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Research Article

Investigation of the Surface Properties of Glass-ceramic Materials During their Binding with Albumin

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Abstract

The study of the interaction between physicochemical and hemocompatible characteristics of biomaterials is extremely important in the field of creating medical devices that contact with blood, including implants. It is known that the adsorption of proteins is the first stage of blood interaction with a foreign surface.

The aim of the study is to observe the influence of surface structure of bioactive glass materials on their ability to bind to albumin.

Materials and Methods: The production of the test material took place in accordance to standardized instructions in the conditions of a certified laboratory as part of the at the Department of Ceramics, Refractory Materials, Glass and Enamels Technology of the National Technical University "Kharkiv Polytechnic Institute". The surface energy of solid bodies cannot be evaluated directly by the value of the surface tension parameter, as there is no movement of molecules on their surface. The wetting edge angle was measured statically and calculated by the sediment drop method.

Toxicity of implants based on developed materials was assessed at the Sytenko Institute of Spine and Joint Pathology, "National Academy of Medical Sciences of Ukraine" on the basis of dynamics study of content in raw materials of blood of the common protein, urea and activity of enzyme alanin-aminotransferase (ALT). The ALT was determined by the kinetic method, the total protein content - biuret method, urea content - enzymatic method.

Statistical analysis of the obtained indicators was performed using software packages Microsoft Excel and Statsoft Statistica 6.0.

Results and Discussion: Experimental glass crystalline materials were synthesized on the basis of calcium phosphate silicate glasses with a chemical composition, where modifying introduced to increase the crack resistance of the material in the amount of 5 wt. hours per 100 wt. including frit.

As an indicator of glass resorption and the level of its bioactivity *in vivo*, depending on the composition, the criterion glass reaction was calculated, the values of which indicate the possibility of formation of apatite-like layer on the surface of materials is an important manifestation of their biocompatibility.

Conclusion: It is established that the local redistribution of calcium ions and their binding to albumin on the surface of the developed material AC3-5 are determined by the characteristics of their surface, the nature of their resorption, features of their structure, the presence of surface crystallization hydroxyapatite. Ensuring the hemocompatibility of the developed glass-crystalline material, which is associated with the formation of an adsorption protein layer on its surface, is an important factor in their reliable operation *in vivo*.

Keywords: Bioactive Glass Materials; Surface Structure; Albumin; Hemcompatibility

Abbreviations

AHS: Adsorbed Human Serum Albumin; ALT: Alanin-aminotransferase; GR: Glass Reaction; GAP: Hydroxyapatite; HAS: Human Serum Albumin

Introduction

The study of the interaction between physicochemical and hemocompatible characteristics of biomaterials is extremely important in the field of creating medical devices that contact with blood, including implants. It is known that the adsorption of proteins is the first stage of blood interaction with a foreign surface. Most of biomaterials hemocompatibility properties studies are related to ascertaining the processes of adsorption of the protein layer formation and changes in its properties over time. Until now the influence of physicochemical properties of materials (chemical composition, amorphous and crystalline structure, hydrophilicity or hydrophobicity of the surface, etc.) on the nature of the adsorption-desorption processes of proteins are contradictory. Thus, there is no consensus on the chemical composition, structure and energy and adsorption properties of the material, which would provide the necessary hemocompatibility of products [1].

It is considered that biological effects on the surface of the biomaterial are associated with the phenomenon of "protein adsorption on a solid surface", coming from the blood plasma and directed to the implant during the first minutes of the interaction "blood-tissue". Based on the theoretical model of plasma proteins competitive adsorption on the surface of the endoprosthesis material, the pattern of changes in protein composition in the following order is established: album*i*n, γ-globulins, fibrinogen. The role of these proteins in the development of the inflammation process is not equal. When applied albumin to the biomaterial, decrease in the cells growth of the inflammatory row is observed and deficiency of fibrinogen did not allow to develop a normal inflammatory response until the implant was not covered by protein data. Albumin, which is presented in the largest amount in plasma, can "shield" the surface of the biomaterial, preventing the adhesion of other proteins and blood cells. Therefore, exactly the peptides on the surface of the implant, that allows the biomaterial to be identified as foreign, initiates immune responses. This theory explains why inert non-immunogenic material triggers the inflammatory process.

Experiments with model systems have shown a special character in the hemocompatibility of preliminary adsorption of albumin. On the one hand, adsorbed human serum albumin (HSA) inhibits platelet adhesion, and on the other hand, it prevents the binding of fibrinogen to the surface. When carrying out passivation in conditions close to those realized by contact of the biomaterial with blood (prescription of the surface with blood plasma, increasing the concentration of protein in solution, the use of a mixture of proteins), succeeded significantly reduce the difference between the adsorption and energy properties of hemocompatibility of hydrophilic and hydrophobic materials. Therefore, to increase the hemocompatibility of medical devices, it is advisable to passivate the surface with serum, plasma, model solutions with physiological concentrations of proteins, which is better in comparison with the widely used surface treatment with a solution of albumin.

The probability of hemocompatibility of a medical device with short-term or prolonged contact with blood increases if the primary stages of the interaction of the surface of the product with blood are characterized by:

- Rapid formation of strongly bound to the surface of the homogeneous (uniformity of coating) multilayers of protein (the case of hydrophobic materials) or the formation of a monolayer coating, the proteins of which are weakly bound to the surface and able to exchange with non-adsorbed blood proteins (case of hydrophilic materials);
- Minimal activation of blood enzyme systems, including the complement system;
- Minimal activation of cellular components of the blood, including aggregation, adhesion, release reaction and lysis of cells.

Protein adhesion on the surface of the implanted material is the initial stage in the cascade of interdependent processes that inaugurate each other and occur in the tissues after the implant is placed. It is followed by activation of the coagulation system and the complement system, thrombosis and migration of leukocytes [2].

Polymorphonuclear leukocytes are the first to appear in the site of inflammation and play a major role in intercellular interaction. Their migration begins 1-3 hours after the onset of the inflammatory reaction, after 6-12 hours around the source of irritation a pronounced leukocyte shaft is formed.

In the next phase of the inflammatory reaction, the main type of cells are macrophages, which take the role of a key regulatory cell - the "conductor of the cell ensemble". They limit the foreign body, consistently forming neutrophilic macrophage, macrophage and macrophage-fibroblastic barriers that precede the formation of granulation tissue. It is believed that macrophages play a key role in the interaction of "implant-tissue". On the surface of macrophages there are specific integrins that are responsible for adhesion on the surface of the implant, namely - interact with the proteins adsorbed on it [2]. The process following the accumulation of phagocytes is specific to this type of inflammatory reaction - the fusion of macrophages with the formation of giant foreign body cells [12].

After implantation of the synthetic biomaterial, the typical pathophysiological reaction which is expressed in an inflammation develops. Its features are the formation of granulomas containing giant cells of a foreign body - macrophage derivatives, as well as the formation of a connective tissue capsule that separates the implant from the surrounding tissues. The nature of the reaction to a foreign object is determined by the reactivity of the organism and the properties of the implant material and, to a greater extent, the characteristics of the surface.

Until now, scientist's development in the field of medical materials, science has determined the direction of development and implementation of implants with "specified biocompatible properties" - bioinert, bioactive, resorbable. Among these materials, the most hopeful for the creation of hemocompatible materials are bioactive glass crystalline materials with adaptable solubility and the capacity to fuse with bone tissue in a short time period [3]. This can be realized, including by treating their surface in an albumin solution. The synthesis of composite glass materials of this type will increase the hemocompatibility of the implant and prevent tissue inflammation during implantation *in vivo*, which determines the main current direction development of biocompatible calcium phosphate materials for bone arthroplasty.

The Aim of the Study

The purpose of this work is to observe the influence of surface structure of bioactive glass materials on their ability to bind to albumin.

To achieve this goal, the following tasks were established:

- To establish concentration of calcium ions after exposure of experimental glass-crystal materials in 10 mass. % of albumin solution;
- To investigate the redistribution of calcium fractions in albumin solution after contact with test samples, taking into account the peculiarities of their structure;
- To determine the hemocompatibility of the developed materials and to assess their prospects in the development of bone endoprostheses.

Materials and Methods

Order of experiment

Concentration of calcium ions in 10 mass. % solution of albumin after exposure of experimental glass-crystal materials during 5 days determined by calorimetric method [4,11]. Into a clean test tube was filled 1.0 ml of distilled water, 2.0 ml of serum and 0.5 ml of sodium hydroxide solution with C = 0.4 mol/L. Solution is mixed and after 5-10 minutes 2,0 ml solution of reactive (morexide) is added. At the same time etalon and the solution of comparison is prepared (Table 1). All solutions are re-mixed and the optical density of the sample and the standard with the comparison solution is measured. The measurement is carried out in 1 cm of a cell at length waves 540 nm not earlier than in 5, but no later than 15 minutes after adding the solution of the reagent. Evaluation of results. By the optical density of the samples (A) and the standard (B) of the free calcium (x) is calculated in mmol/l of biological material by the formula:

$x=2,5 \cdot A/B$; mmol/l.

Components	Sample (A)	Comparison solution	Standard (B)
Distilled water, ml	1,00	1,02	1,00
Experimental material, ml	0,02	-	-
Calibration solution, ml		-	0,02
Sodium hydroxide, ml	0,50	0,50	0,50
Reagent solution (murexide), ml	2,00	2,00	2,00

Table 1: Distribution scheme of components.

To determine the total content of calcium up to 1 cm^3 of solution added 4 cm^3 of nitric concentrated ch.h acids and 5 cm^3 of distilled

water was placed in the microwave distribution system Ethos Easy and conducted decomposition of the sample under the action of microwave radiation at temperature 185°C.

The obtained solutions were quantitatively transferred to the measuring flasks and brought to 15 cm^3 with water distilled. To determine the free calcium to 0,5 cm^3 of solution added 4,5 cm^3 ethyl alcohol 96%, to remove the formed sediment samples stayed for 6 days. Determination of mass fraction of calcium, and prepared solutions were conducted by the method of flammable atomic absorption spectrometry on the device Agilent AA 240FS.

*C*alcium-phosphatosilicate glass crystalline material was manufactured using traditional technology, which includes charge of raw materials, cooking model glass in corundum crucibles with a temperature of 1250-1350 ° C for 6 hours and holding at these temperatures for 0.5 hours.

Samples of glass, crushed to a residue of not more than 5% on a sieve № 008, were prepared by semi-dry isostatic pressing, formed into cylinders with a diameter of 4 mm and a height of 10 mm, from which then for experiment grind cylinders with a diameter of 1 mm and a height of 3 mm.

The surface energy of solid bodies cannot be evaluated directly by the value of the surface tension parameter, as there is no movement of molecules on their surface. The wetting edge angle was measured statically and calculated by the sediment drop method.

Toxicity of implants based on developed materials was assessed in the Sytenko Institute of Spine and Joint Pathology, "National Academy of Medical Sciences of Ukraine" on the basis of dynamics study of content in raw materials of blood of the common protein, urea and activity of enzyme alanin-aminotransferase (ALT). The ALT was determined by the kinetic method, the total protein content - biuret method, urea content - enzymatic method.

Statistical analysis of the obtained indicators was performed using software packages Microsoft Excel and Statsoft Statistica 6.0. The Mann - Whitney method was used to determine the differences between the comparison groups. The difference under conditions $p < 0.05$ was considered significant. In biochemical studies, group comparisons were performed according to the nonparametric Wilcoxon criterion with the calculation of the median (Me) and percentiles (25-75 ‰).

Results and Discussion

Experimental glass crystalline materials were synthesized on the basis of calcium phosphate silicate glasses with a chemical composition (Table 2), where R_2O_3 - Al_2O_3 R_2O_3; R_2O - Li_2O, Na_2O, K_2O; RO_2 - ZrO_2 TiO_2, CeO_2, MnO_2; RO - CaO, MgO, ZnO; modifying components CaO, V_2O_5, MoO_3, La_2O_3, Cu_2O, SiO_2 and filler ZrO_2, which was introduced to increase the crack resistance of the material in the amount of 5 wt. hours per 100 wt. including frit.

Oxides	Marking				
	AC3-1	AC3-2	AC3-3	AC3-4	AC3-5
	Differences in chemical composition, wt. %				
SiO_2	50,0	50,0	47,0	50,0	47,0
R_2O_3	7,0	7,0	7,0	7,0	6,0
R_2O	11,4	12,4	12,0	12,0	10,9
RO_2	3,0	1,0	4,0	2,5	4,0
P_2O_5	9,0	9,0	10,0	9,0	10,0
RO	19,0	17,0	20,1	18,4	19,6
CaF_2	0,5	0,5	1,0	1,0	2,4
Σ CoO, V_2O_5, MoO_3, La_2O_3, Cu_2O, SrO	0,1	0,1	0,1	0,1	0,1
ƒSi	0,30	0,30	0,28	0,30	0,28
GR	3	3	4	3	4

Table 2: Differences in the chemical composition of experimental glasses and structural indicators.

Glass with AC3 marking was manufactured according to the traditional technology, which includes loading of raw materials, welding of model glasses in corundum containers at temperatures of 1250°C - 1350°C for 6 hours and exposure to these temperatures for 0,5 hours.

Glass-crystalline materials were obtained by ceramic technology in the conditions of one-stage low-temperature (750-800°C) high-speed (15 min) thermal processing. Complex calcium phosphate-silicate glass-crystalline coatings containing yttrium oxide-stabilized zirconium dioxide filler were labeled AC3-1, AC3-2, AC3-3, AC3-4 and AC3-5.

The formation of polyphosphates in the glass structure, which is determined by the ratio of R_2O: $P_2O_5 > 1$, will allow the formation of

cybotactic groups of future crystalline phases [5], that, along with ensuring high reactivity of glass materials at fSi <0.32, is important manifestation of their bioactivity [6].

As an indicator of glass resorption and the level of its bioactivity *in vivo*, depending on the composition, the criterion glass reaction (GR) was calculated [7], the values of which indicate the possibility of formation of apatite-like layer on the surface of materials (GR = 4) is an important manifestation of their biocompatibility.

The formation of bonds between bioactive glass [8] and bone is carried out by a condensation reaction between ≡ Si - OH groups of acidic nature and polar groups of protein adhesive molecules. It is also possible the appearance of electrostatic bonds between protein molecules and the surface of the glass material. The strength of these bonds in comparison with the valence is insignificant, but as a result of their number, as well as due to the reaction condensation, the protein molecule is quite firmly fixed on the surface of bioactive glass. These phenomena can lead to a local change in the concentration of ionized calcium near the surface of the sample.

This is confirmed by the fact that after exposure to 10% solution of albumin test materials (A/AC3) for 5 days, the total calcium content is highest for albumin solution after exposure to material AC3-2 (A/AC3-2), the lowest - for A/AC3-5 (Figure 1). However, in the process of interaction of the experimental materials with the albumin solution in comparison with distilled water, the yield of free (ionized calcium) changes significantly. Only for A/AC3-3 solutions and to a greater extent for A/AC3-5, the content of bound calcium increases slightly compared to the albumin solution, and free calcium decreases. For solutions A/AC3-1 A/AC3-2 A/AC3-4 the specified characteristic is inversed. Therefore, the highest amount of bound calcium is observed for the material AC3-5, which is characterized by fSi = 0.28, and GR = 4.

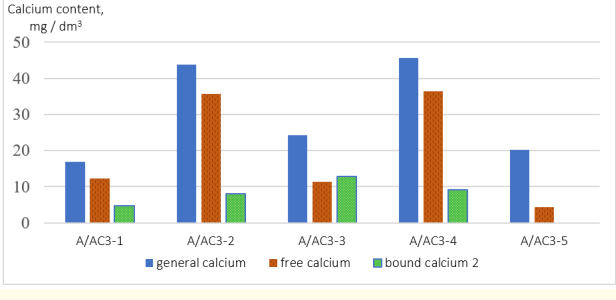

Figure 1: The content of various forms of calcium in the albumin solution after exposure to experimental materials.

Characteristics of glass-crystalline material

Material marked AC*3*-5 is made at the Department of Ceramics, Refractory Materials, Glass and Enamels Technology of the National Technical University "Kharkiv Polytechnic Institute". It is a strengthened bioactive calcium-phosphatosilicate glass crystalline material, which is composed of frit containing oxides of 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, further containing alumina, zirconium, cerium, cobalt, vanadium, lanthanum and lanthanum molybdenum. Increasing the crack resistance of the experimental materials was realized by introducing a filler of zirconium dioxide stabilized with yttrium oxide in the amount of 5 mass fractions per 100 mass fractions of frit.

From a thermodynamic point of view, the surface of the implant material must provide spontaneous adsorption of proteins, which significantly depends on the free energy of the surface for the processes of osseointegration. The values of free surface energy (55.37 ÷ 74.59 mJ/m^2) (Figure 2) for the original experimental materials indicate the possibility of intermolecular interaction at the boundary of the phase distribution and the formation of new phase embryos.

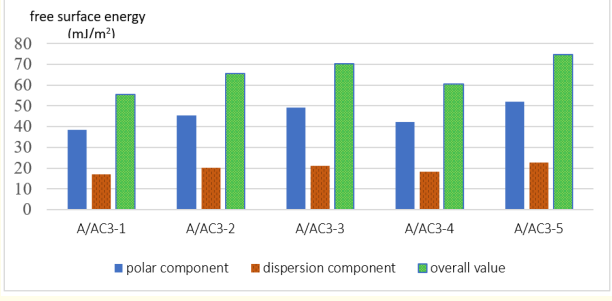

Figure 2: The value of the free energy of the surface of the original experimental materials and after their exposure in albumin.

In connection with experimental hydrophilic glass materials, it shows astringent ability, contains lipophilic and hydrophilic bonds. In addition, hydroxyapatite (GAP), which has pronounced osteoconductive properties and is present in the structure of experimental materials, provides adhesion of proteins and cells of bone tissue, is actively involved in ion exchange and metabolism of the bone matrix, maintains ionic and covalent bonds with bone minerals [9].

After exposure to albumin solution, free surface energy for experimental materials increases (62.9-82.3 mJ/m^2) (Figure 2). This is explained by the intensification of the adsorption process due to the formation on the surface of the test materials of hydrophilic and hydrophobic areas of albumin. Albumin adsorption is closely related to the process of redistribution of calcium fractions as a result of bioactive materials contact with blood serum [10].

The process of binding of calcium to albumin for experimental materials is circumscribed by the characteristics of their surface (free surface energy), the nature of their resorption (GR, the content of bound calcium in solution), the characteristics of their structure (fSi, the presence of surface crystallization of hydroxyapatite).

As a result of toxicity investigations of implants on the dynamics of the content in the serum of total protein, urea and ALT enzyme activity, the content of total protein on the 7th day after implantation (79.13 ± 1.71) g/l, on the 14th - (70.15 ± 2.23) g/l, 30th - (73.15 ± 0.45) g/l, 90th - (71.57 ± 3.00) g/l.

The urea content on the 7th day was (5.08 ± 0.34) mmol/l, on the 14th - (3.93 ± 0.25) mmol/l, on the 30th - (4.25 ± 0, 55) mmol/l, the 90th - (4.94 ± 0.43) mmol/l. ALT activity was equal to (44.25 ± 3.16) U/L, (48.00 ± 4.91) U/L, (70.00 ± 14.00) U/L, (41.56 ± 5.81)) U/L respectively. There was no statistically significant difference between the studied parameters in terms of the experiment, which indicates the absence of the effect of AC3-5 implants on the functional state of the liver and kidneys of experimental animals. This indicates the hemocompatibility of the experimental materials, including due to the preliminary adsorption of albumin, and their capacity to function effectively in a living organism after implantation.

Conclusion

It is established that the local redistribution of calcium ions and their binding to albumin on the surface of the developed material AC3-5 are determined by the characteristics of their surface (free surface energy = 74,59), the nature of their resorption (GR = 4, the content of bound calcium in solution) 15,3 mg/dm^3, features of their structure (fSi, = 0,28), the presence of surface crystallization hydroxyapatite (GAP). Ensuring the hemocompatibility of the developed glass-crystalline material, which is associated with the formation of an adsorption protein layer on its surface, is an important factor in their reliable operation *in vivo*.

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Conflict of Interest

The authors declare no conflict of interests.

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