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O.V. Savvova^a, *O.I. Fesenko*^a, *H.K. Voronov*^a, *O.V. Babich*^b, *V.V. Bitiutska*^a,
Yu.O. Smyrnova^a, *A.O. Hopko*^a

STUDY OF MINERALIZATION OF LITHIUM CALCIUM PHOSPHOSILICATE GLASS CERAMICS *IN VIVO* DURING BONE TISSUE REGENERATION

^a O.M. Beketov National University of Urban Economy in Kharkiv, Kharkiv, Ukraine

^b Research Institution «Ukrainian Research Institute of Environmental Problems», Kharkiv, Ukraine

Prospective directions for the creation of biologically active substitutes for bone tissue were analyzed. The effectiveness of the use of calcium phosphosilicate materials modified with CuO, ZnO, Ag₂O, Fe₂O₃, TiO₂, SrO and Nb₂O₅ to ensure high biocompatibility and antibacterial properties of bone endoprostheses has been established. The prospective use of lithium calcium phosphate silicate glass ceramics for obtaining strengthened, biologically active bone implants was substantiated. The main criteria for the development of biocompatible glass-ceramic materials regarding their composition, structure, texture, and surface properties have been established. The influence of differences in the structure and resorption of calcium phosphosilicate glass ceramics on the mechanism of formation of an apatite-like layer *in vivo* was analyzed. The features of mineralization of calcium phosphosilicate glass ceramics *in vivo* during bone tissue regeneration were determined, and the effectiveness of the use of glass ceramics based on hydroxyapatite and lithium disilicate in bone tissue replacement was established to reduce the rehabilitation period and long-term use of endoprostheses under variable loads. The developed OS-7 calcium phosphosilicate glass-ceramic material is characterized by the content of crystalline phases of 10 vol.% lithium disilicate and 55 vol.% hydroxyapatite with a ratio of CaO/P₂O₅=1.67, surface microrelief of 6 μm, surface free energy value of 75 mJ/m² and crack resistance of 6.0 MPa·m^{1/2}. This material is biocompatible due to the formation of carbonate hydroxyapatite crystals already on the 14th day, which allows us to consider its promising use in the treatment of fractures, defects of long bones and in the replacement of short or tubular bones.

Keywords: calcium phosphosilicate glass-ceramic material, hydroxyapatite, lithium disilicate, mineralization, regeneration, bone tissue.

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Introduction

Successful regeneration of bone tissue when using biologically active bone tissue substitutes is determined by their adapted ability to biological decomposition, mineralization, tissue mechanical properties, structural similarity to the extracellular matrix, appropriate bioactivity and cytocompatibility. In recent years, scaffolds, injectable hydrogels based on calcium silicophosphate materials have attracted increasing attention due to their high biocompatibility and adjustable physicochemical properties in response to the environment [1].

Calcium silicate ceramics have excellent bioactivity and biomineralization indicators due to the release of Ca²⁺ cations and SiO₃²⁻ anionic groups. The process of ions exchange leads to an increase in pH and the formation of ≡Si–OH groups. H⁺ ions released from the ≡Si–OH groups due to the pH variation, as a result of which a negatively charged surface is formed for recombination with Ca²⁺ (Fig. 1). Importantly, the results of the cytological response also showed that a thicker layer of calcium phosphate minerals with lamellar morphology covered on the CaSiO₃ ceramic surface played a more prominent

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Study of mineralization of lithium calcium phosphosilicate glass ceramics in vivo during bone tissue regeneration

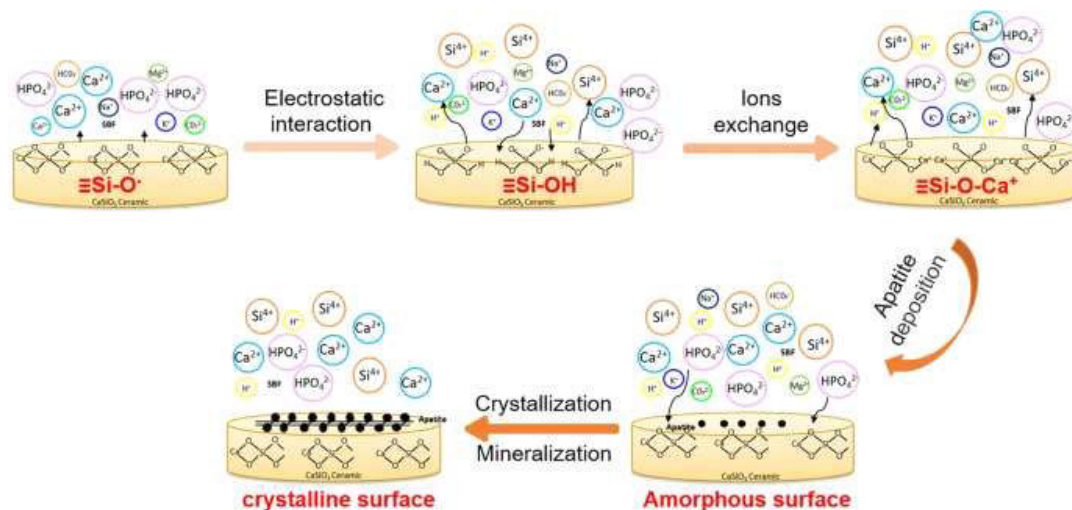


Fig. 1. Mechanism of biom mineralization

role in increasing the expressions of genes related to osteogenesis [2]. All this determines the exceptional effectiveness of the use of calcium silicate materials as substitutes for bone tissue regeneration.

Considerable attention is paid to alloying their compositions and surface modification to ensure simultaneous biological activity and the ability to withstand significant dynamic loads, when developing new types of bioactive materials for restorative medical use. The prospect of using lithium disilicate glass ceramics with a stoichiometric composition, which is subject to ion exchange in pure NaNO_3 or mixed $\text{NaNO}_3+\text{KNO}_3$ molten salt baths below the glass transition temperature (T_g) is explained not only by the predicted increase in strength and fracture toughness to 546 MPa and $4.31 \text{ MPa}\cdot\text{m}^{1/2}$, respectively, and by increasing its bioactivity. The formation of a Na^+ -rich gradient layer on the glass-ceramic surface, which was caused by Li^+/Na^+ exchange, is favorable for the formation of HAP (hydroxyapatite) with nano-sized pores after contact with SBF (Simulated Body Fluid) [3].

It is known that the modification of bioactive glasses with CuO , ZnO , Ag_2O , Fe_2O_3 , TiO_2 and SrO increases their biological compatibility. The degradation period of such glasses is from hours to years, depending on the composition, which indicates the perspective of their use as a scaffold for tissue regeneration [4].

Several new bioactive glass compositions have been developed, including active ions such as Sr^{2+} , Zn^{2+} , Co^{2+} , F^- and Mg^+ . Metal ions, Cu^{2+} , Sr^{2+} , and Co^{2+} , are considered as a possible alternative to growth factors and genetic approaches in tissue engineering because of their easy processing, stability at high temperatures, and tunable release kinetics.

It is known that increasing the content of zirconium dioxide in bioactive glass increases the density, bending strength, compressive strength and microhardness, which determines the prospects of using such bioactive glasses in the replacement of bone tissue in a short time [5]. This can be achieved by the heterogeneous nucleation of apatite crystals [6], namely due to the presence of Si-OH- , Ti-OH- , Zr-OH- structural elements on the surface of the material. It has been confirmed that Ti-OH , Zr-OH , Nb-OH and Ta-OH groups are considered to be the most effective agents for apatite nucleation. Ti-OH or Zr-OH tetragonal/monoclinic structures provide an effective nucleation site for the apatite layer [7].

Aluminum and boron oxides play an important role in the processes of phase formation in bioactive glasses. The effect of Al_2O_3 on the crystallization of calcium silicophosphate glasses is determined by its composition and is based on its ability to increase or, accordingly, decrease the viscosity of the glass mass. The introduction of Al_2O_3 oxide, which is localized in the tetrahedron, into the composition of glass materials creates conditions for the formation of a single aluminum-phosphorus-oxygen framework, which has effect on the increase in the chemical resistance of materials. With increasing boron content in the glass composition, a faster glass dissolution in a native media was observed [7,8]. However, due to the complicated structural nature of bioactive glasses, in particular the change in boron coordination in the composition under heat treatment, a detailed study of the structure of calcium borosilicate glasses is necessary [9].

The introduction of niobium oxide into the composition of glasses allows intensifying the

processes of mineralization of the apatite-like layer [10]. Niobium-containing bioactive glasses promoted bone formation comparable to that of the autogenous bone without compromising the quality of the formed bone [10]. It was established that Nb^{5+} ions create cross-links between several oxygen sites, breaking Si–O–Si bonds to form a range of polyhedra $[\text{Nb}(\text{OM})_{6-y}(\text{OSi})_y]$, where $1 \leq y \leq 5$ and $M = \text{Na}, \text{Ca},$ or P . Bioglass particle dispersions prepared by incorporating up to 1.3 mol.% Nb_2O_5 by replacing P_2O_5 or up to 1.0 mol.% Nb_2O_5 by replacing SiO_2 in 45S5 Bioglass® (BG45S5) using deionized water or solutions buffered with HEPES showed a significant increase in the pH during the early steps of the reaction, compared using the rate and magnitude during the earliest stages of BG45S5 dissolution [11].

It is known that Bioglass 45S5 (BG45S5) and 1 mol.%-Nb-containing-bioactive glass (BGSN1) were able to grow apatite layer on their surfaces within 3 h, while glasses with higher concentrations of Nb_2O_5 (2.5 and 5 mol.%) took at least 12 h. Nb-substituted glasses were shown to be compatible with bone marrow-derived mesenchymal stem cells (BMMSCs). Moreover, the bioactive glass with 1 mol.% Nb_2O_5 significantly enhanced cell proliferation after 4 days of treatment. Concentrations of 1 and 2.5 mol.% Nb_2O_5 stimulated osteogenic differentiation of BMMSCs after 21 days of treatment. According to analyzed morphometric parameters (the thickness of the newly formed bone layer and the area of the newly formed subperiosteal bone), it was established that BGNb bioactive glass is osteoconductive and osteostimulative [12].

According to the data given in ref. [13], a nanocomposite material characterized by an intrinsic antibacterial activity with tissue regeneration properties, in order to minimize invasive dental therapies, is consist of narrowly disperse $\text{Na}_2\text{O}-\text{CaO}-\text{P}_2\text{O}_5-\text{SiO}_2$ bioglass-ceramic nanoparticles doped with antibacterial and osteogenic zinc and niobium ions, and hybridized with chitosan.

Chitosan increased the hydration capacity of the bioglass-ceramic and it formed a continuous interface around the bioglass-ceramic nanoparticles, devoid of micropores. This intimacy of the interface was confirmed by the downshift of the critical Si–O(–Si) vibration modes in the bioglass-ceramic upon hybridization with chitosan. The addition of zinc ions hampered the partial recrystallization during annealing by interfering with the Si–O network restructuring, in direct proportion with its concentration. Niobium ions produced a similar structure-breaking effect, which was evidenced, as in the case of zinc, by upshifting the antisymmetric

Si–O–Si stretch of the bridging oxygen and increasing the full-width at half maxima for all the major Si–O(–Si) vibration modes. The effective electrostatic attraction between the aminated hydrocarbon chains of chitosan and the negatively charged silanol groups of silica may extend to the interaction with dentin collagen fibrils decalcified due to caries, making the material of potential interest for adhesive fillers of cariogenic lesions in teeth. Both the undoped and the doped bioactive glass ceramics interacted favorably with odontoblast-like cells, accentuating their potential for further research for applications in minimally invasive reparative dentistry.

Addition of Nb_2O_5 up to 2 wt.% in diopside ($\text{CaMgSi}_2\text{O}_6$) glass ceramics exhibits a near monotonous effect on improving the bending strength. The maximum value of it can reach as high as 236 MPa. The crystallization of diopside crystals was more likely enhanced through the mechanism related to the Nb_2O_5 -induced phase separation in the base glass rather than the normally reported heterogeneous nuclei mechanism reported for ferric oxide (Fe_2O_3), chromium trioxide (Cr_2O_3), and titania (TiO_2).

The above data are an important factor in the design of a new type of biocompatible materials for replacing bone tissue in the diaphysis, which can withstand a significant load. The development of bioactive glass-ceramic materials based on hydroxyapatite and lithium disilicate (DL) and modified with Nb_2O_5 and ZnO will significantly increase their biological compatibility under significant dynamic loads [14]. The study of mineralization of lithium-calcium-phosphate-silicate glass-ceramics in vivo during bone tissue regeneration is a determining factor in the further introduction of the developed materials into medical practice. This was the purpose of this work.

Experimental

Complementary methods of physicochemical analysis (X-ray phase (DRON-3M diffractometer), petrographic (NU-2E optical microscope)) were used to study the processes of phase formation, structure and phase composition of materials. The microstructure of glasses was studied using REMMA-2000.

The degradation of experimental glass materials was determined by the loss of mass after exposure to extreme citric acid solution (L_{ES}) and simulated solution (L_{SS}) according to ISO 10993-14-2011 and the state standard DSTU EN ISO 10993-14:2015.

The microrelief of the surface was evaluated by arithmetic mean value of surface profile deviation (R_a) using a Surtronic 3+ profilometer.

The surface free energy (SFE) of the experimental materials was determined by the Owens-Wendt-Rabel-Kable method, according to which the surface energy of a solid body includes two components: dispersive and polar. This method involves calculating the SFE based on the contact angle between the surface of the material and various liquids, followed by the calculation of its two surface components using Mathcad computer software.

Clinical and biological testing of materials was carried out in accordance with the National Standards of Ukraine for biological evaluation of medical devices according to the state standards sGOST ISO 10993-6-2011, and DSTU EN ISO 10993-6:2019 (Biological evaluation of medical devices. Part 6. Tests for local effects after implantation (EN ISO 10993-6:2016, IDT; ISO 10993-6:2016, IDT)).

The index of crack resistance (fracture toughness) (K_{IC} , MPa·m^{1/2}) of the test samples was determined using a TMV-1000 hardness tester.

During the development of biocompatible glass-ceramic materials, the following necessary criteria for their structure, texture, and surface properties were established:

- flow of finely dispersed volume crystallization with the simultaneous content of lithium disilicate crystalline phases from 5 to 10 vol.% and calcium phosphates from 45 to 55 vol.%;
- occurrence of surface crystallization with the presence of crystals 1–10 mm in size to ensure microroughness $R_a \geq 3.5$ mm;
- provision of SFE=60.0–120.0 mJ/m² for protein adsorption;
- ensuring the certain level of resorption necessary for the release of Ca²⁺ cations and PO₄³⁻ groups;
- formation of an apatite-like layer on the

surface of materials *in vivo* with a ratio of Ca/P=1.0–1.67.

When developing a bioactive glass matrix, the following synthesis criteria were defined:

- selection of compositions in the area of metastable liquation of the CaO–P₂O₅ and SiO₂–Li₂O systems with the possibility of its implementation by the spinodal mechanism;
- content of phase-forming oxides in the range of CaO+P₂O₅=21.5–33.5 mol.% and SiO₂+Li₂O=57.5–66.25 mol.% with the ratios CaO/P₂O₅=1.67 and SiO₂/Li₂O=4;
- the formation of structurally formed sybotaxic groups, which are the nuclei of the crystalline phase, when the crystallinity coefficient $K_{cr} > 3.5$ is ensured;
- provision of favorable conditions for nucleation during cooling and growth of crystals upon heat treatment while ensuring the transparency coefficient $K_{tr} > 2.1$;
- ensuring non-toxicity, taking into account the implementation of high structural strength at ψ_B and $\psi_{Al} > 1$;
- high reactivity at $f_{Si} \leq 0.32$.

Taking into account the above criteria for the glass matrix, the compositions of the model glasses of the Na₂O–K₂O–Li₂O–CaO–ZrO₂–TiO₂–MgO–ZnO–Al₂O₃–B₂O₃–P₂O₅–Nb₂O₅–SiO₂ system (OS series) in the area of metastable liquation were designed, which are characterized by the ratio CaO/P₂O₅=1.67 and SiO₂/Li₂O=4 (Table 1) for the simultaneous crystallization of hydroxyapatite and lithium disilicate as crystalline phases that provide both biocompatibility and high mechanical strength.

The calculated factors of K_{cr} and K_{tr} and ψ_B and $\psi_{Al} > 1$ for the developed glasses indicate the possibility of developing crystallized glass materials with a high degree of structural strength and

Table 1
Chemical composition of the OS series model glasses and indicators of their structure and solubility

Indicators		Glass composition								
		1	2	3	4	5	6	7	8	9
Components	SiO ₂ +Al ₂ O ₃ +B ₂ O ₃ +Nb ₂ O ₅	60.0	51.0	50.0	55.0	50.0	55.0	55.0	52.5	52.5
	Li ₂ O+CaO+P ₂ O ₅	35.0	35.0	45.0	40.0	40.0	35.0	37.5	40.0	37.5
	Na ₂ O+CaF ₂	1.0	3.0	1.0	1.0	2.0	2.0	1.5	1.5	2.0
	RO	2.0	6.0	2.0	2.0	4.0	4.0	3.0	3.0	4.0
	RO ₂	2.0	5.0	2.0	2.0	4.0	4.0	3.0	3.0	4.0
Content of crystalline phase, vol.%	calcium hydroxyapatite	45	50	60	55	55	50	55	60	55
	lithium disilicate	15	10	15	10	5	10	10	10	5
L _{SS} , wt.%		2.5	3.5	2.0	3.0	2.5	2.0	2.5	2.0	3.0
Calculated structure factors	f_{Si}	0.29	0.26	0.26	0.28	0.26	0.28	0.28	0.27	0.27
	$\psi_{Al/B}$	7.46	9.74	13.95	12.63	13.31	8.26	10.0	12.9	8.8
	K_{tr}	2.70	2.90	2.88	2.82	2.91	2.83	2.80	2.87	2.88
	K_{cr}	14.15	8.83	18.11	19.02	12.26	11.06	14.74	14.43	11.0

simultaneous high reactivity at $f_{Si} \leq 0.32$ on the basis of these glasses (Table 1).

Experimental glasses with OS marking in the given system were melted in corundum crucibles at the temperatures of 1623–1723 K under the condition of oxidizing atmosphere, and heat treated at the temperatures of 1079–1373 K.

Experimental glasses after melting were characterized by a transparent and microheterogeneous structure. Investigations of the phase composition and solubility of the synthesized glass-ceramic materials made it possible to establish that among the obtained glasses the OS-7 material is characterized by simultaneous presence of hydroxyapatite HAP 55 vol.% and lithium disilicate LD 10 vol.% (Table 2) and solubility in the simulated solution 2.5 wt.%. These parameters will ensure high strength properties and biological activity. That is why the OS-7 glass material was chosen for the following studies.

Results and discussion

Previously synthesized and introduced into medical practice materials ASZ-5 and BSZ-11 (modified with zirconium oxide (5 wt.%) stabilized with yttrium oxide) which are characterized by a certain structure and degradation, were selected to compare the formation features of an apatite-like layer on the surface of glass-ceramic material of the OS series (Table 2). These materials are indicated for surgical treatment of patients with bone tissue defects, which, in combination with splinting of the fracture with metal retainers, allows early loading. According to the results of histological and biochemical studies, the materials are characterized by high biocompatibility and the absence of toxic effects on the body, as confirmed by biochemical studies. Moreover, they have high osteoconductive and osteoinductive properties, incorporate into the bone and allow their use in the treatment of fractures and defects of long bones [15].

During the morphological study, it was

established that under the conditions of introducing cylindrical blocks made of glass-ceramic calcium phosphosilicate materials into the distal metaphysis of the femur of rats, osteoreparation around them takes place according to well-known stages with the formation of bone tissue of a lamellar structure by the end of the study (90 days). A feature of the ASZ-5 material, compared to BCZ-11, is its gradual resorption with formation along the perimeter and in the outer parts of the bone tissue (BT), penetration into the internal areas of tissue fluid, poorly differentiated cells, fibroblasts, and at the end of the study (90 days) – connective tissue. However, the faster degradation of ASZ-5 samples makes it mechanically unstable under loads, which can be a limitation for its use in loaded areas of the skeleton. Therefore, this material can be recommended for dental prosthetics and jaw prosthetics.

The advantage of the developed OS-7 material, which is characterized by intermediate indicators of degradation, when compared with ASZ-5 and BS-11, and high indicators of SFE and R_a of the surface, is its possible simultaneous high bioactivity and ensuring biocompatibility and the strength of the structure, due to the presence of lithium disilicate, already within one month.

The analysis of changes in the surface structure of the studied materials was carried out after their exposure *in vivo* for 28 days. In the first hours of contact, the processes of hydrolysis and condensation take place on the surface of the materials. The developed materials are hydrolyzed with the formation of a gel-like silica-rich layer saturated with OH^- groups. This layer plays an important role in the mechanism of apatite formation. It is believed that Si–OH structural elements provide sites for heterogeneous nucleation of apatite crystals.

After 14 days of implantation, BCZ-11 glass-ceramic material (GCM) is a multiphase system consisting of individual spherical and lamellar crystals non-stoichiometric hydroxyapatite (nHAP) with a

Table 2

Characteristics of experimental materials

Sample marking	Structural indicators and differences in chemical composition			Characteristics of surface morphology and structure of material			Degradation according to ISO 10993-14-2001		Mechanical properties
	SiO ₂	CaO/P ₂ O ₅	f_{Si}	R_a , μm	SFE ₂ , mJ/m^2	Type and content of crystalline phase	L_{ES} , wt.%	L_{SS} , wt.%	K_{1C} , $\text{MPa}\cdot\text{m}^{1/2}$
BS-11	55	4	0.28	6	51.5	HAP 55 vol.%	0.2	2.00	3.0
ASZ-5	47	1.67	0.28	2	74.59	HAP 60 vol.%	0.44	2.96	2.8
OS-7	50	1.67	0.29	4	75.0	HAP 55 vol.%, LD 10 vol.%	0.32	2.5	6.0

size from 0.5 to 5 μm (Fig. 2,a), and their clusters of about 10 μm , which form a single crystal block. The presence of a significant number of spherulites is evidence of the formation of amorphous calcium phosphate (ACP) (Fig. 2,a), which is a precursor to the formation of native bone.

For ASZ-5 GCM, inhomogeneities are represented by spherulites, which form ridges and chips (Fig. 2,b). This process is accompanied by a phase rearrangement of ACP, followed by surface leveling and the formation of a layered structure of the material with the presence of lamellar nHAP crystals (Fig. 2,b).

For OS-7 GCM, lamellar nHAP crystals form splices (Fig. 2,c), which are similar to aggregates of natural bone HAP crystals, on the 14th day of exposure due to the defined surface and structure

characteristics of the material.

The formation of hydroxyapatite aggregates nHAP (Fig. 2,d,e,f), which are similar to crystals for mature lamellar bone HAP (Fig. 2,d,e,f), was observed after 28 days of implantation in the structure of BCZ-11, ASZ-5 and OS-7 GCM implants. At the implant-bone interface, the formation of a transition layer was observed, which, like the material of the implant, contains crystals of the lamellar structure of carbonate hydroxyapatite (CHAP). This is due to the incorporation of carbonate ions into the apatite lattice, which affects the mineralization process.

The nucleation intensification for ASZ-5 and OS-7 GCM allows the formation of CHAP, which is crystals of hexagonal syngonia from prismatic to acicular, assembled into aggregates (Fig. 2,e,f),

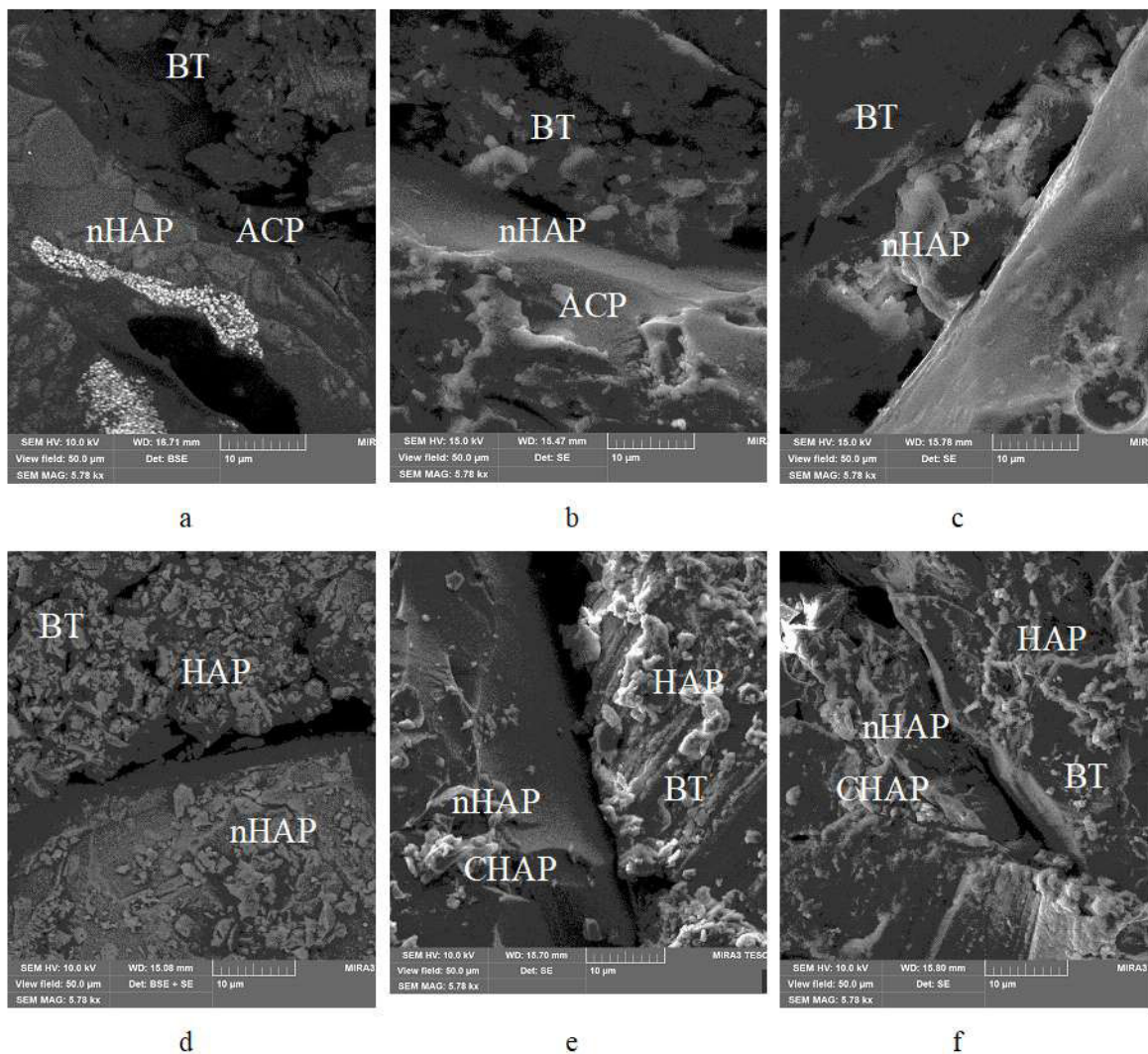


Fig. 2. Surface structure of the experimental materials: BT – bone tissue; ACP – calcium phosphate; nHAP – non-stoichiometric hydroxyapatite; and HAP – hydroxyapatite

already on the 28th day *in vivo* on their surface. Carbonate hydroxyapatite crystals are present in the form of plates measuring 5020×550 nm, which are oriented in a certain way in relation to the axis of collagen fibers. Regeneration has a more favorable course, when using the developed OS-7 material as an implant, as evidenced by the restoration of the bone structure on the 28th day of observation, when part of the regenerate (50–70%) in the defect is represented by mature lamellar bone.

The results of the study of the cross-section of the GCM BCZ-11 (hereinafter the implant), which was implanted in the bone tissue, made it possible to establish the following: after 14 and 28 days of exposure *in vivo* under conditions of static and dynamic loads, the implant tightly adheres to the bone tissue, which indicates the integrity of the formation connection implant-bone tissue. The structure of the implant does not lose its strength after dynamic loads: it does not contain cracks, fractures and the presence of debris (Fig. 3,a,b). This indicates the correspondence of elastic and mechanical properties to those of bone tissue. This material is characterized by $K_{IC}=3$ MPa·m^{1/2}. It is the K_{IC} crack resistance index that is one of the determining factors when using glass-ceramic materials as an implant under loads.

In the cross-section of the ASZ-5 GCM sample, which is implanted in bone tissue after 14 days *in vivo*, its strong fixation in the contact zone is observed (Fig. 3,c). After 28 days *in vivo*, the ASZ-5 GCM sample is characterized by minor surface fractures (Fig. 3,d), which indicates its fragility ($K_{IC}=2.8$ MPa·m^{1/2}). This can cause the difficulty of extracting the implant during repeated operations. However, due to the fact that this sample is characterized by the ability to accelerate the formation of an apatite-like layer within one month, the mineralization process of this implant will ensure its durability during operation.

In the cross section of the OS-7 GCM sample after 14 and 28 days (Fig. 3,e,f) of exposure, its destruction and damage to the adhesion interface between the implant and the bone were not observed ($K_{IC}=6$ MPa·m^{1/2}). The formation of a strong transitional apatite-like layer allows for long-term and reliable functioning of the implant. This is an important factor in the formation of a single structure in the implant-bone tissue system, and it will allow the implant to be used successfully for a long time under variable loads on the bone tissue. The formation of a strong framework and the manifestation of bioactivity of the OS-7 material occurs through the formation of a dendritic structure

of lithium disilicate and due to the intensification of the nucleation of hydroxyapatite crystals and the presence of osteogenic zinc and niobium cations in its structure.

When cross-sectioning the samples to a depth of ≈ 200 μ m, a cluster of hemispheres ACP 20–100 μ m in size (Fig. 3,a,c,e) and grains of CHAP crystals (Fig. 3,b,d,f) were observed both after 14 and after 28 days of their exposure *in vivo*. However, the intensity of the mechanism and crystal nucleation is excellent for the experimental samples. The cross-section of the BCZ-11 GCM surface structure is characterized by a small number of CHAP nuclei and crystals, which increases by 28th day (Fig. 3,a,b). For ASZ-5 and OS-7 GCM, a significant presence of both spherical inhomogeneities and CHAP was observed. A feature of the OS-7 structure both on the 14th and 28th day is the intense overgrowth of pores with CHAP crystals (Fig. 3,e,f). This is the result of the final formation of the mineralized bone matrix.

According to the data of the morphological study, it was established that OS-7 GCM is characterized by high biocompatibility, has osteoconductive and osteoinductive properties, and is incorporated into the bone. This will make it possible to use the OS-7 material when replacing short or tubular bones, where significant bone mobility is combined with a large mechanical load (carpal and metatarsal bones).

Conclusions

The factors of the biocompatibility manifestation of GCM for replacing bone tissue were established: selection of the bioactive glass matrix compositions in the area of metastable liquation, which is characterized by $K_{cr}>3.5$; $K_{tr}>2.1$; ψ_B and $\psi_{Al}>1$; $f_{Si}\leq 0.32$, with a crystal size of 1–10 μ m to ensure the formation of a sintered structure with a content of 55–60 vol.% during heat treatment; the surface crystallization to ensure indicators $R_a=2$ –6 μ m and SFE 50–75 mJ/m² for protein adsorption; ensuring the level of resorption according to the ISO 23317:2012 with mass losses in an extreme solution of 2–4 wt.% by the subsequent formation of an apatite-like layer on the surface of the materials *in vivo* within one month.

The peculiarities of the apatite-like layer formation for bioactive calcium phosphosilicate glass-ceramic materials were established, which consist in the formation of a layer-by-layer structure of the material with the presence of lamellar nHAP crystals on the 14th day and the subsequent formation of a lamellar structure of carbonate hydroxyapatite, which on the 28th day is similar to the structure of crystals

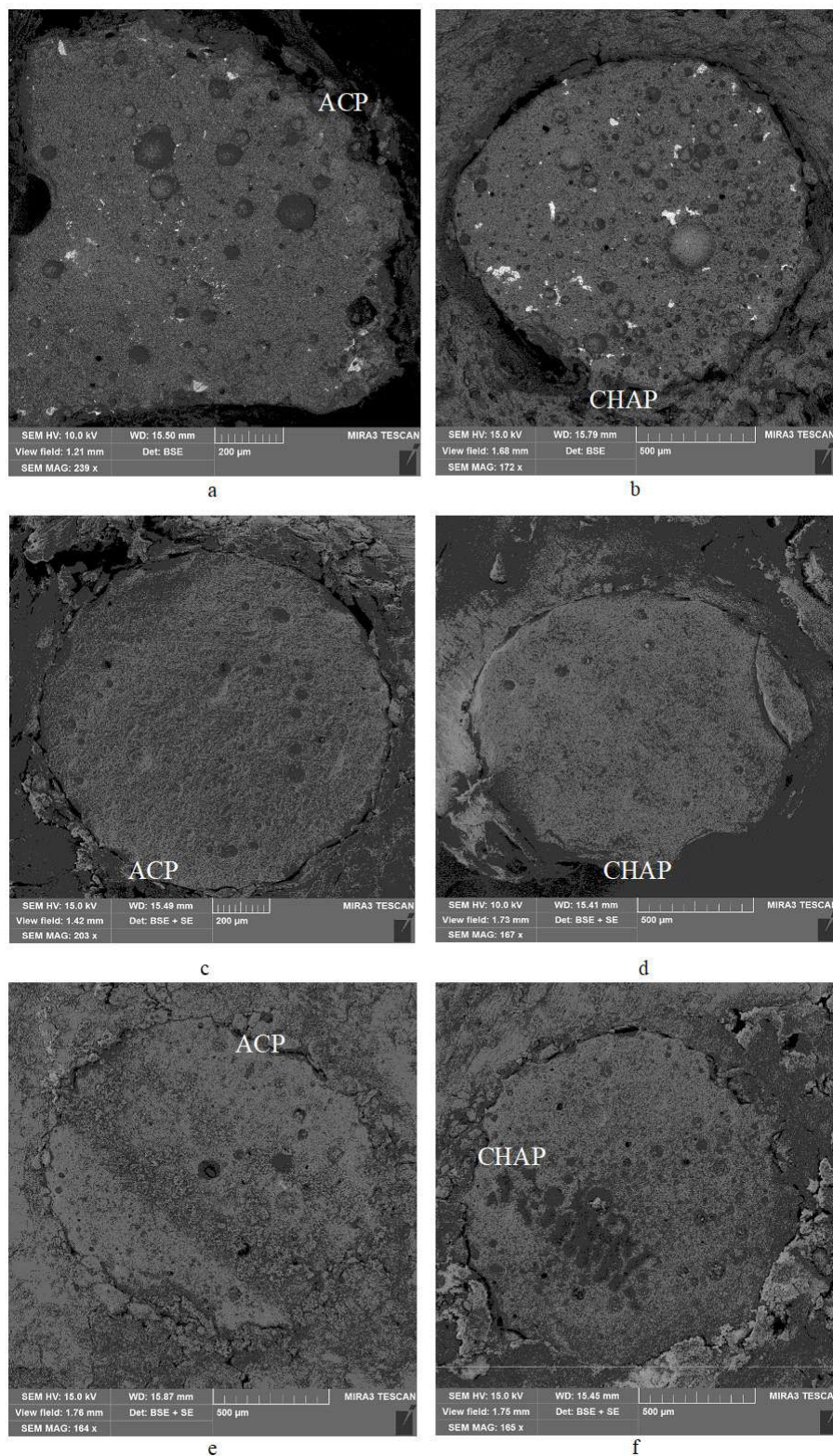


Fig. 3. Cross-section of developed glass-ceramic materials implanted in bone tissue after 14 and 28 days of exposure *in vivo*: BCZ-11 (a, b); ASZ-5 (c, d); OS-7 (e, f). BT – bone tissue; ACP – calcium phosphate; and CHAP – carbonate hydroxyapatite

for mature lamellar bone. Differences in the mechanism of apatite formation for materials with different content of the crystalline phase were determined, which consist in an increase in the regenerated part from 50 to 70 vol.% while providing a ratio of CaO/P₂O₅ from 4.0 to 1.67 for the formation of mature lamellar bone. It was established that the formation of an interconnected dendritic structure with the content of hydroxyapatite and lithium disilicate for the glass-ceramic material modified by Nb₂O₅ and ZnO leads to the intensive growth of CHAP crystals oriented in a certain way in relation to the axis of collagen fibers. The final formation of the mineralized bone matrix on the 14th and 28th day due to the simultaneously high bioactivity and structural strength of the glass-ceramic material based on hydroxyapatite and lithium disilicate allows accelerating the rehabilitation of patients and successfully operating the implant under variable loads on the bone tissue for a long time.

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**ДОСЛІДЖЕННЯ МІНЕРАЛІЗАЦІЇ
КАЛЬЦІЙФОСФАТОСИЛІКАТНОЇ СКЛОКЕРАМІКИ *IN VIVO*
ПРИ РЕГЕНЕРАЦІЇ КІСТКОВОЇ ТКАНИНИ**

*О.В. Саввова, О.І. Фесенко, Г.К. Воронов, О.В. Бабіч,
В.В. Бітюцька, Ю.О. Смирнова, А.О. Гопко*

Проаналізовано перспективні напрями створення біологічно активних замінників кісткової тканини. Встановлено ефективність застосування кальцієсилікофосфатних матеріалів, які модифіковано CuO, ZnO, Ag₂O, Fe₂O₃, TiO₂, SrO і Nb₂O₅ для забезпечення високої біосумісності і антибактеріальних властивостей кісткових ендопротезів. Обґрунтовано перспективність застосування літійкальцієфосфато-силікатної склокераміки для одержання зміцнених, біологічно активних кісткових імплантатів. Встановлено основні критерії при розробці біосумісних склокристалічних матеріалів щодо їх складу, структури, текстури та властивостей поверхні. Проаналізовано вплив відмінностей структури та резорбції кальцієсилікофосфатної склокераміки на механізм формування апатитоподібного шару *in vivo*. Визначено особливості мінералізації кальційфосфатосилікатної склокераміки *in vivo* при регенерації кісткової тканини та встановлено ефективність застосування склокераміки на основі гідроксиапатиту та дисилікату літію при заміщенні кісткової тканини для скорочення строків реабілітації та тривалої експлуатації ендопротезів при змінних навантаженнях. Розроблений кальцієфосфатосилікатний склокристалічний матеріал ОС-7 характеризується вмістом кристалічних фаз дисилікату літію 10 об.% та гідроксиапатиту 55 об.% при співвідношенні CaO/P₂O₅=1,67, мікрорельєфом поверхні 6 мкм, значенням вільної енергії поверхні 75 мДж/м² та тріщиностійкістю 6.0 МПа·м^{1/2}. Даний матеріал є біосумісним за рахунок формування кристалів карбонатгідроксиапатиту вже на 14 добу, що дозволяє вважати перспективне його використання при лікуванні переломів, дефектів довгих кісток та при заміні коротких або трубчастих кісток.

Ключові слова: кальцієсилікофосфатні склокристалічні матеріали, гідроксиапатит, дисилікат літію, мінералізація регенерація, кісткова тканина.

**STUDY OF MINERALIZATION OF LITHIUM CALCIUM
PHOSPHOSILICATE GLASS CERAMICS *IN VIVO*
DURING BONE TISSUE REGENERATION**

O.V. Savvova^a, O.I. Fesenko^a, H.K. Voronov^a, O.V. Babich^{b,},
V.V. Bitiutska^a, Yu.O. Smyrnova^a, A.O. Hopko^a*

^a O.M. Beketov National University of Urban Economy in Kharkiv, Kharkiv, Ukraine

^b Research Institution «Ukrainian Research Institute of Environmental Problems», Kharkiv, Ukraine

* e-mail: lenysjababich@gmail.com

Prospective directions for the creation of biologically active substitutes for bone tissue were analyzed. The effectiveness of the use of calcium phosphosilicate materials modified with CuO, ZnO, Ag₂O, Fe₂O₃, TiO₂, SrO and Nb₂O₅ to ensure high biocompatibility and antibacterial properties of bone endoprostheses has been established. The prospective use of lithium calcium phosphate silicate glass ceramics for obtaining strengthened, biologically active bone implants was substantiated. The main criteria for the development of biocompatible glass-ceramic materials regarding their composition, structure, texture, and surface properties have been established. The influence of differences in the structure and resorption of calcium phosphosilicate glass ceramics on the mechanism of formation of an apatite-like layer *in vivo* was analyzed. The features of mineralization of calcium phosphosilicate glass ceramics *in vivo* during bone tissue regeneration were determined, and the effectiveness of the use of glass ceramics based on hydroxyapatite and lithium disilicate in bone tissue replacement was established to reduce the rehabilitation period and long-term use of endoprostheses under variable loads. The developed OS-7 calcium phosphosilicate glass-ceramic material is characterized by the content of crystalline phases of 10 vol.% lithium disilicate and 55 vol.% hydroxyapatite with a ratio of CaO/P₂O₅=1.67, surface microrelief of 6 μm, surface free energy value of 75 mJ/m² and crack resistance of 6.0 MPa·m^{1/2}. This material is biocompatible due to the formation of carbonate hydroxyapatite crystals already on the 14th day, which allows us to consider its promising use in the treatment of fractures, defects of long bones and in the replacement of short or tubular bones.

Keywords: calcium phosphosilicate glass-ceramic material; hydroxyapatite; lithium disilicate; mineralization; regeneration; bone tissue.

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