Development of calcium phosphate coatings with regulated porous structure as drug carrier systems

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Abstract. The calcium phosphate coatings were formed by the micro-arc oxidation method under different applied voltage. It was shown that the thickness, surface roughness, porosity and sizes of the structural elements of the coatings increased with increasing of the applied voltage. In addition, the increase in the voltage led to the structural-phase transformation in the coatings from the amorphous state to the amorphous-crystalline state with incorporation of CaHPO$_4$, $\alpha$-Ca$_2$P$_2$O$_7$, $\beta$-Ca$_2$P$_2$O$_7$ and TiO$_2$ (anatase) nano-sized phases. The micro-porous amorphous coatings formed at a low voltage of 200 V showed the highest adsorption of the doxorubicin. The formed coatings with regulated porous structure and specified phase composition can be used as anticancer (e.g., doxorubicin) drug carrier systems.

1. Introduction
A promising direction in a biomedical materials science is the development of new composite biomaterials based on calcium phosphate (CaP) ceramic with a specified porous interconnected structure as drug carriers (antibacterial, anticancer, etc.) [1]. The success of developing such CaP-based specified systems as a matrix for controlled drug delivery and release is largely determined by their characteristics, such as biocompatibility, adsorption properties, mechanical strength, specific composition and structure, preventing uncontrolled release of the drug and sufficiently studied methods of creating porous structures of a wide range of morphology [2]. CaP ceramics are used in the different forms, such as powders; granules; bulk materials; as coatings on metals; as components of composite materials in the form of a crushed or continuous matrix [3]. The most promising and technologically advanced method for forming on the metal surface of the CaP coatings with a wide range of physical and chemical properties, varying crystallinity, thickness, roughness and porosity is the micro-arc oxidation (MAO) [4]. By varying the process electrophysical parameters, changing the electrolyte composition and the substrate material, it can be control the properties, structure and composition of the formed coatings. The aim of the work was to produce the CaP coatings with a regulated porous structure as anticancer (e.g., doxorubicin) drug carrier systems, and investigate their morphology, structure, composition and absorption properties.

2. Materials and methods
The experimental specimens with sizes of 10×10×1 mm$^3$ were cut from the billets of commercially pure titanium (Ti, grade 2). The specimens were polished with a series of increasingly finer abrasive papers up to 1200 grit. Then, they were ultrasonically cleaned in the distilled water and ethanol. The Micro-Arc 3.0 installation with the pulsed electrical source was used to synthesize the MAO
biocoatings [4, 5]. The electrolyte contained 30 % H₃PO₄, 100 g/l CaCO₃ and 60 g/l hydroxyapatite (HA, Ca₅(PO₄)(OH)₂) nano-powders. The MAO processing carried out in the anodic potentiostatic regime at the fixed pulse frequency of 50 Hz, pulse duration of 100 µm and processing time of 10 min. The MAO voltage was varied from 200 to 300 V with the step of 50 V.

The morphology and microstructure of the coatings were analyzed by the scanning electron microscopy (SEM, Zeiss LEO EVO 50) and transmission electron microscopy (TEM, JEOL 2100), respectively, in the “Nanotech” center at ISPMS SB RAS, Tomsk, Russia. The phase composition was determined with X-ray diffraction (XRD, Shimadzu XRD 6000) in the angular range of 2θ = 10–90° with a scan step of 0.02° using Cu Kα radiation The surface roughness was measured with a Profilometer-296 via the average roughness parameter (R̴a).

Impregnation of CaP coatings was performed by the direct immersion of the coated samples into the solution of doxorubicin (DOX) (TEVA Pharmaceutical Industries, Netherlands/Israel) with a concentration (C₀) of 0.103±0.001 mg/ml. Each pre-weighted CaP coated sample coating was placed in 30 ml tube, and then 10 ml doxorubicin solution was added. All samples were immersed in the solution at room temperature for 12 hours. The doxorubicin concentration in the solution (C) after impregnation was measured using spectrophotometer (SP-2000) with wavelength of 480 nm and the cuvette optical path length of 10 mm [6]. After immersion in the solution, the samples were dried at 100 °C for 2 hours, and then the amount of doxorubicin adsorbed by the samples was determined.

3. Results and discussion
The SEM-images of the surface and cross-section of the CaP coatings deposited at 200 and 300 V is presented in figure 1. It can be seen that the coating surface morphology is represented by the spheroidal shaped structural elements (spheres and hemispheres) with the internal micro-pores and the micro-pores in the intergovernmental spaces (figure 1(a), (c), (e)). The SEM images of the cross-sectional coatings show the complex porous structure including the multiple branched round and elliptic pores inhomogeneously distributed over the coating thickness (figure 1(b), (d), (f)). With increasing of the applied voltage from 250 to 300 V the intensity of micro-arc discharges and the current density increase as was shown previously [4, 5]. It leads to the linear increase of the coating thickness from 40 to 90 µm and the average roughness from 3.0 to 7.5 µm. In addition, with increasing of the voltage the spheres on the coating surface increase in sizes and partially destruct, the plate-shaped crystals (up to 15 µm) are formed in the destroyed hemispheres, and the local micro-pores with large sizes (15–30 µm) are formed in the interface between the substrate and the coating (figure 1(d), (f)). Such morphological transitions lead to the increase of the surface porosity from 20 to 35 %.

XRD analysis revealed that the MAO coatings formed at a low voltage of 200 V are predominantly in the X-ray amorphous state (figure 2). It is confirmed by the presence of diffused halo in the small angles (2θ = 20–38°) and reflections from single Ti phase of substrate in the corresponding XRD pattern. XRD patterns of the coatings formed at high voltages of 250–300 V include diffused halo as well as the reflections from crystalline phases of dicalcium phosphate (monetite, CaHPO₄) and β-calcium pyrophosphate (β-Ca₃P₂O₇). The intensity of the reflections from these CaP phases increases with increasing voltage (figure 2). Previously [5] we described the mechanism of the crystalline phases formation in the coatings occurred due to the growth of the temperature inside the micro-arc discharges. Thus, the increase of the MAO voltage leads to the coating structure transformation from X-ray amorphous state into the amorphous-crystalline state. These XRD data are in the agreement with the SEM results indicating the incorporation of plate-shaped crystals corresponding to the crystalline CaHPO₄ phase in the coatings formed at the high voltages (figure 1(c), (d)).

Figure 3 shows bright field (BF) and dark field (DF) TEM images of selected area diffraction (SAD) patterns for fragments of the CaP coatings formed at 200 and 300 V. TEM studies showed that the coatings deposited at lowest voltage of 200 V have mainly amorphous microstructure (figure 3(a)–(c)). It is confirmed by the SAD pattern including diffused halos from amorphous CaP substance and very weak pinpoint reflections (figure 3(b)). However, with increasing of the applied voltage the microstructure in the coatings transforms into the crystalline-amorphous state (figure 3(d)–(h)). The
SAD pattern of the coating formed at 300 V includes numerous heavy pinpoint reflections as well as weak diffused halos (figure 3(e)). The indexing of the SAD patterns revealed the following crystalline phases in the coatings: CaHPO₄ with triclinic lattice; α-Ca₃P₂O₇ with orthorhombic lattice; β-Ca₃P₂O₇ with tetragonal lattice; TiO₂ (anatase) with tetragonal lattice. The DF TEM images show that the crystallites of these phases have nano-sizes less than 100 nm (figure 3(c), (f), (g), (h)).

Figure 1. SEM-images of the surface (a), (c), (e) and cross-section (b), (d), (f) of the CaP coatings formed under different applied voltages: 200 V (a), (b); 250 V (c), (d); 300 V (e), (f).

Figure 2. XRD patterns of the CaP coatings formed under different applied voltages.
Figure 3. BF TEM (a), (d) and DF TEM (c), (f)–(h) images and SAD patterns (b), (f) of the particles of the CaP coatings deposited at 200 V (a)–(c) and 300 V (d)–(h).

The impregnation studies is represented in Table 1. It can be seen that the CaP coatings formed at lowest voltage of 200 V (samples No. 1 and No. 2) have highest adsorption capacity in relation of doxorubicin. After 12-hours immersion, these coatings adsorbed the doxorubicin in amount of 0.0126±0.0002 mg/ml.

Table 1. Amount of doxorubicin adsorbed by the coated samples.

| Sample | Coated sample mass, g | Coating mass, mg | \( C_0 \), mg/ml | C, mg/ml | DOX amount, mg | DOX amount on coated sample, mg/g | DOX amount on coating, mg/mg |
|--------|-----------------------|------------------|-----------------|----------|---------------|----------------------------------|-----------------------------|
| No. 1 (U=200V) | 0.5027 | 14.9 | 0.0840 | 0.190 | 0.3779 | 0.0128 |
| No. 2 (U=200V) | 0.5292 | 14.2 | 0.0855 | 0.175 | 0.3307 | 0.0123 |
| No. 3 (U=250V) | 0.5337 | 20.9 | 0.0836 | 0.194 | 0.3635 | 0.0093 |
| No. 4 (U=250V) | 0.5773 | 21.2 | 0.0847 | 0.183 | 0.3170 | 0.0086 |
| No. 5 (U=300V) | 0.5321 | 29.4 | 0.0845 | 0.185 | 0.3477 | 0.0063 |
| No. 6 (U=300V) | 0.5354 | 27.1 | 0.0847 | 0.183 | 0.3418 | 0.0068 |
In opposite, the coatings formed at high voltages (samples No. 5 and No. 6) have lowest adsorption of doxorubicin (0.0065±0.0002 mg/ml). It can be due to the difference in the structural and morphological properties of the coatings deposited under different applied voltages. The coatings formed at 200 V have amorphous structure and contain numerous pore channels on the coating surface both inside the solid spheres and in the interstructural spaces. While, the coatings applied at a high voltage of 300 V are characterized by an amorphous-crystalline structure, and the surface contains numerous solid destroyed spheres, fragments and monetite crystals preventing the drug adsorption.

4. Conclusions
The calcium phosphate coatings were formed by the micro-arc oxidation method under different applied voltage. It was shown that with increasing of the applied voltage from 200 to 300 V the coating thickness (40–90 µm), surface roughness (3.0–7.5 µm), porosity (20–35%) and sizes of the structural elements (spheres and pores) increased. In addition, the increase in the voltage led to the structural-phase transformation in the coatings from the amorphous state to the amorphous-crystalline state with incorporation of CaHPO₄, α-Ca₂P₂O₇, β-Ca₂P₂O₇ and TiO₂ (anatase) nano-sized phases. The micro-porous amorphous coatings formed at a low voltage of 200 V showed the highest adsorption of the doxorubicin. The formed coatings with regulated porous structure and specified phase composition can be used as anticancer (e.g., doxorubicin) drug carrier systems.

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