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Bone substitute material on the basis of natural components

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Abstract. The creation of regenerative materials remains a problem for rehabilitation medicine, but the obtaining of initial substances that can cause bone tissue regeneration, possessing biological activity and creation on their basis of composite materials with specified physical and mechanical characteristics is an important scientific problem. This paper presents the investigation of physical-chemical and biological properties of bioresorbable composite material that can restore their own bone tissue of the body.

Keywords: hydroxyapatite, biopolymer, regeneration, bone resorption, composite material, natural components.

1. Introduction

Modern medical experience of traumatology and orthopedics is mainly based on a mechanical replacement implant bone defect with the creation of complex biomechanical structure. At the same time, intensive research is carried out to develop methods to restore damaged bone by physiological regeneration processes.

In past, materials, intended for implantation, are designed as "bioinert." Nowadays materials are increasingly used, which integrate with biological molecules or cells and regenerate tissue. Application of regenerative biodegradable composite material eliminates the necessity for re-operation to remove of the implant from the patient due to its resorption and eliminates the need for additional fixation of bone fragments (debris), as well as allows to regenerate lost or destroyed bone.

Despite the considerable amount of bone replacement materials are used in regenerative medicine in the world, searching and the development of new ones, is still an urgent problem. It is possible to solve it if compounds are natural and they can be a part of the metabolic processes of the human organism.

Bone tissue is a mineral-organic composite material with a porous structure, which is subjected by constant remodeling. Mineral matrix of bone is hydroxyapatite but larger part of organic matrix is collagen.

In order to create a material based on calcium salts and biological polymers with porous bone structure and which has the special strength, it's necessary to combine technology and the components so that the implant is made of this material does not destroy bone and bone does not destroy the implant. Moreover, in the case of loose implant contact and native bone regeneration process slows or stops altogether. To meet these conditions it is necessary that the material was a viscous fluid or plastic at the beginning of work and then at the end of work it became hard and strength.

It is established that increasing the surface area and porosity of biocomposite materials affects positively on the kinetics of bone formation and improves the bioactivity (complex of material properties, allowing creating a direct contact with natural bone [1, 2]). To understand the relationship between structure and bioactivity, as well as to design better implants is very important to control of total porosity, pore size and internal structure of the porous biomaterials [1, 2].

2. Material and methods

The structure of a bone replacement material includes only natural components. The basis of the composite material is ultrafine hydroxyapatite obtained by the original technology [3] from the bones of farm animals. Its main feature is the presence of trace elements equal to bone mineral, and stable chemical composition.

Biopolymer matrix collagen is used as the organic binder. Shredded tendons of cattle are used to make collagen. They were subjected to acid- saline treatment and purification of non-collagenous proteins. Then, an ultrafine powder of hydroxyapatite is mixed with the liquid polymer in specific ratios to form the plastic cement paste.

The technology permits to make a bone replacement composite material to fill bone defects by different ways. The finished product is self-curing cement mass. Seizing instantaneously occurs under room temperature. Pre-curing of the material occurs within 15-20 minutes, and full seizing occurs after 24 hours.

Studies of micro- and macrostructure are carried by a scanning microscope JSM-7500FA. Investigation of the phase composition and structure parameters was made by diffractometer XRD - 6000 CuKα - radiation. Analysis of the phase composition, the size of coherent scattering of internal elastic stresses is made by databases PCPDFWIN, as well as full-profile analysis program POWDER CELL 2.4. The composition was determined by X-ray fluorescence spectroscopy (XRF) with spectrometer VRA- 30. The specific surface area was determined with multipoint BET method by hydroxyapatite particles tool named Sorbtometer M ver 1.0.0.0. The mechanical properties of bone cement were tested by electromechanical testing machine Instron 3369.

Biological tests included preliminary toxicological and preclinical studies. Investigation of the cytotoxicity of cell culture was carried out by direct contact (ISO 10993-5, 1999) by using rabbit fibroblast cells.

Bone regeneration was investigated by rabbits weighing 1.8-4 kg. In the experiment, all requirements of the international standard of quality assurance GLP (Good Laboratory Practice) were taken into account. Under anesthesia by intramuscular injection into the femoral muscle and after skin treatment with 70% alcohol, skin incision was made parallel to the long axis of the femur to 10 cm. Then the thigh muscle bands were cut and muscle was diluted exposing the outer surface of the femur. A hole with diameter of 7 mm and depth of 0.5 cm was performed in the shaft of the femur. The hole of the animals from the first group was filled with composite material. Muscle tissue is sutured over the site of filling, and then the wound was sutured. Bone defect wasn't filled in the control group.

The level of bone regeneration was tested by X-ray computed tomography named Somatom AR.HP produced by "Siemens".

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3. Results and discussion

Micrographs presented in Figure 1 clearly show that the bone replacement composite material is highly porous with different pore sizes and has a rough surface.

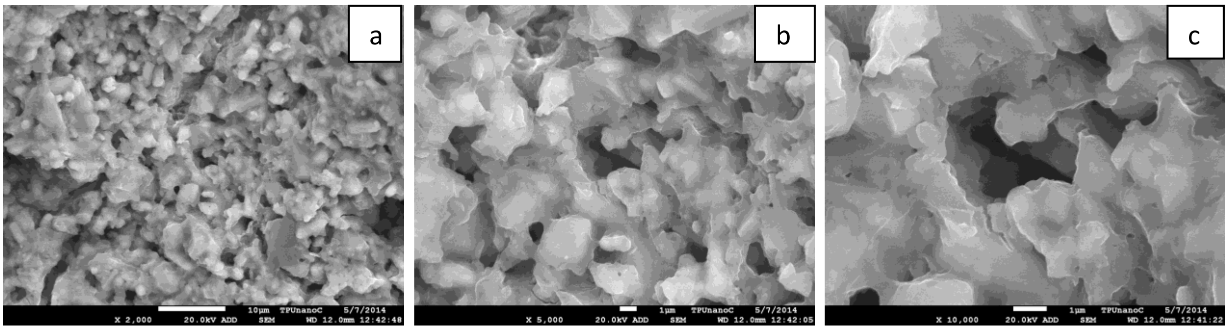

Figure 1. The micrographs of composite material, made with different zooming: a) x 2000; b) x 5000; c) x 10000

Research of bone replacement material composition indicates the presence only the main elements and trace elements. There are no uncharacteristic bone impurities. It is confirmed by spectral diagram in Figure 2.

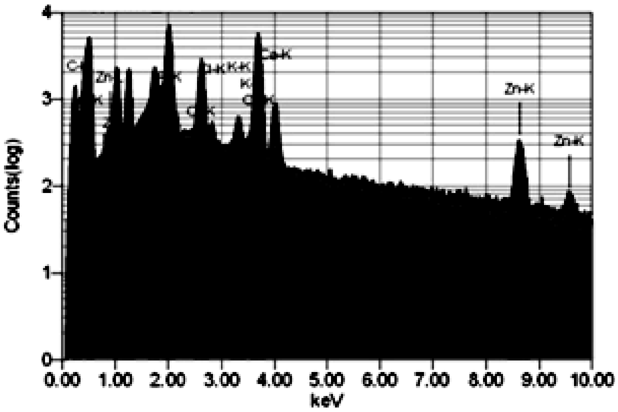

Figure 2. The spectral diagram of composite material composition

According to X-ray analysis results, which are given in Table 1, the composite material is represented by 30% amorphous phase; a crystalline phase is composed of hydroxyapatite monoclinic (about 23%) and hexagonal (about 47%) modifications. It corresponds to a ratio of hydroxyapatite-polymer in the structure of composite material. So, polymer and hydroxyapatite chemically unrelated, and biopolymer only envelops particles of hydroxyapatite.

Table 1. Results of X-ray diffraction

Sample	Detected phase	Contents phases of the %	Lattice parameters, A
Hydroxyapatite	$Ca_5(PO_4)_3(OH)$	89,4	A=9,522 C=6,875
	$Ca_{10}(PO_4)_6(OH)_2$	10,5	A=9,519 C=6,850 B=18,748 β =120,66

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	$Ca(HPO_4)(H_2O)$ - - Brush	0,1	a=6,389 β=118,95 B=15,227
	Amorphous	1	–
	$Ca_5(PO_4)_3(OH)$	23	*a*=9,5218 *c*=6,9631
Composite material	$Ca_{10}(PO_4)_6(OH)_2$	47	*a*=9,3582 *c*=6,850 *в*=18,748 *β*=120,66
	Amorphous	30	–

Mechanical tests of composite material show high strength properties close to the dense bone strength. The results of mechanical tests are given in Table 2.

Table 2. Results of mechanical tests of composite material

Test	Strain rate, (mm/min)	Area, (mm^2)	Voltage, (MPa)	Load, (кН)	Young's modulus, (GPa)
1 Compression	0,2±0.001	1713±1	11,9±0.03	1,95±0.005	0,513±0.003
2 Bend	0,2±0.001	1951±1	40±0.2	106±0.3	8,605±0.04

To assess the effect of implant on a body, pre-tests were carried out on rabbits.

Figure 3 shows the results of histological studies.

Figure 3a (after 14 days) shows the traces of a polymer as homogeneous red- brownish masses, which have the formation of bone trabeculae on the periphery. There is the proliferation of osteoblasts among them. There are foci of fibrosis presented by fibroblasts layers of collagen in a side of the medullary canal. Bone marrow is celled; the cells are modified by decalcification. From the side of the periosteum there is phenomena osteogenesis presented by mature beams with layers of soft collagen fibers.

Figure 3b shows the histological section through the month. In the cortex of the femur there is a slight defect which is closed by newly bone beams formed perpendicularly to longitudinal axis of bone. There are newly formed vessels and fibroplastic reaction from the periosteum.

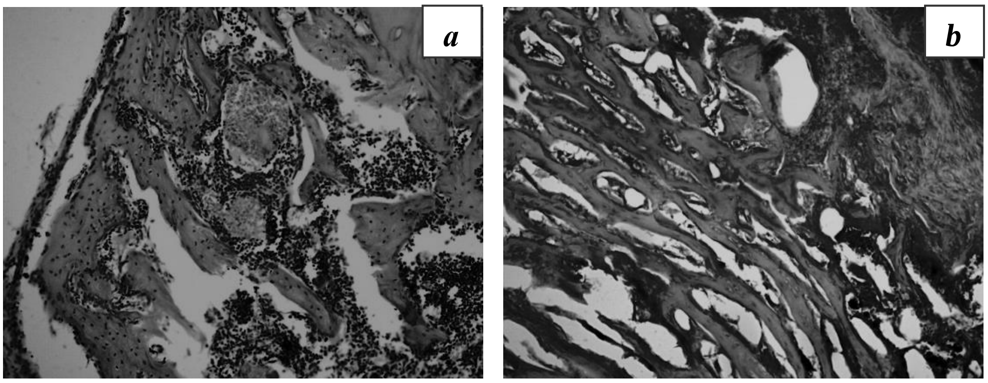

Figure 3. Staining by hematoxylin-eosin (uv. x 100):
a) mature bone beams; the period of 14 days; b) newly formed bone beams with large blood vessel between them, after1 month

As a result of histological examination of composite material, it was found that the newly formed tissue surrounding and sprouting in a matrix there are vessels of different diameters, and nerve fibers. Across the thickness of the implant newly formed bone trabeculae changed material in a result of biodegradation. The vessels are filled with blood. It indicates that the vessels are connected to the bloodstream. The results of X-ray studies are given in Table 3.

Table 3. Change the index of bone tissue regeneration

Research Group	Index bone regeneration		
	one week after the surgery	two weeks after the surgery	four weeks after the surgery
Control	28.2 ± 0.3	28.8 ± 0.2	30.7 ± 0.3
Composite material	29.2 ± 0.1	44.3 ± 0.2	46.8 ± 0.3

Conclusions

Thus, obtained composite material has high specific surface and porosity, which is one of the basic conditions for the formation of new bone tissue. The constituents of the composite material (hydroxyapatite and collagen) included in the connective tissues, such as bone, cartilage, skin, tendons, ligaments and blood vessels.

In vivo studies have shown that the investigated composite material proved to be a biocompatible, biodegradable, has osteogenic properties. Therefore, it may be used for bone tissue engineering.

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