

Crystallization of modified hydroxyapatite on titanium implants

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Abstract. Carbonated-hydroxyapatite (CHA) and Si-hydroxyapatite (Si-HA) precipitation have been synthesized from the model bioliquid solutions (synovial fluid and SBF). It is found that all the samples synthesized from the model solutions are single-phase and represent hydroxyapatite. The crystallization of the modified hydroxyapatite on alloys of different composition, roughness and subjected to different treatment techniques was investigated. Irradiation of the titanium substrates with the deposited biomimetic coating can facilitate further growth of the crystal and regeneration of the surface.

1. Introduction

Currently, much attention is paid to improving the quality of life and lifespan [1]. In medical practice, to save lives and restore important physiological functions of patients we have to resort to reconstruction of bone and tooth tissue through replacing the defect with an implant [2]. Every year, about 2 million of these operations are made throughout the world, while in Russia the number of operations such as joint prosthetics per year is ten times smaller than it is required.

To date, the research, development and production of medical supplies and proper implants are the most urgent issues for material scientists, physicists, chemists and engineers [3–8]. Modern trends in the development of high technologies and laboratory base of research centers require advances in the technology of medical products and expansion of their diversification to meet the requirements of international standards and to reduce their prime cost.

The solution of this problem is production of a bioactive coating on metal implants [9–13]. Bioactive implants can reduce the time of treatment for severe diseases and eliminate loosening and rejection (due to toxicity, inflammation and foreign body response). One of the important trends of modern material science is the development of biomaterials based on calcium phosphates [3–13]. The advantage of these materials is absolute biocompatibility due to the identity of chemical composition and crystal structure of hydroxyapatite (HA) of the natural bone. Thus, the hydroxyapatite coating on the implant surface ensures rapid and effective engraftment in the bone structure due to high level of biological activity of the surface.

In biomimetic synthesis, identification of optimal conditions for producing powders of highly dispersed phase with high resorption and biocompatibility with the human body are relevant. Therefore, the study and development of new methods for synthesis of fine crystalline hydroxyapatite, development of low-cost and technologic techniques of formation of bioactive coatings made of composite material on titanium are of critical importance.



The paper aims to investigate the possibility of crystallization of modified hydroxyapatite on the titanium alloy surface with different surface roughness and processing techniques applied.

2. Materials and methods

Synthesis of the modified hydroxyapatite was carried out in two ways:

1. From the model medium similar to the human synovial fluid in its ionic and electrolyte composition, pH and ionic strength [14]. To produce materials with properties close to those of the bone apatite, carbonate ions (CO_3^{2-}) were added to the synovial fluid model solutions. Their concentration was varied from 0 to 32 mmol/l. Crystallization of the solid phase was carried out within 30 days. The carbonate-hydroxylapatite prepared via biomimetic synthesis in the form of 5.00 mass% suspension was deposited on the sample surface subjected to different treatment techniques.

2. From the model medium similar to the human synovial fluid in its ionic and electrolyte composition, pH and ionic strength [15]. Na_2SiO_3 was chosen as the basic compound, the modifier of the silicate groups, for the synthesis from the intercellular fluid model solution. The synthesis time was 48 hours. The silicon-containing hydroxyapatite produced by biomimetic synthesis in the form of 1.00 mass% suspensions was deposited on the sample surface subjected to different treatment techniques. Upon completion of the solid phase crystallization in the supernatants, pH and Ca^{2+} concentration was measured by potentiometric method. The photometric method was used to determine the residual concentration of the phosphate ions in the solution [RD 52.24.382-2006], the residual concentration of the silicate ions (RD 52.24.382-2006) was measured with the colorimeter CPC-2. The solid phases were filtered, dried and analyzed by FTIR spectroscopy and X-ray diffraction analysis (XDA). IR spectra were recorded with the spectrometer FT-801 (samples were prepared in the form of KBr pellets). XDA was performed with the Bruker D8 Advance X-ray diffractometer (Germany) under monochromatic $\text{Cu-K}\alpha$ radiation. Statistical data processing was carried out using StatSoft Statistica 6.0. To deposit hydroxyapatite on the VT1-0 titanium surface, the titanium plates 15 mm*15 mm*1.2 mm in size were pretreated: polished; etched (etching mixture HNO_3 :HF 1:1 enlarging the surface of the titanium substrates was used); exposed to power ionization beam (the setup "Temp" was used for irradiation); perforated (etched, not subjected to mechanical impact and chemical attack, subjected to laser ablation) and plates which were not subjected to mechanical impact and chemical attack.

After that, the plate was immersed into the model prototypes of the synovial and intercellular fluids [14,15]. This system was soaked for 3, 6 and 12 days (the original SBF solution being changed and not changed), and after that, the plates were removed from the model solution and dried at room temperature. Optical microscopes Neophot 2 and MBS-9, and the scanning electron microscope JEOL JSM-6610LV were used to study the surface morphology.

3. Results and discussion

The results obtained by XDA indicate that throughout the range of varied carbonate ion concentration, the produced hydroxyapatites are of low crystallinity, for which the lattice parameters are evaluated [14]. The value of the parameters a and c of the elementary cell of the synthesized samples differ from that of the bone tissue and stoichiometric hydroxyapatite. The a parameter of the produced samples compared to the bone apatite is larger, and the c parameter, on the contrary, is smaller. Such characteristics of the crystal lattice are typical of the non-stoichiometric calcium-deficient hydroxyapatite, carbonate-bearing hydroxyapatite among them [14]. Thus, A-type carbonated-hydroxyapatite (CHA) was synthesized [14], which is currently widely used in surgery [16].

The carbonated-hydroxyapatite produced through biomimetic synthesis in the form of 5.00 mass% suspension was deposited on the prepared titanium sample surface subjected to different treatments. Fig. 2 (as an example) shows the surface morphology of the samples coated with the suspension (CHA). The coating formed on all the titanium samples contained cracks regardless of the pretreatment technique.

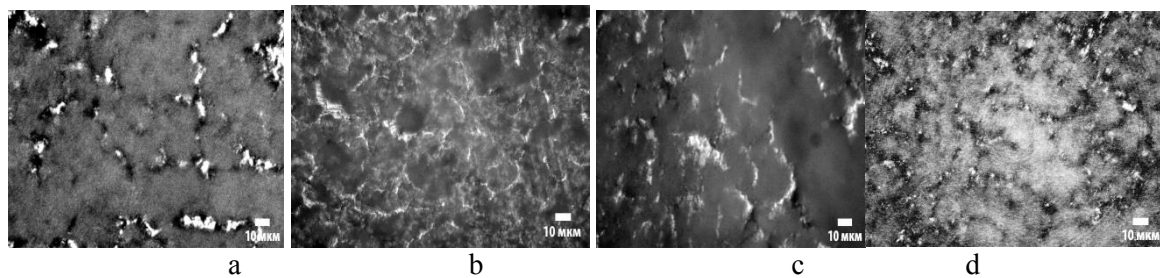


Figure 1. Morphology of the BT1-0 surface of different roughness coated with the hydroxyapatite suspension: polished surface (a), perforated surface (b), etched surface (c), ground surface (d).

Fig. 2 shows the morphology of the VT1-0 titanium sample obtained via scanning electron microscopy. It indicates CHA particles of a rounded shape clearly seen on the titanium alloy surface.

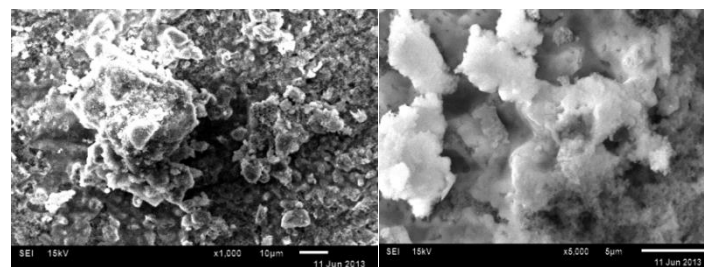


Figure 2. Surface morphology of the perforated surface of the VT1-0 titanium alloy coated with hydroxyapatite suspension.

A thickness gauge was used to measure the thickness of the deposited CHA layer. The measurement error was $\pm 3 \mu\text{m}$. The thickness of the deposited layer was approximately identical for all the samples and equaled $30 \mu\text{m}$. After producing the CHA layer, the samples were exposed to a power ion beam (PIB) with the "Temp" setup. Under PIB action, the deposited layer and the substrate interfused and partially intermixed due to high temperatures and gradients on the surface. Figure 3 shows the morphology of the CHA layer on the sample surface after irradiation obtained via scanning electron microscopy. It indicates intermixed portions of the substrate and CHA (Fig. 5) and shows the film structure mixed with the metal formed on the surface of VT1-0 titanium in some areas with cracks along the cleavage planes (Fig. 3b).

The study of crystallization of the metal samples from the synovial fluid model solution under irradiation with the varying number of pulses ($n = 3$ and 10) has shown that as the number of pulses increases, the CHA crystal growth activates. It is apparent that when $n=10$, the sample surface becomes more developed which contributes to an increase in the number of nucleation sites on its surface (Table. 1) and the area of the CHA layer coating the titanium alloy enlarges.

Table 1. Coated areas on the irradiated sample surface during crystallization

Alloy	Exposed to PIB with $j = 150 \text{ A/cm}^2$ $n=10$	Exposed to PIB with $j = 150 \text{ A/cm}^2$ $n=3$
Titanium VT1-0	28 %	20 %

It was found that under irradiation by PIB, the layers of the coatings based on the CHA suspension get fused and reliably fastened. This increases the biocompatibility of the titanium implant which is confirmed by further crystallization of CHA from the model solution on the surfaces of the synovial fluid samples. The surface morphology is shown to affect the coating uniformity and the rate of crystal growth. The more developed the surface morphology, the more active the crystal growth (Table. 3).

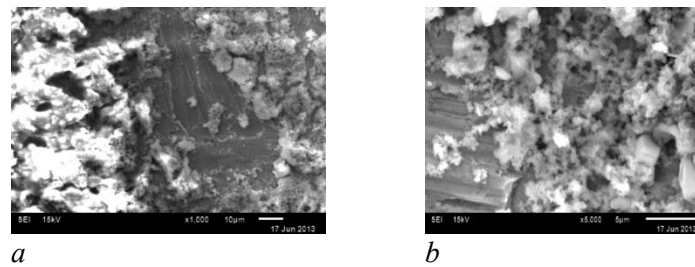


Figure 3. Surface morphology of the perforated VT1-0 titanium alloy surface coated with hydroxyapatite suspension after exposure to PIB.

For a group of samples with different surface roughness, deposition on the surface was performed from the model solution of the human synovial fluid and the solid phase crystallization lasted for 40 days. The dependence of the CHA crystal growth and the areas of the coated surface on the method of the titanium alloy treatment were studied using optical microscopy at different time intervals (7, 11, 18 and 40 days), and each time the initial solution for crystallization was renewed. After crystallization, the coated area of the CHA was determined with respect to the sample surface (Table. 2).

Table 2. Areas of the sample surface coated with CHA

Alloy	Polished	Perforated	Etched	Ground
Titanium VT1-0	15 %	85 %	26 %	40 %

The intensive crystallization of the solid phase was found to occur on the VT1-0 titanium samples with highly perforated surface. The coated area of this sample is about 85%, whereas, on the polished surface of the titanium alloy it is smaller.

The next stage involved synthesis of the modified hydroxyapatite silicate ions from the model medium of the human intercellular fluid. XDA has revealed that all the samples synthesized in the medium of the model solution of the extracellular fluid under varying concentration of silicate ions are single-phase and represent hydroxyapatite [15]. The sizes of the produced unmodified crystallites are 6.3 nm for HA and 6.0÷7.1 nm for Si-HA, which indicates the formation of nanocrystalline compounds. The parameters of the crystal lattice of the solid phase increase as compared to the unmodified hydroxyapatite, which confirms possible substitution of the orthophosphate-ion by the silicate ion (distances Si-O = 1.66 Å, P-O = 1.55 Å).

The study of the surface and morphological characteristics of the produced phosphate coatings modified by silicate ions identified more complete deposition of Si-HA on the titanium substrate surface for etched samples (Fig. 4).

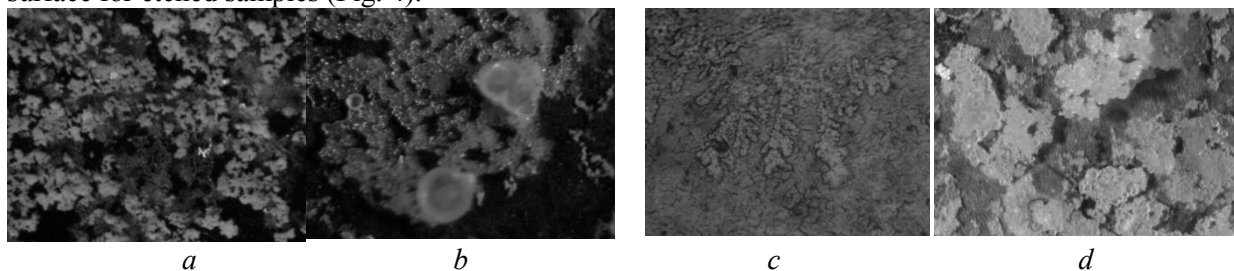


Figure 4. Deposition of Si-HA on titanium substrates (etched surface) within 3 days (a); 3+3 days, the model solution being changed (b); 6 days without changing the solution (c); 3+3+6 days, the model solution being changed (d).

This treatment technique provides the coating which is uniform, dense, highly dispersed, and the HA crystals grow in the form of dendrites. The crystals tend to grow in a more structured form. Increase in the time of titanium soaking in the model sample solution leads to non-uniform growth of columnar-shaped crystals, which indicates the start of Si-HA surface structuring. At this stage, the height of the formed aggregates is found to be different (Fig.4b). Further increase in the time of the soaking in the model solution for 6 days is characterized by the formation of non-uniform coating, and the crystal grow in the form of dendrites. The crystals are recorded to grow in the form of cylindrical

columns that characterizes the start of Si-HA structuring on the substrate surface (Fig.4c). During longer soaking without changing the model solution, the crystals accumulate in the form of aggregates and crystal growth goes beyond the substrate (Fig.7d).

The analysis of the coatings produced on titanium implants under power ion beam (PIB) shows that after this treatment followed by soaking in the SBF model solution, the coating of the titanium surface is uniform, and the Si-HA residuum on the samples is dense and highly dispersed (Fig. 5 *a*). After synthesis of the Si-HA layer on the titanium substrates, the samples were subjected to PIB with $j=50 \text{ A/cm}^2$ (Fig. 5 *c-d*). As a result, the coating was found to be uniform and dense with the edges fused. Crystals in the form of dendrites were observed on the substrates, which in our opinion, will contribute to further growth and renewal of the implant surface in physiological conditions.

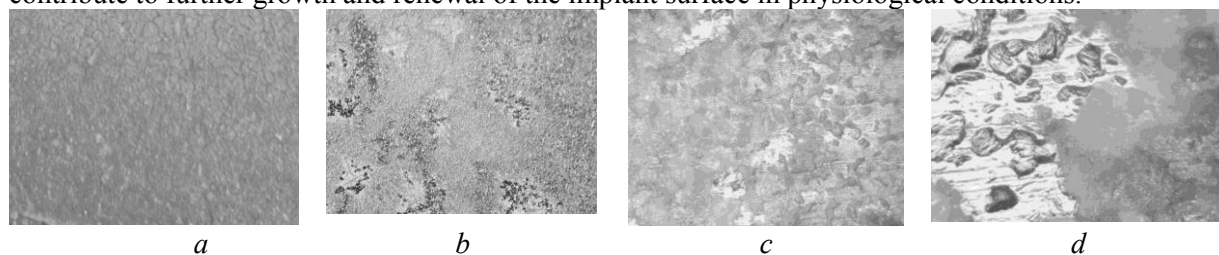


Figure 5. Surface morphology of the of hydroxyapatite crystal grown on the surface of VT1-0 Ti: exposure of the surface to PIB before deposition (*a*); after exposure to PIB with $j = 50 \text{ A/cm}^2$ (*b-d*)

While determining the coated area (Table 4), it was found that the highest percentage of deposition on the titanium substrate proceeds within the first three days. This is attributed to the fact that Si-HA dissolution in a static condition occurs mainly within three days, after which the dissolution of Si-HA is not observed. As can be seen in Table 3, to attain the best characteristics of the coatings, they are to be synthesized with the change of the suspension, which results not only in larger coated surface of the substrate, but in more uniform coating in terms of size and homogeneity of the crystals.

Table 3. The area of the coated surface depending on the technique of the titanium substrate treating

Treatment	S _c (%)				
	3 days	6 days	3+3 days	3+6 days	3+3+6 days
Etching	72	75	79	-	85
No treatment	60	67	70	-	78
Perforation etching	30	-	-	73	-
Perforation without treatment	25	-	-	79	-
PIB	-	60	-	-	-
Perf. with laser n=1	19	-	-	26	-

The analysis of the data provided in Table 3 shows satisfactory coating of the Si-HA surface for non-treated titanium samples, which confirms that the substrates made of the titanium alloy are highly biocompatible, non toxic, resistant to corrosive attack, possess characteristics are close to the mechanical properties of the bone tissue. The coatings are uniform, dense, with characteristic cracking indicating high thickness of the coating; large and small Si-HA aggregates can be found (Fig. 6 *a, d*). As the time of soaking in the model solution increases, the growth of columnar-shaped crystals is found to be non-uniform that characterizes the start of Si-HA surface structuring. No significant difference is observed in the height of the newly grown crystals (Fig. 6, *b*), the crystal growth goes beyond the substrate surface, and the bottom HA Si layer crystal lattice takes the form of the titanium crystal lattice. During long-term soaking of the titanium substrates in the SBF model medium, the coating is uniform, dense and highly dispersed across the whole surface, no agglomerates of individual Si-HA can be found (Fig. 6 *c, d*).

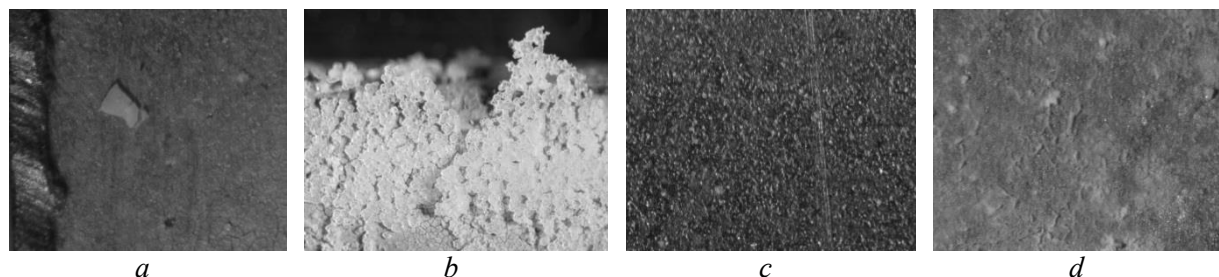


Figure 6. Deposition of Si-HA on titanium substrates without surface treatment: 3 days (a); 3+3 days, the model solution being changed (b); 6 days (c); 3+3+6 days, the model solution being changed (d)

4. Conclusions

The results of the study allow us to draw the following conclusions:

- varying the content of carbonate ions in the synovial fluid solution model can provide carbonate-containing materials of different crystallinity; the surface morphology affects the uniformity of the coating and the CHA crystal growth rate; the more developed the surface, the more active the crystal growth; perforated surface of the VT1-0 titanium alloy is most favorable for CHA solid phase crystallization; exposure of the sample surface coated with hydroxyapatite suspension to PIB contributes to fixation of the layer and formation of the nucleation sites for the subsequent crystal growth;
- all the samples synthesized in the model solution of the extracellular fluid under varying concentration of silicate ions are single-phase and represent hydroxyapatite; deposition of Si-HA on the titanium substrate surface preferably occurs on the etched samples; it is shown that the produced coating on the titanium is formed in several stages: growth in the form of the titanium crystal lattice; formation of crystals in the form of dendrites; crystal growth upwards in the form of cylindrical columns to form islands; it is found that after exposure of the titanium substrates to PIB with $j=50 \text{ A/cm}^2$, further growth of crystals and the regeneration of the surface are possible.

Acknowledgments

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