MICROSTRUCTURALLY MODIFIED ZINC CONTAINING PHOSPHOSILICATE BIOGLASSES APPLICABLE IN COMPOSITE SCAFFOLDS FOR TISSUE REGENERATION

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Abstract

Aims. *Bioactive glasses have attracted significant attention in recent years, especially for their insertion in composite biomaterials to serve as support in tissue engineering. These biomaterials have clinical applications also as a coating for implants, bone filling and, in a porous form they are used for bone regeneration. Different bioactive glasses and glass-ceramics have been synthesized in order to get desired properties conferred by required microstructure. The aim of this work is to obtain and characterize the structure and surface of a new bioglasses in zinc containing phosphosilicate system, applicable as scaffold for tissue regeneration.*

Methods. *The new* SiO_2*-ZnO-*P_2O_5 *bioglass system, with four different molar ratios, was prepared following the sol-gel route. The precursors used were TEOS (*$Si(OC_2H_5)_4$*), zinc nitrate hexahydrate (*$Zn(NO_3)2 \cdot 6H_2O$*) and dibasic ammonium phosphate (*$(NH_4)_2HPO_4$*), while* HNO_3 *was used as catalyst. The obtained gels were dried for 24 hours at 110 ºC. For the characterization of samples was used differential thermal analysis (TGA / DTA), X-ray diffraction (XRD) as well as atomic force microscopy (AFM).*

Results. *According to thermal analysis results, heat treatments at 300 ºC and 750 °C are necessary for removal of water and residual groups from the investigated samples. XRD data show that all samples are prevalent amorphous, but they also contain nanometric crystals of zinc silicates and phosphates developed during sol-gel synthesis. After the heat treatments performed at 300 ºC and 750 °C, the XRD patterns prove that the samples are still predominantly amorphous. AFM analysis of samples shows a rough surface with high specific area.*

Conclusions. *The structure of the new ZnO-*P_2O_5*-*SiO_2 *bioglass processed by sol-gel route remains predominantly amorphous after the heat treatments applied at 300 ºC and 750 °C, and in the prevalent vitrous matrix are developed nanosized crystallites. AFM analysis reveals a rough surface with high surface area. Both the nanostructure and the large surface area of these samples are promising for the attachment of biomolecules.*

Keywords: biomaterials, sol-gel, scaffold, bone.

BIOSTICLE FOSFOSILICATICE CU ZINC MODIFICATE MICROSTRUCTURAL, APLICABILE CA SUPORT PENTRU REGENERARE TISULARĂ

Rezumat

Scop. *Sticlele bioactive au atras o atenţie semnificativă în ultimii ani, mai ales pentru introducerea lor în biomaterialele compozite servind ca suport în ingineria tisulară. Aceste biomateriale au aplicaţii clinice ca înveliş pentru implanturi, în umplere osoasă şi, într-o formă poroasă, acestea sunt folosite pentru regenerarea* *osoasă.*

Scopul lucrării este obţinerea şi caracterizarea structurii şi suprafeţei unor noi biosticle într-un sistem fosfosilicatic cu zinc, aplicabile în biomateriale compozite ca suport pentru refacerea ţesutului osos.

Metode. *Noul sistem de biosticlă SiO_2-ZnO-P_2O_5, cu patru raporturi molare diferite, a fost preparat urmând tehnica sol-gel. Precursorii folosiţi au fost TEOS ($Si(OC_2H_5)_4$), azotat de zinc hexahidratat ($Zn(NO_3)2 \cdot 6H_2O$) şi fosfat de amoniu bibazic ($(NH_4)_2HPO_4$), folosind HNO_3 ca şi catalizator. Gelul obţinut a fost uscat 24 ore la 110 °C. Pentru caracterizarea probelor s-a folosit analiza termică diferenţială (TGA/DTA), difracţia de raze X (XRD), precum şi microscopia de forţă atomică (AFM).*

Rezultate. *Din analiza termică a probelor rezultă că, pentru eliminarea apei şi a elementelor reziduale, sunt necesare tratamente termice la 300 ºC şi 750 ºC. Rezultatele XRD au arătat că toate probele uscate la 110 °C au un caracter predominant amorf şi apar cristale nanometrice de silicaţi şi fosfaţi de zinc formaţi în timpul sintezei probelor. În urma tratamentelor efectuate la 300 ºC şi 750 ºC, din difractograme reiese faptul că probele sunt încă predominant amorfe. Analiza AFM a probelor evidenţiază o suprafaţă rugoasă, cu arie specifică ridicată.*

Concluzii. *Noua biosticlă SiO_2-ZnO-P_2O_5 obţinută prin metoda sol-gel, tratată termic la 300 ºC şi 750 °C are o structură predominant amorfă, în care se formează cristalite nanometrice. Analiza AFM pune în evidenţă o suprafaţă rugoasă. Atât nanostructurarea probelor, cât şi suprafaţa specifică mare sunt caracteristici urmărite în vederea ataşării de biomolecule.*

Cuvinte cheie: biomateriale, sol-gel, suport, os.

INTRODUCTION

Bones are very important in our lives because they support our bodies and enable us to perform various motions. When the bone is damaged and if an area of damaged bone is too large for self-repair, the damaged bones must be repaired by using alternative materials, such as autografts, allografts and artificial materials [1]. Autografts, which are transferred from healthy parts of the bones of the same patient, are widely used because they show high performance. Although allografts, which are transferred from other people, are also used, they have problems related to foreign body reactions and infections. However, sufficient material cannot always be derived from the patient or a suitable donor and current medical technologies are not yet able to synthesize these materials. As a consequence, artificial materials are substituted. Initial, this materials implanted in bony defects were encapsulated by fibrous tissue and do not bond to living bone. To solve the problem of the foreign body reaction, bioactive glasses have received much attention. For repairing bony tissues, the bioactivity is regarded as the capability to make direct contact with living bone after implantation in bone defects [2-3]. Different bioactive glasses and glass-ceramics have been synthesized in order to get desired properties conferred by required microstructure. The properties that are of interest in the characterization of biomaterial surfaces include the chemical composition, the hydrophilicity or hydrophobicity, the presence of ionic groups, the morphology (i.e. the domain structure), and the topography (i.e. the surface roughness, planarity, and feature dimensions) that can influence the attachment of bio-organic molecules such as enzymes, proteins, antibodies or even cells [4-6]. Common components used for synthesis of 45S5 and S53P4 registered bioglasses are Na_2O, CaO, P_2O_5, SiO_2 [7]. In addition to these are often added K_2O, MgO, B_2O_3. There are some other glasses and glass-ceramics which also include ZnO, Ag_2O and Al_2O_3. A major disadvantage of bioglass is its very high solubility because most of the released ions might be transported away from the surroundings of the implantation site by body fluid before new bone can form. Control of the surface reactions and, therefore, of the biodegradation and bioactivity of implanted materials can be achieved by rational design of the glass composition. In fact, by varying the chemical nature and/or concentration of the bioglass constituents, new important mechanical and biological properties can be tailored for specific clinical applications [8]. The bioactivity and the bioresorption of ceramics is governed by the reaction between the ceramic and the body fluid. Zinc oxide addition to the phosphosilicate matrix was considered because it is know that zinc stimulates cell proliferation, its stimulating effect being demonstrated *in vitro* [9]. In addition, Zn slows the glass dissolution. From the literature it is known that ZnO-P_2O_5-SiO_2 system has not been studied until now.

Techniques for preparing bioactive glasses include both traditional melting methods and sol-gel route, the latter being covered in this paper [10]. By comparison with

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typical melt-derived bioactive glasses, sol-gel bioactive glasses have a larger surface area and OH groups are present in their structure. Therefore, sol-derived bioglasses show higher bioactivity than melted ones by faster formation of a strong bond with the bone tissue as well by the stimulation of living bone to faster regeneration [11]. Other advantages of the sol-gel process are well known: it takes place at low temperatures, and gives homogeneous mixtures in the final glass composition.

The aim of this work is to obtain and characterize the structure and surface of a new bioglass in zinc containing phosphosilicate system, applicable as scaffold for tissue regeneration**.**

METHODS

The new SiO_2-ZnO-P_2O_5 bioglass system, with four different molar ratios (table no I.), was prepared following the sol-gel route.

Table no I. Molar ratios of sol-gel derived SiO_2-ZnO-P_2O_5 bioglasses.

Sample code	SiO_2:ZnO:P_2O_5 ratio	SiO_2 (mol %)	ZnO (mol %)	P_2O_5 (mol %)
S-414	4:1:4	44.5	11	44.5
S-412	4:1:2	57	14	29
S-411	4:1:1	66	17	17
S-4103	4:1:0.3	75	19	6

The precursors used were TEOS ($Si(OC_2H_5)_4$), zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$) and dibasic ammonium phosphate ($(NH_4)_2HPO_4$). The catalyst used was HNO_3. TEOS was stirred with C_2H_5OH, the other two precursors were stirred separately with distilled water for 1/2 h. Then both solutions were added to TEOS solution under continuous stirring. Solution was left overnight for gelation at the room temperature. The gel was kept for 7 days at 37 ºC for aging and then was dried for 24 h at 100 ºC.

Differential thermal analysis (DTA) and thermogravimetric analysis (TGA) were performed on Shimadzu type derivatograph DTG-GOH with a heating rates of 5 ºC/min using alumina open crucibles, in order to investigate the thermal behavior of the dried samples.

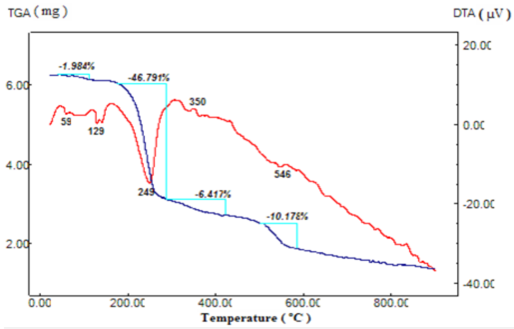
After DTA/TGA analysis, the samples were heat treated at 300 ºC and 750 ºC.

The structure of the dried and heat treated samples were examined using a X-ray Shimadzu XRD6000 with a Ni – filter, using CuK_α radiation (λ = 1.5418Å). The operation voltage and current were 40 kV and 30 mA. The measurements were performed at a scan speed of 1º/min in a 2θ scan range from 10º to 80º.

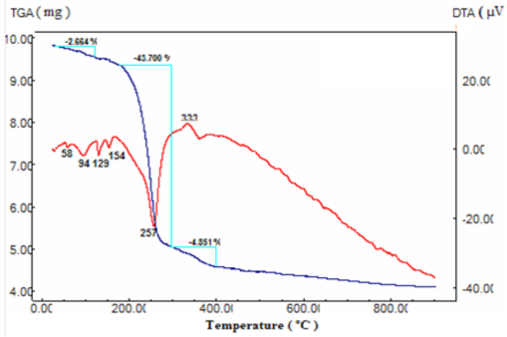
Topographic study of samples pellets for SiO_2-ZnO-P_2O_5 system was performed with an NT-MDT atomic force microscope in tapping-mode. Bi-dimensional topographic images with 10μm x 10 μm area were obtained and analyzed three-dimensional images with a resolution of 256 pixels.

RESULTS

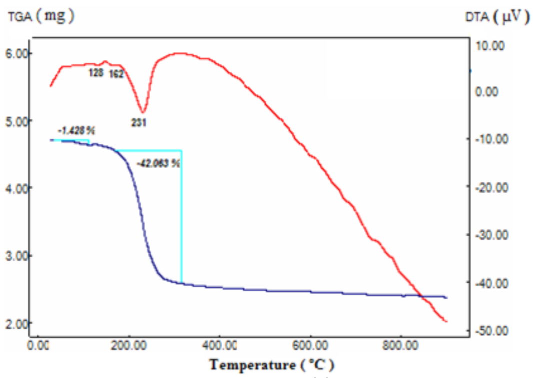
The TGA/DTA curves of 110 ºC dried samples are shown in figure no 1.



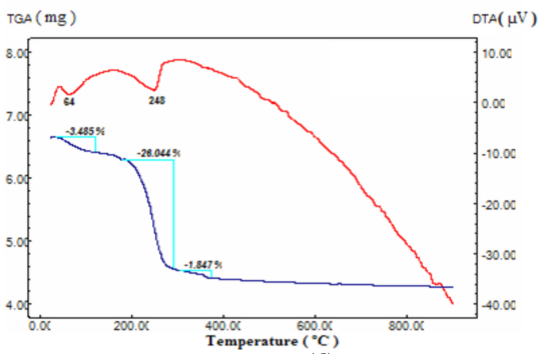
(a)



(b)



(c)



(d)

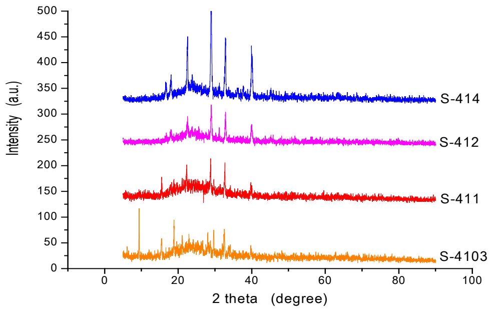
Fig. 1. DTA and TGA runs of sol-gel derived S-414 **(a)**, S-412 **(b)**, S-411 **(c)**, S-4103 **(d)** bioglasses.

The TGA/DTA events temperature and weight losses are presented in table no.II.

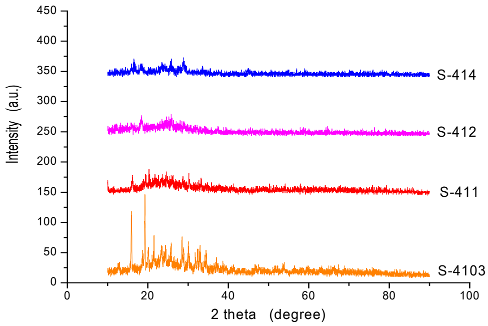
Table no II. Temperature and weight losses related to DTA and TGA events in sol-gel derived SiO_2-ZnO-P_2O_5 bioglass.

Sample code	Endothermic events temperature (°C)	Weight losses (%)
S-414	59, 129, 249, 546	1.984, 46.791, 6.417, 10.178
S-412	58, 94, 129, 154, 275	2.664, 43.700, 4.851
S-411	128, 162, 231	1.428, 42.063
S-4103	64, 248	3.485, 26.044, 1.847

The XRD patterns of the 110 ºC dried bioglass sample and 300 and 750 ºC heat treated samples are illustrated in figure no. 2.



(a)



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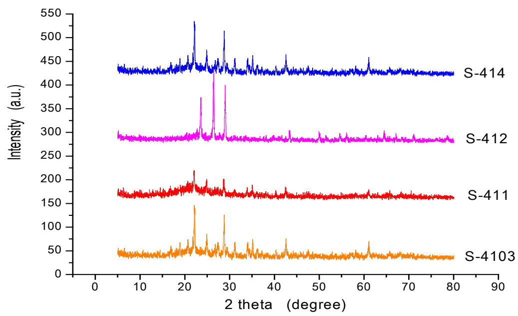
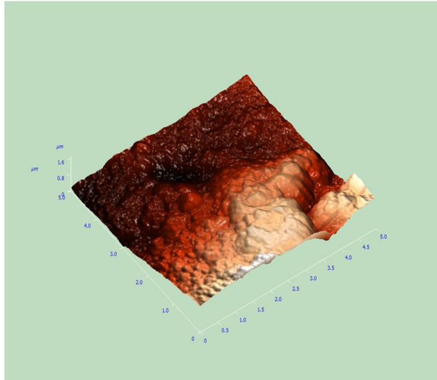


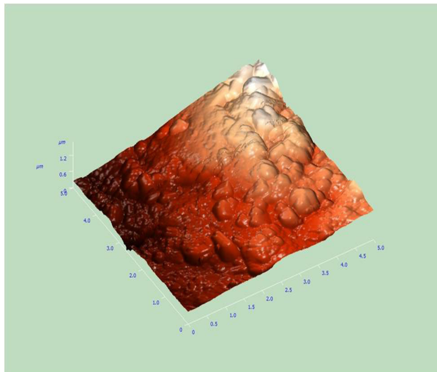
(c)

Fig. 2. XRD pattern of 110 °C dried **(a)**, 300 °C **(b)** and 750 °C **(c)** heat treated sol-gel samples.

The three-dimensional (3D) views of the surface morphology of 750 ºC heat treated samples pellets are shown in figure no 3.



(a)



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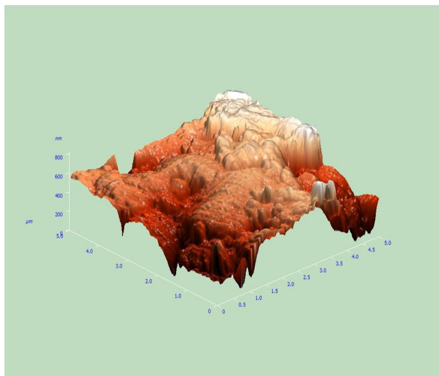

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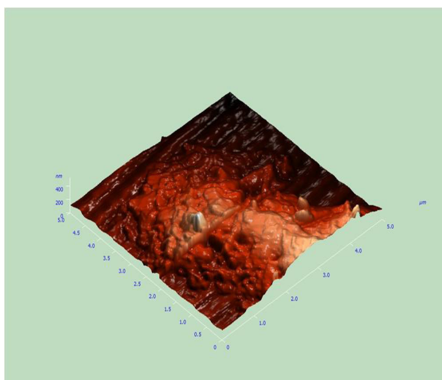

(d)

Fig. 3. AFM tapping mode 3D image of 750 °C heat treated S-414 (a), S-412 (b), S-411 (c), S-4103 (d) samples.

DISCUSSIONS

The TGA curves for all the samples present more regions of weight loss. The first weight loss that occurs coincides with an endothermic peak in DTA signal and it can be associated with the remove of free water molecules. A second big weight loss with a large corresponding endothermic peak in the DTA curve can be observed around 250 ºC. This loss can be associated with the decomposition of ammonium nitrate and the elimination of water caged in the pores. The others weight losses are small and the DTA signal is diminished constantly and can correspond to the elimination of the residual elements from precursors resulted during the synthesis of samples. Thermal analysis indicates that the elimination of the residual elements take place up to around 750 ºC temperatures, which can be considered as thermal treatment temperature.

The XRD patterns of the 110 ºC dried samples indicate a prevalent amorphous state of all glasses. Beside the large diffraction peak that dominates the XRD patterns, there are peaks indicating the presence of a minor crystalline structure, that could be assigned to residual zinc nitrate and phosphate crystals zinc silicates and phosphates crystals formed during the samples synthesis [12,13]. After thermal treatment at 300 °C it can observe of no peaks in the patterns that is indicative of their amorphous nature, except S-4103 that still has nano-crystals. The XRD results of 750 ºC heat treated samples indicate that the samples are still amorphous, except S-412.

Surface morphology is well revealed in AFM images. Their analysis denotes a rough surface with high specific area [13].

CONCLUSIONS

The structure of the new ZnO-P_2O_5-SiO_2 bioglass processed by sol-gel route remains predominantly amorphous after the heat treatments applied at 300 ºC and 750 °C, and in the prevalent vitrous matrix are developed nanosized crystallites. AFM analysis reveals a rough surface with high surface area. Both the nanostructure and the large surface area of these samples are promising for the attachment of biomolecules.

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