Vacuum 83 (2009) 569–574

Contents lists available at ScienceDirect

Vacuum

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

Development of sputtered nanoscale titanium oxide coating on osseointegrated implant devices and their biological evaluation

Yunzhi Yang [a,*], Sangwon Park [b], Yongxing Liu [a], Kwangmin Lee [c], Hyun-Seung Kim [b], Jeong-Tae Koh [d], Xianwei Meng [e,f], Kyohan Kim [f], Hongbin Ji [g], Xiaodu Wang [g], Joo L. Ong [h]

[a] Department of Biomedical Engineering and Imaging, University of Tennessee Health Science Center, Memphis, TN 38163, USA
[b] Department of Prosthodontics, College of Dentistry, Chonnam National University, Gwangju 504-190, Korea
[c] Department of Materials Science and Engineering, College of Engineering, Chonnam National University, Gwangju 500-757, Korea
[d] Department of Pharmacology and Dental Therapeutics, Chonnam National University, Gwangju 504-190, Korea
[e] Technical Institute of Physics and Chemistry, Chinese Academy of Science, Beijing 100080, China
[f] Department of Dental Biomaterials, Kyungpook National University, Daegu 2-188-1, Korea
[g] Department of Mechanical Engineering, University of Texas at San Antonio, San Antonio, TX 78249, USA
[h] Department of Biomedical Engineering, University of Texas at San Antonio, San Antonio, TX 78249, USA

Keywords:
Magnetron sputtering
Nanoscale coating
Titania
Titanium implant devices
In vitro cell culture
In vivo animal study

A B S T R A C T

Nanoscale titanium oxide (TiO_2) coating was deposited on titanium (Ti) disks and Ti dental implants using r.f. magnetron sputtering technique. The coating was characterized using grazing angle X-ray diffraction (XRD), scanning electron microscopy (SEM), atomic force microscopy (AFM), contact angle measurement, profilometry and nano-scratch test. The coating also was evaluated with *in vitro* cell culture and *in vivo* dog femur model. Three groups of samples were prepared, including as-sputtered Ti (AS-Ti), sputtered Ti with a post-deposition heat treatment at 600 °C (SH-Ti) and machined Ti (MA-Ti) as controls. The AS-Ti and SH-Ti were dense surfaces consisting of nanoscale grains of 40 nm and 80 nm, respectively. Post-deposition heat treatment increased the coating adhesion. The SH-Ti and AS-Ti significantly decreased the water contact angles compared to the MA-Ti. The nanoscale AS-Ti and SH-Ti significantly improved cell adhesion within the first hour of incubation compared with the MA-Ti. No significant differences were observed in osteoblast proliferation and differentiation *in vitro* as well as reverse torque and histology *in vivo* among the three groups. In the present study, it was not observed that the nanoscale dense TiO_2 coating improved the osseointegration compared to the microscale dense Ti.

1. Introduction

More than one million titanium (Ti) dental implants have been placed into patients annually in the world. [1,2] The number of total hip arthroplasty (THA) is projected to grow 174% by 2030. For knees, a growth of 673% is expected [3]. The success of dental and orthopedic implant devices is believed to be based on osseointegration, which is defined as the direct histological bone-implant contact [4–6] which allows anchorage of the implants in bone tissue and their functional loading [7,8]. Although dental and orthopedic endosseous implant devices have been successfully used in clinics for decades, there are still many aspects of peri-implant bone healing that remains unclear.

The performance of an implant material depends not only on its physicochemical nature but also on its surface [9]. Recently, sintered nanoscale alumina (Al_2O_3) [10–15], titania (TiO_2) [10–15], and hydroxyapatite (HA) [14] ceramics have shown promising results for osteoblast cell adhesion [11–15] and cell function [11,12]. Osteoblast adhesion on nanoscale Al_2O_3 (23 nm grain size) and TiO_2 (32 nm grain size) was significantly greater than on conventional Al_2O_3 (177 nm grain size) and TiO_2 (2.12 μm grain size), respectively [12]. Further investigation indicated the presence of a critical grain size for osteoblast adhesion between 49 nm and 67 nm for Al_2O_3 and 32 nm and 56 nm for TiO_2 [12]. *In vitro* cellular models also have shown that osteoblast proliferation, differentiation and mineralization were significantly greater on nanoscale Al_2O_3, TiO_2 and HA than on conventional microscale formulations of the same

* Corresponding author. Department of Biomedical Engineering and Imaging, College of Medicine, University of Tennessee Health Science Center, 920 Madison Avenue, Suite 1005, Memphis, TN 38163, USA. Tel.: +901 448 1742; fax: +901 448 1755.
E-mail address: yyang19@utmem.edu (Y. Yang).

0042-207X/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vacuum.2008.04.062

ceramics [13]. In addition to sintered nanoscale ceramics, nanoscale metals including titanium (Ti) [16,17], Ti alloy [16,17] and cobalt alloy [16,17] also promote cell adhesion when compared to conventional metals with bigger particle sizes. [17] All the data have suggested that nanoscale surfaces are more biocompatible with respect to osteoblast cell adhesion [11–15,18,19] and cell function [11,12,16,17] compared to conventional microscale materials. However, most of the above results were *in vitro* data. The objective of this project is to develop nanoscale surfaces and test the hypothesis if the nanoscale surfaces on Ti implant enhance osseointegration. In this study, we fabricated and characterized the sputtered nanoscale dense titanium oxide (TiO_2) coatings and evaluated the developed coating using *in vitro* cell culture and *in vivo* animal study.

2. Materials and methods

2.1. Materials preparation and characterization

In this study, we designed three groups of samples: (A) machined Ti as controls (MA-Ti); (B) as-sputtered TiO_2 coated Ti (AS-Ti); and (C) sputtered TiO_2 coated Ti with a post-deposition heat treatment (SH-Ti). The commercially pure Ti disks (diameter 15 mm and thickness 5 mm) were used as substrates. The Ti disks were wet-ground and passivated in 30% nitric acid (ASTM F86-76). One-third of the Ti disks were used as controls (Group A). The rest of the Ti disks were coated with a CMS-18 radiofrequency magnetron sputtering system (Kurt J. Lesker Company, Clairton, PA, USA). The target was the titanium oxide (TiO_2). The sputtering deposition was performed at 300 W, 1.3–1.6 Pa, for 3 h at the deposition rate of 70 nm per hour. After the deposition, one half was as-sputtered TiO_2 coated Ti (Group B), the other half was heat-treated at 600 °C for 1 h (Group C). The surfaces of samples were characterized by scanning electron microscope (SEM, JSM-6301F), grazing angle X-ray diffractometer (XRD, D8 Advance, Bruker), water contact angle measurement (VCA OPTIMA, AST Products, Inc., Boston, MA), and profilometry (Surtronic 25, Taylor-Hobson, Precision, UK). The scratch critical load for adhesion failure was evaluated by nano-scratch test (MTS Nano Indenter XP, MTS Nanoinstruments, Oak Ridge, TN), which was used to characterize the adhesion of the thin coatings on the Ti substrates. Scratch test input parameters were as follows: scratch length: 1000 μm; scratch velocity: 20 μm/s; the normal load was increased from 0 mN to maximum load up to 100 mN; the scratch tests were performed in edge-forward mode.

2.2. In vitro *cell culture*

ATCC CRL 1486 human embryonic palatal mesenchymal (HEPM, American Type Culture Collection, Manassas, VA, USA) cells, an osteoblast precursor cell line was used to evaluate the cell response *in vitro*. The cells were incubated in Dulbecco's modified Eagle's medium (DMEM) containing 7% FBS, penicillin (5000 units ml^{-1}), streptomycin (5000 g ml^{-1}), and fungizone (250 g/ml) in a 5% CO_2 humidified incubator at 37 °C. The osteoblasts from pre-confluent cultures were harvested with 0.25% trypsin – 1 mM EDTA (GibcoBRL, life Technologies, NY), centrifuged, followed by re-suspension in the DMEM.

The initial cell adhesion was conducted within 4 h using a coulter counter (Z2, Beckman Coulter. Inc.). The cell response to the samples was conducted for 3 weeks in the incubator. The amount of dsDNA and alkaline phosphatase produced by the cells on the samples were measured to evaluate the cell proliferation and differentiation. The alkaline phosphatase activity was normalized by the amount of dsDNA.

2.3. In vivo *dog femur model*

Forty-eight commercially available dental implants with machined surface (diameter 4 mm and length 8 mm) were used in this project (ExFeel, MEGAGEN, Korea). The one-third of the dental implants was used as controls (Group A). The rest of dental implants were sputtering coated with TiO_2 coating. The dental implants were rotated 120° between each of three coated periods to cover the entire 360° surface of the specimens. After sputtering deposition, one half was left as as-sputtered coating (Group B), and the other half was subjected to a post-deposition heat treatment at 600 °C for 1 h (Group C).

Four healthy adult dogs, with body weights ranging from 15 to 20 kg were used in this study. The dogs were anesthetized with the combinations of ketamine (5 mg/kg, Yu-han, Gunpo, Korea) and rompun (0.3 mg/kg, Bayer Korea, Ansan, Korea) intramuscularly. The dental implants were placed into the bilateral femur following a protocol approved by the university animal care committee. Four and 8 weeks after implantation, the animals were euthanized, and the bone-implant blocks were harvested. The interfacial strength was measured with reverse torque test and the interface at the bone-implant was observed using SEM and histology. The implants and surrounding bones were fixed in the 10% neutral buffered formalin, dehydrated with ascending concentrations of ethanol for 1 or 2 weeks at each stage. The samples were embedded in polymethylmethacrylate and processed for undecalcified sectioning. The samples were serial sectioned with a Buehler Isomet saw (Buehler, Lake Bluff, IL, USA) and ground to the final thickness of approximately 30–50 μm for subsequent analyses. In this manner, 3–4 sections were obtained buccolingually for the implants. The sections were stained with hematoxylin and eosin. These sections were used for histological qualitative analysis of the bone-implant interface.

3. Results and discussion

3.1. Materials and characterization

The surfaces of the three groups of samples were observed to be scratched and look similar at a low magnification (not shown). At a higher magnification, the Ti controls exhibited dense smooth surface (Fig. 1A), but the as-sputtered TiO_2 surface (AS-Ti) consisted of dense 40 nm grains (Fig. 1B) and the heat-treated sputtered TiO_2 surface (SH-Ti) consisted of dense 80 nm grains (Fig. 1C). It has suggested that the post-deposition heat treatment increased the grain sizes of TiO_2 due to thermal diffusion. Cross-section surface showed that the coating was in the columnar structure and very dense. No pores and microcracks were observed on the cross-section surface (Fig. 1D).

Fig. 2 shows the XRD patterns with an incident grazing angle at 1°. Compared to the Ti control, the 600 °C heat treatment caused a rutile phase on the Ti substrate surface. The as-sputtered TiO_2 coating (AS-Ti) did not exhibit any peaks, suggesting amorphous structure. The 600 °C heat-treated TiO_2 (SH-Ti) coating exhibited a mixture of rutile and anatase phases. We also deposited the TiO_2 coating on glass substrates to avoid the influence of the peaks from the Ti substrates, and the XRD data confirmed the above results. The as-sputtered TiO_2 is amorphous because the eroded target atoms deposited on the substrate on an atom-by-atom basis and the previous crystal structure of the target material was lost during the sputtering process. The amorphous structure will be re-crystallized by the post-deposition heat treatment.

Surface roughness and contact angles of the samples are listed in Table 1. The sputtering deposition increased the surface roughness and the post-deposition heat treatment reduced the surface