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Biocompatible glass composite system – some physical-mechanical properties of the glass composite matrix system

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In this work there are discussed the physical-mechanical properties of the glass $CaO-SiO_2-P_2O_5-Na_2O$ system (FB3) assigned for the glass composite matrix system using the following research methods: spectral chemical analysis (XRF), S_{BET} specific surface area analysis, XRD investigation, observation with a scanning electron microscope (SEM), wettability of the submicrocrystalline sintered corundum (ssc) by the glass system, microhardness test and DTA measurement. It was found that theoretical oxide chemical composition was close to that obtained from the spectral chemical analysis (XRF), the prolongated high energetic milling of the glass system did not have any significant influence on the specific surface area of grains (from 0.9159 m^2/g after 5-hour milling to 1.9241 m^2/g after 20-hour milling process only), in comparison to the specific surface area of the ssc, wettability investigation of the submicrocrystalline sintered corundum by the glass FB3 system showed the value of contact angle ($\theta < 45°$), and the microhardness value of about 6 GPa. On the basis of DTA results the sintering temperature of bioglass composite with the strengthening phase from the submicrocrystalline sintered corundum was determined and, using the previous experience, the way of producing composite was proposed. The calculation of the thermodynamic stability of the glass system-strengthening phase by VCS algorithm showed the presence of 4–5 solid compounds. The results of the fibroblast (cell line CCL 110, Promochem LG) preliminary culture investigation on the bioglass composite substrate were positive. The best results were obtained in the case of the biocomposite with the smallest amount of strengthening phase.

Keywords: glass of $CaO-SiO_2-P_2O_5-Na_2O$ system, submicrocrystalline sintered corundum, bioglass composite, XRF, XRD, DTA, contact angle, VCS algorithm, fibroblast culture.

1. Introduction

Glassy biocompatible composites form a new generation of ceramic materials for use in tissue engineering [1–5]. Inorganic, polymer and hybrid biomaterial substrates can form two- or three-dimensional scaffolds on which cells (*e.g.*, fibroblasts) are

planted and bred in vitro and subsequently this material-cell product is implanted. The fundamental criteria for suitability of the substrates have been thus formulated [4, 6–8]:

– The substrate should contain interconnected pores of such sizes that would favor integration of the cells, and subsequently tissues and their vascularity.

– They should have appropriate chemical properties of bioactivity and non-toxicity which favor the attachment of cells to the substrate, their differentiation and multiplication. Also, mechanical properties similar to the natural ones are required, specifically, resistance to stretching and twisting, hardness and Young's modulus.

– They must not produce unwanted reactions such as inflammation.

– They should be easily produced in various shapes and sizes.

Taking account of all these requirements, substrate materials were synthesized, including biocompatible glassy composites. Glassy composites, comprising properties of their constituent materials, allow the attainment of unique properties, such as: high mechanical strength, resistance to fracture, high biocompatibility and bioactivity. The biological activity of glasses and glass-ceramics depends on their chemical composition and results from the specific nature of the glassy substance contained [8–11].

The ability of bioactive glasses and glass-ceramic implants to bond to bone tissue forms the subject of numerous investigations involving in vitro observations of changes on the material surface caused by solutions simulating human blood plasma (SBF). It was shown that for materials such as Bioglass, Ceravital, and glass-ceramics, hydroxylapatite Cerabone, bonding with the living bone material starts by the formation of a surface layer enriched in calcium and phosphorus, which forms a hydroxyapatite containing a carbonate molecule with a deformed structure, similar to that of bone apatite [3, 7, 12, 13].

2. Subject and research methodology

The research aims at a glass matrix composite from the glass of $CaO–SiO_2–P_2O_5–Na_2O$ system and the bioglass composite with the strengthening phase of submicrocrystalline sintered corundum added with the amounts of 10, 20, and 30 vol% designed for the substrate of human skin fibroblast culture.

The glass of $CaO–SiO_2–P_2O_5–Na_2O$ system (FB3) was obtained by traditional method (heat treatment and fritting process) from previously precisely mixed raw materials.

Further, the glass system powder was subjected to the high energetic milling for 5, 10, 15, 20 hours in the Fritsch type milling grinder in the weight proportion of balls to grains 10:1 and the addition of water as a sliding substance. The glass samples after specific time (5, 10, 15, 20 hours) were removed from the chamber and subjected to further research procedures.

The measurement of the specific surface area S_{BET} was done using the special multifunctional apparatus of ASAP2010 of Micrometrix. The specific surface area

S_{BET} was determined by the physical adsorption of nitrogen at nitrogen's liquefaction temperature (77 K) with the Brunauer–Emmett–Teller equation.

The specific density measurements of the glass FB3 system were done in the helium picometer of Accu Pyc 330.The average grain size based on the specific density and specific surface area data was calculated using the following equation:

$$2r = \frac{6}{S_{BET}\,\rho}$$

where: $2r$ – diameter of the grains, S_{BET} – specific surface area of grains, ρ – specific density of the glass FB3 system.

The obtained glass powder was subjected to spectral analysis by XRF method. The X-ray investigation was carried out on the X'Pert diffractometer of Panalytical Company using the Cu lamp in the 2θ angle range of 10–90°.

Microscopic observation of the glass system and bioglass composite was carried out on the scanning electron microscope of the Joel Company of JSM6460 LV at low vacuum and accelerating voltage of 20 kV at magnifications of 10, 100, 1000.

DTA investigation of the glass FB3 system was carried out using the Derivatograph 1500 D apparatus by heating the sample in the platinum crucible with 10 °C/min speed to 1000 °C in the air atmosphere.

The wettability investigation of the submicrocrystalline sintered corundum substrate by the glass FB3 system was carried out under the high temperature microscope of MHO2 Leitz–Watzler type by the sessile-drop method in the air atmosphere.

Microhardness test was performed using the FM7 detector under a 100 g loading. On the basis of DTA of the glass FB3 system investigation the sintering temperature of glass matrix composite system with strengthening phase was carried out and using the previous experience the way of obtaining the bioglass composite was proposed. Thermodynamic stability of the glass FB3 system-strengthening phase (10, 20, 30 vol% of submicrocrystalline sintered corundum) was calculated using VCS algorithm.

On the samples of the bioglass composite (ϕ 10×4 mm), after proper preparation of surface area (quasi-polished section) and sterilization (12 hours in a 70% alcohol solution and 2 hours of exposure of each side of the sample to UV lamp), fibroblasts from human skin (cell line No CCL 110, Promochem LG) were cultured in DMEM medium (Dulbecco's Modified Eagle Medium, Sigma) containing 5% of fetal bovine serum and a 1% mixture solution of antibiotics (streptomycin, neomycin and penicillin). The results of investigation are discussed below.

3. Results and discussion

The glass of the $CaO–SiO_2–P_2O_5–Na_2O$ system (FB3) obtained by fritting process at a temperature of 1350 °C was characterized by low viscosity, absence of gas bubble and milk-amber color (Fig. 1).

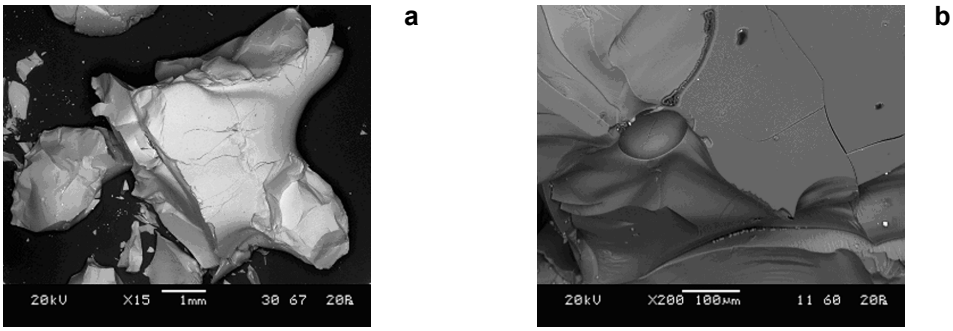

Fig. 1. The scanning electron microscope images of the glass $CaO-SiO_2-P_2O_5-Na_2O$ (FB3) system after fritting process; magnified 15× (**a**), magnified 200× (**b**).

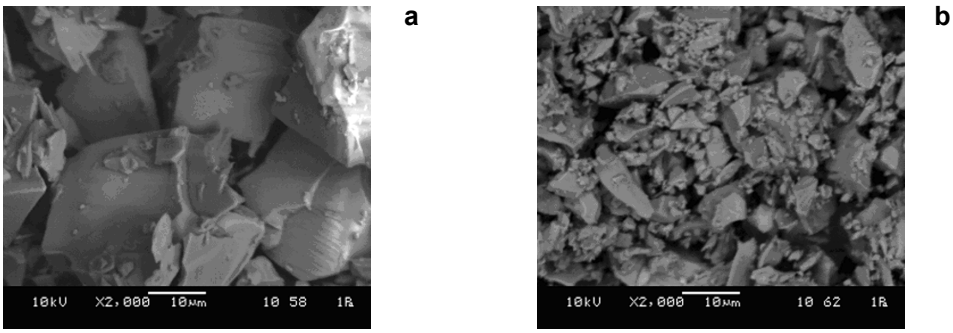

Fig. 2. The scanning electron microscope images of the glass $CaO-SiO_2-P_2O_5-Na_2O$ (FB3). FB3 glass system after 5-hour milling (**a**), FB3 glass system after 20-hour milling (**b**).

The glass powder was milled for 5, 10, 15, 20 hours (Fig. 2) in the Fritsch planetar ball grinder. It was found that prolongated high energetic milling of glass FB3 system did not have a significant influence on the increase of specific surface area of the glass FB3 system (S_{BET} 0.9159 m^2/g after 5-hour milling, 1.9241 m^2/g after 20-hour milling) in comparison to the specific surface area changes of the submicrocrystalline sintered corundum (0.1 m^2/g for unmilled sample and 16.4 m^2/g after 30-hour milling). This can be explained by the specific structure of the glass FB3 system and big cohesive interactions.

Because of this fact the granulometric analysis of grains was difficult to perform. The calculations of average grains size from the equation showed that the grain size of the glass FB3 system was decreased 2.5 times only (Tab. l).

The density of the glass FB3 system was determined by the helium method at the AccuPyc 330 picometer and had the value of 2.6554 g/cm^3.

Microhardness test was done using the FM7 detector under a 100 g loading. In Tab. 2 and Fig. 3, the results of microhardness of the glass FB3 system (about 6 GPa) are presented.

T a b l e 1. The results of specific surface area and average grain size after prolongated high energetic milling of the glass FB3 system.

Milling time [h]	Specific surface area [m^2/g]	Average grain size [μm]
5	0.9159	2.47
10	0.9553	2.36
15	1.1964	1.89
20	1.9241	1.17

T a b l e 2. The microhardness measurement of glass FB3 system.

Sample	HV	HVav	Standard deviation of single measurement	Average standard deviation	Uncertainty of HVav for $t(\alpha = 0.005, n-1 = 4)$	
	[–]	[–]	[–]	[–]	+/–	%
FB3	560	568	8.4	3.7	10.4	1.8

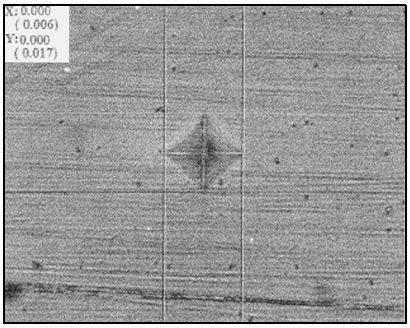

Fig. 3. The microhardness indents of the glass FB3 system.

To fulfill the requirements of the XRF apparatus assigned for spectral chemical analysis, the glass powder milled for 10 hours was used. The method, which is simple and cheap, allowed the chemical oxide composition to be precisely determined (Tab. 3). The obtained values of spectral chemical oxide composition were close to those of the theoretical calculations.

The X-ray investigation showed absolutely amorphic structure with increasing the background in the low angle range.

T a b l e 3. Chemical oxide compositions of glass system, spectral chemical analysis [wt%].

Oxide composition of glass system	Calculated composition	Spectral chemical analysis
CaO	19.200	19.0
SiO_2	54.145	52.9
P_2O_5	5.912	5.23
Na_2O	20.706	20.0
Additives	< 0.37	< 2.67

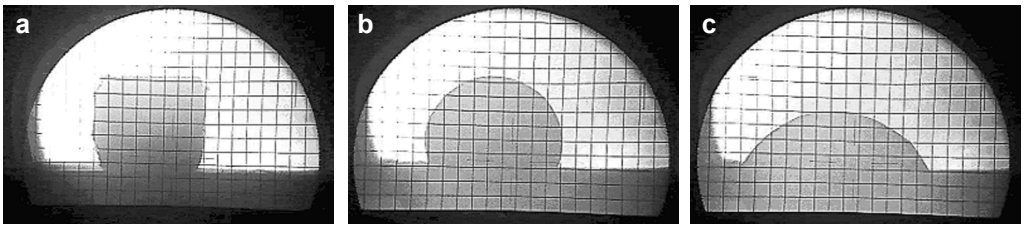

Fig. 4. The wettability of submicrocrystalline sintered corundum by glass FB3 system: 101 °C (**a**), 958 °C (**b**), 1010 °C (**c**), $\theta < 45°$.

The wettability investigation of submicrocrystalline sintered corundum substrate by the glass FB3 system showed that contact angle was proper and low at elevated temperature ($\theta < 45°$, Fig. 4).

The DTA research (Fig. 5) allowed determination of the vitrification temperature (T_g = 525.2 °C) and dilatometric point temperature (T_d = 711.9 °C). On the basis of the above investigation the sinter temperature of bioglass composite with the strengthening phase was initially estimated.

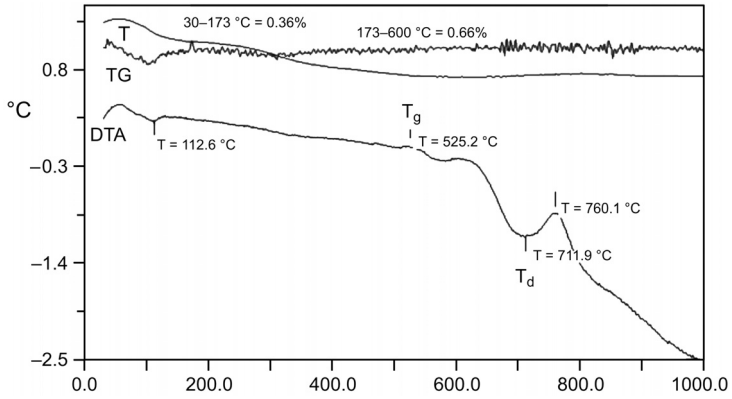

Fig. 5. DTA of the glass $CaO-SiO_2-P_2O_5-Na_2O$ (FB3) system.

A production technology comprising cold pressing and traditional heat treatment or cold pressing, isostatic densification and traditional heat treatment was developed.

The theoretical calculation of the thermodynamic stability of the glass FB3 system-strengthening phase with the submicrocrystalline sintered corundum (ssc-amount of 10, 20, 30 vol%) was carried out by the VCS algorithm (Tab. 4).

Using the VCS algorithm, theoretical calculations were made of the thermodynamic stability of the system FB3 glass – 10, 20 and 30 vol% submicrocrystalline sintered corundum strengthening phase. It was found that amongst some 100 possible compounds, probably stable are 4–5 compounds in the solid phase (Tab. 4). From both hypotheses it follows that, for all of the three types of composite present,

T a b l e 4. The thermodynamic stability of bioglass composites with additives of ssc – VCS algorithm calculation.

Glass FB3 system (90 vol%) + ssc (10 vol%)				
Temperature	570	570	590	590
Assumption	I	II	I	II
Na_2SiO_3	2.868	2.868	2.868	2.868
$CaSiO_3$	4.104	4.104	4.104	4.104
$Ca_3(PO_4)_2$	0.786	0.786	0.786	0.786
$NaAlSiO_4$	6.59	6.59	6.59	6.59
$NaAlSi_3O_8$	1.148	1.148	1.148	1.148
Glass FB3 system (80 vol%) + ssc (20 vol%)				
Temperature	570	570	590	590
Assumption	I	II	I	II
$Ca_3(PO_4)_2$	0.698	0.698	0.698	0.698
$NaAlSiO_4$	11.975	11.975	11.975	11.975
$Ca_2Al_2SiO_7$	0.241	0.241	0.241	0.241
$Ca_3Al_2Si_3O_{12}$	0.965	0.965	0.965	0.965
Glass FB3 system (70 vol%) + ssc (30 vol%)				
Temperature	570	570	590	590
Assumption	I	II	I	II
Al_2O_3	4.554	4.554	4.554	4.554
$Ca_3(PO_4)_2$	0.611	0.611	0.611	0.611
$CaAl_4O_7$	0.448	0.448	0.448	0.448
$NaAlSiO_4$	10.478	10.478	10.478	10.478
$Ca_3Al_2Si_3O_{12}$	0.914	0.914	0.914	0.914

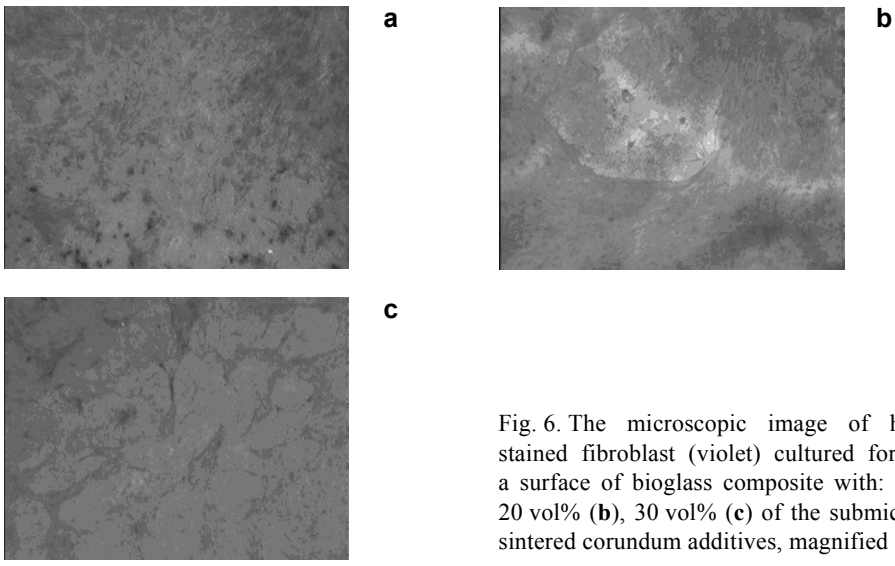

Fig. 6. The microscopic image of haematoxylin stained fibroblast (violet) cultured for 14 days on a surface of bioglass composite with: 10 vol% (**a**), 20 vol% (**b**), 30 vol% (**c**) of the submicrocrystalline sintered corundum additives, magnified 6×.

there are the following compounds: $NaAlSiO_4$ solid state and $Ca_3(PO_4)_2$ in the liquid state.

Practical verification of the presence of these compounds in the biocomposites will be made by X-ray methods in the next stage of the work.

The fibroblasts from human skin (cell line No CCL 110, Promochem LG) were cultured, after the appropriate surface preparation, on the three variants of bioglass samples (ϕ 10×4 mm). After 14 days of growth, the cells were fixed using cold acetone (4 °C) for 10 minutes and stained with haematoxylin to visualize cell nucleolus. The optical microscope images showed the largest number of fibroblast cells to be present on the surface of the sintered composite containing 10 vol% of submicrocrystalline corundum. A felt-like layer of fibroblasts was established, which, during the cleaning process, was not separated from the surface. With increasing amount of the strengthening phase, submicrocrystalline sintered corundum (20%, 30%), the number of the cultured fibroblast cells decreased (Fig. 6). This observation will be possible to explain after examination of the surface topography (roughness, open and closed porosity) of the sintered glassy biocomposites.

4. Resume

The glass of $CaO-SiO_2-P_2O_5-Na_2O$ system (FB3) has proper physical-mechanical properties (microhardness – 6 GPa, contact angle to submicrocrystalline sintered corundum substrate $\theta < 45°$) and biocompatibility (the fibroblast culture results) which fulfill the application criteria for biocomposites.

The theoretical oxide chemical composition of the glass system was the same as that resulting from the spectral chemical analysis (XRD) taking into consideration volatility of phosphates at a 10% level.

It was found that prolongated high energetic milling of glass system, did not have any significant influence on the specific surface area of grains (from 0.9159 m^2/g after 5-hour milling, to 1.9241 m^2/g after 20-hour milling process) and decrease of the average value of grains (from 2.47 μm to 1.17 μm).

The wettability (wetting) investigation of the submicrocrystalline sintered corundum by the glass system showed the value of contact angle ($\theta < 45°$).

On the basis of DTA results the sintering temperature of biocomposite was determined and using the previous experience the way of producing biocomposite was proposed.

The calculation of the thermodynamic stability of the glass system strengthening phase from submicrocrystalline sintered corundum by VCS algorithm showed the presence of 4–5 solid compounds, whose verification by XRD method will be performed in the next stage of the work. The results of the preliminary fibroblast growth on the surface of bioglass composite substrates were positive showing the biocompatibility of these surfaces. The best results were obtained in the case of the biocomposite substrate with the smallest amount of the strengthening phase.

5. Conclusions

On the basis of the research work performed it can be concluded that:

– The proposed glass of the $CaO-SiO_2-P_2O_5-Na_2O$ system (FB3) fulfills the application criteria for glass matrix composite because of the proper physical--mechanical properties (microhardness, wettability of submicrocrystalline sintered corundum, temperature stability) and biocompatibility (the fibroblast culture results).

– Verification of the thermodynamic stability of compounds calculated by VCS algorithm based on XRD and DTA investigation is necessary.

– Investigation of the influence of the way of producing bioglass composite (cold sintering and traditional heat treatment or cold sintering with isostatic densification and traditional heat treatment) on the topography of the surface area (roughness, porosity) is necessary.

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