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Influence of Germanium on Some Properties of Calcium Phosphate Coatings on Titanium Implants

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Abstract

A limited life span of endoprostheses in the absence of other complications is explained by the aseptic implant instability arising and developing as a result of insufficient osteointegration. The surface of implants made of the VT-6 alloy was treated to form calcium phosphate coatings including those containing germanium (0.4 and 2.5 mass %), for the purpose of carrying out a comparative analysis of the response of an organism. The surface of the resulting coatings was investigated by means of scanning electron microscopy. It was established that the introduction of germanium has a substantial effect on the surface morphology of the coatings. It was shown with the help of the light microscopy of histological sections that osteointegration processes are most intense with the group of implants containing germanium at a level of 2.5 mass % in the calcium phosphate coating.

Keywords: endoprosthesis, calcium phosphate coatings, implants, germanium, osteointegration, bone tissue, microarc treatment

INTRODUCTION

Degenerative-dystrophic diseases of joints account for 25.8 % in the structure of the diseases of the musculoskeletal system of adults, and a trend to an increase in the incidence of these diseases is observed during the recent years [1]. The diagnostics of osteoarthrosis, as a rule at late stages, the absence of the integrated approach to the treatment and many other factors lead finally to endoprosthesis replacement operations.

In 2017 in the Russian Federation, endoprosthesis hip replacement was carried out for 70 316 patients, 65.5 % of which are older than the employable age; replacement of knee joints was made for 42 904 patients, among which the persons of employable age accounted for 18.5 %. In spite of a broad range of the models of endoprosthetic devices and improvements of surgery techniques, the structure of post-operative complications remains almost unchanged; a substantial number of surgical operations (38.8–56.5 %) are carried out for the reason of the aceptic instability of the components of endoprostheses [2].

One of the methods for the prophylactics of this instability is the creation of biocompatible coatings on the surface of an endoprosthesis, which would prevent the diffusion of the materials into the surrounding tissues and would stimulate osteointegration processes. The most promising materials for obtaining the coatings of this kind are the materials based on calcium phosphates. Among them, the leading position is occupied by hydroxyapatite (HAP) as the major bone tissue metabolite.

Investigations aimed at the improvement of the characteristics of these materials through the introduction of different microelements or substituents are actively carried out during recent years. For example, to enhance the strength characteristics of calcium phosphate materials, zirconium dioxide (ZrO_2) particles are introduced. It was established that the strength of the coatings increases substantially with an increase in the concentration of ZrO_2. A similar effect is observed after the introduction of aluminium oxide (Al_2O_3), silicon carbide (SiC), as well as polyethylene. At the same time, it is known that polyethylene is a biologically inert material and causes a decrease in the osteointegrative capacity of the coatings [3–6].

In [7], the materials based on anion-modified HAP were ranged according to their biological activity in the following sequence: SiO_4^{2-}-HAP > CO_3^{2-}-HAP > HAP. The stimulating effect of silicon with respect to chondrocyte proliferation was also detected.

I. A. Skripnikova with co-authors stresses the role of silicon in re-modeling of bone tissue and substantiates the possibility to use silicon-containing additives in the integrated prophylactics of osteoporosis. In addition, the participation of silicon in the development of connective tissue and in the formation and mineralization of bone tissue was confirmed. A positive effect of silicon-containing additives on the bone mass and the markers of bone metabolism was detected [8].

It was proposed to use germanium as an additive to the calcium phosphate coating (CPC) because germanium is most close to silicon in chemical structure and possesses a broader range of biological effects. The confirmations of the antitumour, hematopoetic, bacteriostatic effect of germanium on the organism were reported, as well as its ability to stimulate fibroblast proliferation [9]. It was confirmed experimentally that composite coatings based on germanium-modified HAP enhance the cohesion of implants with bone tissue and stimulate regeneration processes [10, 11].

One of the most promising methods to form CPC is microarc treatment, which leads to the formation of porous coatings with the high degree of osteointegration [12, 13]. A specific feature of the method is the use of the energy of electric microdischarges moving chaotically over the surface under treatment. These microdischarges cause a plasmachemical and thermal action on the coatings. The thickness of the coatings may reach 80–100 μm. Investigation of the properties of coatings formed through the microarc treatment appears to be urgent and retains significance both in the fundamental and applied aspects.

The goal of the present work was to carry out a comparative analysis of the response of an organism to calcium phosphate coatings without modifiers and those modified with germanium.

EXPERIMENTAL

Procedure for obtaining coatings

Experimental plates 35 × 5 × 2 mm in size were made of the VT-6 alloy. The surface of implants was prepared by defatting with an alkaline solution, and chemical etching in a mixture of HNO_3 and HF before removing the oxide film from the plate surface. After multiple washing in distilled water, the coatings were deposited onto the implants using the microarc method [12, 13]. Inert gas was bubbled through the electrolyte solution in order to form high-quality coatings. So, three groups of coatings with particle size 20 nm to 400 μm in the aqueous solution of orthophosphoric acid H_3PO_4 (15 mass %) and HAP (9 mass %) were prepared:

without the addition of nanodisperse germanium (II) oxide GeO powder into the electrolyte solution (I group);

with the addition of nanodisperse germanium (II) oxide GeO powder (1 mass %) into the electrolyte solution (II group);

with the addition of nanodisperse germanium (II) oxide GeO powder (5 mass %) into the electrolyte solution (III group).

Methods of investigation

Investigation of the surface microstructure of the resulting samples and analysis of the chemical composition of coating were carried out at the Laboratory of Electron Microscopy at the equipment sharing Regional Centre for Probe Microscopy of the Ryazan State Radiotechnical University with the help of the scanning microscope JSM-6610LV (Jeol, Japan) with X-ray energy-dispersive analyzer Inca X-Max20 (Oxford Instruments, UK).

Investigation of osteointegration of the implants with coatings was carried out with 29 outbred cats at the age of 1.5 to 4 years, 2500–3400 g in mass. Animals were kept according to the requirements of the European Convention for the protection of vertebrate animals used for experiments or for other research purposes (Strasbourg, March 18, 1986), methodical recommendations for keeping laboratory animals in the vivaria of research institutes and educational establishments, order of the Ministry of Public Health of the USSR of 12.08.1977 No 755, and the World Declaration of the Rights of Animals of 23.09.1977. Operations were carried out under intramuscular anesthesia with zoletil in the dose of 15 mg/kg with preliminary medicamentous preparation using atropine sulphate in the dose of 0.04 mg/kg and a 0.2 % solution of rometar in the dose of 0.1 mL per 100 g of body mass. Skin and underlying tissues were cut in the upper one-third of the hip to open the intertrochanteric pit, which a channel 20 mm long was drilled using an auger 4 mm in diameter in the distal direction of the thigh bone. A pin was placed in the channel with the help of an impactor. A similar surgical intervention was carried out at the opposite hind limb, and then the wounds were stitched up layer by layer.

The animals were drawn out of the experiment after 90 days in agreement with the rules and recommendations of veterinary and biomedical ethics. Cross plates 5 mm thick were prepared on the basis of the proximal parts of thigh bones. The plates were then decalcified, dehumidified in ethanol solutions, and clarified in xylene. The material was poured with plasticized paraffin and cut with a microtome. Histological sections 7–10 μm thick were stained with hematoxylin and eosine and studied by means of optical microscopy using Carl Zeiss AXIO Observer.Alm (Germany) and LOMO Biolam I (Russia).

RESULTS AND DISCUSSION

One can see in the microphotographs taken by means of scanning electron microscopy that the coatings on the implants of the I group form a relief structure composed of hollow spherolite-like globules of calcium phosphates ~ 10–40 μm in size (Fig. 1, *a*).

Coatings on the implants of II and III groups exhibit a similar relief structure with substantial deformation of globules: the particles with prolate shapes localized on the surface appear in the spaces between the globules. In the II group of implants, the particles are (2–3) × (5–10) μm in size (see Fig. 1, *b*). The sizes of the particles on the surface of implants of III group are somewhat larger, they are (2–5) × (5–20) μm (see Fig. 1, *c*). Analyzing the microphotographs of the surface of implants of II and III groups, we may conclude that the prolate particles are distributed non-uniformly. One can see that an increase in germanium content has a substantial effect on the surface morphology of the coating.

The elemental composition of the coatings was assessed on the basis of the data of energy-dispersive spectra at the regions between the globules. According to the data obtained (Table 1), germanium is present in the implants of the II and III groups: 0.4 and 2.5 mass %, respectively. It may be assumed that germanium partially compensates for the deficiency of calcium in the coating.

Results of histological studies showed that calcium phosphate material of the I group implants (Fig. 2, *a*) is composed of the fragments of the tubular bone with the accumulations of young bone trabeculas on the inner surface. Haversian canals formed by the bone trabeculas have an intermittent nonuniform layer of fine-grained basophilic substance, which points to the start of the inclusion of the material into the regenerating bone tissue.

Calcium phosphate material with germanium content 0.4 mass % (implants of the II group, see Fig. 2, *b*) is characterized by more pronounced changes accompanying the rearrangement and biological resorption of the young bone tissue. A layer of fine-grained basophilic substance was discovered on the whole inner surface of the tubular bone, it is included into the inner surface of Haversian canals. The outer part of the tubular bone is mature bone tissue.

Calcium phosphate osteoplastic material with the maximal studied germanium content on the surface of the newly formed bone trabecula is shown in Fig. 3. Massive accumulations of coarse-grained fragments of the composite coating are present on the bone surface. They are integrated without any layers of the connective tissue directly into the bone matrix of the newly formed trabeculas of reticulofibrous bone tissue. Osteocytes of these trabeculas are disordered, which points to the

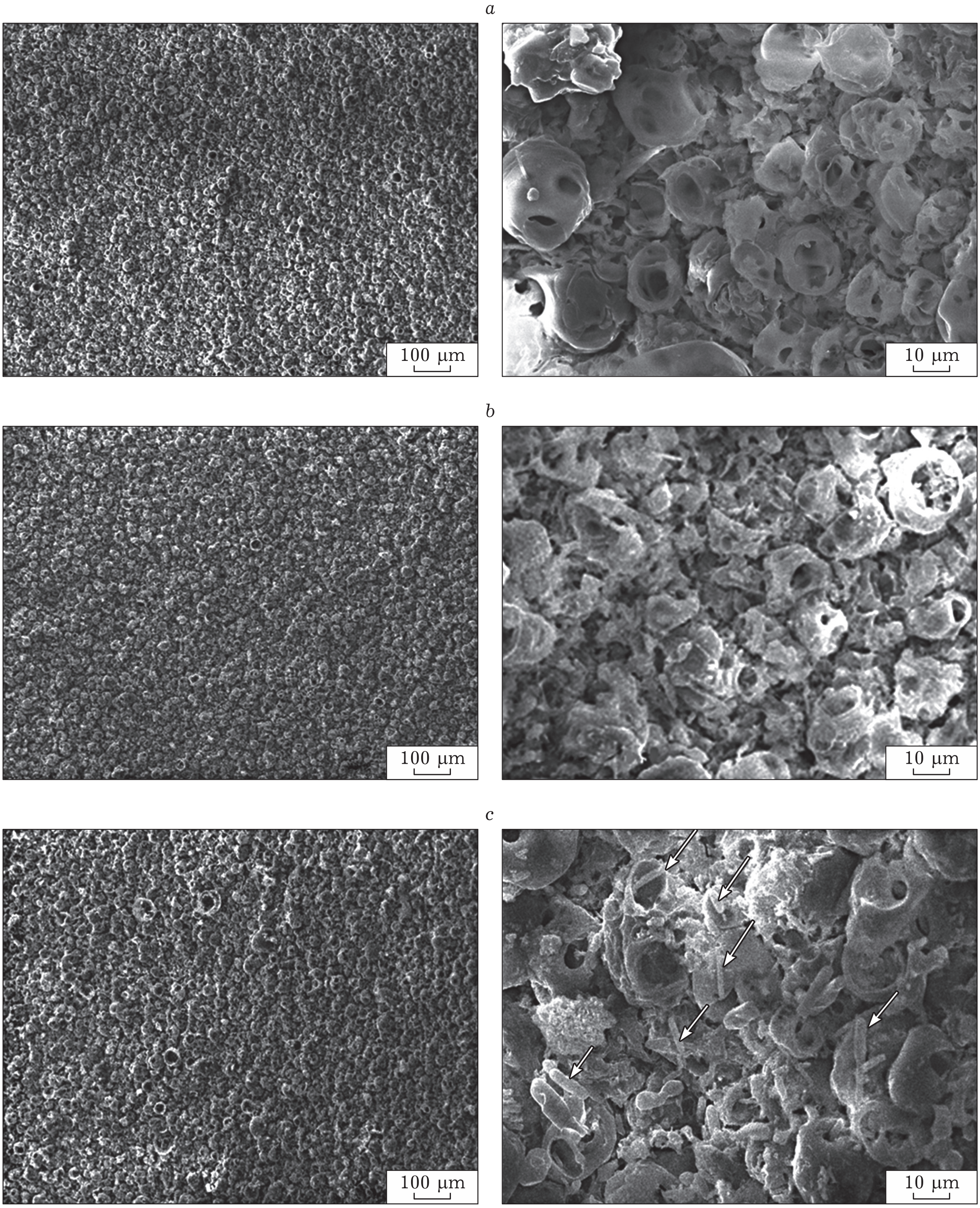

Fig. 1. Microphotographs of the surface of implants of the I (*a*), II (*b*) and III (*c*) groups. Arrows indicate prolate particles containing germanium.

continuing growth and remodeling of the bone tissue. Young fibroblasts are in the layer of the coating material. The data obtained in the investigation provide evidence in favour of the complete osteointegration of the material, which confirms our assumptions concerning the feasibility of the introduction of germanium into the coating to stimulate fibroblast proliferation. The intro-

TABLE 1

Elemental composition of the coatings on the implants of three groups

Group of implants	Electrolyte composition, mass %	Element content, mass %					
		O	P	Ca	Ge	Al	K
I	H_3PO_4 (15) + HAP (9)	57.6	28.4	12.5	Abs.	1.3	0.2
II	H_3PO_4 (15) + HAP (9)+ Ge (1)	56.0	25.4	16.9	0.4	1.0	0.3
III	H_3PO_4 (15) + HAP (9) + Ge (5)	54.4	24.7	17.1	2.5	1.0	0.3

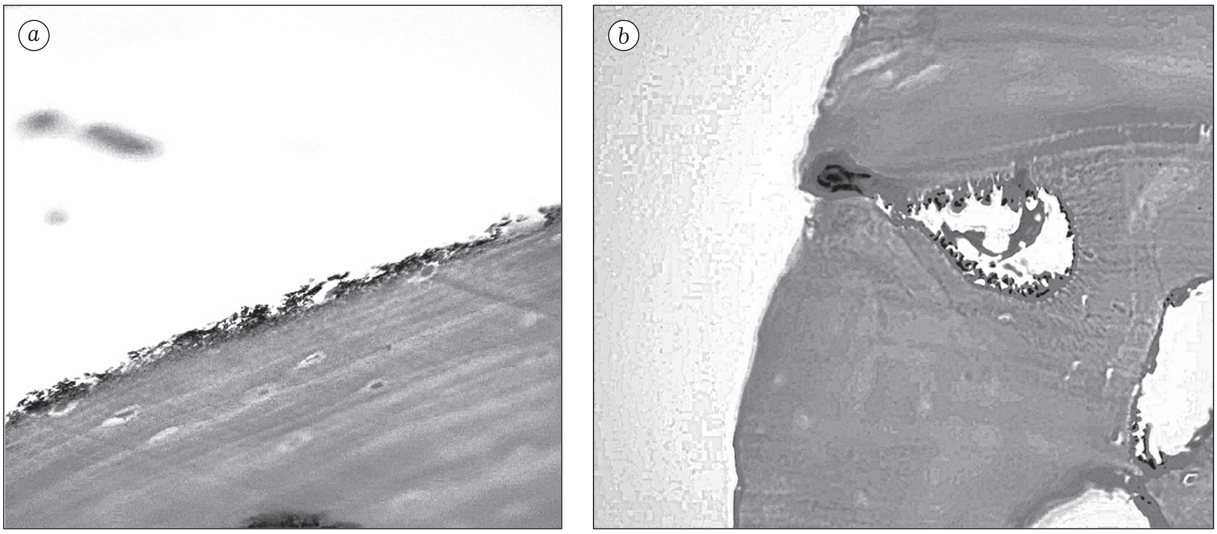

Fig. 2. Internal surface of the tubular bone with the nonuniform layer of a fine-grained basophilic substance (implant of the I group) (*a*); inclusion of the fine-grained basophilic substance into the internal surface of the Haversian canals of the tubular bone (implant of the II group) (*b*).

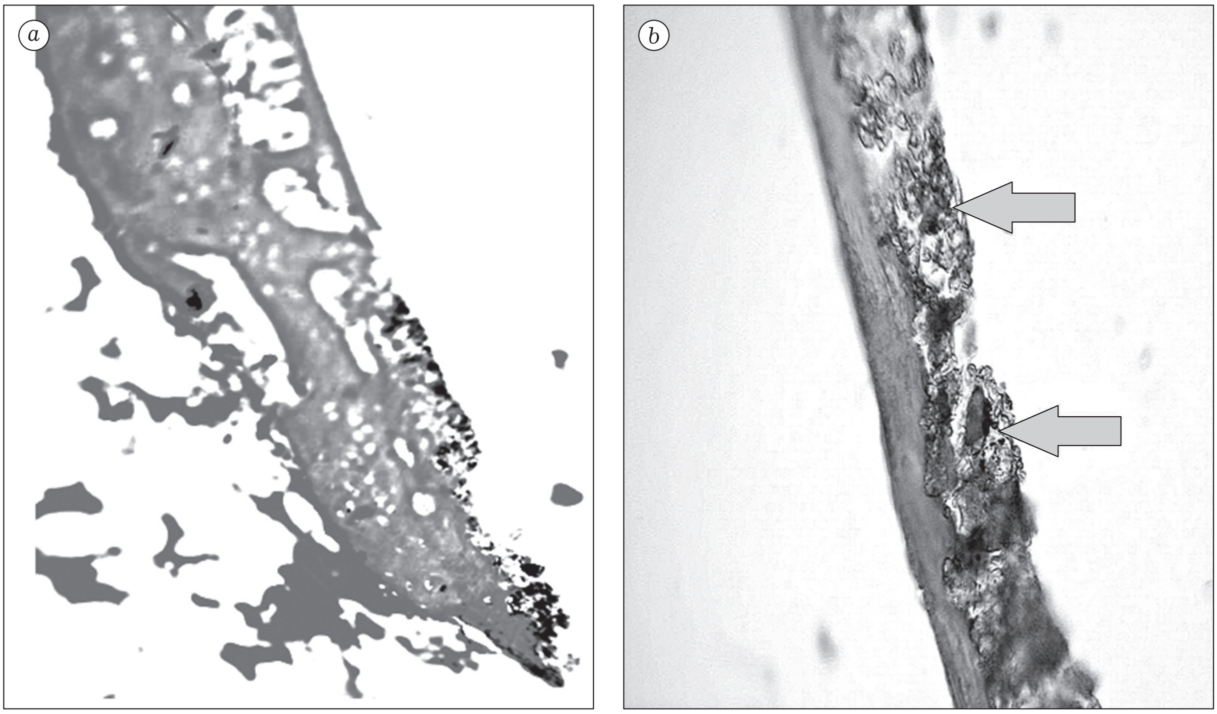

Fig. 3. Calcium phosphate osteoplastic material with germanium content 2.5 mass % on the surface of the newly formed bone trabecula, magnification: 200 (*a*) and 400 (*b*).

duction of germanium activates the osteointegration process.

The best characteristics of the biological response for the implants with the coatings of the III group (the average number of cells, proliferative activity, cell morphology) provide evidence of the higher free surface energy for this group of implants in comparison with the implants of the I and II groups. The data obtained are in agreement with the results of studies confirming the fact that the free surface energy, which is a fundamental characteristic of the surface properties of materials, has a substantial positive effect on the adhesion of fibroblasts, the rate of cell proliferation and cell activity [14].

CONCLUSION

The introduction of germanium into the CPC has a substantial effect on the structure of this coating. Histological studies show the appropriateness of the introduction of germanium into CPC for the stimulation of cell proliferation. It was established that osteointegration proceeds most intensely at the sites of implants with coatings containing germanium in the amount of 2.5 mass %.

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