© (2015) Trans Tech Publications, Switzerland
doi:10.4028/www.scientific.net/AMR.1085.394
Revised: 04.09.2014
Accepted: 12.09.2014

Bioactivity and Physico-Chemical Properties of Composites on Basis of Hydroxyapatite with Lactic and Glycolic Acids Oligomers

RASSKAZOVA Lyudmila A.[a], LYTKINA Darya N.[b], SHAPOVALOVA Yelena G.[c], BOTVIN Vladimir V.[d], POZDNYAKOV Maksim A.[e], ZHUK Ilya V.[f], FILIMOSHKIN Anatoliy G.[g], KOROTCHENKO Natalya M.[h], KOZIK Vladimir V.[i]

Tomsk State University, Russia, 36, Lenina avenue, Tomsk, 634050, Russia

[a]ly_2207@mail.ru, [b]darya-lytkina@yandex.ru, [c]elena.shapovalova@ro.ru, [d]botvinilo1991@gmail.com, [e]mks123@yandex.ru, [f]zhukiv1993@mail.ru, [g]filag05@rambler.ru, [h]korotch@mail.ru, [i]vkozik@mail.ru

Keywords: lactic and glycolic acids oligomers, hydroxyapatite, microwave synthesis, bioactive composites, synthesis *in situ*, SBF investigation.

Abstract. A new method *in situ* for producing of biocompatible composites based on hydroxyapatite and oligomers of lactic and glycolic acid is described. Their thermo-mechanical, physical-chemical and biological properties are studied as well. The formation of bonds between hydroxyapatite with lactic and glycolic oligomers in composites are confirmed by IR spectroscopy of the samples, including those obtained in the Soxhlet apparatus. The formation of calcium-phosphate layer on the surface of composite containing hydroxyapatite was shown by SBF-investigation. The scaffolds based on lactic acid oligomer do not stimulate formation of a calcium-phosphate layer and they are subjected to destruction by the way of hydrolysis.

Introduction

Growing interest in design of artificial materials for bone restore and replacement is the consequence of increasing incidences associated with bone tissue damage (World Health Organization). However, the high cost and low biocompatibility and bioactivity of some orthopedic implants hinder their widespread introduction in clinical practice. Therefore there is a need to create effective bioactive materials to replace bone tissue with minimal material, energy and labor costs. It is known that hydroxyapatite (HA) – $Ca_{10}(PO_4)_6(OH)_2$ is an attractive biomaterial for creating bone implants [1–3]. It is an inorganic component of the chemical bone formula. This fact, in turn, leads to the occurrence of such properties as excellent biocompatibility and bioactivity. Usage of bioceramics based on HA is complicated due to the lack of sufficient flexibility, elasticity and strength of the material, so the development and application of composites of HA with biopolymers is urgent [4–6]. Oligolactic (OLA) and oligoglycolic (OGA) acids are selected as biopolymers capable of dissociate in the human body to CO_2 and H_2O via Krebs cycle. HA in the matrix of OLA and OGA is actively involved in the regeneration of bones as a substrate for the growth of a new bone tissue. Nowadays a large number of studies are conducted to determine the optimal compositions of these composites.

The aims of the work are devoted to the preparation of biocompatible composites based on OLA, OGA and HA *in situ* and investigation of thermo-mechanical, physical-chemical and biological properties.

Experimental part

Hydroxyapatite was obtained at pH = 11 by microwave technology [7] according to the reaction:

$$10Ca(NO_3)_2 + 6(NH_4)_2HPO_4 + 8NH_4OH \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 20NH_4NO_3 + 6H_2O.$$

The reactants were mixed rapidly in stoichiometric ratio and the mixture was subjected to microwave exposure in order to obtain HA. To prepare the solutions we used chemically pure calcium nitrate tetrahydrate, ammonium hydrogen phosphate, as well as aqueous ammonia solution (ρ = 0.907 g / ml), and carbon dioxide-free distilled water.

HA was used to make the composites. To synthesize the polymer matrix of the composite we used aqueous 80 % solutions of L-lactic (PURAC) or 67 % of glycolic (Across Organics) acids and *p*-toluenesulfonic acid (catalyst). Composites were prepared *in situ* (catalyst added) in a rotary evaporator at 5 mm Hg (Table 1). *In situ* method unlike the method of mechanical mixing of HA with the previously synthesized oligomers means formation of the above composites, which takes place simultaneously with polycondensation of lactic (or glycolic) acid in the reaction medium containing dispersed HA particles.

Table 1. Composites of HA/oligolactic acid, HA/oligoglycolic acid.

Labels	Composition of the samples
P1	Oligomer of Glycolic acid (OGA)
P2	Composite of Hydroxyapatite (HA) and OGA (25:75)
P3	Composite of Hydroxyapatite (HA) and OGA (50:50)
P4	Composite of Hydroxyapatite (HA) and OGA (75:25)
P5	Oligomer of Lactic acid (OLA)
P6	Composite of Hydroxyapatite (HA) and OLA (25:75)
P7	Composite of Hydroxyapatite (HA) and OLA (50:50)
P8	Composite of Hydroxyapatite (HA) and OLA (75:25)

Research methods

Properties of the ingredients and some composites were investigated by the following physico-chemical methods: IR, SEM and TGA. Coverage of the experimental data of all investigated samples does not make sense because of great variety of obtained samples obtained, so the most interesting results are given in this paper.

Infrared absorption spectra of the original samples (without further treatment) were recorded on a FTIR 6700 spectrometer Nicolet in the range 4000–500 cm^{-1}. Identification of the absorption bands was carried out by the characteristic frequencies.

Morphology of the surface of the samples was determined by scanning electron microscope Quantex 70, magnification 1000 times.

The thermal stability and the number of stages of the thermolysis of the samples were ascertained in accordance with the results of thermogravimetric analysis performed on TGA Q5000 instrument in ceramic crucibles (Al_2O_3) under nitrogen in the temperature range 25–700 ºC (10 ºC/min).

Biological activity of the synthetic HA, P3 and of the P7 composites was carried out *in vitro* by means of ageing of the SBF-modeling solution (Simulated Body Fluid) [8]. The total concentration of calcium and magnesium ions in the remaining SBF-solution was determined by daily trilonometry in ammonia buffer with *eryochrom black* T as indicator. Solubility of the HA as a part of the composites at pH = 7, I = 0.1 (NaCl), T = 20 ºC was determined by trilonometry.

Results and discussion

The absorption bands of stretching (950–1200 cm^{-1}) and deformation (560–610 cm^{-1}) vibrations of phosphate groups in the IR-spectrum are characteristic for HA (Fig.1 a, b). The stretching vibrations of carbonyl groups in the oligomers spectra are registered as 1730–1750 cm^{-1} bands (Fig.1 a, b).

New bands, which correspond to the $–COO^-$ vibrations in the IR spectra of the composites, appear in the region 1580–1630 cm^{-1} (Fig.1 a, b). The $–COO^-$ groups appear as a result of oligomers grafting to HA particles according to the reaction:

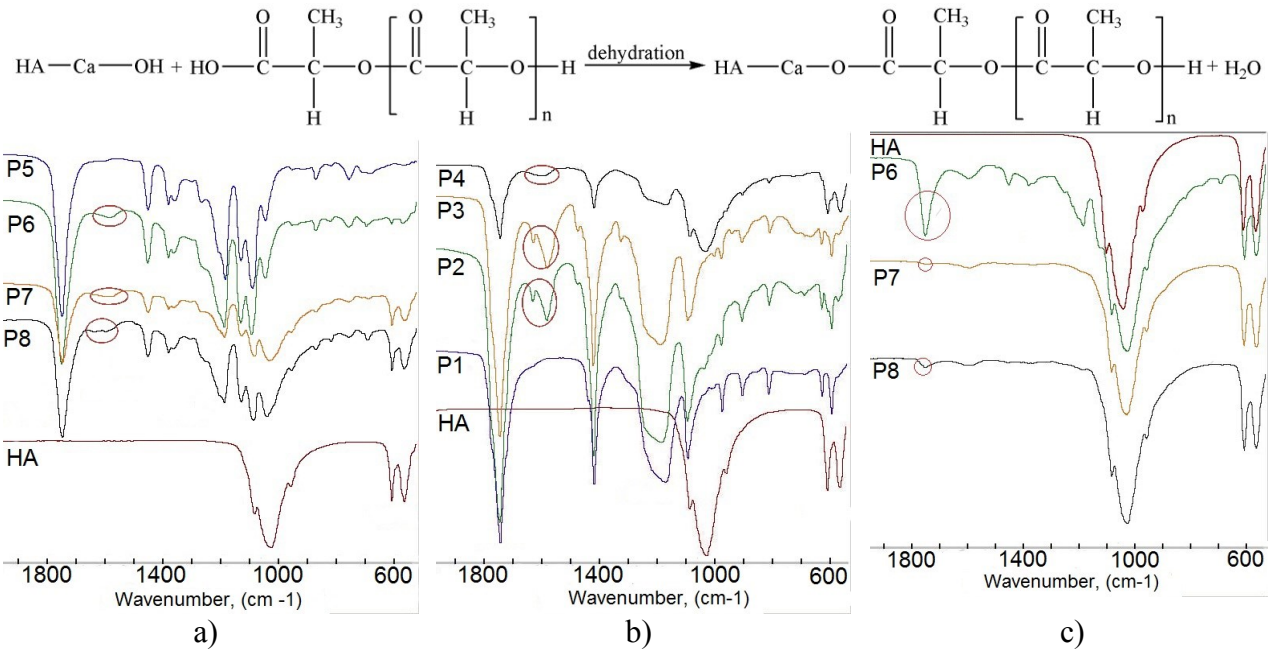

Fig. 1. IR spectra of the composites P6, P7, P8, OLA and HA (a), composites P2, P3, P4, OGA and HA (b) and insoluble fraction obtained by extraction of the composites P6, P7, P8 and HA (c).

Comparison of the bands intensity (*I*) of the internal standard method leads to the conclusion that *I*, corresponding to vibrations of the carboxylate ion (I_{-COO^-}), varies inversely with the content of HA in the composite, thus the amount of the grafting in the composite oligomer is more in the P6 species.

Composites P6, P7 and P8 were subjected to extraction with chloroform in a Soxhlet apparatus, and the separated fractions were investigated by IR spectroscopy (Fig. 1, c).

The spectra of the insoluble fraction of P6, P7 and P8 composites have 1750 cm^{-1} bands corresponding to OLA, which indicates that grafting of the oligomer to HA takes place.

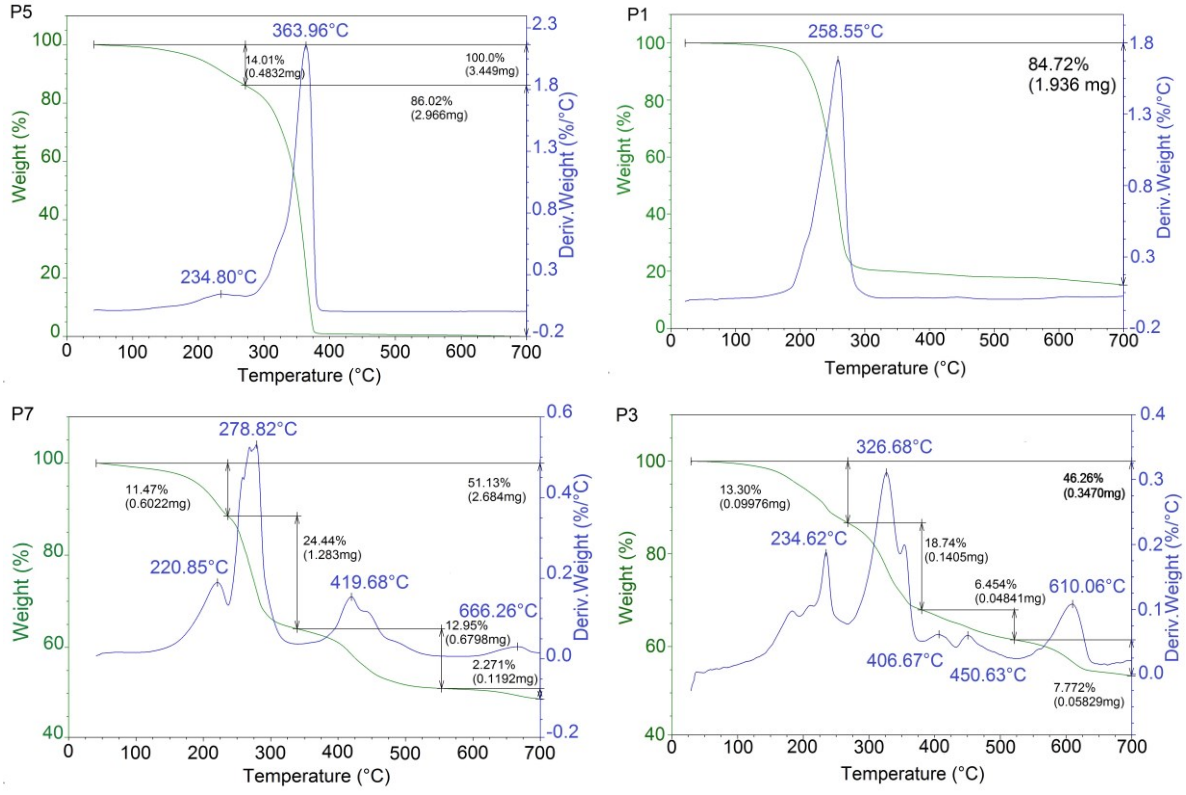

Fig. 2. Thermograms of oligomers (P1, P5) and the composites (P3, P7).

The thermal analysis results show that the main weight loss of the oligomers (Fig. 2) occurs in one stage as it is shown by the TG curves. The weight loss of the composites occurs in wide temperature range compared with that of the oligomers. The weight reduction of the composites P3 and P7 is 10–15% (Fig. 3) in the range of 100–150 ºC and it is due to the removal of adsorbed water. Most part of the weight loss (35–40%) at 150–700 ºC is associated with complete thermal decomposition of the organic matrix in several stages involving relevant *exo*-effects. In addition, chemical reactions between the components of the polymer matrix of the composites increase the total number of stages. It is interesting to note that the mass of the samples after the thermal analysis is equal to the initial mass of HA in the composites.

Tensile strength of the samples was measured under 500 N and the test speed 0 – 1 mm/s. Maximum values of Young modulus (E) of the P6 and P7 composites turned out to be 2279.1 and 2179.9 MPa, respectively.

Biological activity *in vitro* was estimated in SBF solution. The results of the measurements are represented as kinetic curves of cumulative total concentration of Ca^{2+} and Mg^{2+} ions on the surface of the substrates (Fig. 3).

Analysis of the kinetic curves shows that the surface of synthetic HA (1) adsorbs Ca^{2+} and Mg^{2+}, as evidenced by the large cumulative total concentration of adsorbed ions and high speed process (Table 2, $\Delta C/\Delta\tau$, mol/(L·days)). Adsorption of Ca^{2+} and Mg^{2+} on the surface of pure OLA (3) does not take place, and the substrate is destroyed in due course. It is important to note that when the HA/OGA (2) and HA/OLA (4) substrates being aged in the SBF solution, partial dissolution of HA occurs first of all (for the first two days), and a new calcium-phosphate layer is being formed for the next 26 days.

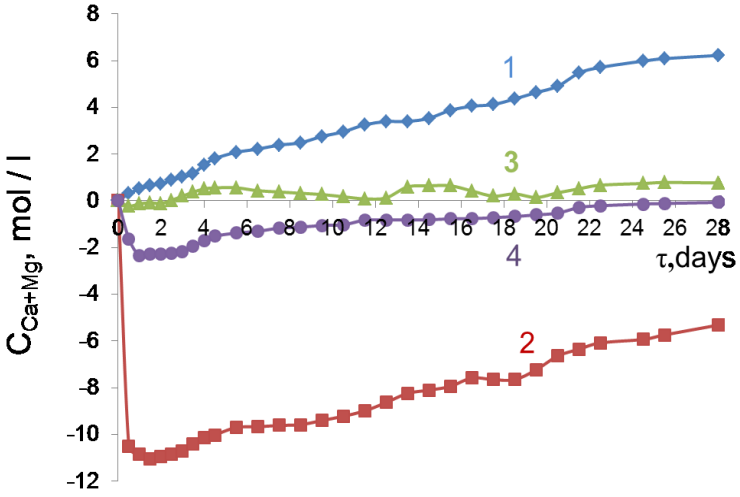

Fig. 3. Cumulative concentration of Ca^{2+} and Mg^{2+} ions on the substrate surface of the synthetic HA (1), of the P3 composite (2), of the pure OLA P5 (3) and of the P7 composite (4).

Table 2. The average rate of calcium-phosphate layer formation on the scaffolds of the synthetic HA, of the P3 composite (2), OLA P5 (3) and P7 composite (4).

Labels	$\Delta C/\Delta\tau$, [mol/(L·days)]		
	1–7 days	8–21 days	22–28 days
Pure HA	0.343	0.196	0.175
P3	0.185	0.216	0.185
P5	0.064	0.003	0.057
P7	0.186	0.057	0.058

It is notable that during the first seven days the adsorption rate of calcium and magnesium ions from the SBF solution on the surface of the samples containing HA is high, then the rate is being reduced. The concentration of Ca^{2+} and Mg^{2+} ions in the presence of the P5 composite in the SBF solution does not change for all the time of the experiment. This indicates that the pure oligomer is not capable of forming on its own surface a new calcium-phosphate layer. Moreover, SEM investigation (Fig. 4 b, d) proves this conclusion to be valid.

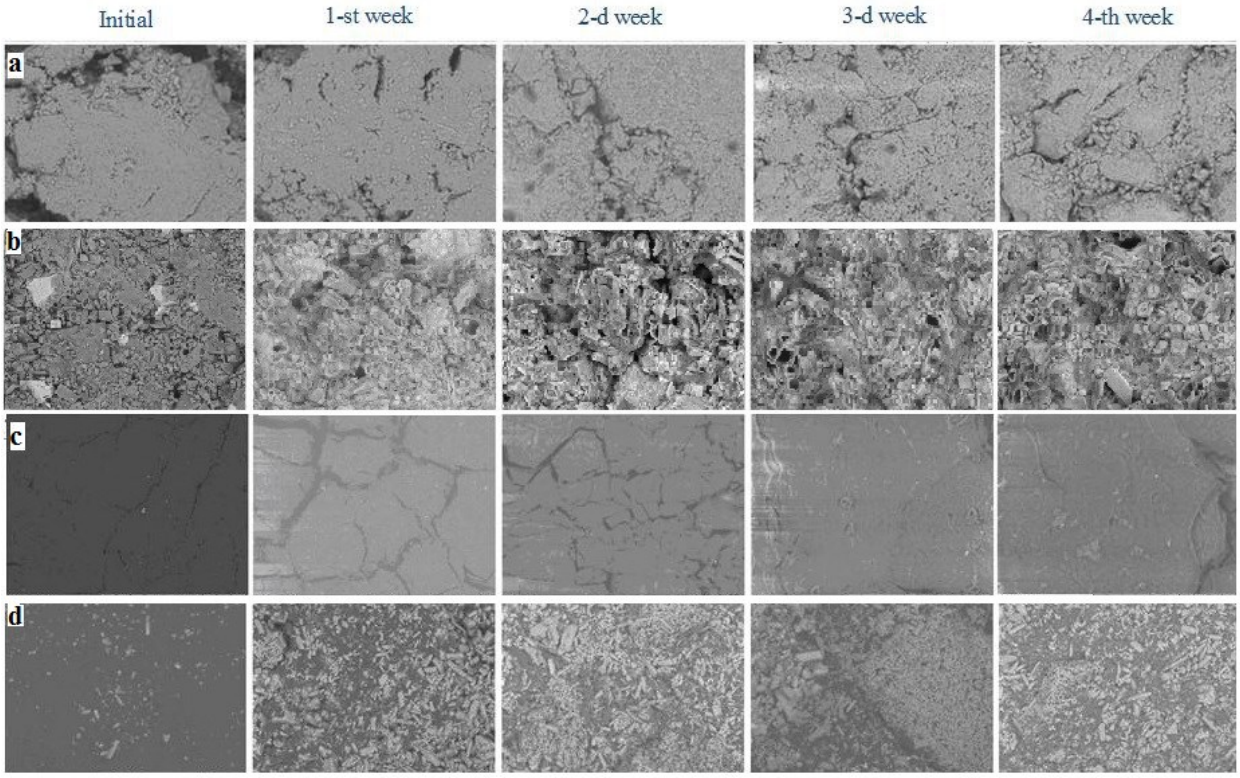

Fig. 4. Electron micrographs of the substrate surfaces; magnif. 3000. Dynamics of the growth of calcium phosphate layer: a – synthetic HA; b – P3 composite; c – pure lactic acid oligomer P5; d – P7 composite.

The SEM images testify that formation of the calcium-phosphate layer takes place on the substrate of the composites in 7 days of aging in the SBF solution. A new layer is being formed with the course of time especially actively on the surface of the composite (Fig. 4 b, d). Dissolution of composites containing oligomer grafted on HA, occurs simultaneously. Really, an increase of Ca^{2+} and Mg^{2+} ions concentration in the SBF solution in the first two days was detected. Lactic acid oligomer does not promote formation of a new layer, but it undergoes degradation due to hydrolysis (Fig. 4 c).

Summary

Bioactive composites based on hydroxyapatite and oligomers of lactic and glycolic acids are prepared. Composition, structure, bioactivity, thermal and certain mechanical properties of the composites are investigated. At least two oligomer macromolecules "sit themselves" on the HA molecular fragments with formation of the ester (*i.e.* ~–COO^-) linkages during the *in situ* synthesis.

The composites possess of biological activity *in vitro* (SBF investigation). It is established that the calcium phosphate layer is being actively formed for 28 days at 37 °C on the surface of the samples containing hydroxyapatite, while pure lactic acid oligomer in the SBF solution was destructed, undergoing the hydrolysis. The composites will meet all the requirements of biocompatibility and promote the growth of bone tissue. The tests *in vivo* are to follow.

Acknowledgments

The research was supported by Laboratory of catalytic Research TSU (Russia, Tomsk), ICT Fraunhofer (Germany, Pfinztal).

References

[1] M. Sadat-Shojai, M. Khorasani, E. Dinpanah-Khoshdargi, A. Jamshidi, Synthesis methods for nanosized hydroxyapatite with diverse structures, Acta Biomaterialia. 9 (2013) 7591-7621.

[2] S.V. Barinov, V.S. Komlev, Bioceramics based on calcium phosphates, Science, Moscow, 2005.

[3] Ben-Nissan B. Advances in Calcium Phosphate Biomaterials, Spinger, 2014.

[4] M. Shtilman, The polymers of medical and biological applications, Akademkniga, Moscow, 2006.

[5] B.I. Sevastyanov, M.P. Kirpichnikov, Biocompatible materials, Medical informational agency, Moscow, 2011.

[6] L.Hench, J.Jones, Biomaterials, artificial organs and tissue engineering, Woodhead Publishing, Cambrige, 2005.

[7] N.M. Korotchenko, L.A Rasskazova, Rus. Fed. Patent 2,507,151. (2014).

[8] T. Kokubo, H. Takadama, How useful is SBF in predicting in vivo bone bioactivity? Biomater. 27 (2006) 2097-2915.