Applied Surface Science 256 (2010) 2018–2024

Contents lists available at ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

Effect of frequency on the structure and cell response of Ca- and P-containing MAO films

Yingjun Wang [a,b,*], Lin Wang [a,b], Huade Zheng [a,b], Chang Du [a,b], ChengyunNing [a,b], Zhifeng Shi [a,b], Caixia Xu [a,b]

[a] College of Materials Science and Engineering, South China University of Technology, Guangzhou 510640, PR China
[b] Key Lab of Specially Functional Materials, Ministry of Education, South China University of Technology, Guangzhou 510640, PR China

ARTICLE INFO

Article history:
Received 23 July 2009
Received in revised form 5 September 2009
Accepted 13 September 2009
Available online 19 September 2009

Keywords:
Micro-arc oxidation
Film
Titanium
rMSC
Amorphous calcium phosphate

ABSTRACT

The Ca- and P-containing MAO films were prepared on titanium substrate at different frequencies (100–5000 Hz) and were characterized by SEM, XRD, XPS and contact angle goniometer. For in vitro test, the rat bone marrow mesenchymal stem cells (rMSCs) were seeded on the films. The fluorescence microscopy and the PicoGreen assay were used to determine the cell initial adhesion and proliferation. It shows that the frequency of the MAO affected the crystallinity, composition, morphology and wetting ability of the oxidation film. At a high frequency, the crystallinity decreased, and the content of Ca and P increased. The structure formed at a high frequency – there were many smaller pores on the wall of the larger ones and many inner pores in the film – could improve the connectivity of the film. The wetting ability of the film was also improved by increasing the frequency. The mechanism of how the frequency of the MAO process could influence the oxidation film was discussed. It could be explained by the theory of electron avalanche and the phenomenon of secondary breakdown. In vitro test showed that the film formed at 5000 Hz was more favorable for the initial cell attachment and proliferation.

1. Introduction

As important materials for orthopedic implants, titanium and titanium alloys have excellent biocompatibility, corrosion resistance, mechanical properties, and light weight [1]. In spite of the excellent biocompatibility of Ti, bonding between Ti implant and bone tissue is normally physical attachment rather than bioactive fixation [2,3]. The surface properties of Ti implants play important roles in ensuring their long-term anchorage to the tissue [4,5]. There have been many ways to improve the osteointegration of the titanium implants by enhancing osteoconduction on their surfaces through topological and/or chemical modifications, such as grit blasting, acid-etching, plasma spraying and so on [6–11].

Micro-arc oxidation (MAO) has been used to form a TiO_2 surface layer by applying a positive voltage on Ti specimen immersed in an electrolyte [12]. As reported, the titanium oxide layer is of importance for providing bone-bonding ability of Ti implants, i.e. osteoconductivity [13]. The oxide layer formed by anodic reaction has a barrier type structure inside and a porous structure outside [14,15]. One of the advantages of MAO process is the possibility of incorporating Ca and P ions into the surface layer, by controlling the composition and concentration of the electrolyte [16]. The incorporated Ca and P ions can further crystallize into hydroxyapatite or other calcium phosphate minerals by hydrothermal treatment [17,18].

There are some researches on the effect of frequency on MAO films. It shows that frequency can affect the morphology of the films [19,20]. The film formed at low frequency was rougher and have larger pore size than that at high frequency. Thereby, coatings produced at 100 Hz perform relative low corrosion resistance for their rough structure [19]. And recently, the TiO_2-based films containing amorphous calcium phosphate have also been noticed [21,22]. Nagano et al. [23] suggested that amorphous calcium phosphate may support the initial fixation of porous materials due to its excellent osteoconductive property. The researchers prepared the amorphous films on the titanium by changing the content of the EDTA2Na in the electrolyte during the MAO process [24]. The low crystallinity could accelerate the ion, such as PO_4^{3-} and Ca^{2+}, to release from the film. This could increase the ion concentration of the medium around the sample, and was favorable for depositing the bone-like apatite [21,25]. However, there is no report concerning the effect of frequency on the crystallinity and bioactivity of the films on the titanium, and the frequency was always limited to 800 Hz.

* Corresponding author at: College of Materials Science and Engineering, South China University of Technology, Guangzhou 510640, PR China.
E-mail address: imwangyj@scut.edu.cn (Y. Wang).

0169-4332/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.apsusc.2009.09.041

Thus, the present study attempts to investigate the mechanism of how the frequency of MAO process could influence the oxidation film. In the experiment, titania-based films containing Ca and P ions with different crystallinity were prepared at different frequencies ranging from 100 to 5000 Hz during the MAO process, with calcium acetate ($Ca(OOCCH_3)_2$) and calcium dihydrogen phosphate ($Ca(H_2PO_4)_2$) as electrolyte components, and the morphology, composition, phase and wetting ability of the oxide layer were characterized by SEM, XRD, XPS and contact angle goniometer with respect to the applied frequency. In vitro test of the film containing calcium phosphate with different crystallinity was also carried out.

2. Experimental

2.1. Sample preparation

Commercial pure Ti, machined into disks with dimensions of 10 mm × 10 mm × 2 mm, was used as substrate. These disks were polished with 240-grit SiC sandpaper, and then ultrasonically cleaned in acetone, ethanol and distilled water, respectively, for 20 min. After being cleaned, the samples were etched with the mixture of 49% H_2SO_4 and 18% HCl at room temperature for 20 s, rinsed thoroughly with deionized water, and air dried.

2.2. MAO process

MAO of the specimens was carried out in an aqueous solution with 0.082 M $Na_2(EDTA)$ as chelating agent, 0.06 M $Ca(OOCCH_3)_2$, and 0.02 M $Ca(H_2PO_4)_2$, the pH value of which was adjusted to 7 with 2 M NaOH solution, by applying a pulsed DC field to the specimens. The duty of the pulsed DC power was 30%, the voltage was 450 V, and the treatment time was 5 min. To obtain different oxide layers, the frequency of the pulsed DC power was changed in a wide range of 100–5000 Hz, to observe the influence of the frequency on the oxide films of MAO.

2.3. Characterization of oxide layer

The phases of the specimens were evaluated by thin-film X-ray diffraction analysis (TF-XRD; X'Pert Pro, PANalytical B.V., The Netherlands) with Cu Kα (λ = 0.15418 nm) incident radiation. The morphology of the specimens was characterized by scanning electron microscopy (SEM; Quanta 200, FEI, The Netherlands). The compositions of the samples were determined by energy dispersive spectroscopy (EDS; INCA, Oxford, England) and X-ray photoelectron spectroscopy (XPS; Axis Ultra DLD, Kratos, England) with an Al Kα (BE = 1487.3 eV) X-ray source and an anode powder of 150 W (10 kW, 15 mA). The wetting angle of the surface ($n = 3$) was measured via the liquid drop method on a contace angle goniometer (OCA15, Dataphysics, Germany).

2.4. Cell culture and seeding

The rat bone mesenchymal stem cells (rMSCs) labeled with humanized renillar green fluorescent protein (hrGFP) were cultured (5% CO_2 at 37 °C) in L-DMEM containing 10% newborn bovine serum. The culture medium was changed every 2 days. Ti disks were immersed in 75% ethanol for 24 h. After sterilization, the samples were placed individually into the 24-well plate and the cells were inoculated onto them (20,000 cells/10 μl droplet culture). The cell culture plate was used as the positive control. After 2 h incubation, 750 μl of complete medium was added to each well. They were cultured for either a few hours or days according to the different regimes.

2.5. Initial cell attachment

After incubating the samples with cells at 37 °C for 6 h, the substrates were washed with PBS carefully to remove unattached cells and were investigated with the fluorescence microscopy (AXIOSKOP 40, ZEISS, Germany).

2.6. DNA quantification

The PicoGreen assay was used to measure the DNA content of the samples incubated with MSCs for cellular adherence and proliferation. The kinetic points were at 1 (6 h), 3 and 7 days after MSCs incubated onto the samples. To remove culture medium residues, the samples and their adherent cells were rinsed with PBS three times. Then the samples were incubated in 1 ml digestive solution (50 mM Na_3PO_4, 20 mM N-acetyl cysteine, 28 μg/ml papain). The specimens were vortexed for 10 min and the supernatant was stored at −80 °C. According to the manufacturer's information, the assay displays a linear correlation between dsDNA concentration and fluorescence with a detection range from 25 pg/ml to 1 μg/ml dsDNA. Afterward, the 10 μl supernatant was mixed with 10 μl PicoGreen dsDNA Quantitation in darkroom for 5 min, the DNA content was quantified using a fluorospectrometer (NanoDrop 3300, Thermo, USA). Results were compared to standard curves of λDNA dilution series. To exclude measurement errors, all experiments were carried out in triplicate.

2.7. Statistics

Data are presented as means and standard deviation. The statistical software SPSS 11.0 was used for repeated measurement variance analysis (the wetting angle measurement and the PicoGreen assay), and the Tukey–Kramer procedure for post hoc comparison, with $p < 0.05$ being regarded as significant.

3. Results

The variation of current with time at different frequencies is shown in Fig. 1. It reveals that as the time increased, the values of the current decreased quickly in the initial stage, and remained stable after about 1 min. It shows that with the increase of the applied frequency, the value of current also increased. The stable current at 100 Hz was only about 1.5 A, but that at 5000 Hz was up to 5.7 A.

Fig. 2 shows the morphology of the surfaces formed at the frequency of 100, 500, 2000, and 5000 Hz. It can be seen that the oxide film exhibited porous microstructure with micro-pores. The pores were relatively well separated and homogeneously

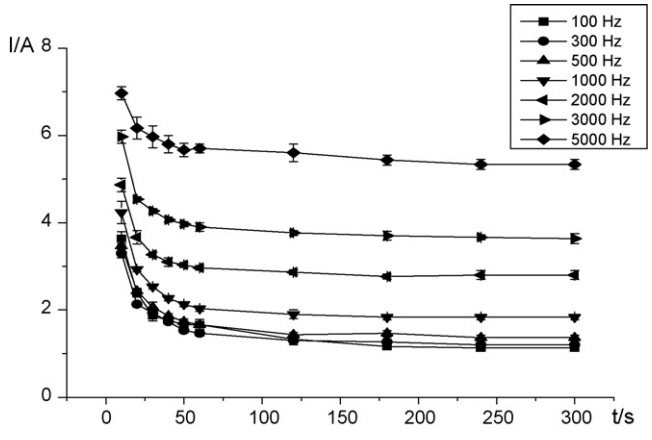

Fig. 1. The variation of current with time at different frequencies.