

# THE SYNTHESIS OF TITANIUM-ORGANIC NANOSTRUCTURES ON NANOTITANIUM SURFACE FOR BIOCOMPATIBLE COATING DEVELOPMENT

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**Abstract.** The work reports the development of titanium-organic nanostructure synthesis on a nano-titanium surface. The conditions for reproducible manufacturing of the brush-type titanium-organic nanostructures with calcium-phosphate inclusions via ML-ALD technique are studied. The technique for composite coating synthesis of titanium-organic brush-type nanostructures on nano-titanium surface with calcium-phosphate structures inclusions acting as osseointegration promoters is developed. The adhesion properties of composite nanocoating to osteoblasts MC3T3-E1 cell line were evaluated. The developed composite coating can be a promising material for implants with accelerated osseointegration.

## 1. INTRODUCTION

Implanted materials are widely used in a routine clinical practice in various medicine fields. When it comes to dentistry and orthopedics, many materials are in the direct contact with a bone tissue. That necessitates the development of new materials with the specific surface types, intentionally designed for the implant – bone tissue interaction enhancement [1,2].

The survival of cell tissues upon the contact with metallic materials usually does not depend on the bulk material properties, while the surface properties and the availability of biocompatible coatings on it are the key factors here. For this reason, manufacturing of the materials with adjusted surface properties is one of the most important goals for the modern material science [2]. This area attracts many researchers all over the globe and still up-to-date during last decades. Many researches focus on the development of bioactive materials; among these works, the investigations dealing with titanium surface modification should be mentioned. They include

various methods of titanium surface modification coupled with the addition of osseointegration promoters, such as calcium phosphate or hydroxyapatite [3-5]. Hydroxyapatite (biological calcium phosphate) is conventionally used here due to the fact that its chemical structure is close to the bone composition. During the matrix synthesis, osteoblasts produce orthophosphate anions from organic precursors due to high alkaline phosphatase activity, these anions undergo condensation as a result of the reaction with calcium cations. Thus, hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ) is formed, which is precipitated as a solid in the intercellular space.

The design of the coating with incorporated hydroxyapatite promoters on the titanium should lead increased ability of the implant surface to interact with biological fluids and cell elements. That is of particular importance on the early stages of osseointegration as it favors faster implants biocompatibility to the bone tissue. This general statement was proved in a number of studies [6-9], it was shown that bone implants covered by hydroxyapatite demonstrate the shorter interaction time

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of biological substance and metallic implant surface.

However, it should be noted that mineral phase is dispersed in the bone in a complex way because a bone itself is a composite material that has a dynamically changing multilevel organization. That is why it is absolutely logical to expect the cell response not only to the chemical composition but also to the coating structure. The reference data analysis shows that some authors report the effect of coating particle size, its porosity, and a number of other structural parameters on implants biocompatibility [10-12]. The goal of present work was the development of nanotitanium based biomaterial having an accelerated osseointegration due to the hybrid approach coupling both the surface geometry and chemical composition of superficial layer change. The evaluation of adhesion properties of titanium-organics based composite nanocoating with calcium-phosphates nanostructures inclusions acting as osseointegration promoters for osteoblasts MC3TZ-E1 cell line was also the task of the research.

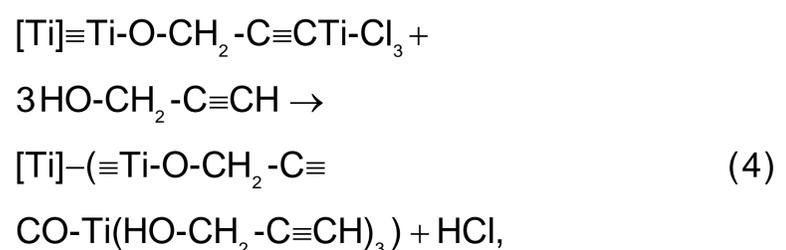
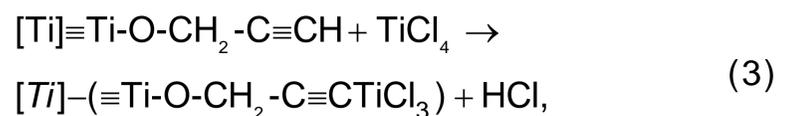
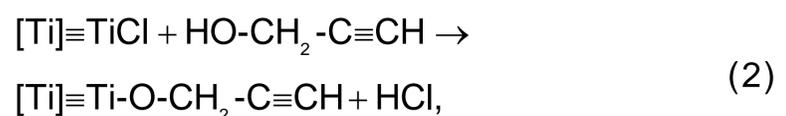
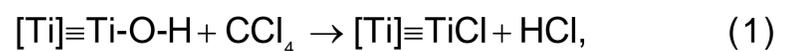
## 2. EXPERIMENTAL

In order to obtain nanostructured coating, the step-by-step synthesis of brush-type titanium-organic structures with calcium phosphate nanoinclusions on titanium surface was performed. To change nanotitanium surface relief specifically, the titanium-organic structure synthesis was carried out. Molecular layering technique also known as atomic layer deposition – ALD [13-18] was used for the synthesis here. Its main advantage is the possibility to obtain nanostructures (nanofilms) with the precise size (up to angstrom) and with high coating continuity [19-21]. That feature is achieved by consequent and cyclic low molecular weight chemisorption from the gas phase. The synthesis of calcium-phosphate nanostructures was performed into two steps: phosphate groups synthesis in the gas phase and  $\text{Ca}^{+2}$  sorptional addition in the liquid phase.

Nanotitanium samples were prepared in LLC “Nanomet” (Ufa, Russia) from titanium Grade 4. Titanium Grade 4 rods of 1 m length were deformed using ECAP-Conform machine in the temperature range of 200-450 °C; the number of passes was varied from 1 to 5, as a result, the value of the accumulated true strain varied from 0.7 to 3.5, respectively [22]. The average nanotitanium grain size was ~ 50-100 nm. After ECAP-Conform processing, the billets were subjected to extrusion at 200 °C. At the final step, wafers with 1x1 cm surface were manufactured.

### 2.1. Brush-type titanium-organic nanostructure synthesis on the nanotitanium surface

The preliminary surface treatment and the design of chemically active sites are required to obtain brush-type titanium-organic nanostructures on nanotitanium via ML-ALD. The two-step washing by organic solvents was used for the surface treatment. On the first step, nanotitanium surface was purified from lubricants and impurities in the turbulent acetone flow at 25 °C. On the second step, the surface was dewatered in the turbulent ethanol flow at 25 °C for 15 min. Then, the samples were treated by carbon tetrachloride (see reaction (1) at 200 °C in order to activate nanotitanium surface. The synthesis of brush-type titanium-organic nanostructures on the nanotitanium surface via ML-ALD technique was performed in the gas phase reactor in argon atmosphere according to surface chemical reactions (2-4).



where [Ti] - nanotitanium.

Nanotitanium treatment according to reactions (2-4) is considered as a single cycle of titanium treatment. One cycle allows to obtain titanium-organic nanostructures of minimal length (height). If it is necessary to increase the nanotitanium surface roughness and to perform the synthesis of long chains of titanium-organic groups, the product of reaction (3) is treated by propargyl alcohol vapor according to reaction (4). Then treatments by  $\text{TiCl}_4$  and propargyl alcohol are repeated. What is more, the number of  $\text{TiCl}_4$  and propargyl alcohol treatment cycles determine the length of as-synthesized one-dimensional titanium-organic nanostructures.

In the present work, the samples containing titanium-organic nanostructures were synthesized via nanotitanium treatment of 20 surface reactions

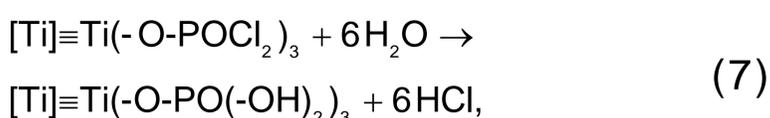
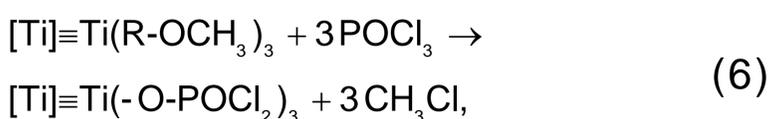
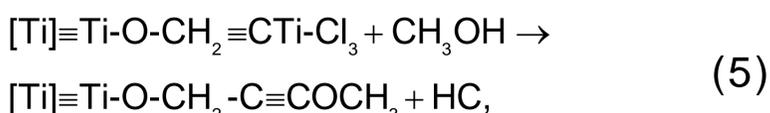
cycles. That choice is due to the necessity to get brush-type titanium-organic nanostructures with roughness with the height up to 200 nm. The preliminary evaluation of adhesion properties of osteoblasts cells MC3T3-E1 showed that 20 ML-ALD cycles and the height of titanium-organic nanostructures of ~200 nm are optimal for sufficient titanium nanolayer formation for further cell monolayer design on it.

## 2.2. Calcium-phosphate nanostructure synthesis on brush-type titanium-organic nanostructures surface

Calcium-phosphate nanostructure synthesis was performed included (i) phosphate groups synthesis in the gas phase; (ii) calcium phosphate synthesis based on  $\text{Ca}^{+2}$  addition via sorption in the liquid phase.

### 2.2.1. Phosphate groups synthesis

Phosphate groups synthesis on the surface of titanium-organic nanostructures was carried out in argon ambiance at 200 °C according to reactions (6) and (7). The product of reaction (5) was treated by  $\text{POCl}_3$  according to reaction (6). Phosphate groups were obtained in the final samples according to reaction (7).



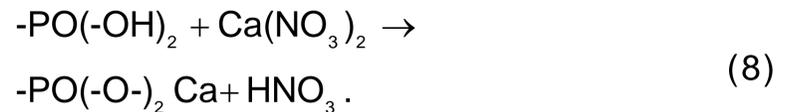
where  $\text{R} = -\text{O-CH}_2\text{-C} \equiv \text{C-} (-\text{O-CH}_2\text{-C-})$ .

The presence of phosphate groups in samples was verified by X-ray emission spectroscopy and X-ray fluorescence analysis.

### 2.2.2. Calcium phosphate synthesis

Calcium phosphate synthesis was performed via ion exchange of proton of  $-\text{PO}(\text{-OH})_2$  groups on the sample surface to calcium kation of  $\text{Ca}(\text{NO}_3)_2$  in a liquid organic phase. To do this, the sample with the phosphate groups monolayer with  $-\text{PO}(\text{OH})_2$  composition was placed into 1 M calcium nitrate solu-

tion for 5 minutes under stirring, the solution temperature was 35 °C, see reaction (8).



After the sorption, samples were removed and placed into a glass reactor for drying in the argon at room temperature. As a result, the following structure on nanotitanium surface with as-synthesized titanium-organic nanostructures was obtained:  $[\text{Ti}]-(\equiv(\text{Ti-OR})_n\text{-PO}(\text{-O-})_2\text{Ca})$ , where  $[\text{Ti}]$  - nanotitanium,  $(\text{Ti-OR})_n$  - titanium-organic nanolayer,  $\text{PO}(\text{-O-})_2\text{Ca}$  - calcium-phosphate groups. The phosphate groups and calcium content in the as-synthesized calcium phosphate layer were controlled by ESCA.

X-ray photoelectron spectra (XPS) were registered with a "Thermo Fisher Scientific Escalab 250Xi" spectrometer at the Resource Centre of "Physical Methods of Surface Investigation". The samples were excited by  $\text{Al K}\alpha$  (1486.7 eV) X-rays in a vacuum of  $7 \times 10^{-8}$  Pa. The sample charging was automatically compensated. The binding energy scale has been referenced using the C1s carbon line (284.5 eV). The information on calcium-phosphate structures was obtained using the equipment of the Center for Innovative Technologies of Composite Nanomaterials of Research park of St. Petersburg State University. Energy dispersive X-ray fluorescence spectrometer EDX Series 800 HS (Shimadzu) was used for the analysis.

## 2.3. The evaluation of osteoblasts MC3T3-E1 cell monolayer formation in experimental samples

The evaluation of osteoblasts MC3T3-E1 cell adhesion properties and cell monolayer formation in the samples were analyzed by scanning electron microscopy (SEM). The investigations were performed in the Institute of Cytology of the Russian Academy of Sciences (Saint Petersburg). All investigated samples were placed in Petri dishes and underwent sterilization via ozonation. The application of cell line MC3T3-E1 suspension on the titanium surface was performed in a small volume of the culture medium so that the "drop" formed on the sample surface did not flow down. The cell concentration under sample sowing was  $1 \cdot 10^5 \text{ cm}^{-2}$ .

Samples with cell suspension thus applied on their surface, were placed into  $\text{CO}_2$ -incubator at 37 °C for 3 hours. After that, when the cell adhesion

**Table 1.** Synthesis conditions for brush-type titanium-organic nanostructure synthesis on nanotitanium surface.

Surface pre-treatment temperature, °C	Synthesis temperature, °C	Number of treatment cycles
400	200	20

**Table 2.** AFM statistical analysis data of the experimental sample (program “Grain Analysis (Threshold)”).

Parameter	Value
Maximal height [nm]	200
Average height [nm]	150
Average width [nm]	120
Relative standard width deviation [%]	10
Surface concentration [1/sm <sup>2</sup> ]	$8 \times 10^7$

should likely take place on sample surface, the nutrient medium was added into Petri dishes. For comparison and control, the cell suspension was also applied into dishes surface. After 5 days of cells cultivation, the nutrient medium was removed, washed three times by phosphate PBS buffer and fixed in 20- $\mu$ l aliquot volume of 2.5% glutaraldehyde solution. The evaluation of cell condition (adhesion character and cell spreading on samples surface) was performed by SEM (JSM-35.7, Japan).

### 3. RESULTS AND DISCUSSION

#### 3.1. The investigation of the nanotitanium surface relief

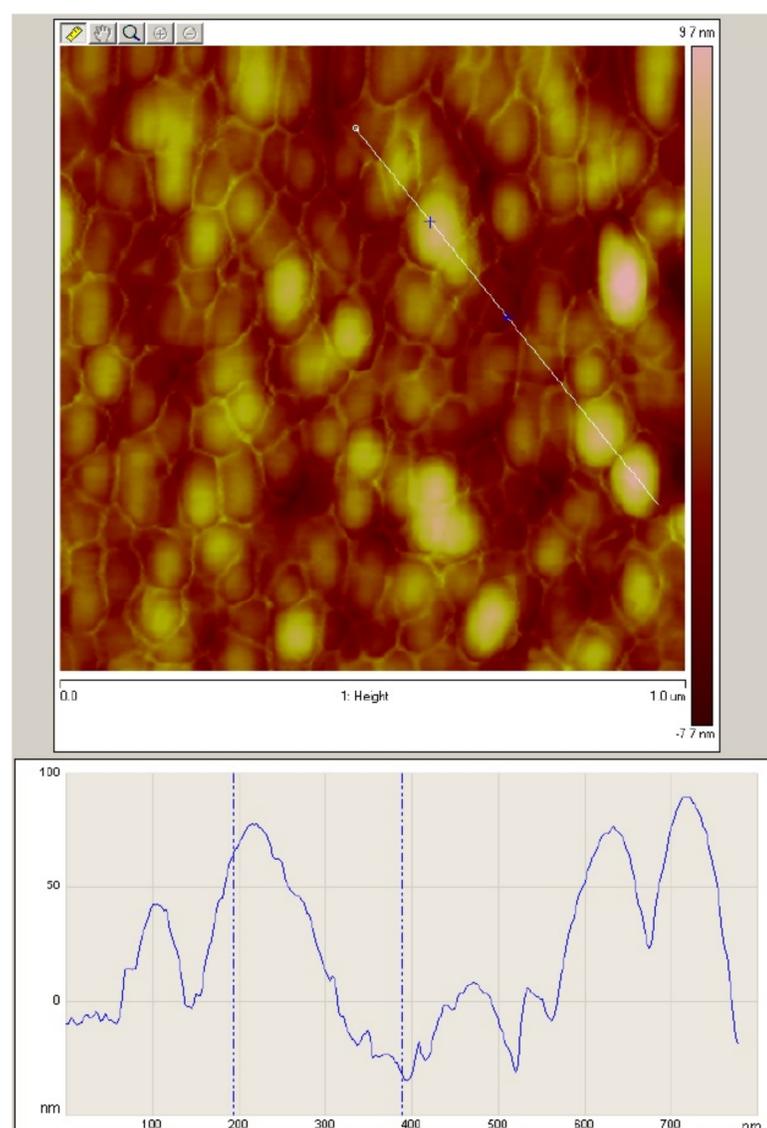
The nanotitanium surface relief was modified by means of brush-type titanium-organic nanostructures synthesis. The roughness height lied in the range 75-200 nm. As calcium-phosphate nanostructures height is  $\sim 1$  nm, their contribution into the total relief change can be neglected. The synthesis conditions for brush-type titanium-organic nanostructures synthesis on nanotitanium matrix surface are listed in Table 1.

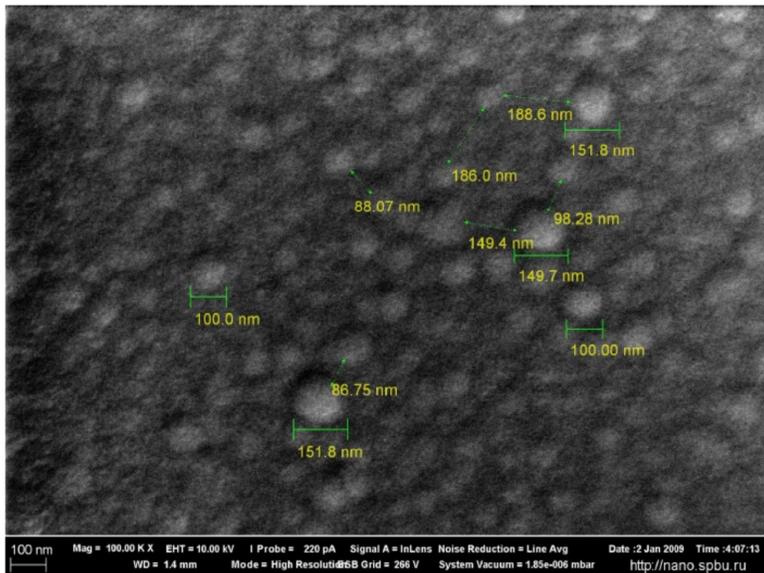
The brush-type titanium-organic nanostructure growth was controlled by atomic force microscopy (AFM) and SEM. It should be noted that SEM data gives a possibility to determine titanium-organic nanostructures size (width) and the spacing between them. The titanium-organic nanostructures height (surface roughness) can be determined by AFM.

The relief of the samples surface was studied using a Solver P47 Pro (NT-MDT, Russia) probe

microscope in the tapping mode. AFM study was conducted at 4–5 points on the sample surface. SEM microphotographs were obtained using the SEM Zeiss Merlin at the “Nanotechnology Interdisciplinary Resource Centre”, SPbSU. Microscope spatial resolution was  $\sim 1$  nm at the magnetization up to 200000. In-lens SE and SE2 regimes were used.

Fig. 1 depicts AFM data on surface topography of nanotitanium with titanium-organic nanostructures after 20 ML-ALD cycles. Average statistical height and width values of titanium-organic nanostructures are listed in Table 2. As it can be seen from these data, a complete overlapping of nanotitanium initial surface relief by synthesized structures takes place after 20 ML-ALD treatment cycles. The relief of the

**Fig. 1.** AFM data on 2D microrief; linear profile scan (real roughness), 20 cycles of reactions (2) and (3).



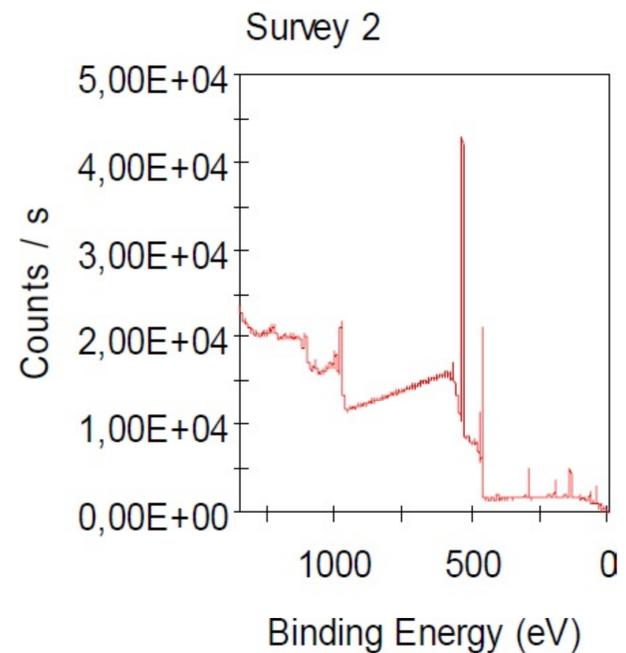
**Fig. 2.** SEM microphotograph of nanotitanium surface with layered titanium-organic structures. Number of reactions(2) and (3) cycles is 20.

modified surface is made up of structures with average height up to 200 nm and width up to 120 nm.

SEM analysis was performed to determine the size of titanium-organic structures and spacing between nanostructures as-synthesized samples were investigated via SEM, typical SEM image is shown in Fig. 2. The investigation of as-synthesized samples proved the complete overlapping of the initial surface by as-synthesized titanium-organic nanostructures after 20 ML-ALD cycles (stated above from AFM data). The space between nanostructures lies in the range 75–200 nm; average nanostructure size is 120 nm.

### 3.2. The investigation of as-synthesized calcium-phosphate nanostructures

In order to obtain calcium-phosphate nanostructures, the synthesis conditions allowing to derive samples with maximal phosphate groups content were founded. The use of surface  $-Ti_3$  groups in the synthesis was the main condition for it. Synthesized phosphate groups content in nanolayers was investigated by ESCA (see Table 3 and Fig. 3). As seen from these data, the average amount of phosphorus



**Fig. 3.** ESCA spectra of phosphate groups monolayer on titanium modified by titanium-organic nanostructures.

in the discussed nanolayers (phosphate groups on nanotitanium with titanium-organic nanostructure inclusions) is ~8.8 at. %. This fact agrees with our assumption on phosphorus oxychloride sorption on as-synthesized titanium-organic nanolayer surface.

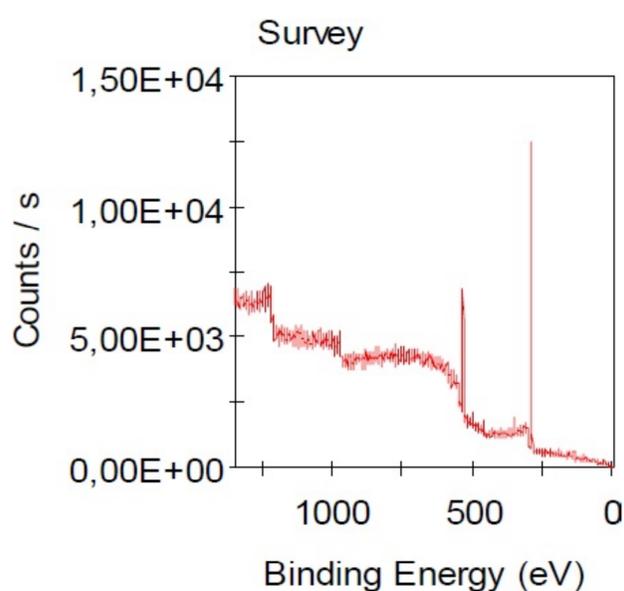
It should be noted that the position of the peaks maximums (134.3-134.6 eV) allows to us consider that phosphorus does not exists in the elemental state, P (with peak position 129.9-130 eV) but, likely, is a part of a phosphate group because the characteristic maximum of such groups lies in the similar range of 133-134.5 eV depending on the chemical environment. The absence of chlorine confirms the complete phosphorus oxychlorine hydrolysis along phosphorus-chlorine bonds with following P-OH group formation. The presence of titanium (15.5%), carbon, and oxygen (oxygen content is quite high here) is due to titanium-organic nanostructures present on the sample surface. Phosphate groups and calcium content in as-synthesized calcium phosphate monolayer were also investigated by ESCA, see Fig. 4 and Table 4. As seen from these data, all samples contain carbon, titanium, oxygen, calcium, and phosphorus. This composition char-

**Table 3.** Elemental composition of surface nanolayer on titanium modified by both titanium-organic nanostructures and phosphate groups monolayer.

Element	Peak BE	WHM eV	Area (P) CPS.eV	At. %
O1s	531.35	2.90	104525.12	58.78
Ti2p	459.23	2.00	63895.60	15.47
C1s	285.09	2.08	9708.54	14.17
P2p	134.34	2.13	8600.14	8.81
N1s	402.12	2.46	1874.85	1.71

**Table 4.** ESCA data on the element composition of the surface monolayer, containing phosphate groups and calcium phosphate on nanotitanium with titanium-organic nanostructures inclusions.

Name	Peak, BE	FWHM, eV	Area (P) CPS,eV	At. %
C1s	284.57	1.16	5028.02	72.43
O1s	531.95	2.99	3732.96	19.50
N1s	399.61	1.62	328.85	2.84
P2p	133.18	1.85	258.72	2.61
Ti2p	458.64	1.19	662.38	1.51
Ca2p	346.73	1.29	456.08	1.09

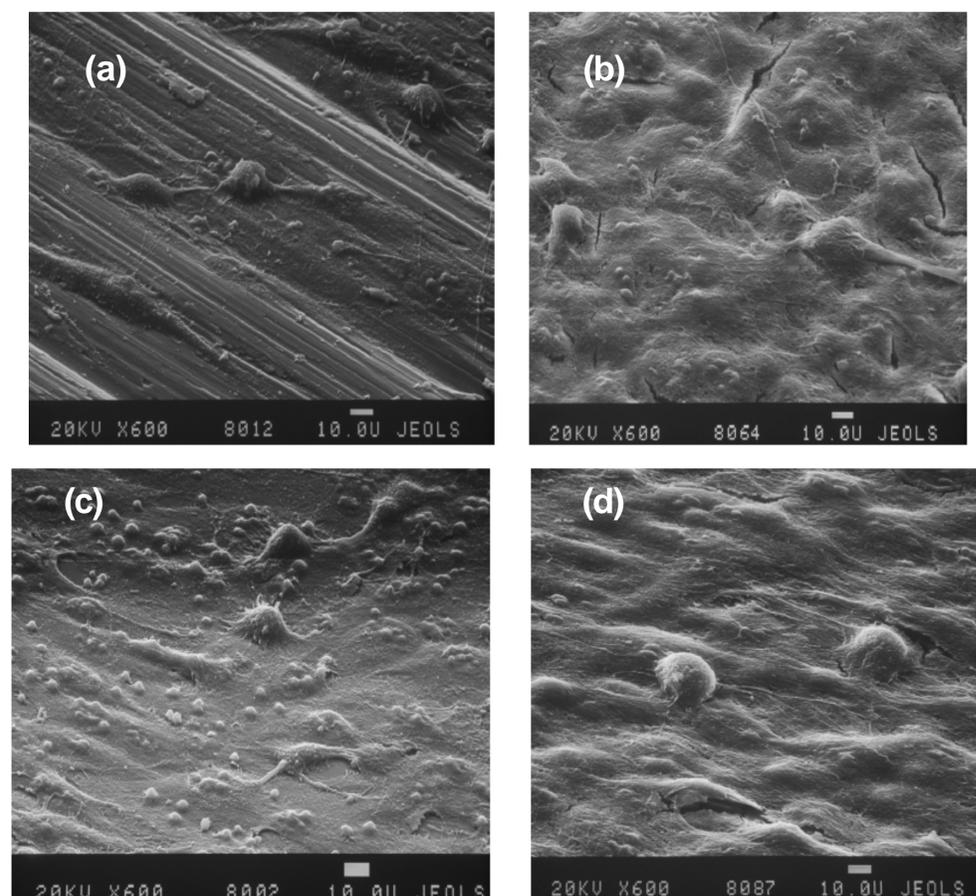


**Fig. 4.** ESCA spectra of synthesized calcium phosphate nanolayer on nanotitanium with titanium-organic nanostructures inclusions.

acterizes both titanium nanostructures and calcium-phosphate monolayer on it. The data shows that calcium phosphate structures were synthesized on the top of titanium-organic nanostructures.

### 3.3. Adhesive and spreading properties of the human fibroblasts MG-63 on the samples

It is known that the enhancement of the titanium surface bioactivity is the necessary requirement for further effective implant fabrication [2]. Usually it is achieved by the coatings fabrication on its surface and further osseointegration promoter inclusion (i.e. calcium phosphate and hydroxyapatite) on it. On the first step of the coating bioactivity investigation,



**Fig. 5.** Characteristic SEM images of samples #1-4 after human osteoblasts MG-63 adhesion and spreading: a - polished coarse grained titanium; b - polished nanotitanium; c - polished nanotitanium with a layer of applied phosphate groups; d - polished nanotitanium with brush type titanium-organic nanostructures on the surface and calcium-phosphate structures inclusions.

the experiments on the evaluation of cell lines adhesion properties to the sample surface are performed. That is a necessary but not the sufficient condition. The evaluation of the adhesion properties of the composite nanocoating based on titanium-organic nanostructures with inclusions of calcium-phosphate structures for osteoblasts MS3T3-E1 cell line was one of the tasks of the present work.

The following samples were chosen for the investigation:

- sample # 1, polished titanium – average grain size ~ 25  $\mu\text{m}$ ;
- sample # 2, polished nanotitanium – average grain size ~ 100 nm;
- sample #3, polished nanotitanium with applied phosphate groups layer;
- sample #4 polished nanotitanium with brush type titanium-organic nanostructures on the surface and calcium-phosphate structures inclusions.

The evaluation of cell state (adhesion character and cell spreading on sample surface) was investigated via SEM (see Fig. 5). In spite of the fact that cell concentration was the same for all the samples, only single osteoblasts cell were identified on sample #1 surface (microtitanium). It gives the evidence of low adhesion properties of that sample to investigated cell line (see Fig. 5a). In case of nanotitanium sample #2, the formation of osteoblasts cell monolayer on its surface is characterized by sufficient adhesion. However, cell differentiation is not observed (see Fig. 5b). Only single spread cells are revealed on the surface of sample #3. Besides, small rounded inclusions may serve the evidence of either poor cell adhesion or their possible death. Such cell behavior leading to their death is related to phosphoric acid formation in the nutrient medium because of phosphate groups hydrolysis (see Fig. 5c).

In this sample series, one should distinguish sample # 4 with brush type titanium-organic nanostructures and calcium-phosphate structures inclusions. Clearly marked monolayer of osteoblasts with a negligible amount of rounded attached cells on their division step (see Fig. 5d) are observed on the surface of that sample. In case of the investigated cell line, the sample possesses high surface adhesion. The initial cell differentiation is observed simultaneously with the high adhesion. That phenomenon indicates the ability of the discussed sample to accelerated osseointegration.

## 5. CONCLUSIONS

A technique for the synthesis of the composite coating on nanotitanium with brush-type titanium-organic

nanostructures on the surface and calcium-phosphate structures inclusions acting as osseointegration promoters was developed. The investigation of synthesized samples shown that the complete overlapping of the initial surface by synthesized titanium-organic nanostructures is observed after 20 ML-ALD cycles. The spacing between nanostructures varies from 75 to 200 nm. Mean nanostructures size is 120 nm. Surface roughness height lies in the range 75 - 200 nm. As calcium-phosphate nanostructures height is ~ 1 nm, their contribution into surface relief change was neglected.

The evaluation of the adhesion properties of composite nanocoating based on titanium-organic nanostructures for osteoblasts MS3T3-E1 osteoblasts cell line was performed. It was found that on the polished titanium surface modified by the nanolayer of phosphate groups only, the formation of cell monolayer does not take place. Single spreaded cells are revealed onto the surface which indicates both poor cell adhesion and their possible death. Such cell behavior leading to their death, is related to phosphorous acid in the nutrient medium because of phosphate groups hydrolysis.

The conditions for nanotitanium chemical modification (the design of composite coating) favoring the formation of clearly distinguished cell monolayer and high surface adhesion properties for osteoblasts MS3T3-E1 cell line are founded. It was shown that on nanotitanium sample surface with brush-type titanium-organic nanostructures and calcium-phosphate groups inclusions, the initial osteoblasts cells differentiation is observed simultaneously with high adhesion. That phenomenon indicates the ability of the discussed surface to the accelerated osseointegration.

## ACKNOWLEDGEMENT

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