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Stability of Hydroxyapatite–TiO_2–Ti Structures in Physiological Solutions of KH_2PO_4–Na_2HPO_4 at Varied pH

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Abstract—Thermally annealed structures of hydroxyapatite–TiO_2–Ti prepared by high-frequency magnetron spraying have been studied before and after incubation in the 0.02 mol/l buffer solutions of KH_2PO_4–Na_2HPO_4 at pH 6.0, 7.0, and 8.0. Atomic force microscopy has revealed that the surface relief and mean roughness are not changed in the course of incubation. No sharp changes in the calcium to phosphorus ratio have been observed according to local X-Ray spectral microanalysis. The stability of hydroxyapatite–TiO_2–Ti towards buffer solutions has been confirmed by X-Ray powder diffraction studies.

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Titanium and its alloys are used as biomaterials due to biological inertness and corrosive stability. To supply them with necessary properties under the conditions of functioning human body, they are covered with hydroxyapatite coating mimicking the inorganic part of dental and osseous tissues of mammals. Biocompatibility of hydroxyapatite makes it a widely spread material applied in medicine, in the forms of powder and dense or porous ceramics. Poor strength of hydroxyapatite-based implants, however, complicates their *in vivo* applications in the conditions of mechanical stress. Therefore, hydroxyapatite-coated metal (in particular, titanium) implants are intensively studied. Such coatings improve the reliability of titanium-based devices and their osteointegration. Various procedures to coat titanium alloys are known; among them, magnetron spraying is one of the most readily available in the cases of biocompatible calcium phosphate coatings [1]. The drawback of the method is poor adhesion of the coated layer to metal substrate. High-temperature annealing of Ti-hydroxyapatite structures improves the adhesion, at the cost of risk of significant volume of titanium dioxide phase formed. The latter considerably increases the layer thickness and may lead to the film peeling off the support. The peeling can be prevented by a thin intermediate layer if TiO_2 formed by magnetron spraying as well. Titanium dioxide acts as a chemical barrier preventing the metal release and its oxidation with oxygen contained in hydroxyapatite, thus the bioactive film disruption is avoided [2].

Previous studies of the hydroxyapatite–Ti structures have shown [3] that thermal annealing of hydroxyapatite film improves its stability towards physiological solutions at various pH; thus, one of the major questions related to potential application of the hydroxyapatine–TiO_2–Ti system implies the study of its behavior in contact with physiological media.

In this work, we studied the changes of physicochemical properties of the solid samples (surface morphology, phase and elemental composition) and physiological solutions (pH and pCa) after hydroxyapatine–TiO_2–Ti incubation in KH_2PO_4–Na_2HPO_4 solutions (0.02 mol L^{-1}, pH = 6.0, 7.0, and 8.0) to conclude on the potential of the application of examined structures as bioimplants.

After thermal annealing under argon hydroxyapatite–TiO_2–Ti compositions were crystalline, with a pronounced surface roughness. After incubation in the KH_2PO_4–Na_2HPO_4 solutions, the agglomerates size decreased due to partial dissolution, and the surface became smoother (the highly developed surface in the case of the samples not incubated in the physiological solutions was also confirmed in [4]). The morphology changes were less pronounced at pH 7.0 and 8.0, whereas in the acidic solution, at pH = 6.0, the surface erosion was more significant (Fig. 1). The changes of

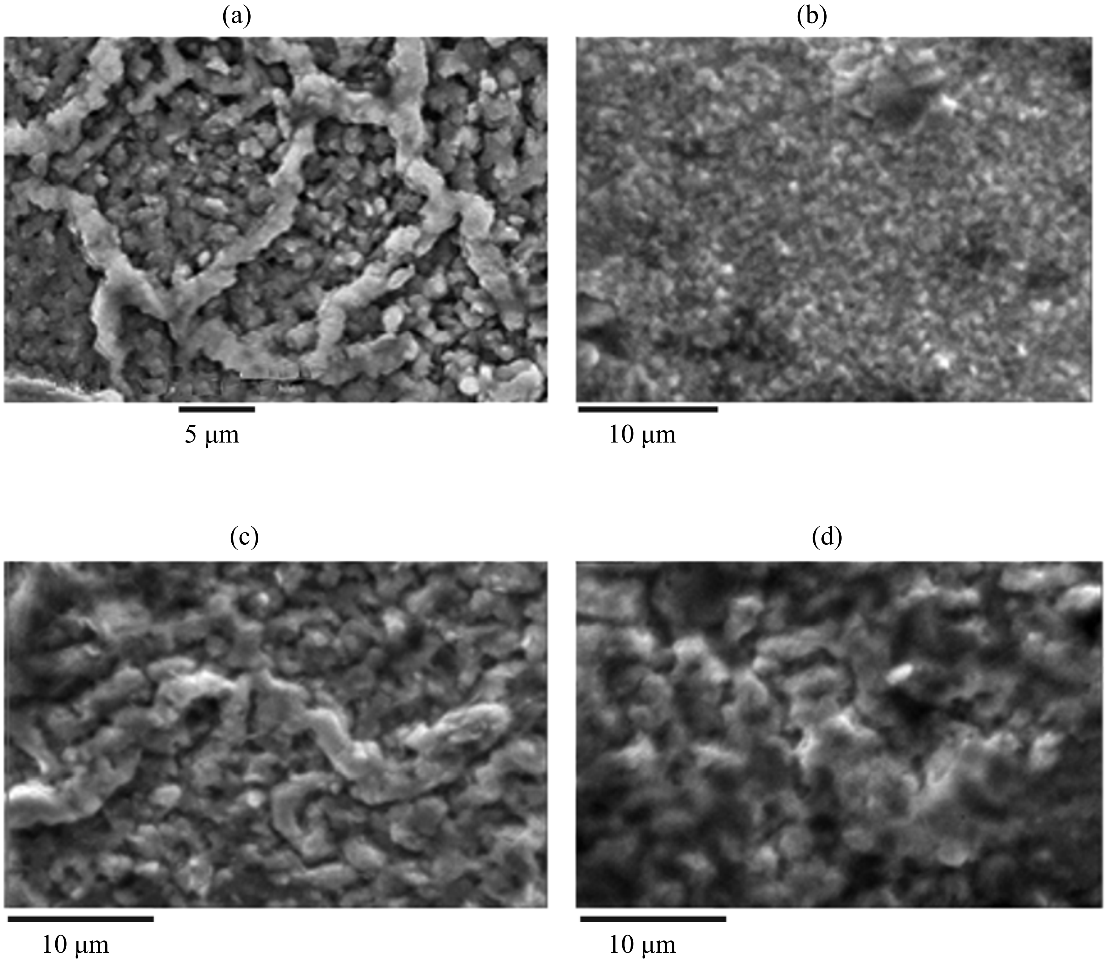

Fig. 1. SEM pictures of the hydroxyapatite–TiO_2–Ti structures with hydroxyapatite layer of 1 μm annealed at 950°C during 60 min, (a) initial sample, (b–d) incubated in the KH_2PO_4–Na_2HPO_4 solution during 96 h; (b) at pH 6.0, (c) at pH 7.0, (d) at pH 8.0.

morphology in the cases of samples with intermediate TiO_2 layers demonstrated that they were less eroded than the hydroxyapatite–Ti under the same conditions [3]. That conclusion was confirmed by AFM studies: the mean surface roughness of hydroxyapatite–TiO_2–Ti sample (3×3 μm^2 scan) was of 3–4 nm. The mean

Table 1. Results of X-ray structural microanalysis of thermally annealed (950°C, 60 h) and incubated samples of hydroxyapatite–TiO_2–Ti with hydroxyapatite layer of 1 μm and TiO_2 layer of 0.2 μm

pH of KH_2PO_4–Na_2HPO_4 solution	Incubation time, h	O, wt%	Ti, wt%	Ca, wt%	P, wt%	Ca/P
–	No incubation	43.28	26.25	20.61	9.86	2.09
6.0	2	33.39	65.28	0.79	0.54	1.46
6.0	24	32.30	66.26	0.82	0.63	1.30
6.0	96	40.09	48.98	6.49	4.21	1.54
7.0	2	42.76	47.12	6.81	3.31	2.06
7.0	24	46.43	52.09	1.10	0.38	1.9
7.0	96	45.98	44.65	5.26	2.81	1.87
8.0	2	47.56	51.97	0.31	0.16	1.94
8.0	24	44.27	19.40	24.38	11.51	2.12
8.0	96	37.23	25.15	24.69	12.53	1.97

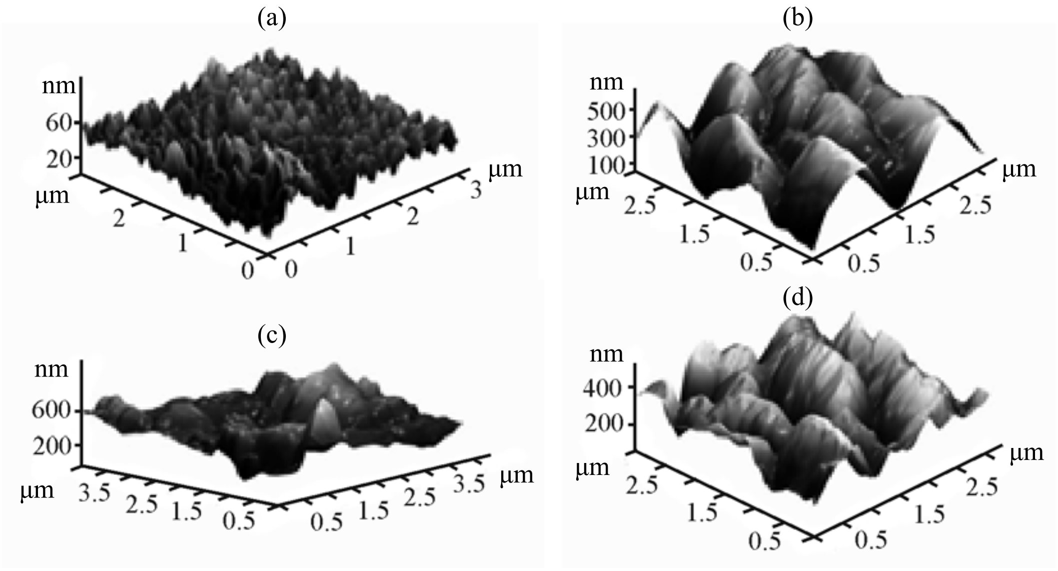

Fig. 2. AFM pictures of the hydroxyapatite–TiO_2–Ti structures with hydroxyapatite layer of 1 μm annealed at 950°C during 60 min, (a) initial sample, (b–d) incubated in the KH_2PO_4–Na_2HPO_4 solution during 96 h; (b) at pH 6.0, (c) at pH 7.0, (d) at pH 8.0.

roughness of the samples was changed upon incubation in the solutions, the sharp-peak relief got smoother (Fig. 2). Relief of the film prepared via high-frequency magnetron spraying before the incubation represented anisotropic juts of up to 50 nm wide and 400 nm high.

According to the local X-Ray spectral microanalysis, the Ca/P ratio was calculated (this parameter is important to evaluate the sample biocompatibility) (Table 1). For the biocompatible hydroxyapatite, Ca/P ≈ 1.5–1.7 [5].

The Ca/P changes of the studied samples upon their incubation in the buffer solutions were more pronounced than those in the case of hydroxyapatite–Ti, and their direction was condition-dependent (Fig. 3). At pH = 7.0, that parameter was virtually constant within the studied incubation duration, thus proving the sample stability towards neutral solution. At pH 6.0 and 8.0, the Ca/P ratio initially decreased and then increased at later stages of incubation; however, the net change of the ratio was negligibly low at pH 8.0, and noticeable under acidic conditions, at pH of 6.0. The sharp decrease of Ca/P at the initial stage of incubation was due to rapid dissolution of the phosphate phases [4]. After the longest incubation (96 h), with decreasing pH the Ca/P ratio went somewhat lower. In general, the Ca/P parameter changed much less in the cases of hydroxyapatite–TiO_2–Ti structures as compared to that in the case of hydroxyapatite–Ti compositions. Thus, the introduction of the intermediate titanium dioxide layer prevented the bioactive layer erosion.

To estimate the samples biocompatibility more reliably, pCa = –log [Ca^{2+}], of the surrounding solutions was measured. From Table 2, pCa slightly decreased upon incubation by only 0.06–0.08 log units. The stability of the samples towards the buffer solution was improved in the course of their thermal annealing, as demonstrated by lower calcium release into the solutions. The trend of pCa change was similar in the cases of hydroxyapatite–TiO_2–Ti and hydroxyapatite–Ti, however the change was less pronounces when TiO_2 interlayer was present. Thus, the stability improvement due to formation of the intermediate layer was additionally confirmed.

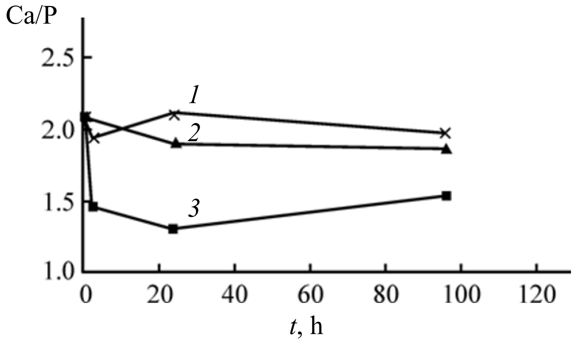

Fig. 3. Ca/P (from X-Ray structural microanalysis) as function of incubation time at pH (*1*) 6.0, (*2*) 7.0, and (*3*) 8.0.

Table 2. Data of measuring pCa = –log $[Ca^{2+}]$ in the buffer solutions after incubation of the hydroxyapatite–TiO_2–Ti samples with hydroxyapatite layer of 1 μm and TiO_2 layer of 0.2 μm

Treatment procedure	pH	Incubation time		
		2 h	24 h	96 h
950°C, 60 min	6.0	3.98	4.00	3.81
950°C, 60 min	7.0	5.82	5.84	5.76
950°C, 60 min	8.0	5.86	5.80	5.81
No thermal treatment	6.0	4.17	3.91	3.75
No thermal treatment	7.0	5.67	5.49	5.35
No thermal treatment	8.0	5.97	5.78	5.56

In the thermally annealed hydroxyapatite–TiO_2–Ti prior to incubation, the peaks assigned to the Ti_3O, Ti_2O, TiP_2O_7, and $CaTiO_3$ phases were revealed by X-ray powder diffraction, besides those of the initial materials [4]. Of titanium oxides, the underoxidized ones prevailed (Ti/O > 0.5), thus, the oxygen concentration gradient was directed from hydroxyapatite layer to Ti. From the X-Ray studies, it was concluded that incorporation of the intermediate TiO_2 layer prevented titanium oxidation, as the major part of oxygen was transported from TiO_2 phase rather than from $Ca_{10}(PO_4)_6(OH)_2$, thus, destruction of the bioactive layer was avoided.

Diffraction patterns of the incubated samples showed minor phase changes at pH 6–8 (Fig. 4), thus proving high biological stability of thermally annealed hydroxyapatite–TiO_2–Ti compositions.

To conclude, in the course of the samples incubation in KH_2PO_4–Na_2HP0_4 solutions at pH 6.0 to 8.0, the hydroxyapatite surface was somewhat eroded, however, the phase changes of the bioactive layer were

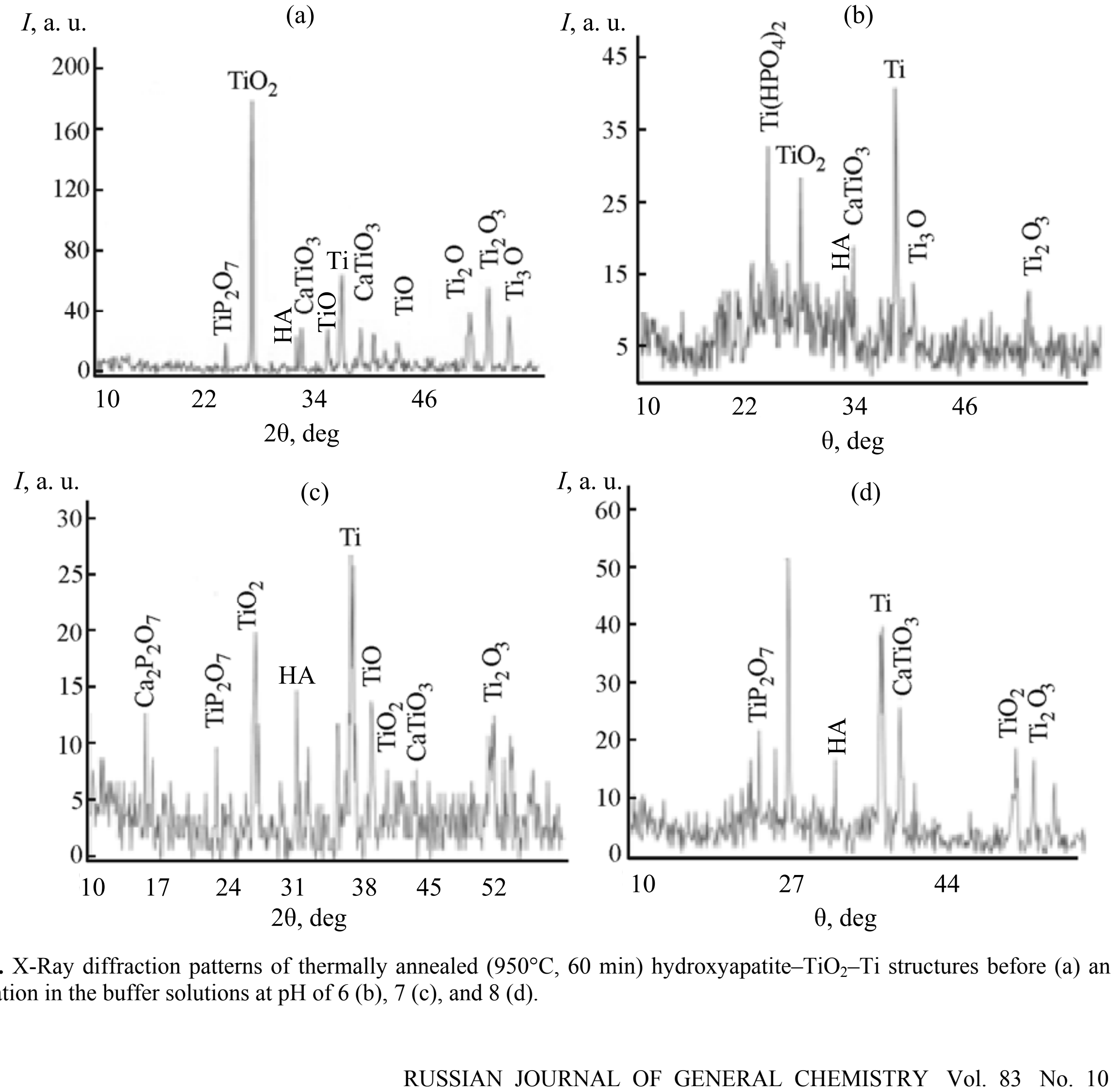

Fig. 4. X-Ray diffraction patterns of thermally annealed (950°C, 60 min) hydroxyapatite–TiO_2–Ti structures before (a) and after incubation in the buffer solutions at pH of 6 (b), 7 (c), and 8 (d).

not observed. From the comparison with the previously published results, the incorporation of TiO_2 intermediate layer improved the stability of hydroxyapatite-based layered composition.

EXPERIMENTAL

The Ti–TiO_2–hydroxyapatite structures were prepared by the formation of hydroxyapatite and titanium dioxide films via high-frequency magnetron spraying. Oratoriya-40 device [6] operation in argon medium (0.1 Pa) at the magnetron specific power of 40–70 W cm^{-2} resulted in the film growth rate of up to 0.7 nm s^{-1}.

Polished foil (99% Ti and 1% Al) was used as supporting layer. The foil was mechanically polished with the diamond paste and then treated with an ion source under argon atmosphere during 30 min to remove the corrupted layer. The samples with hydroxyapatite layer thickness of 1 μm and that of the intermediate TiO_2 layer of 0.2 μm were prepared.

Thermal annealing conditions (argon, 950°C, 60 min) were selected based on the previously reported results [7]. Physiological solution of KH_2PO_4–Na_2HPO_4, isotonic and non-cytotoxic, was chosen because of their wide use in the biological studies.

Solutions, 0.02 mol L^{-1}, were prepared from the weighted samples of KH_2PO_4 and Na_2HPO_4 (both of chemical pure grade), then, the solution pH was appropriately adjusted. In the typical experiment, a sample of hydroxyapatite–TiO_2–Ti was fully immersed in 4 ml of the freshly prepared buffer solution, and incubated at 37°C during 2, 24, or 96 h.

Morphology of the annealed films was studied by SEM (JEOL JSM-6380LV) and AFM (Solver-47). The composition of the samples was determined by means of local X-Ray spectral microanalysis (JEOL JSM-6380LV). Phase structure was determined by X-ray powder diffraction (DRON-3). Concentrations of ions (H^+ and Ca^{2+}) were measured with Ekspert-001 ionomer.

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