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Oksana Savvova

BIOCIDE APATITE GLASS-CERAMIC MATERIALS FOR BONE ENDOPROSTHETICS

Chair of Technology of Ceramics, Refractories, Glass and Enamels,
National Technical University "Kharkov Polytechnic Institute"
21, Frunze str., 61002 Kharkiv, Ukraine; savvova_oksana@ukr.net

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Abstract. Availability of chitosan for obtaining of biocide apatite glass-ceramic materials for bone endoprosthetics has been confirmed. Comparative assessment of biocide properties against *Candida albican*, and *Escherichia coli* of materials used for bone endoprosthetics has been made. The use of implants on the base of calcium silicophosphate glass-ceramic materials saturated with chitosan will allow to decrease the possibility of inflammatory processes caused by infection and to shorten the terms of resorption of biomaterial with a bone.

Keywords: calcium silicophosphate glass-ceramic materials, bone endoprosthetics , biocide properties.

1. Introduction

The first step on the way to creating a concept of materials for bone endoprostethics is development of the set of chemical, mechanical, technological, medical, and biological requirements, which are to be met by the material from the perspective of its successful use in implant surgery [1].

An implant is affected by the complex of factors in human body at physiological temperature – solutions of salts and organic acids, dissolved oxygen, etc. which create actively corrosive medium, the effect of which may be different depending on the composition of used material [2]. For this reason the provision of biological compatibility, *i.e.* absence of immune, carcinogenic, bacteriological reactions of tissues, injuries of them, is an important factor for the choice of the implant material.

Since important factors that influence recommendation of a material for medical applications are the results of morphological, toxicological and cell researches, conducted to prevent the possibility of inflammation processes in adjacent tissues, changes of blood count and pathological changes of internal organs, researches of materials biocide properties used in contemporary bone endoprosthetics are necessary.

Nowadays materials used for bone endoprosthetics with biocide properties are known. Biocide properties of titanium alloys are caused by the formation of titanium dioxide on their surface, antibacterial properties of which are due to its photo catalytic effect [3-5].

Prospectivity of the use of biocompatible materials on the base of calcium phosphates is explained, along with the high level of bioactivity, absence of inflammation reactions, pathologies of the organs and mutagenic effects, by imparting of high level of biocide properties. The authors [6] have developed material on the base of silver-alloyed hydroxyapatite, which is used for treatment of patients with chronic generalized periodontitis. Antibacterial properties of apatite composite materials on titanium substrate are provided by immobilization of decametoxine and ethonium on their surface [7].

The use of chitosan in the material structure on the base of calcium phosphates allows obtaining the implants in therapeutic, surgical and orthopedic dentistry and implantology with high biocompatibility, anticarcinogenity, antioxidativity, bactericidity, bacteriostaticity, non-toxicity, non-allergenicity, and haemostaticity [8]. It is known that chitosan affects pathogenic staphylococci, streptococci, enterobacteria, colon bacilli, corynebacteria, micrococci, and *Candida fungi*. Termination of growth of pathogenic flora is explained by agglutination of microbial bodies by chitosan. Mechanism of agglutination is identical to coagulation of erythrocytes by polycations. Bonding of chitosan by receptors of saccharides on cell membrane provides bacteriostatic effect [9, 10].

2. Experimental

The purpose of this work is the synthesis of biocide bioactive composite glass-ceramic material on the base of

calcium silicophosphate glass and chitosan. Solubility of investigated material was determined by mass loss (P_m, %) after exhibition in 10 % albumin solution during 30 days. The presence of crystalline phase in experimental glasses was determined by X-ray diffraction analysis conducted with DRON-3M unit. Water absorption (W, %) and porosity (P_{real}) were determined with the use of apparatus for soaking of specimens with water in vacuum and scales for hydrostatic weighing. Surface characteristics of the material were assessed by scanning electron microscopy on PEMMA101A.

Bactericidal and fungicidal properties were determined with the use of liquid medium by quantitative method, which is based on the calculation of growth level of biotest microorganisms inoculated to liquid nutritious medium in the presence of test specimens and without them.

To obtain cumulative biotest cultures selective nutritious media were used:

2-% meat-peptone agar (MPA) and meat-peptone broth for cultivating of bacteria of colon bacillus group.

Liquid and agarised Czapek-Docks medium for cultivating of microfungi.

To obtain *Escherichia coli* inoculate cell suspension, which is original for cell culture used for placing to nutritious medium, bacterial cultures in exponential growth phase were used. For that, daily strain culture grown *in vitro* on 2 % MPA at 308 ± 2 K washed with distilled water and obtained suspension was diluted to the concentration of 10^4 microbial cells in 1 ml.

To obtain *Candida albicans* inoculate, fungal cultures were grown *in vitro* on the agarised selective Czapek-Docks medium at 299± 2 K. For determination of fungicide properties of material towards vegetative cells the fungi cultures were grown during 72 h until exponential growth phase. After that time, fungi biomass was transferred to distilled water with the use of bacterial loop and the suspension of fungi with the concentration of (C)10^6 microbial cells in 1 ml was obtained by serial dilutions.

Standardization of inoculants was carried out by direct calculation in Goryayev chamber or with the use of turbidimetric method on photoelectrical colorimeter KFK-2 by optical density (D).

Each of the test specimens was placed into the test-tube with respective nutritious media, where the suspension of biotests was pre-inoculated. The tubes were hermetically sealed with cotton-gauze plugs and placed for incubation at room temperature with periodic shaking. Exposition time for *Escherichia coli* was 24 and 96 h, *Candida albicans* – 7 and 14 days.

3. Results and Discussion

In order to obtain antibacterial bioactive apatite glass-ceramic material the glass-ceramic material BS-11 was chosen, which was synthesized earlier on the base of calcium silicophosphate glass in Na_2O – CaO – R_2O_3 – RO_2 – P_2O_5 – SiO_2 system, where RO_2 – TiO_2, ZrO_2; R_2O_3 – B_2O_3, Al_2O_3 with content of SiO_2 from 45 to 55, P_2O_5 from 5 to 10; CaO from 15 to 25, content of TiO_2, ZrO_2, B_2O_3, Al_2O_3 was from 0 to 5 mol % and had the CaO/P_2O_5 rate of 4.

X-ray diffraction analysis showed that glass-ceramic material BS-11 is characterized by intensive hydroxyapatite crystallization after melting and thermal treatment and by low amounts of rutile and quartz. This material has P_m in albumin of 0.2 % and belongs to III hydrolytic class [12].

With the use of polymer matrix duplication method, a porous material PBSM-11 with developed structure, P_{real} = 56 %, W = 27 % and pore sizes of 100–700 μm (Fig. 1) was obtained on the base of the investigated glass-ceramic material.

After saturation of the investigated material PBSM-11 with 50 % chitosan aqueous solution for 1 day, porous composite material PBSM-11X was obtained to provide antibacterial properties.

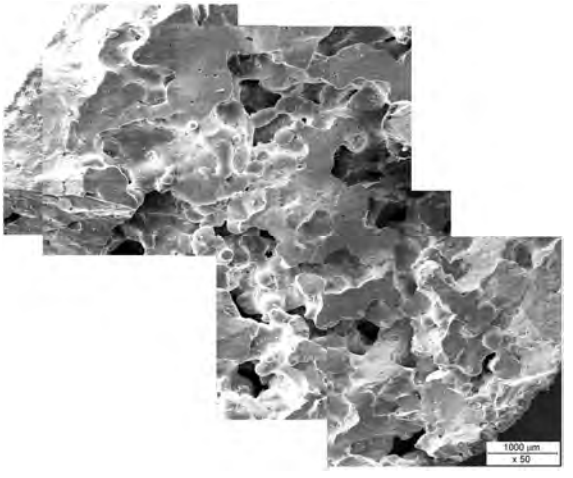

Fig. 1. Structure of investigated material PBS-11 by REM

Biocidal activity researches of the investigated porous composite material PBSM-11, material PBSM-11X saturated with chitosan solution and, for comparison, of corundum ceramics and titanium alloy OT4 used in bone endoprosthetics were conducted by quantitative method in liquid medium have shown that the investigated test-specimens manifest inhibiting properties towards *Escherichia coli* and vegetative cells of *Candida albicans* fungi.

The highest bactericidal properties towards *Escherichia coli* bacteria were shown by corundum ceramics and PBSM-11X. The concentration of the organism increased after 24 h by 8.8 and 5.3 times, and after 96 h – by 5 and 3.3 times (Table 1). For the test-specimens of titanium alloy OT4 and PBSM-11 the concentration of *Escherichia coli* bacteria increased after 24 h by 16 and 10 times and after 96 h – by 110 and 65 times, respectively. In the nutritious medium of tubes without test specimens the concentration of *Escherichia coli* $C_{culture}$ increased after 24 and 96 h by 42 and 280 times, respectively.

Fungicidal properties of the investigated melted corundum specimens and PBSM-11X were also the highest for the test materials. After 7 days of exposition concentration of vegetative cells of *Candida albicans* fungi in nutritious medium of tubes with test specimens increased by 9.4 and 8 times, respectively (Table 2). For the test-specimens of titanium alloy OT4 and PBSM-11 the concentration of *Candida albicans* fungi vegetative cells increased by 76 and 127 times, respectively.

After 14 days of exposition concentration of vegetative cells of *Candida albicans* fungi in nutritious medium of tubes with the test specimens of melted corundum increased by 16.9 and 14.4 times, respectively. For test-specimens of titanium alloy OT4 and PBSM-11 the concentration of *Candida albicans* fungi vegetative cells increased by 151 and 250, times respectively. By the nutritious medium of tubes without test specimens the concentration of *Candida albicans* vegetative cells $C_{culture}$ bycreased after 7 and 14 days by 140 and 260 times, respectively.

Table 1

Escherichia coli cell growth control in contact with investigated test specimens

Test specimen variant	Exposition start (0 h)		Exposition time, h			
			24		96	
	D	*C*, cells/ml	*D*	*C*, cells/ml	*D*	*C*, cells/ml
Corundum ceramics	0.05	$1.0 \cdot 10^4$	0.44	$8.8 \cdot 10^4$	0.024 (x10)	$0.5 \cdot 10^5$
Titanium alloy OT4	0.05	$1.0 \cdot 10^4$	0.08 (x10)	$1.6 \cdot 10^5$	0.055 (x100)	$1.1 \cdot 10^6$
PBSM-11X	0.075	$1.5 \cdot 10^4$	0.41	$8.0 \cdot 10^4$	0.025 (x10)	$0.5 \cdot 10^5$
PBSM -11	0.05	$1.0 \cdot 10^4$	0.052 (x10)	$1.0 \cdot 10^5$	0.32 (x10)	$6.5 \cdot 10^5$
$C_{culture}$	0.05	$1.0 \cdot 10^4$	0.21 (x10)	$4.2 \cdot 10^5$	0.14 (x100)	$2.8 \cdot 10^6$

Table 2

***Candida albicans* cell growth control in contact with investigated test specimens**

Test specimen variant	Exposition start (0 days)		Exposition time, days			
			7		14	
	D	*C*, cells/ml	*D*	*C*, cells/ml	*D*	*C*, cells/ml
Corundum ceramics	0.40	$2.35 \cdot 10^6$	0.38 (x10)	$2.23 \cdot 10^7$	0.68 (x10)	$3.99 \cdot 10^7$
Titanium alloy OT4	0.40	$2.35 \cdot 10^6$	1.0 ($x10^2$)	$1.78 \cdot 10^8$	1.72 ($x10^2$)	$3.55 \cdot 10^8$
PBSM-11X	0.40	$2.35 \cdot 10^6$	0.32 (x10)	$1.88 \cdot 10^7$	0.6 (x10)	$3.4 \cdot 10^7$
PBSM -11	0.40	$2.35 \cdot 10^6$	0.5 ($x10^2$)	$2.99 \cdot 10^8$	1.0 ($x10^2$)	$5.88 \cdot 10^8$
$C_{culture}$	0.40	$2.35 \cdot 10^6$	0.60 ($x10^2$)	$3.60 \cdot 10^8$	1.05 ($x10^2$)	$6.18 \cdot 10^8$

4. Conclusions

It was found that biocide properties of investigated glass-ceramic material PBSM-11X are caused by chitosan in its composition, which has inhibiting action towards pathogenic organisms. Antibacterial and fungicidal properties of corundum are related to its high bioinertness and, presumably, to its porous structure, which absorbs microorganisms. The presence of titanium dioxide on the surface of titanium alloy OT4 insignificantly increased its biocidal activity. The lowest biocidal properties of PBSM-11 glass-ceramic material are explained, in contrast, by its high bioactivity, namely by the content of calcium phosphates, which are the nutritious medium for microorganisms.

The investigated chitosan saturated apatite glass-ceramic materials, which have biocidal properties, are promising at production of bone endoprostheses in maxillofacial surgery. The use of implants on the base of the obtained materials will allow to decrease the possibility of inflammation processes through infection and shorten the terms of resorption of biomaterial with bone.

Inhibiting influence of chitosan in the composition of calcium silicophosphate glass-ceramic materials is manifested by active prolonged effect against pathogenic microorganisms *Candida albican* and *Escherichia coli.*

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БІОЦИДНІ АПАТИТОВІ СКЛОКРИСТАЛІЧНІ МАТЕРІАЛИ ДЛЯ КІСТКОВОГО ЕНДОПРОТЕЗУВАННЯ

Анотація. *Підтверджена перспективність використання хітозану при одержанні біоцидних апатитових склокристалічних матеріалів для кісткового ендопротезування. Проведена порівняльна оцінка біоцидних властивостей по відношенню до Candida albican, Escherichia coli матеріалів, які використовуються в кістковому ендопротезуванні. Використання імплантатів на основі кальційсилікофосфатних склокристалічних матеріалів, насичених хітозаном, дозволить знизити ймовірність запальних реакцій внаслідок інфекційного зараження та скоротити строки резорбції біоматеріалу з кісткою.*

Ключові слова: *кальційсилікофосфатні склокристалічні матеріали, кісткове ендопротезування, біоцидні властивості.*