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Research of formation of apatite-like layer on the surface of glass-ceramic coatings for dental implants

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The prospects of application of calcium phosphate-silicate coatings on titanium alloys for dental implantology have been analyzed. The calcium silicophosphate glass was developed in the system R_2O–RO–CaF_2–R_2O_3–P_2O_5–SiO_2 and it was obtained using a scraper technology under low temperature short-term thermal treatment of glass-crystalline titanium coatings with given mechanical and thermal properties. Solubility of glass-crystalline coatings in distilled water and physiological fluids was investigated. The peculiarities of forming the apatite-like mineralized layer on the surface of calcium phosphate silicate coatings in vitro for one month, in connection with the *pH* of the solution and leaching of calcium and phosphate cations in distilled water, are formulated. The obtained physicochemical and microbiological data can be used for the development of bioactive glass-crystalline coatings for titanium alloys with a shorter term for resorption, for dental endoprosthetics.

Keywords: calcium phosphate silicate glasses, glass-crystalline coatings, solubility, structure, apatite-like layer.

Проанализирована перспективность применения кальцийфосфатосиликатних покрытий по сплавам титана для дентальной имплантологии. Разработаны кальцийсилико-фосфатные стекла в системе R_2O–RO–CaF_2–R_2O_3–P_2O_5–SiO_2 и получены по шликерной технологии в условиях низкотемпературной кратковременной термической обработки стеклокристаллические покрытия по титану с заданными механическими и термическими свойствами. Исследована растворимость стеклокристаллических покрытий в дистиллированной воде и физиологических жидкостях. Сформулированы особенности формирования структуры апатитоподобного минерализированного слоя на поверхности кальцийфосфатосиликатних покрытий в условиях *in vitro* в течение одного месяца, во взаимосвязи с *pH* раствора и выщелачивания катионов кальция и фосфатных групп в дистиллированной воде. Полученные физико-химические и микробиологические данные могут быть использованы при разработке биоактивных стеклокристаллических покрытий по сплавам титана с сокращенным сроком резорбции, для дентального эндопротезирования.

Дослідження формування апатитоподібного шару на поверхні склокристалічних покриттів для дентального протезування. *О.В.Саввова, О.І.Фесенко, О.В.Бабіч.*

Проаналізовано перспективність застосування кальційфосфатосилікатних покриттів по сплавам титану для дентальної імплантології. Розроблено кальційсилікофосфатні стекла у системі R_2O–RO–CaF_2–R_2O_3–P_2O_5–SiO_2 та одержано за шлікерною технологією в умовах низькотемпературної короткочасної термічної обробки склокристалічні покриття по титану з заданими механічними та термічними властивостями. Досліджено розчинність склокристалічних покриттів у дистильованій воді та фізіологічних ріди-

нах. Сформульовано особливості формування структури апатитоподібного мінералізованого шару на поверхні кальційфосфатосилікатних покриттів в умовах *in vitro* впродовж одного місяця, у взаємозв'язку з *pH* розчину та вилуговуванням катіонів кальцію і фосфатних груп у дистильованій воді. Одержані фізико-хімічні та мікробіологічні дані можуть бути використані при розробці біоактивних склокристалічних покриттів по сплавам титану зі скороченим терміном резорбції, для дентального ендопротезування.

1. Introduction

Unimpaired functioning of human bone tissue, in particular after restorative surgeries and dental implanting, can be assured by developing new, more advanced materials based on modern scientific contributions of materials science and medicine. Development and introduction of bio-compatible materials with shortened terms of fusion with bone, in particular functional silicate materials, will allow to solve a crucial task of combining the material's bioactivity with its high mechanical characteristics.

Silicate bioactive materials and glasses on their base take the leading position in the field of application of synthetic materials for replacement surgery due to their ability to bind bone tissue with subsequent formation of the surface layer of hydroxyapatite (HA) and their osseo-conductive properties [1].

Bioactive calcium phosphate-silicate materials do not exist in the body as separate phases. They form a dynamic system with heavy mineral turnover, occurring under the influence of physiological factors. The bone tissue is a buffer system that maintains continuous concentration of calcium ions and phosphate groups in a human body. Resorptive vitreous materials have the highest reactivity and potential to form strong bonding between an implant and bone due to formation of the apatite-like layer. They also have mechanical strength properties similar to those of human bone tissue.

The first bioactive silicate material, 45S5 Bioglass, was developed on the base of Na_2O–CaO–P_2O_5–SiO_2 system by L.Hench et al. [2] in the late 1960-s as an attempt of creating a filler for bone defects. However, this material had low affinity to the bone tissue, which had limited its fields of application. Subsequently, bio-glasses on the base of 45S5 with hydroxyapatite and tricalcium phosphate were suggested [3].

Known bio-glasses on the base of MgO–CaO–SiO_2 system have the high reactivity and bioactivity defined by the high rate of the reaction of hydroxyapatite formation when exposed to bodily fluids [4, 5]. Release of Na^+ and Ca^{2+} increases the value of *pH* of the system and increases the rate of the crystal formation. According to the authors [5], MgO–CaO–SiO_2 based bio-glass with the content of MgO = 10 wt. %, CaO = 40 wt. % and SiO_2 = 50 wt. % is reported to start forming surface hydroxyapatite layer already on the third day.

Well known calcium phosphate glass-ceramic cellular material BAK and KF glass-ceramics used as a coatings on titanium alloys developed in RKhTU named after Mendeleev under the supervision of P.D.Sarkisov have the terms of bone union of up to 7–8 months and 455 days, respectively, and can be used in orthopedy and maxillofacial surgery for the purpose of endoprosthetics [6]. The authors of the work [7] have developed bioactive glass-ceramic materials, which exhibit biocompatibility and non-toxicity after being implanted into a bone for 90 days. However, the abovementioned terms of union with bone are quite long for dental implants, which must provide formation of strong mineralized layer (shear strength > 15 MPa) during 1–3 months.

Complexity of providing the high strength of the implant-bone bond in short-term period depends mostly on the bioactivity of material and its ability to form the thin apatite-like layer according to the following mechanisms:

— *chemical*, providing dissolution of the implant material in bodily fluid due to the process of diffusion [8];

— *biochemical*, which considers corrosion of biomaterial as the outcome of diffusion and the action of macrophages on the implant surface. Macrophages seize and break off separate elements of the implant structure, facilitating its dissolution [9–11].

Transition zone is observed *in vitro* on the surface of calcium phosphate-silicate vitreous materials or glass-ceramics. It has non-uniform thickness: formation of the silica-containing layer is observed directly on the surface of the implant, under it lies a calcium-phosphate layer with amorphous structure. This structure crystallizes during 7–10 days with formation of the apatite-like polycrystalline phase [6, 12]. Important factor of formation of strong adherence in

Table 1. Chemical composition of model glasses

Indicator of chemical composition	Content of main components, wt. %					
	FAR 1	FAR 2	FAR 3	FAR 4	FAR 5	FAR 6
$K_2O + Al_2O_3$	14	17	14	15	15	14
$CaO + P_2O_5$	15	15	26	15	20	21
$Na_2O + Li_2O$	12	9	9	11	9	10
$CaF_2 + ZnO$	11	3	3	7	3	7
$B_2O_3 + SiO_2$	48	56	48	52	53	48

the implant-bone system is the provision of necessary formation rate and structure of transition layer, matching the rate of growth of natural bone.

Amorphous calcium phosphates (ACP) are considered to be precursors of non-stoichiometric hydroxyapatite (nHA) in the complex sequence of transformations. They are, in turn, formed upon the buildup of calcium ions in intercellular space of bone matrix. According to the findings of O.V.Zagorodko [13], formation of HA on the surface of bioactive crystalline phases occurs via formation of nHA crystallization precursors, namely ACP and octacalcium phosphate (OCP) crystalline phases. Formation of the apatite-like layer on the base of nHA on the surface of materials occurs in conditions of pH = 7–11 to the point when Ca:P ratio reaches 1.71 by the following reaction [14]:

$$\begin{gathered} \mathrm{ACP(Ca/P = 1.0)} \rightarrow \\ \rightarrow \mathrm{OCP(Ca/P = 1.33)} \rightarrow \mathrm{nHAP(Ca/P < 1.67)} \rightarrow \\ \rightarrow \mathrm{HAP(Ca/P = 1.67)}. \end{gathered} \quad (1)$$

Implementation of the above mechanism will allow to develop the biocompatible resorptive glass-ceramic calcium phosphate-silicate coatings on titanium which form strong mineralized layer of apatites during one months and will allow obtaining the competitive dental implants on their base, with mechanical properties that match those of the bone tissue. This determines the relevancy of this work.

2. Aim setting and research methods

The aim of the work is to research the formation of apatite-like layer on the surface of calcium phosphate-silicate coatings on titanium *in vitro* to forecast their behavior *in vivo*.

Distilled water (DW) was used as a control solution to determine concentration of ions based on pH change. Resorption level of experimental vitreous materials was determined by the loss of mass after exposure to DW ($L_{D.W}$, wt. %) and physiological solution ($L_{P.S}$, wt. %) for 30 days. It should be noted, that pH_0 index after DW is 7, and decrease of this index is due to intensive absorption of carbon dioxide by DW.

Determination of the behavior of the experimental glass-ceramic coatings in solutions with different values of pH was selected as a base of the research. Assessment of biological action was performed using the method of extreme solution (express investigation of destruction) and modelling solution (real-time destruction) according to ISO 10993-14-2001. To forecast the behavior of the material in vivo, simulated body fluid (SBF) according to ISO 23317:2012 was selected.

To perform the assessment of chemical composition of the surface layer of the experimental glass-ceramic coatings before and after the *in vitro* exposure, concentrational distribution of element was investigated using wavelength dispersive X-ray fluorescence spectrometric method (XFM), based on change of the element concentrations (C, wt. %) in the initial material (C_{init}, wt. %) and after the exposure to SBF during 1, 7, 14, 21, 28 and 35 days (C_{exp}, wt. %). Concentration of the elements with ZAF correction (atomic number, absorption, fluorescence) on the surface of the materials before and after the exposure to SBF was assessed based on element's line intensity change (I) imp/s, compared to the initial material.

Structure of the surface layer was investigated with the by scanning electron microscope REM Tesla 3 LMU with resolution of 1 nm, using Oxford X-max 80 mm energy-dispersive spectrometer.

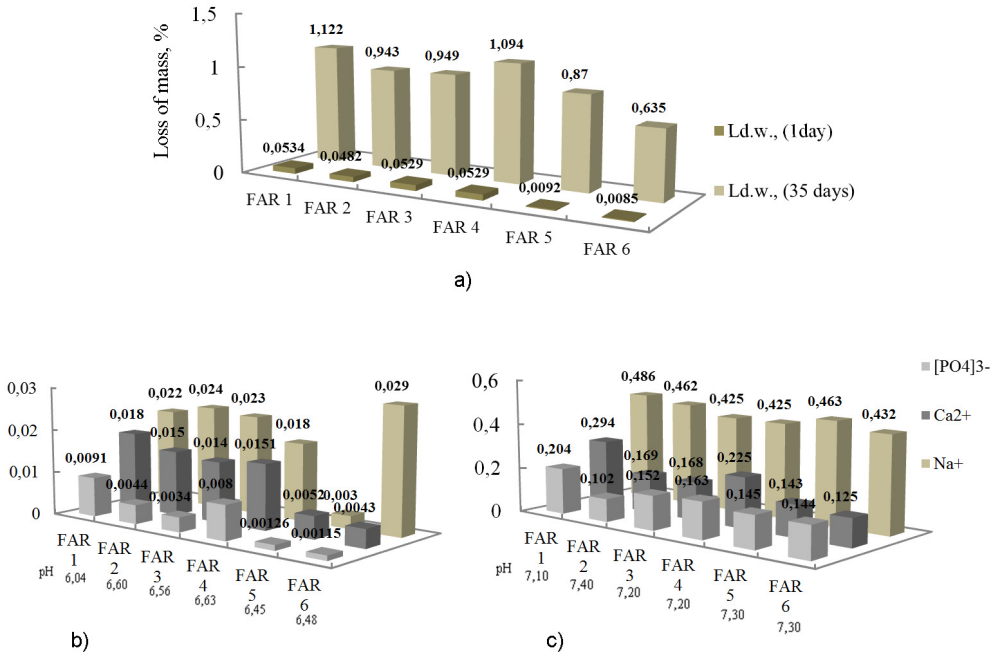


Fig. 1. Destruction of experimental coatings to DW (a) and the yield of sodium, calcium and phosphate ions after 24 h (b) and 35 days (c) aging in it.

Table 2. Structural characteristics, mechanical and thermal properties of research coatings

Glass marking	Indicator			Amount of crystalline phase, vol. %		H, GPa	HV, GPa	K_{1c}, MPa·m$^{1/2}$	E, GPa	$\alpha \cdot 10^7$, deg^{-1}
	f_{Si}	RO/P_2O_5	Na_2O/P_2O_5	HA	FAP					
FAR 1	0.27	2.48	1.27	24	1	6.35	6.20	1.5	70.0	47.1
FAR 2	0.30	1.85	1.50	33	2	7.25	7.32	1.8	75.0	76.0
FAR 3	0.26	1.81	0.74	32	3	7.20	7.15	2.5	76.0	95.9
FAR 4	0.28	2.16	1.39	26	2	6.45	6.40	1.7	72.0	75.5
FAR 5	0.28	1.83	1.01	37	3	6.80	6.60	2.6	80.0	89.3
FAR 6	0.26	2.05	0.93	40	4	6.90	6.85	2.0	85.0	84.9

3. Experimental

3.1 Development of compositions of calcium phosphate-silicate glasses for obtaining glass-ceramic coatings

To obtain glass-ceramic coatings for dental implantology, Na_2O–K_2O–Li_2O–CaO–ZnO–CaF_2– B_2O_3–Al_2O_3–P_2O_5–SiO_2 system was selected (Table 1). Model glasses of FAR series with the ratio of phase-forming components $CaO/P_2O_5 = 1.67$ and content of $CaF_2 = 1.5 \div 6.5$ wt. % were selected for the crystallization of HA and FAP (fluorapatite) bioactive phases in the area of determined concentration limits.

The glasses were melted in fireclay crucibles in a furnace with silicon carbide heaters at 1300÷1350°C, with following cooling on a steel sheet. Frit grinding was done in a ball mill during two hours to the fineness that corresponds to 9 wt. % remaining frit on 0.065 mm sieve.

Coatings on the base of developed glasses were obtained by wet method. 0.2 wt. % solution of xanthan gum (XG) was selected as an optimal dispersion medium for obtaining the defect-free coatings. Obtained slips were applied on VT1-00 bioinert titanium alloy by submerging after sand blasting. Specimens with applied coatings were dried

under the temperature of 80÷120°C and fired at 700÷780°C during 1.0 min.

As a result of investigations of the single-stage low-temperature short-term treatment of FAR 5 model glass, monodirectional glass-ceramic structure with presence of fine HA and FAP crystals in total amount of 40 vol. % has been identified. Such structure will allow to have according values of mechanical and thermal properties (Table 2), which indicates the ability of using the experimental glass as a base in creating the glass-ceramic coatings on titanium for the dental implantology applications.

3.2 Research of solubility of experimental glass-ceramic coatings in physiological media

Intrinsic feature of the experimental coatings of FAR series is their significant solubility in distilled water, which is related to the content of resistive crystalline phases with the size of up to 30÷45 vol. % and significant content of the resorptive phosphate-silicate glass phase. Developed coatings possess resemblance to the resorptive glasses [6] by chemical properties and high reactivity $f_{Si} < 0.32$ (Table 2) [13] which is an important factor in obtaining the bioactive glass-ceramic coatings on titanium alloys for the dental implants with short-term resorption.

The loss of mass in DW (Fig. 1a) is the highest for the coating FAR 1 and is due to the lowest level of connectivity of its silicon-oxygen network, with the value of $f_{Si} = 0.27$ and amount of HA and FAP of only 30 vol. %. The increase in the amount of HA and FAP crystalline phases up to 35 vol. % significantly influences the solubility of the coatings FAR 5 and FAR 6 in DW. For FAR 2 experimental coating with $f_{Si} = 0.30$, its solubility is limited by water-resistant glassy matrix. Reversely, for the coatings FAR 3 and FAR 4, high values of solubility indices are caused by the high amount of phosphorus oxide in the glass phase, along with $f_{Si} = 0.26$ and 0.28, respectively.

An interesting phenomenon is the combined influence of sodium and phosphorus oxides in the glass on solubility of the experimental glasses. Thus, for FAR 1 experimental coatings with the amount of Na_2O = 7.16 and of P_2O_5 = 5.62 wt. %, the high solubility is observed due to its composition is located within the area of invert glasses with the ratio of $Na_2O/P_2O_5 = 1.27$ and SiO_2 content of 42.5 wt. %. In this case, according to P.D.Sarkisov [6], reallocation of sodium ions from silicon-oxygen groups of $(SiO_3)O^-$ to phosphorus-oxygen groups $(P_2O_4)O_2^-$ and $(PnO_{4+n})O_n^-$ occurs to compensate their negative charge. Silicon-oxygen groups, $(SiO_3)O^-Na^+$ are converted to $[SiO_4]^-$ groups, increasing the connectivity of the glass structure. Due to the high polarization of non-bridging oxygen in the phosphorus-oxygen tetrahedra, bond strength of sodium cations with anion component in the groups like $(P_2O_4)O_2^-Na^+$ is lower than in $(SiO_3)O^-Na^+$ groups, which determines higher solubility of FAR 1 coating with the lower amount of Na_2O. Another confirmation of this is the fact that the increase of Na_2O content in FAR 2 coating to 8.42 wt. %, with the ratio of Na_2O/P_2O_5 = 1.50 and in FAR 4 and FAR 5 coatings to 7.79 wt. % with the ratio of Na_2O/P_2O_5 = 1.39 and 1.01, respectively, does not lead to the increase in their solubility. The fact of the decrease in solubility is also explained by "seaming" of structural phosphate elements with modifying Al^{3+} ions and stabilization of the glass network via formation of $[AlPO_4]$ groups as a result of introducing 7.87 and 7.23 wt. % of Al_2O_3 to the coatings FAR 4 and FAR 5. This is very important from the point of view of proving non-toxicity and biocompatibility of the coatings.

Release of sodium and calcium ions and phosphate groups of the experimental glasses into DW is determined primarily by the structure and composition of the residual vitreous phase. The coatings FAR 1, FAR 2, FAR 3 and FAR 4 with reduced water resistance have the higher amount of Na^{2+}, Ca^{2+} ions and phosphate groups compared to FAR 5 and FAR 6 coatings, both after 1 day of the exposure (Fig. 1b) and after 35 days of the exposure (Fig. 1c). Main contributors into the loss of mass of the coatings after one day of the exposure are sodium and calcium ions, which are released almost 100 times more, compared to the release of $[PO_4]^{3-}$ groups. After thirty days of the coatings exposure in DW, the losses of sodium and calcium ions are increased by approximately 20 times and are leveled for calcium ions and phosphate groups. This denotes the prevailing influence of the $[PO_4]^{3-}$ groups release on the increase of acidity of the medium in specified time period.

The higher content of calcium ions as vitreous phase solubility inhibitors q (cal-

Table 3. Destruction of research coatings in physiological fluids

Indicator	Glass marking					
	FAR 1	FAR 2	FAR 3	FAR 4	FAR 5	FAR 6
$L_{ph.s}$, (30 days), %	1.531	1.432	1.523	1.515	0.986	0.834
$L_{c.a}$, (120 h), %	1.649	1.424	1.486	1.624	1.3701	1.242
$L_{b.s}$, (120 h), %	6.541	6.352	6.402	6.514	6.321	5.804

Table 4. Peculiarities of chemical composition of the initial model and corrected glasses, structural parameters and mass losses after aging in DW

Indicator	Glass marking			
	FAR 5	FAR 5.1	FAR 5.2	FAR 5.3
	Content of the main components, wt. %			
$\sum (K_2O + Al_2O_3)$	15.27	21.85	16.49	16.49
$\sum (CaO + P_2O_5)$	20.50	17.13	17.02	15.67
CaO/P_2O_5	1.67	1.21	1.20	1.40
$(Na_2O + Li_2O)$	8.99	9.47	9.34	6.34
$\sum (CaF_2 + ZnO)$	3.01	6.56	6.55	10.90
$\sum (B_2O_3 + SiO_2)$	52.23	44.99	50.61	50.61
Na_2O/P_2O_5	1.01	1.07	1.05	0.79
RO/P_2O_5	1.83	1.37	1.36	2.25
$L_{D.W.}$, 30 day, %	0.87	0.887	0.875	0.890

cium ions can interchange with sodium ions in the hydrated layer) in the solution after a day of the exposure for the coatings FAR 1, FAR 2, FAR 3 and FAR 4 compared to FAR 5 and FAR 6 has a significant effect on the decrease of phosphate groups release after 30 days of the exposure, which may adversely affect the process of formation of the apatite-like layer. This process is based on the fact that the solid phase is in equilibrium state with more acidic solution compared to the solid phase composition, because of this, the calcium phosphates are partially dissolved with formation of more basic phosphates and phosphoric acid. The evidence of this process is change in *pH* of the solutions, from $pH_0 = 6.41$, after one day of the exposure, to *pH* in the range of 6.0÷6.6.

Durability of the residual phosphate-silicate vitreous phase of the experimental coatings depends significantly on the solvent *pH*. It is known, that solubility of P_2O_5 in acid solution increases with growth of RO/P_2O_5 ratio. Optimal release of the phosphate groups into DW after 30 days from FAR 5 and FAR 6 coatings with $RO/P_2O_5 = 1.83$ and 2.05, respectively, results in *pH* ~ 7.3, which is required for the formation of the apatite-like layer. It should be noted that binding of calcium ions in the structure of the crystalline phase allows to provide both adhesion and inhibition of the phosphate groups dissolution process.

The mass losses of the experimental coatings increase in physiological solution due to surface dissolution processes intensification as a result of the medium increased activity. The direction of change for this parameter is the same as in DW for the experimental coatings, the only peculiarity is that the difference between the losses of the more soluble coatings FAR 1, FAR 2, FAR 3 and FAR 4 and the less soluble ones, FAR 5 and FAR 6, is more substantial.

After the exposure to buffer solutions during 120 h, the experimental glass-ceramic coatings have the values of $L_{c.a.} = 1.242 \div 1.649$ wt. % and $L_{b.s.} = 5.804 \div 6.541$ wt. % (Table 3), which allows to make a conclusion about their low level of destruction and

possibility of using in the medical items according to ISO 10993-14-2011.

Considering the optimal amount of calcium and phosphorous ions and *pH* (Fig. 1), which required for deposition process, the coatings FAR 5 and, for comparison purposes, FAR 2 have been selected for the further research.

Mechanism of the bonding of the biomaterial with a bone can be assessed by the material mass growth in SBF, which is based on the occurrence of the following surface phenomena and processes: dissolution, i.e. transfer of the material's components into the outer medium, and deposition of primarily calcium and phosphorus on the surface of the material when outer medium is oversaturated with these components [6].

The losses of mass of the experimental coatings during the 21-days exposure to SBF is correlated with their solubility in DW and physiological mediums. The mass growth curve generally changes by parabolic law. This process occurs the most intensively from the day 21 to day 28. Insignificant decrease in the mass loss for the coating FAR 5 compared to FAR 2 in the first day (Fig. 2a) allows to have the significant mass growth on day 7 and day 14. This can be due to intensive formation of amorphous silicon-oxygen layer during the first day for the coating FAR 2 with the amount of $SiO_2 \approx 50.0$ wt. % and, as a result, decrease in diffusion of calcium cations and phosphate groups to the solution and decrease of deposition rate on the surface of the coating during the following 7 days. On the 14th day of the coatings exposure in SBF, a more intensive mass growth by module is observed for FAR 5 specimen. Despite the fact that on 21st day the mass growth for these coatings differs insignificantly, the phase composition of their surface layer will have differences.

For the FAR 5 coating, the total mass growth after 28 days (L_{SBF} = 2.45 wt. %) (Fig. 2b), may provide intensive formation of the apatite-like layer on its surface. The change in deposition kinetics in SBF for FAR 2 coating in the period of 1–21 days will cause the increase of the silicon-phosphate layer formation duration.

With the purpose of intensification of the process of the calcium-phosphate layer formation in early stages of implanting, the composition of FAR 5 model glass was modified by decreasing CaO/P_2O_5 ratio and increasing RO/P_2O_5 ratio (Table 4).

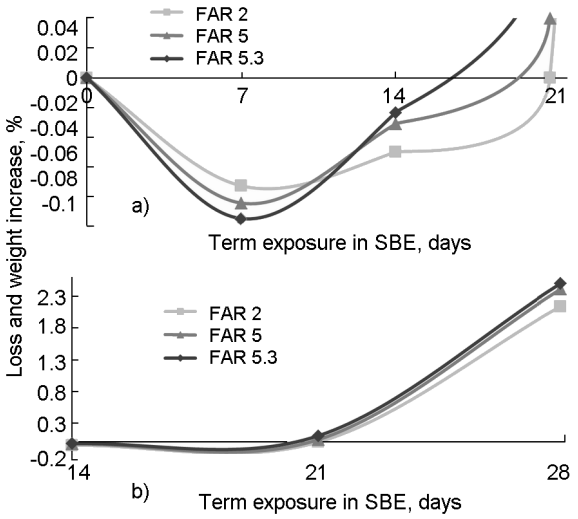


Fig. 2. Loss and weight gain developed coatings after exposure to SBF during the first weeks (a) and after one month exposure (b).

XRD results of the developed coatings allow to establish that FAR 5.1 specimen is characterized by presence of cuspidine and calcium fluoride, which may negatively affect biocompatibility of the coating. For the experimental coating FAR 5.2, the increase in the content of sodium oxide to 8.13 wt. % compared to FAR 5.3 coating (5.13 wt. %) resulted the decrease of mass losses in DW (Table 4). Modified glass-ceramic coating FAR 5.3 is characterized by the presence of HA and FAP with the total amount of 40 vol. %, required release of sodium, calcium ions and phosphate groups ($Na^+_{35\ days}$ = 0.401, Ca^{2+}_{35days} = 0.126, $[PO_4]^{3-}_{35days}$ = 0.162) and *pH*, which makes possible its consideration in further researches.

Mass loss and growth for the developed FAR 5.3 coating is similar to those of FAR 5, however a slight difference in the mass loss on the first day significantly influences mass loss on day 7 and its growth on day 14 due to phosphate component. At this point, the intensive formation of nHA crystallization precursors occurs — crystalline phases of APC and OCP. This allows to approximate the condition of the apatite-like layer formation to conditions of natural regeneration of bone and growth of the young bone, which is a prerequisite of the long-term use of the implant *in vivo*.

3.3 Research of chemical composition of the glass-ceramic coating surface layer after in vitro exposure

Research of chemical composition change of the surface layer of the coatings after

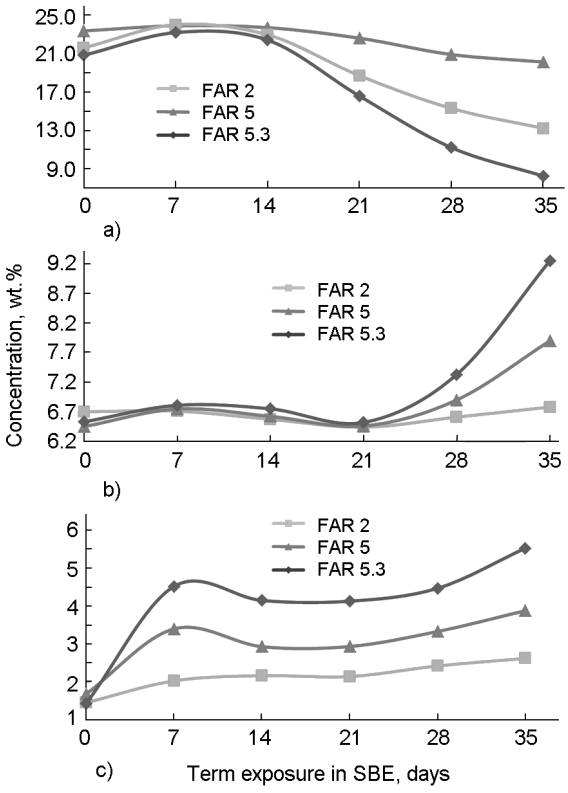


Fig. 3. The concentrations of silicon (a), calcium (b) and phosphorus (c) in the surface layers of test materials after aging in SBF.

the exposure to SBF is important to establish the possibility of the apatite-like layer formation on the coatings surface, as well as to provide their non-toxicity.

Research of aluminium concentration in the surface layers of the experimental glass-ceramic coatings FAR 5.3 and, for comparison, FAR 2 and FAR 5 after the SBF exposure insignificantly increases on the 7th day of exposure and has the minimal value on the 35th day, is important based on its toxicity to human. Similar picture is observed for zinc. Potassium content is decreasing in the general element content on the experimental coating surface. This is due to the fact that the intensive leaching of alkaline elements occurs in the first hours of exposure to SBF, with emergence of the respective maximum.

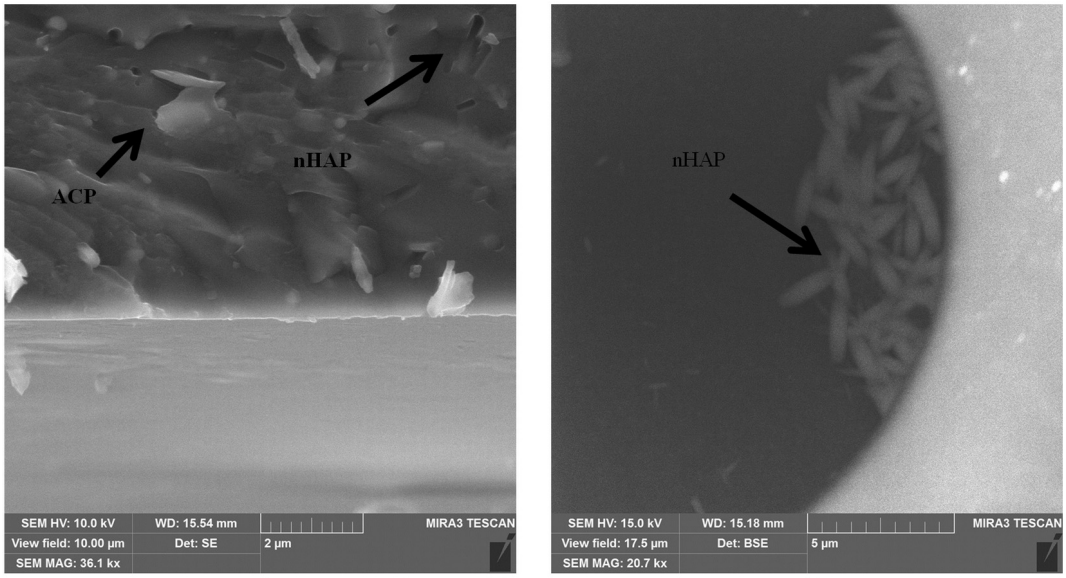
The increase in content of carbon on the surface of the coating and occurrence of chlorine is explained by anionic substitution of phosphate groups to carbonate and hydroxyl groups by chloride in DHA through immobilization of extrinsic $[CO_3]^{2-}$ and $[Cl]^-$ anions from the SBF [6, 10].

Evidence of the apatite-like layer formation on the surface of experimental glasses after the exposure to SBF is the change in concentrations of silicon, calcium and phosphorus elements. For the experimental coatings, the concentration of silicon significantly increases on the 7th day, which is the evidence of silicon gel layer formation on its surface during mentioned period. Presence of silicon in the surface layer of the experimental coatings creates conditions for the formation of silanol groups on their surface, which are required for HA nucleation [6]. Difference in intensity change of concentration of this element for coatings FAR 5, FAR 5.3 and FAR 2 will lead for the latter to blocking the diffusive mobility of modifying cations, and the release of calcium ions on the first day inhibits the process of phosphate vitreous phase dissolution. After the exposure during 14, 21 days, the silicon concentration gradually decreases, and on the 28th day it is close to the minimum values (Fig. 3a), with the lowest ones for FAR 5.3 coating.

Concentration and ratio of calcium and phosphorus on the surface of experimental glass-ceramic coatings *in vitro* is paramount in formation of the apatite-like layer on the implant surface *in vivo*. For the mentioned elements, the growth in their concentration on the experimental coatings surface with different dynamics is observed on day 7 (Fig. 3b, Fig. 3c). For FAR 2 coating, the concentration of calcium and phosphorus is significantly less than for FAR 5.2 and FAR 5 coatings. This fact is the evidence of the simultaneous formation of the silica gel and calcium phosphate layer on the surface of the experimental glass-ceramic coating with different ratio of components. On the days 14, 21, the phosphorus concentration increases for all coatings. For calcium the

Table 5. Change in ratio of Ca/P on surface of coatings after aging in SBF

Glass marking	The Ca/P ratio at different stages of aging in SBF					
	0 days	7 days	14 days	21 days	28 days	35 days
FAR 2	4.62	3.31	3.04	3.01	2.73	2.59
FAR 5	3.84	1.98	2.26	2.20	2.07	2.04
FAR 5.3	4.57	1.50	1.63	1.58	1.64	1.67



a) b)

Fig. 4. Nanostructure of glass-ceramic coating FAR 5.3 after 21 (a) and 35 days (b) aging in SBF.

reverse is true, when significant decrease of intenity is observed on the day 14 and at subsequent exposure to SBF on the day 21. The period of days 28 and 35 of the exposure is characterized by the maximal increase in phosphorus and calcium concentration, the highest one being for FAR 5.3 coating, which indicates on the possibility of active formation of apatite on their surface in vivo through the occurrence of the precursors.

The value of Ca/P of experimental coatings before the exposure corresponds to the ratio of these elements in the initial coating. The value of this parameter significantly decreases for all experimental coatings after the exposure to SBF during 7 and 14 days (Table 5). However, only for FAR 5.3 the ratio Ca/P = 1.51, which can be the evidence of APC crystallization. On the day 21, the proximity of Ca/P ratio to 1.58 for this coating indicates the possibility of DHA formation on the surface during the specified period. Upon the further exposure, on day 28, the ratio of Ca/P is approaching 1.64. This fact indicates that the HA crystallization occurs not directly, but through the stages of deposition of intermediate phases — precursors of non-stoichiometric hydroxyapatite (nHA), which has the Ca/P ratio value of ≤1.67. Research of the surface structure (Fig. 4) confirms the fact that the formation of nHA occurs via formation of amorphous calcium phosphate (APC) (Ca/P = 1.0÷1.67), through the formation of spherulites on the initial stages with their further coalescence into crests (Fig. 4a) and gradual levelling of the structure, and further hydroxyapatite crystallization (Fig. 4b).

Maintaining Ca/P = 1.67 ratio on the surface of the glass-ceramic coating FAR 5.3 after 35 day exposure to SBF is due to the excessive $[HPO_4]^{2-}$ groups absorbed by the surface. In this case, formation of the non-stoichiometric HA is observed, which is a series of solid solutions with $[HPO_4]^{2-}$ groups substituted by $[PO_4]^{2-}$ groups. The increase in stoichiometricity level of calcium-deficient HA occurs by immobilization of calcium ions from the solution and leads to the formation of HA with the Ca/P > 1.67 ratio.

The researches conducted on the base of the Institute for Problems of Cryobiology and Cryomedicine of NAS of Ukraine confirm the bioactivity of the glass-ceramic coating FAR 5.3 and possibility of its use for creating the bioengineered constructions with stem cells.

4. Conclusions

This, dynamics of dissolution of the developed glass-ceramic coatings in distilled water and bodily fluids has been investigated. It has been established that provision of the ratios $CaO/P_2O_5 = 1.4$, $Na_2O/P_2O_5 = 0.79$, $RO/P_2O_5 = 2.25$ in the structure of the experimental coatings creates conditions for HA crystallization in the amount of 37 vol.% and fluorapatite in the amount of 3 vol. %. The above structural features of

the coatings establish conditions that allow *pH* 7.3 of the medium by leaching out 0.126 wt. % of Ca^{2+}, 0.401 wt. % of Na^{+} and 0.162 wt. % of P^{5+}. This allows to provide formation of apatite-like layer on the surface of the experimental glasses upon *in vitro* deposition of components after 35 days of exposure to SBF through the following stages of deposition: formation of amorphous calcium phosphate spherulites (Ca/P = 1.5); coalescence of spherulites into crests intrinsic to nHA (Ca/P = 1.58÷1.64); levelling of the structure with the following crystallization of HA (Ca/P = 1.67).

References

1. L.L.Hench, *J. Mater. Sci. Mater. Med.*, **17**, 967 (2006).
2. L.L.Hench, *J. Mater. Sci. Mater. Med.*, **26**, 86 (2015).
3. L.L.Hench, J.M.Polak, *Science*, **295**, 1014 (2002).
4. C.Ohtsuki, T.Kokubo, K.Takatsuka et al., *J. Ceram. Soc. Jpn.*, **99**, 1 (1991).
5. K.Tsuru, C.Ohtsuki, A.Osaka, *Chinese Ceram. Soc.*, **5**, 85 (1995).
6. P.D.Sarkisov, Mendeleyev PkhTU (1997), p. 218 [in Russian].
7. O.V.Savvova, L.L.Bragina, G.N.Shadrina et al., *Glass Ceram+*, **74**, 29 (2017).
8. E.E.Stroganova, N.Y.Mikhailenko, O.A.Moroz, *Glass Ceram+*, **60**, 315 (2003).
9. B.I.Beletskii, N.V.Sventskaya, *Glass Ceram+*, **66**, 104 (2009).
10. Y.L.Chen, X.F.Zhang, Y.D.Gong et al., *J. Colloid Interface Sci,*, **214**, 38 (1999).
11. S.Ozawa, S.Kasugai, *Biomaterials*, **17**, 23 (1996).
12. J.Vogel, P.Wange, P.Hartman, Verlag der Deutschen *Glastechnichen Gesellschaft*, **70**, 220 (1997),
13. W.Suchanek, M.Yashimura, *J. Mater. Res.*, **13**, 94 (1998).
14. S.V.Dorozhkin, *J. Colloid Interface Sci*, **191**, 489 (1997).