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Surface modifications and cell–materials interactions with anodized Ti

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Abstract

The objective of this study was to investigate in vitro cell–materials interactions using human osteoblast cells on anodized titanium. Titanium is a bioinert material and therefore becomes encapsulated after implantation into the living body by a fibrous tissue that isolates it from the surrounding tissues. In this work, a bioactive TiO_2 layer was grown on commercially pure titanium substrate by an anodization process using different electrolyte solutions, namely H_3PO_4, HF and H_2SO_4. These electrolytes produced bioactive TiO_2 films with a nonporous structure showing three distinctive surface morphologies. Human osteoblast cell growth behavior was studied with as-received and anodized surfaces using an osteoprecursor cell line (OPC 1) for 3, 5 and 11 days. When anodized surfaces were compared for cell–materials interaction, it was noticed that each of the surfaces has different surface properties, which led to variations in cell–materials interactions. Colonization of the cells was noticed with a distinctive cell-to-cell attachment in the HF anodized surface. Good cellular adherence with extracellular matrix extensions in between the cells was noticed for samples anodized with H_3PO_4 electrolyte. The TiO_2 layer grown in H_2SO_4 electrolyte did not show significant cell growth on the surface, and some cell death was also noticed. Cell adhesions and differentiation were more pronounced with vinculin protein and alkaline phosphatase, respectively, on anodized surfaces. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium assays also showed an increase in living cell density and proliferation with anodized surfaces. It was clear that rough surface morphology, high surface energy and low values of contact angles were important factors for better cell materials interaction. A mineralization study was done in simulated body fluid with ion concentrations nearly identical to those of human blood plasma to further understand biomimetic apatite deposition behavior. Similar to cell–materials interaction, variations in mineral deposition behavior were also noticed for films grown with different electrolytes.

Keywords: Anodization; Cell–material interactions; Oxide film

1. Introduction

Titanium and its alloys are widely used for orthopedic and dental implants for their superior mechanical properties, low modulus, excellent corrosion resistance and good biocompatibility. Titanium, being bioinert, cannot directly bond to bone. After implantation into the living body it becomes encapsulated by a fibrous tissue that isolates it from the surrounding bone [1]. Studies have demonstrated that bone tissue can form on the titanium surface with a very thin oxide cementum layer in between [2,3]. This phenomenon is known as osseointegration [4]. It takes several months for titanium implants and bone tissue to reach integration. Hence, there is a growing interest in shortening the process toward osseointegration and thereby reducing surgical restrictions. Various surface modifications, including chemical treatment [5–7], thermal treatment [8], electrochemical method [9] and anodization [10], have been applied to form a bioactive titanium oxide layer on the metal surface. Evaluation of the above-processed films in a simulated body fluid (SBF) demonstrated that apatite deposition depends on chemical composition, surface properties, concentration of the electrolytes and crystalline phases present in the film [11].

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Several in vitro and in vivo studies have shown that modified surfaces have a higher early level of cell attachment than the untreated Ti surface. It has been shown that micro-arc-oxidized and hydrothermally synthesized surfaces showed higher cell adhesion and proliferation rates than did the untreated Ti surface [12]. Hydrothermal treatment of anodized Ti-substrate with calcium glycerophosphate and calcium acetate as electrolytes also led to higher protein production than with untreated Ti surface [13]. It was also shown that a titanium oxide surface had a greater number of osteoblasts with higher cell activity than did bare Ti surface [14].

The objective of this study was to understand the bone cell materials interactions on a commercially pure Ti (cp-Ti) surface that has been modified via anodization in acid electrolytes. A titanium oxide layer on the Ti surface was grown by an anodization process using H_2SO_4, HF and H_3PO_4 electrolytes at an anodizing voltages of 20 V. The biological properties of these anodized surfaces were evaluated in SBF for 3, 7, 14 and 21 days, and in vitro with the osteoblast precursor cell line (OPC1). Apatite precipitation on substrates was observed for all anodized surfaces after incubation in SBF, and each surface gave rise to a different cellular response due to variations in the surface properties.

2. Materials and methods

2.1. Preparation and characterization of anodized Ti

A cp-Ti (99.6 at.%) sheet was used as the starting material. Each of the samples was 12 mm in diameter and 0.5 mm in thickness. Samples were abraded by silicon carbide paper in successive grades from 600 to 1200 grit (Leco Corporation, MI), then ultrasonically cleaned in distilled water and dried at room temperature. Final polishing was performed using a cotton polishing cloth with a 1 μm alumina suspension. Polished samples were used as controls for the experiments. For the anodization process, the electrolytic cell consisted of a three-neck round bottomed flask. A cp-Ti anode was suspended from the centre neck of the cell, a platinum cathode was suspended from one of the necks by a platinum wire and a thermometer was placed through the other neck. A DC power supply (Hewlett Packard 0–60 V/0–50 A, 1000 W) was used to vary the applied voltage. Three different solutions were used as electrolyte: 1 (N) sulfuric acid (H_2SO_4), 1 (N) phosphoric acid (H_3PO_4) and 0.25 (N) hydrofluoric acid (HF). All anodization experiments were performed at 20 V. Temperature of the electrolyte was maintained at 30 °C. The temperature rise due to exothermic anodic reaction was controlled by using large amount of electrolyte and changing the solution after every 1 h. TiO_2 anodized film was characterized using a scanning electron microscope (SEM; Hitachi's-570) and a field emission scanning electron microscope (FESEM; FEI, SIRION, OR) fitted for energy-dispersive X-ray analysis. The operating voltage for both SEM and FESEM was 20 kV. Energy-dispersive spectroscopy (EDS) was performed to qualitatively identify the composition of the film. Oxide film thicknesses were measured in SEM from a cross-sectional view of the Ti-foils. Three samples were used to give the average thickness value for each anodized condition.

Glancing angle X-ray diffraction (GAXRD) was conducted using a Bruker/Seimens platform system at University of Wisconsin, Madison. GAXRD studies were carried out from a sealed Cu tube operating at 40 keV and 20 MPa. Glancing angles of 5° and 10° were used for all the samples. Each of these samples was scanned in the 2θ range of 20–80°.

2.2. Surface analysis

2.2.1. Surface roughness

The surface roughness of each Ti-control and anodized sample was measured using a surface profilometer (SPN Technology, Goleta, CA). The scan was performed on each sample three times, with a scan distance of 4 mm, at different places on the sample. For each anodized condition three samples were used. The stylus radius was 25 μm. The roughness data for 4 mm scan was based on 5000 points on the tip of the profilometer. From all of these 5000 points, the root mean square data (r.m.s.) were calculated. The average of 9 r.m.s. values (three from each sample) for each anodized condition was determined along with the standard deviation.

2.2.2. Contact angle measurement

Contact angles were measured using the sessile drop method with a face contact angle set-up equipped with a microscope and a camera. A 0.5–1.0 μl droplet of distilled water and McCoys 5A solution at pH 7.4 (cell culture medium) was suspended from the tip of the microliter syringe. The syringe tip was advanced toward the disk surface until the droplets made contact with the disk surface. Images were collected with the camera and the contact angle between the drop and the substrate was measured from the magnified image. Three samples for each anodized conditions were used to collect the contact angle data in water and in cell media. The apolar liquid diiodomethane and two polar liquids, formamide and glycerol, were used in the following equation to calculate the surface energy:

$$\gamma_L(1+\cos\theta) = 2\left(\gamma_S^{LW}\gamma_L^{LW}\right)^{1/2} + 2\left(\gamma_S^{+}\gamma_L^{-}\right)^{1/2} + 2\left(\gamma_S^{-}\gamma_L^{+}\right)^{1/2} \quad (1)$$

In Eq. (1), θ is the contact angle of liquid L and solid S, γ^{LW} is the apolar component of the surface energy, γ^{+} is the Lewis acid component (electron acceptor) and γ^{-} is the Lewis base component (electron donor) [15].

2.3. Cell–materials interaction studies

2.3.1. Cell morphology

For the cell–materials interaction studies, human osteoblasts were used. Cells were derived from an osteoblastic

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