Materials Science and Engineering C 49 (2015) 159–173

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Bioactivity of coatings formed on Ti–13Nb–13Zr alloy using plasma electrolytic oxidation

Maciej Sowa [a], Magdalena Piotrowska [a], Magdalena Widziołek [b], Grzegorz Dercz [c], Grzegorz Tylko [b], Tadeusz Gorewoda [d], Anna M. Osyczka [b], Wojciech Simka [a],*

[a] Faculty of Chemistry, Silesian University of Technology, B. Krzywoustego Street 6, 44-100 Gliwice, Poland
[b] Faculty of Biology and Earth Sciences, Jagiellonian University, Gronostajowa Street 9, 30-060 Kraków, Poland
[c] Institute of Materials Science, University of Silesia, 75 Pulku Piechoty Street 1a, 41-500 Chorzow, Poland
[d] Institute of Non-Ferrous Metals, Sowińskiego Street 5, 44-100 Gliwice, Poland

ARTICLE INFO

Article history:
Received 18 July 2014
Received in revised form 9 December 2014
Accepted 20 December 2014
Available online 23 December 2014

Keywords:
Titanium alloy
Surface modification
Anodic oxidation
Osteogenesis
Adult mesenchymal stem cells

ABSTRACT

In this work, we investigated the bioactivity of anodic oxide coatings on Ti–13Nb–13Zr alloy by plasma electrolytic oxidation (PEO) in solutions containing Ca and P. The bioactive properties of the films were determined by immersion in simulated body fluid (SBF), and their biocompatibility was examined using adult human bone marrow derived mesenchymal stem cells (hBMSCs). The oxide layers were characterised based on their surface morphology (SEM, AFM, profilometry) as well as on their chemical and phase compositions (EDX, XRF, XRD, XPS). We report that anodic oxidation of Ti–13Nb–13Zr led to the development of relatively thick anodic oxide films that were enriched in Ca and P in the form of phosphate compounds. Furthermore, the treatment generated rough surfaces with a significant amount of open pores. The surfaces were essentially amorphous, with small amounts of crystalline phases (anatase and rutile) being observed, depending on the PEO process parameters. SBF soaking led to the precipitation of small crystals after one week of experiment. During culturing of hBMSCs on the bioactive Ti–13Nb–13Zr surfaces the differentiation of human mesenchymal stem cells toward osteoblasts was promoted, which indicated a potential of the modified materials to improve implant osseointegration.

1. Introduction

Currently, bone reconstruction strategies often involve the use of metallic biomaterials. Titanium and its alloys are commonly used because of their good mechanical strength, low density, chemical inertness in the tissue environment, biocompatibility, ability to osseointegrate and the lowest elastic modulus (i.e., 48–112 GPa) compared to other metallic biomaterials [1–3]. Ti–6Al–4V alloy is widely used, despite the fact that it was initially developed for aeronautical use. However, problems regarding the use of this alloy in biomedical engineering have been reported. Poor wear resistance and a relatively high elastic modulus (110 GPa) can be listed among these problems [4,5]. Moreover, Ti–6Al–4V alloy is considered to be toxic due to the presence of carcinogenic vanadium and its oxide (V_2O_5) and possibly neurotoxic aluminium [6–10]. As a result, a new generation of vanadium-free alloys has been engineered [11–13], including Ti–13Nb–13Zr, which has been identified as a potentially non-toxic alloy that is capable of supporting bone growth.

Anodic oxidation is a relatively non-expensive method for surface modification. It can be performed via two distinct modes of operation. The conventional mode, whereby the metal workpiece is polarised anodically at voltages that do not exceed the potential corresponding to dielectric breakdown of the oxide layer, and the second mode — plasma electrolytic oxidation (PEO) or micro-arc oxidation (MAO). The latter name comes from the plasma- and thermochemical reactions that occur on the surface of the treated metal, when the threshold of dielectric breakdown voltage is breached. Relatively thin, homogeneous oxide coatings of barrier type can be produced by conventional anodising. In contrast, PEO coatings are highly porous and considerably thicker than the conventional anodic films. The control of thickness and surface morphology of the oxide coating permits indirect manipulation over corrosion resistance of the anodised material. Thick (micrometer range), tight and compact oxide layer, free from deeply penetrating cracks, slows down the rate of corrosion of underlying metal or metal alloy. Furthermore, increased amounts of electrolyte species can be incorporated into the structure of the oxide layer in the course of PEO treatment [14–18].

Adult human bone marrow derived mesenchymal stem cells (hBMSCs) are the multipotential cells capable of differentiating into osteoblasts, adipocytes, chondrocytes and myoblasts and other cell types [19–21]. The process of bone regeneration requires the recruitment and migration of osteoblast progenitors via the blood and epithelium of blood vessels to specific locations within the organism or to the MSC niche in bone marrow [22]. Next, osteoprogenitors

---

* Corresponding author.
E-mail address: wojciech.simka@polsl.pl (W. Simka).

http://dx.doi.org/10.1016/j.msec.2014.12.073
0928-4931/© 2014 Elsevier B.V. All rights reserved.

begin to produce collagen-rich extracellular matrix, which is subsequently mineralised [22,23]. Biomaterials designed to be used in bone reconstruction should at least be osteoconductive or osteoinductive. Thus, they should not only stimulate the migration of osteoprogenitor cells toward bone defects, but also support or stimulate the differentiation of these progenitors into osteoblasts to repair the damaged bone tissue [2,19,24,25].

Surface of a biomaterial which has been enriched with calcium and phosphorus can be characterised in terms of its chemical composition (Ca/P ratio), surface topography and crystallinity. It is believed that calcium phosphate coatings with Ca/P atomic ratio are similar to that of stoichiometric hydroxyapatite (approximately 1.67) in order to induce apatite precipitation in simulated body fluid (SBF). However, it was determined that higher Ca/P ratios increase the adsorption of adhesion proteins onto modified surface. Nanotopography of material has a direct impact on adsorption of adhesion proteins, which in turn affects cell adhesion and proliferation, while microtopography provides so-called niches with conditions suitable for cell growth. High crystallinity of CaP in the coatings promotes slower dissolution, which has a detrimental effect on cell attachment, spreading and proliferation [26].

Although Ti–13Nb–13Zr alloy has been widely described in the recent literature [16,27–29], to the authors' best knowledge, this is the first report on the ability of this alloy to support the osteogenic differentiation of hBMSCs in vitro. The surface modification of Ti–13Nb–13Zr alloy using PEO in solutions containing calcium and phosphorus compounds was performed to produce anodic oxide layers, which exhibited bioactive properties. The obtained coatings were characterised based on their surface morphology as well as their chemical and phase compositions. The bioactivity of these coatings was determined by an SBF immersion test, and the biocompatibility of the coatings was studied in short- and long-term osteogenic hBMSC cultures.

2. Materials and methods

2.1. Materials and pretreatment

Rectangular samples of Ti–13Nb–13Zr alloy (BIMO metals, Wrocław, Poland) were prepared from a sheet of alloy, and their chemical composition is presented in Table 1. The surface area of the face that was modified was equal to 5 cm^2. In addition, disc-shaped samples were cut from Ti–13Nb–13Zr alloy rods (diameter = 8 mm). The specimens were mechanically ground using waterproof abrasive paper up to a #800-grit size. They were then chemically etched in a solution that contained 1 mol dm^{-3} HF and 4 mol dm^{-3} H_2SO_4 for 1 min. Afterwards, the samples were rinsed with distilled water and ultrasonically cleaned in a deionised water/2-propanol mixture for 5 min. Then, the titanium alloy specimens were electropolished in a solution composed of concentrated H_2SO_4 (10 mol dm^{-3}), ethylene glycol (5.4 mol dm^{-3}) and NH_4F (0.4 mol dm^{-3}). The process was carried out at a constant current density of 1 A cm^{-2} for 210 s. Then, the samples were cleaned ultrasonically.

2.2. Anodisation of the Ti–13Nb–13Zr alloy

The sample labels and process conditions are summarised in Table 2. The pretreated specimens (TNZ-EP) were subjected to anodisation in electrolytic baths I–IV, which contained different amounts of $Ca(H_2PO_2)_2$ and H_3PO_4 (Table 2). The anodic oxidation was performed under a constant current of 0.31 A cm^{-2} and up to final voltage of either 200 or 400 V for 10 min. The anodised specimens were rinsed with distilled water and ultrasonically cleaned in a deionised water/2-propanol mixture for 5 min. A DC power supply (PWR 800H, Kikusui, Japan) was used throughout these treatments. The process was performed in a water-cooled electrolysis cell with a titanium mesh cathode and magnetic stirrer. The titanium alloy specimen served as the anode.

Table 1
Chemical composition of Ti–13Nb–13Zr alloy (wt.%).

Nb	Zr	O	Fe	C	H	N	Ti
13.3	13.0	0.10	0.08	0.05	0.009	0.004	Balance

Table 2
Sample labels, treatment conditions and roughness parameters of the titanium alloy specimens.

Sample	$Ca(H_2PO_2)_2$, mol dm^{-3}	H_3PO_4, mol dm^{-3}	*U*, V	*Ra*, µm
TNZ-I-200	0.01	–	200	0.10 ± 0.03
TNZ-I-400			400	0.14 ± 0.02
TNZ-II-200	0.1	–	200	0.09 ± 0.02
TNZ-II-400			400	0.43 ± 0.09
TNZ-III-200	1	–	200	0.51 ± 0.08
TNZ-III-400			400	1.50 ± 0.18
TNZ-IV-200	0.1	0.1	200	0.13 ± 0.03
TNZ-IV-400			400	0.91 ± 0.15

2.3. Surface property characterisation

2.3.1. Scanning electron microscopy and energy-dispersive X-ray analysis

The modified Ti–13Nb–13Zr surfaces were examined in terms of their elemental compositions and morphology by the use of a scanning electron microscope (SEM, Hitachi S-3400N, Japan) operating at an accelerating voltage of 25 kV.

2.3.2. Surface roughness characterisation

The *Ra* parameter of the oxide layers on the Ti–13Nb–13Zr specimens was determined using a Mitutuyo Surftest SJ-301 profilometer, in accordance with the EN ISO 4287:1997 standard [30]. The *Ra* parameter was calculated according to the formula:

$$Ra = \frac{1}{l}\int_0^l |Z(x)|dx, \tag{1}$$

where *l* is the elementary length in the *x* coordinate, used to determine the irregularity of the investigated surface, and $|Z(x)|$ is the absolute ordinate value inside *l*.

2.3.3. Atomic force microscopy (AFM)

The nanoscale surface topography of selected samples was examined using an atomic force microscope (NanoScope E, Digital Instruments) operating in contact mode. Standard silicon nitride cantilevers with a spring constant of 0.12 N m^{-1} were used.

2.4. Characterisation of the anodic oxide film composition

Four samples were selected based on the preliminary surface studies (described above), for detailed characterisation. Microscopic and spectral methods were used, as described below.

2.4.1. Cross-sectional analysis

The cross-section of the anodic oxide films on the titanium alloy samples was examined using a high-resolution field emission scanning electron microscope (HR-FESEM, Inspect F, FEI Company, USA) equipped with an EDX system (EDAX, Oxford Instruments, UK).

2.4.2. X-ray diffractometry

The phase compositions of the oxide layers formed on the Ti–13Nb–13Zr alloy specimens were determined using an X-Pert Philips PW 3040/60 diffractometer operating at 30 mA and 40 kV. A vertical goniometer and Eulerian cradle were used throughout the experiments. The wavelength of the radiation source (λCuKα) was 0.154178 nm.