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VARIOUS TECHNOLOGIES

Bioactive Composites Produced In Situ on the Basis of Calcium Phosphates and Lactic Acid Oligomers

L. A. Rasskazova, D. N. Lytkina, Ye. G. Shapovalova, V. V. Botvin, M. A. Pozdnyakov, A. G. Filimoshkin, N. M. Korotchenko, and V. V. Kozik

National Research Tomsk State University, pr. Lenina 36, Tomsk, 634050 Russia
e-mail: ly_2207@mail.ru

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Abstract—Composites based on lactic acid oligomers, calcium hydrophosphate and hydroxyapatite synthesized under the action of microwave radiation were obtained in situ. The appearance of a new band associated with stretching vibrations of >C=O in spectra of the chloroform-insoluble fraction is indicative of the chemical interaction between lactic acid and hydroxyapatite. To determine whether a calcium phosphate layer can be formed on the surface of composite samples, biomimetic studies in a physiological SBF solution were carried out during 28 days at 37°C. It was found that all samples containing calcium phosphates promote active formation of a new calcium phosphate layer, whereas lactic acid oligomer in samples containing no inorganic component undergoes destruction in the SBF solution as a result of hydrolysis. The estimate of the resorption rate demonstrated that the solubility of calcium phosphates contained in the composites at 20°C in the physiological solution is 3–7 times that of pure hydroxyapatite.

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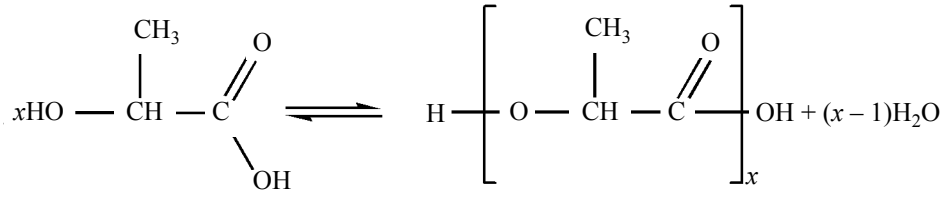
The search for optimal materials for implants [1–4], development and fabrication of various medicinal composites based on calcium phosphates (CPs) [5–7] and on polymers and oligomers of lactic acid (OLA) are promising areas of research at the confluence of chemistry and medicine. Implants based on biocomposites of this kind, whose mechanical and other important properties are comparable with the properties of the bone tissue, possess a sufficient strength and cause no negative responses of the immune system of an organism. The capacity for biodegradation in the course of a time optimal for healing of a bone defect and the absence of an undesirable biological activity of decomposition products are the necessary requirements to materials of this kind [8, 9].

The problem of development of ways to improve the strength-related, biochemical, and other important properties of CP-based composites includes three main tasks: (1) synthesis of composites based on components capable of binding CPs and reinforcing the material, (2) search for the optimal ratio between the chosen components, and (3) study of physicochemical interactions between the components in order to control the functional properties of the materials. Studies in this area have been carried out by scientists in the Russian Federation and other countries during several decades [10–16].

An important principle in development of biomaterials for implantation consists in reproducing the basis characteristics of the natural bone tissue because it is the unique bone structure (chemical composition, morphology, structure) that strongly affects regeneration processes. Because of the poor solubility of synthetic hydroxyapatite $Ca_5(PO_4)_3OH$ (HA), a new bone grows on the surface of the implant, while its material is not yet dissolved, which frequently necessitates repeating

Scheme 1.



the same procedure. Thus, it is essential to create a material with the optimal time of dissolution in an organism, and the growth rate of the new bone tissue should correspond to the dissolution rate of the implanted material (resorption) [17]. The ability of materials to form on their surface a calcium phosphate layer from a model SBF (Simulated Body Fluid) solution imitating the mineral composition of human plasma is evaluated by the procedure suggested by Kokubo [18].

In this study, we used as a CP the mixture of hydroxyapatite $Ca_5(PO_4)_3OH$, which is the main component of the bone tissue, and calcium hydrophosphate $CaHPO_4$, which improves the resorption capacity of materials on its basis. Calcium phosphates in the composites are assigned a part of an active source of elements necessary for construction of the bond tissue; these compounds also improve the strength of the materials. Composites containing CPs (bioactive component) and LAO (binder) are regarded as starting materials for obtaining implants based on polylactic acid (PLA). It is known that PLA-based materials are close to bone in mechanical properties and do not cause its disintegration under cyclic dynamic loads [1, 2]. The optimal resorption time, controlled degradation rate of the polymeric matrix, and absence of any adverse biological activity in decomposition products are the indispensable properties of lactic acid polymers, which determines their being in demand as the polymeric component of biodegradable implants [3, 4]. The goal of our study was to synthesize in situ biologically active composites as materials for fabrication of bone implants based on calcium phosphates and lactic acid oligomers and to evaluate the ability of these materials to form on their surface a calcium phosphate layer in a model SBF solution.

EXPERIMENTAL

Lactic acid oligomers were produced by polycondensation of lactic acid in an inert atmosphere (Scheme 1).

LAOs were synthesized from a 80% aqueous solution of L-lactic acid (PURAC, chemically pure grade). To vary the molecular weight (MW) and the degree of polydispersity of the oligomers, the synthesis was performed both in the presence of para-toluenesolfonic acid (p-TSA) as catalyst and without a catalyst. In the first stage of obtaining CP/LAO composites, an aqueous suspension of HA at pH ~10 is prepared by using microwave radiation in accordance with the equation

$$5Ca(NO_3)_2 + 3(NH_4)_2HPO_4 + 4NH_4OH \rightarrow Ca_5(PO_4)_3OH + 10NH_4NO_3 + 3H_2O.$$

The starting solutions were prepared from reagents of chemically pure grade: calcium nitrate tetrahydrate, disubstituted ammonium phosphate ($\rho = 0.907$ g mL^{-1}), and distilled water free of carbon dioxide. Freshly prepared solutions were mixed and subjected to a microwave irradiation for 40 min. After a 48-h settling, the precipitated HA was filtered off on a Buchner funnel, washed with distilled water to pH ~7, and dried at 90°C to constant mass.

In the second stage of obtaining the composites, dried HA was added to a lactic acid solution in which *p*-TSA was preliminarily dissolved. The polycondensation of lactic acid occurred in the vessel of a rotary evaporator in the course of 5 h, with the simultaneous in situ formation of the composite.

The molecular masses of the LAOs were determined by gel-permeation chromatography (GPC) on a GPC Agilent System 1100 instrument equipped with a DAD Agilent 1100 UV-Detektor (230 nm) on a set of PSS SDV polymer columns with pore sizes of 50 to 105 Å. Tetrahydrofuran (THF) served as the eluent with a flow rate of 1 mL min^{-1} at 35°C; the oligomer was dissolved in the course of 3 h. The calibration line was determined using polystyrene reference samples with MW in the range from 162 to 246 000 g mol^{-1}. An oligomer sample

Table 1. Concentration of ions in the SBF solution and in human plasma

Medium	Concentration, mM							
	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	HCO_3^-	HPO_4^{2-}	SO_4^{2-}
SBF solution	142.0	5.0	1.5	2.5	148.8	4.2	1.0	0.5
Human plasma	142.0	5.0	1.5	2.5	103.0	27.0	1.0	0.5

with a concentration of 3 g mL^{-1} was purified on being passed across a filter with pore size of 0.45 μm.

An X-ray phase analysis (XPA) of HA and the composites was made on a Shimadzu XRD 600 diffractometer with CuK_α radiation (1.5406 Å) in the 2θ range 20–140°. The phases were identified with a PCPDF WIN-1.3 database.

To confirm the interaction of HA with lactic acid in the course of the polycondensation, samples of the composites were extracted with chloroform in a Soxhlet apparatus. A part of the composite remaining in the paper cartridge was kept in a vacuum-desiccator over P_2O_5 until water and $CHCl_3$ were completely removed. IR spectra of the samples, obtained before and after the extraction in the Soxhlet apparatus, were recorded for powders in the range 4000–500 cm^{-1} with a Nicolet 5700 IR spectrometer having a FTIR attachment with a Ge crystal.

To evaluate the integral solubility of calcium phosphates contained in the composites, the total concentration of calcium ions Ca^{2+} was determined in a saturated physiological solution (0.9% NaCl) at 20°C (trilonometric titration in the presence of Eriochrome black T with an ammonia buffer solution) [21].

The biological activity of the samples was evaluated as the ability to form a calcium phosphate layer on their surface in a model SBF solution by the procedure described in [18]. The substrates fabricated by compaction into pellets with a diameter of 5 mm were placed in an SBF solution identical in the mineral composition and concentration of ions to the human plasma (Table 1).

The substrates were kept in the SBF solution at 37°C for 28 days, with daily renewal of the solution. It is difficult to selectively determine Ca^{2+} ions by trilonometric titration [22] in the presence of Mg^{2+} ions (the stability constants of the complexes of calcium and magnesium with EDTA are close: log K = 10.7 for CaY^{2-} and log K = 8.7 for MgY^{2-}) [23]. Therefore, the formation rate of the calcium phosphate layer on the surface of the substrates was evaluated by the decrease in the total concentration of calcium and magnesium ions ($\Delta c_{Ca^{2+}+Mg^{2+}}$, mM) in the SBF solution on the assumption that the concentration of magnesium ions is fixed. The surface morphology of substrate on which a calcium phosphate layer was formed was examined by scanning electron microscopy (SEM) on a Hitachi TM-3000 microscope at an accelerating voltage of 15 kV in the charge-up reduction mode (electron gun: 5×10^{-2} Pa; sample chamber: 30–50 Pa).

RESULTS AND DISCUSSION

The mass-average degree of polymerization ($\bar{X}_w$) of LAO samples produced by polycondensation in the absence of a catalyst reaches a value of 20 at a degree of polydispersity $\bar{X}_w/\bar{X}_n \approx 2$ ($\bar{X}_n$ is the number-average degree of polymerization). To raise $\bar{X}_w$, the reaction was performed in the presence of *p*-TSA. The influence exerted by the catalyst and its amount on the mass-average MW $\bar{M}_w$ and on the polydispersity of products was judged from the data in Table 2. With increasing content of the catalyst, $\bar{M}_w$ of the oligomers grows. It is known that, as the mass-average MW degreases and the degree of polydispersity becomes larger, the physicomechanical properties of polymers are, as a rule, deteriorated.

According to the GPC data, the optimal amount of the catalyst is 2 wt % *p*-TSA. Use of oligomers with a larger MW and smaller degree of polydispersity must improve the mechanical properties of the composite and have a positive effect on the biodegradation of the polymeric matrix [25].

The composition and conventional designations of the composites obtained in situ with various contents of CPs and LAOs are presented below:

Sample	Composite A	Composite B	Composite C
CP/LAO, wt %	25/75	50/50	75/25

According to the results of the X-ray phase analysis, the main phase of composites A and B is calcium hydrophosphate, and composite C contains two phases, hydroxyapatite $Ca_5(PO_4)_3OH$ and $CaHPO_4$. Figure 1

Table 2. Values of $\bar{M}_w$, $\bar{X}_w$, and $\bar{X}_w/\bar{X}_n$ for lactic acid oligomers at different contents of catalysts in the reaction mixture

Content of *p*-TSA in LAO, wt %	$\bar{M}_w$	$\bar{X}_w$	$\bar{X}_w/\bar{X}_n$
Without a catalyst	1200	20	2.09
0.5	11000	150	1.89
1.0	14000	200	1.89
2.0	14000	200	1.66
5.0	15500	215	1.85

shows as an example X-ray diffraction patterns of composites B and C.

The IR spectra of LAOs (Fig. 2) contain bands associated with stretching vibrations of carbonyl groups

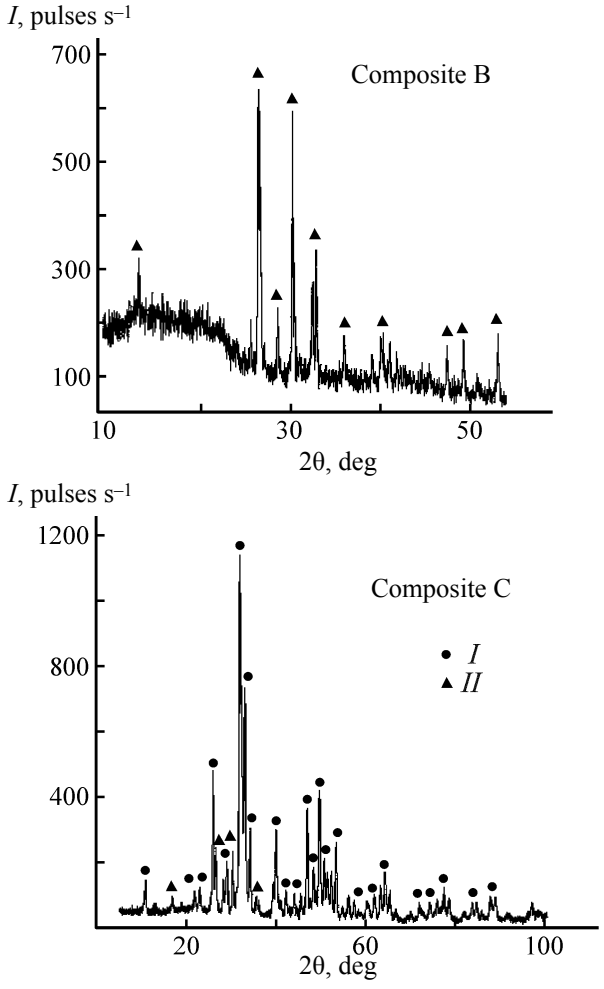


Fig. 1. X-ray diffraction patterns of composites B and C. (*I*) Intensity and (2θ) Bragg angle. (*I*) $Ca_5(PO_4)_3OH$ and (*II*) $CaHPO_4$.

ν(>C=O) (1730–1750 cm^{-1}) and ν(C–H) in methyl groups (2990–2880 cm^{-1}); at 1100–1400 cm^{-1}, there are bands associated with deformation vibrations δ(C–H) in methine groups. The IR spectra of the composites show bands that are characteristic of both calcium phosphates [stretching (1085–960 cm^{-1}) and deformation (560–605 cm^{-1}) vibrations of phosphate groups ν(PO_4^{3-}, HPO_4^{2-}), δ(PO_4^{3-}, HPO_4^{2-})] and LAOs. In the range 1630–1580 cm^{-1}, there appear new high-intensity bands associated with vibrations of $–COO^-$ groups formed as a result of the interaction of HA with lactic acid to give calcium hydrophosphate (Scheme 2).

The integral concentrations of Ca^{2+} (mM) in the physiological solution saturated with calcium ions as a result of the prolonged (for 7 days) keeping of HA and composites in this solution (Table 2) make it possible to judge about the expected resorption capacity of the composite. The integral solubilities of calcium phosphates in composites A–C (pH 7, I = 0.15, T = 20°C, P = 0.95) are given below:

Sample[a]	$c_{Ca^{2+}}$, mM
A	4.72 ± 0.06
B	3.39 ± 0.06
C	7.09 ± 0.07

[a] For comparison: the solubility of HA, determined under the same conditions is 0.97 ± 0.01 mM.

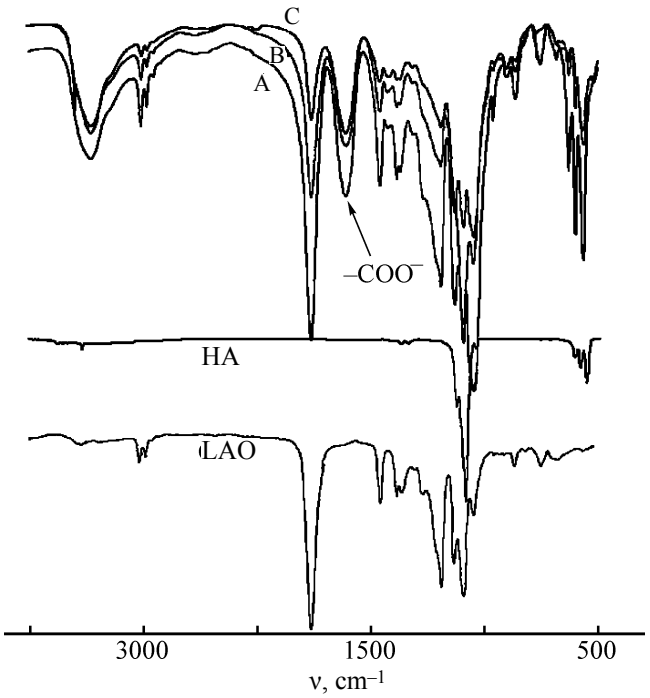
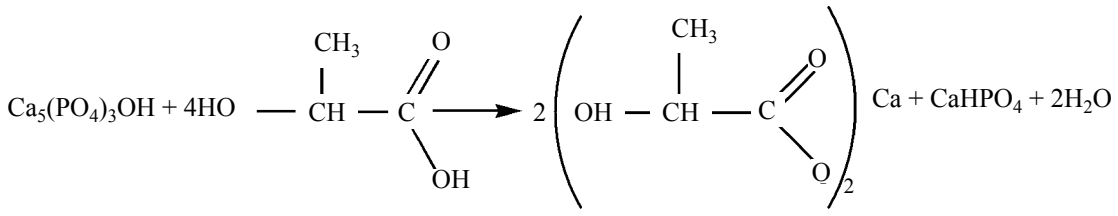


Fig. 2. IR spectra of pure LAOs, HA, and composites A–C. (ν) Wave number.

Scheme 2.



It can be seen that the solubility of calcium phosphates in the composites is 3–7 times the solubility of pure HA. These changes in solubility must promote a faster resorption of the biomaterial.

The results obtained in measurements of the total concentration of Ca^{2+} and Mg^{2+} ions in solution were used to plot the kinetic curves $\Delta c_{Ca^{2+}+Mg^{2+}}$ (mM)–τ (days) of their accumulation on substrate surfaces from the SBF solution (Fig. 3).

Analysis of the kinetic curves shows that Ca^{2+} and Mg^{2+} ions are actively adsorbed from the SBF solution on the surface of pure HA during the whole time of substrate keeping in solution (Fig. 3, curve *1*). The noticeable loss of calcium ions from the surface of composite B (Fig. 3, curve *3*) during the first 48 h of substrate keeping in solution is due to the comparatively high solubility of calcium hydrophosphate contained in the composite. Beginning from the second 24 h, there occurs adsorption of calcium ions from solution on the surface of composite B substrates, which is due to the presence of the HA phase in the composite. The rate of the process in which a calcium phosphate layer is formed on the surface of pure HA exceeds than on the surface of composite B, as indicated by the larger slope ratio of dependence (1). On the surface of pure LAO, there occur insignificant processes adsorption–desorption of calcium ions from the SBF solution (Fig. 3, curve *2*), which is evidenced by only slight changes in the concentration of Ca^{2+} and Mg^{2+} ions in solution.

SEM data (Fig. 4) clearly confirm the results of biomimetic studies and indicate that (i) calcium phosphate layers are formed on HA and composite B substrates (Fig. 4, *1* and *2*) already after 7 days of their keeping in the SBF solution and (ii) no calcium phosphate layer is formed on the purely polymeric substrate (Fig. 4, *3*).

The calcium phosphate layer is formed on the substrate surface by the mechanism suggested in [26]. The substrate surface containing hydroxy groups and bearing a partial negative charge attracts Ca^{2+} ions from solution, with their simultaneous adsorption on the surface and gradual change of the surface charge to partial positive. The process ends in addition of PO_4^{3-} ions. As a result of these serial-parallel events, layers of poorly soluble calcium phosphates are formed on the surface. Thus, a decrease, compared with pure HA, in the content of OH^- groups in composite B leads to a lower overall rate of adsorption of calcium and magnesium ions from the SBF solution on the surface of the composite.

CONCLUSIONS

(1) Biologically active composites were produced by the in situ method on the basis of calcium phosphates and lactic acid oligomers. The composition and structure of the composites were confirmed by IR spectroscopy and X-ray phase analysis. The composites are formed as a result of the interaction of hydroxyapatite with lactic acid oligomers.

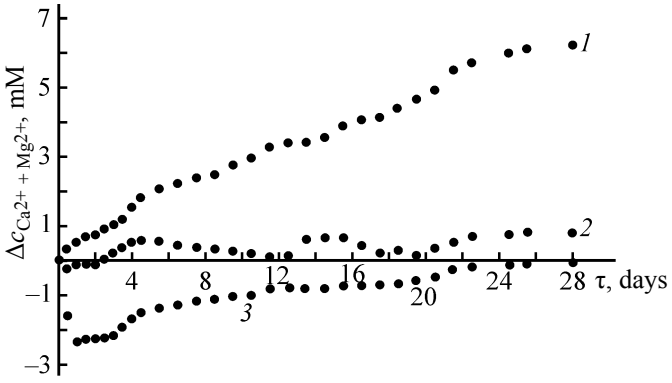


Fig. 3. Kinetic curves of accumulation of Ca^{2+} and Mg^{2+} ions on the surface of (*1*) HA, (*2*) LAO, and (*3*) composite C from the SBF solution. (τ) Time.

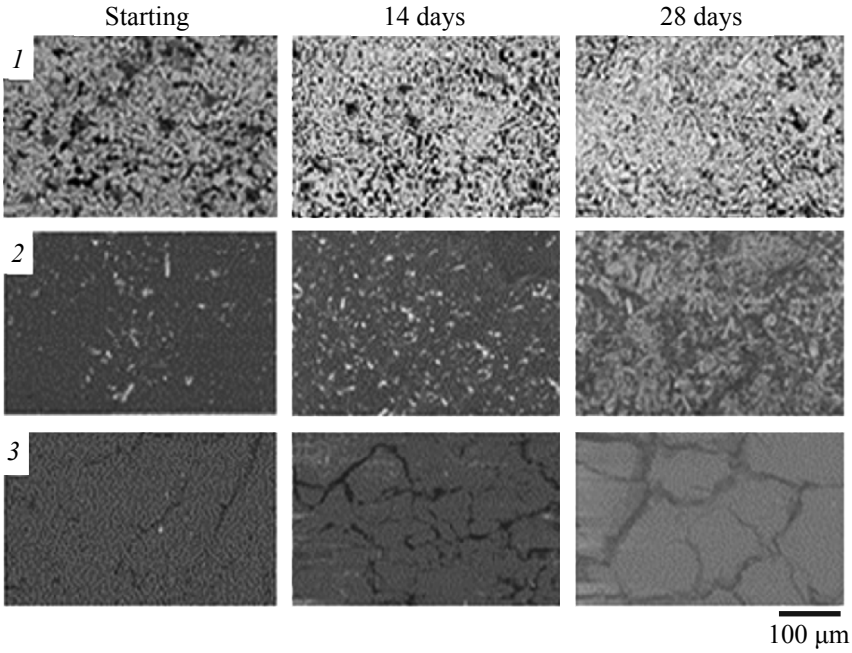


Fig. 4. Electron micrographs of the substrate surface. Magnification ×3000. Growth dynamics of the calcium phosphate layer on the surface of (*1*) hydroxyapatite, (*2*) composite C, and (*3*) lactic acid oligomer.

(2) It was found that the catalyst affects the molecular weight and the degree of polydispersity of lactic acid oligomers. The optimal ratio between the molecular mass and degree of polydispersity of lactic acid oligomers is obtained at a catalyst content of 2 wt %.

(3) The integral solubility of calcium phosphates contained in the composites in the physiological solution is several times the solubility of synthetic hydroxyapatite, which gives reason to expect an increase in the resorption capacity of composites of this kind in an organism.

(4) It was found that a calcium phosphate layer is actively formed (28 days, 37°C) in the SBF solution on the surface of samples containing calcium phosphates, whereas a pure lactic acid oligomer only undergoes destruction as a result of hydrolysis.

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