Hybrid Calcium Phosphate Coatings for Implants

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Abstract. Monophasic biomaterials cannot provide all the necessary functions of bones or other calcined tissues. It is necessary to create for cancer patients the multiphase materials with the structure and composition simulating the natural bone. Such materials are classified as hybrid, obtained by a combination of chemically different components. The paper presents the physical, chemical and biological studies of coatings produced by hybrid technologies (HT), which combine primer layer and calcium phosphate (CaP) coating. The first HT type combines the method of vacuum arc titanium primer layer deposition on a stainless steel substrate with the following micro-arc oxidation (MAO) in phosphoric acid solution with addition of calcium compounds to achieve high supersaturated state. MAO CaP coatings feature high porosity (2–8%, pore size 5–7 μm) and surface morphology with the thickness greater than 5 μm. The thickness of Ti primer layer is 5–40 μm. Amorphous MAO CaP coating micro-hardness was measured at maximum normal load F_{max} = 300 mN. It was 3.1 ± 0.8 GPa, surface layer elasticity modulus E = 110 ± 20 GPa, roughness R_a = 0.9 ± 0.1 μm, R_z = 7.5 ± 0.2 μm, which is less than the titanium primer layer roughness. Hybrid MAO CaP coating is biocompatible, able to form calcium phosphates from supersaturated body fluid (SBF) solution and also stimulates osteoinduction processes. The second HT type includes the oxide layer formation by thermal oxidation and then CaP target radio frequency magnetron sputtering (RFMS). Oxide-RFMS CaP coating is a thin dense coating with good adhesion to the substrate material, which can be used for metal implants. The RFMS CaP coating has thickness 1.6 ± 0.1 μm and consists of main target elements calcium and phosphorus and Ca/P ratio 2.4. The second HT type can form calcium phosphates from SBF solution. In vivo study shows that hybrid RFMS CaP coating is biocompatible and produces fibrointegration processes.

Key words: radio frequency magnetron sputtering, biocompatibility, micro-arc oxidation, hybrid technology

INTRODUCTION

It is known that calcium phosphates due to the great chemical similarity to the inorganic part of bones and teeth appear to be very attractive compounds for the biomedical applications [1–4]. However, since bulk calcium phosphates have a ceramic nature, they are mechanically weak (brittle) and cannot be applied as load-bearing implants in human body. Therefore, for many years, the clinical applications of CaP have been limited to non-load bearing parts of the body [5–8]. Nevertheless, the idea to combine the advantages of various materials appeared several decades ago. Scientists applied biocompatible CaP ceramic as a coating onto the surface of mechanically strong but bioinert or biotolerant materials [9, 10]. For instance, metals are used in endoprosthesis for total hip joint replacements and artificial teeth sockets because of its sufficient mechanical stability. To increase the biocompatibility of metallic implants they are covered by CaP coating to create biomechanically stable links between the implant and bone tissues. Many different physical and chemical methods were developed to create CaP coating for biomaterials, such as sol-gel, pulsed laser deposition, electrochemical deposition, biomimetic deposition, physical vapor deposition (PVD) techniques [11–15].

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All single-phase materials used in implantology are imperfect and have their drawbacks. It is necessary for the needs of personalized medicine to create biomaterials with a multiphase structure and composition imitating natural bone. Such materials are classified as hybrid, which are obtained through combining various chemical components. Hybrid materials include composite materials, multilayer systems, coatings, particles and fibers with the modified surface [1].

Experience shows that a universal method of producing coatings suitable for all medical applications and fully satisfying the whole medical and technical requirement complex does not exist. Another approach is to use hybrid technology, combining existing technologies and methods [5]. For example, there are some methods to provide corrosion resistance of metals in organism environment including anodization and oxide coatings deposition [16, 17]. At the same time, it is important to provide bioactivity by applying CaP coating. Therefore, the decision is preliminary oxide metallic implants before applying biocompatible coating to simultaneously attach more stable anticorrosive properties and greater adhesion of CaP coating to metallic implants and also bioactivity.

HYBRID TECHNOLOGIES

Titanium Primer Layer with MAO CaP Coating

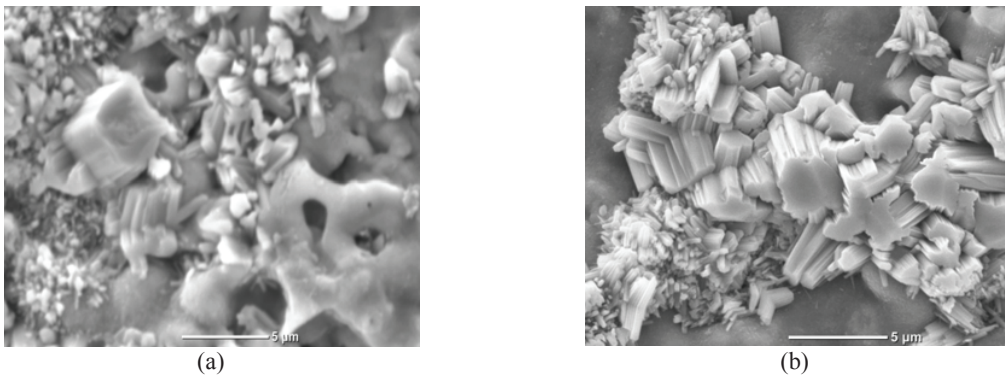
Micro-arc oxidation is an economical and widely used method for producing CaP coatings on titanium implants. The disadvantages of CaP coating produced by MAO are low elasticity and high brittleness. In addition, MAO method allows applying coatings only on the gate material group. On the other hand, it is preferable to use stainless steel and its alloys as the metallic implant base because of its good strength characteristics. It is the most expeditious to create a composite structure, in which steel is used as a strong basis. The steel surface is coated by gate material primer layer, for example, titanium with the following MAO in electrolyte containing calcium and phosphorus compounds [18]. Formed Ti film which is preferably applied by vacuum ion -plasma method for proposed HT must be thick enough and have high adhesion to the basis material.

Titanium coating thickness was varied in the range of 5–40 μm. X-ray fluorescence (XRF) analysis results have shown that no peaks corresponding to base material elements were detected. According to Auger spectroscopy the coating contains: 91.1 at. % Ti, 2.2 at. % C, 3.1 at. % N, 3.6 at. % O. CaP coating formation by MAO was carried out in a saturated CaO solution with 10% H_3PO_4 and hydroxyapatite dispersion phase with particle size 70 μm. The voltage was 200 V and process duration was 15 minutes.

Since the hybrid Ti–MAO CaP coating demonstrated very similar physical and chemical properties to initial CaP MAO coating, it can be supposed that biological research results of hybrid Ti–MAO CaP coating will be similar.

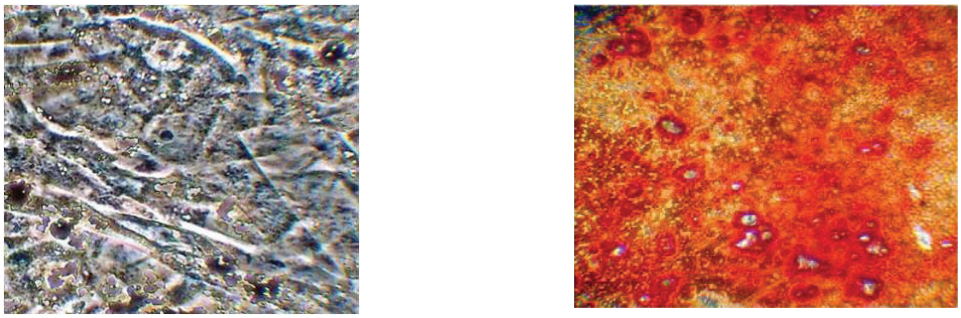
Hybrid materials bioactivity was studied via simulated body fluid test on CaP MAO coating, described in [19]. Figure 1 shows CaP MAO coatings image before and after SBF test.

The thickness of the hybrid MAO CaP amorphous coating is approximately 7–15 μm, porosity 2–8%, pore size 5–7 μm, its surface has a typical MAO structure (Fig. 1a). Micro-hardness of amorphous MAO CaP coating as measured at maximum normal load F_{max} = 300 mN is 3.1 ± 0.8 GPa, surface layer elasticity modulus E = 110 ± 20 GPa, roughness R_a = 0.9 ± 0.1 μm, R_z = 7.5 ± 0.2 μm, which is less than the titanium primer layer roughness. According to energy dispersive analysis the coating includes 44.7 at. % Ti, 6.8 at. % Ca, 6.9 at. % P, Ca/P ratio = 1.



(a) (b)

FIGURE 1. The morphology of MAO CaP coating: (a) before and (b) after SBF test. Scale line 5 μm



(a) (b)

FIGURE 2. Preparations histological sections obtained during in vitro experiment: (a) 14 hours, ×400, (b) 30 hours, ×100 [20]

The chemical composition after SBF test has changed: 12.3 at. % Ca, 20.15 at. % P, indicating that CaP MAO coating has the ability to form calcium phosphates from SBF solution. The biocompatibility of CaP MAO coating was investigated in vitro using PCR analysis to identify specific markers of bone osteopontin. The mesenchymal stem cell monolayer formation was observed on CaP coating surface on day 14 (Fig. 2a). The appearance of bone tissue was observed on day 30 of the experiment (Fig. 2b). Thus, CaP MAO coating is biocompatible and has the ability to stimulate osteoinduction processes.

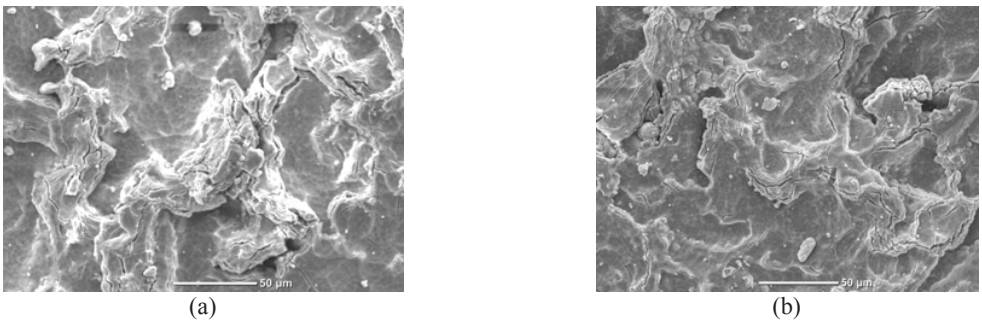
Oxide Primer Layer with RF Magnetron CaP Coating

In some cases, implants require thin CaP coatings which are not destroyed during operation and improve adhesive strength of the implant with bone tissue due to its osteointegration properties. A promising method to form nonporous, highly adhesive CaP coating is radio frequency magnetron sputtering. Most metallic materials are exposed to corrosion in biological fluids. Metallic implant must be protected from corrosion after CaP coating dissolution. It can be provided by chemically inert dielectric coating formation on implant surface. It was proposed to oxidize metal implant thermally in pure oxygen atmosphere.

Multilayer coating consists of oxide primer layer formed by thermal oxidation method at temperature 600°C for 30 min. The second layer is CaP with thickness 1.6 ± 0.1 μm, which is formed by RFMS of hydroxyapatite target at 13.56 MHz in argon and oxygen gas mixture with ratio 1 : 1 at pressure 0.3 Pa, the power density 20 W/cm^2.

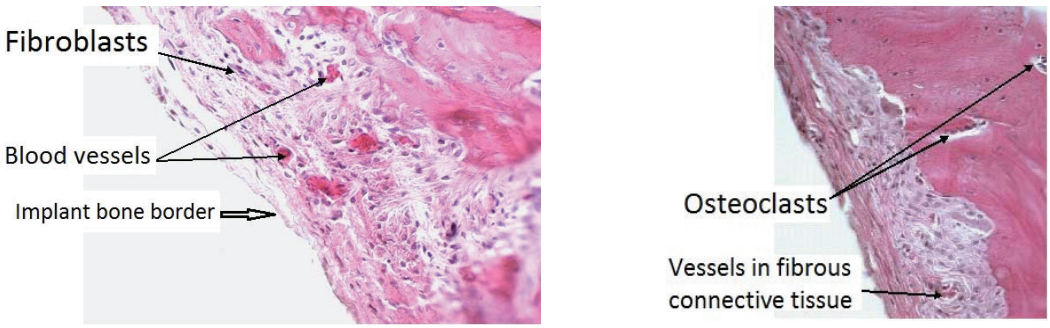
Adhesion studies demonstrated a significant increase in the adhesion strength of CaP coating to preliminary oxidized steel surface compared to non-oxidized steel samples with CaP RFMS coating. High adhesion strength of CaP hybrid coating to oxidized steel is the result of strong chemical bonds formation between oxide layer and CaP coating under high temperature (300°C), which occurs during RFMS process. Hybrid coating consisting of oxide primer layer and CaP coating provides the best corrosion protection compared to RF magnetron CaP coating on steel. Hybrid coating integrity was maintained after isotonic solution (0.9% NaCl) immersion during 35 days.

The ability to form calcium phosphates of hybrid CaP RFMS coatings studies according to SBF test procedure show that significant differences in coating morphology before and after the SBF test were not observed (Fig. 3).



(a) (b)

FIGURE 3. Oxide CaP RFMS hybrid coating morphology: (a) before and (b) after SBF test. Scale line 50 μm



(a) (b)

FIGURE 4. Rat blade bone part in implanted place: (a) 1 month after implantation, ×100; (b) 2 months after implantation, ×100

According to energy dispersive analysis the coating included 22.7 at. % Ca, 9.4 at. % P, Ca/P ratio 2.4. After SBF test the content of elements was 23.9 at. % Ca, 12.7 at. % P. Since CaP RFMS coating is thin (1.6 ± 0.1 μm), SBF calcium phosphate layer formed on its surface is thinner than SBF layer formed on MAO CaP coating.

Biological in vivo studies of hybrid CaP RFMS coatings were carried out with the help of the colleagues from Samara State Medical University in accordance with [21, 22]. The typical histological picture of the implant installation location with hybrid coating after 1 month of implantation is shown in Fig. 4a. Reclaim filling the bone defect is presented by loose irregular connective tissue with a large number of cellular elements and with a large amount of full-blooded vessels. Fibroblast-type cells are arranged densely and have large nuclei. All these facts show fibrointegration processes of implant material. The typical histological picture of the implant installation location with hybrid coating after 2 month of implantation is shown in Fig. 4b. Reclaim on the implant to bone border is still represented by loose irregular connective tissue, which includes many blood vessels.

The edge of the rat blade bone at the implant site of testing material is rough with a large amount of gaps containing osteoclasts with an elongated and flattened shape. There are groups of osteoblasts in some areas. These facts indicate the active reconstruction processes of bone tissue in test material implantation zone (Fig. 4b). Cells that characterize inflammation processes or tissue necrosis, such as polymorphonuclear neutrophils, lymphocytes, plasma cells, eosinophils, macrophages and multinucleated cells have not been identified. All these facts show the normal process of the implant to bone osteointegration.

CONCLUSION

Two types of hybrid technologies were proposed: vacuum arc titanium primer layer with following micro-arc oxidation in phosphoric acid solution with calcium compounds addition formed on stainless steel surface and thermal oxide primer layer with calcium phosphate coating deposited by radio frequency magnetron sputtering of hydroxyapatite target. The first hybrid technology type has titanium primer layer thickness of 5–40 μm and calcium phosphate coating thickness of 7–15 μm, porosity 2–8 %, pore size 5–7 μm. It was shown that hybrid MAO CaP coating is biocompatible, able to form calcium phosphates from simulated body fluid and also stimulates osteoinduction processes . The second hybrid technology on the opposite has a thickness of 1.6 ± 0.1 μm and the calcium phosphate layer formed on coating surface from simulated body fluid solution is less than MAO CaP layer. However, RFMS CaP with oxide primer layer has great adhesion to substrate and maintained integrity after being in isotonic solution (0.9% NaCl) during 35 days. RFMS hybrid CaP coating is biocompatible and has the ability to stimulate fibrointegration processes.

Thus, the proposed methods of multilayer coating formation and hybrid materials can be used for personalized approach to treating cancer patients. Hybrid technologies allow expanding the scope of materials for medical application, including various cell technologies and tissue engineering strategies.

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The study reported in this article was conducted according to accepted ethical guidelines involving research in humans and/or animals and was approved by an appropriate institution or national research organization. The study is compliant with the ethical standards as currently outlined in the Declaration of Helsinki. All individual participants discussed in this study, or for whom any identifying information or image has been presented, have freely given their informed written consent for such information and/or image to be included in the published article.

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