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Ordering in sol-gel-derived bioactive glasses and its influence on the dissolution/precipitation behavior in a complex culture medium

Silmara Caldas Santos[a], Gilderman Silva Lazaro[a], Euler Araujo dos Santos[a,b,*]

[a] Programa de Pós-Graduação em Ciência e Engenharia de Materiais (P²CEM), Universidade Federal de Sergipe, Av. Marechal Rondon, s/n, São Cristóvão 49100-000, Sergipe, Brazil

[b] Departamento de Ciência e Engenharia de Materiais (DCEM), Universidade Federal de Sergipe, Av. Marechal Rondon, s/n, São Cristóvão 49100-000, Sergipe, Brazil

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ABSTRACT

Sol-gel-derived bioactive glasses obtained via sol-gel can exhibit inherent structural ordering even below the glass-transition temperature (T_g) depending on the levels of modifier and intermediate oxides that are included in their composition. To answer whether this previous structural ordering can affect their surface reactivity, we synthesized two distinct systems that are known to present ordering below the T_g: a standard bioactive glass without MgO [Na_2O-CaO-P_2O_5-SiO_2] and another that contained different MgO proportions in substitution for CaO [Na_2O-CaO(MgO)-P_2O_5-SiO_2]. The bioactive glass without MgO suffered an ordering process below the T_g with the formation of nanocrystalline apatite domains. The insertion of MgO caused the ordering of nanocrystalline Mg^{2+}-containing β-tricalcium phosphate domains. After immersion in a complex medium (McCoy's 5A modified culture medium), the MgO-containing bioactive glass had a higher apatite precipitation rate than the bioactive glass without MgO. Although the MgO reduced the T_g, the increased precipitation was not caused directly by the decrease in T_g but rather by the previous presence of the ordered phase. Additionally, the layer precipitated onto the MgO-containing bioactive glass presented a higher level of Mg^{2+} and CO_3^{2-} ions, suggesting the formation of an Mg^{2+}-containing carbonate apatite similar to that observed in bone tissue. Therefore, the inherent ordering of sol-gel-derived bioactive glasses below the T_g can define their bioactivity, which constitutes a consistent way of controlling their surface reactivity.

1. Introduction

Mg^{2+} ions are known to participate actively in the osteogenesis process, promoting excellent biocompatibility and osteoconductivity to biomaterials in which these ions are present [1]. Mg^{2+} ions can establish bonds between proteins and several types of biomaterial surfaces, directly affecting the adsorption of important biological molecules [1–5]. Thus, the use of MgO in the composition of bioactive glasses is a tool for controlling their bioactivity and, consequently, the corresponding cellular behavior.

The transformations observed on the bioactive glass surface upon implantation into the body or immersement in a simulated body fluid are directly related to its surface reactivity. Two processes are involved: dissolution/degradation of the bioactive glass, and the subsequent precipitation of a biological apatite that is very similar to that initially mineralized into new bone tissue [6–8]. The attachment of proteins, growth factors and cells will always be influenced by the composition and structure of this precipitated layer.

There are two manners for controlling the surface reactivity of bioactive glasses: manipulating their composition, and changing their average surface area. Sol-gel-derived bioactive glasses can present significant structural differences when compared to melting-derived glasses. In the melting process, the fast cooling procedure reduces the mobility of ions, avoiding their migration to specific regions as a result of free energy reduction [9–11]. In general, the obtained glass structures are very dense, with an insignificant level of porosity [10,11]. On the other hand, sol-gel-derived glasses can range from extremely dense structures to mesoporous [12,13]. Additionally, the gelification process can create very specific short- and medium-range atomic ordering. The gel is initially formed by a network of globular SiO_4 tetrahedral units evolved by a liquid environment that contains several ions, mainly alkaline and alkaline earth ions (e.g., Na^+, K^+, Ca^{2+}, Mg^{2+}) [14]. This environment provides higher mobility for the ions, which are confined in a nanoporous structure. The ions are generally stabilized close to the non-binding oxygens at the surface of the clustered SiO_4 chains. As a consequence, channels that are rich in alkaline and alkaline earth ions

* Corresponding author at: Departamento de Ciência e Engenharia de Materiais (DCEM), Universidade Federal de Sergipe, Av. Marechal Rondon, s/n, São Cristóvão 49100-000, Sergipe, Brazil.
E-mail address: euler@ufs.br (E.A. dos Santos).

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are generated between these silica clusters [15]. This structural arrangement is quite favorable to the crystallization of phases composed of these ions.

In a recent study, we showed that a standard sol-gel-derived bioactive glass [Na_2O-CaO-P_2O_5-SiO_2] exhibited an inherent crystallization of a nanostructured calcium apatite [9]. This crystallization was directly associated with the heterogeneities in the distribution of alkaline and alkaline earth ions, and phosphate specimens in the glass structure. To confirm the crystallization of this nanostructured calcium apatite phase, we used MgO as a probe oxide by inserting it into the bioactive glass composition. We knew that in the presence of Mg^{2+} ions, calcium apatites are transformed into whitlockite [16]. These crystallization processes occurred below the glass-transition temperature (T_g) as a direct result of structural arrangement during the gelification process.

The main issue raised from these previous results was how this inherent crystallization affects the glass bioactivity, specifically, the dissolution and precipitation processes that occur when these materials are implanted into the body or immersed in simulated body fluids. To answer this question, we used a bioactive glass composition that is similar to that previous studied: [Na_2O-CaO(MgO)-P_2O_5-SiO_2]. The CaO was replaced by MgO at three different ratios (0.7, 1.4 and 2.1%). The effects of MgO on the bioactive glass structure and its thermal behavior were evaluated by wavelength dispersive X-ray fluorescence (WDXRF), thermogravimetric analysis (TGA), differential thermal analysis (DTA) and X-ray diffraction (XRD). The dissolution/precipitation assays were conducted by immersing the samples in McCoy's 5A culture medium, a complex medium used to culture human cells [17] that has a composition that is not merely inorganic but enriched by amino acids and sugar [7], which makes it closer to the composition of blood plasma. The surface transformations after immersion were followed by inductively coupled plasma optical emission spectrometry (ICP-OES), diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy, scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS).

2. Materials and methods

2.1. Bioactive glass synthesis

2.1.1. Materials

Tetraethylorthosilicate (TEOS, $Si(OC_2H_5)_4$), nitric acid (HNO_3) 0.1 mol L^{-1}, triethyl phosphate (TEP, $OP(OC_2H_5)_3$), sodium nitrate ($NaNO_3$), calcium nitrate tetrahydrate ($Ca(NO_3)_2.4H_2O$) and magnesium nitrate hexahydrate ($Mg(NO_3)_2.6H_2O$) were purchased from Sigma and had purity grades above 98%.

2.1.2. Standard bioactive glass

TEOS was diluted with nitric acid and mixed until the solution became clear. Then, the other reagents were added according to time until the solution became clear again. The synthesis was performed at room temperature. The solution was stirred for an additional 1 h and then stored in closed containers at room temperature for 10 days to promote gelification. The obtained gel was dried at 60 °C and 120 °C for 72 and 40 h, respectively. After drying, the xerogel was grounded, sieved and stored. Pellets with a diameter of 9.0 mm and a height of 1.5 mm were prepared by uniaxial pressing of the xerogel powders under a load of 1.5 tons. After pressing, the obtained tablets were sintered at 700 °C for 2 h (heating rate of 10 °C/min). The synthesis was performed in triplicate, and independent assays were used to assess the reproducibility of the method.

2.1.3. MgO-containing bioactive glass

The modified glass systems were prepared by substituting CaO for MgO in the following proportions: 0%, 0.7%, 1.4% and 2.1% (mol%). The synthesis of MgO-containing bioactive glass was conducted under the same conditions described above, and magnesium nitrate hexahydrate ($Mg(NO_3)_2.6H_2O$) was used as the source of magnesium.

2.2. Composition

The elemental compositions of the bioactive glasses were determined by WDXRF. Sample analyses were performed after heat treatment at 700 °C in an X-ray fluorescence spectrometer (S8 Tiger, Bruker). The amounts of the identified elements were calculated for each sample and normalized by the total quantification of the elements (mol%) using a calibration curve made with standard oxides.

2.3. Thermal analysis

The thermal behaviors of the bioactive glasses were studied by TGA and DTA using STA 449 F3 Jupiter (Netsch) equipment. Analyses were performed with approximately 60 mg of sample after drying in an N_2 atmosphere with a flow rate of 50 mL min^{-1}. The samples were heated in a platinum crucible at a 10 °C/min heating rate from room temperature to 1100 °C.

2.4. Structure

Samples in powder form were thermally treated at 700 °C and then analyzed by XRD. The diffraction patterns were obtained on an XRD 6000 (SHIMADZU) diffractometer using a CuKα source (λ = 1.5405 Å) operating at 40 kV and 30 mA in a 2θ range between 5° to 60° with a step size of 0.02° and a scanning speed of 2° min^{-1}.

2.5. Bioactivity assay

To assess the surface transformations onto the glass systems, only samples without MgO (BV 0.0% Mg) and samples containing 0.7% MgO (BV 0.7% Mg) were used. The 0.7% proportion is exactly the ratio of the replacement of Ca^{2+} by Mg^{2+} in bone-like hydroxyapatite.

The pellets were immersed in McCoy's 5A medium for periods of 1, 4 and 7 days under sterile conditions at 37 °C. After the immersion period, the pellets were washed with distilled water and dried at 120 °C/24 h. The concentrations of Ca^{2+}, PO_4^{3-}, SiO_4^{4-} and Mg^{2+} in the culture medium that were in contact with the pellets were monitored throughout the test by ICP-OES on a Varian 720-ES spectrometer. The pH variations were also monitored over time.

All the morphological transformations that occurred after immersion in McCoy's 5A medium were monitored by SEM using a JEOL 5700 microscope. The samples were recovered by a carbon conductive layer before the analyses. The ratios of emission X-ray lines obtained from the elements Ca, Mg, P and Si were calculated via EDS. These surface changes were also followed by DRIFT spectroscopy. Spectra were acquired directly on the pellet surface in the transmittance mode from 4000 to 400 cm^{-1} with a resolution of 4.0 cm^{-1} using a Perkin Elmer 1720× spectrometer.

2.6. Statistical analyses

Quantitative analyses were performed in triplicate using samples from different syntheses ($n = 3$). The values are expressed as the means ± standard deviations. The statistical significance of the obtained data was evaluated by analysis of variance ANOVA followed by Tukey's test. p values ≥ 0.05 were considered statistically insignificant. Linear regressions were used to identify correlations between variables, where the statistical parameters r^2 and p were always calculated.