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The Comparative Characteristic of Physical, Chemical and Bioactive Properties of the Synthesized Hydroxyapatites

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Abstract: Nanodimensional samples of Si-GA have received from water solution by the sedimentation method. In the real work researches of physical and chemical properties and bioactivity synthesized by a sedimentation method from water solution of a nanodimensional hydroxyapatite (GAP) and a hydroxyapatite, modified silicate ions (Si-GAP) are conducted. It is established that synthetic modified GAP are biocompatible, possess a high rezorbtion and don't cause degenerate changes in surrounding connecting fabrics. Results of research suggest that (Si-GAP) can be used as osteoplastic materials as in orthopedics and dental prosthetics.

Key words: Nanodimensional hydroxyapatite · Modification silicate ions · Replacement coefficient · Size of particles · Specific surface · Rezorbtion · Bioactivity · Biocompatibility

INTRODUCTION

In recent years in medical practice for the purpose of replacement, restoration or restoration of the injured bones and joints as biocompatible implants are very effectively used calcium - phosphatic materials.

At the same time, limited sources of autogenic materials and also the risks connected with use of allogeny or xenogeny materials, made actual broad application in dental and orthopedic practice of synthetic materials. Decisive factor at a choice of similar synthetic materials is that they have to be the most biocompatible and biologically active materials capable to full integration into natural process of remodeling of bone fabric [1].

Among the biomaterials used in bone surgery and dental a hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) takes a special place, it is analog of a mineral component of tissues of a skeleton of animals and in this regard is biologically compatible to a human body.

However the last results of clinical approbation of preparations of the medical appointment prepared with use of GAP, show that such material along with clear advantages possesses shortcomings: slow speed of a bioresorption of *In vivo* and weak stimulating impact on growth of new bone fabric. Bioactivity is the complex characteristic of the materials compatible to an organism, considering, besides biological processes of growth and differentiation of cages, physical and chemical characteristics of an implant, such as: the size of particles, diameter of a time, speed of dissolution of a material, sedimentation speed GAP from interfabric liquid of an organism on material surfaces and also existence of the osteostimulating action, being accompanied acceleration of processes of restoration and full replacement of an implant with new bone fabric [2-4].

Calcium-phosphatic materials is the perspective direction of increase of a rezorbtion and an osteoinduction chemical modifying of a hydroxyapatite by biocompatible anions. It is known that silicon plays a positive role in a calcification of bones and a metabolism. Active zones of growth, such as osteoides of mice and rats, contain about 5% of silicon and its deficiency leads to abnormal development of a skeleton. Thus, chemical modifying of ortho-phosphates of calcium by silicon positively influences a rezorbtion of implantation materials [5, 6].

It is established that GAP, the modified silicate-anions, promotes the improved proliferation ostoblasts and to growth extracellular matrix, the accelerated mineralization of bone fabric. However, receiving similar monophase and stable synthetic Si-GAP, as appears from these authors, still remains an unresolved technical

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problem [7,8]. Formally for synthesis of Si-GAP of this structure in alkaline solution with pH more than 9 (as a source of OH-groups) have to be brought or be present at a demanded ratio free ions of calcium, phosphate ions and silicate ions. However silicate ions, unlike Ca^{2+} and PO_4^{3-}, have special chemical behavior in water solutions, namely high tendency to polymerization with formation of oligomer and further particles of colloidal silicon dioxide. And polymerization speed other things being equal parameters increases in process of increase in concentration of ions of SiO_4^{4-} and decrease in alkalinity of the environment.

The purpose of this work is comparative research of bioactivity of synthesized GAP and Si-GAP.

MATERIALS AND METHODS

The nanodimensional GAP and Si-GAP were received by a sedimentation method from water solution. As reagents used saturated solution of hydroxide of calcium and solution of ortophosphoric acid. As reagent – "supplier" of SiO_4^{4-}anion used tetraethoxysilan. Amounts of reagents determined by data of stoichiometrical calculations, proceeding from a painting ratio of Ca / (P+Si) = 1.67. The replacement coefficient in samples of GAP made x = 0,5; 1,0; 1,5; and 2,0. The received precipitation separated from uterine solution filtering and dried. For achievement of the greatest degree of crystalline and removal of by-products of reaction powders subjected to heat treatment in the muffle furnace [9].

Researches of morphology of samples of GAP and Si-GAP carried out on a translucent electronic microscope of JEM 2100 (JEOL Ltd. Japan) with resolution – 0,2 nanometers. The accelerating tension of an electronic gun made 200kV, a cathode material a monocrystal LaB_6, leak current at the switched-on cathode – 101,5mkA. For research in a translucent mode samples put on a copper grid with round openings diameter 0,1mm. Images in a translucent mode received at increases to 40000x and registered with use of digital plates and SSD-cameras.

Radiographic researches conducted on the Rigaku Ultima IV (Japan) with the detector D/teXUltra. Filming carried out in quartz ditches in a mode on reflection (Bregga-Brentano's geometry) with use of Cu $K_á$ of radiation [lambda] = 1.54178 Å. Parameters of operation of the generator: the accelerating voltage of 40 kV, current of a tube 40mA. Shooting parameters an interval of corners 2θ = 5-85°, a step on 2θ = 0.02°, the speed of registration of ranges 3°/min. The qualitative analysis of the received roentgenograms and the profile analysis of ranges carried out by means of the PDXL Qualitative Analysis program when using the ICDD databases (PDF 2008). The size of crystals determined by the Williamson-Hall method on the basis of RFA data.

Definition of a specific surface on the BET method is realized on the automated sorption TriStar II 3020 installation. Used volume option of a sorption method. Specific surface counted on an isotherm of low-temperature sorption of vapors of nitrogen on the BET single-point method in P/Po point = 0.3189. Samples were sustained in inert gas of nitrogen and helium with simultaneous ensuring heating of samples at a temperature 350°C.

For an assessment of bioactivity of Si-GAP were selected: 1) a dynamic method – change fixation pH at dissolution of samples of Si-GAP in the diluted hydrochloric acid, 2) a static method – analytical determination of concentration of calcium at dissolution of samples of Si-GAP in acetate buffer solution with different time of an exposition.

For an assessment of biocompatibility of the synthesized powders GAP used a method "Hypodermic introduction " according to state standard specification P ISO 10993-6-2009 requirements "Products medical. Assessment of biological action of medical products. Part 6. Researches of local action after implantation". Experiments are made on 18 not purebred laboratory white mice (12 animals – skilled groups and 6 animals – in a false manner operated). All works in a vivarium performed according to the international requirements [10]. Researches conducted in research laboratory "Physiology of Adaptation Processes" of NIU "BelGU".

Samples seated under skin in the layer of connecting fabric located on a back of experimental animals. At first, carried out the following actions: animals were anesthetized with aether, area for implantation processed antiseptic solution, further cut skin of an experimental animal, by a stupid section did one pocket 2 mm wide and 4 mm in depth. In a pocket placed one of samples of a dental material, a wound processed BF-6 glue. Animal of control group surgery and all manipulations carried out according to the scheme used in work with skilled groups, without introduction of samples. Reaction of surrounding fabrics to an introduction of materials estimated in 7 days after operation. Extent of reaction determined by distance measurement from a surface of contact of an implant with fabric to the sites having characteristics of intact fabric with normal blood circulation. Having conducted macroscopic research of a zone of defect, carried out a resection of an introductionly material.

Table 1: Specific surface, the size of a time of samples of not replaced and modified GAP

		Specific surface, m^2/g	Volume of a time, cm^3/g	Average size of a time, Å
	GAP	27,7	0,1	171,8
Si-GAP	0,5	59,1	0,3	278,1
	1,0	65,9	0,5	316,9
	1,5	108,9	0,6	212,2
	2,0	122,2	0,7	240,4

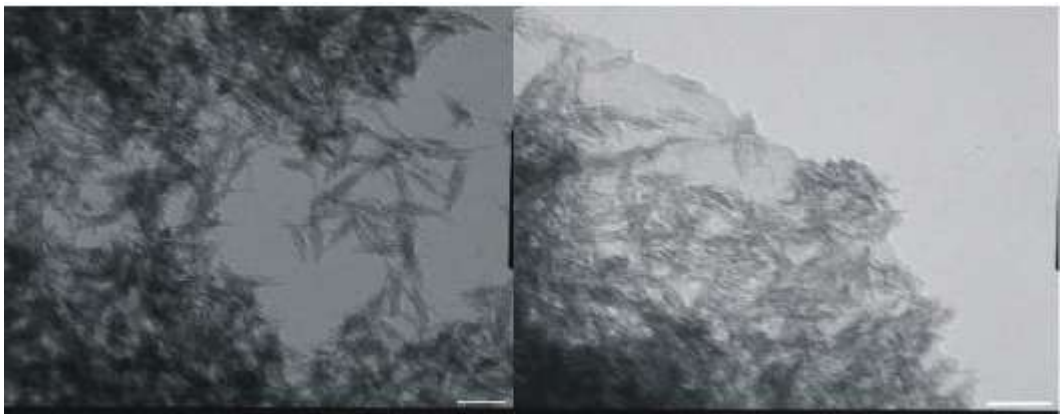

Fig. 1: PEM-mikrofoto of nanoparticles of GAP (a) and Si-GAP (b) 2100 (JEM JEOL Ltd. Japan)

Histology preparations of connecting fabrics of a zone of defect prepared the standard methods. Estimated the following parameters: a degree of fibrosis and inflammations; degeneration of surrounding fabrics; necrosis existence; extent of integration of a material of an implant with connecting fabric of an introductionly zone. The volume of regenerator process (in %) defined by means of a grid with 100 points (81 squares = 100%), inserted into an eyepiece of a stereomicroscope of Leica EZ4D. With the help it is hardware - a program complex the Video Test Size (a microscope of Axioplan plus of Zeiss firm) the histologic preparations, painted studied hematoxylin-eozinom.

The Main Part: The size of particles and their specific surface are defining factors of osteoplastic materials. From the tab. it is visible that the size of crystals of Si-GAP is much less, than at GAP and at increase of the content of silicon in samples of Si-GAP reduction of the size of crystals and, respectively, increase in their specific surface is naturally observed. It is positively reflected in an implant resorbtion.

It is possible to draw a conclusion that introduction silicate ions in a crystal lattice of GAP causes increase in a time and a specific surface of Si-GAP that implant resorbtions at the expense of active proliferation of cages promote. Si2,0-GAP that is 4 times more at GAP has the highest specific surface of equal 122,2 m^2/g (Table 1).

By method of the power dispersive x-ray analysis it is established that besides calcium, phosphorus, oxygen at samples of Si-GAP there is a silicon and the relation of Ca / (P+Si) makes 1,66 ± 0,02. On PEM-microphotography (Fig. 1) it is visible that particles of Si-GAP have a needle formula with a length of 60-95 nanometers, 4-8 nanometers wide and at GAP particles length and width of a crystal make the 100-130th and 20-30 nanometers, respectively. It is explained by charge change GAP surface at introduction silicate ions in its crystal lattice. Thus samples of Si-GAP possess a big charge on a surface of particles that interferes with their aggregation and formation of conglomerates. Reduction of the size of crystals at increase of the content of silicon in samples of Si-GAP leads to increase in their specific surface. Such structure and the size of crystals of Si-GAP can provide resorbtion increase and, therefore and bioactivities of a material in comparison with GAP.

For determination of purity, parameters of an elementary cell, degree of crystalline and the crystal size and also spatial group of the received powders by RFA (Rigaku Ultima IV). The received results are presented in fig. 2. It is visible that peaks of pure GAP and Si-GAP are identical. It allows to claim that the received product is single-phase. Big broadenings of peaks at samples of Si-GAP are caused by weak degree of crystalline of a product.

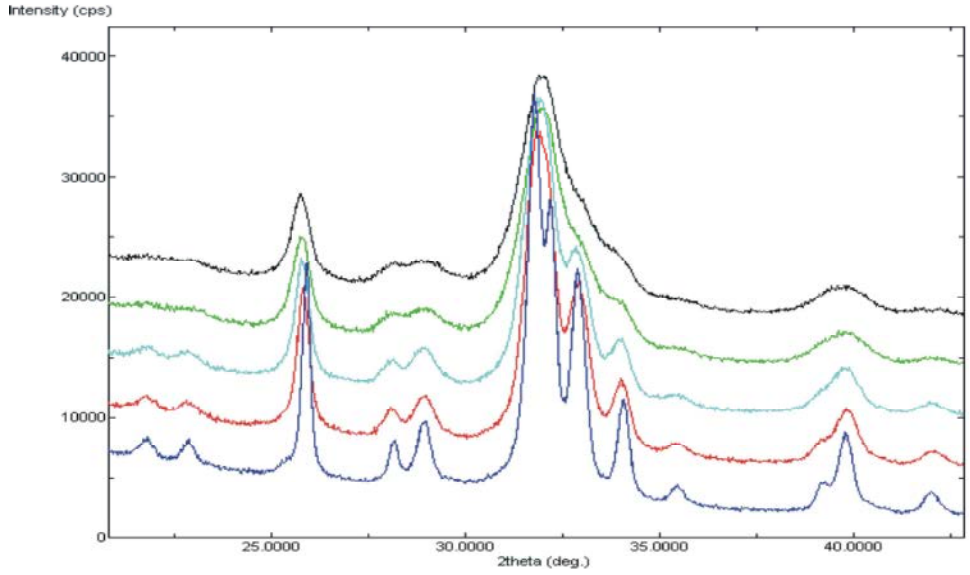

Fig. 2: X-ray powder GAP and Si-GAP is model

Table 2: X-ray qualitative characteristics of the received samples

			Constant of lattice, Å			
		Number of phases	a=b	c	Crystallinity, %	Size of a crystal, nanometer
	GAP	1	9,414	6,865	91,00	65,51
Si- GAP	x=0,5	1	9,440	6,916	92,49	19,29
	x=1,0	1	9,423	6,902	90,25	12,78
	x=1,5	1	9,424	6,904	85,39	11,37
	x=2,0	1	9,420	6,908	88,43	11,68

Table 3: Dynamics of dissolution of samples of GAP and Si-GAP in HCl and acetate buffer solution

	Hcl solution 0,001m						acetate buffer solution				
x	0	0,5	1,0	1,5	2,0	x	0	0,5	1,0	1,5	2,0
minutes	pH					days	Ca^{2+}, %				
0	3,00	3,00	3,00	3,00	3,00	0	5,00	8,13	11,50	14,00	14,38
5	6,07	6,40	6,92	7,13	8,29	1	9,38	12,00	13,75	15,63	17,13
10	6,28	6,48	7,14	7,50	8,67	2	10,63	12,00	15,00	16,88	17,63
15	6,36	6,52	7,23	7,66	8,79	3	10,88	12,25	16,00	16,88	18,75
20	6,43	6,57	7,35	7,82	8,90	4	12,13	13,38	17,13	18,88	20,00
25	6,46	6,59	7,41	7,93	8,96	5	9,38	13,13	16,88	18,13	20,50
30	6,49	6,62	7,48	8,04	9,01	7	6,88	12,50	13,75	18,13	18,88
35	6,52	6,63	7,51	8,08	9,04	8	8,00	13,13	13,75	17,50	18,13
40	6,54	6,65	7,55	8,13	9,07	9	8,13	13,13	14,00	16,50	18,13
50	6,57	6,67	7,60	8,20	9,10	14	8,13	12,50	14,13	16,25	18,13
60	6,58	6,68	7,61	8,22	9,11	20	8,13	12,25	13,75	16,25	18,13

On shift of peaks at Si-GAP concerning GAP it is possible to draw a conclusion on change of volume of an elementary cell at the expense of embedding in a lattice silicate ions. Thus the size of crystals of modified GAP decrease approximately by 5 times.

According to RFA it is established that the received samples belong to spatial group P63/m of hexagonal system and are single-phase. GAP and Si-GAP crystallize for only 90-92%.

The resorbtion of samples of GAP and Si-GAP determined by a static and dynamic method, is presented in table 3. It is visible that both methods yield comparable results. Samples of Si-GAP have the raised resorbtion in comparison with GAP and with increase in the content of

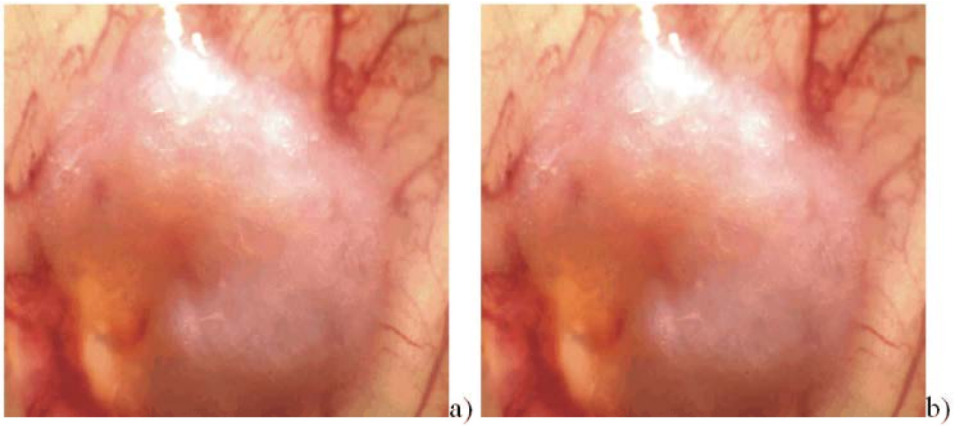

Fig. 3: The capsule formed round a conglomerate of powders GAP (a) and Si-GAP (b)

silicon in samples of Si-GAP their resorbtion raises. The highest resorbtion Si2,0-GAP possesses and Si1,0-GAP has close value with not modified GAP resorbtion.

In vivo experiences as a result of macroscopic studying of area of defect on skin of animal external signs of local inflammatory reaction it isn't revealed. Histology research of a zone of defect in group of in a false manner operated animals showed that layers lying under skin have no degeneration and necrosis signs. The formation of capillary of fabrics moderate, small vessels are full-blooded, penetrate all layers to cross striated muscles. Fibroblasts prevail over other cellular elements i n defect zones, the parenchyma is presented by fibrous structures. Extent of reaction of connecting fabric on surgery was weak and made less than 0,4 mm. The volume of regenerator process made 10% of the area of a zone of defect.

Research of a zone of defect and underlying layers in group which was has introduction GAP, showed existence of a capsule round a sample. The capsule has a thin dense wall and repeats contours of an material. Capsule parenchyma has more capillary than its bed (Fig. 3a). Extent of reaction of fabric on an formation of capillary of powder GAP was moderate and made 1,5 mm. The volume of regenerator process - 70% the area of a zone of defect.

The dense capsule is formed in skilled groups. The capsules created round a sample of Si-GAP, has a dense consistence, their parenchyma and a bed are penetrated by a dense network of blood capillaries (Fig. 3b). There are fibers of friable connecting fabric which surrounds as a particle of an osteoplastic material. Extent of reaction of fabric a sample of Si-GAP was moderate and made 1,5 mm. The volume of regenerator process made 80% of the area of a zone of defect.

The histology analysis of preparations showed that all introduction samples of osteoplastic materials are biocompatible and don't cause degenerate changes in surrounding fabrics. However processes of regeneration of connecting fabrics proceed more intensively in groups to which Si-GAP samples are implanted.

CONCLUSION

The method of sedimentation received nanodimensional samples of Si-GAP. The synthesized products of Si-GAP are single-phase and biocompatible, possess a high resorbtion and don't cause degenerate changes in surrounding fabrics. Introduction in a crystal lattice of GAP leads silicate ions to reduction of the size of particles and increase in surface area that raises a material resorbtion. The received results of research suggest that nanodimensional siliceous GAP - a perspective biomaterial for orthopedic and dentally prosthetics.

Inference:

1. The sedimentation method from water solution received nanodimensional samples of Si-GAP with replacement coefficient x = 0,5; 1; 1,5; 2.
2. It is established that the received samples of Si-GAP belong to spatial group P63/m of hexagonal system and degree of crystalline of 88,4-92,5%, the size of crystals of 11,68-29,6 nanometers are single-phase.
3. The received samples surpass in the physical and chemical characteristics of GAP: the volume of a time the volume of a time on the average increased by 4 times, the average size of a time – twice, a specific surface – more than by 4 times.

4. It is established that introduction in a lattice of not modified GAP leads silicate ions to increase in its resorbtion. The histology analysis of preparations showed that all samples are biocompatible and don't cause degenerate changes in surrounding fabrics. Processes of regeneration of connecting fabrics proceed more intensively in groups to which Si-GAP samples are implanted.

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