) were optimized using Taguchi Design of Experiment (DoE) approach to obtain appreciable deposition yield following our previous study[2]. The synthesized samples were characterized by Scanning electron microscopy (SEM), X-ray diffraction (XRD), Atomic force microscopy (AFM), Fourier transform infrared spectroscopy (FTIR). Drug release kinetics was studied using Inductively coupled plasma/optical emission spectrometry (ICP-OES). A comparative study of Ag ion-release from TNTs was conducted for different (in terms of thickness) Ag-coatings. Furthermore, adhesion strength, corrosion and wear behavior, antibacterial testing and *in-vitro* cell testing was conducted for coated samples.

Results: TNTs of 56 nm (average diameter) and 526 µm (average length) were obtained as shown in Figure 1A. 2 hours of anodization at 20 V resulted in sufficiently long and wide TNTs for drug loading application. Figure 1B depicts the uniform dispersion of Ag particles on the surface of TNTs. The cross-sectional SEM image shown in Figure 1C confirms the loading of Ag (green color) inside TNTs. The color mapping shows that Ag particles are not only present on the surface of the TNTs but are also loaded inside the TNTs through the entire length of the tubes. This validates the selection of TNTs as drug carrier in orthopedic implants for targeted and sustained drug release. The EDX and XRD of Ag sputtered TNTs also confirmed the presence of Ag (Figure 1D and E, respectively) in samples.



(Figure 1: (A) Morphology of TNTs, (B) Ag-sputtered TNTs, (C) Color mapping confirms loading of Ag inside TNTs, (D) EDX and (E) XRD results of Ag-sputtered TNTs. Results are shown for 50 nm thick Ag layer).

Antibacterial test was conducted against *E.coli* and *S.aureus* via disk diffusion test. The Ag-TNTs showed antibacterial effect against both strains. A very thin antibacterial zone was observed around the sample which confirms the antibacterial effect of Ag. The thin zone formation could be attributed to the few nanometers thick layer of Ag as Ag is toxic above a certain limit in the human body, thus 25 and 50 nm were selected. However, antibacterial effect was obtained as required. The EPD coating of MCL-PHA on Ag-sputtered TNTs is in progress. The osteogenic effect of MCL-PHAs is well reported in the literature.

References: ([1]Rai R. Mat. Sci. & Eng. R: Rep. 2011;72:29-47.) ([2]Rehman M. A. U. Mat. & Des. 2017;130:223-230.)