Antimicrobial properties of nanostructured plasma condensates from medical implants

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Abstract. Thin film coatings of titanium nitrides and hafnium for implants were investigated. The HfN + TiN coatings were condensed from the plasma phase. Analysis of the structure and composition revealed multilayers of the coating, the composition of the layers, and the presence of columnar crystals. The coating thickness is 2-5 μm, with a characteristic size of grains 20-50 nm. Antimicrobial properties for *staphylococcus* and *pseudomonas aeruginosa* have been established, a technique for determining the antimicrobial properties of coatings has been demonstrated, and medical approbation of implants with a coating has been carried out.

Superhard thin-film coatings made of nitrides Ti, Al, Cr, Zr, Mo are widely used in the production of metal-cutting tools made of heat-resistant steels and sintered metal-ceramic alloys. The world leaders in the development of technology and equipment in this area are Oerlikon Balzers AG and Diener Elektronik gmbh, Ion Bond gmbh. Condensation of coatings is performed on magnetron installations and installations with electric arc evaporation of metal.

The use of coatings for medical devices is limited, due to the diversity and specificity of medical and technical requirements [1], including the assessment of biological effects in accordance with GOST (ISO) 10993-2011. The technology of plasma condensation with ion bombardment (CIB) and surface cleaning with high-energy metal ions Ti, Cu, Cr allows to significantly increase the adhesion of the coating to the substrate. Prospective is the use of thin-film coatings to protect the human body from the migration of toxic elements Cr, Mo, Co from steel V and Mo from titanium implant alloys, which can cause negative reactions of the organism with the development of metallurgy, allergic conditions and inflammation [2].

The condensation of the investigated coatings [3] was carried out in a nitrogen atmosphere at a pressure of about 10⁻¹ Pa in an installation with three electric arc evaporators. Surface cleaning was carried out with titanium ions to a temperature of titanium implants not exceeding 600 °C, i.e. the beginning of the phase changes leading to the growth of the grain of the β-structure. For single-use skeletal traction locks of Cr18N9 steel, the temperature during cleaning was reduced to 300-400 °C.
Measurement of the microhardness of the coating showed a value of 14-30 GPa, with a thickness of 2-5 μm. The thickness was measured both on the samples from the polycorundum, and on the bends of the spokes, screws, osteosynthesis plates, and the cleavage of the coating, fixed by an electron microscope. Estimation of porosity showed that the pore volume in the coating does not exceed 1-2% at a thickness of 2 μm. The pores were fixed on the scaffold by microscopic survey and are seen in Figure 1. It should be noted that the thickness of the coating on the inner surface of the hole in the osteosynthesis plates was understated and amounted to about 1 μm. Multilayer nanostructured coatings consist of columnar crystals of titanium and hafnium nitrides with hetero-thermal chemical bonds and have characteristic dimensions of 5-20 nm.

![Figure 1. SEM image of fracture of the coating and the type of closed pores.](image1)

Investigations of the structure of the coating, both on the surface and on the thickness, revealed the characteristic dimensions of the HfN + TiN crystals of 20-50 nm and the height in the thickness of the coating (Figures 2 and 3).

![Figure 2. SEM image of column crystals of nitrides.](image2)

The layer composition in the electron spectrum was analyzed by a high-resolution energy dispersion spectrometer and is shown in Figures 4 and 5. The coating cross-section in the thickness of the Ga ion flux established a multi-layer coating consisting of nitride layers with a different layer composition and a layer thickness of 20-30 nm. At the same time, the integrity of the crystal is not disturbed, but a transition is visible, a thin layer that is transitional in composition. X-ray diffraction analysis recorded the phases of HfN, TiN₀.₉, and TiHfN₂ with crystal growth by the crystallographic plane 111 parallel to the base. The composition analyzer established a molar ratio of titanium to hafnium in equal parts with a deviation of 10%.

The guarantee of the absence of toxicity is the study of the migration of hafnium and titanium ions from the coating surface and toxic vanadium from the titanium alloy through the coating. The concentration of hafnium ions from the 20-day stretch coating was less than 10⁻⁹ mol / liter, and the
vanadium concentration decreased by 5 times in comparison with the uncoated sample [4]. This characterizes the barrier effect of the coating, and a small migration of ions - biocompatibility.

![Figure 3. Coating section and composition analysis point.](image)

![Figure 4. Spectrum of the energy of electrons at the point of analysis.](image)

Preclinical tests on animals showed the main effects and advantages of implants coated with titanhafnium nitrides:
1) Accelerated wound healing;
2) Absence of allergic reactions;
3) Decreased reactive protein values that do not exceed the normative values in comparison with the increased index from the materials of other implants.

The absence of toxic effects of coatings containing titanium nitride and hafnium on the hepatobiliary system, biochemical indicators of blood give rise to further introduction of nitride coatings, expansion of their application spectrum and types of osteosynthesis with intramedullary, bone and extra-focus fixation [7]. Positive biological activity of nitride titanium-garnet coatings is related to their antimicrobial properties.

At present, there are no standardized methods for reliable determination of the degree of antibacterial action of metal-like antimicrobial surfaces. Testing the degree of sensitivity of microbial culture to antimicrobial drugs is carried out in accordance with MUK 4.2.1890-04 "Determination of the sensitivity of microorganisms to antibacterial drugs." The degree of sensitivity of bacteria to antimicrobial drugs is determined by the diameter of the inhibition zone (absence) of growth of test strains on the surface of the Muller-Hinton culture medium. When measuring the zones, growth retardations are oriented towards the zone of complete suppression of visible growth. The initial suspension of the inoculum contains $1.5 \times 10^5$ colony forming units - CFU / ml, which corresponds to 0.5 units according to the McFarland standard. Applying student criterion 0.95, you can compare the results of studies and obtain reliable indicators.

The essence of the technique developed for metal and metal-like coatings is the direct contact of the coating with the culture suspension of the microorganism throughout the experiment, i.e. the coating is immersed in a suspension of the culture suspension of the test microorganism. From special conditions it is necessary to allocate: 1) the concentration of microflora is reduced to $10^5$ CFU / ml; 2)
The metal of the substrate for coatings is limited by the titanium alloy VT6 and steel Cr18N9; 3) in the experiment, tenfold dilutions of the tested microbial culture are used; After incubation, a seeding is carried out on a solid nutrient medium followed by counting the colonies. The effect is on the multiplying microflora in the growth stage.

In the experiment, titanium substrates of VT6 alloy, 20X20 mm in size, were used with a two-sided coating of nitrides of titanium and hafnium with their ratio in the coating 50% to 50% by weight of metals and a coating thickness of 3-4 μm. In control - in the absence of coatings on the plates, a normal growth of culture takes place in the nutrient medium with a sharp increase in its concentration exceeding $10^8$ CFU / ml. The results are illustrated in Figure 6. Growth starts quickly within 4-6 hours and lasts several days until the nutrient medium is completely depleted, after which the death of microorganisms begins (decrease in CFU). The last increase in microflora growth, necrotic growth, is caused by reproduction of the culture on the products of dead bacteria. A completely different picture is observed in the presence of nitride coated plates in a medium with a microflora. In the first hours there is an increase in the number of microflora by about an order of magnitude, but then there is an intensive dying out of microorganisms up to the complete destruction of the culture. This is the case with staphylococcus and, to a lesser extent, Pseudomonas aeruginosa, which is due to the difference in the structure of the cell wall.

Hospital strains of microflora showed similar sensitivity to the coating material in the experiment. The antimicrobial effect of the coating was confirmed, despite the known resistance of hospital strains to antibiotics. Antimicrobial effect from coatings from titanium nitride is not revealed.

The fungicidal effect of the titanium naphtha coating [5] was evaluated on samples of genuine leather with this coating. The coating had a scaly, rather than a continuous structure and the dimensions of its structural elements did not exceed 2 μm [6]. Peculiarities of experiments with pathogenic cultures of fungi consist in a low rate of their growth and resistance to external factors. In the experiment, a pathogenic mycotic culture from the trichophyton family was used. The evaluation results of the experiment showed the presence of fungicidal properties in the nitride titanium-garnet coating.

According to the experimental data, the estimated antimicrobial efficacy of the nitride titanate coating is approximately 40 times lower than the antimicrobial activity of benzylpenicillin.

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