BIOGLASS DISSOLUTION: A COMPARISON BETWEEN SBF SOLUTION AND HYDROLYTIC ETCHING

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ABSTRACT

The evaluation of chemical dissolution phenomena of the glasses in general is important because it is related to the bioactivity. This paper aims a comparative study of the bioglass dissolution between in vitro bioactivity test in SBF (Simulated Body Fluid) solution and the chemical durability test, in glasses on SiO_2-Na_2O-CaO with 6wt% de P_2O_5 system. The glasses were obtained by melting at 1500°C/2h and annealed at 500°C/2h followed by natural cooling. To in vitro test, the samples were immersed in SBF solution in different periods (1, 3, 7 and 14 days) at 7,25 pH and 37ºC. For the hydrolytic resistance test were performed in a Soxhlet column. The physic-chemical and morphological characterization of the samples before and after both tests was realized through XRD, DRIFT and SEM. The samples presented a difference in kinetics dissolution for both tests that allow an improved understanding of the glasses bioactivity mechanism.

Key Words: Biomaterials, Bioglass, Simulated Body Fluid, Chemical Durability

1. INTRODUCTION

Bioglasses, calcium phosphate based ceramics and A-W glass ceramics are examples of bioceramics that can promote a suitable bonding with biological tissue and promote the osteointegration process [(1)].

The first publication of bioglass with osteointegration characteristics was successfully reported in 1971, by Larry Hench [(2)]. Lately, the reactions that can

occurs in material-tissue interface were systematically *in vitro* predicted. With this model, the interaction between bioglass (as biomaterial) and simulated body fluid is important in order to predict the apatite surface layer formation, which is able to chemically interact with bone tissue. This process is in general regulated by five stages [3], comprising (a) faster change of ions Na^+ or K^+ from materials with H^+ and H_3O^+ ions in solution; (b) the soluble silica is released for solution, with the formation of silanol groups due to the breakage of Si-O-Si bonding; (c) Condensation and re-polymerization of SiO_2 rich surface layer on the surface which is poorly in alkali and alkali earth ions; (d) the Ca^{2+} e PO_4^{3-} ions are released (by a silica rich layer), forming a phosphorous and calcium rich layer, and a subsequently amorphous calcium phosphate layer, forming a double-layer structure; (e) crystallization of the amorphous calcium phosphate layer by the OH^-, CO_3^{2-} ions incorporation. This layer corresponds to the carbonated hydroxyapatite crystalline phase.

In order to simulate these reactions, Pentano et al.[4] proposed an acellular solution composed by chloridric acid and tris-hydroxymetil-aminopentano solution able to mimic the body fluid. After that, Kokubo et al. [5,6] suggested the simulated body fluid (SBF) solution, with ionic concentration similar as those presented in human fluids. The composition of this solution was more suitable to understand the reaction mechanisms of such interaction.

Recently, some researchers are being performed in order to study the reaction mechanisms proposed by Hench. In a review article, Cerruti [7] present some characterization techniques, either traditional or advanced ones, used to study the initial formation of the hydroxyapatite surface layer on the surface of bioglass, which involves more complex mechanisms than those proposed by Hench. Jones et al. [8] have showed that calcium ions are constant released and re-deposited on the surface of bioglass, forming an octa-calcium phosphate layer and a subsequently amorphous calcium phosphate layer. This layer is in general crystallized into a carbonated hydroxyapatite phase. Fitz Gerald et al [9] had observed the formation of both tricalcium phosphate and carbonated hydroxyapatite layers on their bioglass surface samples, after different experimental times in SBF.

Besides the hydroxyapatite surface formation, the bioglass degradation process when in contact with body fluid is also important in order to predict the biological behavior of bioglass *in vivo*, as bioactive or resorbable. The first behavior is associated with bioglass which present suitable characteristics to forms the hydroxyapatite surface layer. The resorbable behavior is related to glasses that can be completely degradable in the body, inducing the formation of a bone matrix through their osteoinductor effects [10]. The products of bioglass dissolution can also influence on osteoblasts proliferation, expression and cellular differentiation, collagen production and calcification of a new bone extracellular matrix [11].

On the other hand, the interest into corrosive phenomena that is presented on glass surface is not exclusive to biomaterials science field. As example, several glass technological field study the reactions on interface silicate glasses and the products of degradation are being carefully controlled [12, 13]. The chemical durability test is proposed in standard procedures (Product Consistency Test, ASTM 1285-94)[14] in order to evaluate the degradation process of vitreous materials and to analyses the vitreous structure during water immersion. Within overall characterization techniques, the Fourier Transformed Infrared Spectroscopy (FTIR) is widely used to identify silicon bonding, which helps on vitreous structure characterization [12, 13].

In this work, the structure of vitreous surface was analyzed through chemical durability and simulated body fluid in vitro tests after different periods. These analysis were associated in order to better characterize the structural arrange of different composition of bioglasses. The comprehension of such mechanisms can helps on the bioglass processing with controlled bioactivity and degradation rates. These behaviors certainly influence on biological response of such materials when *in vivo* applied.

2. MATERIALS AND METHODS

Three composition of bioglass were chosen in a systematic way on the Na_2O-CaO compatible line of Na_2O-SiO_2-CaO ternary phase diagram with fixed

6% de P_2O_5, containing resorbable, bioactive and resorbable/bioactive behavior transition glasses (BG-Na, BG and BG-Ca, respectively). The glass processing was described by Borges et al. [15]. Briefly described, the raw materials were fused in vertical electrical furnace at 1500°C/2h, poured into a brass mold and subsequently annealed at 500°C/2h, followed by natural cooling. The glasses were cut into 0,5 x 0,5 x 0,1cm specimens using a high precision equipment (ISOMET 4000, Buehler)

For the bioactive in vitro tests, the specimens were immersed on 3 ml of a prepared SBF solution (controlled at 7.25 pH) [6] for different periods (1, 3, 5, 7 and 14 days) using an incubator system (40rpm, 37°C). During the procedure, the SBF solution was changed every 2 days, in order to optimize the in vitro simulation. In order to evaluate the chemical durability, the specimens were immersed in an aqueous solution during different periods (3, 5, 7 and 14 days) using a Soxlet distillation equipment controlling the temperature of the system.

In order to understand the bioglasses structure, before and after in vitro tests, the samples were characterize using X-ray diffraction (XRD), diffuse reflectance infrared fourier transform spectroscopy (DRIFT) and scanning electron microscopy (SEM) techniques.

3. RESULTS AND DISCUSSION

The x-ray diffractograms after in vitro experiments during different periods were presented in Fig. 1. After chemical durability during 14 days (Fig. 1a), no crystalline structure was observed. On the other hand, after SBF in vitro tests during 5 days (Fig. 1 c-d) the crystalline phases carbonated hydroxyapatite (ICDD 01-084-1998) and calcium silicate (ICDD 00-036-0642) were identified, respectively in BG and BG-Ca samples.

FTIR spectra of bioglasses after in vitro tests are presented in Fig. 2. Considering BG-Na and BG samples after different SBF immersion periods (Fig. 2, 2d and 2e), there is a dislocation on peak position in 960 e 1080 cm^{-1} region. These region corresponds to the silicates formation (peak 5), related to the silica gel layer on the surface of glasses during dissolution process.

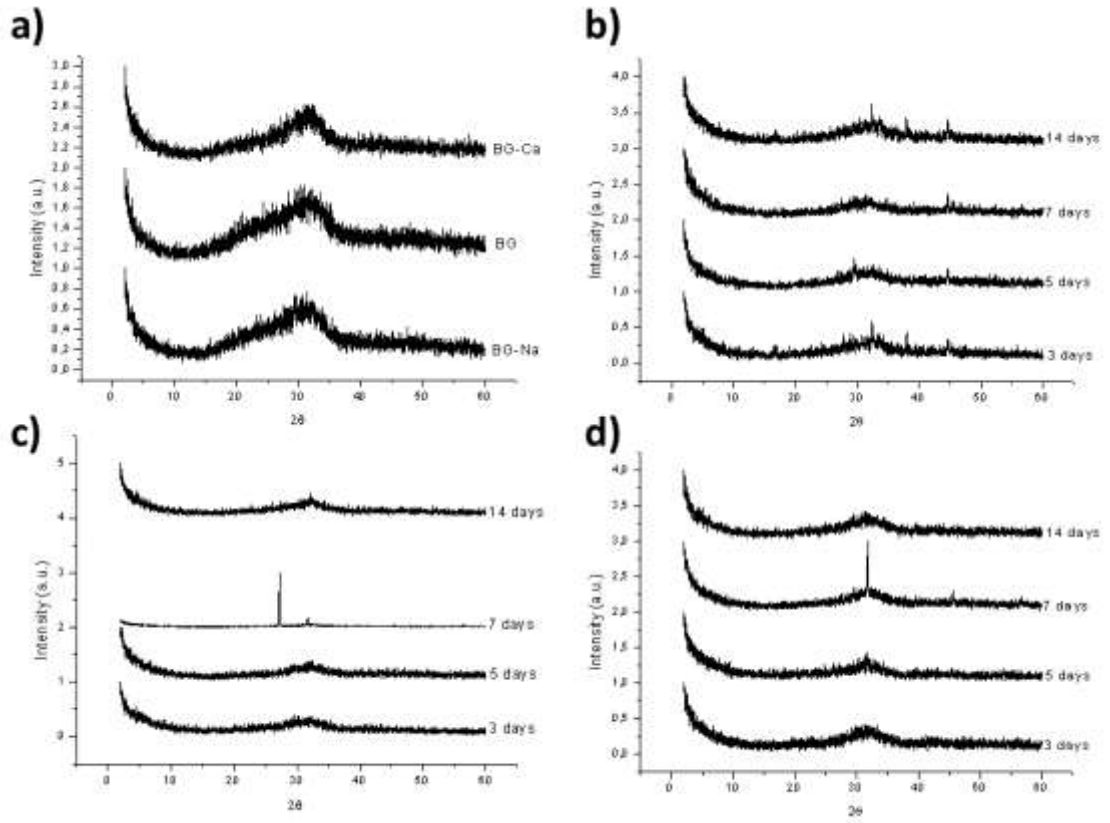

Figure 1: XRD: after chemical durability test (a); BG-Na (b), BG (c) and BG-Ca (d) samples after different periods of immersion in SBF solution

Before in vitro tests, the higher peak can be associated with arranges containing higher amounts of silica Q^3 (at 1080 cm^{-1}). However, during the SBF immersion tests, this peak shifts to higher silica Q^2 arrange (at 960 cm^{-1}). During chemical durability experiments (Figs. 2a and 2b), this region of both glasses seems to be a Q^3 arranges. This fact can indicates a diffusion process of R^{+X} ions (such as Na^+; K^+; Ca^{2+}; Mg^{2+}) on glass surface, which were in SBF solution during experimental procedures.

Such ions were acting as a charge balance in vitreous lattice of silica gel, so that silica chains less depolymerized and more stable to OH^- etching can be formed. Without these ions, the FTIR spectra after chemical durability tests ((fig 2a and 2b) suggests that in order to keep a lattice equilibrium after OH^-, the phosphate ions plays an important role. The percolation of such ions for the surface would be damaged, hindering the formation of calcium and phosphorous compounds on the gel layer. This hypothesis is supported considering that the Q1 and Q^0 peaks related to segregate silica (at 900-920

cm^{-1} and 850-880 cm^{-1}, respectively) and peaks corresponding to segregated metals oxides (480-510 cm^{-1}) had their intensity decreased as the SBF experimental period was increased. Moreover, the P-O bonding is presented as amorphous (550-560 cm^{-1}) or crystalline. When immersed in SBF solution, the crystalline bonding (515-530 cm^{-1}) tends to a region that is associated with a smaller participation on the vitreous structure than the peak located at 600-610 cm^{-1}, which is observed with higher intensity during chemical durability tests. The behavior of P=O peaks seems independent of the other ones.

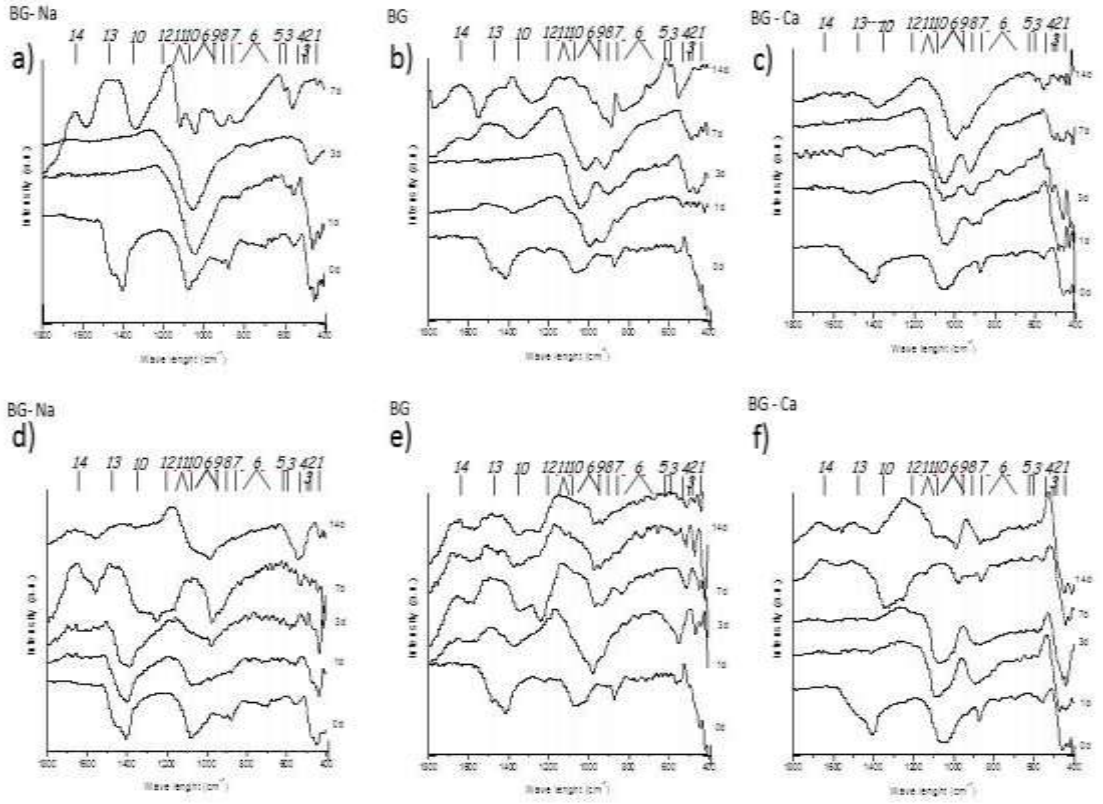

Figure 2 – FTIR before and after different periods in: a,b, and c, chemical durability test; d,e and f, SBF solution. 1.Si-O-Si [Q^4]; 2. Metal Oxides segregated; 3. P-O Crystalline; 4. P-O Vitreus**; 5.** OH Hydroxyapatite**; 6.** Silicates**; 7.** Si-O[NB],[Q^0]; 8. Si-O[NB][Q^1]**; 9.** Si-O[NB],[Q^2]**; 10.** P=O**;** 11. Si-O[NB],[Q^3]**;** 12. Si-O,[Q^4]**; 13.** Na-O**; 14,** structural water.

On the other hand, FTRI of BG-Ca samples after in vitro tests (Fig. 2f) can show same behavior considering chemical durability tests (Fig. 2c) but another behavior considering the SBF in vitro test (Fig. 2f) when compared to BG and BG-Na samples. During SBF in vitro procedures, the presence of peaks related to segregated metals oxide, silica formation Q^0 and Q^1 and the shifting gel

layer containing silicates for Q^2 associations, are observed only in longer periods (7 and 14 days). This behavior leads naturally in the formation of lower amounts of calcium and phosphorous compounds on the gel surface. Such observation can be explained by the higher CaO amount on BG-Ca sample, acting as a lattice modifier more stable than R+, which helps the charge equilibrium of the vitreous lattice on the gel layer and, consequently, reduce the cation diffusion from the solution. This mechanism, which is associated with ion diffusion from SBF solution can control the phosphorous percolation rate for the gel surface. Moreover, leads to interpretation that the presence of calcium and phosphorous compounds on such surface can be associated with the attraction of R^+ cations, when associated with silica groups, for the charges compensation on the gel layer.

Scanning electron micrographs of BG-Na, BG and BG-Ca samples after in vitro tests are presented in Figs. 3 and 4.

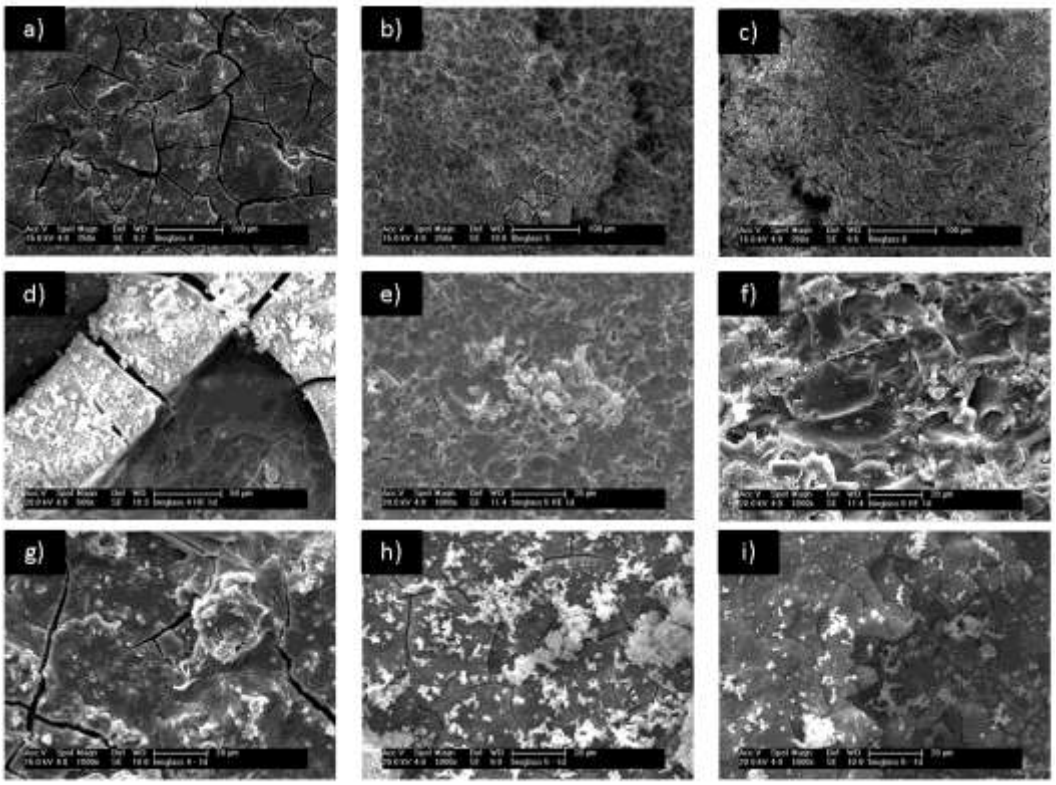

Figure 3 – MEV: BG-Na sample before tests (a), after one day in hydrolytic etching (d) and after one day in SBF solution (g); BG sample before tests(b), after one day in hydrolytic etching (e) and after one day in SBF solution (h; BG-Ca sample before tests (c), after one day in hydrolytic etching (f) and after one day in SBF solution (i).

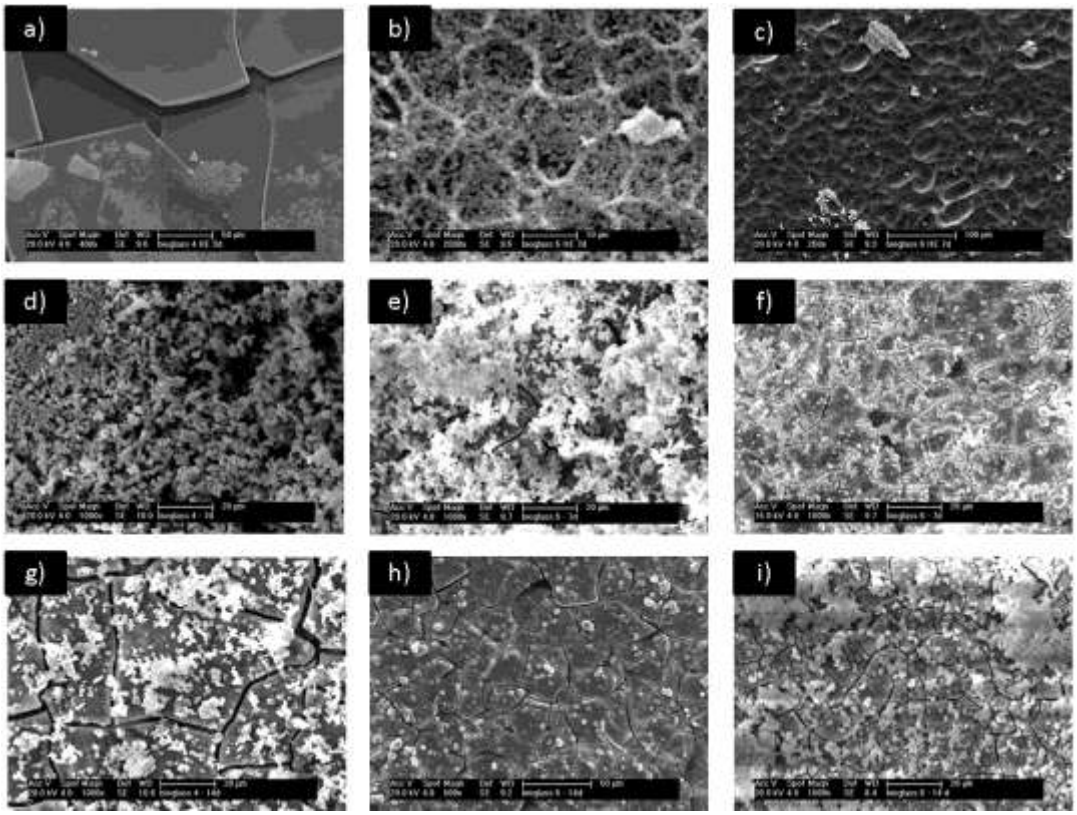

Figure 4 – MEV: BG-Na sample after three days in hydrolytic etching (a), after 7 (d) and 14 days (g) in SBF solution; BG sample after three days in hydrolytic etching (b), after 7 (e) and 14 (h) days in SBF solution; BG-Ca sample after three days in hydrolytic etching (c), after 7 (f) and 14 (i) days in SBF solution.

Considering BG-Na sample, the formation of amorphous calcium phosphate compounds is observed after third day SBF immersion test (Fig 4d). However, after chemical durability tests (Fig 4a) this formation was not observed. This results are supported from FTIR analysis (Fig 2a and 2d). For BG sample, with a similar composition that the 45S5 bioglass developed by Hench [2] the formation of such compounds occurs after only one day SBF immersion (Fig 3h), also in accordance with FTIR results (Fig 2e). The formation of calcium phosphate compounds during chemical durability tests seems to be formed after 7 days (Fig. 4b). For BG-Ca samples, the calcium phosphate compounds formation is observed only after 14 days in SBF immersion (Fig. 4i), confirming the overall results.

4 . CONCLUSIONS

The dissolution tests of bioglasses containing three different compositions close to 45S5 commercial bioglass, through SBF immersion or chemical durability procedures, have demonstrated distinct behavior after samples characterization. As the chemical durability is a non usual procedure for biomaterials characterization, the association with a usual SBF in vitro test can be successfully applied on the understanding of the dissolution kinetics and bioactivity of several bioglasses. Considering the studied compositions, the indication of an interesting mechanism of ion dissolution from SBF solution to etched surface of bioglass was observed. This behavior is an important factor on formation and remaining of calcium phosphate compounds on bioglass surface, acting also on the materials bioactivity. A dependency of R^{+}/R^{2+} relation on bioglass composition, as an inductor of such mechanism, was also observed.

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