Low-temperature-derived composites of bioactive nanoglass for biomedical applications

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INTRODUCTION

The subject of this work is a method of producing nanometric composite material based on bioactive glass (bioglass) in the three-component SiO2-CaO-P2O5 system, obtained by a low-temperature method without heating in a furnace and modified with titanium, zirconium and hafnium phthalocyanine complexes. This material, which is an example of bioactive ceramics, can be used as a material for implantation applications in orthopedics and dentistry to fill bone defects and tooth canals. Bioactive glasses (BGs), considered as the third generation biomaterials, are a promising type of materials for bone tissue regeneration due to their generally excellent osteoconductivity, osteostimulation, and degradation rate. The four-component glass, called 45S5 bioglass reported by Hench is still considered to be the gold standard for bioactive glasses, although bioactive glass obtained by the traditional melt-quenching method has a number of limitations. The need to use a high temperature (greater than 1300 °C) during production and the lack of microporous structure inside the materials with a low specific surface area represent some of them. Today, bio-glasses are mainly obtained by the sol-gel method, which allows to obtain a material with the same properties, but at much lower temperatures. The first reports of obtaining bioglass using the solvothermal method have appeared recently. But even when using these methods, the temperatures at which the final processing of the materials takes place after synthesis remain quite high, above 500ºC, which prevents their possible modification with organic compounds (including, for example, antibiotics or other bioactive compounds that suppress osteosynthesis). In this work, we propose a completely new approach to obtaining SiO2-CaO-P2O5 ternary bioglass based on the sol-gel technology but on the reverse micelle method, and the material obtained in this way does not require additional thermal treatment, while maintaining the requirements for this type of materials. . An additional novelty is that these materials have been additionally modified with group IV metal chloride phthalocyanine complexes (Ti, Zr, Hf), which are distinguished by high thermal and chemical stability, and as most metal phthalocyanine complexes are capable of generating singlet oxygen under the influence of light red and near infrared. By this we wanted to achieve two main goals: 1. to accelerate the formation of apatite when the material is exposed to light within the "biological window"; 2. creating, also under the influence of light in the range of 650-950nm, an environment unfavorable for the development of pathogenic microflora (antibacterial photodynamic therapy).

SYNTHESIS OF NANOSIZED CaO

Nanosized CaO was obtained by standard Peccini method from $Ca(NO_3)_2$. General procedure was the next: 5g of calcium nitrate was dissolved in 10ml of demineralized and deionized water. Then 10g of citric acid and 5 ml of ethylene glycol were added do reaction mixture under vigorous stirring on magnetic stirrer. The reaction mixture was left on the magnetic stirrer for another 2h, then it was transferred to a ceramic crucible, placed in a laboratory dryer at 90°C and allowed to gel. After gelling, the gel was fired at a temperature of 700°C for 2h.

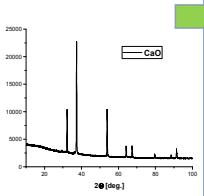


Fig. 1 XRD pattern of obtained CaO nanopowder.

SYNTHESIS OF MPc MODIFIED 70%SiO_2-25%CaO-5%P_2O_5 BIOGLASS BY INVERTED MICELLE METHOD

0.5g of CaO was dispersed in 12,3 ml of cyclohexane by ultra sonification

50ml round bottom flask, magnetic stirrer

+ 1ml Triton X100 1,04ml 1-hexanol

Stirring 15 min

+ 0,096ml H3PO4 dissolved in 0.3ml oh H2O

Stirring 15 min

+ 0,5 mmol of appropriate PcM was dispersed in 1 ml of TEOS by ultrasonification; added dropwise

Stirring 15 min

+ 1ml 25 solution of NH_4OH

Stirring 5 h

Keep in 5°C 72h

Settle the product with acetone (break the emulsion), separate with the ultracentrifuge, and wash first three times with ethanol, then three times with water.

The product was dried in a laboratory dryer with forced air circulation at 90°C for 72 h.

SYNTHESIS OF PHTHALOCYANINE COMPLEXES ($PcMCl_2$)

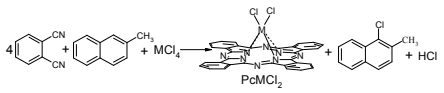


Fig. 2 Scheme of template synthesis of $M(IV)Cl_2$ phthalocyanine complexes, where M=Ti, Zr, Hf

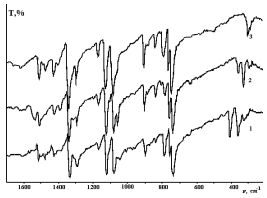


Fig. 3 FTIR spectra of $M(IV)Cl_2$ phthalocyanine complexes, where M=Ti (1), Zr (2), Hf (3) in KBr pellets

Substance	Abs. λmax (nm) in H_2SO_4	FTIR (cm^{-1})
$PcTiCl_2$	312, 435, 808	410, 370 ($\nu_{as,s}$ Ti – Cl)
$PcZrCl_2$	310, 445, 800	355, 330 ($\nu_{as,s}$ Zr – Cl)
$PcHfCl_2$	309, 445, 798	310, 285 ($\nu_{as,s}$ Hf – Cl)

Tab. 1 Absorbance and FTIR band maxima of $M(IV)Cl_2$ phthalocyanine complexes, where M=Ti (1), Zr (2), Hf (3)

STRUCTURE AND MORPHOLOGY MEASUREMENTS - *XRD*

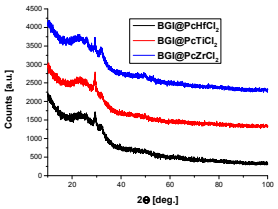
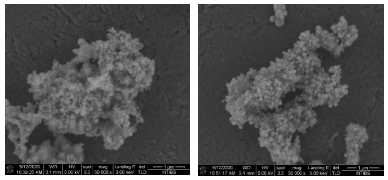


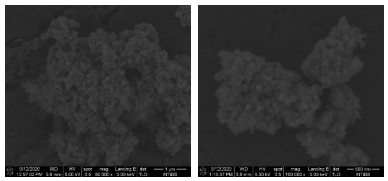
Fig. 4 X-ray diffraction patterns of the obtained modified bioglass

STRUCTURE AND MORPHOLOGY MEASUREMENTS – *SEM/EDX*

Figs. 5 SEM images of $BGl@PcTiCl_2$.



Figs. 6 SEM images of $BGl@PcZrCl_2$.



Figs. 7 SEM images of $BGl@PcHfCl_2$.

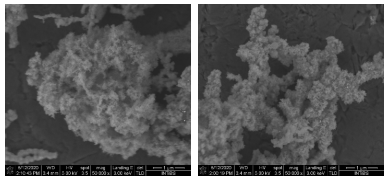


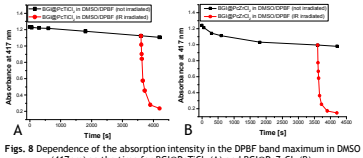
Table 2 Average Wt% values of individual elements determined in various fields.

BGl@$PcTiCl_2$	Calculated	Field 1	Field 2	Field 3	Found (average)
O	47.23	47.53	46.11	47.13	46.92
Si	32.73	35.11	36.23	33.77	35.04
P	1.15	1.05	0.92	1.01	0.99
Ca	17.85	16.26	16.72	18.03	17.01
Ti	0.05	0.05	0.02	0.06	0.04
BGl@$PcZrCl_2$	Calculated	Field 1	Field 2	Field 3	Found (average)
O	47.23	47.34	46.98	48.29	47.55
Si	32.73	36.97	29.30	34.45	33.57
P	1.15	1.25	2.25	1.46	1.65
Ca	17.85	14.43	21.23	15.75	17.14
Zr	0.1	0.11	0.14	0.05	0.1
BGl@$PcHfCl_2$	Calculated	Field 1	Field 2	Field 3	Found (average)
O	47.23	46.24	45.21	47.44	46.30
Si	32.73	37.10	29.81	35.41	34.10
P	1.15	0.69	3.04	0.60	1.44
Ca	17.85	15.78	21.67	16.35	17.93
HfZr	0.2	0.18	0.27	0.2	0.22

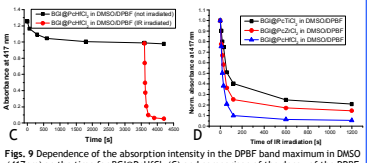
SPECTROSCOPIC PROPERTIES:

Detection of ROS generation

The generation of reactive oxygen species (ROS) in the suspension of the investigated materials was carried out using the reaction in which the 1,3-Diphenylisobenzofuran (DPBF) dye is discolored when interacted with singlet oxygen, and these changes can be registered using absorption spectroscopy. It should be noted that the tested modified bioglass has a mesoporous structure with a very well-developed specific surface. That is why, the decrease in DPBF absorption can also be caused by the adsorption of the dye in the pores of the material. Therefore, the material was first incubated in the presence of DPBF for 1 hour (the absorption spectra were also recorded during the incubation), after which the sample was irradiated with an infrared lamp. Philips 150 W lamp with wavelength range between 550 and 900 nm with the maximum at around 700 nm was used as red/NIR irradiation light source.



Figs. 8 Dependence of the absorption intensity in the DPBF band maximum in DMSO (417nm) on the time for $BGl@PcTiCl_2$ (A) and $BGl@PcZrCl_2$ (B).



Figs. 9 Dependence of the absorption intensity in the DPBF band maximum in DMSO (417nm) on the time for $BGl@PcHfCl_2$ (C) and comparison of the decay of the DPBF absorption band at irradiation with an IR lamp for all obtained materials. .

As shown in Figure 9D, $BGl@PcHfCl_2$ showed the highest ability to generate ROS among the tested materials. This is why the material was transferred to the next stages of research. But before that, we also checked whether this material would also generate ROS in a buffer that would be used downstream to biotransform bioglass. In aqueous solutions, the DPBF spectrum has a different shape, at the maximum of the characteristic band it shifts towards longer wavelengths (from 417 nm for DMSO to 456-465 nm for aqueous solutions). However, as shown in Fig. 10, in DPBS, i.e. in conditions similar to those we have in the body, $BGl@PcHfCl_2$ is also characterized by a fairly intense generation of ROS under red light irradiation.

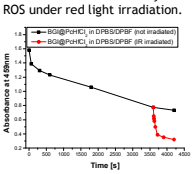


Fig. 10 Dependence of the absorption intensity in the DPBF band maximum in DPBS (459nm) on the time for $BGl@PcHfCl_2$.

SPECTROSCOPIC PROPERTIES:

Excitation and emission spectra

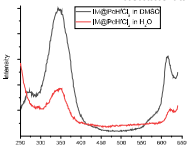


Fig. 11 Excitation spectra of $BGl@PcHfCl_2$ in DMSO and water suspension. A of detection was 697nm for DMSO and 707 nm for water.

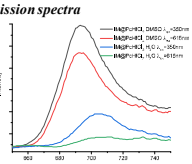


Fig. 12 Emission spectra of $BGl@PcHfCl_2$ in DMSO and water suspension. Excitation wavelengths was 350nm and 615 nm.

ANTIMICROBIAL ACTIVITY:

Contact bactericidal test for $BGl@PcHfCl_2$

General procedure: glass samples were "soaked" with a small volume of bacterial suspension (E. coli, S. aureus). BGl @ $PcHfCl_2$ showed very high bactericidal activity, where more than 95% of bacteria in the IR irradiated test were killed in <30 min. Without IR irradiation, bactericidal activity was also observed for this material, but in a much lower degree.

BIOGLASS BIOTRANSFORMATION TESTS

Immersion of SiO_2-CaO-P_2O_5-Pht powder in DPBS for 3, 7, 14 and 21 days at 37 °C

Placed under IR lamp for 15 min 3 times per day (2 samples without irradiation)

Replacement of DPBS every 3.5 days

After incubation time, washingof powder 3 times by deionized water and dried at 37 °C for 1 day

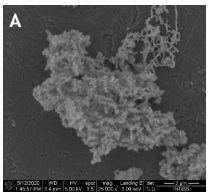
XRD and EDS analysis

1g of $BGl@PcHfCl_2$ was put into sealed 100ml plastic containers and 67ml DPBS was added. The tests were performed using the static method with DPBS (Dulbecco's Phosphate Buffered Saline) buffer exchange every 3.5 days in one series: 3, 7, 14 and 21 days and irradiated with an infrared lamp 3 times per day for 15 minutes, and for comparison, two samples were incubated without irradiation: 3 and 21 days. Buffer exchange: gently pipette the supernatant solution and add fresh DPBS buffer or if the solution above precipitate was not clear - centrifuged 4,000 rpm for 4 min. After the incubation time, the solution was pipetted or centrifuged at 4,000 rpm for 4 minutes; precipitate was washed 3 times with deionized water and dried in an oven at 37°C overnight. After that, the powder structure was analyzed by XRD method, and the material composition evaluated by EDS method on a scanning electron microscope.

Note: The samples fade after irradiation (turns from blue to white. After two rinses, buffer salts were not rinsed off, and still present in probes, which significantly hinders the assessment of hydroxyapatite signals in X-ray diffraction patterns.

Table 3 EDX determined At% values of individual elements and their ratio in time of biotransformation.

		With IR irradiation				Without IR irradiation	
	0	3	7	14	21	3	21
Si	56,97667	54,59667	47,61667	26,55333	7,976667	47,22333	6,23
P	5,43	23,65	31,39667	45,24667	54,10333	27,78667	57,12333
Ca	37,59667	21,75333	20,98333	28,2	37,91667	24,98667	36,64667
Changing the atomic ratio of the basic elements in the material during biotansformation tests							
	0	3	7	14	21	3	21
Ca : P	6,92388	0,919803	0,66833	0,62325	0,700819	0,899232	0,641536
Ca : Si	0,659861	0,398437	0,440672	1,062014	4,753448	0,529117	5,88229
P : Si	0,095302	0,433177	0,659363	1,703992	6,7827	0,58841	9,169074



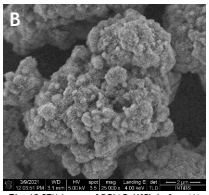
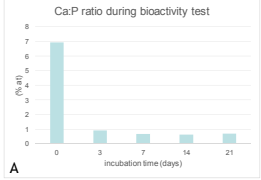


Fig. 13 SEM image of $BGl@PcHfCl_2$ before (A) and after (B) biotransformation tests



Figs. 14 Diagrams of changes in atomic ratios (At%) of the main components of the bioglass Ca:P (A) and P:Si (B) in the tested material during biotransformation.

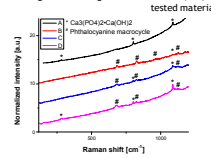


Fig. 15 Raman spectra of $BGl@PcHfCl_2$ durig biotransformation with IR irradiation: before (A), after 3 days (b), 7 days (C) and 21 gays (D)

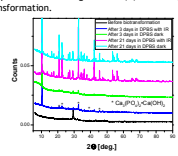


Fig. 16 XRD patterns of $BGl@PcHfCl_2$ durig biotransformation with and without IR irradiation

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