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Features of the apatite-like layer formation on the surface of bioactive glass-ceramic materials *in vivo*

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Strengthened bioactive glass-ceramic materials based on hydroxyapatite and lithium disilicate with high crack resistance, corresponding to bone tissue, have been developed. The features of the formation of an apatite-like layer on the surface of bioactive glass-ceramic materials in vivo, namely, the intensification of the nucleation and growth of calcium phosphate crystals due to the developed surface structure, have been established. It has been established that the formation of a crystallized structure of a glass-ceramic material based on nano- and submicron crystals of hydroxyapatite and lithium disilicate in an amount of 50 and 10 vol. %, respectively, can provide its high strength (flexural strength 180 MPa, crack resistance index 6.0 MPa·m$^{1/2}$) and bioactivity (presence of accumulations of hydroxyapatite crystals). It is these indicators that are a necessary condition for the accelerated formation of a strong apatite-like layer on the surface of prototypes in the environment of a living organism. The active formation of bone tissue on the surface of lithium calcium silicate glass-ceramic materials was confirmed by biochemical markers. The results obtained can be used in the development of bioactive glass-ceramic materials with shortened resorption periods up to one month for the replacement of bone defects.

Keywords: lithium calcium silicate glass-ceramic materials, hydroxyapatite, lithium disilicate, crack resistance, apatite-like layer, biochemical markers.

Особливості формування апатитоподібного шару на поверхні біоактивних склокристалічних матеріалів *in vivo*. *Оксана Саввова, Олексій Фесенко, Олена Бабіч, Геннадій Воронов, Юлія Смирнова*

Розроблено зміцнені біоактивні склокристалічні матеріали на основі гідроксиапатиту та дисилікату літію. Встановлено особливості формування апатитоподібного шару на поверхні біоактивних склокристалічних матеріалів *in vivo*, які полягають у інтенсифікації зародкоутворення та росту кристалів фосфатів кальцію завдяки розвиненій структурі поверхні. Встановлено, що формування кристалізованої структури склокерамічного матеріалу на основі нано- та субмікронних кристалів гідроксиапатиту та дисилікату літію в кількості 50 та 10 об. %, відповідно, забезпечить його високу міцність (міцність на вигин 180 МПа, показник тріщиностійкості 6,0 МПа·м$^{1/2}$) та біоактивність (наявність скупчень кристалів гідроксиапатиту). Саме ці показники є необхідною умовою для прискореного утворення міцного апатитоподібного шару на поверхні прототипів у середовищі живого організму. Підтверджено активне формування кісткової тканини на поверхні літійкальційсилікатних склокристалічних матеріалів за біохімічними маркерами. Одержані результати можуть бути використані при розробці біоактивних склокристалічних матеріалів зі скороченими строками резорбції до одного місяця для заміщення кісткових дефектів.

1. Introduction

Today, the priority direction in the development of science, engineering and technology in the world is the development and implementation of innovative approaches in the field of medical materials science. It is especially important to solve the main problems of modern prosthetics of organs and tissues, aimed at creating biocompatible materials that are safe for the body and can fully reproduce not only their shape, but also their functions.

An innovative aspect of the development of tissue engineering is the creation of new bioactive materials, which should be characterized by a long service life of the implanted biomaterial without revision surgery, which improves the quality of life of patients. Therefore, the biomaterial must be characterized by certain properties for a specific application, for example: biological activity, adequate mechanical strength, high corrosion and wear resistance, biocompatibility, low friction and mechanical compatibility [1].

Bone endoprosthetics, which is aimed at creating an "intelligent" material for replacing bone tissue, occupies a special place in the system of prosthetics [2]. For this, the following functional materials have been widely used for a long time [3]: nearly inert ceramics (sapphire or zirconia), porous ceramics (hydroxyapatite), glass (Bioglass(r)), glass ceramics (apatite-wollastonite glass-ceramic), or composites (polyethylene-hydroxyapatite).

Of decisive importance in the application of bioactive materials for the replacement of bone defects is their ability to form a mineralized apatite-like layer on the surface of materials *in vivo* in a short time (from 6 to 12 months). At the same time, an important condition is the exclusion of toxic effects on the body and possible calcification of organs due to the rapid release of calcium and heavy metal cations during the dissolution of resorption glass-ceramic materials with accelerated fusion with bone. Also, rapid resorption can cause a decrease in structural strength and, as a result, a decrease in the mechanical properties of bioactive materials. The traditionally used bioactive ceramics based on calcium phosphates and bioactive glasses have low mechanical properties that do not allow them to be used in dynamically loaded areas of the skeleton (Table 1).

In special conditions, during accidents, hostilities and catastrophes, the greatest need for bone endoprostheses is observed in cases of injuries to the bones of the skull, limbs of the arms and legs, functioning under a significant dynamic load. Therefore, the strengthened bioactive materials based on calcium silicophosphate glass-ceramic materials modified by: CeO_2, Cu_2O, V_2O_5, MoO, CoO, Na_2O, K_2O, Ru_2O, Cs_2O, Fr_2O, SrO, Bi_2O_3, ZnO, Ag_2O, B_2O_3, MnO_2, Fe_2O_3 and TiO_2 are used to ensure both high bioactivity and appropriate mechanical properties for substitution of bone tissue [4, 5]. These materials become biocompatible with living bone within a few weeks, do not weaken either mechanically or histologically, and exhibit good osteointegration and therapeutically relevant mechanical properties, such as fracture toughness and flexural strength [3] (Table 1).

Various types of bioactive glass-ceramic materials known on the market are developed on the basis of the $CaO–P_2O_5–SiO_2$ system. with various modifying additives (Table 1). However, with an increase in their bioactivity, for example, for the Ceravital material, the mechanical properties decrease. The decrease in the strength of the structure of materials can be explained by the presence of mobile Na^+ or K^+ cations in resorption glass-ceramic materials.

Bioactive glass ceramics Bioverit with MgO and Al_2O_3 additives have increased mechanical properties (Table 1), and the addition of CaF_2 contributes to the approximation of the composition of materials to the bone tissue. Also the compositions of bioactive chloroapatite, (ClAp) glass-ceramics in the system of $SiO_2–P_2O_5–CaO–CaCl_2$ are also known. Fluor/Oxyapatite-Wollastonite Glass-Ceramics has developed an apatite-wollastonite (A–W) (β-$CaSiO_3$) system based on $SiO_2–P_2O_5–CaO–MgO–CaF_2$, also known under the commercial name Cerabone(r). The β-wollastonite phase in the A–W system enhances mechanical performance of the glass-ceramics. Compositions of fluorapatite-leucite ($KAlSi_2O_6$) (A-L) glass-ceramics in the $SiO_2–Al_2O_3–Na_2O–K_2O–P_2O_5–F$ system and compositions of fluorapatite-anorthite-diopside ($Ca_5(PO_4)_3F–CaAl_2Si_2O_8–CaMgSi_2O_6$) glass-ceramics in the system of $SiO_2–Al_2O_3–P_2O_5–CaO–CaF_2–MgO$ [6] also possess high mechanical properties due to high-strength crystalline phases; this allows applying them on the dynamically loaded plots bone tissue. However, the above materials are characterized by low crack resistance, which is a significant obstacle to the reliable long-term operation of these materials when replacing bone tissue.

Table 1. Bioglass-ceramics typical characteristics [3, 4, 6]

Bio-ceramics	System/additives	Flexural strength, MPa	K_{1C}, MPa·m$^{1/2}$	E, GPa	Bioactivity IB 1/4, 100/t50	Load to failure, kg	Machina bility
45S5	Na_2O–CaO–P_2O_5–SiO_2	70	0.6	50	12	2.8	Poor
Hydroxy apatite	CaO–P_2O_5	40–70	< 1	120	2.5	6.2	Low
Cerabone	CaO–P_2O_5–SiO_2/MgO, CaF_2 $Ca_{10}(PO_4)_6(OH)_2$, β–$CaSiO_3$	215	2.00	220	3	7.4	Low
Ceravital	CaO–P_2O_5–SiO_2/Na_2O, K_2O, MgO, $Ca_{10}(PO_4)_6(OH)_2$	150	–	150	6	3.5	Low
Bioverit	Na_2O–MgO–CaO–AlO_3–SiO_2/ P_2O_5, F_2, $Ca_{10}(PO_4)_6(OH)_2$, $_{Mg3}K[AlF_2O(SiO_3)_3]$	160	1.00–2.00	90	3	–	Good
AZS	Na_2O–K_2O–Li_2O–CaO–MgO–P_2O_5 –B_2O_3–SiO_2/ZnO, TiO_2, MnO_2, Cu_2O, SrO, CaF_2, 160	2.80	85	10	10	Good	
BS-11Z	Na_2O–CaO–Al_2O_3–B_2O_3–P_2O_5–SiO_2	160	2.22	90	8	8	Low
Bone	CaO–P_2O_5/Mg^{2+}, Na^+	120–180	6.00	15–18	–	–	–

Known fluorapatite-mullite glass ceramics contaning crystal phases in the fluorapatite-mullite system show elongated needle-like microstructure and exceptional mechanical properties, particularly flexural strength and fracture toughness of up to 330 MPa and 3.3 MPa·m$^{1/2}$. However, these ceramics are characterized by a low resorption rate, which increases the period of implant fusion with bone tissue. The strengthened bioactive glass-ceramic material AZS developed by the authors [4] (Table 1) is characterized by the formation of lamellar bone tissue around the implantation site already after 30 days. At the same time, the crack resistance index for this material is at the level of hardened glass-ceramic materials, but does not reach such an index for bone. An actual solution to this problem can be the creation of bioactive glass-ceramic materials with enhanced resorption, high mechanical properties and a significant level of bioactivity of such materials based on modified lithium-calcium phosphate silicate glasses using a three-stage short-term low-temperature heat treatment.The aim of this work is to study the features of the formation of an apatite-like layer on the surface of bioactive glass-ceramic materials *in vivo*.

2. Experimental

2.1 Research methodology

To achieve the goal, the following tasks were set:

1. Establishment of a set of criteria for a glass matrix — the basis of bioactive strengthened glass-ceramic materials;
2. Selection of compositions and synthesis of glass-ceramic materials ;
3. Establishment of the relationship between the phase composition of lithium calcium silicophosphate materials and their biological activity;
4. Evaluation of the ability to form an apatite-like layer on the surface of bioactive glass-ceramic materials *in vivo*;
5. Evaluation of the bone formation ability of the developed glass-ceramic material after implantation *in vivo*.

The crystallization ability was assessed by petrographic (EMV 100 AK microscope) and X-ray phase (DRON-3M diffractometer) analyses of glass-ceramic coatings.

The structure of the surface layer was studied using complementary methods of physicochemical analysis: X-ray fluorescence (SPRUT spectrometer) at a depth of about 1 μm from the surface, X-ray spectral (RES Tesla 3 LMU scanning electron micro-

scope with a separation power of 1 nm using an Oxford X-max 80 mm energy dispersive spectrometer) at a depth of 1.7–15.0 μm from the surface.

Formation of bone tissue was assessed by analyzing the dynamics of alkaline phosphatase activity in the blood serum of rats on days 7, 14 and 28 after implantation of the glass ceramic material into a perforated defect of the distal metaphysis femur.

2.2 Set of glass matrix criteria for bioactive strengthened glass-ceramic materials

The combination of high performance properties of bioactive glass-ceramic materials is the basis for biocompatible materials for bone tissue replacement. It can be achieved by forming a sitallized nano- and submicron structure of the glass matrix during heat treatment in the following stages:

1. Volumetric crystallization of finely dispersed glass during two-stage low-temperature (I stage — 773–823 K, II stage — 873–923 K, III stage — 773–823 K) and short-term (total duration 1–2 h) heat treatment. This stage includes:

— design of compositions based on Li_2O–CaO–P_2O_5–SiO_2 in the area of metastable liquation and crystallization of hydroxyapatite (HAP) and lithium metasilicate;

— ensuring the formation of stoichiometric groups $[PO_4]^{3-}$ and $[SiO_4]^{4-}$ in the melt;

— use of ZrO_2 and TiO_2 crystallization catalysts to enhance nucleation;

— use of glass-forming Al_2O_3 and B_2O_3 in order to provide a strengthened structure of the glass matrix;

2. The appropriate level of solubility and bioactivity is ensured by:

— certain composition and content of phosphate silicate glass phase ≤50 wt. %;

— certain composition and content of the crystalline phase ≥50 wt. % (lithium disilicate (LD) 10–20 wt. %, calcium phosphates 30–40 wt. %);

— presence of modifying components Li_2O, MgO, SrO, ZnO, Nb_2O_5, CeO_2, Ga_2O_3.

2.3 Choice of compositions and synthesis of glass materials

The choice of a system for obtaining bioactive glass-ceramic materials was based on the main principles of their designing, taking into account the above requirements for the chemical composition, structure, and properties of materials.

Synthesis of glass-ceramic materials based on the lithium-calcium phosphate-silicate system must satisfy the requirements of biocompatibility and mechanical strength due to the simultaneous crystallization of the bioactive crystalline phases of calcium phosphates and the high-strength phase of lithium disilicate. The presence of $Li_2Si_2O_5$ crystals with sizes reaching several microns and firmly fixed in the glassy matrix, will provide high strength (360–400 MPa) [7] and a crack resistance coefficient of up to 12.0 MPa·$m^{1/2}$ [8] due to the formation of a dendritic interconnected structure.

A prominent role in providing bioactivity of phosphatosilicate glass is played by polar ≡Si–OH groups of an acid nature, which are adhesive islands. Osteoblast cells are attached to them by the condensation reaction of a protein molecule and a silanol group. Bioactive glasses based on silica are characterized by antioxidant activity due to wettability, zeta potential, chemical composition and reactivity of glasses. The addition of Ce^{4+} or Sr^{2+} ions to their composition limits inflammation and reaction to the foreign body of the implant [9].

Microadditives were chosen taking into account the role of trace elements in bone tissue remodeling. The introduction of niobium oxide into the composition of bioactive materials can significantly increase the ability to form bone tissue. The niobium-containing bioactive materials developed in [10], after 28 days of implantation, demonstrate a higher level of mineralization of the transition layer and a higher viability of living cells on their surface compared to the original materials that do not contain niobium. Li-BGC has been found to directly promote the pro-angiogenic ability of HUVECs *in vitro* and the in-growth of new blood vessels *in vivo*. Moreover, Li-BGC activated Wnt/β-catenin, AKT and NF-κB signaling pathways, while AKT signaling pathway can function as the upstream of Wnt/β-catenin and NF-κB signaling pathways. More importantly, Li-BGC further facilitated the pro-angiogenic capacity of HUVECs by eliciting the expression of exosomal pro-angiogenic miR-130a in BMSCs-derived exosomes, which subsequently leading to the downregulation of PTEN protein and activation of the AKT pathway, ultimately resulting in the elevated proliferation, migration and tube formation of endothelial cells, as well as the upregulated expression of pro-angiogenic genes [11].

The angiogenic properties of biomaterials are of great importance in bone regeneration, as they directly correlate with the

Table 2. Chemical compositions of the initial glasses of the OS series

Indicators		Compositions, wt. %								
		1	2	3	4	5	6	7	8	9
Components	glass forming $SiO_2 + Al_2O_3 + B_2O_3 + Nb_2O_5$	60.0	51.0	50.0	55.0	50.0	55.0	55.0	52.5	52.5
	phase-forming $Li_2O + CaO + P_2O_5$	35.0	35.0	45.0	40.0	40.0	35.0	37.5	40.0	37.5
	modifying $Na_2O + CaF_2$	1.0	3.0	1.0	1.0	2.0	2.0	1.5	1.5	2.0
	$MgO + ZnO + SrO$	2.0	6.0	2.0	2.0	4.0	4.0	3.0	3.0	4.0
	crystallization catalysts $TiO_2 + ZrO_2 + SnO_2 + CeO_2$	2.0	5.0	2.0	2.0	4.0	4.0	3.0	3.0	4.0

osteogenic potential of the biomaterial through the mechanisms of "angiogenic-osteogenic conjugation". Bioactive glasses can affect vascularization by incorporating biologically active ions such as boron (B) [12]. The incorporation of gallium into bioactive materials has been reported to enhance osteogenesis, to influence blood clotting, and to induce anti-cancer and anti-bacterial activity. The addition of gallium to biomaterials has great potential for treating bone-related diseases since it can be efficiently transported to the desired region at a controllable rate. Besides, it can be used as a potential substitute for antibiotics for the inhibition of infections during the initial and advanced phases of the wound healing process [13]. Nanoparticles of mesoporous bioactive glass containing Ce^{4+} and Ga^{3+} cations showed antibacterial activity against S. Aureus and E. Coli without cytotoxicity against MG-63 osteoblast cells. These features of bioactive glasses doped with Ce^{4+} and Ga^{3+}, indicate their promise for the creation of bioactive fillers for bone tissue engineering [14].

With this in mind, a glass matrix based on the $Li_2O–CaO–ZrO_2–TiO_2–MgO–ZnO–Al_2O_3–B_2O_3–P_2O_5–SiO_2$ system was developed; compositions of glasses of the OS series with the content of the main components (Table 2) and modifying additives, wt. %: CaF_2 0.5–2.5; CeO_2 0.01–0.05; SrO 0.01–0.05; Nb_2O_5 0.01–0.10; $\Sigma(Ga_2O_3 + SrO)$ 0.01–0.1 and ratios Ca:P = 1.67 and SiO_2:Li_2O = 4.0 were chosen to obtain glass-ceramic materials operating under conditions of variable dynamic loads.

Experimental glasses of the OS series were melted under the same conditions at the temperatures of 1523–1623 K in corundum crucibles, followed by cooling on a metal sheet to avoid leaching of alkali and alkaline earth metal ions. To obtain glass-ceramic materials, powders of experimental glasses were used, crushed to a residue on sieve No. 008 of no more than 5 %. Samples were prepared by semi-dry isostatic pressing (P = 35–40 MPa), molded into cylinders 4 mm in diameter and 10 mm high, using xanthan gum solution (4 wt. %) as a temporary binder.

Thermal treatment of experimental glass-ceramic materials based on OS glasses was carried out in three stages (stage I — 773–823 K, stage II — 873–923 K, stage III — 773–823 K) in a short-term (1–2 hours) treatmrnt mode (TM). These regimes were chosen for crystallization of lithium metasilicate — at stage I, its transition to lithium disilicate — at stage II, and its transition to hydroxyapatite — at stage III. This mode of heat treatment allows you to control the amount of crystalline phase of each type, and their competitive crystallization does not allow a significant increase in the size of lithium disilicate crystals at the stage III of heat treatment.

3. Results and discussion

3.1 Study of crystallization ability and structure of experimental glass

After melting, the experimental glasses were characterized by varying degrees of transparency lost, depending on the content of glass-forming, phase-forming, modifying components and crystallization catalysts. After melting, the experimental glasses of the OS series contain only hydroxyapatite crystals (Fig. 1) in an amount from 10 to 35 wt. %, which was identified according to the data of X-ray phase and petrographic analyzes. After three-stage heat treatment of experimental glasses, simultaneous crystallization of HAP and LD is observed; this is a prerequisite for ensuring the biological

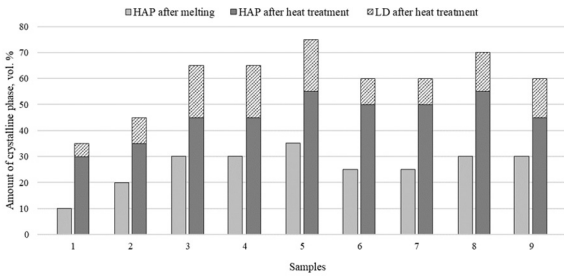


Fig. 1. Content of crystalline phases in experimental samples.

activity and strength of glass-ceramic materials based on them.

Thus, OS-3, OS-5, OS-8 and OS-9 glasses containing glass-forming components (GC) 50.0–52.5 wt. % and phase-forming components (PhC) 37.5–45.0 wt. %, are characterized by the highest content of the crystalline phase of HAP after melting, and they are nontransparent. The result of this can be a significant increase in the crystalline phase after heat treatment, which can lead to a decrease in the resorption of the material and, as a result, an increase in the time of fusion with the bone.

For OS-2 glass while maintaining the GC content up to 51 wt. %, with a decrease in PhC up to 35 wt. % and the maximum content modifying components (MC), a decrease in the content of the crystalline phase of HAP is observed even at the maximum content of crystallization catalysts (CC) both after melting and after heat treatment. This can increase the level of resorbability of the material and reduce the ability to form an apatite-like layer on the surface of experimental materials. Ensuring the content of GC for glasses OS-6 and OS-7 in the amount of 55 wt. % with a content of PhC up to 37.5 wt. % and CC 3–4 wt. % plays an important role in the formation of strengthened bioactive structures due to the general ratio of HAP:LD ≈ 5:1. However, an increase in the CC content up to 4 wt. % for OS-6 glass leads to the appearance of crystals with a size of more than 1 μm in the structure, which can adversely affect the mechanical properties of the glass-ceramic material.

The very formation of sitallized structure in OS-7 glass-ceramic material based on nano- and submicron crystals of HAP and lithium disilicate in the amount of 50 and 10 vol.%, respectively, will provide strength and bioactivity; this is a necessary condition for the accelerated formation of a strong apatite-like layer on the surface of the experimental materials in the environment of a living organism. Arbitrarily oriented and interconnected small needle-like crystals of a flat shape reinforce the structure of the developed OS-7 glass-ceramic material (Fig. 2). The presence of small acicular crystals (0.5 μm) in the structure of the material leads to a deviation of the direction, branching, or cessation of the growth of emerging microcracks. Thus, lithium disilicate crystals block the development of microcracks in the structure of the material, which leads to a significant increase in the bending strength of the material up to 180 MPa and the crack resistance index (K_{IC}) up to 6.0 MPa·m$^{1/2}$.

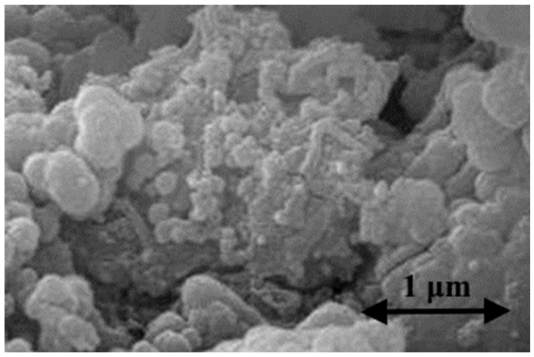


Fig. 2. Microstructure of the developed OS-7 glass-ceramic material.

This glass-ceramic material was chosen for further study of its behavior *in vivo*.

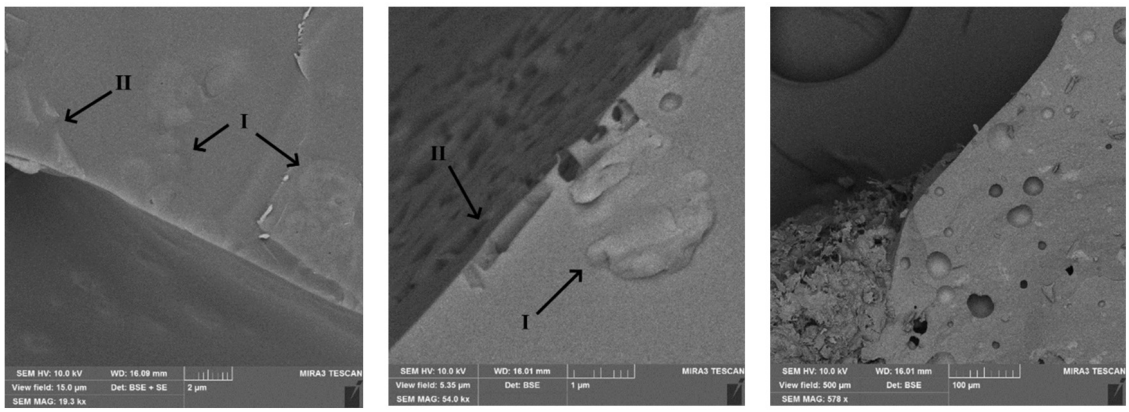
3.2 Features of the formation of an apatite-like layer

The main difference of the OS-7 material is the formation of hydroxyapatite crystals 3–4 μm in size on its surface, which are potential nucleators during the formation of an apatite-like layer, which are potential nuclei in the formation of an apatite-like layer under *in vivo* conditions. The changes in the content and in the ratio of calcium and phosphorus elements were used to determine the features of the formation of an apatite-like layer on the surface of the material *in vivo*.

During the first day of exposure, due to the presence of active hydroxyl groups, negatively charged and electrostatically adsorbing Ca^{2+} ions on the surface of the experimental material, an increase in the content of the Si element in the near-surface layer at a depth of 2.1 μm is observed if compared to deeper layers of 6.3 and 9.1 μm, without changing *in vivo* over the indicated period (Table 3). This indicates the formation of a silica layer and smoothing of the material structure: clusters of

Table 3. The content of elements in OS-7 material at different depths from the surface after *in vivo* exposure

Depth from the surface, microns	Element concentration, wt. %													Ca:P ratio
	O	F	Li	Na	Mg	Al	B	Si	P	Cl	Zr	Ca	Zn	
after one day of exposure														
2.1	60.35	0.41	3.47	0.45	0.30	1.57	1.56	16.83	5.26	0.14	2.00	7.00	0.66	1.33
6.3	61.87	0.56	4.49	0.77	0.48	1.90	1.67	15.74	2.97	–	2.27	6.44	0.84	2.17
9.1	62.38	0.58	4.51	0.78	0.52	2.01	1.74	15.92	2.66	–	2.32	5.76	0.82	2.17
after 14 days of exposure														
1.7	59.96	0.78	2.47	0.32	0.17	1.12	0.92	13.54	7.21	0.29	1.12	11.63	0.47	1.61
6.7	61.43	0.54	4.30	0.51	0.28	1.73	1.38	15.58	3.60	–	2.20	7.65	0.80	2.13
15.0	62.90	0.52	4.30	0.69	0.52	2.06	1.79	15.89	2.64	–	2.32	5.52	0.85	2.09
after 28 days of exposure														
1.7	58.38	0.72	1.56	–	–	–	–	6.12	11.90	0.32	1.00	19.90	0.10	1.67
6.7	59.55	0.53	1.61	–	–	–	–	9.21	10.12	0.44	1.82	16.55	0.17	1.64
15.0	61.91	0.59	1.72	–	–	–	–	9.96	8.66	–	2.25	14.72	0.19	1.70

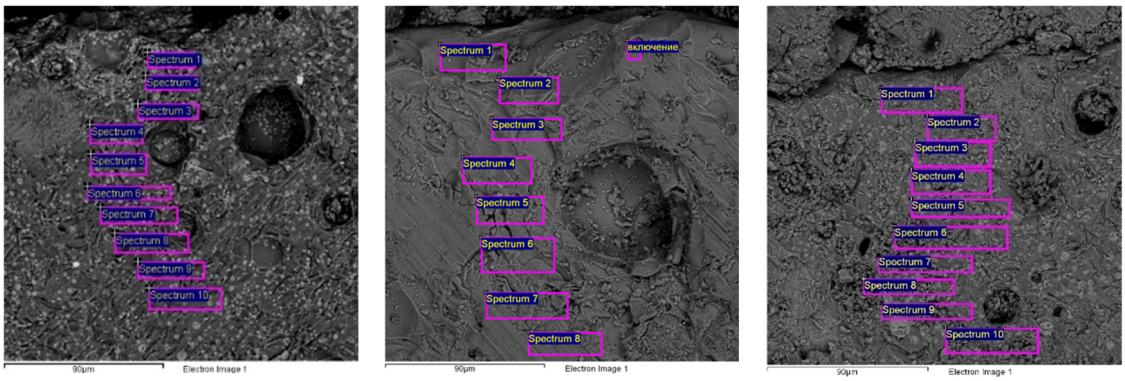


a *b* *c*

Fig. 3. Microstructure of the experimental material after *in vivo* exposure — 1 day of exposure; b — 14 days of exposure; c — 28 days of exposure.

lithium disilicate crystals are noticeable on the body of the amorphous structure (Fig. 3a I) and hydroxyapatite (Fig. 3a II). The formation of a thin jelly-like layer (gel) of silicic acid in the form of spheres on the surface of the experimental coating during one day is an important manifestation of the bioactivity of the materials. It is known that already at the stage of synthesis of collagen fibers and at the initial stages of bone biomineralization, silicon is associated with calcium, initiating the deposition of bone minerals, being an important transitional element in the formation and development of cartilage and bone structures. In general, an increase in the content of P and Ca elements indicates supersaturation of blood plasma with calcium phosphates and the formation of calcium orthophosphate nuclei (Fig. 4a) in the near-surface layer of the material, as evidenced by a decrease in the Ca:P ratio to 1.33 when compared with this ratio for the original glass Ca:P = 1.67.

After 14 days of exposure of the OS-7 experimental material, the calcium concentration increases sharply. As a result of this process, an excess of $[HPO_4]^{2-}$ groups is formed, which are adsorbed by the surface. This leads to an increase in the Ca/P = 1.61 ratio on the surface of the experimental material. In this case, the formation of non-stoichiometric calcium — deficient HAP (Ca:P <<1.67) is observed, representing a series of solid solutions, where the $[HPO_4]^{2-}$



a *b* *c*

Fig. 4. The character of the formation of an apatite-like layer and the distribution of elements on the surface of the experimental material after *in vivo* exposure. a — 1 day of exposure; b — 14 days of exposure; c — 28 days of exposure.

groups are replaced by the $[PO_4]^{2-}$ groups. In general, bone mineralization is characterized by the interaction of three factors: a local increase in the concentration of phosphate ions; adsorption of Ca^{2+} ions; and pH shift.

Articulated plates of calcium-deficient HAP are presented on the surface (Fig. 3b I) and deep into the sample by 1.7 µm (Fig. 3b II). Such mineral crystal lattices are observed on the sample surface in the zones located in regular intervals between collagen fibrils (Fig. 4b). The localized first crystals become nucleation centers for the deposition of hydroxyapatite in the space between the collagen fibers. The change in the concentration of elements to a depth of 6.7 and 15.0 µm is almost the same, which indicates insignificant changes in the structure of the coating during the specified period *in vivo*.

On the 28th day of the in vivo exposure, both the ratio and concentration of elements change dramatically both on the surface and in the depth of the material. The degree of stoichiometry of calcium-deficient HAP increases due to the immobilization of calcium ions from the solution and leads to the formation of HAP with the Ca:P ≈ 1.67 ratio. An apatite-like layer based on nHAP (nonstoichiometric hydroxyapatite) is formed on the surface of materials when pH = 7–11 until Ca:P reaches 1.67. The formation of HAP crystals in the form of plates or rods (8–15)×(20–40)×(200–400) A on the surface of the material is observed (Fig. 3c), which is typical for natural bone tissue.

For the experimental material, the accelerated formation of an apatite-like layer is associated with a developed surface with large pores and caverns that formed in place of more soluble ionogenic regions of the glass phase. This character of the surface structure of the coating contributes to the intensive adsorption of biologically active substances by the apatite layer. Pores in the structure of the experimental material are a kind of reactors, in which the formation of apatite crystals is intensified (Fig. 4a) on the first day, followed by the growth of nHAP crystals (Fig. 4b) on the 14th day and their formation in the form of rosettes on the 28th day (Fig. 4c). The presence of such accumulations of hydroxyapatite crystals is an indicator of the intensive formation of an apatite-like layer within one month.

3.3 Biochemical analysis of rat blood serum after implantation of the experimental materia

The processes of bone tissue formation at the implant-bone interface with subsequent complete mineralization of the material can be fully assessed using biochemical markers of bone tissue metabolism, in particular, by alkaline phosphatase activity. In bone tissue, alkaline phosphatase is formed in osteoblasts — special cells involved in the formation and renewal of bone. With an increase in the activity of these cells, the level of alkaline phosphatase in the blood increases. The presence of chondroitin sulfates indicates the regeneration of articular cartilage and the production of intra-articular fluid to increase the mobility of the affected joints.

The influence of the immobilization in halloysite on the enzyme activity was measured using standard alkaline phosphatase (ALP) activity assay. Immobilized ALP effectively induced the bone mineralization process. As a result, calcium phosphate was

produced in the form of hydroxyapatite cauliflower-like structures, with a slight content of calcium hydroxide [15].

According to the results of biochemical analysis of blood serum of rats, after implantation of the OS-7 glass-ceramic material in preparations of the distal metaphysis of the femur, a significant increase in the activity of alkaline phosphatase in the blood serum was found in comparison with intact rats from the 7th to the 30th day of observation. At the same time, on the 7th day, the difference was 1.5, on the 14th day — 1.8, on the 30th day — 3.0 times. It indicates an active formation of an apatite-like layer on the surface of experimental material already on the 30th day.

The dynamics of the increase in the content of chondroitin sulfates in the blood persisted from the 7th to the 30th day of observation, which indicates possible destructive changes in the bone tissue at the site of implantation. The maximum increase by 67.8 % compared with intact rats was recorded on the 7th day. The presence of chondroitin sulfates indicates an acceleration of the processes of bone tissue restoration and inhibition of cartilage degeneration.

4. Conclusions

The priority of creating resorption bioactive glass-ceramic materials with high mechanical properties and a significant level of bioactivity using a three-stage short-term low-temperature heat treatment has been determined. The main types of hardened apatite-containing glass-ceramics are analyzed and the prospects for the use of high-strength crystalline phases for bone arthroplasty are established.

The following criteria have been established for a glass matrix as the basis of biocompatible materials for bone tissue replacement: they consist in the synthesis of bioactive glass-ceramic materials characterized by a crystallized nano- and submicron structure, containing both bioactive and high-strength crystalline phases to ensure high performance properties. The choice of a lithium-calcium phosphate-silicate system for the synthesis of glass-ceramic materials under the conditions of three-stage short-term heat treatment is substantiated, and the compositions of model glasses are synthesized.

The effect of the content and ratio of glass components on its phase composition during heat treatment is analyzed. The factors that determine the formation of a glass-ceramic structure with a ratio of HAP/LD ≈ 5:1 under conditions of three-stage heat treatment (melting — 1823 K, 6 hours; stage I — 773–823 K, stage II — 873–923 K, stage III — 773–823 K, with a total crystallization time of 1–2 hours): the content of glass-forming Σ (SiO_2, Al_2O_3, B_2O_3, Nb_2O_3) = 55.0 wt. %, phase-forming oxides Σ(Li_2O, CaO, P_2O_5) = 37.5 wt. %, crystallization catalysts Σ(TiO_2, ZrO_2, CeO_2) = 3.0 wt. % and modifying additives Σ(Na_2O, CaF_2, ZnO, SrO, MgO) = 4.5 wt. %.

Formation of the sitallized structure of OS-7 glass-ceramic material based on nano- and submicron crystals of HAP and lithium disilicate in the amount of 50 and 10 vol. %, respectively, may provide the strength of the material in bending 180 MPa and crack resistance index (K_{1C}) 6.0 MPa·m$^{1/2}$ and bioactivity necessary for the accelerated formation of a strong apatite-like layer.

It has been established that the features of the accelerated formation of an apatite-like layer are associated with a developed surface and the presence of pores in the structure of the experimental material; these are reactors in which, on the first day, the nucleation of crystals of octacalcium phosphate nuclei is intensified at Ca:P = 1.33, followed by the growth of nHAP crystals on the 14th day at Ca:P = 1.61 and their formation into rosettes on the 28th day at Ca:P = 1.67, which is an indicator of the formation of natural bone on the surface of the experimental material.

The results of biochemical analysis of blood serum of rats after implantation of samples of the OS-7 glass-ceramic material into the distal metaphysis of the femur revealed a significant increase in the activity of alkaline phosphatase, which indicates activity in osteoblasts during the formation and renewal of bone tissue. The dynamics of the increase in the content of chondroitin sulfates in the blood indicates an acceleration of the processes of bone tissue restoration and inhibition of cartilage degeneration.

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