Individual and combined effects of the elements Zn, Mg and Sr on the surface reactivity of a $\text{SiO}_2\cdot\text{CaO}\cdot\text{Na}_2\text{O}\cdot\text{P}_2\text{O}_5$ bioglass system

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**Abstract**
This study had two aims: to determine how the elements Zn, Mg and Sr can affect the reactivity of a bioglass system, and to determine the mechanism of dissolution/precipitation when this material is immersed in a complex medium such as cell culture medium. To answer these questions, we synthesized a standard bioglass ($\text{SiO}_2\cdot\text{CaO}\cdot\text{Na}_2\text{O}\cdot\text{P}_2\text{O}_5$, BV), a bioglass containing Zn (BV-Zn), Mg (BV-Mg) or Sr (BV-Sr), and a bioglass containing all three elements (BV-SrZnMg) by the sol–gel route. A typical cell culture medium (McCoy’s 5A modified medium) was used for the dissolution/precipitation assays. Thermogravimetric analysis (TGA), differential thermal analysis (DTA), X-ray diffraction (XRD) and $\text{N}_2$-adsorption measurements were performed to characterize the obtained glasses. Inductively coupled plasma optical emission spectrometry (ICP-OES), diffuse reflectance infrared Fourier transform (DRIFT), scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) analyses were performed to determine the bioglass reactivity after immersion in McCoy’s 5A modified medium. The mechanism of ionic change responsible for the initial increase in pH after immersion in cell culture medium was independent of the modifier elements (Zn, Mg and Sr) present in the samples. The addition of Sr to the bioglass composition did not significantly change the $\text{SiO}_4^{4-}$ release compared to the control. The presence of Zn increased $\text{SiO}_4^{4-}$ release by decreasing the crystallization temperature $T_c$. In contrast, the $\text{SiO}_4^{4-}$ release decreased upon the addition of Mg to the glass system, despite a remarkable decrease in $T_c$. The presence of Mg in the McCoy’s 5A medium most likely generated a saturation state close to the surface, avoiding $\text{SiO}_4^{4-}$ release. While $\text{SiO}_4^{4-}$, $\text{Zn}^{2+}$ and $\text{Sr}^{2+}$ were released from the bioglasses upon immersion, $\text{Ca}^{2+}$, $\text{Mg}^{2+}$ and $\text{PO}_4^{3-}$ were captured from the medium. As a consequence, the formation of a Ca–Mg phosphate layer containing $\text{CO}_3^{2-}$ was observed for all samples, regardless of which elements were present.

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1. Introduction

The surface reactivity of a bioglass is a determining factor in its ability to participate in the tissue regeneration process [1,2]. Surface reactions with physiological media may induce the precipitation of a bone-like apatite layer, providing appropriate biocompatibility [1–4]. These surface reactions are also responsible for the degradation of the bioglass after implantation, which can directly affect its replacement by a new tissue. This degradation should occur in a controlled manner, with balance always maintained between degradation and new tissue formation. Several methods have been used to establish the equilibrium between degradation and new tissue formation to improve the performance of existing systems [5]. One of these methods involves the use of sol–gel instead of the melt–quenched process. The basic difference between the glasses obtained by these two methods is that there is a higher nanoporosity and surface area achieved by sol–gel [5,6]. The increase in the surface area consequently increases its reactivity/solubility. At the same time, the presence of nanoporosity can improve cellular response, stimulated by the presence of a nanotopography [5,6].

Another usual method involves the insertion of intermediate and/or network modifier elements into the bioglass structure. These elements control the surface reactivity and stimulate cellular activity, accelerating the regeneration of damaged tissue [7–9].

From the biological point of view, the use of the elements Mg, Sr and Zn in bioglass systems is generally justified by their ability to improve cellular metabolism [7,9–12]. The gradual release of these elements from bioglass can affect the mechanism of bone formation by stimulating the growth and proliferation of osteoblasts, the synthesis of extracellular matrix, and thus bone remodeling by osteoclasts. At the same time, these elements can act as intermediate or network modifiers in the amorphous structure of a bioglass system, directly affecting its surface dissolution and precipitation behavior after immersion in physiological media, such as Kokubo solutions. Several studies have shown that the presence of Sr in the bioglass system may increase apatite precipitation [5,13,14]. Conversely, the presence of Zn has been shown to induce a decrease in apatite precipitation [15,16]. Increasing concentrations of Mg seem to reduce the bioglass degradability and the formation of apatite [17–19].
In most of these studies, the bioactivity assays are conducted by immersing the samples in Kokubo solutions [20,21]. However, cell culture assays are performed in typical cell culture media that exhibit a more complex composition than the Kokubo solutions. This means that the samples are exposed to completely different environments during the two assays that could lead to different surface transformations. In this sense, a solution for characterizing the surface bioactivity must warrant an adequate correlation between solution-mediated transformations occurring onto material surface and cell behavior [22]. Besides, it has the role of reducing the false extrapolations in the comparison of in vitro experiments and in vivo performance [22–24]. Therefore, to improve the value of bioactivity studies, we studied the surface reactivity of bioglasses containing Mg, Sr and Zn in McCoy’s 5A medium, a typical medium used for the culture of osteoblastic cells [25]. Our goal here is a) to determine how individual elements or combinations of the elements affect the reactivity of a bioglass system, and b) to elucidate the mechanism of dissolution/precipitation in a more complex medium.

2. Materials and methods

2.1. Materials

Tetraethyl orthosilicate (Si(OC₂H₅)₄, TEOS), triethylphosphate (OP(OC₂H₅)₃, TEP), calcium nitrate tetrahydrate (Ca(NO₃)₂·4H₂O), sodium nitrate (NaNNO₂), magnesium nitrate hexahydrate (Mg(NO₃)₂·6H₂O), strontium nitrate (Sr(NO₃)₂), zinc nitrate hexahydrate (Zn(NO₃)₂·6H₂O) and 0.1 mol L⁻¹ nitric acid were used as precursors. All were obtained at >98% analytical grade.

2.2. Preparation of the bioglass systems

The quaternary glass system SiO₂-CaO-Na₂O-P₂O₅ was prepared by the sol–gel method [26] and used as the control material. Initially, 0.1 mol L⁻¹ nitric acid was added to the TEOS in the presence of stirring for 30 min to complete its hydrolysis and polycondensation. Then, TEP and other salts were added consecutively to the TEOS, with 30 min intervals between each new addition. After the last addition, the mixture was stirred for an additional 1 h to complete the reaction. The obtained solution (sol) was kept in sealed polymer plates at room temperature for 10 days to allow the gel to form. Then, the gel was heated in two steps to promote the complete elimination of the water: 1) at 70 °C for 10 days and 2) at 120 °C for 48 h. The dried gel (xerogel) was milled and sieved to obtain particle sizes smaller than 44 µm.

The modified glass systems were prepared by substituting CaO for the other oxides (ZnO, SrO and MgO) individually or in combination (Table 1). The other precursors were kept at the same concentration of each inserted element followed the proportions observed in bones [27]. Tablets with a diameter of 9.0 mm and a height of 1.5 mm were prepared by uniaxial pressing of xerogel powders under a load of 1.5 ton. After pressing, the obtained tablets were sintered at 700 °C for 2 h.

Three independent syntheses were performed to evaluate the reproducibility of the analyses used in this work.

Table 1
Theoretical composition of the bioglasses studied in this work.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Composition (wt.%)</th>
<th>SiO₂</th>
<th>P₂O₅</th>
<th>Na₂O</th>
<th>CaO</th>
<th>ZnO</th>
<th>MgO</th>
<th>SrO</th>
</tr>
</thead>
<tbody>
<tr>
<td>BV</td>
<td></td>
<td>64.42</td>
<td>5.95</td>
<td>5.20</td>
<td>24.43</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>BV-Sr</td>
<td></td>
<td>64.42</td>
<td>5.95</td>
<td>5.20</td>
<td>24.31</td>
<td>–</td>
<td>–</td>
<td>0.12</td>
</tr>
<tr>
<td>BV-Mg</td>
<td></td>
<td>64.42</td>
<td>5.95</td>
<td>5.20</td>
<td>22.48</td>
<td>1.95</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>BV-Zn</td>
<td></td>
<td>64.42</td>
<td>5.95</td>
<td>5.20</td>
<td>24.42</td>
<td>0.01</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>BV-SrZnMg</td>
<td></td>
<td>64.42</td>
<td>5.95</td>
<td>5.20</td>
<td>22.36</td>
<td>0.01</td>
<td>1.95</td>
<td>0.12</td>
</tr>
</tbody>
</table>

2.3. Thermal behavior of the glass systems

2.3.1. Thermal analyses

The thermal properties of the glass systems were studied by thermogravimetric and differential thermal analyses (TGA/DTA) in a STA 449 F3 Jupiter Netzsch instrument. Non-isothermal experiments were carried out by heating 70 mg of each xerogel glass system (10 °C/min) in a Pt crucible under a N₂ atmosphere (50 mL/min) up to 1100 °C.

2.3.2. X-ray diffraction

X-ray diffraction (XRD) was used to confirm the glass state and to identify the presence of crystalline phases after thermal treatment. These tests were performed from 2θ = 5° to 60° (with a step size of 0.02°) and a scanning speed of 2°/min in a SHIMADZU (model XRD 6000) powder diffractometer (CuKα λ = 1.5405 Å).

2.4. Specific surface area

The N₂ adsorption and desorption measurements were performed using the Barrett–Joyner–Halenda (BJH) method in a BET Surface Area Analyzer-Quantachrome Nova® 1200e. Before analysis, bioglass samples were degassed at 300 °C for 4 h.

2.5. Bioactivity

2.5.1. Bioactivity assay

To assess the surface transformations onto the glass systems, the samples (tablets) were placed in standard 24-well cell culture plates...
Temperatures performed in the absorbance mode from 4000 to 400 cm
The diffuse re and energy dispersive spectroscopy (EDS) in a JEOL 5700 microscope.

on the surface were observed by scanning electron microscopy (SEM)

3. Results and discussion

2.6. Statistical analyses

All the quantitative experiments were performed in triplicate using specimens from different syntheses (n = 3). The values were expressed as the mean ± standard deviation. The statistical significance of the obtained data was assessed using ANOVA variance analysis followed by Tukey test. Differences of p ≥ 0.05 were considered to be statistically insignificant.

3. Results and discussion

3.1. Thermal behavior of the glass systems

The mass loss observed in the TGA/DTA curves occurred in three distinct steps (I, II and III) for all samples (Figs. 1, 2). The first loss occurred at approximately 150 °C and is directly associated with an endothermic process. According to the literature, this mass loss is attributed to the loss of physically adsorbed water and ethanol evaporation. The second loss is associated with the elimination of chemically adsorbed water and occurred at approximately 260 °C as an endothermic process. The third loss occurred in the range of 500–600 °C and is also associated with an endothermic process generated by the condensation of silanol groups and the elimination of the remaining nitrates and organic compounds from the precursors [6,26,28,29].

Above 700 °C, the mass loss becomes insignificant, indicating the complete release of the volatile reaction products. The absence of mass loss above that temperature allowed us to associate the observed thermal events to those typically described for glass systems, such as the glass transition temperature Tg and the crystallization temperature Tc. A small deviation of the baseline of the curves was observed at approximately 700 °C, characterizing the range of Tg for each glass system (Table 2). Other remarkably exothermic events were observed at approximately 897 °C for the samples BV-Zn, BV-SrZnMg and BV-Mg and at approximately 950 °C for BV and BV-Sr. These events are associated with the Tc for each glass system. For some samples, other exothermic peaks were verified at higher temperatures, suggesting additional crystallization events.

The variations in Tg among glass systems are directly related to the mobility of the inorganic chains forming the glass network, and therefore, to the viscosity of these materials at a certain temperature. Especially for the systems studied in this work, these variations in Tg indicate how each new element added to the control glass system can affect the mobility of the chains formed by SiO4 and PO4 tetrahedra.
However, no significant variations in $T_g$ were observed for the systems studied here. This can be related to the fact that the substitution of Ca for the other elements was performed at very low concentrations. In fact, most studies in the literature reporting significant variations in $T_g$ after the substitution of other elements for the Ca (at the same heating rate) used higher concentrations than used here [15,30].

The crystallization temperatures $T_c$ of the glass systems were easily observed by DTA. Crystallization processes in glasses are driven by the mobilities of the atoms and their capacity to form a crystalline network. Once the activation energy for ordering atoms in a lattice is reached, the crystallization process will take place. Indeed, the presence of each different oxide remarkably changed the crystallization temperatures $T_c$ and the onset crystallization temperatures $T_i$ (Table 2). In all cases, the insertion of the oxides decreased $T_i$ and $T_c$ at $\Delta T > 10^{°}\text{C}$, except for the Sr-containing sample (BV-Sr). In this case, the temperature was similar to that observed for the control glass system (BV), suggesting that Sr and Ca played a similar role in the thermal behavior of these samples [13].

Mg, Sr and Ca oxides are considered network modifiers in glass systems or fondants [31]. Therefore, in contrast to SiO2 or P2O5 (network formers), after addition to glass systems, these oxides will break the chains of SiO4 and PO4, interrupting the bonds of bridging oxygens ($\text{Si}-\text{O}\rightarrow\text{Si}$ and $\text{P}-\text{O}\rightarrow\text{P}$) and generating non-bridging oxygens at the ends of the chains ($\text{Si}\rightarrow\text{O}^{\text{M}^{2+}}\text{O}\rightarrow\text{Si}$) [31]. Several criteria have

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Fig. 6. Schemes of the surface reactions onto the bioglasses after immersion in physiological solutions.
been defined for predicting the role of oxides in glass formation [32,33]. In a simplified approach, one can assume that whether an element acts as a modifier, an intermediate or a glass network former is defined by the type of chemical bond established with oxygen (ionic or covalent). It is accepted that the higher the ionic character, the greater the tendency of an element to act as a network modifier [34]. Zachariasen described a set of rules that determine how an element will act [31,35]. Based on the ionic field strength (1 = Z / r²), where Z and r are the cation charge and its radius, respectively, McMillan found that a network modifier cation must have I less than 5 Å⁻² [33,36]. It has also been reported that this behavior may have a significant compositional dependence [37], as the same element may act either as a modifier or a network former in accordance with their close chemical environment. Thus, elements such as Zn and Al are frequently considered intermediates between the modifier and network former categories.

The effect of Sr on the bioglass network is controversial because the results are influenced by the method used to calculate the substitution of Ca (weight or molar percent) [13,38]. When the SrO content substituted for CaO in the system is increased, as SrO has a higher molecular weight than CaO. In this case, the glass network would tend to be stabilized, and the glass transition temperature Tg would increase. Similarly, a decreased rate of dissolution would be observed. When the molar quantity of SrO is increased, a contrary effect would be observed for Tc and dissolution. The substitution of CaO for SrO could be assumed to decrease Tg and, consequently, increase the rate of dissolution. In our case, the insertion of Sr in the glass system did not significantly affect Tc or Tg (Table 2 and Fig. 2). This result can most likely be explained by the low quantity of Sr added to the glass (0.12 wt.%).

Mg was the most effective element in decreasing Tc (Table 2 and Fig. 2), with a difference of more than 50 °C observed compared to the control bioglass (BV). This element is considered a network modifier, as is Sr. However, the dissociation energy of the Ca – O bond (257 kcal mol⁻¹) is higher than that observed for Mg – O (222 kcal mol⁻¹) [31,39]. A lower dissociation energy means a greater tendency to disrupt the SiO4 and PO4 chains, increasing the chance that Mg generates non-bridging oxygens. That led to a decrease in the glass viscosity, and therefore a decrease in Tg, which was observed for the samples BV-Mg and BV-SrZnMg (Table 2 and Fig. 2). Watts et al. [39] demonstrated this using MAS NMR analyses. Their results showed that 86% of the MgO added to a SiO2·CaO·Na2O·P2O5 system acted as a network modifier, while the rest entered the silicate network as MgO4 tetrahedral units. The charge imbalance was compensated by other modifier elements available in the bioglass such as Ca. This behavior is the main reason for the weakness of the glass network, as observed in our work.

Mg and Zn are similar with respect to charge and ionic radius (0.72 Å and 0.74 Å, respectively) which means that these two elements are expected to exhibit a similar behavior [39,40]. In fact, Zn is typically defined as an intermediate element and can function either as a modifier or as a network former, according the assumed coordination number. If the coordination number is 4 (tetrahedral units), then Zn must act as a network modifier. In this case, the dissociation energy of the Zn – O bond is 144 kcal mol⁻¹ lower than the energy of the Ca – O bond. This seems to explain why even a very low concentration of Zn compared to the concentration of Ca (BV) and Mg (BV-Mg) leads to a remarkable change in Tc of approximately 49 °C (Tables 1–2 and Fig. 2).

The crystallization behavior of the bioglasses was studied by X-ray diffraction (XRD) after thermal treatment at various temperatures: 600 °C, 700 °C, 800 °C and 900 °C. These thermal treatments were performed under the same conditions used in the DTA analyses. Therefore, the crystallization events observed by DTA could be associated with those verified by XRD.

According to the DTA analyses, the onset crystallization events for our samples occurred above 850 °C. In fact, XRD demonstrated that the samples had an amorphous character until 800 °C (Fig. 3). At 900 °C, crystalline phases appeared in the BV-Mg, BV-Zn and BV-SrZnMg samples (Fig. 4). The crystallizations of the BV and BV-Sr samples were not pronounced at this temperature. Indeed, the onset crystallization temperatures obtained by DTA for those samples were above 930 °C, which can explain this low level of crystallization.

Two different crystalline phases were identified in the samples treated at 900 °C: Na2Ca2Si3O9 (hexagonal calcium and sodium silicate, JCPDS card no 22-1455) and Na2CaSiO4 (cubic calcium and sodium

Table 4

<table>
<thead>
<tr>
<th>Sample</th>
<th>Surface area (m²·g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BV</td>
<td>44 ± 7</td>
</tr>
<tr>
<td>BV-Sr</td>
<td>41 ± 15</td>
</tr>
<tr>
<td>BV-Mg</td>
<td>41 ± 14</td>
</tr>
<tr>
<td>BV-Zn</td>
<td>54 ± 15</td>
</tr>
<tr>
<td>BV-SrZnMg</td>
<td>41 ± 16</td>
</tr>
</tbody>
</table>

Table 3

<table>
<thead>
<tr>
<th>Sample</th>
<th>Released (mmol)</th>
<th>Entrapped (mmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SiO₄⁻</td>
<td>Sr²⁺</td>
</tr>
<tr>
<td>BV</td>
<td>6.8 ± 0.4</td>
<td>–</td>
</tr>
<tr>
<td>BV-Sr</td>
<td>6.9 ± 0.1</td>
<td>3.2 ± 0.4</td>
</tr>
<tr>
<td>BV-Mg</td>
<td>5.6 ± 0.2</td>
<td>–</td>
</tr>
<tr>
<td>BV-Zn</td>
<td>7.4 ± 0.3</td>
<td>–</td>
</tr>
<tr>
<td>BV-SrZnMg</td>
<td>6.3 ± 0.5</td>
<td>2.5 ± 0.4</td>
</tr>
</tbody>
</table>

| a | | 10⁻⁴ |
| b | | 10⁻³ |
| c | There is a significant difference when compared with BV (n = 3; p < 0.05).
| d | There is a significant difference when compared with BV-Zn (n = 3; p < 0.05).

Fig. 7. Si concentration in the cell culture medium (McCoy’s 5A) after immersion of the samples.
silicate, JCPDS card no 03-0831) (Fig. 4). These two phases are typically observed during the crystallization of type 45S5 bioglasses [41,42]. Zn and Mg facilitated the crystallization process compared to Ca and Sr. However, these elements did not affect the phase composition, as the same phases were observed for all samples. It was not possible to identify any other crystalline phases that could be associated with the segregation of the modifier/intermediate elements from the bioglass matrix. This suggests that the modifiers either remained on the amorphous phase or were inserted into the lattice of the crystalline phases formed after the thermal treatment.

Fig. 8. SEM images obtained from bioglasses surface before and after 7 days of immersion in the McCoy’s 5A modified medium.
3.2. Bioactivity of the glasses

3.2.1. Dissolution

After the bioglasses were immersed in McCoy’s 5A medium, the pH was monitored for 7 days. In all cases, the initial pH of 7.4 increased remarkably to approximately 9 on the first day (Fig. 5). According to several studies [1,43–45], this increase in pH is associated with a rapid ionic exchange between Na+ ions from the bioglass and H+ from the aqueous medium. This ionic exchange leads to the formation of silanol groups (Si-OH) on the surface through the reaction shown in Fig. 6a.

The pH was not affected by the composition of the bioglass, as no significant differences were observed throughout immersion between BV and the other samples. In fact, the Na concentration was the same for all the samples. Even considering the other modifier/intermediate elements (Ca, Mg, Sr and Zn), the sum of their concentrations was similar in all the samples. These similar concentrations could explain why no significant changes in pH were observed for any of the samples.

McCoy’s 5A medium has a very low concentration of SiO4^{4−} [22], so the increase in its concentration can be directly associated to the dissolution of the bioglass. The bioglass degradation began immediately after its first contact with the medium through the reaction between water and the SiO4 tetrahedral units from the silicate chains (Fig. 6b).

The hydration-induced disruption of these silicate chains causes the release of soluble isolated SiO4^{4−} or even small chains to the medium. The increase in pH is also known to affect its dissolution by promoting several reactions between Si–OH and OH^− [43].

In our assay, the quantity of SiO4^{4−} in solution was determined by ICP-OES analyses, and a continuous increase was observed throughout immersion for all samples (Fig. 7). After 7 days, the quantity of SiO4^{4−} in solution for the BV-Sr samples was similar to that for BV (Table 3). Therefore, the addition of Sr to the bioglass composition (BV-Sr) did not significantly change the SiO4^{4−} release compared to the control (BV). However, the presence of Zn increased SiO4^{4−} release. According to the literature, decreases in T_g and T_c generally imply easier dissolution [15]. Even at low concentrations, the addition of Zn remarkably reduced T_c (Table 2), explaining the more pronounced SiO4^{4−} release. The BV-Zn samples also had the highest surface area among the samples tested here (Table 4), resulting in a greater susceptibility to dissolution.

In contrast, the addition of Mg to the glass system (BV-Mg) decreased SiO4^{4−} release despite the remarkable decrease in T_c (Table 2 and 3). Considering that the reductions in T_c and the surface area are similar between the BV-Mg and BV-SrZnMg samples, it seems that the mechanism of dissolution cannot be simply described as resulting from a reduction in T_c and an increase in the surface area. Other aspects

![Fig. 9. EDS spectra obtained from BV surface before and after immersion in the McCoy's 5A modified medium.](image)

![Fig. 10. EDS spectra obtained from BV-SrZnMg surface before and after immersion in the McCoy's 5A modified medium.](image)
must be taken into account. For instance, if we consider the three elements studied here (Mg, Zn and Sr), Mg is the only one included in the original composition of the McCoy’s 5A medium [22]. The Mg present in the bioglass reduced $T_c$, remarkably increasing the motility of the chains in the glass structure and consequently increasing its susceptibility to release to the medium. However, the presence of Mg in the McCoy’s 5A medium must have generated a saturation state close to the surface that prevented that release, stabilizing the SiO$_4^-$ chains and decreasing the SiO$_4^2-$ release.

The opposite effects of Zn and Mg on SiO$_4^2-$ release seem to balance one another in the BV-SrZnMg sample, where the dissolution was similar to that measured for the BV and BV-Sr samples.

Except for Mg, the concentration of Zn and Sr increased in solution after immersion (Table 3). As discussed in Section 3.1, the Zn ion displays an intermediate character and is better stabilized in the glass network than Na and Ca [46]. Therefore, its release during the dissolution process is gradual and does not reach high rates but rather confirms the continuous degradation of the bioglass. The low quantity of Sr added to the bioglass system, and therefore the lower probability of entrapment to form an Sr-apatite layer, also induced a gradual release of this element over time. This particular aspect is very important, as one of the motivations for inserting Sr and Zn into bioglasses is to control their release to the surrounding tissue during regeneration. In this way, we can ensure that the cells will be stimulated by the gradual release of these ions during the entire replacement/regeneration process.

3.2.2. Precipitation

The bioactivity of the samples was confirmed by the MEV–EDS analyses. Indeed, the morphological changes on the surfaces started after the 1st day of immersion with the formation of a characteristic coating that became more pronounced over time (Fig. 8). The compositions of the samples did not affect the morphologies of the coatings. Considering that changes in composition generally imply changes in crystal morphologies during growth, we can assume that regardless of the original composition of the bioglass, a coating with similar physical–chemical characteristics was deposited. This could be confirmed by EDS analyses (Figs. 9, 10). Thus, the compositions of the samples did affect neither the morphology nor the final composition of the coatings. For this reason, we opted to show only the BV-SrZnMg and the control sample (BV) in Figs. 8–10.

The quantity of Si on the surfaces decreased over time of immersion, while the quantities observed for Ca and P significantly increased. In the case of the BV-SrZnMg sample, the quantities of Ca and P even surpassed that observed for Si after 7 days, indicating a strong precipitation process. Thus, a CaP-rich layer was formed on both the BV and BV-SrZnMg samples.

Another finding was the increase in Mg over time on the sample surfaces. According to our assays, this element clearly came from the McCoy’s 5A medium, as this process was also observed for the BV samples (without Mg in its original composition). Thus, regardless of the presence of Mg in the bioglass composition, the coating formed after immersion in the medium always contains this element. This has been shown in several works in the literature, even when Kokubo solutions are used instead of culture media [6,45].

The DRIFT analyses confirmed the presence of phosphate and carbonate on the coatings (Fig. 11, 12). Absorption bands at approximately 870 cm$^{-1}$ corresponding to the vibration modes from the CO$_3^-$ groups were observed for both the BV and BV-SrZnMg samples after 1 day of immersion. Similarly, phosphate absorption bands at 570 cm$^{-1}$ appeared after immersion. These findings show that after immersion, a coating composed of a calcium phosphate containing Mg$^{2+}$ and carbonate was precipitated.

To better understand these surface transformations, we monitored the quantity of the ions Ca$^{2+}$, PO$_4^{3-}$, SiO$_4^{2-}$, Zn$^{2+}$, Mg$^{2+}$ and Sr$^{2+}$ in solution during the course of immersion. Despite the high quantity of Ca$^{2+}$ in McCoy’s 5A medium, changes in its concentration over time could be evaluated by ICP-OES. In fact, the level of Ca$^{2+}$ decreased by the end of the 1st day, suggesting a strong process of precipitation onto the bioglasses (Fig. 13). After the 1st day, its concentration increased again.

One of the accepted mechanisms that explains the precipitation of apatites onto bioglasses is the rapid entrapment of Ca$^{2+}$ by the hydrated silica-rich layer. As previously described, the pH increases after the initial release of Na$^+$ ions, leading to the development of a negative charge on the surface. This negative charge is able to easily attract Ca$^{2+}$ to the surface, again changing the surface charge and generating the conditions necessary to entrap PO$_4^{3-}$ (Fig. 6c–e).

Therefore, the precipitation of a calcium phosphate layer starts at the same time as the increase in pH. Indeed, the Ca$^{2+}$ concentration in the medium quickly decreased by the end of the 1st day in our experiments (Fig. 13). In a similar way, the Mg$^{2+}$ and PO$_4^{3-}$ concentration also decreased, corroborating the formation of a Mg-containing calcium phosphate layer as suggested by the EDS and DRIFT analyses (Figs. 9, 10 and Figs. 11, 12, respectively).

The Ca$^{2+}$, Mg$^{2+}$ and PO$_4^{3-}$ precipitations exhibit distinct dynamics: while the Ca$^{2+}$ is abruptly precipitated on the 1st day, Mg$^{2+}$ and PO$_4^{3-}$ are gradually precipitated throughout the immersion period. According to Harding et al. [47], the PO$_4^{3-}$ groups will predominate on the apatite surface during the precipitation process. This has a tendency to stabilize the surface charge during the crystal growth, as PO$_4^{3-}$ is adsorbed by the Ca$^{2+}$ sites. Therefore, after the rapid capture of Ca$^{2+}$ ions by the reactive
silica gel layer, the PO$_4^{3-}$ ions are adsorbed, stabilizing the surface charge (Figs. 14 and 15).

After the 1st day, the concentration of Ca$^{2+}$ in solution again increased. One hypothesis to explain this behavior is the probable competition between the release of Ca$^{2+}$ from the bulk and the capture of Ca$^{2+}$ from the culture medium. During the 1st day, all Ca$^{2+}$ ions released from the bulk and even those in solution were likely captured by the reactive silica gel layer on the bioglass surface. After the 1st day, all the adsorption sites became occupied by Ca$^{2+}$ ions, the ions released from the bulk were no longer captured by the reactive silica gel layer, leading to an increase in its concentration in the culture medium.

4. Conclusions

The mechanism of ionic exchange responsible for the initial increase in pH after the immersion in cell culture medium was independent of the modifier elements (Zn, Mg and Sr) present in the samples. In all cases, the insertion of the oxides decreased $T_C$ and $T_R$, except for the Sr-containing sample (BV-Sr). Additional crystallization events were induced by the presence of Mg and Zn. The SiO$_4^{4-}$ release presented a good correlation with $T_C$ changes. The addition of Sr to the bioglass composition did not significantly change the SiO$_4^{4-}$ release compared with the control. The presence of Zn increased the SiO$_4^{4-}$ release, as the crystallization temperature $T_C$ decreased. In contrast, the SiO$_4^{4-}$ release decreased when Mg was added to the glass system, despite a remarkable decrease in $T_C$. In this case, the presence of Mg in the McCoy's 5A medium most likely generated a saturation state close to the surface, limiting SiO$_4^{4-}$ release. While SiO$_4^{4-}$, Zn$^{2+}$ and Sr$^{2+}$ were released from bioglasses after immersion, Ca$^{2+}$, Mg$^{2+}$ and PO$_4^{3-}$ were captured from the medium. Therefore, the formation of a Ca–Mg phosphate layer containing CO$_3^{2-}$ was observed for all samples, regardless of which elements were present in the sample.

Acknowledgments

The authors acknowledge financial support from the Brazilian research agencies FAPITEC/SE, CAPES and CNPq.

References
