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# Investigation of diamond biocompatible coatings for medical implants

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Despite the advantages of diamond-like carbon films that are used as wear-resistant coatings for implants, they may have a number of disadvantages such as the high level of internal tension, low adhesive durability and high sensitivity to environment conditions. These problems can be overcome by application of the new carbon nanocomposite coatings that can be deposited from $C_{60}$ ionic beam. It was found that the proposed diamond-like nanocomposite coatings increase implant material resistance to electrochemical corrosion processes due to shift of its electrode potential to area of positive values, and also promote a complex of reparative and adaptation and compensatory reorganizations that will allow to accelerate processes of healing and postoperative adaptation of organism in zone of implant inputting.

**Keywords**: diamond-like films, nanocomposite, implant coating, structure, electrode potential, biocompatibility.

Незважаючи на переваги діамантоподібних вуглецевих покриттів, що використовуються в якості зносостійких захисних покриттів для імплантатів, вони можуть мати ряд недоліків, таких як високий рівень внутрішніх напружень, низька адгезійна міцність, висока чутливість до умов навколишнього середовища. Ці проблеми можуть бути подолані в разі застосування нових вуглецевих нанокомпозитних покриттів, нанесених з іонного пучка С60. Встановлено, що запропоновані діамантоподібні нанокомпозитні покриття підвищують опірність матеріалу імплантату до електрохімічних корозійних процесів за рахунок зміщення його електродного потенціалу в область позитивних значень, а також сприяють комплексу репаративних і адаптаційно-компенсаторних перебудов, що дозволить прискорити процеси загоєння та післяопераційної адаптації організму в зоні введення імплантату.

**Ключові слова**: діамантоподібні плівки, нанокомпозит, покриття на імплантаті, структура, електродний потенціал, біосумісність.

Несмотря на преимущества алмазоподобных углеродных пленок, которые используются в качестве износостойких защитных покрытий для имплантатов, они могут иметь ряд недостатков, таких как высокий уровень внутреннего напряжения, низкая адгезионная прочность, высокая чувствительность к условиям окружающей среды. Эти проблемы могут быть преодолены в случае применения новых углеродных нанокомпозитных покрытий, нанесенных из ионного пучка С60. Установлено, что предлагаемые алмазоподобные нанокомпозитные покрытия повышают сопротивляемость материала имплантата к электрохимическим коррозионным процессам за счет смещения его электродного потенциала в область положительных значений, а также способствуют комплексу репаративных и адаптационно-компенсаторных перестроек, что позволит ускорить процессы заживления и послеоперационной адаптации организма в зоне введения имплантата.

**Ключевые слова**: алмазоподобные пленки, нанокомпозит, покрытие на имплантате, структура, электродный потенциал, биосовместимость.

## Introduction

The choice of material for production of medical implants always represented certain difficulties. The majority of the used metals don't possess sufficient chemical inertness and necessary mechanical properties [1, 2]. One of problem solutions is creation and use of carbon coating of wide functional purpose on metal implants [3, 4]. In this plan diamond-like carbon (DLC) coatings [5, 6] are of great interest. Such coatings possess unique mechanical, chemical and thermal characteristics. The combination of low friction coefficient and high wear resistance allows to increase repeatedly durability of friction couples, for example, when forming artificial joints [7]. The DLC coating shows high biocompatibility [2, 7]. It unlike other types of coatings doesn't cause blood coagulation, serves as the effective barrier preventing diffusion of metal ions and can effectively be used on the implants contacting to bone and soft body tissues [8].

Validity of carbon films use as coating at production of endoprostheses and implants and their application in medicine is defined by ability of carbon materials to grow together quickly with surrounding tissues, and also to stimulate formation of a new bone tissue. Carbon materials differ in high biochemical and mechanical compatibility. Besides, they possess the biostimulating action, promoting regeneration of the tissues surrounding an implant. Products of their wear or destruction don't make harmful effects on surrounding tissues, lymph nodes and organism in general. Thus, these materials are very perspective for application as implants or coatings on them.

DLC coatings can be deposited on different types of substrates by means of several methods, including sputtering [9], pulse and laser deposition [10] or ion-beam deposition [11]. Unlike other methods the ion-beam deposition of fullerene $C_{60}$ instead of atomic carbon has allowed to receive new diamond-like material with unique nanocomposite structure and low level of residual tension [12, 13]. The synthesized films consisted of the graphite nanocrystals injected in amorphous diamond-like matrix [14]. Graphite nanocrystals had primary orientation of the graphene planes which are settling down perpendicularly to surface [13]. Such structure of nanocomposite coatings led to the high ratio of hardness to edacity module that allowed to expect the best combination of mechanical properties of coating and metal substrate in comparison with the DLC coatings received by traditional methods with use of nuclear streams of carbon. Primary orientation of graphite nanocrystals can play an important role at necessary of transition from biocompatibility of coverings to their biological activity as there is a distinction in chemical and biochemical properties of graphite crystals depending on the crystallographic directions [15]. Besides, thin DLC coatings usually have a smooth surface and reproduce initial topography of substrate [2]. The new type of diamond-like nanocomposite coatings had own topography [14] in addition to excellent mechanical and frictional properties. The similar nanodimensional topography can be useful to various biological applications [16, 17].

Thus, research of biocompatibility of carbon nanocomposite coatings created by means of ion-beam deposition of $C_{60}$ can lead to development of new class of the carbon coatings having optimum mechanical properties. Research of electrochemical activity and biocompatibility of the diamond-like nanocomposite carbon coatings deposited on cobalt-chromium alloy in comparison with titan and Co/Cr alloy without coating was the purpose of work.

## Object and methods of research

Two types of metal alloys were used for preparation of the samples: cobalt-chromium alloy Vitallium (Co 62%, Cr 30%, Mo 5%, and C 0.4%) and titanium alloy VT1-0 (Ti 99% and Fe 0.18%).

$C_{60}$-fullerene powder (99.5% purity; NeoTechProducts, St. Petersburg, Russia) was used as the source material for the deposition of the carbon nanocomposite coatings. Coatings were deposited on metal substrates from $C_{60}$ ionic beam which was generated by means of source with saddle electric field. The discharge was fired in fulleren vapor at accelerating voltage 6 kV. From source chamber the ionic beam went to a magnetic mass separator and further to substrate. For films structure formation the single-charged ions with average energy 5 кэВ were used. For this purpose, at the exit of mass spectrometer the mobile aperture which spatially limited a beam has been established. Deposition of carbon films from $C_{60}$ ion beam was carried out on substrates at temperature of 100 °C. Speed of films deposition was 0,1 nm per second, thickness of the investigated films – 20-100 nm [18].

Microscopic investigations of the DLC coating structure were made on transmission electron microscope TEM-125K with resolution of 0,2 nm. The samples-witnesses were prepared on NaCl substrates. After separating the films from substrate they were washed with deionized water and placed on a copper grid.

Electrode potentials were measured using the standard AgCl reference electrode [1] for rating of initial activity of metals. The measurements were conducted in an electrochemical cell filled with 0,9% aqueous solution of NaCl.

To carry out model tests in a biological environment the metal plates imitating implants were placed into solution with a composition of inorganic components close to blood plasma. The solution composition involves the creation of calcium phosphate sediments and subsequently the formation of hydroxyapatite crystals. Entering implants into solution induces sludge formation and its quantity and distribution over the surface of samples was estimated. It was supposed, that if the implant after anode processing will activate the formation of calcium phosphate on its surface, the stimulation of similar processes at the introduction of the implant to real bone tissue will lead to faster restoration of tissue in the implant site and, therefore, to faster healing of the wound. A solution for tests was prepared as an equivalent blood plasma at T = 36.5 °C and pH = 7.4. Molar concentration of ions in solution was the following: $Na^+$ - 142.0; $K^+$ - 5.0; $Mg^{2+}$ - 1.5; $Ca^{2+}$ - 2.5; $Cl^-$ - 147.8; $HCO^{3-}$ - 4.2; $HPO_4^{2-}$ - 1.0; $SO_4^{2-}$ - 0.5 mmol. The solution was prepared by the dissolving of reagents: NaCl, $NaHCO_3$, KCl, $K_2HPO_4 \bullet 3H_2O$, $MgCl_2 \bullet 6H_2O$, $CaCl_2$ and $Na_2SO_4$ in distilled water [19]. To adjust for the necessary pH was added $(CH_2OH)_3CNH_2$ and one-molar solution of hydrochloric acid.

Infrared (IR) spectra of the sediment in the solution were taken using Fourier spectrometer Perkin Elmer Spectrum One. Processing and analysis of spectra were carried out by means of software attached to the device.

The X-ray research of phase structure was carried out by diffractometer DRON-2,0 according to Bragg-Brentano scheme θ-2θ. To eliminate reflections from $K_\beta$-radiation a manganese filter was used. The survey was conducted point-wise with step 0.1° and the exposure at each point was 10 seconds in the angular range 2θ = 15 - 120 °.

Biological estimation of the carbon nanocomposite coatings integration was carried out in animal experiments. Rabbits (20 months old with body mass of approximately 3 kg) were separated into three groups. Subperiosteal implants made of Co/Cr alloy, titanium alloy and nanocomposite-coated Co/Cr alloy were implanted in rabbits from the first, second and third groups, respectively. Rabbits were sacrificed by air embolism at 12 weeks. The animal tests were performed according to the requirements of the European Convention and Ukrainian law.

After the rabbits were sacrificed, a fragment of the mandible, including the implant and a portion of the adjacent bone comprising an outer and an inner compact bone and spongy substance, was extracted. The extracted material was visually investigated using an optical microscope (Leika Axiostar Plus). For the microscopic investigation, the extracted fragments were fixed in 5 vol.% nitric acid, then dehydrated in 96° ethanol and embedded into celloidin [20]. Cross-sections with thicknesses of 7-10 µm were coloured with haematoxylin, eosin, and van Gieson's stain.

Morphometric investigations were concentrated on the following characteristics: an estimation of the newly formed tissue between the parent compact bone and the implant; the presence of necrosis on the surface of the tissue adjacent to the implant; and assessment of the nature of the restructuring of the compact and trabecular bone [21].

**Results and discussion**

At investigation of carbon nanocomposite coatings by transmission electron microscopy the contrast inherent to amorphous films and microdiffraction consisted of two halos with peaks characteristic of amorphous carbon could be seen (Fig. 1). Transformation structure, mechanical, electrical and optical properties of such coating at different temperatures of substrate is described in [18]. These coatings have high values of mechanical properties: elasticity modulus - 340 GPa, hardness - 46 GPa.

Time dependences of electrode potentials of all three samples types are presented in Figure 2. Length of measurements was determined by going into constant value of potential. It can be seen, the lowest potential had sample of Co/Cr alloy. At the beginning this electrode potential was E = -0,32 V, and after slight growth and stabilization it came to E = -0,15 V. The electrode potential of titanium practically unchanged during measurement and was E = 0,05 V. The Co/Cr alloy with DLC coating had maximum potential (E = 0,2 V) that was indicated about maximum of its initial resistance to degradation in electrochemical corrosion process.

Test of Co/Cr sample with carbon nanocomposite coating in the solution imitating blood plasma has shown

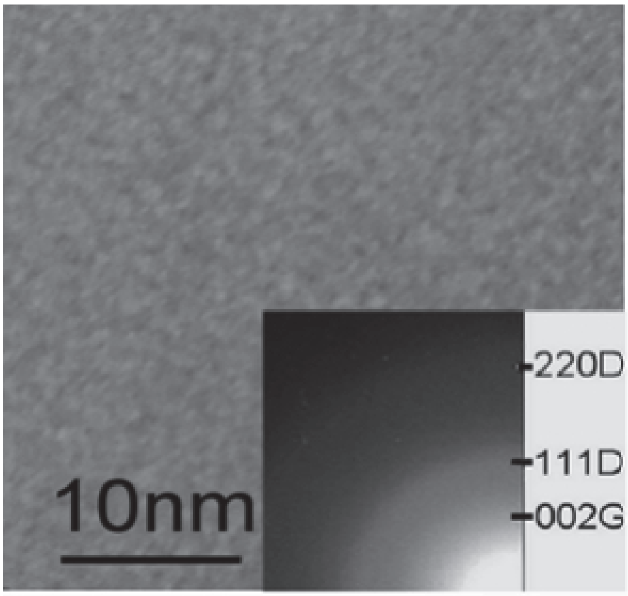



*Fig. 1.* Micrograph and electron diffraction pattern of diamond-like nanocomposite film. On the electron diffraction pattern it was marked the corresponding position for the family of graphite (G) and diamond (D) planes.

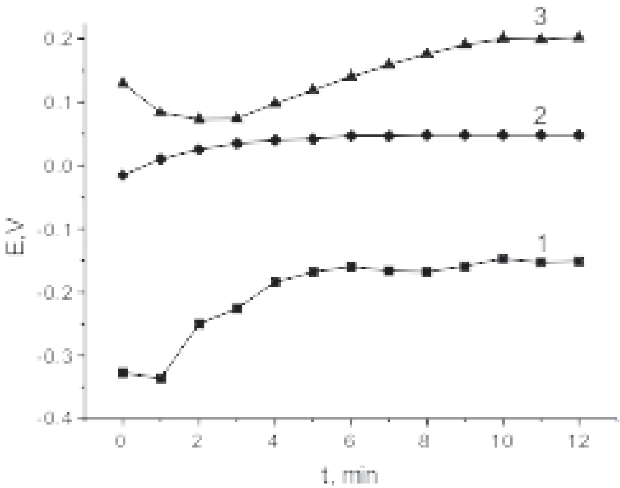



*Fig. 2.* Time dependence of electrode potential for Co/Cr implant (1), Ti implant (2) and Co/Cr implant with carbon diamond-like nanocomposite coating (3).


*Fig.3.* Photo of diamond-like film surface with sediment from solution.

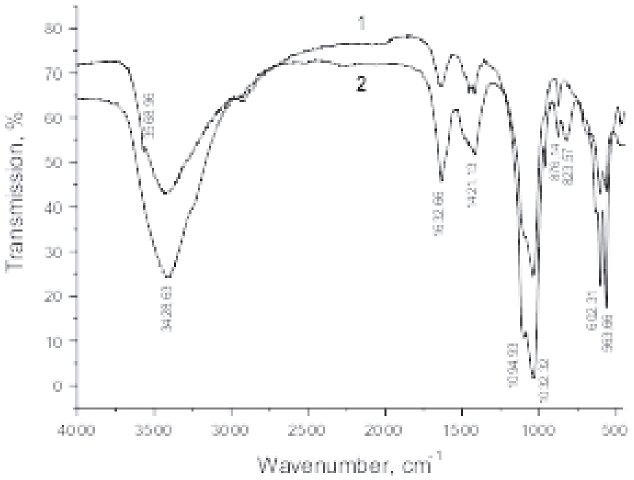



*Fig. 4.* IR spectra of native bone tissue (1) and calcium phosphate sediments (2) from solution imitating blood plasma.

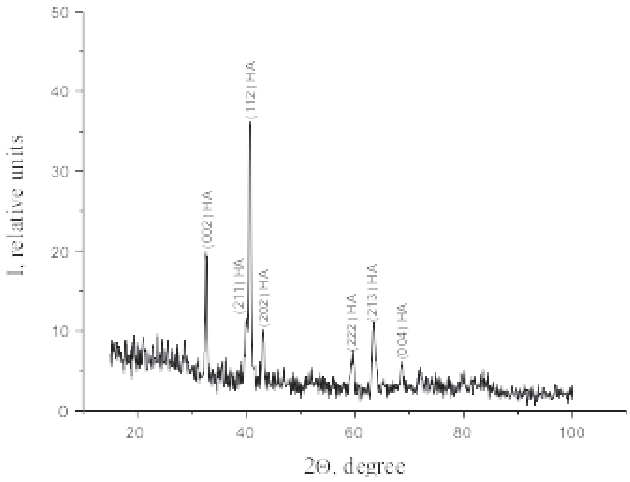



*Fig. 5.* X-ray diffraction pattern of the calcium phosphate sediments from solution imitating blood plasma.

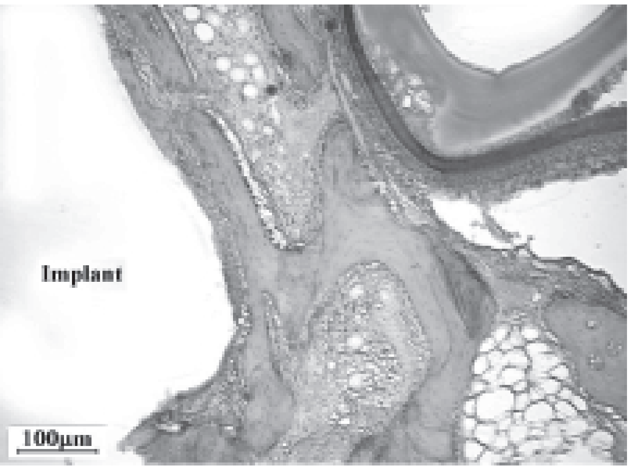
that on the sample surface there is formation of sediment and crystals (Fig. 3).

Analysis by IR-spectrometer was taken and was compared with that of native bone tissue (Fig. 4). On both spectra there are lines at 3428,63 $cm^{-1}$ corresponding to valent vibrations of hydroxyl groups $OH^-$; lines 1632.66 $cm^{-1}$ corresponding to deformation molecular vibrations of $H_2O$; lines 1421.13 $cm^{-1}$ corresponding to carbonate groups $CO_3^{2-}$, lines 876,14 $cm^{-1}$ и 823,57 $cm^{-1}$ corresponding to hydrophosphate groups $HPO_4^{2-}$ or carbonate groups $CO_3^{2-}$ and also lines 1094,93 $cm^{-1}$, 1032,32 $cm^{-1}$, 602,31 $cm^{-1}$ and 563,66 $cm^{-1}$ corresponding to phosphate groups $PO_4^{3-}$. The main IR absorption lines of bone tissue coincide with the lines of calcium phosphate sediments on the sample

*Table 1*

Severity gradation of morphological parameters of the mandible body for animals in 12 weeks after implantation

| Indicator | Gradation | Implant type | | |
|---|---|---|---|---|
| | | Co/Cr alloy | Titanium alloy | Co/Cr with DLC coating |
| | | Severity | | |
| Newly formed tissue between parent compact bone and implant | Connective tissue, % | 89,67±0,88 | 76,33±4,30 | 69,33±4,80 |
| | Coarse-fibered bone,% | 10,33±0,88 | 23,67±4,40 | 31,66±1,76 |
| Foci of necrosis at the tissue surface adjacent to the implant | Absence | | | + |
| | Isolated foci | + | + | |
| | 1/3 of the contact surface | | | |
| The nature of the compact bone restructuring | Osteocyte-free area | 0.33 | 0.33 | 0.25 |
| | Resorption cavity% | 50,70±2,60 | 44,67±2,60 | 41,67±3,33 |
| | Demineralization foci | single | single | absent |
| Characteristics of trabecular bone reconstruction | Density of Osteocytes | 119,33±1,20 | 122,00±6,43 | 158,00±1,15 |
| | Expanded intertrabecular space % | 61,00±4,36 | 57,30±4,40 | 43,67±2,60 |

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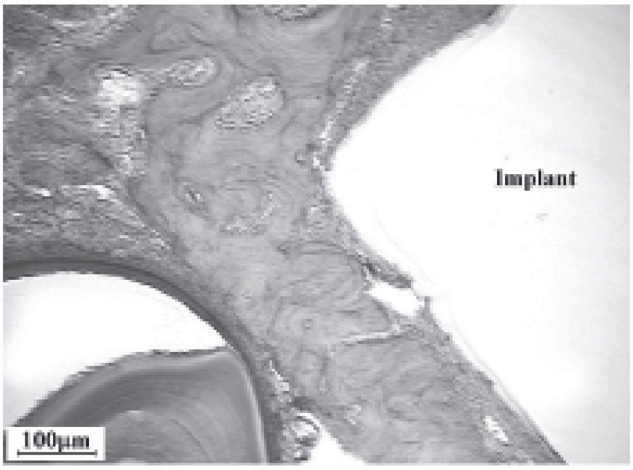


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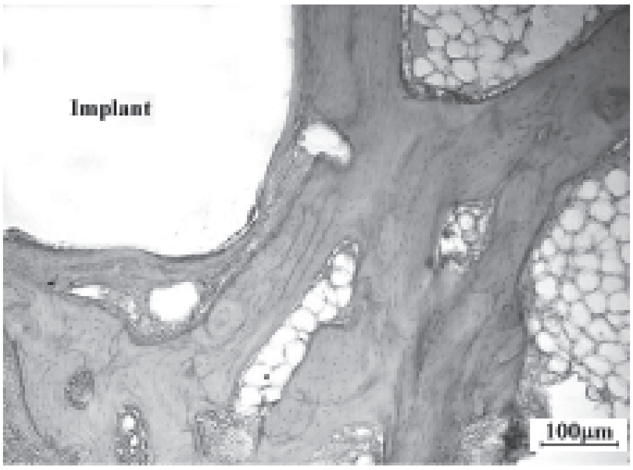


*c*

*Fig. 6.* Microscopy of contact zones of implants and bone: a – Co/Cr implant; b – Ti implant; c - Co/Cr implant with carbon diamond-like nanocomposite coating.

surface. This indicates that the coating composition which is formed from the prepared solution is similar to the composition of native bone tissue wherein the main part is hydroxyapatite.

The phase structure of sediments has been also investigated. Large crystals which are visible on the surface represent chlorides and oxychlorides. Structure of thin continuous coating layer, which was scratched out from sample surface, was more interesting. The received X-ray diffraction pattern is presented in Figure 5. As can be seen, calcium phosphate sediments from solution are generally presented by single phase – hydroxyapatite that contains, according to IR spectra, significant amount of carbonate and other impurity. Formation of hydroxyapatite crystals on the nanocomposite carbon coating surface testifies to high biointegration qualities of diamond-like film and potential efficiency of its application as a coating on medical implants.

Figure 6 shows the contact zones of bone and implants made from Co/Cr alloy, titanium and Co/Cr alloy with nanocomposite DLC coating in three months after surgery.

Microscopic investigation revealed that the Co/Cr alloy implant was firmly adhered to the surface of the bone and was partially covered by connective tissues. Connective tissue sections with a high density of fibroblasts were observed in the areas between the implant and compact jaw bone (Fig. 6a). At other locations, the implant was in direct contact with the parent bone. In these areas the bone tissue exhibited signs of destructive disorders such as absence of osteocytes, presence of demineralisation niduses, and chaotic arrangement of basophilic resting lines. Also, small foci of cellular detritus were observed, as compact bone restructuring areas with small resorption cavities. These cavities were filled with loose connective tissues. Narrow basophilic resting lines of bone remodelling foci and strata between the bone tissues were also observed. The morphological parameters of the mandible after implantation of the Co/Cr alloy plates are shown in Table 1.

The microscopic investigation of compact bone sites adjacent to the Ti alloy implant revealed areas of newly formed, mature bone. This bone was observed with connective tissues, a low density of fibroblasts and bundles of collagen fibres (Fig. 6b). Isolated foci of necrosis between the implant and the newly formed tissue were observed. Evidence of restructuring processes was observed in the compact bone of the jaw near the surface of the implant. These processes required the formation of resorption cavities, which were filled with friable connective tissue with a high density of capillary-type blood vessels. The density of osteoblasts was increased in the edge regions of trabecula of the cancellous bone. Intertrabecular spaces were also filled with friable connective tissues.

The microscopic investigation for Co/Cr alloy with nanocomposite DLC coating showed that edge surfaces of the implant were covered with connective tissues. Fields with rough bone were primarily observed between the implant and the compact body of the jaw bone. Only small areas containing connective tissues with collagen fibres arranged parallel to the surface of the implant were evident.

Only single, mature fibroblasts were observed (Fig. 6c). Resorption cavities were observed on compact bone areas located under the implant. These cavities exhibited various shapes and sizes and were filled with friable connective tissues or bone marrow fat. Some signs of restructuring, which included the formation of basophilic resting lines, were observed. These resting lines separated the strata and the newly formed bone. These areas contained a high density of osteoblasts (Table 1).

Thus, the comparative analysis of bone reconstruction was performed in the fixation implants of various materials to the compact bone of the mandible. It was found that in all cases after implantation the complex reparative and compensatory-adaptive restructurings, the severity of which depended on the implant material, occurs in compact and trabecular bone. It was revealed that in terms of "Newly formed tissue between parent compact bone and implant" most favorable results were observed for the implant made of Co/Cr alloy with nanocomposite DLC coatings. In this case it is observed a maximum formation of coarse fiber bone and minimal presence connective tissues, as well as more favorable value of other parameters. Average position was marked for implant made of titanium, a more pronounced negative rates were monitored for Co/Cr implant without coating.

**Summary**

Results of experiment have shown that the deposition of DLC nanocomposite coatings on Co/Cr alloy considerably increases biological compatibility of implant that reduces risk of fibrous tissue formation in zone of implant and bone contact and, therefore, increases primary stabilization and duration of implant use. Such coating increases the implant resistance to electrochemical corrosion processes at the expense of shift of its electrode potential to the positive values area. In the physiological plan the action of coating is defined by behavior of complex of reparative and adaptation-compensatory reorganizations that allows to accelerate processes of healing and postoperative adaptation of organism in implant introduction zone.


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