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# Preparation of Composite Materials Based on Hydroxyapatite and Lactide and Glycolide Copolymer

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**Abstract.** Composite materials for the restoration of bone tissues based on hydroxyapatite and a copolymer of lactide and glycolide were obtained. The composition of materials at different stages of production is estimated by X-Ray diffraction method. It has been established that during the production of materials a new phase of chlorine-substituted hydroxyapatite ( $\text{Ca}_{9,7}(\text{P}_6\text{O}_{23,81})\text{Cl}_{2,35}(\text{OH})_{2,01}$ ) was formed, which promoted an increase of hydroxyapatite solubility in the composition of materials. The quantitative estimation of micro- and macroporosity of materials is given. It was found that the use of sodium chloride as the pore-forming agent delivers porosity of the composites required for use as bone substitutes.

## INTRODUCTION

Restoration of bone defects arising from injuries, inflammatory processes, after removal of tumors, is an urgent problem for reconstructive surgery. Operations performed using bone implants are constantly increasing causing a high demand for modern materials and technologies. Annually in Russia, injuries are received by about 15 million people, 70% of them are seniors. It should be borne in mind that almost 90% of all bone fractures occur in the musculoskeletal system; the number of patients requiring treatment is more than 60% [1]. Due to the high frequency of relapses and low satisfaction with the results of traditional treatment, the development of new and improvement of existing implants becomes one of the primary tasks of medicine, biology, and biotechnology.

In clinical practice, the problem of repairing bone tissue defects in recent years is being addressed through the development and introduction of new techniques for reconstructive surgeries using materials replenishing the lost bone volume and factors that improve its restorative properties [2]. Practical experience accumulated by clinicians in the field of maxillofacial surgery and traumatology shows that the currently used osteoplastic materials have both their advantages and disadvantages. This is why it is necessary to search for new materials - bone substitutes with the following advantages: relative simplicity of surgical intervention, expansion of modeling capabilities, structure stability, high mechanical strength, biocompatibility, osteoconductive and osteoinductive properties, absence of infectious agents, resorption in the body without the formation of toxic products as the restoration of new tissues in the place of the defect, etc. [3].

The use of artificial materials for the reconstruction of bone defects, identical to the mineral component of bone substance, attracts close attention of clinicians. Such materials are for example calcium phosphate composites and hydroxyapatite (HA) and its combination with other substances in the form of powders, granules, microparticles, wafers, etc. At present, many different modifications of hydroxyapatite are used to replace bone defects in surgical dentistry, orthopedics and traumatology, differing in shape and size of the particles [2]. Macroporous materials are of particular interest, since their structure is close to bone tissue. The open pore structure can provide cell migration and vessel sprouting in the integration with the bone tissue material [4]. The size of osteoblasts - bone-forming cells - is 15-20 microns, however, it is known from the literature that for free circulation of bone cells in bone and their vital activity, the diameter of macropores should

reach 50-200 microns [5]. Hydroxyapatite is the main mineral constituent of bones (about 50% of the total bone weight) and teeth (96% in enamel), it can be synthetic or natural type. Synthetic hydroxyapatite is used as a filler, replacing parts of lost bone (in traumatology and orthopedics, hand surgery), and as a covering of implants, which contributes to the growth of a new bone [4,6].

Despite a number of advantages, HA has a significant drawback - low mechanical strength [3]. This problem can be solved by introducing a polymer component and production composite materials based on HA and biocompatible polymers. Such polymers are, for example, copoly(lactide-glycolide)s (CLG), which are worldwide used in medicine because of their biocompatibility and the ability to decompose in a living organism without the formation of toxic compounds. In the body, such polymers degrade to lactic and glycolic acids, which are normal metabolites and do not have a toxic effect on the body. The rate of hydrolysis depends on the size and hydrophilicity of the polymer material, the degree of crystallinity, the pH and temperature of the medium, and other factors [7]. The introduction of a comonomer and the synthesis of copolymers of various compositions based on lactide and glycolide makes it possible to control the rate of biodegradation. Varying the molecular weight of the macromolecule structure and allows to obtain a range of properties of copolymers and composites in which they are [7]. The aim of the work is to produce porous biocompatible composites as materials for the production of bone implants based on hydroxyapatite and a copolymer of lactide and glycolide, as well as an investigation of their structural properties and surface morphology.

## EXPERIMENTAL PART

### Materials

A lactide-glycolide copolymer was prepared by a ring-opening ionic polymerization method from L-lactide and glycolide (Fig. 1):

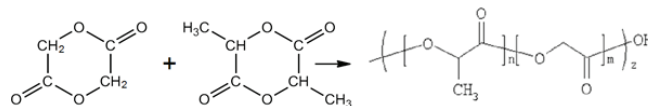


FIGURE 1. Scheme for the copolymerization of lactide and glycolide

More details of the synthesis of copoly (lactide-glycolide) are described in [8]. Liquid-phase synthesis of HA was carried out under microwave conditions [9,10] by precipitation of the product at pH =10. By X-Ray phase analysis it was found that the obtained HA contains the phase of  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ , the other phases are not registered.

Composites were obtained in several stages. The first stage was the production of tablets from HA and pore-forming agent. Sodium chloride was used as the pore-forming agent. HA and sodium chloride powders were grinded in a mill to form a homogeneous mixture, then from a sample of a 1 g mixture and the tablets were compressed (P = 200 atm). Tablets were calcinated in a muffle furnace at 700 ° C for three hours. A different content of NaCl (Table 1) was taken to produce composites C1, C2, and C3 with different porosities.

The second stage was the preparation of a porous framework for the composite. The tablets were soaked in distilled water for three days with the water refreshed every 12 hours. Then the obtained porous framework of hydroxyapatite was dried under vacuum to constant weight. As a result, substrates with different porosity, which increases from C1 to C3, were obtained. The third stage was impregnation of the carcass with a polymer. For this purpose, CLG was dissolved in chloroform (concentration 0.1 g/ml). The tablets were immersed in the resulting solution and sonicated to remove air bubbles from the substrate to obtain uniform coatings for 20 minutes. The third stage is impregnation of the carcass with a polymer. For this purpose, SLG was dissolved in chloroform (concentration 0.1 g / ml). The tablets were immersed in the resulting solution and sonicated to remove air bubbles from the substrate to obtain uniform coatings for 20 minutes.

### Research Methods

The molecular masses of SLG were determined by gel permeation chromatography (GPC) using a GPC Agilent System 1100 apparatus equipped with a UV-Detector (230 nm) DAD Agilent 1100 detector using a series of PSS SDV polymer columns with a pore size of 50 to 105 Å. As the eluent, tetrahydrofuran (THF) was used at a flow rate of 1 ml·min<sup>-1</sup> at 35 °C; copolymer was dissolved for 3 hours. The calibration line was established using polystyrene standards with Mw from 162 to 246.000 g·mol<sup>-1</sup>. A sample of a 3 g/ml<sup>-1</sup> copolymer was purified by passing through a filter with a pore size of 0.45 µm. The weight average molecular

weight of CLG was 26.800 g/mol, with a polydispersity of 2.2.

X-ray phase analysis (XRD) of HA and composites was performed on a Shimadzu XRD 6000 diffractometer with  $\text{CuK}\alpha = 1.5406 \text{ \AA}$  radiation in the range 20-140° (2 $\theta$ ). The phases were identified using the PCPDF WIN-1.3 database. IR spectra were recorded on an Agilent Cary 630 FTIR spectrometer. The porosity and morphology of samples were evaluated from images obtained with a Hitachi TM3000 scanning electron microscope (SEM), magnification  $\times 3000$ .

The specific surface area (SSA) of the C1-C3 composites was analyzed on a Micromeritics 3Flex analyzer, and nitrogen was used as the gas. Degassing was carried out on the degasifier VacPrep 061 under degassing conditions:  $t = 2 \text{ h}$ ,  $T = 130 \text{ }^\circ\text{C}$ ,  $P = 1 \text{ atm}$ . The content of the polymer component in the composites (five parallel samples) was determined by the gravimetric method (the mass of the impregnated tablets was compared with the weight of the original porous substrates of the same area).

The total volume of macropores was measured by immersing pre-weighed samples of materials in distilled water and held for 5 hours (to constant weight). The sample was then removed from the water and weighed. The difference was determined by the mass of absorbed water. Based on the fact that the density of distilled water is  $1 \text{ g / cm}^3$ , the total pore volume was found. The volume of the samples was calculated as for the cylinder with radius and height of 0.952 and 0.1 cm, respectively. Based on the obtained results, the volume fraction of the pores for composites and initial unimpregnated substrates was calculated.

## RESULTS AND DISCUSSION

Analysis of the obtained composites (Table 1) by IR spectroscopy showed that bonds characteristic for both HA and CLG are observed in the spectra. The IR spectra of the composites contain bonds ( $1800\text{-}1700 \text{ cm}^{-1}$ ) corresponding to the vibrations of bonds in the CLG structure  $\nu(\text{C}=\text{O})$ , as well as valence ( $1085\text{-}960 \text{ cm}^{-1}$ ) and deformation ( $560\text{-}605 \text{ cm}^{-1}$ ) vibrations phosphate groups  $\nu(\text{PO}_4^{3-})$ ,  $\delta(\text{PO}_4^{3-})$  HA.

**TABLE 1.** Ratio of components and weight loss during obtaining of composites HA:CLG.

Composite	C1	C2	C3
Ratio of HA:NaCl, wt. %	90:10	75:25	50:50
Ratio of HA:CLG, wt. %	95:5	93:7	83:17
$\omega_{\text{loss}}$ due to chlorine-substituted HA, relative %	3.20 $\pm$ 0.06	3.7 $\pm$ 0.02	4.1 $\pm$ 0.09

The results of XRD indicate the presence of the HA phase during the whole process of obtaining composites. The CLG phase is not detected because of the low crystallinity of the CLG (Fig.2a). After calcination of the HA substrate with NaCl, a new phase of the chlorine-substituted HA is formed (Fig.2b), which is obviously more soluble. Then the new phase was completely washed out of the substrate (Fig.2c) that was confirmed by the loss of weight (Table 1).

On the SEM photomicrographs of composite's surface (Fig.3a, Fig.3b), it is seen that the composite is peppered with pores up to 7  $\mu\text{m}$  in size, we can also observe both particles of HA (parallelepipeds) and SLG (spheres), which indicates that CLG penetrated into the entire volume of the substrate from Hydroxyapatite, i.e. Composites with open porosity were obtained.

Pores up to 200  $\mu\text{m}$  in size are also seen on the photomicrographs of the SEM surface of composites (Fig.3b). Using the method of low-temperature adsorption of nitrogen, the specific surface area of composite materials was determined (Fig.4, Fig.5). The SSA value and the total pore volume in the composite range decreases from K1 to K3, which is associated with a more uniform coating from inside the substrates and filling a part of the micro- and mesopores (Fig.4, Fig.5, Fig.1a, Fig.2a).

Specific surface area of samples sharply decreases during its preparation due to calcination and washing out the NaCl. Further, the SSA also decreases from 3 to 11% after impregnation of the substrate with the copolymer (Fig.6). This is also related to the filling of a part of the micro- and mesopore with a copolymer (Fig.5a, Fig.5b, Fig.5c).

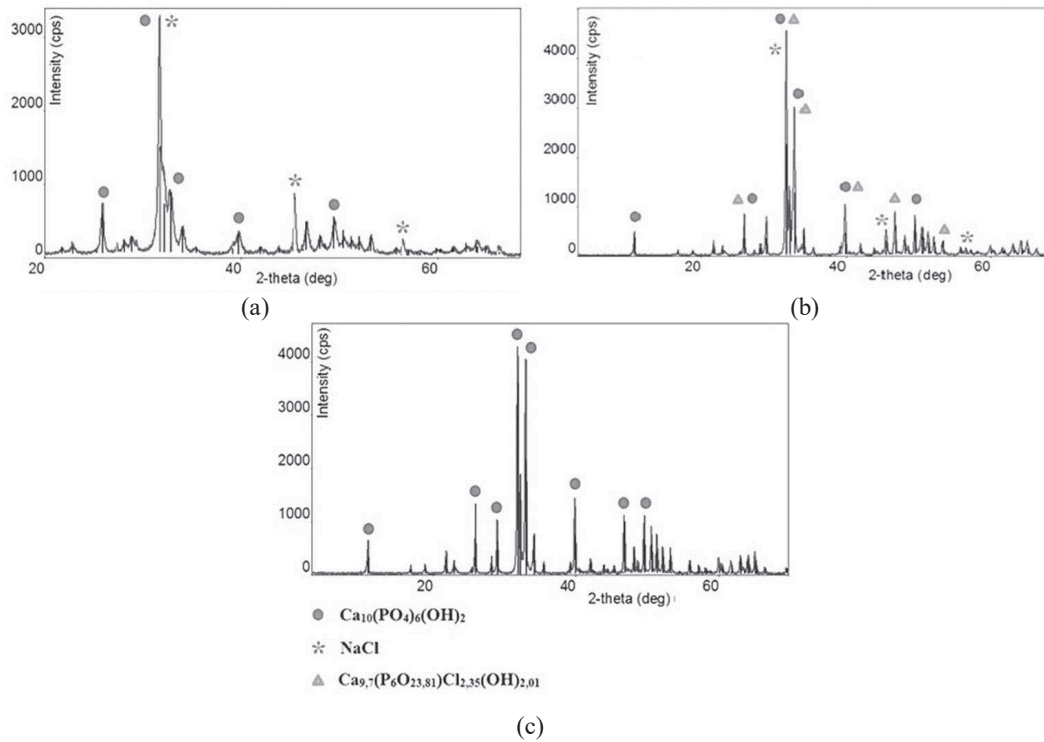


FIGURE 2. Diffractograms of C1 in the process of obtaining: a) substrate HA + NaCl; b) HA + NaCl substrates after calcination; c) HA substrates after calcination and leaching with NaCl

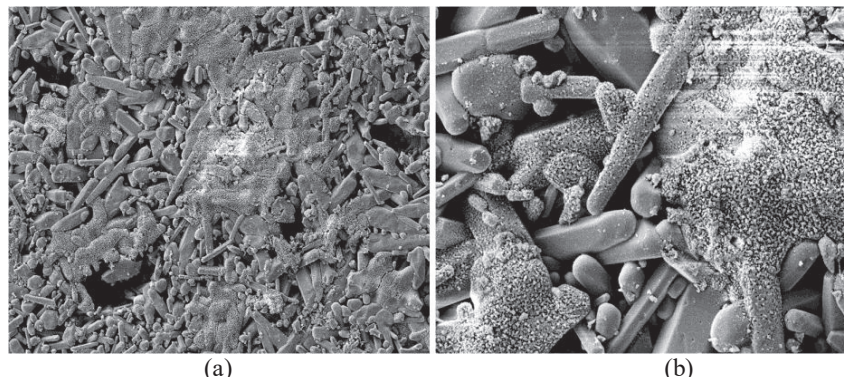


FIGURE 3. SEM images of C2 surface magnification: a)  $\times 990$ , b)  $\times 4000$

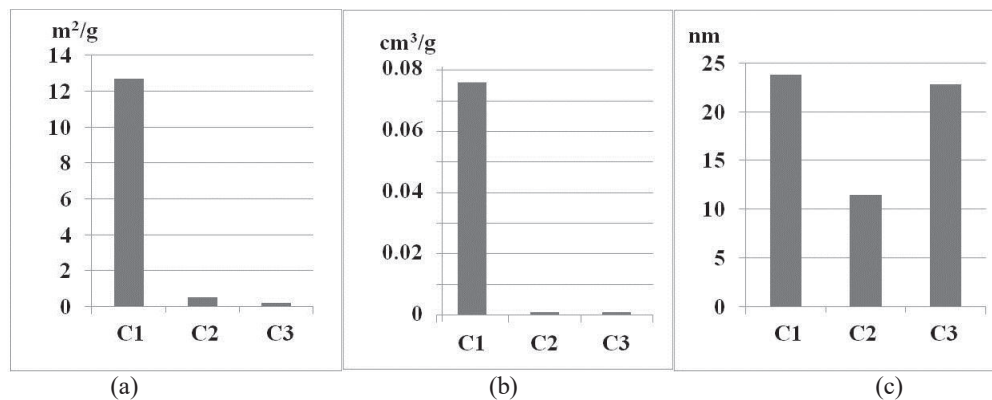
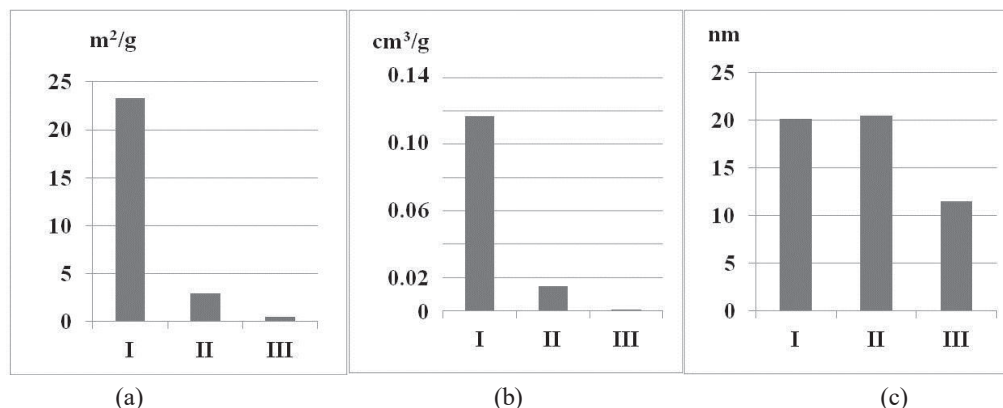
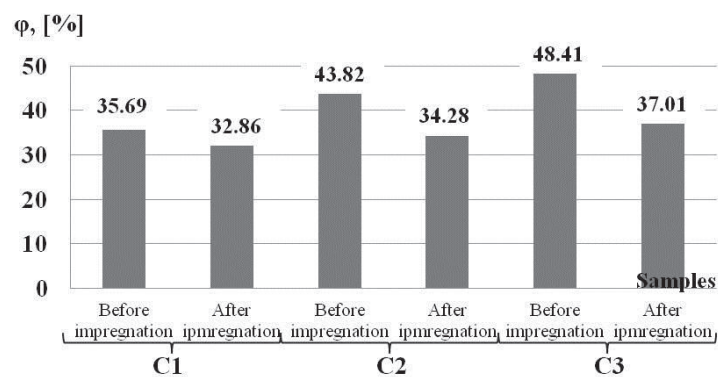


FIGURE 4. Surface characteristics of composites C1, C2 and C3: (a) SSA, m<sup>2</sup>/g; (b) total pore volume, cm<sup>3</sup>/g; (c) average pore size, nm



**FIGURE 5.** Surface characteristics of C2 at different stages of its preparation: I – the first stage of composite preparation (tablets from HA and NaCl); II – the second stage (the skeleton from HA after NaCl washing out); III – the third stage (final composite); (a) SSA, m<sup>2</sup>/g; (b) total pore volume, cm<sup>3</sup>/g; (c) average pore size, nm



**FIGURE 6.** Porosity of composites and initial substrates

## CONCLUSIONS

A technique for the production of porous composite materials based on hydroxyapatite and a copolymer of lactide and glycolide has been developed. The composition and structure of the materials are confirmed by the method of IR spectroscopy and X-ray fluorescence. It is established that during obtaining frameworks of composites  $\text{Ca}_{9.7}(\text{P}_6\text{O}_{23.81})\text{Cl}_{2.35}(\text{OH})_{2.01}$  phase is forming, the presence of which causes a higher solubility of HA. CLG coating allows to reduce the specific surface area of materials significantly, that may improve the biocompatibility of materials. Macroporosity of composites depends on the content of NaCl at the first stage. It also influences to the content of copolymer in the range of composites and to the specific surface area. It was found that synthesis using NaCl delivers porosity of the composites required for use as bone substitutes.

## ACKNOWLEDGMENTS

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