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Full Length Article

The effect of two-step surface modification for Ti-Ta-Mo-Zr alloys on bone regeneration: An evaluation using calvarial defect on rat model

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ABSTRACT

This study was conducted to explore the optimal anodization conditions and to evaluate the bioactivity of the coated-CaP TiO_2 nanotube layer on new Ti-Ta-Mo-Zr alloys. The nanotube was fabricated by anodization process, then anodized samples were treated with cyclic precalcification. Critical agents for nanotube morphology are anodizing voltage, time, and electrolyte compositions. The surface features were analyzed by field emission scanning electron microscopy equipped with an energy-dispersive X-ray spectrometer; X-ray diffractometer and surface roughness tester. The results showed clearer morphology and higher density of the nanotube structure on the surface of 71Ti-20Ta-1Mo-8Zr alloy than that of other alloys. The presence of precursors of hydroxyapatite (TCP, OCP) on the precalcified surface of this alloy inclined a high potential of hydroxyapatite formation. For in vivo test, membranes treated with either anodization (AH) or precalcification-combined anodization (APH) were covered on rat calvarial defects (8 mm of diameter) for 5 weeks, and untreated membranes were used as the control. The newly formed bone was evaluated by microcomputed tomography, histologic and fluorescent analysis. The newly formed bone on the APH membrane showed higher bone mineral density and a contact osseointegration, indicating that the APH treatment promotes durable osteogenesis at the early stage of bone defect repair.

1. Introduction

Titanium and its alloys are considered as essential materials for use in biomedical application [1]. As compared to other biometals used as dental implants, titanium and its alloys have noticeably attracted due to their exceptional biocompatibility, corrosion resistance, and mechanical properties such as high hardness, low elastic modulus and low density [2–4]. The critical factor of an excellent implant device is that its Young's moduli should be similar to that of the human bone. The lower discrepancy in modulus between the implant and adjacent bone, the less risk of bone reabsorption and implant failure [5]. Heretofore, the commercial (α + β)-type Ti-6Al-4V alloy is the conventional material used for dental implants due to its outstanding strength and heat resistance. Nevertheless, this alloy also shows some drawbacks, for instance, high Young's modulus (appx. 110 GPa) causes the weak bonding strength to bone [6], and the vanadium oxides formed on the surface by long-term implantation are toxic [7]. In recent, β-type titanium alloys have been considerably attracted by their lower elastic modulus compared with α- and (α + β)-type titanium alloys due to their structure of body-centered cubic (bcc) where titanium atoms are not densely packed [8]. Also, their elastic modulus can be adjusted up to the bone level approximately [9,10]. Most of the representative β titanium alloys used as biomedical materials ordinarily include lots of superior innocuous and allergy-free elements such as Nb, Ta, Zr, and Mo.

After exposing to air, the presence of titania (TiO_2) layer on the surface makes the substrate switch to bioinert state against the corrosion of the titanium substrate [11]. Despite its above advantages, in some vivo cases, this titania layer is enclosed by fibrous tissue leading to the deficiency of osseointegration [12]. Many various surface-modified methods have been conducted to promote the bioactivity and osseointegration of titanium and its alloys since the early bonding of bone-implant interface is affected by implant surface features such as chemical composition, topography, and

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morphology [13,14]. Electrochemical anodization is mentioned as a relatively straightforward and profitable process that turns titanium implant surface into nanostructure, which can accelerate the growth of hydroxyapatite and provide more active surface areas for cellular interaction [15,16]. Under tailoring anodization conditions such as anodization voltage, time and electrolyte composition, the characteristics of the nanotube involving tube diameter, tube length, and surface roughness can be controlled [17].

Precalcification treatment has been reported as one of surface modifications to induce the precipitation of calcium phosphate which enhances the apatite deposition. This buildup benefits to the rapid bone-forming at the early stage after implantation [18,19]. Many of preceding researchers have reported the outstanding success of the combination of anodization and precalcification treatment in improving cell interaction and in enhancing early contact bone formation [20,21].

In this study, we present the fabrication of titania nanotube layers on new β-type titanium alloys containing Ti and Ta, Zr and Mo elements by anodizing in NH_4F containing electrolytes. The impact of anodization under various conditions on the morphology of the nanotube layers and their surface properties was evaluated. The bioactivity of Ca-P coated nanotube by anodization coupled with precalcification was also explored. A vivo test was undertaken on rat calvaria critical size defects to examine the ability of bone regeneration at the early stage of the healing period.

2. Materials and methods

2.1. Sample preparation

In this work, four new titanium alloys were developed as shown in Table 1 using commercial available Ti rods (Pure Chemical Co., Japan) to develop dental titanium alloys. The new titanium alloys with a low modulus of elasticity were designed based on the DV-Xα cluster method [22,23].

The dissolution of the new low elastic modulus titanium alloy was carried out using a non-consumable arc melting furnace (Arc Skull Melting System, Acevacuum, Korea). The alloy was inverted ten times for making alloy composition uniform and then made into an ingot. The ingot was heat-treated at 800 °C for 2 h and cold-rolled to a diameter of 10 mm.

2.2. Preparation of coatings

2.2.1. Development of the nanotube layers

The order procedures for fabricating nanotube structure were carried out by electrochemical anodization based on a previous study [24]. In brief, the sample disks (10 mm in diameter, 1 mm in thickness) were firstly ground and polished with SiC paper, then ultrasonicated for 5 min, and died at 40 °C. For anodization experiments, the glycerol-based electrolyte was used which contained 20 wt.% distilled water and different concentration of NH_4F ranging from 1% to 4%. Anodization processes were performed using the two-electrode system, where the Ti-Ta-X sample and a platinum plate were linked to the anode and cathode of the power source (SPD 303D, Daininotek, Korea) respectively. The applied voltage was varied from 35 V to 65 V, and anodizing time was altered from 1 min to 60 min. The post-anodized samples were rinsed with distilled water (DW) using an ultrasonic cleaner for five seconds and dried at room temperature.

Table 1
Chemical compositions of prepared Ti-XTa alloys (mass.%).

Alloy code	Ti	Ta	Mo	Zr
1	55.0	52.0	3.0	
2	45.5	48.0	3.5	3.0
3	79.0	13.0	2.0	6.0
4	71.0	20.0	1.0	8.0

2.2.2. Preparation of Ca-P coatings

Cyclic precalcification process was conducted by immersing the post-anodized samples in 0.05 M NaH_2PO_4 solution (80 °C, 1 min) and saturated $Ca(OH)_2$ solution (90 °C, 1 min), respectively. In this study, the samples were experienced 20 cycles following the resembling process in our previous research [21]. Subsequently, the samples were annealed at 500 °C for 2 h to obtain the crystalline structural stability and to eliminate remaining moisture and other impurities. The temperature was increased to a heating rate of 10 °C per minute up to 500 °C and kept for 2 h.

2.3. Surface characterizations

A field emission scanning electron microscope (FE-SEM, S-800; Hitachi, Tokyo, Japan) equipped with an energy-dispersive X-ray spectrometer (EDS; Bruker, Billerica, MA, USA) was used to explore the surface morphology, cross-sectional view of the nanotube arrays and the chemical composition of the resulting surfaces. The arithmetical mean roughness (Ra) was checked using a surface roughness tester (Surftest SV-3000 M4; Mitutoyo Corporation, Kawasaki, Japan). Multi-purpose high-performance X-ray diffractometer (XRD; X'pert Power, PANalytical Co., Japan) was used to determine the crystal phases of the sample surface.

2.4. Animal testing

2.4.1. Materials for the animal experiment

To evaluate bone-material reaction, Ti-Ta-X (X = Mo, Zr) membranes 10 mm in diameter and 0.15 mm in thickness were prepared. Untreated membranes (UT), TiO_2 nanotube membranes (AH) and Ca-P coating TiO_2 nanotube membranes (APH) were set up as described above.

2.4.2. Procedures

To examine the in vivo osseointegration, nine male Sprague-Dawley rats (8 weeks of age and weighing from 210 g to 250 g) were undergone operations. Animal operations were authorised by the Institutional Animal Care and Use Committee of the Chonbuk National University Laboratory Animal Center, Jeonju, South Korea (Approved number: CBNU 2017-0049). Before surgery, the rats were housed at constant temperature and humidity for stabilization. The rats were split into three groups with three rats in each one. The equipment was sterilized by autoclave, then was allowed cooling to room temperature.

General anesthesia was induced by intramuscular administrating 50 mg/kg of Zoletil (Zoletil 50, Virbac Laboratories) mixed with 15 mg/kg Xylazine (Rompun, Bayer). The procedure area was shaved and cleaned using iodine scrubs. Local hemorrhaging was reduced by subcutaneous injection of Lidocaine 1% (1:100,000 epinephrine). The operation started with a midsagittal incision down to periosteum. The incised periosteum was gently lifted and pushed laterally to visualize the calvarium on both sides of the midline. A critical size defect (8 mm in diameter) was created by a trephine bur with an inner diameter of 8 mm under saline irrigation. After rinsing off bone chips, one of three membranes (UT, AH or APH) was alternately placed to enshroud the defect. The raised periosteum and skin were closed over the membrane with bioabsorbable suture and 4-0 silk suture, respectively. For three days post-operatively, the rats received intramuscularly administered antibiotic (300 μL/kg) for preventing infection. The rats were euthanized after five weeks, and biopsies were conducted to take out