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Titanium Nanosurface Modification by Anodization for Orthopedic Applications

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ABSTRACT

Titanium is broadly used in orthopedic and dental applications mainly because of its optimal mechanical properties in load-bearing applications. However, insufficient new bone formation is frequently observed on titanium which sometimes leads to implant loosening and failure. For this reason, the objective of the present *in vitro* study was to modify the surface of conventional titanium to include nanostructured surface features that promote the functions of osteoblasts (bone-forming cells). This study focused on creating nanostructured titanium surfaces since bone itself has a large degree of nanostructured roughness that bone cells are accustomed to interacting with. In this study, the surface of titanium was modified by anodic oxidation techniques. The electrolyte used for anodization was hydrofluoric acid. Depending on acid concentration and anodization time, two kinds of different nano-architectures, either particulate or tube-like structures, were formed on the titanium surface. X-ray diffraction results confirmed that the titanium oxide formed on the surface of titanium was amorphous. Scanning electron microscopy (SEM) and atomic force microscopy (AFM) were used to characterize the surface morphology. Cell adhesion studies showed that the anodized nanostructured titanium surface promoted osteoblast adhesion compared to non-anodized titanium. This result indicated that anodization may be a simple method to modify the surface of titanium implants to enhance bone-forming cell function thereby increasing orthopedic implant efficacy.

INTRODUCTION

Titanium and its alloys are among the most utilized biomaterials and are still the materials of choice for many structural implantable device applications [1]. However, current titanium implants face long-term failure problems due to poor bonding to juxtaposed bone, severe stress shielding and generation of debris that may lead to bone cell death and perhaps eventual necrotic bone [2-5]. Improving the bioactivity of titanium implants, especially with respect to bone cells, is a major concern in the near and intermediate future.

Surface properties such as wettability, chemical composition and topography govern the biocompatibility of titanium. Conventionally processed (e.g., cast, forged, etc.) titanium currently used in the orthopedic and dental applications exhibits a micro-rough surface and is smooth at the nanoscale. Surface smoothness on the nanoscale has been showed to favor fibrous tissue encapsulation [4-6]. An approach to design the next-generation of implants has recently focused on creating unique nanotopography (or roughness) on the implant surface, considering that natural bone consists of nanostructured materials like collagen and hydroxyapatite. Some

researchers have achieved nano-roughness in titanium substrates by compacting small (nanometer) constituent particles and/or fibers; such studies found increased osteoblast functions (specifically, adhesion, proliferation, and deposition of calcium containing mineral) on such nano-rough surfaces relative to conventional materials [7,8]. However, nanometer metal particles can be expensive and unsafe to fabricate. For this reason, alternative methods to create nanometer features on titanium are desirable.

Anodization (anodic oxidation) is a method for producing protective oxide layers on metals. Recently, it has become a promising technique to modify the surface of implants because anodization can easily change both the chemical composition and topography of the metal surface. Some studies incorporated calcium phosphate into the oxide layer by using specific electrolytes like calcium glycerophosphate (Ca-GP) and calcium acetate (CA), or by using hydrothermal treatment after anodization. They found enhanced osteoblast function on such anodized surfaces [9-12]. However, few studies have been conducted examining the effects of nanoroughness on cell function in the absence of changes in chemistry. Following the work by Gong et al. [13,14], anodization techniques used in the present study enabled the fabrication of titanium oxide films with unique nanoarchitectures by using hydrofluoric acid (HF) as the electrolyte. The objective of this *in vitro* study is to synthesize nano-rough surfaces on titanium through anodization and determine osteoblast adhesion on such novel surfaces compared to conventional ones.

EXPERIMENTAL

Anodization of Titanium Substrates

99.2% (metal basis) titanium foil was purchased from Alfa Aesar Inc. and was cut into square pieces (1 cm x 1cm) for cell experiments. Before anodization, all of the substrates were cleaned with liquid soap and DI water, dried in the oven at about 65 °C, and then immersed in an acid mixture (2 ml 48% HF plus 3 ml 70 % HNO_3 and 100 ml DI water; all chemicals were obtained from Mallinckrodt) for 5 min to remove the passivation layer on the surface.

The anodization system is an electrochemical cell consisting of a 30V / 3A power supply, a platinum sheet cathode, and an anode made from the titanium substrates. Samples were anodized in a hydrofluoric acid (HF) solution at room temperature with magnetic stirring. According to previous studies conducted by Gong et al. [13,14], the direct-current voltage was set to 20 V and the distance between platinum cathode and anode was kept at about 1 cm during anodization. One set of titanium samples was anodized in 1.5 wt% HF for 5 min to produce a particulate structure while the other set was anodized in 0.5 wt% HF for 20 min resulting in a tube-like structure.

After anodization, all of the substrates were cleaned in acetone and 70 % ethanol (Mallinckrodt), dried in an oven at 65 °C and finally sterilized in an autoclave at about 121 °C for 30 min.

Material Characterization

The microstructure of the titanium substrates was observed using scanning electron microscopy (JEOL JSM 840). Anodized samples were sputter-coated with a thin layer (less than 10 nm) of gold and observed at 5 V.

Phase analysis of the titanium substrate was carried out by x-ray diffraction analysis using CuKα radiation (Simens D500 Diffractometer).

To quantify the topography and roughness, the samples were examined by Multimode Scanning Probe Microscopy (SPM, Digital Instruments Veeco). The multimode SPM was conducted under tapping mode and Nanoscope imaging software was used to analyze root-mean-square (RMS) roughness values. The scan size was 1 μm^2, the scan rate was 2 Hz and all scans were performed in ambient air.

Experiments were completed in triplicate. Statistical significance was considered at $p < 0.01$.

Cell Adhesion Experiments

Human osteoblasts (CRL-11372 American Type Culture Collection) at population number 7 were cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco) supplemented with 10% fetal bovine serum (Hyclone) and 1% Penicillin/Streptomycin (Hyclone). Substrates were placed in 12-well cell culture dishes and were rinsed with phosphate buffered saline (PBS; Sigma) before seeding cells. The cells were seeded at a density of 3500 cells/cm^2 onto each substrate of interest to this study. Cells were then allowed to adhere under standard cell culture conditions (a humidified, 5% CO_2 / 95% air environment at 37 °C) for 4 hours. After the prescribed time period, the cell culture medium was removed from all wells and the substrates were gently rinsed with PBS three times to remove any non-adherent cells. The adherent cells were then fixed with 4% formaldehyde solution and their nuclei stained with Hoescht 33258 dye (Sigma). The cell numbers were counted under a fluorescence illumination (Leica) at 100X original magnification. Five random fields were counted per substrate with all experiments run in triplicate and repeated three separate times. Data were analyzed using standard analysis of variance (ANOVA) procedures followed by a Student's T-test. Statistical significance was considered at $p < 0.05$. Borosilicate glass, which was etched in 1N NaOH for 1 hour, was used as a positive reference substrate in all cell culture experiments. Etched glass has previously been shown to have numerous nanometer features.

RESULTS AND DISCUSSION

Fig. 1(a) and (b) shows the surface morphologies of unanodized and anodized titanium under low magnifications, respectively. Depending on the different HF concentration and duration described before, two kinds of nanoarchitectures were obtained: nanoparticulate structure shown in Fig. 1(c) and nanotube-like structure shown in Fig. 1(d). X-ray diffraction analysis confirmed that all of the oxide layers were amorphous.

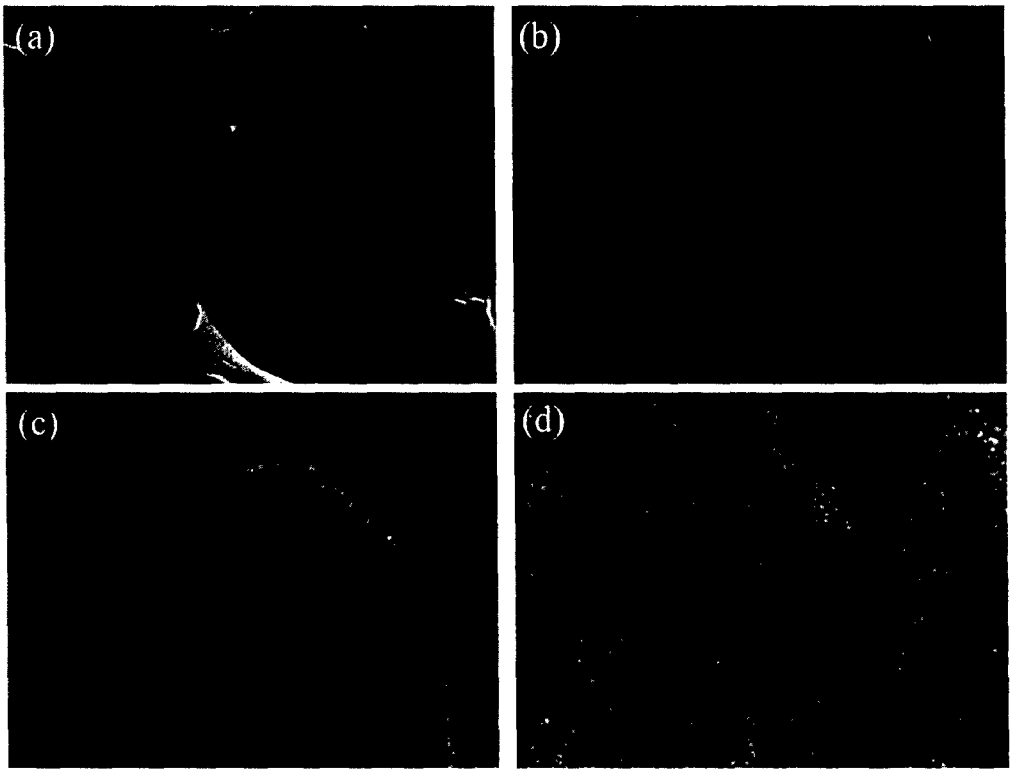

Figure 1. SEM photomicrographs showing the surface morphology of (a) unanodized titanium, bar = 1 μm, (b) anodized titanium under low magnification, bar = 10 μm, (c) anodized titanium under high magnification with nano-particulate structure (treated in 1.5 wt% HF solution for 5 min), bar = 1μm and (d) anodized titanium under high magnification with nanotube-like structure (treated in 0.5 wt% HF solution for 20 min), bar = 1μm.

RMS roughness data obtained from SPM is listed in Table I. Anodized titanium, either nanoparticulate or nanotube-like, possessed increased nanoroughness than unanodized titanium. Further, the anodized titanium surface with a nanotube-like structure was much more rough than the surfaces composed of particulates. Estimated from the SPM image, the diameter of the particles and the inner diameter of the tubes were between 40 and 70 nm.

Table I. Surface Roughness as a Function of Microstructure.

Substrate	Root-Mean-Square (nm), n=3
Unanodized titanium	4.74 ± 1.87
Anodized titanium with nano-particulate structure	10.80 ± 1.73**
Anodized titanium with nano-tube-like structure	25.54 ± 3.02**

** $p < 0.01$ compared to the unanodized titanium.

Significantly enhanced osteoblast adhesion was observed on anodized titanium compared to unanodized titanium (Fig. 2). Specifically, 15% and 20% increases were found for anodized titanium with nanoparticulate and nanotube-like structures, respectively. However, there was no significant difference between the two anodized structures.

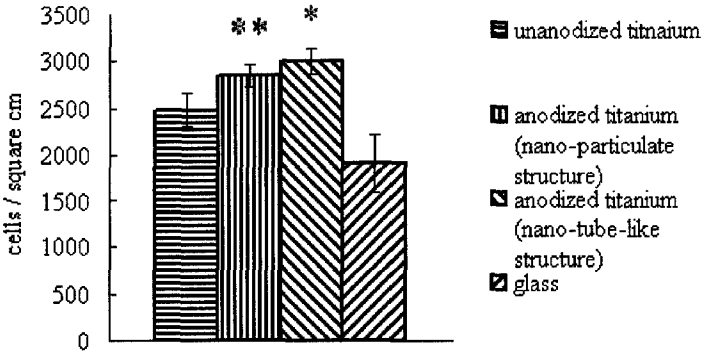

Figure 2. Enhanced osteoblast adhesion on anodized titanium with either nanoparticulate or nanotube-like structures. Cell adhesion tests were conducted under standard cell culture conditions (a 37 °C, 5% CO_2 and 95% air humidified environment) for 4 hours. Samples were anodized in 1.5 wt% HF solution for 5 min and 0.5 wt% HF solution for 20 min to get nanoparticulate and nanotube-like structures, respectively. *$p < 0.05$ compared to the unanodized titanium; **$p < 0.01$ compared to the unanodized titanium. Data are mean ± SEM; n = 3.

As we know, the biocompatibility of titanium comes from its passive oxide film [1]. Similarly, the oxide layer that resulted from the anodization treatment may protect the underlying titanium metal from uncontrolled further oxidation, chemical reaction and corrosion. In this respect, future studies will have to determine whether different amounts of oxidation occurred on the titanium samples due to the various anodization processes employed here.

One critical factor in the present study is that the nanoarchitectures formed by anodization

duplicate the biological size scales that bone cells and related proteins like vitronection and fibronectin are accustomed to interacting with. Many studies have found increased cell function on nanophase materials with nanoscale surface roughness [15-17]. Since cell adhesion is a prerequisite for subsequent functions leading to bone formation, this kind of nanostructure on a titanium surface may help to improve the long term performance of the implants.

CONCLUSION

The present study provided evidence of increased osteoblast adhesion on titanium substrates which were anodized in HF resulting in surface topographies with nanoscale roughness. It is thought that nanoscale roughness played an important role in the attachment of osteoblasts by providing more possible adhesion sites to interact with proteins and cells. The result of the present study indicates that anodization, a quick and inexpensive method of surface modification, is promising for performance improvement of titanium and titanium alloy implants.

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