Formation of Biomimetic Hydroxyapatite Coating on Titanium Plates

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Hydroxyapatite (HA) has long been used as a coating material in the implant industry for orthopedic implant applications. HA is the natural inorganic constituent of bone and teeth. By coating titanium (base material of implant engineering because of its lightness and durability) with hydroxyapatite, we can provide higher biocompatibility of titanium implants, according to HA ability to form a direct biochemical bond with living tissues.

This article reports a biomimetic approach for coating hydroxyapatite with titanium A method of modifying the surface of titanium by organic modifiers (for creating functional groups on the surface), followed by formation "self-assembled" layer of biomimetic hydroxyapatite in simulated body fluid (SBF). FTIR and XPS confirmed the formation of hydroxyapatite coatings on titanium surface. Comparative study of the formation of HA on the surface of titanium plates modified by different functional groups: Ti($\equiv$OH), Ti/($\equiv$Si-OH) and Ti/($\equiv$COOH) is conducted. It was found that the closest to natural stoichiometric hydroxyapatite Ca/P ratio was obtained on Ti/($\equiv$COOH) samples.

Keywords: biomimetic hydroxyapatite, titanium plates, surface modification.

1. INTRODUCTION

The unique properties of biological materials produced by living organisms have always attracted the attention of researchers from the point of view of the possibilities of their practical use [1].

In modern medicine, biotechnical products and systems, in which the various elements and parts interact with biological fluids, soft and hard tissues of the body, are widely used and investigated. Transplantation of foreign material is inevitably accompanied by suppressive therapy, which severely limits the recovery of damaged tissue [2].

The bioceramics are widely used for the repair and re-construction of diseased or damaged parts of the skeleton. They can be used in a variety of shapes and forms: single crystals (sapphire), polycrystalline (alumina or hydroxyapatite (HA)), silicate glass, glass-ceramics and composites (polyethylene-hydroxyapatite). The implanted materials selected as inert, bioactive, or resorbable materials according to their reaction with living tissue. One of the attractive materials used in clinical field as orthopedic and dental implants is hydroxyapatite, which falls into the categories of bioactive (high density) and resorbable (porous HA) materials. The bioactive materials form direct bio-chemical bonds with living tissues [3].

Although numerous synthetic bone substitutes using metals, ceramics, and polymers have been developed to promote bone regeneration for several decades, there exists a lack of confidence in their biological performances, particularly related to long term, in vivo safety and efficacy [4]. As a result, over the last few years, many biomaterials have been suggested as bone substitutes. HA has been studied as a possible substitute material for hard tissue due to its high biocompatibility and osteoconductiv-ity, and it has been used clinically as artificial bone and for dental implants substitute [5].

Various techniques have been developed for producing HA coatings on implant surfaces, such as plasma spraying, hot isostatic pressing, sol-gel technique and biomimetic precipitation. Unfortunately, subsequent heat treatment at high temperature results in cracking and poor bond strength between the hydroxyapatite coating and metal substrate. Further, an HA coating of high crystallinity, which is desirable for optimal biocompatibility, could not be achieved through these methods. [6]

The most important areas of research and development of these materials, can be considered a composite nanomaterial synthesis, similar in structure and properties of human bone, hybrid organic-inorganic phases involving calcium phosphates for use in regenerative surgery – a engineered bone using cellular technology. In addition, the material must have a surface capable of supporting cell adhesion, proliferation and differentiation [3].

Systematization of experimental data and the basic principles of design and modeling of natural biomineral structures reveal the potential to determine the relationship of biological and mineral component that provides structure of biological composites at the nano-, micro-and macro-levels.

The basic principles of construction of biomineral structures are hierarchical structural organization, multi-functionality, self-organization and self-assembly. In this article we studied the main ways of achieving the result: biochemical and chemical. Biochemical simulation is based on finding and isolating the organic matrix (protein or active fragments), and the subsequent synthesis biomineral structures on their basis.

Chemical direction of biomimetics based on a synthesis of materials that simulate the specific properties of natural biomineral structures.

A promising direction of biomimetic modeling is the use of self-organization and self-assembly – the basic

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principles of living systems [4]. Currently it was developed a simple and potentially effective method for biomimetic synthesis of nanostructured materials by self-organization of polymers and/or inorganic nano-sized particles on the surface of the substrate [7 – 11].

A comprehensive study of the characteristics of natural biological mineral is essential for the development of technologies for new biomimetic structures and nanocomposite materials with desired properties and characteristics.

Currently nucleation mechanisms and the role of the functional group in the formation of an artificial bone remain controversial.

Formation of the crystalline HA requires an organized hydrophilic and negatively charged surface [12].

Crystal formation in the aqueous solution depends on many factors including the concentration of ions, temperature, degree of saturation and the interfacial energy.

The strong influence of surface properties on coating formation means that the key step in any biomimetic method is the chemical modification of the surface to achieve the optimum conditions for heterogeneous nucleation.

Self-assembled monolayers (SAM) – compact and highly organized monolayers, which are formed by chemical adsorption (Fig. 1). The structure of one molecule SAM includes three main elements: a functional group and an anchor end between alkyl chains [13].
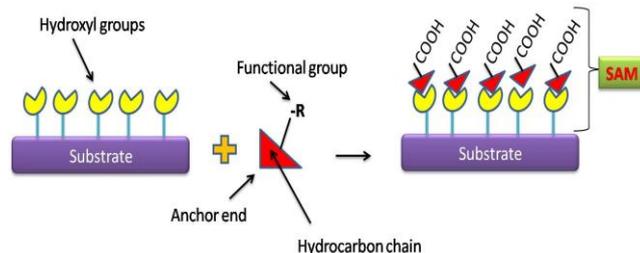

Fig. 1. Formation of self-assembled layers (SAM)

Functional groups for the SAM can be: $-CH_3$, $-NH_2$, $-OH$, $-SH$, $-SO_3H$, $-COOH$, $-PO_4H_2$.

With a high affinity anchor group with hydroxylated substrate formed a strong bond due to polar covalent or ionic bond.

The authors [14] have studied the surface: Si–OH, Ti–OH, Zr–OH, Nb–OH and Ta–OH. It was shown, that the hydroxyl functional groups, due to their hydrophilic properties, have good ability to initiate growth in of the investigated HA surfaces. However, the best results were obtained after silanization of surface with 3-alkylsilyl, trichloro-or trialkosysilane [15].

The aim of this work is comparative studies of the influence of the nature of the surface functional groups on the process of biomimetic synthesis of hydroxyapatite on the surface of the titanium, creating reactive groups on its surface, effective for the subsequent initiation of the deposition of apatite.

In connection with this, a promising solution to the problem can be the formation of the biocompatible nanostructured coatings based on hydroxyapatite (HA) and oxide compounds, the most compatible with biological tissues in the body for all parameters (physical, chemical, biological, biomechanical) [2].

The strategy of the method is based on the formation of hydroxyapatite layer by coating the surface of the titanium by biologically active functional groups. The basis of the method is supposed to simulate the process of mineralization, which occurs in the human body [16].

2. EXPERIMENTAL DETAILS

To simulate the natural properties of hydroxyapatite and its formation on titanium layer biomimetic method which consists in the creation of nucleation sites on the metal surface by modifying its surface by functional groups, and further the process of mineralization in the simulated body fluid, similar to human plasma, were examined.

Procedure for the synthesis of hydroxyapatite layer on the titanium surface consists of the following stages:

1. Pre-treatment of titanium samples (cleaning, oxidation);

2. Preparation of the reactive functional groups on titanium surface by chemical modification;

3. Preparation of simulated body fluid medium, a chemical analogue of human plasma, which is responsible for formation of hydroxyapatite;

4. Formation of a layer of hydroxyapatite on titanium as a result of the interaction of its biomimetic surface functionalized with the surrounding medium.

Pre-treatment of models of implants - titanium plates, was performed as follows. For the studies, we used chemically pure titanium strip. Firstly, cutting titanium plates (1.5 cm × 2 cm) and their mechanical cleaning was carried, then degreased with acetone and kept in ultrasonic bath for 6 – 10 minutes to complete remove the organics. Next, the surface of samples was hydroxylated with the reaction mixture of H_2O_2 (30 %) : H_2SO_4 (conc.) = 1 : 1 (by volume) with stirring for 10 – 15 minutes at room temperature. After preparing, the plate washed with distilled water.

In order to create reactive functional groups on pre-treated titanium plates they were modified with tetraethylortosilicate (TEOS) to produce silanol coating $\equiv$ Si–OH and (triethoxysilylpropyl-carbamoyl)-butyric acid (TESPCBA) for creating functional carboxyl (–COOH) groups on the surface.

Sample 2 was synthesized by keeping titanium surface in anhydrous toluene (35 ml) and tetraethylortosilicate (15 ml) for 24 hours at room temperature (Figure 2). Plates were placed in the reaction mixture so that the modification reaction was held uniformly over the entire area of the plate. At the end, the plate washed thrice with distillate water.
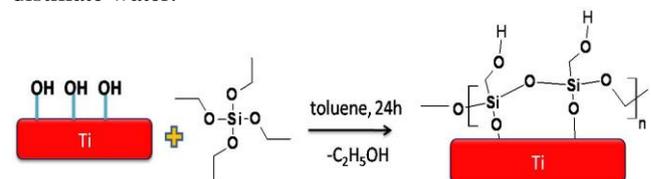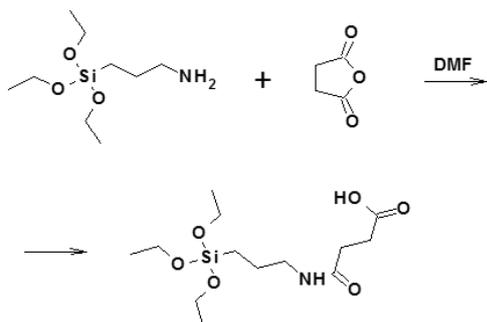

Fig. 2. Surface modification of titanium by $\equiv$Si–OH functional groups

Synthesis of the reactive carboxyl groups on the surface of titanium (sample 3) was performed by modifying triethoxysilylpropyl-carbamoyl butanoic acid (TESPCBA), which is obtained by reacting γ-aminopropyltriethoxysilane with glutaric anhydride.

In 20 ml of anhydrous dimethylformamide (DMF) was dissolved γ-aminopropyltriethoxysilane (4.7 ml) and glutaric anhydride (2.28 g). The reaction formed TESPCBA:

Modification was carried out by keeping the titanium plates in TESPCBA solution in DMF for 24 hours at room temperature (Fig. 3). Samples were washed with ethanol and distillated water.
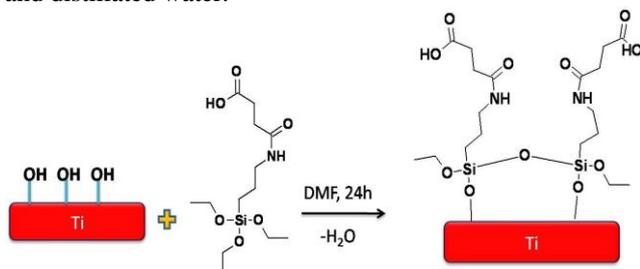

Fig. 3. Surface modification of titanium by ≡COOH functional groups

Thus, the following samples were synthesized: Ti/(≡OH) (sample 1), Ti/(≡Si–OH) (sample 2) and Ti/(≡COOH) (sample 3).

The simulated body fluid, which is a chemical analog of human plasma, was prepared by dissolving in distilled water (1 liter) such salts: 8 g NaCl, 0.353 g of $NaHCO_3$, 0.224 g KCl, 0.174 g of K_2HPO_4, 0.305 g of $MgCl_2·6H_2O$, 0.278 g of $CaCl_2$, and 0.071 g of Na_2SO_4. pH was adjusted to 7.4 by adding 0.1 M HCl.

For formation of a layer of hydroxyapatite, titanium plates containing various functional groups, was immersed in a simulated body fluid for the process of mineralization. It took several weeks.

Studying of the surface of samples was carried out by Fourier transform infrared spectroscopy (FTIR) («Perkin Elmer», model 1720H) and X-ray photoelectron spectroscopy (XPS) (electron spectrometer ES-2402 with energy analyzer PHOIBOS-100, SPECS).

The XPS spectrometer is equipped with an ion gun IQE-11/35 and a source of slow electrons FG-15/40 to compensate for charging the surface of insulators. The radiation source was a ray gun with a magnesium anode (E MgK$_\alpha$ = 1253.6 eV, P = 300 W). The calibration of the spectrometer was carried out at three points of the energy scale for the position of the maxima and Au4f7/2- Ag3d5/2- Cu2p3/2- lines.

Ca2p-level spectra have been placed on the interrelated components of the parameters $\Delta E_{3/2-1/2} = 3.56$ eV and $I_{1/2}/I_{3/2} = 0.5$, their width at half maximum was $\Delta E = 1.9$ eV. P2p-level spectra were decomposed into individual components with $\Delta E = 2$ eV. The decomposition on components was carried out by the Gauss-Newton. This area component was determined after background subtraction with Shirley method [16–18].

The surfaces of titanium samples were examined after 3 and 21 days after immersion in the SBF solution.

3. RESULTS AND DISCUSSION

In nature, the formation of the mineral phase of bone is subject to prevail heterogeneous nucleation over homogeneous. Modification of the biologically inert metal substrate by organic functional films mineralization induces apatite crystals growth [19].

Given the above, this study investigated the titanium samples with a hydroxylated surface – Ti/(≡OH) and the samples modified with tetraethylortosilicate – Ti/(≡Si–OH) and triethoxysilypropyl-carbamoyl-butyric acid – Ti/(≡COOH) to form –OH and –COOH functional groups (Fig. 4).
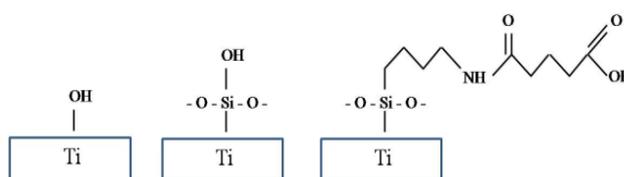

Fig. 4. Modification and functionalization of the titanium surface

Formation of biologically equivalent apatite can be simulated in experiments using the SBF. Biomineralization processes include the nucleation and growth of crystals of the new mineral phase, and these processes are controlled by the degree of supersaturation of the liquid and local concentrations of components. Titanium samples, containing various functional groups were immersed in a physiological fluid model for the process of biomineralization. Crystallization coating was formed by weeks [19, 12].

Coatings formed on the titanium surface in SBF using self-assembled monolayers containing hydroxyl (–OH), a silanol (≡ Si–OH) and carboxyl (–COOH) terminal groups were investigated by IR spectroscopy. Functional groups, associated with the structure of hydroxyapatite, are identified in all IR spectra of the synthesized samples (Figures 5–7).

The absorption bands (AB) at the (1000–1100) cm^{-1} indicate the stretching vibrations of PO_4^{3-}, AB at (870–880) cm^{-1} – bending vibrations of phosphate groups, and the AB 560 cm^{-1} and 605 cm^{-1} – is the bending vibrations of the PO_4^{3-}-tetrahedra.

A broad band absorption spectrum in the region (3400–3100) cm^{-1} is associated with the presence in the sample of water molecules, and a weak absorption band at (2300–2400) cm^{-1} indicates the presence of potassium bromide, which is used for sample preparation. Stretching vibrations of OH groups also corresponds to a broad band at (2800–3700) cm^{-1}, indicating the presence of hydrogen bonds. AB at (1400–1500) cm^{-1} and 871 cm^{-1} indicate stretching vibrations of CO_3^{2-} [20].

Analysis Ti-OH of the spectra (Fig. 5) shows only major peaks of absorption bands that appeared after immersion in SBF, it is (650–720) cm^{-1} and 1000 cm^{-1} to 1100 cm^{-1}. These peaks correspond to the OH$^-$ and PO_4^{3-} groups. In this spectrum, absorption bands appeared slightly, which can be attributed to CO_3^{2-} ions.
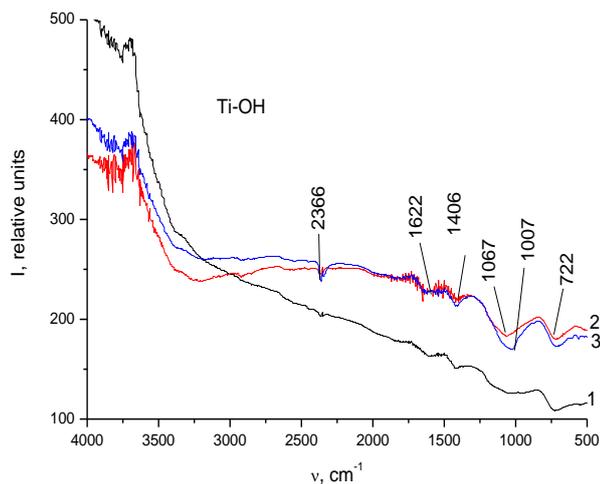
Fig. 5. FTIR spectrum of samples: 1 – Ti/≡OH, (initial sample), 2 – three days-layer, 3 – layer after three weeks exposure in SBF
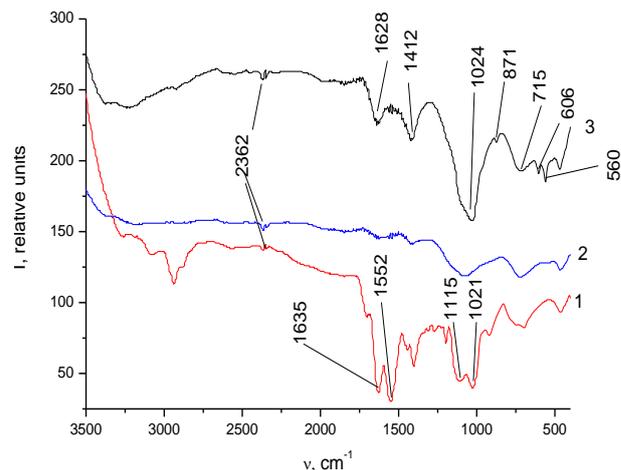

Fig. 6. FTIR spectrum of samples: 1 – Ti/(≡Si–OH) (initial sample), 2 – three days-layer, 3 – layer after three weeks exposure in SBF

Fig. 7. FTIR spectrum of samples: 1 – Ti/(≡COOH) (initial sample), 2 – three-day layer, 3 – layer after three weeks exposure in SBF
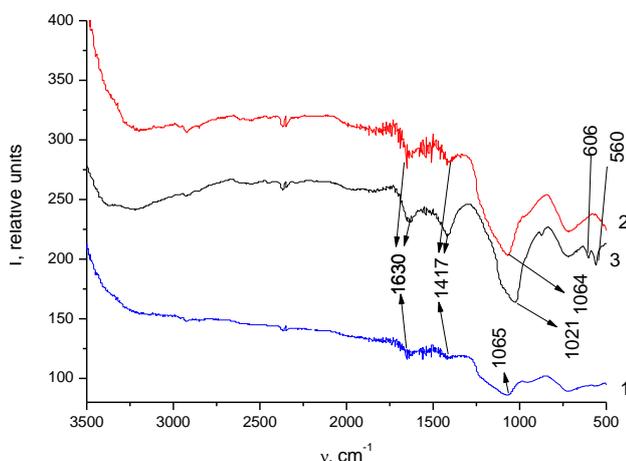
Analysis of XPS spectra of Ca2p and P2p levels of HA samples, containing various functional groups, indicate, that P2p spectra contain two nonequivalent states of phosphorus ions. Component with $E = 133.7$ eV corresponds to phosphorus ions of HA [23], and component at $E = 134.6$ eV could be associated with hydrated HA phase with increased content of OH groups on the surface. The relationship between equivalent phosphorous states depending on the synthesis conditions.
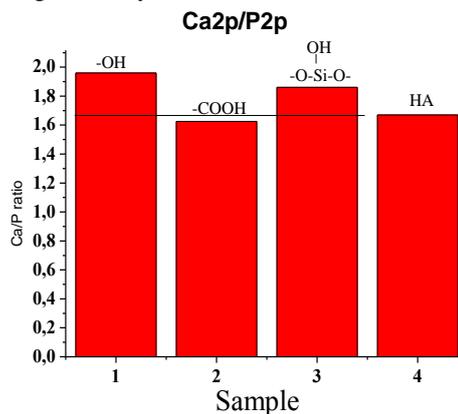
Figures 6 and 7 show, as gradually (depending on the time), appears the additional absorption bands at $(1400 – 1500)$ cm^{-1}, which indicate the stretching vibrations of CO_3^{2-}, i.e. forming hydroxyapatite layer on the sample surface with groups ≡Si–OH and ≡COOH [21].

In case of Ti/≡COOH, AB, corresponding to carboxylic groups at 1635 cm^{-1} and 1552 cm^{-1}, decrease after 3 days immersion in SBF. In can be due to growth of small layer of HA, and it is overlapping with AB of carboxylic and Si–O–Si groups at 1115 cm^{-1} and 1021 cm^{-1}, respectively. After 3 week immersing in SBF, HA-layer clearly identified in IR-spectra, by characteristic AB at 1024, 715 and 606 cm^{-1}.

Furthermore, investigations [22] show that the HA of crystallization can be measured by splitting characteristic peak at 565/605 cm^{-1} and 1070/1150 cm^{-1}. [22] Assume that the sharpness of the band at 606 cm^{-1} and 560 cm^{-1} also shows the crystallinity of the HA. It should be noted, that crystallinity of the HA observed in ≡Si–OH modified surface. This is especially characterizing titanium samples with modified by carboxyl groups surface (Fig. 7).

The electronic structure of the samples surface after three-week exposure in SBF also was investigated by X-ray photoelectron spectroscopy (XPS).

Fig. 8. Ca/P ratio in the obtained materials, compared to native hydroxyapatite

The binding energy of Ca2p3/2 levels in all samples was 347.8 eV corresponds to the calcium ions in the HA. In our efforts to create HA on the titanium surface [24, 25] was recorded much higher energies for the P2p and Ca2p lines (respectively, 137 eV and 351 eV) that can be associated with a reactive surface-active plates.

It is well known, that in natural HA, Ca/P ratio = 1.67, and it is very important for biocompatibility. We studied Ca/P ratio in samples 1, 2, 3 using intensities of elements (calcium and phosphorus) in XPS spectra. As it can be seen from the diagrams (Fig. 8), the most effective is the method of formation of HA on the surface of titanium with the carboxyl groups by using TESPCBA.

According to the XPS, the ratio of the integrated intensities of Ca2p-/P2p-lines, shown in Fig. 8, on COOH–modified titanium surface corresponds to the stoichiometric hydroxyapatite.

The investigations show that coatings formed in the SBF using self-assembled monolayer's with a functional ≡COOH group, show more consistent performance of hydroxyapatite crystallinity than coatings formed with the ≡ Si–OH and OH– functional groups.

4. CONCLUSIONS

It was found that on surface of non-modified Ti, process of HA formation is very slow, and non-specific. It confirms the low intensity of PO_4^{3-} peaks in FTIR spectra and high Ca/P ratio (~1.96).

In case of ≡Si–OH-modified surface, this process occurs more rapidly, but Ca/P ratio is worse than desired (~1.86). It should be noted, that we observe indicators of crystallinity at $606/560\ cm^{-1}$, in contrast with unmodified Ti/OH sample.

It was show, that the modification of titanium surface by –COOH groups could form a layer of hydroxyapatite with the morphological characteristics, Ca/P ratio and the degree of crystallinity the most similar to natural hydroxyapatite.

Fourier transform infrared spectroscopy and XPS confirmed the formation of the biomimetic hydroxyapatite coating, on the surface of titanium plates, with different quantity and "quality" of HA, according to chemical nature of samples surface.

All obtained data show that developed materials is perspective for use in medicine as implants with biocompatible surface, similar in composition to the natural HA.

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