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Characteristics of multi-layer coating formed on commercially pure titanium for biomedical applications

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ABSTRACT

An innovative multi-layer coating comprising a bioactive compound layer (consisting of hydroxyapatite and calcium titanate) with an underlying titanium oxide layer (in the form of anatase and rutile) has been developed on Grade 4 quality commercially pure titanium via a single step micro-arc oxidation process. Deposition of a multi-layer coating on titanium enhanced the bioactivity, while providing antibacterial characteristics as compared its untreated state. Furthermore, introduction of silver (4.6 wt.%) into the multi-layer coating during micro-arc oxidation process imposed superior antibacterial efficiency without sacrificing the bioactivity.

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1. Introduction

As an implant material, commercially pure titanium (Cp-Ti) and Ti6Al4V alloy are attractive materials because of their low elastic modulus (closer to that of bone), lightweight (low density), non-magnetic properties, low thermal conductivity, high corrosion resistance and good biocompatibility [1–3]. Their enhanced corrosion resistance and biocompatibility arise from instantaneous formation of a compact nanometer thick titanium oxide (TiO_2) layer on the surface when exposed to any oxygen containing environment [4]. However, this native oxide layer cannot resist against destructive mechanical effects and prevent release of alloying elements into the body fluid. In fact, excess concentrations of the alloying elements lead to detrimental biological responses [5,6]. For instance, toxic effect of vanadium along with its contribution to cardiac and renal dysfunction associated with hypertension, Parkinson's disease and depressive psychosis has been established [7–11]. Moreover, aluminum has a high potentiality to cause neurological dysfunctions, anemia, epileptic disease and osteomalacia [9,10]. In this respect, Cp-Ti appears as the most convenient implant material for dental applications, where high mechanical strength is not a priority. Despite the release of alloying elements, Ti6Al4V alloy is generally preferred for manufacturing load bearing orthopedic implants due to the high strength requirements [12].

Mechanical and chemical processes (i.e. sand blasting and etching, respectively) are usually employed to increase the surface area of the implants, because rough surfaces with high surface area induce mechanical interlocking between the bone and the implant [13]. As a matter of fact, rough surfaces slightly stimulate osseointegration which would take place in several months after implantation. It has been reported that bioactive surfaces play a significant role in early stages of implantations due to better osteoconductive properties promoting fast attachment and proliferation of osteoblast cells [14].

One of the key issues in long term success of implantation is the development of infections leading to inflammation around the implants. It is well known that, infections increase the risk of implant failure not only at early stages of implantation but also after complete osseointegration. A clinical study demonstrated that about 7.7% of dental implantations faced with failures in five years related to infections caused by various bacteria [15]. Although, rough surfaces provide better binding between dental implant surface and bone, high roughness increases in the risk of peri-implantitis, which may even result in tissue destruction and bone loss [16–19]. In this respect, surfaces of implants should provide efficient solutions against poor osteoconductivity and insufficient cell attachment as well as infections related to bacterial bio-film formation. There is a possibility for reducing the bacteria

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colonization around the implants by introducing antibacterial agents onto their surfaces. Since silver (Ag) has been known for years as an antibacterial agent, an extensive number of reports focusing on incorporation of Ag into the coatings deposited on titanium and its alloys are available in the open literature [20–23]. Antibacterial efficiency of Ag against bacteria arises from inactivation of DNA replication and vital bacterial proteins [24].

Among the surface modification techniques employed to enhance the bioactivity of the implants, micro-arc oxidation (MAO) is a promising one for titanium-based materials [25–27]. MAO is an electrochemical process similar to conventional anodizing, but it employs higher potentials in non-toxic, environmentally friendly electrolytes. It has a potential to generate thick, porous, rough, adherent and bioactive TiO_2 layer containing biocompatible compounds comprising calcium and/or phosphorous (such as hydroxyapatite, calcium phosphate and calcium titanate) on titanium and its alloys. Its porous nature provides good mechanical fixation when the bone cells grow into the pores. Biocompatible compounds induce much better biochemical bonding between the TiO_2 layer and the surrounding tissues. There are many reports in the literature demonstrating superior cell integration to porous TiO_2 layer in comparison to the untreated surfaces of titanium-based materials [20,28–32]. Recently, some attempts have been made to enhance the antibacterial activity of MAO treated titanium and its alloys upon adding nano-Ag particles and Ag containing chemicals into the relevant electrolytes [20,28–30].

In this study, MAO process was employed in an electrolyte containing calcium, phosphorous and Ag ions with an aim to impart antibacterial activity while enhancing the biocompatibility of Cp-Ti. Silver acetate was chosen as the source of Ag ions due to its high solubility in water and presence of acetate in its chemical structure. After characterization of the surfaces, bioactivity of the MAO exposed samples was evaluated in simulated body fluid. Antibacterial activity of the samples was analyzed against Gram positive and Gram negative bacteria, which play a crucial role in development of infections related to bio-film formation around implants.

2. Materials and methods

2.1. Sample preparation

Coatings were deposited on Grade 4 quality Cp-Ti discs (10 mm in diameter and 4 mm in thickness). Prior to deposition, disc samples were ground with SiC sand paper up to 1200# and cleaned in acetone and distilled water. They were subjected to MAO with an applied voltage of 380 V in an electrolyte containing calcium acetate hydrate (VWR BDH Prolabo) and disodium hydrogen phosphate anhydrous (Alfa Aesar). The pulse frequency and duty cycle were 500 Hz and 60%, respectively. During processing, variation of current density and applied voltage was continuously recorded with a sampling time of 750 ms by using a memory recorder system (Hioki, Memory Hicorder 8808). Each sample was oxidized for 5 min and then cleaned ultrasonically in ethanol and distilled water. Another group of samples was oxidized under the same MAO conditions after adding 0.0025 mol/l silver acetate anhydrous (Alfa Aesar) into the base electrolyte. Here after, samples oxidized in the base electrolyte and silver acetate containing base electrolyte will be referred as MAO and MAO-Ag samples, respectively.

2.2. Coating characterization

The morphology and mean elemental composition of the MAO exposed surfaces were evaluated by utilizing scanning electron microscopes (SEM, Hitachi TM-1000, Jeol JSM-6510 and Jeol NeoScope JCM-6000) equipped with energy dispersive spectrometers (EDS). The phase composition of the coatings was identified by conducting X-ray diffraction (XRD, GBC, MMA 027) analysis using Cu-Kα radiation at 35 kV and 28.5 mA. Scan range was between 20–80° and scanning speed was 2°/min at a scan step of 0.020°. The average surface roughness (Ra) of the samples was measured by using a surface profilometer (Veeco Dectac, 6 M) under 5 mg load, with a scan distance of 2000 μm. Ten measurements were done on each sample. The thicknesses of the oxide layers were determined during SEM examination by imaging the cross-sections of the samples after gentle grinding and polishing. The spectrum of the coatings on MAO and MAO-Ag samples was investigated in ATR mode by a Fourier transform infrared spectroscope (FT-IR, Bruker Alpha-T). Background was calibrated by a reference sample coated with Au. Analysis was performed directly on the coated surfaces to identify the chemical bonding groups in MAO-treated samples, and the spectra were collected over the spectral range of 4000–500 cm^{-1} using a polychromatic irradiation with a resolution of 4 cm^{-1} and sample scan rate of 24 scan/min.

2.3. Apatite forming ability test

In order to evaluate and compare the bioactivity characteristics in terms of apatite forming capability, untreated and MAO treated samples were immersed in 1.5× simulated body fluid (SBF). Samples were vertically soaked in 120 ml of SBF in closed screw-capped polypropylene bottles for 1, 3 and 7 days. The 1.5× SBF was prepared by dissolving reagent-grade chemicals of NaCl, $NaHCO_3$, KCl, Na_2HPO_4, $MgCl_2 \cdot 6H_2O$, $CaCl_2 \cdot 2H_2O$ and Na_2SO_4 in deionized water buffered at pH 7.40 with $((CH_2OH)_3CNH_2)$ and 1.0 mol/l HCl at 36.5 °C [33]. The SBF was refreshed every second day to maintain constant ion concentrations. After soaking for 1, 3 and 7 days, samples were removed from SBF and gently rinsed with distilled water. Prior to characterization by XRD, SEM, and EDS, samples were dried in air at room temperature.

2.4. Antibacterial efficiency test

Antibacterial activities of the untreated Cp-Ti and MAO treated samples were evaluated against *Escherichia coli* (*E. coli*) ATCC®25822 and *Staphylococcus aureus* (*S. aureus*) ATCC®6538p strains. At first step, single colony of bacteria was inoculated to 50 ml Nutrient Broth (NB) and incubated overnight at 37 °C. The optical density of incubated bacteria was measured by using a spectrophotometer and adjusted to standard values (1 OD = 10^9 cell). Incubated bacteria were diluted with 1/500 NB and test inoculation value adjusted as 2.5×10^5–10^6 cells. For each test group, 3 samples were used and 3 dilutions of each sample were made with PBPS buffer and the colony forming units (CFUs) were calculated. Firstly, 400 μl of stock cultures was inoculated on each untreated (control) and MAO treated samples and they were immediately washed with 10 ml SCDLP broth (17 g of casein peptone, 3 g of soybean peptone, 5 g of sodium chloride, 2.5 g of disodium hydrogen phosphate, 2.5 g of glucose and 1 g of lecithin in 1000 ml of distilled water) to calculate zero time inoculations. Secondly, 400 μl of stock cultures was inoculated on each of the control (as-received Cp-Ti samples) and experimental (MAO treated samples) groups and they were incubated at 37 °C and 90–100% humidity conditions for 24 h. After incubation they were washed with 10 ml SCDLP broth and 3 dilutions were made with PBPS buffer. 100 μl of these dilutions spread on plates and incubated for 12-16 h for *E. coli*, 16-24 h for *S. aureus* at 37 °C. After incubation CFUs were calculated for each plate. Lastly, antibacterial activity was calculated from reduction of bacteria colonies and they were compared with control group. The experiments were carried out for three identical samples, simultaneously. The antibacterial efficiency (*AE*%) of the samples was evaluated by the following equation [34]:

$$AE\% = \frac{\text{CFUs of control group} - \text{CFUs of experimental group}}{\text{CFUs of control group}} \times 100. \quad (1)$$

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