PREPARATION AND CHARACTERIZATION OF BIOACTIVE GLASSES FROM CUBAN NATURAL RAW MATERIALS

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ABSTRACT

The silica-rich glasses from CaO-SiO_2-P_2O_5-Na_2O system were the first man-made materials which had been found to bond osseous living tissues. The intimate bond was attributed to the formation of a calcium-phosphorous layer at the interface between bone and glass implant. In this work, the bioactive behavior of CaO-SiO_2-Na_2O glass obtained from Cuban natural sources was studied and some properties of this glass were determined. The natural raw materials were characterized by X-ray fluorescence and X-ray diffraction. The results of chemical composition of the glass were very similar to the nominal composition. The diffraction patterns revealed that a crystalline phase identified as $Na_2O.2CaO.3SiO_2$ was formed when the glass was annealed at 500ºC for 2h. The thermal transformations of the glass were determined by differential thermal analysis. The bioactive potential of the glass obtained was demonstrated by the formation of a Ca/P layer on its surface after 15 days immersed in a simulated body fluid solution.

Key-words: silica-rich glasses, mineral deposits, bioactivity, biomimetic method

INTRODUCTION

The orthopedic surgery advances, the increasing expectative of live and a desire of better quality of life of the world population nowadays have been conditioned a growing demand of prosthesis, implants and new biomaterials able to be in contact with living tissues without a significant rejection by the human body.

The main problem with the implant used until 70s, e.g. metals and polymers was that they were designed to be bioinert, triggered fibrous encapsulation after implantation, rather than forming a stable interface or bond with tissues. In this context, ceramic as materials (which its metal element in the maximum state of oxidation) resulted attractive by its lesser possibility of oxidation or corrosion in the physiological medium (1).

Bioceramics such as alumina or zircona are the traditional examples of bioinert ceramics because elicits minimal interaction with its surrounding tissues. Nevertheless bioactive ceramics are represented by variations on bioactive glasses (2), glass–ceramics (3) and ceramics such as synthetic hydroxyapatite (HA) and other calcium phosphates (4).

The first bioactive glass discovered with the ability to bond chemically to the living bone was a silica-based glass tested in 1969 by Hench and colleagues (5). This glass was termed as 45S5 Bioglass® by its composition of 46.1 mol.% SiO_2, 24.4 mol.% Na_2O, 26.9 mol.% CaO and 2.6 mol.% P_2O_5. The mechanism for bone bonding is attributed to a rapid sequence of chemical reactions on the surface of the implant that conduces to the formation of a biologically active carbonated apatite layer on the implant surface that is chemically and crystallographically equivalent to the mineral phase in bone (6). This process enables a collagen matrix to be deposited by osteoblasts over the carbonated apatite layer with a subsequently mineralization of the collagen fibrils resulting in bonding of the living tissue (2,7).

The initial chemical reactions includes a rapid cation exchange of Na^+ and/or Ca^{2+} with H^+ from solution, creating silanol bonds (Si–OH) on the glass surface and a significant variation of pH near to the glass surface. High local pH leads to attack of the silica glass network by OH^-, breaking Si–O–Si bonds, leaving more Si–OH (silanols) at the glass–solution interface where take place the repolymerization of the silica-rich layer near the glass surface. Then migration of Ca^{2+} and PO_3^{4-} groups to the surface through the silica-rich layer and from the solution, forming a film rich in amorphous $CaO–P_2O_5$ on the silica-rich layer and finally the incorporation of hydroxyls and carbonate from solution and crystallization of the $CaO–P_2O_5$ film to hydroxyl carbonate apatite (HCA) (2,8).

Multiples studies have been develop new formulations of glasses and glass-ceramics materials from the original composition. For example, a host of sol–gel reaction-derived metallic oxides, including SiO_2, TiO_2, ZrO_2, Nb_2O_5 and Ta_2O_5 were found to possess in vitro bioactivity after immersing in simulated body fluid (10,11); however, the ability of these metallic oxide gels to bond to bone in vivo has not been demonstrated (12).

This work was aimed to develop a bioactive glass based on $CaO-SiO_2-Na_2O$ system using as source materials the mineral deposits of Santa Teresa (silica sand) and Jaruco (calcite) in Cuba.

MATERIALS AND METHODS

Characterization of natural deposit materials

The glass composition used in this work was prepared using natural raws from Santa Teresa (silica sand) and Jaruco (calcite) deposits owing to the Pinar del Rio and Mayabeque provinces respectively in Cuba. Also it was used sodium carbonate (Aldrich) reagent as received. Both natural deposit materials and sodium carbonate reagent were characterized by X-ray fluorescence and X-ray diffraction.

Preparation of $CaO-SiO_2-Na_2O$ glass

For preparation of $CaO-SiO_2-Na_2O$ glass raw materials were weighed, hand homogenized and placed into a platinum crucible. The following proportions was stablished: silica sand (53 wt.%), calcite (23 wt.%), sodium carbonate (24 wt.%) and water (4-5 wt.%). The sample was heated in air at a furnace temperature until 400 °C for 25 minutes to expel the decomposition gases, afterward at 500°C for 25 minutes again, and finally until 1400 °C for 6 hours, the heating rate was 2 °C/min in all cases. The melts were quenched into preheated steel molds, and moved quickly to an annealing furnace, annealed was carried out at 500 °C for 30 minutes and glass was cooled down to room temperature more than 12 h to remove residual stress. The sample name was $BV_{Combeite}$ because of its composition is very close to the zone of the primary cristallization of combeite phase ($Na_2O.2CaO.3SiO_2$) in the ternary diagram.

X-ray fluorescence analysis

Chemical composition was determined using X-ray fluorescence using espectrophotometer WD-XRF Philips PW 2400 (Germany) with two power supply by rhodium (Rh) and gold (Au) anodes.The elements quantification was realized by a calibration curves obtained since 62 geological patterns.

X-ray diffraction analysis (XRD)

X-ray diffraction analysis were performed on an X-ray diffractometer Bragg-Brentano θ/2θ Siemens with a CuKα (λ=1.5418Å). Voltage and current were selected at 40 kV and 30 mA, respectively. Data were collected from 10-60 ° (2θ) with a step size of 0.05 °.

Differential Thermal Analysis (DTA)

Thermal analysis of the shyntetised glass was realized using Thermogravimetric (TG) and Differential Thermical Analysis (DTA) tecniques. It was employed air atmosphere, a heating rate of 5 °C/min and α-alumine as reference sample in a NETZSCH STA 409 equipment (Germany). The temperature scanned over a range from room temperature to 1400 °C/min. The estimated error in DTA data was about 5 percent and the error of thermogravimetric measures was a maximum of 10 percent of the sample weigth.

In vitro bioactivity study

In vitro bioactivity test were carried out by soaking probes in simulated body fluid (SBF). The SBF was prepared by dissolving reagent-grade chemicals of NaCl, $NaHCO_3$, KCl, $K_2HPO_4·3H_2O$, $MgCl_2·6H_2O$, $CaCl_2$ and Na_2SO_4 (Sigma Aldrich,USA) in deionized water and buffering at a pH of 7.40 with tris(hydroxymethyl) aminomethane ($(CH_2OH)_3CNH_2$) and 1 M hydrochloric acid (Sigma Aldrich,USA) at 36.5 ± 0.1°C. The ratio area/volume was maintained at 0.05 cm^2/mL in all cases. Later, probes were placed at 37 °C in a stove for periods from 3 to 15 days. After the soaking period, samples were taken out from SBF, rinsed with bidestillated water and dryed at air. The superficial changes were characterized by Scanning Electronic Microscopy (SEM) (JEOL JSM 6300) acopled at Energy dispersive X-ray spectroscopy (EDS) system OXFORD LINK (ISIS 300 Model) for chemical analysis of the glass after immersed in SBF.

RESULTS AND DISCUSSION

Chemical characterization of natural deposit materials

The silica-rich glasses are mainly composed by sodium, calcium and silica oxides. Since the 70’s they have has been reported as a bioactive material (material with ability to form apatite layer on their surface when in vitro are soaking in simulate body fluid) and in vivo bond with bone rapidly and also stimulates bone growth away from the bone–implant interface. They have a wide range of compositions SiO_2: 45-75%, Na_2O: 0-40%, CaO: 10-50% (%mol).

In this work it was explored the posibility to prepare silica-rich glass from Cuban natural deposits. The chemical compositions determined of natural raws used are given in Table 1. The silica sand presented an elevated purity (99.85 wt.%). However, the calcite deposit contained about 53.79 (wt.%) of CaO that is a superior content than a minimum required for its utilization in the synthesis of sodium-calcium glasses (51.2 wt.%) (13). The sodium carbonate reagent was 99.25 wt.% of purity with a 57.95 wt.% of Na_2O according to its data sheet provided by the Central Laboratory of Enterprises Group of Cement & Glass, Havana, Cuba.

Table 1. Chemical composition of the minerals utilized in the glass synthesis

Cuban Minerals	**Chemical Compositions (wt.%)**				
	SiO_2	**Al_2O_3**	**Fe_2O_3**	**CaO**	**MgO**
Silica sand	99.85	traces	0.06	-	-
Calcite	traces	traces	0.11	53.79	traces

Experimental and reported XRD patterns of silica sand (a) and calcite (b) are given in Fig. 1. It was obtained an appropriated correspondence between the experimental data and PDFWIN crystallographic date base in all cases. The main crystallographic phases were confirmed as SiO_2, $CaCO_3$ and $NaCO_3$ respectively.

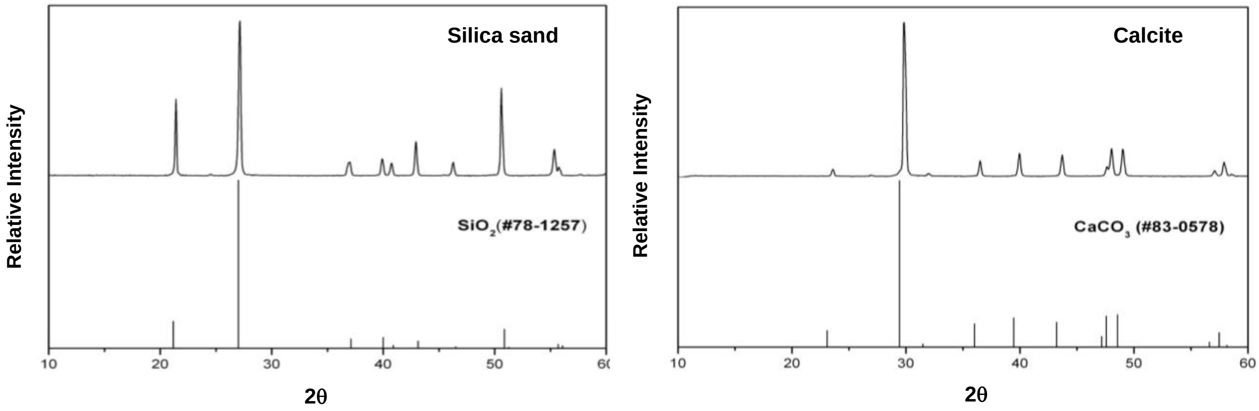

Fig. 1. XRD patterns of silica sand from Santa Teresa deposit, Cuba (a) and calcite from Jaruco deposit, Cuba (b)

Characterization of synthetized glass

a) X-ray fluorescence analysis

The results of the chemical analysis performed to the synthesized glass are summarized in Table 2.

Table 2. Chemical analyses the $BV_{Combeit}$ synthetized and natural silica sand

Oxides content (wt.%)	**Fe_2O_3**	**MnO**	**TiO_2**	**SiO_2**	**K_2O**	**Na_2O**	**Al_2O_3**	**CaO**	**MgO**	**P_2O_5**
$BV_{Combeite}$	0.03	-	0.06	52.13	0.01	23.33	0.26	22.61	0.16	0.02

b) X-ray diffraction analysis of $BV_{Combeite}$

The XRD patterns of the glass after annealing treatment are shown in Fig. 2. Before annealing treatment the XRD pattern is characteristic of amorphous materials with wide bands, indicating the glass powder after stabilization is mainly amorphous. The quenching process follows that the glasses do not have a grid system, as their constituent ions are irregularly arranged to form a more or less distorted structure.

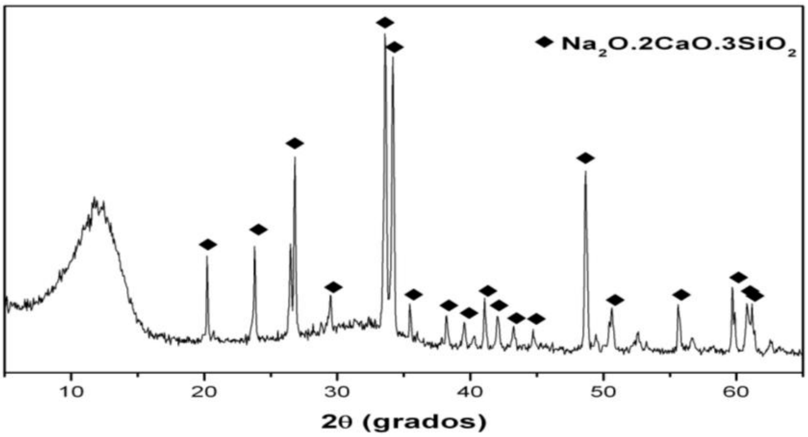

Fig. 2. XRD patterns of the synthesized glasses after annealing

After annealing at 500 °C the amorphous diffraction bands disappeared; meanwhile, some new sharp crystal peaks appeared, indicating some crystallization. The sample pattern was in agreement with the XRD patterns for a crystalline phase combeite ($Na_2O.2CaO.3SiO_2$, PDFWIN 22-1455).

c) Differential thermal analysis

Figure 3 shows the thermogravimetric and differential thermal analysis (TG/DTA) curves of the $BV_{Combeite}$ sample. The endothermic effects at 82 °C (A) and 174 °C (B) are associated with the volatilization of humidity water and with the hydroxile (OH^-) rupture in the crystal lattice respectively. The weight loss is less than 1.0 wt.%.

The endothermic transition at 564 ^oC (C arrow, Fig. 3) doesn't have any variation of weight and it is related with the glass transition of the material. Different authors have been reported that the glass transition (Tg) occurred around 560 ^oC (14). In addition, at 681^oC an exothermic transition is detected (D arrow, Fig. 3) associated with the $BV_{Combeite}$ glass cristallization. The system with a low stability and high internal energy changes to a more stable state releasing the corresponding energy of transition. This transformation takes place between 680 and 690^oC temperature as other materials with similar chemical composition (15).

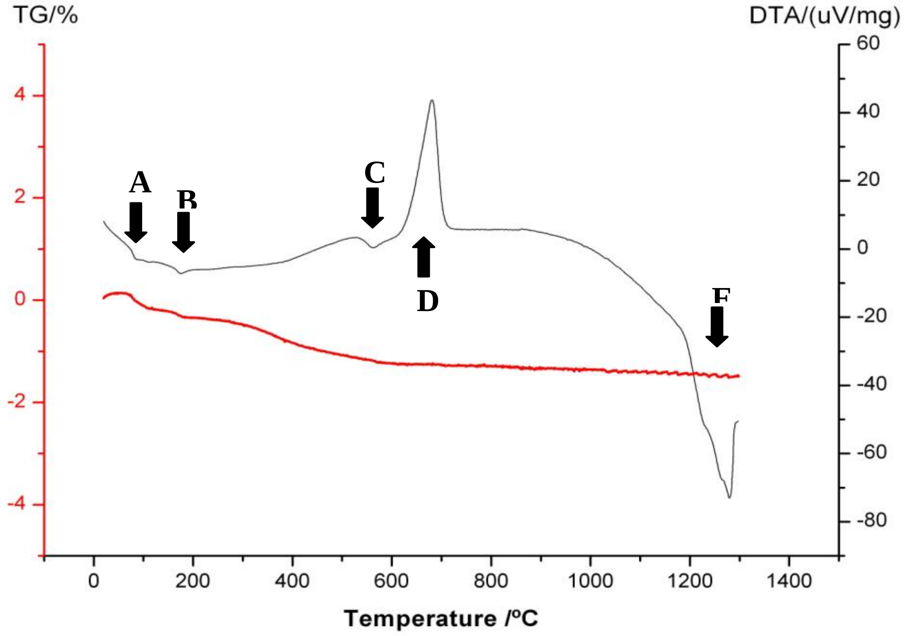

Fig. 3. Differential thermal analysis curves for the phosphate bioglass samples

Finally, the last endothermic effect at 1282 ^oC (E arrow, Fig. 3) corresponds to the melting temperature of the $BV_{Combeite}$ glass.

d) In vitro bioactivity behaviour

As mentioned before bioactive glasses are considered as an appropiated materials for bone substitutes, because they have the ability to form a direct bond with the living bone without the formation of surrounding fibrous tissue. This kind of materials in the presence of the human physiological environment formed a hydroxyl carbonate apatite layer on their surfaces, which provide the bonding interface with bone (16).

In this work the in vitro BV$_{Combeite}$ bioactivity behaviours was analyzed by soaking probes of sample in simulated body fluid. The superficial morphological changes were explored by SEM after 3 days of soaking in SBF indicating the incipient formation of a layer on the surface of the material. After 15 days the nucleation and precipitation of a layer conformed by agglomerates with globular morphology is more evident and the surface is completely coated, Fig. 4 a).

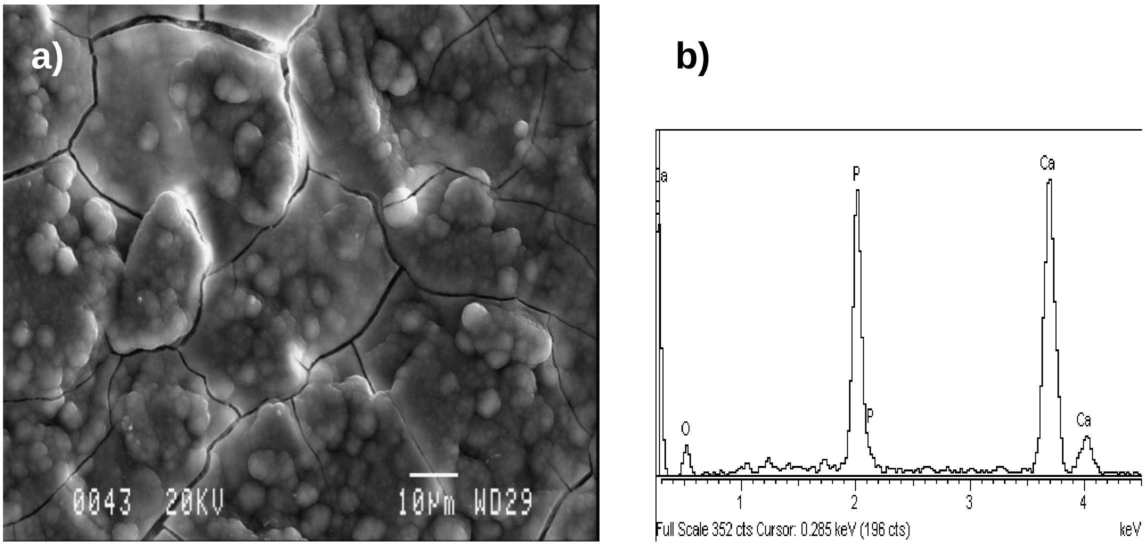

Fig. 4. a) SEM micrograph of the surface of BV$_{Combeite}$ sample after soaking in SBF after 15 days; b) EDS spectrum of calcium phosphate layer on surface of glass recorded after immersion in SBF solution for 15 days

According to other authors this appearance is the tipical morphology of the hydroxyl carbonate apatite formed during in vitro assays in bioactive materials (17).

The energy dispersive X-rays spectroscopy analysis revealed the elements of the coating layer. Figure 4b) shows the EDS for the sample after 15 days of soaking. It was detected Ca and P elements as major constituents of the superficial layer indicating that the calcium phosphate deposit occurred.

Conclusions

In this work was explored the posibility of development a bioactive glass from Cuban natural raws. The silica sand and calcite from Santa Teresa and Jaruco deposit demonstrated higth purity for this purpose. The chemical composition of the glass obtained was SiO_2 (52.1 wt.%)-CaO (22.6 wt.%)-Na_2O(23.3 wt.%) and resulted very similar to the nominal composition. The diffraction patterns revealed that a crystalline phase identified as $Na_2O.2CaO.3SiO_2$ was formed after annealed. The differential thermal analysis determined that Tg value is 560 °C. The in vitro study revealed that the $BV_{Combeite}$ glass exhibited nucleation and growth of Ca/P layer at their surface after immersed in SBF for 15 days. The results suggested that this glass could be appropriated for biomedical applications.

Acknowledgements

The authors acknowledge to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the financial support through the project CAPES-MES/Cuba No.163/12.

References

(1) Jones, J.R. Review of bioactive glass: From Hench to hybrids. Acta Biomaterialia, v.9, p.4457-4486, 2013.
(2) Hench, L.L. The story of Bioglass, J. Mater. Sci. Mater. Med., v.17, p.967-978, 2006
(3) Kokubo, T., Bioactive glass ceramics properties and applications, Biomaterials, v.12, p.155-163, 1991.
(4) LeGeros R.Z., Properties of osteoconductive biomaterials: calcium phosphates. Clin. Orthop. Relat. Res., v.395, p.81-98, 2002.
(5) Hench, L.L.; Splinter, R.J.; Allen, W.C; Greenlee, T.K. Bonding mechanisms at the interface of ceramic prosthetic materials. J. Biomed. Mater. Res. Sym 334, p.117-141, 1971.
(6) Sanz-Herrera, J.A.; Boccaccini, A.R. Modelling bioactivity and degradation of bioactive glass based tissue engineering scaffolds International Journal of Solids and Structures, v.48, p. 257–268, 2011.
(7) Farooq, I.; Imran, Z.; Farooq, U.; Leghari, A. Bioactive Glass: A Material for the Future. World Journal of Dentisty, v.3, p.199-201, 2012.
(8) Martin, R.A.; Twyman, H.; Qiu, D.; Knowles. J.C. A study of the formation of amorphous calcium phosphate and hydroxyapatite on melt quenched Bioglass using surface sensitive shallow angle X-ray diffraction. J. Mater. Sci. Mater. Med., v.20, p. 883–888, 2009.
(9) Hong Z.; Reis, R.L.; Mano, J.F. Preparation and in vitro characterization of novel bioactive glass ceramic nanoparticles. J. Biomed. Mater. Res. A, v.88, p.304-313, 2009.
(10) Thonglem S.; Rujijanagul, G.; Eitssayeam, S.; Tunkasiri, T. Fabrication of P_2O_5–CaO–Na_2O glasses doped with magnesium oxide for artificial bone applications. Ceramics International, v.39, p. 537-540, 2013.
(11) Li, H.C.; Wang, D.G.; Hu, J.H. Effect of various additives on microstructure, mechanical properties, and in vitro bioactivity of sodium oxide-calcium oxide-silica-phosphorus pentoxide glass–ceramics. J. of Colloid and Interface Science, v. 405, p. 296-304, 2013.
(12) Kokubo T. Novel bioactive materials. Anales de Química, v.93, p. 49-55, 1997.

(13) Geotti-Bianchini, F.; Brown, J.T.; Faber, A.J.; Hessenkemper, H.; Kobayashi, S.; Smith, I.H. Influence of water dissolved in the structure of soda-lime-silica glass on melting, forming and propperties: state of the art and controversial issues. Glass Sci. Technol., v.72, p.145-152, 1999.

(14) Fokin, V.; Nascimento, M.; Zanotto, E. Correlation between maximum crystal growth rate and glass transition temperature of silicate glasses. J. of Non-Cryst. Solids, v.375, p.789-794, 2005.

(15) Chandra, S.; Delbert, E. An Analysis of Nucleation-Rate Type of Curves in Glass as Determined by Differential Thermal Analysis. J. Am. Ceram. Soc., v.80, p.3100-3108, 1997.

(16) Sainz, M.A.; Pena, P.; Serena, S.; Caballero, A. Influence of design on bioactivity of novel $CaSiO_3$–$CaMg(SiO_3)_2$ bioceramics: in vitro simulated body fluid test and thermodynamic simulation. Acta Biomater., v.6, p. 2797-2807, 2010.

(17) Jones J.R.; Hench, L.L. Biomedical materials for new millenium: perspective on the future. Materials Science and Technology, v.8, p.891-900, 2001.