STUDY OF BIOLOGICAL PROPERTIES IN IN VIVO EXPERIMENTS OF Ti-29Nb-13Ta-4.6Zr ALLOY SAMPLES WITH A POLYMER COATING

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Abstract. This paper shows the technology of creating a composite material from a Ti-29Nb-13Ta-4.6Zr mass% alloy, which does not contain toxic elements, with a polymer coating based on PLA. Primary in vivo studies were carried out to confirm the non-toxicity of the obtained material and the prospects for further studies.

1. Introduction

Problems of existing materials (a group of alloys based on Co-Cr (Co-Cr-Mo, Co-Ni-Cr-Mo, Co-Cr-Ni-W, Co-Ni-Cr-Mo-W-Fe), titanium, Ti-6Al-4V, Ti-15Mo-5Zr-3Al; Ti-5Al-2.5Fe; Ti-6Al-17Nb, etc.) for the manufacture of hip joint endoprostheses remain unresolved. Not a single material has managed to collect the set of properties required for these materials: high strength properties, corrosion and fatigue resistance, an acceptable reaction of the body or its complete absence. The main problem is insufficient biocompatibility [1-2]. The content of toxic elements (nickel, vanadium, aluminum, etc.) in alloys leads to neurological disorders, allergic reactions and other postoperative complications. At the same time, alloys that do not contain them have insufficient mechanical properties. The solution may be beta titanium alloys, which have corrosion resistance, high strength and fatigue characteristics in combination with a low modulus of elasticity, corresponding to the behavior of living tissues. The use of non-toxic elements (tantalum, zirconium, niobium) as beta titanium stabilizers can lead to the creation of an alloy that meets all the requirements for materials for endoprostheses. However, the process of integration in the human body when interacting with the internal environment can be very difficult and lengthy [3-5]. To facilitate integration, various methods are used (surface structuring, application of various types of coatings) [6-8]. It is advisable to use a biodegradable polymer matrix as a coating for the material of the endoprosthesis, which could locally release a drug within the required time, stimulating cell growth and facilitating integration [9-13].

Therefore, this work shows a method for creating a Ti-29Nb-13Ta-4.6Zr mass% alloy that does not contain toxic elements, with a polymer coating based on PLA. Primary in vivo studies were carried out to confirm the non-toxicity of the obtained material and the prospects for further studies.
2. Materials and methods
Smelting of ingots of alloy Ti-29Nb-13Ta-4.6Zr wt.% was carried out in an electric arc vacuum furnace with a non-consumable tungsten electrode LK8 from LEYBOLD-HERAEUS (Germany). Ingots were obtained with a mass of 35 g. 7 remelts were made to achieve uniformity of composition. The shape of the ingot is a biconvex lens, diameter 25-30 mm, height 10-15 mm, from which an ingot 60-70 mm long, 20-25 mm wide, 10-12 mm high was subsequently obtained. The ingots obtained were rolled on a DUO-300 reversing mill. The melted ingots were deformed in air with a step of 0.1 mm to a final sheet with a thickness of 1 mm. Rolling was carried out without preheating the ingot, which indicates the high ductility of the alloy. From the obtained sheet of alloy Ti-29Nb-13Ta-4.6Zr mass% by means of electric discharge cutting, samples with a length of 8 mm and a square section of 1 by 1 mm were made.

The polymer coating was applied by dipping into a PLA solution in chloroform with an injected drug. To prepare this solution in 100 ml of chloroform heated on a magnetic stirrer to 80 °C, a sample of PLA 180kDa weighing 2 g was added. The solution was stirred with an electronic overhead stirrer. After complete dissolution of PLA in chloroform, the solution was cooled to room temperature and 10 wt% drug (glucosamine) was injected. After applying this solution to the samples of the Ti-29Nb-13Ta-4.6Zr alloy, the mass% coating was dried at 370 °C for 48 hours.

To study the biological properties in vivo, samples of the alloy Ti-29Nb-13Ta-4.6Zr mass% with a polymer coating based on PLA were subcutaneously implanted in mice. Mice without implants were used as controls. The animals were withdrawn from the experiment 14 days after the operation. For morphological studies, tissue samples of experimental animals after implantation of samples of the studied alloy were placed in a Mirsky's Fixative (National Diagnostics, USA) for 24 hours at a temperature of +4 °C, while the volume of the fixative was 10-20 times the volume of the fixed tissue. After that, the samples were rinsed in running water for 12 hours, and then rinsed in a PBS-alcohol mixture (1:1). Further, the tissues were dehydrated by passing through aqueous solutions of alcohols of ascending concentration: 70%, 80%, 96% and 100%. After that, the tissues were sequentially passed through xylene-ethanol (1:1) and 100% xylene in a thermostat at 37 °C. In each of the solutions, the samples were kept twice for 60 minutes. Paraﬃn was used as a hydrophobic sealant, for which the fabrics were kept for 60 minutes in a xylene-paraﬃn mixture (1:1), then twice in pure molten paraﬃn in a thermostat at 56 °C. The resulting paraﬃn blocks were left to cool and harden overnight.

The production of paraﬃn sections with a thickness of 3-5 microns was carried out on a Thermo Scientiﬁc Microm HM 325 microtome (Germany). The sections were placed on HistoBond adhesive coated slides (Marienfeld Laboratory Glassware, Germany), straightened on an OTS 40 thermostat (SMT, Germany) at t = 42 °C and dried in a thermostat at t = 37 °C for 3 hours or at room temperature for 12 hours.

For histological staining, the sections were dewaxed on glass slides by sequentially passing xylene and alcohols of decreasing concentration (100%, 96%, 70%) through a battery for 1-2 minutes each. A series of sections were stained with hematoxylin-eosin (VITROSTAIN Biovitrum, Russia) according to the manufacturer’s protocol. After staining, the glasses were washed in running and distilled water, then the preparations were rehydrated and clarified through a fast battery of alcohols of ascending concentration and xylene. The preparations embedded in the Histofluid resin (Marienfeld Laboratory Glassware, Germany) were covered with cover slips (Marienfeld Laboratory Glassware, Germany).

Microscopic analysis of the sections was carried out using a Leica DM 6000 light microscope; photographs were obtained using a Leica DFC 490 digital microscopy camera.

3. Results and discussion
The studied tissue samples after implantation of samples of the studied alloy showed no traces of inflammation or tissue necrosis. Inflammation, edema and hemorrhages were not observed in animals either. The studied animal tissue samples with implanted composite material made of Ti-29Nb-13Ta-4.6Zr alloy, wt% with a polymer coating based on PLA, did not differ from tissue samples of the control group.

Figures 1-3 show the obtained preparations of animal tissue sections.
Figure 1. Preparation of a tissue section surrounding a sample of a composite material made of an alloy Ti-29Nb-13Ta-4.6Zr mass% with a polymer coating based on PLA after subcutaneous implantation in an animal.

Figure 2. Preparation of a tissue section surrounding a sample of a composite material made of an alloy Ti-29Nb-13Ta-4.6Zr mass% with a polymer coating based on PLA after subcutaneous implantation in an animal.
4. Conclusions
A technology has been developed for creating samples of a composite material from an alloy Ti-29Nb-13Ta-4.6Zr mass% with a polymer coating based on PLA. Investigations of the biological properties of in vivo samples of composite materials from the alloy Ti-29Nb-13Ta-4.6Zr mass% with a polymer coating based on PLA were carried out. The absence of differences in the reaction of the surrounding tissue of animals after implantation was shown in comparison with the control group of animals without implantation.

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6. References
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Figure 3. Preparation of a tissue section from an animal from the control group.
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