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Comparative investigations of structure and properties of micro-arc wollastonite-calcium phosphate coatings on titanium and zirconium-niobium alloy

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ABSTRACT

Investigation results of micro-arc wollastonite–calcium phosphate (W–CaP) biocoatings on the pure titanium (Ti) and Zr–1wt.%Nb (Zr–1Nb) alloy were presented. The voltages of 150–300 V generate the micro-arc oxidation (MAO) process with the initial amplitude current of 150–550 A and 100–350 A for Ti and Zr–1Nb substrates, respectively. The identical dependencies of changes of the coating thickness, surface roughness and adhesion strength on the process voltage were revealed for the both substrates. The W–CaP coatings with the thickness of 10–11 μm were formed on Ti and Zr–1Nb under the low process voltage of 130–150 V. Elongated wollastonite particles with the size in the range of 40–100 μm were observed in such coatings. The structure of the coatings on Ti was presented by the X–ray amorphous and crystalline phases. The X–ray reflexes relating to the crystalline phases of Ti and wollastonite were observed only in XRD patterns of the coatings deposited under 130–200 V on Ti. While, the crystalline structure with phases of $CaZr_4(PO_4)_6$, β–ZrP_2O_7, ZrO_2, and Zr was detected in the coatings on Zr–1Nb. FT–IRS, XRD, SEM, and TEM data confirmed that the increase of the process voltage to 300 V leads to the dissociation of the wollastonite. No toxic effect of specimens on a viability, morphology and motility of human adipose–derived multipotent mesenchymal stem cells was revealed *in vitro*.

1. Introduction

The most commonly used alloys in medicine are commercially pure titanium Grade 2 and Grade 4, titanium alloys Ti–6Al–4V, Ti–6Al–4V Eli, and vanadium–free alloys as Ti–6Al–7Nb and Ti–6Al–2.5Fe. However, preferred alloys without any toxic alloying elements, such as A1, V, Mo and others [1,2] that could affect the organism. In this case, the most perspective ones are valve bioinert metals – Ti, Zr, Nb, Hf, Ta and their alloys. The main advantages of these materials are good biocompatibility, hypotoxicity, high corrosion resistance, low thermal linear expansion, low thermal conductivity, non–magnetization, and insignificant specific weight. Commercially pure Ti is the most common material used for manufacturing of orthopedic and dental implants. However, low mechanical strength properties, especially low ultimate strength, limit the further application of pure titanium as implant material. In this case, the zirconium doped with niobium alloys were widely used in medicine due to the high mechanical properties as a result of primarily solid solution hardening. The Zr-1Nb and Zr-2.5Nb alloys have complex properties such as biocompatibility, low thermal conductivity, high fatigue strength and high corrosion resistance that provide their application as implant material [3].

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However, there is the problem of the acceptance of metallic implants in the human body. The solution to this problem is the applications of calcium phosphate (CaP) coatings which has a positive effect on living organism and stimulate the regeneration of bone tissue.

Micro-arc oxidation (MAO) also known as plasma electrolytic oxidation (PEO) is a relatively new technique of the surface treatment based on anodic oxidation, which became well-known for its ability to form *in situ* grown porous and homogeneous oxide coatings on such metals as Ti, Al, Mg, Nb, Zr and their alloys. Additionally, it is one of the most effective methods to modify the metallic surface by the CaP coating formation for the best biocompatibility and bioactivity [4–6]. Besides, the application of micro-arc treatment allows to deposit biocompatible coating with gradient structure, rough and porous morphology [5]. The surface properties of the implant such as surface topography, chemical and phase composition determine its interactions with the surrounding host tissue and are of prime importance for better cell adhesion, spreading and proliferation [7,8].

MAO modification of Ti, Mg, and their alloys has been extensively investigated [4,5,9], whereas studies of MAO modification of Zr and its alloys are still quite limited [6,10–12]. It was demonstrated by Ref. [11], that depending on the anodic oxidation conditions for deposition of oxide coatings on Zr, i.e. voltage, current density, electrolyte composition and temperature, it is possible to grow of oxide layers with thickness of several hundred nanometers. Galvanostatic anodic oxidation of Zr in sulphuric acid leads to stress-induced oxide breakdown under voltages above 120 V. To obtain the layers with higher concentrations of bioactive compounds, the MAO process can be performed in suspensions [13]. The addition of hydroxyapatite (HA), wollastonite (W), tricalcium phosphate (TCP), silica and other bioactive powders into electrolyte may enrich the coatings. A. Kazek-Kęsik and research group [14,15] proposed to add the wollastonite ($CaSiO_3$), and silica (SiO_2) into the electrolyte to deposit the bioactive coatings on Ti–15Mo and Ti–13Nb–13Zr alloy by PEO method. They showed that wollastonite embedded in the coatings provides their improved biological activity. The possibility of the formation of wollastonite-calcium phosphate (W–CaP) coatings on Ti and Ti-40 wt%Nb alloy by the MAO method was shown in our previous reports [16,17].

This paper presents the results of comparative investigations of the structure and properties of wollastonite–calcium phosphate coatings deposited by the MAO method on Ti and Zr–1wt.%Nb (Zr–1Nb) alloy.

2. Materials and methods

Commercially pure titanium (99.58 Ti, 0.12 O, 0.18 Fe, 0.07 C, 0.04 N, 0.01 H wt%) and Zr–1Nb alloy (96.54 Zr, 1.0 Nb, 0.32 Mo, 0.02 Si, 0.1 W, 0.29 Fe, 0.88 Ti, 0.1 O wt%) were used as substrates. The size of the samples was 10 × 10 × 1 mm^3. Samples were prepared with silicon–carbide paper of 120, 480, 600, 1200 grit, in series. Then samples were ultrasonically cleaned for 10 min in distilled water. The average roughness (*Ra*) of the samples was 0.3–0.5 μm. In order to carry out the MAO method the Micro–Arc 3.0 technique was used as in previous works [5,18]. The installation consists of a pulsed power source, galvanic bath with water cooling system, molibdenum electrodes, and software for controlling the deposition process. The electrolyte solution was prepared based on 30% phosphoric acid (H_3PO_4) with pH = 1–2. Then the hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) and wollastonite ($CaSiO_3$) powders were added into the electrolyte in amount of 60 g/l and 75 g/l, respectively. Hydroxyapatite (HA) particles were 10–50 nm in size, and wollastonite (W) particles were 5–100 μm in length with average length of 35 μm. As a result, the homogeneous electrolyte with an ultradispersed phase was produced. The MAO process was carried out with the following parameters: pulse frequency of 50 Hz, pulse duration in the range of 100–500 μs, electrical voltage in the range of 130–300 V, and process duration from 5 to 10 min.

The morphology and microstructure of the coatings were examined by scanning electron microscopy (SEM, Zeiss LEO EVO 50, Germany) and transmission electron microscopy (TEM, JEOL JEM–2100, Japan) in “Nanotech” center at ISPMS SB RAS. In addition, the elemental compositions and distributions of the coatings were analyzed using energy-dispersive X-ray spectroscopy (EDX, Pegasus XM2 and INCA, Oxford Instruments) in combination with the SEM systems. The phase composition was determined by X–ray diffraction analysis (XRD, DRON–7 “Nanotech” center) in the angular range of $2\theta = 10–95^\circ$ with a scan step of 0.02° with Co Kα radiation. Infrared spectra were measured with a Fourier transform infrared spectrophotometer (FT–IRS, BIO RAD FTS 175, Germany) in the wave number range of 400–4500 cm^{-1}. The surface roughness was estimated with a Hommel–Etamic T1000 profilometer (Jenoptik, Germany) by the average roughness (*Ra*). The traverse length and rate of the measured profile were 6 mm and 0.5 mm/s, respectively. To measure the coating adhesion strength to the substrates, two cylinders were glued by the Loctite Hysol 9514 glue to both sides of the coated specimen. The specimens were fixed by grips in the Instron–1185 machine (Instron, USA) for carrying out the tensile test. Adhesion strength was measured as $\sigma_A = F/S$, where *F* is the breakout force and *S* is the area of broken out coating from substrate [19].

Biological tests *in vitro* of specimens with coatings were carried out using human adipose–derived multipotent mesenchymal stem cells (AMMSCs). For biological tests the Ti and Zr-1Nb specimens with W-CaP coatings were dry–heat sterilized with Binder FD53 (Binder GmbH, Tuttlingen, Germany) at 453 K for 1 h. Single specimen per each well was placed in 12–well plastic plates (Orange Scientific, Belgium) with cell culture medium. The control group was a cell culture medium without tested specimens. The AMMSCs culture was prepared from human fat tissue after processing of lipoaspirates (Permission No. 4 from 23.10.2013 of Local Ethics Committee of Innovation Park of Immanuel Kant Baltic Federal University) as particularly described by Ref. [20]. The cell suspension was freshly prepared with a concentration of 5 × 10^4 viable karyocytes/mL of the following culture medium: 90% DMEM/F12 (1:1) (Gibco Life Technologies; Grand Island, NY, USA), 10% fetal bovine serum (Sigma–Aldrich, St. Louis, MO, USA), 50 mg/l gentamicin (Invitrogen, UK) and freshly added L–glutamine sterile solution in a final concentration of 280 mg/l (Sigma–Aldrich). The cell culture was incubated for 7 days in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. The morphology of adherent cells, their motility and ability to form a monolayer in contact (in the interface) with the tested specimens was studied using integrated platforms for continuous visualization of living cells Cell–IQ® v2 MLF (CM Technologies, Finland). Digital images were obtained in 20 min during 7–day culturing. A viability of cells contacted *in vitro* with tested specimens was estimated by 0.4% trypan blue dyeing (Invitrogen, USA) according to ISO 10993-5 with the help of Countess ™ Automated Cell Counter (Invitrogen, USA). To determine a viability adherent AMMSCs were removed in 7 days from plastic wells by standard EDTA-trypsin processing and washed twice by culture medium.

3. Results and discussion

The influence of process voltage and substrate material on structure, morphology, thickness, roughness and adhesion strength of the W–CaP coatings were revealed. The graphs of amplitude current of the coating deposition against the MAO process duration