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Comparative Analysis of the Bioactivity of Materials

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Abstract—Bioactive materials are characterized by a high rate of formation of a carbonate-containing hydroxyapatite contact layer at the implant–bone interface and by a moderate resorption capacity in weakly acidic media. In vitro tests simulating the precipitation of hydroxyapatite from interstitial tissue fluid at the surface of a material are performed to evaluate the bioactivity of a broad range of materials. The data indicate that the silicon-containing material is characterized by the highest bioactivity in the series of calcium hydroxyapatites.

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INTRODUCTION

The development of biomaterials for medicine is an important area in modern inorganic materials science. The first-priority requirements imposed on these materials are as follows: (a) no rejection reaction by contact tissues or by the immune system, (b) a controlled dissolution rate (at a level of tens of micrometers per year), and (c) fast bone union after implantation [1, 2].

Taking into account the above factors, the 60 wt % two-phase material $Ca_{10}(PO_4)_6(OH)_2$–40 wt % $Ca_3(PO_4)_2$ commonly used in clinical practice is currently considered to be optimal. The closeness of the composition of synthetic calcium hydroxyapatite (HAP) $Ca_{10}(PO_4)_6(OH)_2$ to that of bone tissue determines the high biocompatibility of implants made on its basis. HAP-based macrocrystalline ceramics are very slowly replaced by growing bone: blocks of the artificial material can be found after many years have elapsed [3, 4]. On the contrary, ceramics prepared with the use of β-$Ca_3(PO_4)_2$ dissolve so rapidly that the bone growing in its position has no time to fill the appearing cavities [5]. The rate of dissolution of a material depends on its surface area and its physical and chemical characteristics (microstructure, composition, imperfection), which determine the human response to a foreign implant [1, 2, 5, 6].

Materials characterized by rapid union with bone tissue by means of a carbonate hydroxyapatite cement layer formed in the gap between the implant and the bone from the supersaturated interstitial tissue fluid are considered bioactive. In the general case, a practically important procedure of evaluating such an integral characteristic of the biomaterial as bioactivity implies in vivo testing in animals [5, 7]. This procedure is expensive and very time-consuming. By virtue of the noted factors, methods are actively being developed that can rank materials by their bioactivity at the preclinical stage using relatively simple in vitro experiments [7, 8].

A synthetic body fluid (SBF) solution is currently used as a model medium in bioactivity tests of implants at a temperature of 37°C and close-to-physiological pH. This solution is identical in its mineral composition and ion concentration to blood plasma (table). The principal distinction of the majority of model solutions from biological fluids is the absence of organic elements, the low concentration of carbonate ions, and the use of buffer solutions not characteristic of living systems (as a rule, TRIS–HCl).

The aim of this work is to study the bioactivity of materials differing in the chemical nature: steels (Fe) and anion-modified apatites. It is known that unalloyed steel is a toxic material. The precipitation of an isolating apatite layer onto a heterogeneous surface prepared from a supersaturated saline SBF solution can mask the results of corrosion and lead to erroneous conclusions about the biocompatibility of the material. The carbon-

Preparation protocol of an SBF solution

Order of reagent addition	Reagents	Quantity, g/l	Purity, % (Merck grade)
1	NaCl	6.547	99.5
2	$NaHCO_3$	2.268	99.5
3	KCl	0.373	99.0
4	$Na_2HPO_4 \cdot 2H_2O$	0.178	99.5
5	$MgCl_2 \cdot 6H_2O$	0.305	99.0
6	$CaCl_2 \cdot 2H_2O$	0.368	99.0
7	Na_2SO_4	0.071	99.5
8	$(CH_2OH)_3CNH_2$	6.057	99.5

ate-containing material $Ca_9Na_{0.5}(PO_4)_{4.5}(CO_3)_{1.5}(OH)_2$, which more correctly reproduces the composition of bone tissue as compared to unmodified HAP [1], was considered an anion-modified apatite. Silicon-containing hydroxyapatite $Ca_{10}(PO_4)_{6-x}(SiO_4)_x(OH)_{2-x}$ was another object of investigation: it is considered that silicon is favorable to improved bioactivity of a material [2]. Partial substitution of phosphate groups in the hydroxyapatite structure can be used to increase the solubility of the biomaterial with the aim of obtaining resorbed implants [8].

Thus, the main aim of this work is to test the adequacy of the results of bioactivity analysis obtained using model SBF solutions for materials for which the reaction of the human body is known a priori.

EXPERIMENTAL

Hydroxyapatite powders were synthesized by precipitation in aqueous solutions. Chemically pure sodium hydrogen phosphate $Na_2HPO_4 \cdot 12H_2O$ and calcium chloride $CaCl_2 \cdot 2H_2O$ were used to synthesize calcium hydroxyapatite. A solution of sodium phosphate was quickly added to a solution of the calcium salt with intensive stirring. In this case, the following chemical reaction took place:

$$10CaCl_2 + 6Na_2HPO_4 + 8NaOH \longrightarrow Ca_{10}(PO_4)_6(OH)_2\downarrow + 20NaCl + 6H_2O. \quad (1)$$

Carbonate apatite $Ca_9Na_{0.5}(PO_4)_{4.5}(CO_3)_{1.5}(OH)_2$ (CO_3 HAP) was prepared by an analogous HAP procedure in which sodium carbonate was used as an additional reagent. For the synthesis of silicon-modified hydroxyapatite $Ca_{10}(PO_4)_{6-x}(SiO_4)_x(OH)_{2-x}$ (Si HAP), where $x = 0$, 0.1, 0.25, and 0.5, tetraethoxysilane (TEOS) of reagent grade was additionally used.

$$(C_2H_5O)_4Si + 4OH^- = 4C_2H_5OH + SiO_4^{4-}, \quad (2)$$

$$10Ca^{2+} + (6-x)HPO_4^{3-} + xSiO_4^{4-} + (8-2x)OH^- = Ca_{10}(PO_4)_{6-x}(SiO_4)_x(OH)_{2-x} + (6-x)H_2O. \quad (3)$$

After being held for 3 h at 80°C, the obtained precipitates were separated from the mother solution by filtration on a paper filter, washed in water and acetone, and dried in air. To prepare ceramics, apatite powders were pressed and sintered at temperatures of 1100–1300°C for 3–5 h. The geometrical density of the ceramic materials obtained was 93–98%.

A steel nail whose composition, according to x-ray spectral analysis data, corresponded to that of pure iron was used as a toxic sample for comparison.

X-ray investigations of the samples were performed on a DRON-3M diffractometer (NPO Burevestnik, USSR) in the reflection mode (Bragg–Brentano geometry) with the use of CoK_α radiation ($\lambda = 1.79021$ Å, nickel β filter). Electron-microscopic investigations of the samples were carried out using a scanning electron microscope with a LEO SUPRA 50VP (Carl Zeiss, Germany) field emission source. IR absorption spectra of the samples were recorded on a Spectrum One (Perkin Elmer, United States) spectrometer in the range 400–4000 cm^{-1} with a scanning step of 4 cm^{-1}. The potentiometry of the solutions (pCa and pH measurements) was carried out using an Ekoniks-001 potentiometer (NPO Ekoniks-ekspert, Russia). The Z potential of the powder surface was measured by a Zetasizer Nano ZS (Malvern, Great Brittan) device at pH 9.

The precipitation of HAP from solutions on the surface of biologically active materials was investigated during the thermostating of compact samples in a solution modeling the composition of interstitial tissue fluid (SBF solution) supersaturated with respect to HAP (the degree of supersaturation $\log(\sigma_{HAP}) = 9.1$) at pH 7.4.

The Tas-SBF solution used for the bioactivity analysis of the materials in vitro was prepared by dissolving the corresponding quantities of reagents in distilled water strictly according to the preparation protocol (table) [7]. Calcium phosphate tablets (0.3 g) were placed in an SBF solution (20 ml) at 37°C for 1–3 days. The solution was renewed three times a day.

RESULTS AND DISCUSSION

When the bioactivity of the materials in the model solutions was studied, the formation of an openwork calcium phosphate layer on the surface of tablets of the starting material was analyzed. The thickness and the relative area (as a percentage of the total surface area of the samples) of the deposited layer were used as a quantitative measure of the bioactivity of the material. The coating composition was analyzed using x-ray diffraction and IR-spectroscopy data, and the formation of carbonate hydroxyapatite (CHAP) was observed (Fig. 1). The precipitation of a calcium phosphate layer on the surface of the material was caused by spontaneous crystallization from a supersaturated saline solution. If the investigated material is itself one of calcium orthophosphates with a relatively high solubility product, then the new layer can be formed via the dissolution–precipitation mechanism.

The microstructures of CHAP coatings are identical for all the considered biologically active materials: aggregates of scaly crystals up to 2 μm in size were observed (Fig. 2). The perpendicular direction of crystal growth with respect to the sample surface was related to the direction of diffusion flows (dissolution–precipitation mechanism). Among the hydroxyapatites, a silicon-containing material had the highest activity (the largest thickness of the layer deposited on the surface) in spite of the higher solubility of CHAP [9]. Thus, the investigated anion-modified apatites were for the first time ranked by bioactivity using a rather simple in vitro procedure:

$$\text{Si-HAP (2 μm)} > CO_3\text{-HAP (1.5 μm)} \gg \text{HAP (1 μm)}.$$

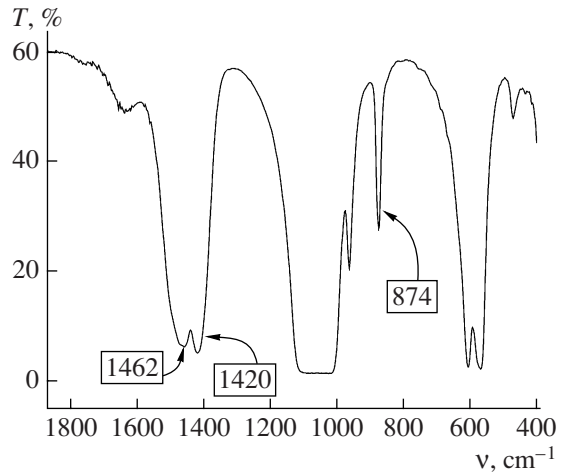

Fig. 1. IR spectrum of carbonate hydroxyapatite.

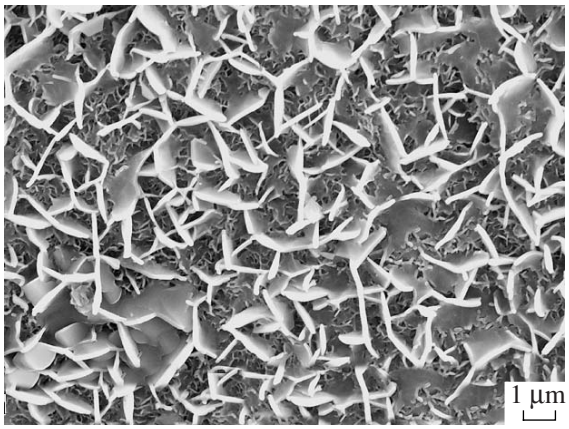

Fig. 2. Characteristic microstructure of a coating formed on the surface of a bioactive material in an SBF solution.

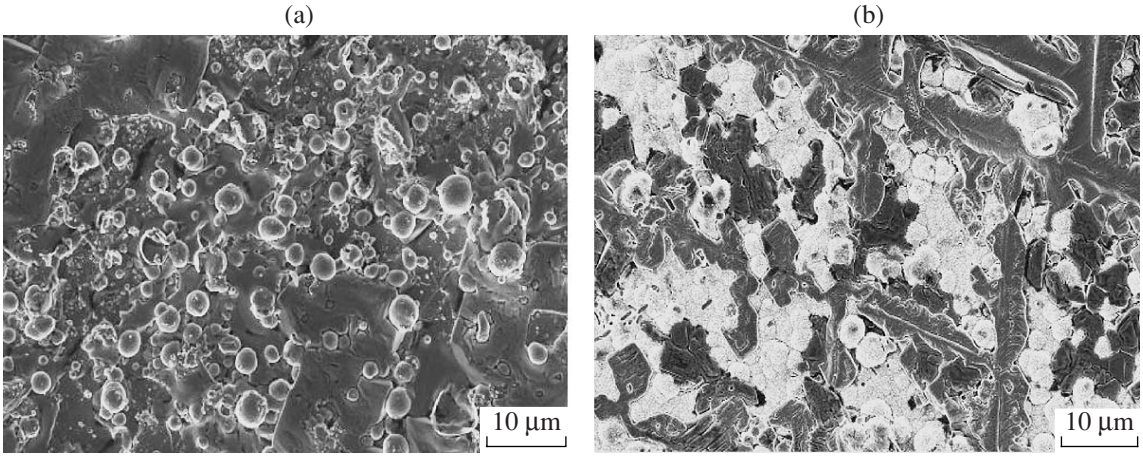

Fig. 3. Photomicrographs of the mineralized surface of samples of silicon-containing $Ca_{10}(PO_4)_{6-x}(SiO_4)_x(OH)_{2-x}$ apatite: (a) $x = 0$ and (b) $x = 0.1$.

The rate of formation of hydroxyapatite deposited on the material surface (coating continuity) increases with increasing silicon content in the $Ca_{10}(PO_4)_{6-x}(SiO_4)_x(OH)_{2-x}$ apatite. The coating area is about 60% of the total surface (after one day hold) at a silicon content of $x = 0.1$ (Fig. 3), as compared to 40% for the unsubstituted HAP material, and about 90% at a silicon content of $x = 0.5$.

According to the Z potential measurements, the surface of particles of the $Ca_{10}(PO_4)_{5.8}(SiO_4)_{0.2}(OH)_{1.8}$ sample has a large negative charge (–17 mV) as compared to the unsubstituted HAP (–1 mV). The high surface activity of Si-HAP, as well as in the case of bioactive glasses, is associated with the formation of silanol SiOH groups on the material surface, which actively participate in the process of surface mineralization. Silanol groups bind calcium, forming apatite nucleation centers.

To check the adequacy of the evaluation of biological activity, an iron nail was used as a reference material (in the early days of bone surgery, steel nails were unsuccessfully used to fasten the sides of extended bone fractures). After 7 days of thermostating the sample in an SBF solution, it was possible to observe the formation of islands of rust of FeO(OH) composition on the surface of the nail, along with the formation of a dense layer of calcium phosphate (Fig. 4). The composition of the formations was confirmed by x-ray diffraction data and by the results of x-ray spectrum microanalysis.

Thus, even a higher increase in the precipitation rate of calcium phosphate on the surface of a noncorrosive steel is required in order that the undesirable formation of iron hydroxide compounds be prevented by means of an isolating coating. However, it is difficult to use solutions with a higher component concentration because of the high degree of supersaturation and, as a result, the instability of these media with respect to the spontaneous crystallization of salts during preparation of the solution. The solution to this problem is complicated by the fact that, according to the literature data, Fe^{3+} iron

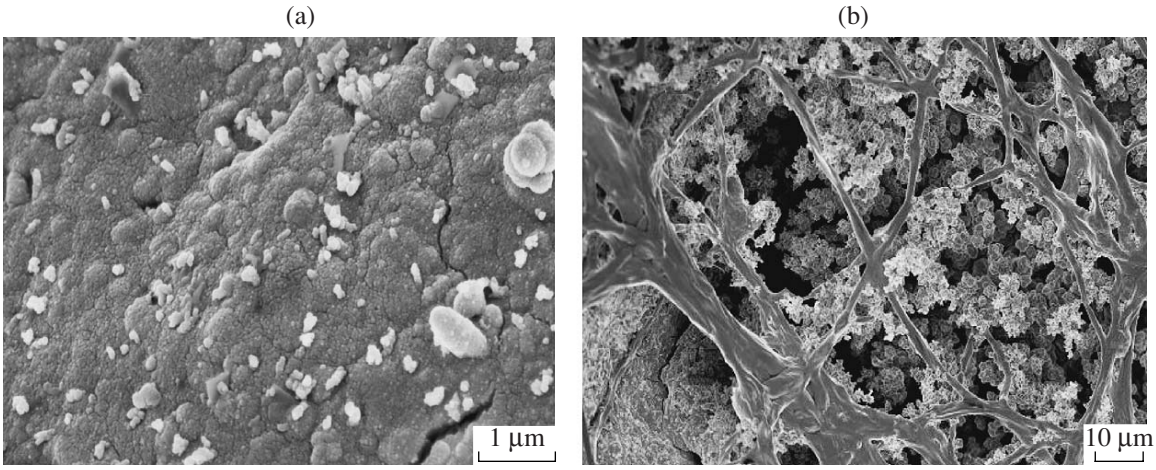

Fig. 4. (a) Apatite layer on a metaland and (b) the corrosion region.

ions inhibit apatite crystallization in aqueous solutions (the amorphous calcium phosphate–apatite transition is decelerated because of the adsorption of iron ions on the particle surface).

CONCLUSIONS

It is shown that the use of model solutions permits materials to be adequately ranked with respect to their bioactivity, the toxicity of unalloyed steel was revealed, and the bioactivity of apatite powders and ceramics was confirmed. Related materials based on anion-modified hydroxyapatite were ranked by their bioactivity by means of using SBF solutions: Si-HAP > CO_3-HAP $\geqslant$ HAP.

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