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The structures, electrochemical and cell performance of titania films formed on titanium by micro-arc oxidation

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ABSTRACT

Through micro-arc oxidation, the titanium dioxide coatings were prepared on titanium at the different applied voltages (in the range of 100–200 V) in an H_2SO_4/H_3PO_4 electrolyte. The morphologies, phase components, corrosion resistances, and biocompatibility of the coatings were investigated. The effect of the applied voltages on the characteristics and properties of the MAO treated coatings were also discussed. The titania coatings formed in acidic electrolyte is mainly composed of anatase phase and the anatase content increases with the applied voltage increasing. It is also found that the applied voltage plays an important role in the characteristics and properties of the composite coatings. With the increase of the applied voltage, the pore size, surface pore density and the roughness of the coatings increase, while the corrosion resistance decreases. Moreover, the MAO coatings treated at a higher voltage (180 and 200 V) exhibited an effect on early osteoblast mineralization.

1. Introduction

Titanium (Ti) is the most widely used implant material for load bearing dental and orthopedic applications because of its excellent mechanical, chemical stability, biological properties and biocompatibility, which are mainly due to the surface oxide layer formed naturally in air or in many aqueous environments [1,2]. Therefore, the properties of the surface oxide layer, such as roughness, topography and composition, play an important role in the biocompatibility of a Ti implant [3,4]. A number of physical and chemical treatments of the titanium surface have been proposed to obtain improved osteointegration [5–8].

Micro arc oxidation (MAO), also called plasma electrolytic oxidation, is a novel technique developed to produce hard ceramic coatings on valve metals such as Al, Ti, Mg and their alloys [9–11]. It can effectively increase wear resistance, corrosion resistance, mechanical strength, and electrical insulation of metals and their alloys. By means of MAO technique, TiO_2 coating can be prepared on pure Ti and Ti alloy. TiO_2 coating is both porous and firmly adhered to the substrate, which is beneficial for the biological performance of the implants [12]. The oxide films formed on titanium via MAO can either be amorphous or crystalline depending on the final anodic potential and the electrolyte used [13–15]. The composition, structure and thickness of the films, to a great extent, determine their stability. It is generally agreed that the oxide film mainly consists of TiO_2, which has three stable forms, that is, rutile, anatase and brookite. Increasing the thickness usually increases crystallinity [16].

Electrochemical impedance spectroscopy (EIS) is a very powerful technique in the analysis of the electrochemical systems. This technique is used in investigations of protective coatings [17–19]. Based on the literatures [19,20], the corrosion resistance of the coating is mainly dependent on its thickness, microstructure and phase composition. Due to its high sensitivity to the coating structure, EIS, thus, can be utilized to analyze the structure of the coating.

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Although many works have prepared micro-arc oxidized titania coatings previously [21–24], the working voltages used in their works were generally high (in the range of 200–500 V). The effect of low working voltage on the microstructure and properties of the MAO coatings has not been reported. In this study, titanium dioxide coatings in H_2SO_4/H_3PO_4 based electrolyte were fabricated by MAO process with the emphasis on the effect of low working voltage on the structure of titanium dioxide coatings. The structure and morphology of the coatings were observed by field emission scanning electron microscope (FE-SEM) and X-ray Diffraction (XRD). The EIS impedance plot was employed to evaluate the properties and construct the structures of these coatings. Finally, immersion tests and cell culture assays were carried out to investigate the bioactivity and biocompatibility of MAO treated coating implants.

2. Materials and methods

2.1. Preparation of MAO coatings

Pure Ti samples of 25 mm × 15 mm × 1 mm were used as the substrate materials for MAO. Prior to the coating, samples were polished with abrasive paper (2000#), degreased with acetone and rinsed with distilled water. The MAO treatment device consists of a potential adjustable DC power supply up to 400 V, a 4 L stainless steel container used as an electrolyte cell, a stirring system and a cooling system. The Ti sample was used as an anode while the wall of the stainless steel container was used as the cathode. An aqueous electrolyte was prepared from a solution of 0.25 M H_2SO_4, 0.25 M H_3PO_4, and a specific amount of additive X. Micro arc oxidation processes were carried out under the conditions of working voltage in the range of 100–200 V. After MAO treatment of 10 min, the coated sample was taken out from electrolyte, rinsed thoroughly with distilled water and dried at room temperature.

2.2. Coating characterizations

The phase composition of the anodic films was analyzed using X-ray diffraction (XRD, Philips powder X-ray diffractometer PW 1710) with Cu Kα radiation (wavelength 1.542 Å) and the scans were performed in the 20–80° range. A field emission scanning electron microscopy (FE-SEM, S-4700, HITACHI, 15 kv) was employed to examine the surface morphology of oxide layer. A three-dimensional (3D) whitelight interference microscopy profiler (ZTGO Chroma Model 7501/7502) was performed to examine the surface roughness and profilers on the substrate. The measured values of the surface roughness were averaged. MAO coatings are multi-phase and their corrosion performance is influenced by the amount and distribution of the different phases. The microstructure of the samples was analyzed prior to the electrochemical tests. The corrosion resistance of the coating was tested by potentialstat (EG&G) and electrochemical impedance spectroscopy (EIS) in Hanks solution. The Hanks solution contains 8000 mg/L sodium chloride, 400 mg/L potassium chloride, 60 mg/L potassium phosphate, 1000 mg/L glucose, 10 mg/L phenol red, 48 mg/L sodium phosphate, 98 mg/L magnesium sulfate, 140 mg/L calcium chloride, and 350 mg/L sodium bicarbonate. All the experiments were carried out at room temperature and a 25 ± 3 °C and a relative humidity of 55% ± 5%.

2.3. Cell culture

D1 cells (Pluripotent mesenchymal cells, American Type Culture Collection, Manassas, VA, USA), cloned from BALB/c mouse bone marrow cells [25], were maintained in bone medium (Dulbecco's Modified Eagle's Medium, Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum and 0.1% sodium ascorbate in a humidified atmosphere of 5% carbon dioxide at 37 °C.

2.4. MTS (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay

D1 cells were seeded on the titania films with/without micro-arc oxidation modification into a 24-well in a density of 100,000 cells/well with culture medium. The cells were harvested on days 1, 4, 7 and 10 to detect the cell proliferation by using MTS tetrazolium (Cell Titer96 Aqueous; Promega, Madison, WI). Briefly, at 3 h before each of the desired time points, 10 μl of the MTS reagent was added into each well and cells were incubated at 37 μC for 3 h. The absorbance was detected at 490 nm with a microplate autoreader (Dynex Technologies, Billingshurst, UK). The whole experiment was repeated three times.

2.5. Alkaline phosphatase (ALP) activity

D1 cells were seeded on the titania films with/without micro-arc oxidation modification into a 24-well in a density of 100,000 cells/well with culture medium. The cells were harvested on days 4, 7, 10 and 14. Sigma–Aldrich Alkaline Phosphatase kit (No. 85, Sigma, USA) was used to detect and stain ALP activity after simvastatin treatments. To prepare the alkaline-dye solution, 2 ml Naphthol AS-MX phosphate alkaline was dissolved in diazonium salt solution which is a fast violet B capsule that dissolves in 48 ml distilled water. Cells were fixed with 10% formalin-saline at room temperature for 10 min. After washing once with ddH_2O, alkaline-dye mixture was added to each well in the 48-well plate and incubated for 15–30 min. The staining solution was removed and the wells were washed with distilled water. The fixed and stained plates were then air-dried at room temperature and the ALP positive stained cells were photographed by microscopy (Nikon, Japan)

2.6. Mineralization assay

Alizarin red S staining was used to determine the level of calcification in the extracellular matrix. D1 cells were seeded on the titania films with/without micro-arc oxidation modification into a 24-well in a density of 100,000 cells/well with culture medium. The cells were harvested on days 4, 7, 10, 14 and 21. The cells were fixed in 10% formalin and phosphate-buffered saline for 10 min. After washing twice with double-distilled H_2O, the fixed cells were stained with Alizarin red S solution for 5 min. After staining, the cells were washed using double-distilled H_2O. The fixed and stained plates were then air-dried at room temperature. The amount of mineralization was determined by dissolving the cell-bound Alizarin red S in 10% acetic acid and measurement at 415 nm.

2.7. Statistical analysis

Each experiment was repeated at least three times, and the data (expressed as the mean ± standard error of the mean) from representative experiments are shown. Statistical significance was evaluated by one-way analysis of variance, and multiple comparisons were performed by Scheffe's method. P, 0.05 was considered to be statistically significant.

3. Results and discussion

MAO is based on the conventional anodic oxidation of processing metals and alloys in aqueous electrolyte solutions accompanied by sparking microdischarge due to dielectric