Colloids and Surfaces B: Biointerfaces 84 (2011) 400–405

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

In vitro corrosion study of different TiO_2 nanotube layers on titanium in solution with serum proteins

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ARTICLE INFO

Article history:
Received 10 October 2010
Received in revised form 18 January 2011
Accepted 22 January 2011
Available online 5 March 2011

Keywords:
Titanium
TiO_2 nanotube layer
Corrosion
Serum proteins

ABSTRACT

Titanium oxide nanotubes prepared by anodization have received considerable attention in the biomaterials domain. The objective of this study was to demonstrate the electrochemical behavior of different diameter TiO_2 nanotube layers on titanium in phosphate buffered saline (PBS) and Dulbecco's minimum essential medium + 10% fetal calf serum (D-FCS) using open circuit potentials (OCP), electrical impedance spectroscopy (EIS), and a potentiodynamic polarization test. The results showed that the nanotubes had higher OCP, higher resistance of the inter barrier layer (R_b), and lower I_{pass} in the two test solutions compared to the smooth Ti, especially the 30 nm diameter nanotubes. The corrosion resistance of the nanotubes in D-FCS was higher than in PBS because of protein adsorption from the D-FCS solution as suggested by scanning electron microscope (SEM) images. In addition, protein aggregates of 30 nm diameter nanotubes caused the model of EIS spectra to transform from two-layer to three-layer. The corrosion behavior of the nanotubes for use as a dental implant material is discussed.

1. Introduction

Titanium is currently one of the most important materials in biomedical and dental implants [1]. Chemical composition and topography of Ti-implant were modified by various methods to improve osseointegration of endosseus implants, which could eventually play an important role in clinical outcomes [2]. Recently, the researchers began to modify the implant surface in nanometer regime because of the in vivo nanometer physiological environment that bone cells are accustomed to [3].

Many studies have shown that the nano-Ti implant can enhance osteoblast adhesion and function more significantly than what the conventional Ti implant dose [4–13]. Corrosion studies are also essential because higher corrosion rate means more ion release, which may interfere with cell metabolism in tissues around implants, even induces implant failure [14,15]. Furthermore, the greater real surface area in nano-Ti implants may exhibit a lower corrosion resistance than the conventional Ti implant [5,6,16]. However, up to now only a few corrosion studies have been carried out on nano-implants [17–19]. Zheng et al. studied the corrosion behavior of Ti–TiC–TiC/diamond-like carbon (DLC) gradient nano-composite films on NiTi alloy in hank's solution. They found that nano composite films coated NiTi showed excellent corrosion resistance properties than uncoated NiTi [17]. Karpagavalli et al. reported that Ti6Al4V with nanostructured TiO_2 films deposited eletrolytically could enhance the corrosion resistance compared to the bare Ti6Al4V [18]. But some reports exhibited contrary corrosion performance. For example, Garbacz et al. found that the nano-Ti by hydrostatic extrusion demonstrated lower corrosion resistance than the micro-Ti in a NaCl solution did [19]. So the corrosion behaviors of nano-Ti in physiological environment need further investigation.

Recently, the biological characteristics of TiO_2 nanotube layers by anodization have been extensively studied and shown promising results in biomaterial application [20–26]. The titanium with nanotube layers can not only significantly increase osteoblast or bone-forming cell adhesion and function in vitro [22–24], but also promote in vivo bone formation around implants compared to their unanodized counterparts [25,26]. However, up to now, few articles about corrosion behavior of nanotubes have been published [27,28]. Saji et al. studied the corrosion behavior of Ti–35Nb–5Ta–7Zr alloy with amorphous nanotubular oxide in Ringer's solution at 37 °C. Their results showed that this nanotube layer had good corrosion resistance due to forming an immediate and effective passivation [27]. Yu et al. tested the corrosion characteristics of titanium with amorphous and anatase nanotubes in Hank's solution and found that this nanotube layer, especially the anatase nanotubes, exhibited better corrosion resistance than smooth-Ti [28]. The Ringer's and Hank's solution only mimic inorganic components of body fluids [29]. Many studies have shown that the serum proteins of body fluids can play an important role in corrosion of the implant materials [30–34]. So it is more rep-

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0927-7765/$ – see front matter.
doi:10.1016/j.colsurfb.2011.01.033

resentative of physiological environment to test the corrosion of TiO_2 nanotube layers in solution with serum proteins. Furthermore, the diameter of nanotubes has been proved to affect the adsorb of serum proteins, which can influence cell behavior [35,36]. It is not known whether the adsorb of serum proteins can change the corrosion of nanotubes on titanium.

The present work was to test corrosion behavior of titanium with different diameter nanotube layers in PBS solution and fluids with serum proteins and detected by scanning electron microscope (SEM), electrochemical impedance spectroscopy (EIS) and potentiodynamic polarization. This study aims to enhance the understanding of corrosion characteristics of nanotubes on titanium in physiological environment.

2. Materials and methods

2.1. TiO_2 nanotube layer preparation

Ti thin foils (0.25 mm thick, 99.5%, Alfa Aesar) were used to prepare the nanotubes. Firstly, titanium foils were immersed in an mixture (2 mL 48% HF, 3 mL 70% HNO_3 (both reagent grade chemicals) and 100 mL deionized water) for 3 min to remove the natural oxide layer, then ultrasonically washed in deionized (DI) water for 5 min and dried by nitrogen stream. The nanotube layers were prepared by anodization in 1 M H_3PO_4 and 0.5 wt% HF solution for 3 h at different voltages (5 V and 15 V) to obtain different diameter nanotubes [35]. The counter electrode was platinum plate. After anodization, the samples were ultrasonically washed in DI water and dried by nitrogen stream. The Ti foil was polished with SiC emery papers No. 240, 400, 600, 800, 1000 and 1200 grit sizes in series for use as a control group sample.

2.2. Surface characterization

Some samples were immersed in 30 mL test solutions at 37 °C for half an hour. Then the samples were picked up and gently washed with DI water. Their microstructures were observed by Scanning Electron Microscopy (Philips, The Netherlands, Sirion200). All specimens were sputter-coated with Au before imaging using a HUMMER I Sputtercoater for 3 min.

2.3. Preparation of corrosion test

The three kinds of test specimens used in this research were (a) smooth Ti (S), (b) the nanotube layers at 5 V (N-5), and (c) the nanotube layers at 15 V (N-15). The mechanical and electrical contact between the specimen and the cupper wire were obtained by electric conduction paste with copper. The cold epoxy resin was used to carefully mount the samples. The exposed test surface (area: 1 cm^2) was ultrasonically washed in deionized water, ethanol, and then deionized water for about 5 min, respectively. Three specimens were prepared for each condition to corrosion test.

Two synthetic solutions simulating physiological environment were used in this study. That is phosphate buffered saline (PBS) and Dulbecco minimum essential medium + 10% fetal calf serum (D-FCS). The solution was prepared from deionized water, analytic grade agents, Dulbecco minimum essential medium, and fetal calf serum. The main components of solution are shown in Table 1 [32].

2.4. Corrosion test

The apparatus for electrochemical measurement consisted of a computer-controlled potentiostat (PARSTAT 2273 Advanced Electrochemical System) with research corrosion software (PowerSuite), an Ag/AgCl with a saturated potassium chloride as a reference electrode, a platinum plate as a counter electrode, and a standard 3-electrode microcell. After the specimen was immersed in PBS and D-FCS solution, the open circuit potential (E_{ocp}) was measured for half an hour at 4 s intervals. The electrochemical impedance spectra (EIS) measurements were taken at the E_{open} achieved in the E vs. t test using a frequency response analyzer coupled with the potentiostat. The frequency span analysed ranged from 10^{-2} to 10^5 Hz with a perturbing signal of 10 mV. The EIS data fitting was done using the suitable equivalent circuit by the ZSimpWin 3.21 software. The potentiodynamic polarization behavior of specimens was recorded after immersed for 90 min in test solution. The scan range of the potentiodynamic polarization was −400 mV to +3000 mV (vs. open circuit potential) at a scanning rate of 1 mV/s. All tests were maintained at 37 °C. All the tests were repeated three times with each sample and solution. The reproducibility of the data by repeating measurements on a series of electrodes was within 5%. All experiment data were statistically analysed using ANOVA and Scheffeĭs test ($\alpha = 0.05$) of SPSS 11.0.

Table 1
The component (mol/l) of solutions.

Component	PBS	D-FCS
NaCl	1.37×10^{-1}	1.16×10^{-1}
KCl	2.68×10^{-3}	5.36×10^{-3}
$CaCl_2$		1.80×10^{-3}
Na_2HPO_4	8.10×10^{-3}	8.98×10^{-4}
KH_2PO_4	1.47×10^{-3}	
$MgSO_4$		8.11×10^{-4}
$NaHCO_3$		$2.38–2.62 \times 10^{-2}$
Amino acid		5.5×10^{-3}
Proteins (g/l)		<10

3. Results

3.1. The morphology of surfaces

The morphology of nanotube layers in different test solutions was shown by Fig. 1. The N-5 and N-15 is the nanotube layers with approximately 30 nm and 70 nm diameter by anodized at 5 V and 15 V (Fig. 1A). The morphologies of all the surfaces after immerged in PBS were the same as before (Fig. 1B). However, SEM images revealed that protein aggregates deposited from D-FCS settle on the surfaces after immerged in D-FCS for half an hour. Few protein aggregates were found in S and N-15 surfaces, but lots of protein aggregates were deposited on N-5 surface so that the pores of nanotubes were sealed.

3.2. E_{ocp} in different solutions

Open circuit potential (E_{ocp}) data after half an hour immersion in PBS and D-FCS solutions were reported in Fig. 2. The E_{ocp} of N-5 was significantly higher than that of S and N-15 in two test solutions ($P < 0.05$). The E_{ocp} of S and N-15 in D-FCS became lower than in PBS test solutions ($P > 0.05$). But the E_{ocp} of N-5 in D-FCS became higher than in PBS test solutions ($P > 0.05$).

3.3. EIS analysis

Fig. 3 shows the electrochemical impedance data for S, N-5, and N-15 in PBS and D-FCS solutions for half an hour. The Bode-phase plots showed clear two time constants in the N-15 surfaces. But only one time constant was seen in the S and N-5 surfaces.

The spectra obtained for S was interpreted by using one time constant due to passive oxide layer. The N-5 in PBS and N-15 in PBS and D-FCS solutions were interpreted by using a model with two constants because of the nanotubes with an outer tube layer and an inner barrier layer [27] (even though only one time constant