In vitro structural changes on the surface of $SiO_2 – CaO – P_2O_5$ bioactive glasses

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Bioactive glasses are of particular interest for biomedical applications. This is due to the surface reactivity of these glasses, especially their ability to form active and strong interface with biological tissue. In this paper we present the results from the synthesis of bioactive glass composition, in the ternary system $CaO-P_2O_5-SiO_2$, obtained by sol – gel process. Also, have been followed the structural changes that occur on the glass surface after soaking in simulated body fluid for 3, 7 and 14 days. Analyses carried out by Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM) to the soaked samples highlights formation of new phosphocalcic phases such apatite on the glass surface. TG/DSC analysis reveals thermal stability of glass powders analyzed at high temperatures.

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1. Introduction

Sol - gel process allowed obtaining a glass with improved bioactivity compared to material made by melting of components. This is caused by the porous nature of the materials used as precursors [1].

Bioactive glass obtain by sol – gel process was applied since the 1990s. This new class of bioactive glasses have a wide range of biocompatibility [2].

Bioactive glasses obtained by sol – gel method can be placed in the binary system $CaO - SiO_2$ [3, 4], ternary $SiO_2 - CaO - P_2O_5$ [5 - 7] or quaternary $SiO_2 - CaO - P_2O_5 – MgO$, $SiO_2 - CaO - Na_2O- P_2O_5$ [8 - 12]. All the glasses have some degree of bioactivity, after immersion in human fluids. Glasses in the system $SiO_2 - CaO - P_2O_5$ can form a hydroxyapatite layer on their surface compositions in terms of the proportion of silica less than 90% [13, 14]. This process involves the synthesis of inorganic network by mixing alkoxide solutions, followed by hydrolysis, gelation and heat treatment at a low temperature in order to obtain a glass [15].

Ability to change the network structure is inherent in this process by monitoring the hydrolysis and polycondensation reactions. Thus, structural changes can be made without changes in the chemical composition, because the glasses can be obtained from gels heat treated at temperatures below 700^0C [16].

Most inconveniences that are the result of heat treatment performed at high temperature can be eliminated through control of purity. Also, the process sol - gel provides potential benefits in obtaining of bulk materials with a wider range of bioactivity and better control of it, by controlling the composition or microstructure, after careful monitoring of heat treatment parameters [17, 18].

Thus, maturation processes that occur at low temperature, have a positive influence on certain characteristics, such as porosity and specific surface of glasses obtained by sol – gel method [19, 20].

The aim of this work was to analyze the structural changes occurring on the surface of glass powders synthesized by sol – gel process, before and after immersion in simulated body fluids (SBF). In this sense have been conducted a series of tests that follow the thermal behavior of glass powders at different temperatures. Also, structural changes on the glass surface were analyzed using Fourier transformed infrared spectroscopy (FTIR) and Scanning electron microscopy (SEM).

2. Material and methods

2.1. Glass synthesis

Raw materials used in the synthesis of 50 SiO_2 – 46 CaO – 4 P_2O_5 (in mol %) glass were tetraethylorthosilicate ($Si(OC_2H_5)_4$), calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$), triethylphosphate ($(C_2H_5)_3PO_4$). As hydrolysis catalyst precursors based on calcium and phosphorus was used hydrochloric acid (HCl).

Experimental procedure that describes the synthesis method of this glass composition has been shown in other works [18, 21].

Briefly, in case of this bioactive glass composition, noted S1, synthesis was performed by hydrolysis and polycondensation of 54 ml tetraethylorthosilicate, 4 ml triethylphosphate and 51.6 g of calcium nitrate tetrahydrate.

Hydrolysis reaction of silicon precursor (TEOS) was achieved by adding Si $(OC_2H_5)_4$ in 28 ml ultrapure water and 5 ml HCl (2N).

Gelation, the second stage of glass synthesis assumed interconnection colloidal particles (sol) in a three-dimensional network. In this case, the sols were placed in appropriate containers, which in turn were placed in a glass container, to obtain a gel under ambient temperature.

In the next stage of the synthesis gelation and maturation are completed. This phenomenon is associated with processes such as: polycondensation structure, gel contraction and expulsion of liquid from the pores of the gel as a result of decreased surface area. Gel forms have been introduced in an oven at a temperature of 60^0C for 2 days.

Gel drying heat treatment was conducted at a maximum temperature of 175^0C for 4 days. Thus, samples were removed from the polystyrene forms, and their drying was done in a covered glass container in the oven, in order to remove alcohol and water retained in the interconnected pores of the silica network. Porous network residual liquid remaining after evaporation is called xerogel. Dried gel was placed in an alumina crucible to be calcined. Calcination was carried out under atmospheric conditions at a temperature of 600^0C. The aim of this last stage was to stabilize the structure and removal of unwanted components.

In order to study the glass bioactivity, have been synthesized a composition of simulated body fluid [18, 21]. The resulting solution has a concentration of ions similar to human extracellular fluid in order to reproduce the germination and growth of apatite layer on the surface of bioactive materials in vitro.

2.2. Characterization techniques

Characterization of glass powders synthesized according to the experimental procedure presented in previous works [18, 21], has been achieved through the following analysis techniques: thermal analysis, Fourier transformed infrared spectroscopy analysis and scanning electron microscopy tehnique. Thermal analysis (thermogravimetry - TG and differential scanning calorimetry - DSC) was achieved through a simultaneous thermal analyzer Netzsch STA 449 F3 JUPITER. Fourier transformed infrared spectroscopy analysis - FTIR - was performed using a Bruker Tensor 27 spectrometer equipped with an ATR device. The infrared spectrum of glass powders analyzed was recorded in 4000 - 400 cm^{-1} range, at a resolution of 2 cm^{-1}. Morphological analysis was achived by using scanning electron microscopy tehnique. Thus, the microscopy tests were performed by using Field Emission - Scanning Electron Microscope FE-SEM Auriga model manufactured by Carl Zeiss. All tests were conducted under INCDIE ICPE-CA Bucharest.

3. Results and discussion

3.1. Thermal analysis of bioactive glass synthesized by sol – gel process

In Fig. 1 are presented the results of thermal analysis for bioctive glass powders synthesized by sol – gel process, in according to the experimental procedure described in previous works [18, 21].

The sample has been subjected to a heating cycle at a rate of 10 K / min., up to 1200^0C.

For data presented in Fig. 1, it is found that the mass loss occurred in four stages, after heating up to 1200^0C. In the first stage there is a mass loss of 2.61%, corresponding to the temperature range 25^0C - 290^0C, in the second stage, the mass loss was slightly increased (4.39%), which corresponds to the temperature between 290^0C and 660^0C, in the third stage there was a mass loss of 1.9% for the temperature 660^0C - 890^0C, and the last stage, the fourth, the mass loss was relatively low (0.48%) and corresponded to 890^0C - 1200^0C.

Thus, in case of bioactive glass denoted by S1, thermogravimetric analysis indicates a total mass loss of 9.38%, after heating cycle between 25 - 1200^0C.

Derivative thermogravimetric analysis (DTG) reveals an endothermic peak at 128.3^0C that corresponds to the loss of physically adsorbed water molecules on the surface of glass powders. Endothermic peak at 570^0C could correspond decomposition of calcium nitrate tetrahydrate $(Ca(NO_3)_2 \cdot 4H_2O)$, present in the glass composition synthesized and elimination of silanol groups, along with the stabilization of the glass structure. At temperatures above 600^0C, derivative thermogravimetric analysis chart has a relatively constant aspect.

3.2. Study of compositional changes on the glass surface

The evolution of apatite (carbonated hydroxyapatite) formation on the surface of synthesized glass powder has been also analyzed by Fourier transform infrared spectroscopy. Was taken into consideration formation and development of molecular groups such as phosphate (PO_4^{3-}) carbonate (CO_3^{2-}) and hydroxyl (OH^-) presents in the hydroxyapatite structure. Also has been analyzed molecular groups such as Si – O – Si, Si – O or Si – OH, specific of synthesized sol – gel glass structure.

In Fig. 2a are presented FTIR spectra of thermally stabilized glass structure at temperature of 600^0C, unsoaked in simulated body fluid. The peak located at 470 cm^{-1} is characteristic to bending vibration mode of Si – O, also peaks at 875 cm^{-1} and 1051 cm^{-1} corresponding to symmetric stretching vibration of Si – O, respectively, asymmetric stretching vibration of Si – O – Si bridges.

The presence of phosphate in glass structure was evidenced by the two peaks located at 568 cm^{-1} and 603 cm^{-1}, both encountered in case of P – O bending vibrations of PO_4^{3-} structure.

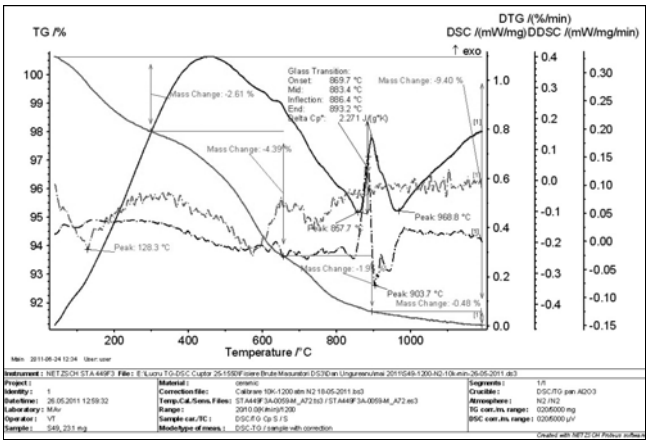

Fig. 1. Thermogravimetric analysis (TG) and differential scanning calorimetry (DSC) in case of glass powders S1.

The broaded band located at 3446 cm^{-1} corresponds to the stretching vibration of hydroxyl (O - H) common especially in case of silanol groups (Si – OH) and chemically bound water presents in the SiO_2 – CaO – P_2O_5 glass structure. The presence of H_2O molecules in the same glass structure has been confirmed by the peak at 1643 cm^{-1}.

Peaks located at 1471 cm^{-1} and 1384 cm^{-1} are characteristic to nitrate groups present in the structure of glass, through the use of Ca $(NO_3)_2 \cdot 4H_2O$ as a precursor in the synthesis of this material.

In case of glass powders immersed for 3 days in simulated body fluid, FTIR spectrum presented in Fig. 2b shows the presence of phosphate groups (PO_4^{3-}) through peaks located at 569 cm^{-1} and 604 cm^{-1}.

These correspond to bending vibration P – O – P of PO_4^{3-} tetrahedra. It is also highlighted the appearance of a new peak located at 962 cm^{-1}, corresponding to symmetric stretching vibration of P – O bonds.

The presence of three dimensional silicate network in the glass structure was evidenced by the peaks at 468 cm^{-1}, encountered in case of symmetrical bending vibration Si - O – Si bridges, 799 cm^{-1}, 873 cm^{-1} and 1093 cm^{-1}, specific to bending and symmetric stretching vibration of Si – O bonds, respectivly asymmetric stretching vibration of Si – O – Si bridges.

Carbonate groups (CO_3^{2-}) and hydration water in glass structure is evidenced by the presence of peaks located at 1427 cm^{-1} and 1643 cm^{-1}.

FTIR spectra of glass powders soaked for 7 days in simulated body fluid are showed in Fig. 2c. Data presented in spectrum shows the presence of silica on the surface of the sample analyzed. Thus, the peaks located at 468 cm^{-1} and 1089 cm^{-1} corresponding to symmetric bending vibration and asymmetric stretching vibration of Si – O – Si bonds, and those at 799 cm^{-1} and 873 cm^{-1}, corresponding to bending vibration and symmetric stretching vibration of Si – O bonds.

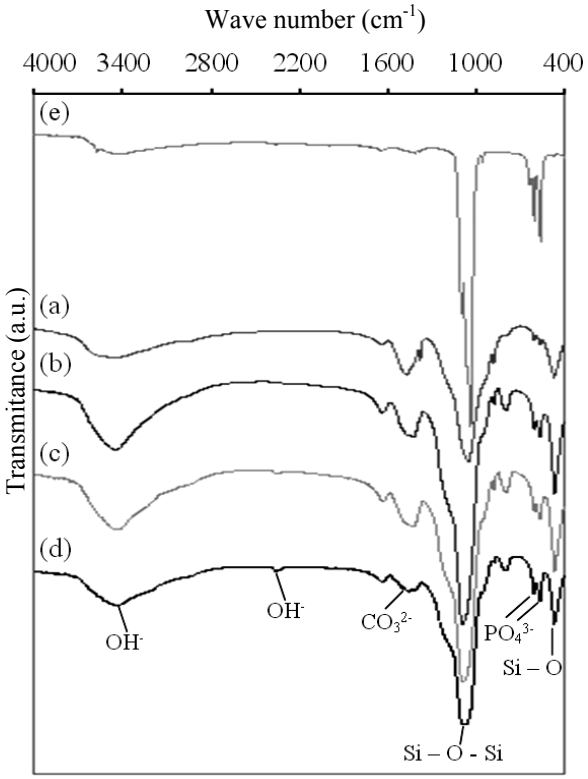

Fig. 2 FTIR spectra of sol –gel powers glass a) unsoaked; after soaked in simulated body fluid for: b) 3 days, c) 7 days and d) 14 days, e) hydroxyapatite.

The presence of phosphate groups on the surface of glass powders was highlighted by the presence of peaks located at 569 cm^{-1} and 603 cm^{-1} both typical for P – O – P bending vibration of PO_4^{3-}, 962 cm^{-1} corresponding to symmetric stretching vibration of P – O bond. Peaks at 3446 cm^{-1}, 2361 cm^{-1} and 1643 cm^{-1} confirm the presence of hydroxyl groups (OH-) and water of crystallization in glass powder structure. Also, the peak located at 1426 cm^{-1} corresponding to asymmetric stretching vibration of C – O bond, that confirms the presence of carbonate groups (CO_3^{2-}) in the same glass structure.

Fig. 2d shows FTIR spectra of S1 glass powders soaked for 14 days in simulated body fluid. Data presented in FTIR spectra shows the presence of large silica content.

This is confirmed by the peak at 468 cm^{-1}, which corresponds to the symmetric bending vibration of silicon - oxygen bonds. Also, can be seen the presence of peaks situated at 800 cm^{-1} and 875 cm^{-1} which correspond to bending vibration and symmetrical stretching vibration of Si – O – Si bridges.

Peaks placed at 2361 cm^{-1} and 1643 cm^{-1} highlights the existence of crystallization water in synthesized glass structure, noteworthy is the presence of these peaks in hydroxyapatite structure.

The peaks located at 568 cm^{-1}, 604 cm^{-1} and 962 cm^{-1} denote the presence of phosphate groups in glass structure. These peaks correspond to flexural vibration of P – O – P bonds, respectively P – O symmetrical stretching vibration of PO_4^{3-}. Moreover, the peak at 1426 cm^{-1} shows the

presence of carbonate groups (CO_3^{2-}) in glass powders structure immersed for 14 days in simulated body fluid.

By comparison in Fig. 2e are presented FTIR spectrum of dried hydroxyapatite.

3.3. Surface morphology analysis

This study was performed by using scanning electron microscopy tehnique (SEM). From SEM image obtained at a magnification of 250X (Fig. 3a), for glass powder soaked for 3 days in simulated body fluid can be observed glass particles characterized by dimensional unevenness and irregular shape. This is the result of insufficient processing of powders, associated with mechanical grinding stage.

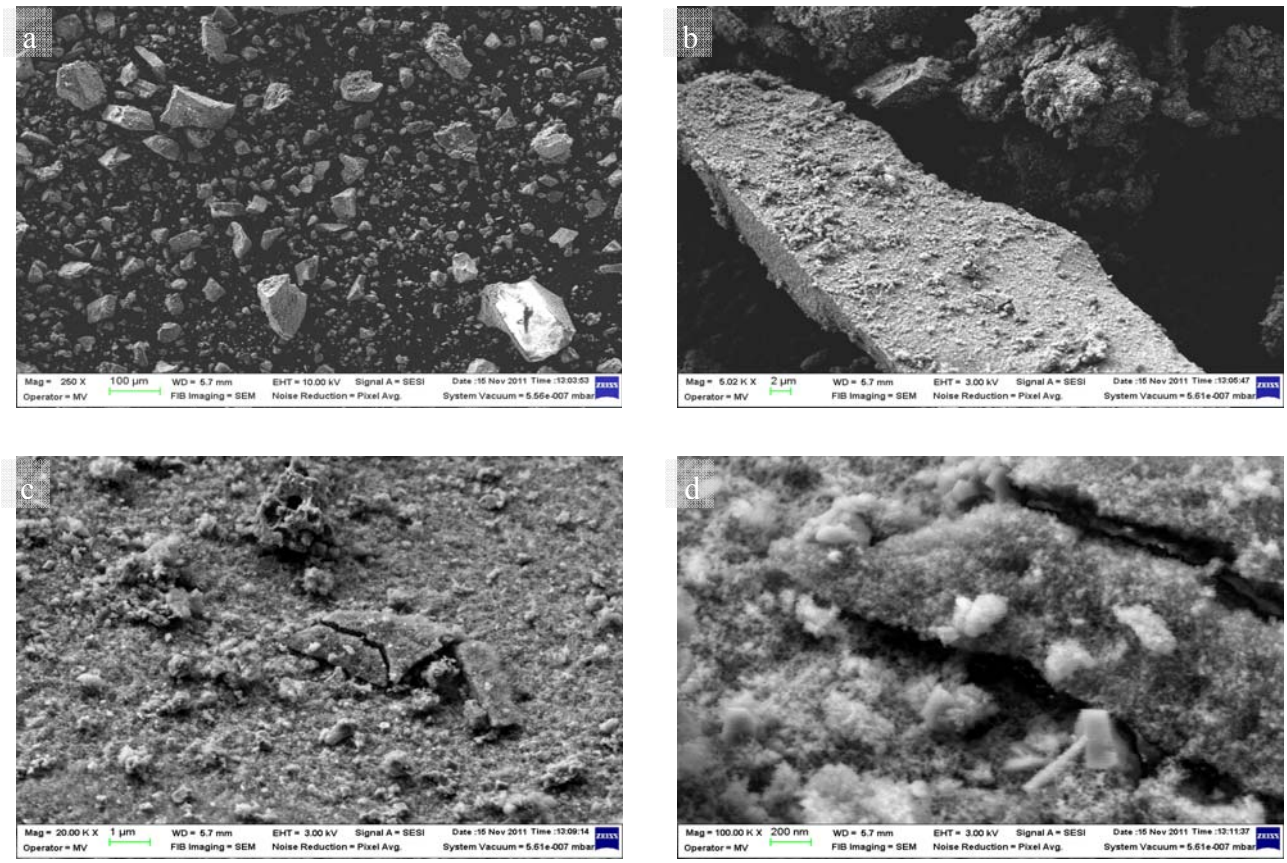

Fig. 3. SEM micrographs of S1 glass powders soaked for 3 days in simulated body fluids: a) Magnification: 250 X, b) Magnification: 5.000 X, c) Magnification: 20.000X, d) Magnification: 50.000X.

Apatite formation on the surface of glass powder has been studied on a particle, after a magnification of 5.000X, 20.000X and 100.000X. From the image shown in Figs. 3b, 3c şi 3d can be observed the irregular shape of the particle surface. This reveals synthesis of glass with high specific surface area, ideal for the germination and growth of apatite layer on the surface of bioactive glasses. At magnifications of 100.000X, SEM images (Fig. 3.d) show apatite formation on the surface of glass powder by the presence of agglomeration of crystals, attributed to formation of the first nuclei characteristic of this phosphocalcic compound.

Micrographs presented in Fig. 4a and 4b correspond to morphological analysis of the S1 surface glass soaked for 7 days in simulated body fluid. From the two figures we can see a significant increase in coverage of the glass surface with apatite crystals. At the same time there is a tendency of apatite crystallites to form agglomerations on the glass surface, with spheroidal shape.

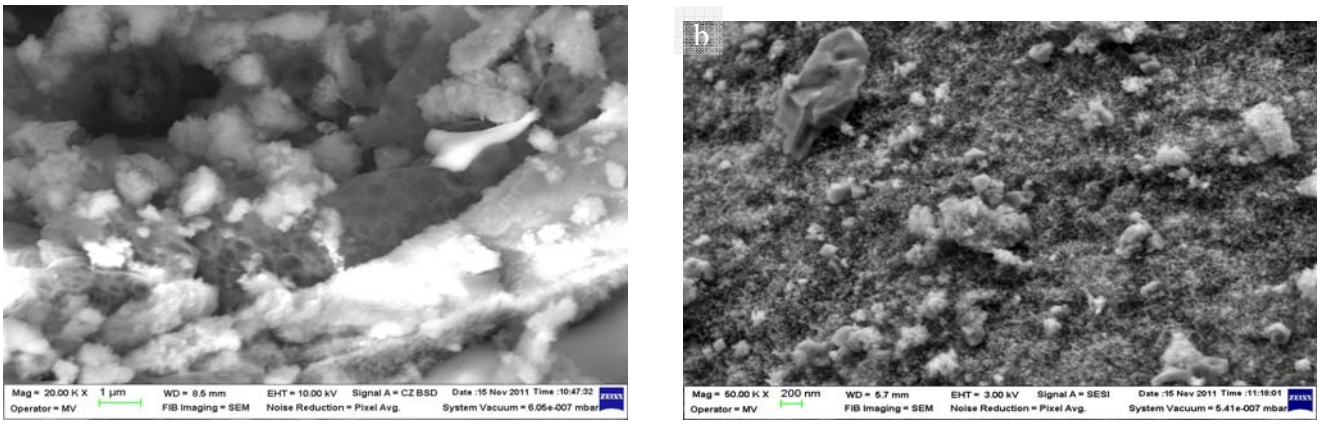

Fig. 4. SEM micrographs of S1 glass powders soaked for 7 days in simulated body fluid: a) Magnification: 20.000X, b) Magnification: 50.000X.

After immersing the S1 glass powders for 14 days in simulated body fluid has been observed large areas of powder particles covered with apatite crystallites (Fig. 5.a, b).

Degree of apatite formation on the glass surface is lower compared to other synthesized glass (SiO_2 = 58%). This is due to the lower reactivity of this material result of lower proportion of silica in composition (SiO_2 = 49%).

This statement is supported by the results obtained for X-ray diffraction analysis, presented in previous works [18, 21].

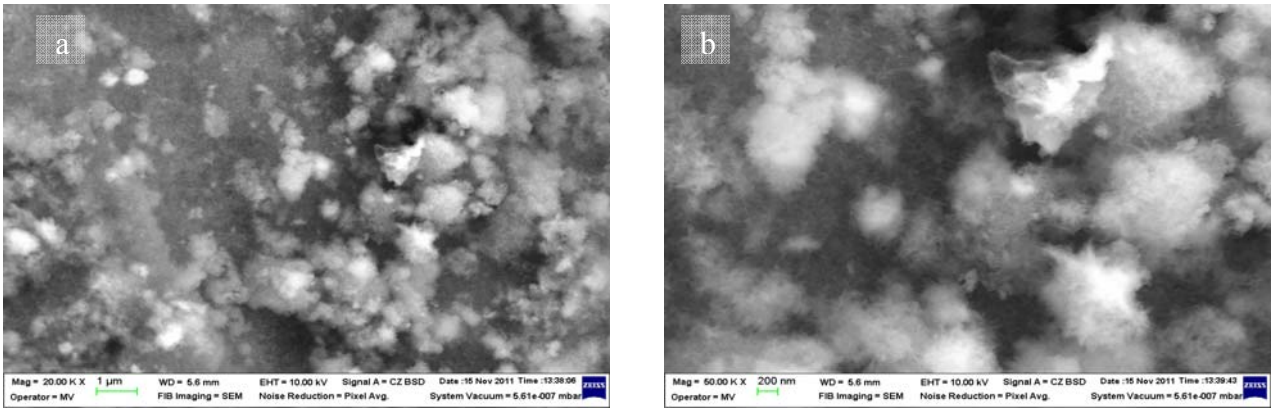

Fig. 5. SEM micrographs of S1 glass powders soaked for 14 days in simulated body fluid: a) Magnification: 20.000X, b) Magnification: 50.000X.

4. Conclusions

In case of bioactive glass synthesis obtained by sol – gel process, has been developed a method which was applied very well on SiO_2-CaO-P_2O_5 system, in conditions of low production costs and minimal use of logistical resources.

It was achieve stabilization of bioactive glass structure synthesized by sol – gel process, afer the heat treatment applied. This aspect was highlighted by TG / DSC analysis. Moreover, performed tests revealed no carbonated hydroxyapatite formation at this stage of the study.

Bioactivity study, namely, the development of apatite (carbonated hydroxyapatite) on glass surface was revealed by the FTIR analysis. After immersion of glass powders in simulated body fluid for 3, 7 and 14 days have been highlighted changes to the glass surface with the appearance or increase of intensity for some peaks typical to phosphate (PO_4^{3-}), carbonate (CO_3^{2-}) also hydroxyl (OH^-) groups specific to this type of biomaterial.

Morphological analysis revealed the appearance of irregular particles of glass surfaces. The result is a material with a high specific surface area, ideal for nucleation and growth of apatite.

Have been emphasized new light colored clusters attributed to the formation of the first nuclei of apatite, after glass powders immersion in simulated body fluid for different periods of time.

The coverage degree of glass particles with apatite increases with immersion time in simulated body fluid, as evidenced in the micrographs presented in this paper.

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References

[1] I. Cacciotti, M. Lombardi, A. Bianco, A. Ravaglioli, L. Montanaro, J. Mater. Sci: Mater. Med. **23**, 1849 (2012).
[2] L. C. Gerhardt, A. R. Boccaccini, Materials **3**, 3867 (2010).
[3] N. Yamaguchi, T. Kanda, H. Fujimori, K. Ioku, S. Goto, J. Mater. Sci. Lett. **20**, 489 (2001).
[4] I. Izquierdo-Barba, F. Conde, N. Olmo, M. A. Lizarbe, M.A. García, M. Vallet-Regí, Acta Biomater. **2**, 445 (2006).
[5] S. Yoshihara, T. Kokubo, N. Nishimura, T. Yamamuro, T. Nakamura, J. Mater. Sci. Mater. Med. **5,** 123 (1994).
[6] M. Mami, A. Lucas-Girot , H. Oudadesse, R. Dorbez-Sridi, F. Mezahi, E. Dietrich, Appl. Surf. Sci. **254**, 7386 (2008).
[7] B. Lei, X. Chen, Y. Wang, N. Zhao, C. Du, L. Fang, J. Non – Cryst. Solids **355**, 2678 (2009).
[8] J. J. Shy, J. M. Wu, J. Mater. Sci. **29**, 3167 (1994).
[9] J. Ma, C.Z. Chen, D. G. Wang, J.Z. Shi, Mater. Sci. Eng. C **30**, 886 (2010).
[10] X. Yan, G. Wei, L. Zhao, J. Yi, H. Deng, L. Wang, G. Lu, C. Yu, Micropor. Mesopor. Mat. **132,** 282 (2010).
[11] R. L. Siqueira, O. Peitl, E. D. Zanotto, Mater Sci Eng C **31,** 983 (2011).
[12] D. Carta. D. M. Pickup, J. C. Knowles, M. E. Smith, R. J. Newport, J. Mater. Chem. **15**, 2134 (2005).
[13] L. L. Hench, J. Mater. Sci. Mater. Med. **17,** 967 (2006).
[14] A. Balamurugan, G. Sockalingum, J. Michel, J. Fauré, V. Banchet, L. Wortham, S. Bouthors, D. Laurent-Maquin, G. Balossier, Mater. Lett. **60** 3752, (2006).
[15] R. Li, A. E. Clark, L. L. Hench, J. Appl. Biomater. **2**, 231 (1991).
[16] B. Lei, X. Chen, Y. Wang, N. Zhao, C. Du, L. Fang, J. Non – Cryst. Solids **355,** 2678 (2009).
[17] P. Saravanapavan, J. R. Jones, R. S. Pryce, L. L. Hench, J. Biomed. Mater. Res. A **66,** 110 (2003).
[18] D. N. Ungureanu, N. Angelescu, D., The Scientific Bulletin of Valahia University - Materials and Mechanics **9,** 115 (2011).
[19] I. I. Barba, M. Vallet-Regí, Solid State Sci. **13,** 773 (2011).
[20] M. M. Pereira, A. E. Clark, L. L. Hench, J. Am. Ceram. Soc. **78,** 2463 (1995).
[21] D. N. Ungureanu, N. Angelescu, A. Catangiu, E. V. Stoian, C. Z. Rizescu, D. Avram, Advanced Materials Research **341-342**, 21 (2012).

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