New Bioactive Glass and Changes in Porosity during the Growth of a Carbonate Hydroxyapatite Layer on Glass Surfaces

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The synthesis of a bioactive glass with a composition (in mole %) SiO_2 (55), CaO (41), P_2O_5 (4) was carried out by the sol−gel method. The in vitro bioactivity of this glass was assessed by following the changes in surface composition and morphology after soaking in simulated body fluid (SBF) for several periods. Also the porosity of the glass was studied by considering the changes of that porosity when a carbonate hydroxyapatite (CHA) layer grows on the glass surface. The glass synthesized in this work shows a high degree of porosity due to its elevated content in Ca(II), which is released from the glass to the media. This dilution of Ca^{2+}, which gives rise to macropore enhancement together with the saturation of the media, leads to a fast growth of CHA on glass surface and pores.

Introduction

Since Hench proved that Bioglass[1] (45% SiO_2, 24.5% CaO, 24.5% Na_2O, and 6% P_2O_5) was able to bond to living bone due to the formation of an apatite-like layer on its surface, some other glass and glass−ceramics compositions have shown similar bioactivity.[2,3] That type of bioactive material can be implanted and reacts chemically with body fluids,[4,5] which aids to tissue reparation process. The apatite-like layer apparition promotes the adhesion to the repaired tissues and avoids the formation of a fibrous capsule and, therefore, decreases the failure possibilities of the prostheses. Therefore, the formation of the mentioned layer of biologically active bonelike carbonate-containing hydroxyapatite on its surface in the body, seems to be necessary for glasses and glass−ceramics to bond to living bone.

The behavior of these biomaterials depends on chemical composition and textural properties (pore size and volume). Glasses belonging to the $Na_2O-CaO-P_2O_5-SiO_2$ system have been synthesized by the melting−quenching method or by the sol−gel method, although an increase in growth rate of the apatite-like layer as well as the wider bioactivity compositional range were obtained when using the sol−gel method.[6] That can be explained in terms of promotion of nucleation sites generation due to the presence of hydroxyl groups (SiOH, TiOH) in the sol−gel-prepared materials, which improves the hydroxyapatite generation.

However, the influence of textural properties in bioactivity has not been thoroughly determined. In fact, there is a small number of previous attempts to relate textural effects (pore size and pore volume) with the in vitro formation of hydroxyapatite in gel−silica surface,[7] and with high surface and porosity.[8] In addition, the influence of the composition on porosity and bioactivity in $SiO_2-CaO-P_2O_5-MgO$ glasses has been recently studied.[9]

In addition, the variations in porosity, as a function of time of the sample in vitro, have not been investigated, although it would be very interesting to follow the evolution of pore volume and pore distribution during the layer formation and growth. In this context, the objective of this work is to prepare a bioactive glass belonging to the $SiO_2-CaO-P_2O_5$ system by the sol−gel method, and once the bioactivity of this new glass is confirmed, to study its porosity and the changes during the assays.

Experimental Section

The glass of composition in mole % of 55% SiO_2−41% CaO−4% P_2O_5 (55S) was prepared by hydrolysis and polycondensation of 62.6 mL of tetraethyl orthosilicate (TEOS), 6.8 mL of triethyl phosphate (TEP), and 48.9 g of Ca $(NO_3)_2 \cdot 4H_2O$, stoichiometric amounts, to obtain the desired composition. TEOS and TEP hydrolysis was carried out by using 2 N HNO_3 to catalyze, using a molecular ratio of $(HNO_3 + H_2O)/(TEOS + TEP) = 8$. After the addition of each reactant the solution was stirred for 1 h, and the resulting sol was introduced in a hermetically sealed cylindrical Teflon container to allow the hydrolysis and polycondensation at room temperature for 3 days. The gel was then aged at 70 °C for 3 days and dried at

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150 °C for 52 h. This latter step was carried out after 1 mm diameter hole was made in the lid, to allow for gas escape. The dried gel was grounded and sieved, taking the grains ranging in size from 32 to 63 μm. Fractions of 0.5 g of powder were compacted at 50 MPa uniaxial pressure and 150 MPa of isostatic pressure to obtain disks (13 mm in diameter and 2 mm in height). To determine the stabilization temperature, thermogravimetric and differential thermal analyses (TG/DTA) of the dried gel were carried out using a Seiko Thermobalance TG/DTA 320, and then gel disks were sintered at 700 °C for 3h.

The assessment of in vitro bioactivity was carried out in simulated body fluid (SBF), which has a composition and ionic concentration similar to human plasma.[10] The SBF was prepared by dissolving the following reagent chemicals in bidistilled water: NaCl (7.996 g), $NaHCO_3$ (0.350 g), KCl (0.224 g), $K_2HPO_4 \cdot 3H_2O$ (0.228 g), $MgCl_2 \cdot 6H_2O$ (0.305 g), 1 N HCl (40 mL), $CaCl_2$ (0.278 g), Na_2SO_4 (0.071 g), and $NH_2C(CH_2OH)_3$ (6.057 g). The solution was kept at 37 °C, and the pH was regulated to be between 7.3 and 7.4. The in vitro assays were carried out by soaking the disks, mounted vertically in a special platinum scaffold, in 45 mL of SBF in polyethylene containers maintained at 37 °C. Ca^{2+} concentration and pH were measured on an ILyte $Na^+K^+Ca^{2+}$ pH system.

The newly formed layer was characterized by FTIR in a Nicolet Magna-IR spectrometer 550, by scanning electron microscopy and energy dispersive spectroscopy (SEM-EDS) in a JEOL 6400 microscope at 20 kV, by XRD in a Philips X'Pert MDP diffractometer, and by transmission EM (TEM) in a JEOL 2000 FX electron microscope working at 200 KV.

The XRD, SEM, and EDS studies were carried out directly on the surface of the glass samples; the FTIR, ED, and TEM analyses were performed on ~1 mg of material scraped from the glass surface with a metallic blade.

Results and Discussion

1. Formation of Apatite-like Layer. FTIR (Figure 1) indicated the formation of an apatite-like layer on the glass surface after soaking in SBF. Silicate absorption bands at about 1085, 606, and 462 cm^{-1} were observed on the spectra of glass before soaking. Phosphate absorption bands at about 1043, 963, 603, 566, and 469 cm^{-1} and carbonate absorption bands at about 1490, 1423, and 874 cm^{-1} were observed on spectra of materials scraped from the surfaces of soaked glass disks. An increase in the intensity of the carbonate bands, which was observed in the spectra of glass, was associated with the length of soaking time in the SBF solution. The phosphate and carbonate absorption bands observed on the glass surfaces after soaking were similar to those observed in synthetic carbonate hydroxyapatite.[11]

The appearance of these bands in the spectra of the materials formed on the glass surface not only confirms the formation of an apatite-like layer, but also determines that the apatite-like layer material is a carbonate hydroxyapatite (CHA) similar to biological apatites, in which a coupled substitution of Na^+ for Ca^{2+} and CO_3^{2-} for PO_4^{3-} is observed.[12,13] Formation of CHA in vivo on the surfaces of calcium phosphate materials (hydroxyapatite and biphasic calcium phosphates), reported previously, has been associated with the reactivity of the materials.[14,15]

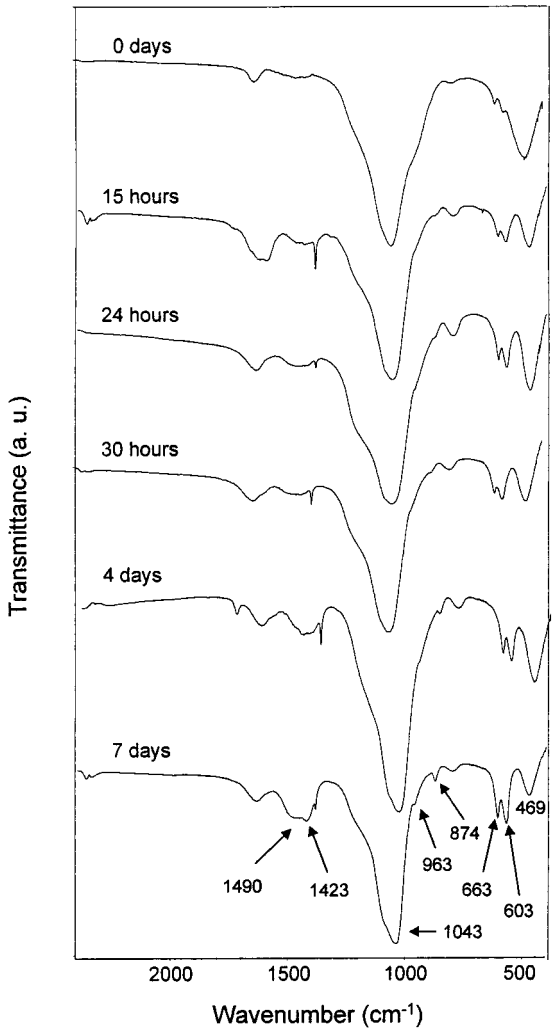

Figure 1. FTIR spectra obtained for glass before and after soaking in SBF.

In Figure 2 the SEM micrographs of 55S glass after different soaking times in SBF are shown. After 15 h of immersion, the glass surface was fully covered by a layer of spherical particles less than 1 μm in diameter. This situation remains constant after 24 and 30 h, although in Figure 2c there is an important change in terms of density of the newly formed layer. This occurs not only at the surface of the glass but at the interior of the pores as well. This effect is observed, as the surface of 30 h sample is more compact and the size of the visible macropores decreases. It is also shown how the formed layer covers the inside and even almost plugs the macropores. However, from 39 h of soaking time in SBF, an important change in both size and morphology of particles is observed. In fact, the spherical particles reach the size of around 2−3 μm and were constituted by hundreds of needlelike crystalline aggregates. After

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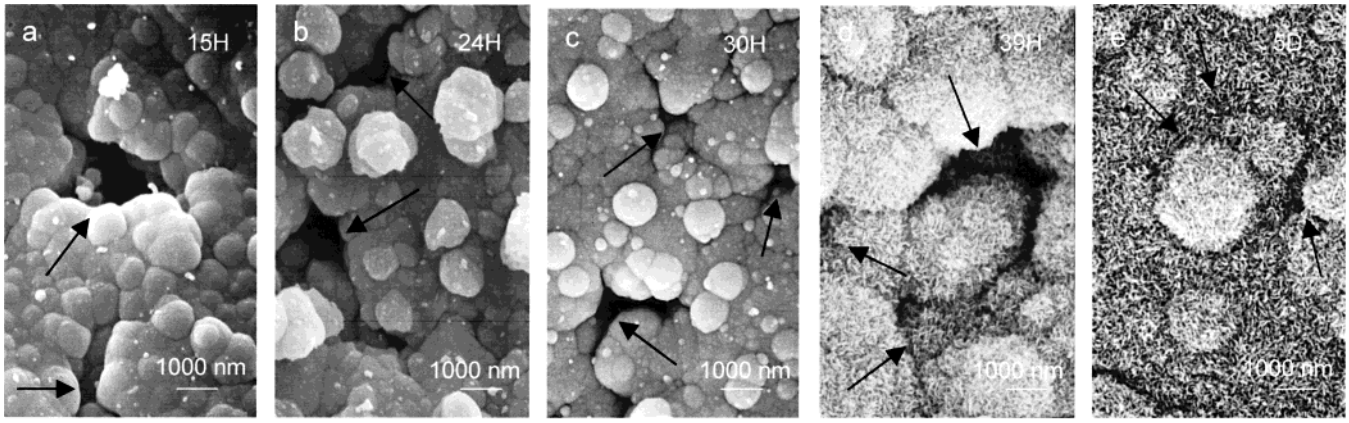

Figure 2. SEM micrographs obtained for the glass before and after soaking in SBF.

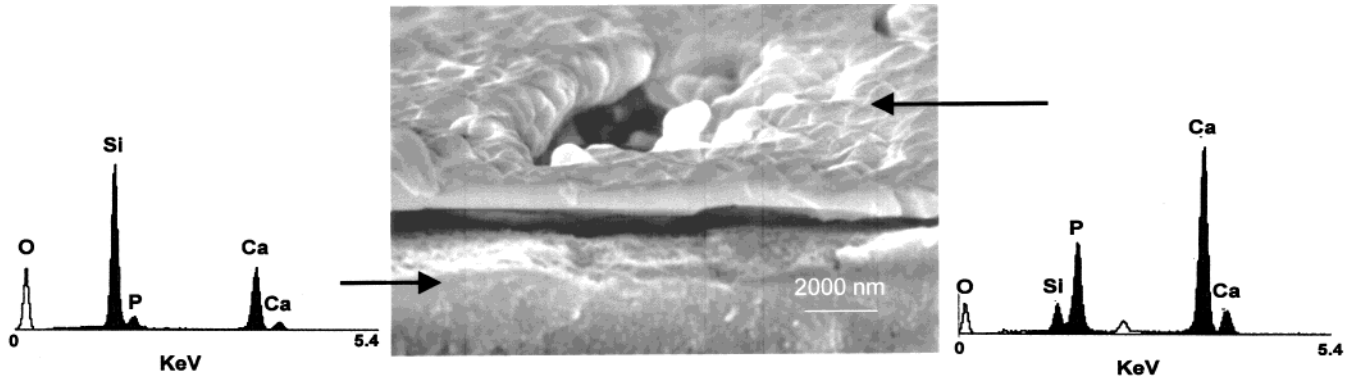

Figure 3. SEM micrograph of the cross section of a glass soaked in SBF for 15 h and the EDS patterns of the inside of the glass and on the layer.

that time, SEM detected no significant changes in the layer morphology. It must be notice that in all cases, these particles grow not only on the glass surface but also in the interior of the pores. In fact, parts a−c of Figure 2 show pores with their interiors covered by round particles, and the evolution of this covering increases as a function of soaking time. In cases of parts d and e of Figure 2, where the particles have become more crystalline, and the presence of needlelike crystalline aggregates is observed, it can be seen how the pores of a sample after 39 h of soaking are covered 5 days after. In summary, the apatite-like layer takes place all over the surface of the glass, including the pore interior.

In Figure 3, the cross section of 55S glass after 15 h soaking is shown. The EDS spectra inside the glass and on the layer are also included. As observed, the obtained analysis of the inner part agrees with nominal glass composition, that is 55% SiO_2−41% CaO−4% P_2O_5 (in mole %). However, in the EDS spectrum of the layer a remarkable increase of Ca and P concentration, together with a significant decrease of Si, was observed. The decrease of Si with increasing Ca and P concentrations indicates the formation of an apatite-like material. On the other hand, the SEM study of the cross section of samples after different soaking times permitted the layer thickness evolution with the soaking time in SBF to be followed. As shown in Figure 4, layer thickness grew from 2 μm after 15 h of immersion to 10 μm after 5 days of assay. It is also observed that there is no difference in layer thickness between 5 and 7 days, which suggests that at least in vitro, the apatite-like layer does not keep growing indefinitely.

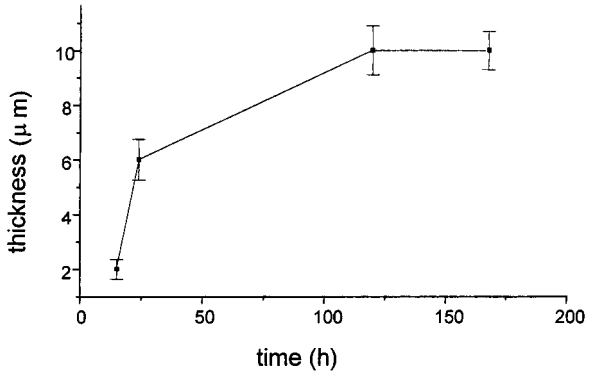

Figure 4. Layer thickness as a function of soaking time in SBF.

To elucidate which phase formed on the glass surface is the calcium phosphate phase, electron diffraction (ED) and transmission electron microscopy (TEM) studies were performed. Figure 5 shows a highly magnified micrograph of the glass after soaking for 7 days in SBF. As can be seen, the phase is formed by hundreds of small crystallites. The ED pattern corresponds to a typical polycrystalline material. By measuring the diameters of the rings observed in the figure and taking in to account the camera constant, the interplanar spacings allowed us to assign the rings to the reflections of an apatite-like phase (002), (210), (211), (222), and (402).

SEM micrograph allowed the particle size of the newly formed layer to be viewed. It is about 2000−3000 nm and consists of thousands of crystalline aggregates that are associated in needlelike particles with sizes below 1000 nm. These needlelike particles are composed

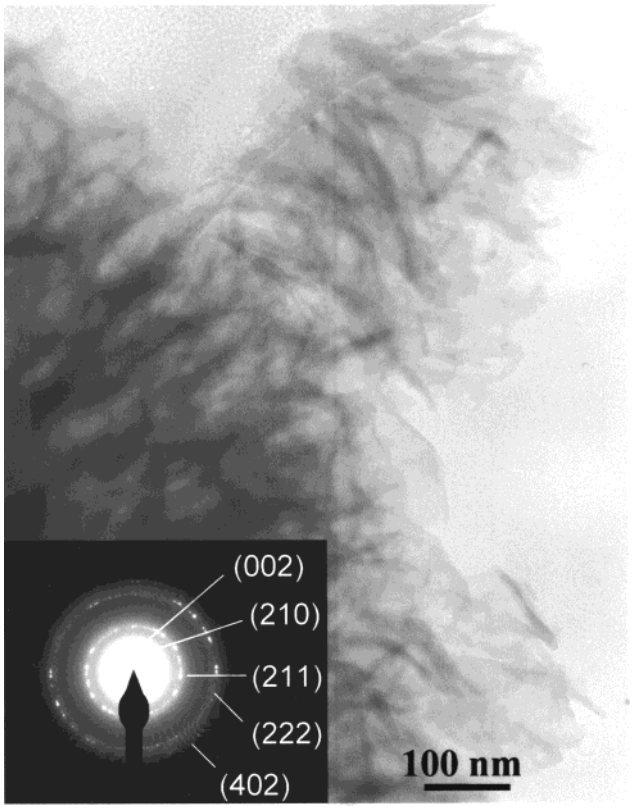

Figure 5. TEM micrograph and ED pattern of the glass after soaking in SBF for 7 days.

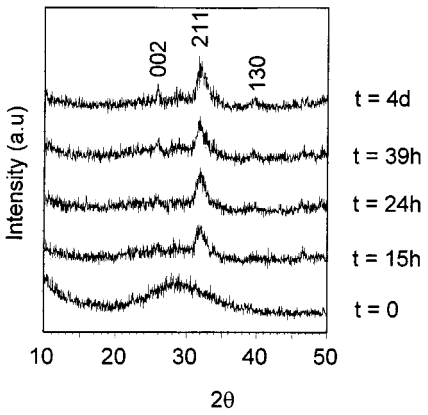

Figure 6. XRD pattern of the glass before and after soaking in SBF.

of hundreds of crystals with sizes below 100 nm, which are observed by TEM.

XRD spectra of sample before and after soaking in SBF are shown in Figure 6. As can be observed, after 15 h of immersion in SBF, a slight reflection (211) of an apatite-like phase starts to appear. Further sharpening of the peak and a slight shifting of the maxima corresponding to the (002) and (130) apatite reflection were observed after 4 days of soaking in SBF.

Hence, FTIR, SEM, TEM, and XRD point to the formation an apatite-like layer on the glass surface after assays in vitro.

2. Changes in SBF Composition. Changes in SBF composition after different soaking times are shown in Figure 7, where variation in Ca concentration and pH are revealed. During the first 24 h, a high release of Ca occurred, reaching values of 452 ppm. Then an important decrease is observed for times between 1 and 3

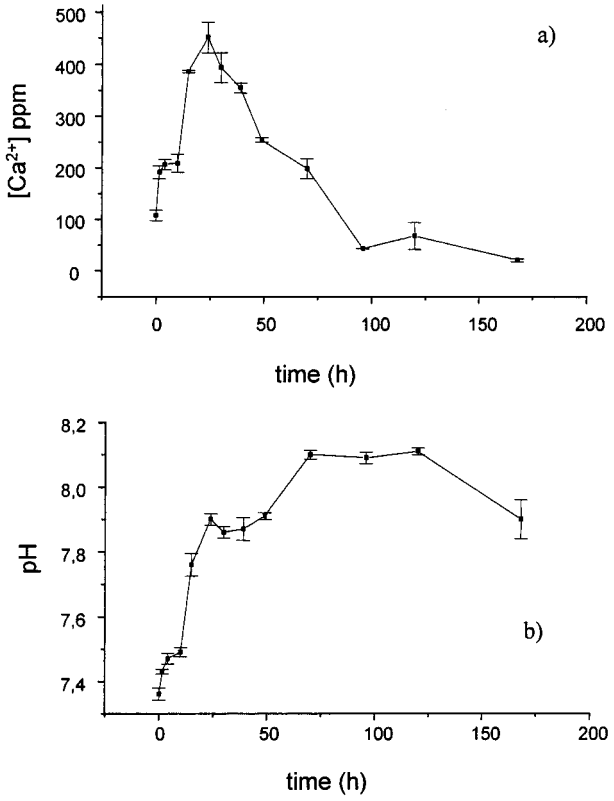

Figure 7. Changes produced in SBF as a function of soaking time: (a) changes in Ca^{2+} content and (b) changes in pH.

days, after which, the decrease becomes smoother, reaching values of 20 ppm after a week. The high concentration of Ca^{2+} detected for the first 24 h reveals the release of Ca^{2+} from the glass to the fluid, while the later reduction in Ca^{2+} concentration of the media can be explained in terms of apatite-like layer formation, which promotes this latter decrease.

In Figure 7b, the changes in pH are shown and a high increase is observed together with the Ca^{2+} release to the media during the first day of soaking. Between 1 and 3 days that increase is slightly smoother, becoming then nearly constant until the seventh day. The observed variations with time of calcium concentration and pH are in agreement with the nucleation and growth mechanism of an apatite-like layer on bioactive glasses containing $CaO-SiO_2$, as first proposed by Kokubo.[5] In such glasses an interchange between the Ca^{2+} ions of the glass and the H_3O^+ of the solution takes place. This gives rise to the formation of Si−OH groups on the glass surface, inducing apatite nucleation. The nuclei thus formed later grow at the expense of the ions in the solution saturated with respect to the apatite.

3. Study of Porosity. To study the porosity of glass before and after soaking in SBF a Hg intrusion assay was carried out by drying the samples at room temperature to avoid any humidity. Obtained results are shown in Figure 8. The variation of intrusion volume is displayed as a function of pore diameter for different soaking times. Alsoshown are two graphs showing the variation of relative intrusion volume with time. In the main graph, the most important change observed is the increase of mesopore size from the in situ sample to the one soaked for 3 h (from 0.0121 to 0.0200 μm), probably due to Ca dilution; then, as soon as the layer starts to grow, the pore size progressively decreases, reaching its initial value at 3−7 days of soaking. In addition, it is

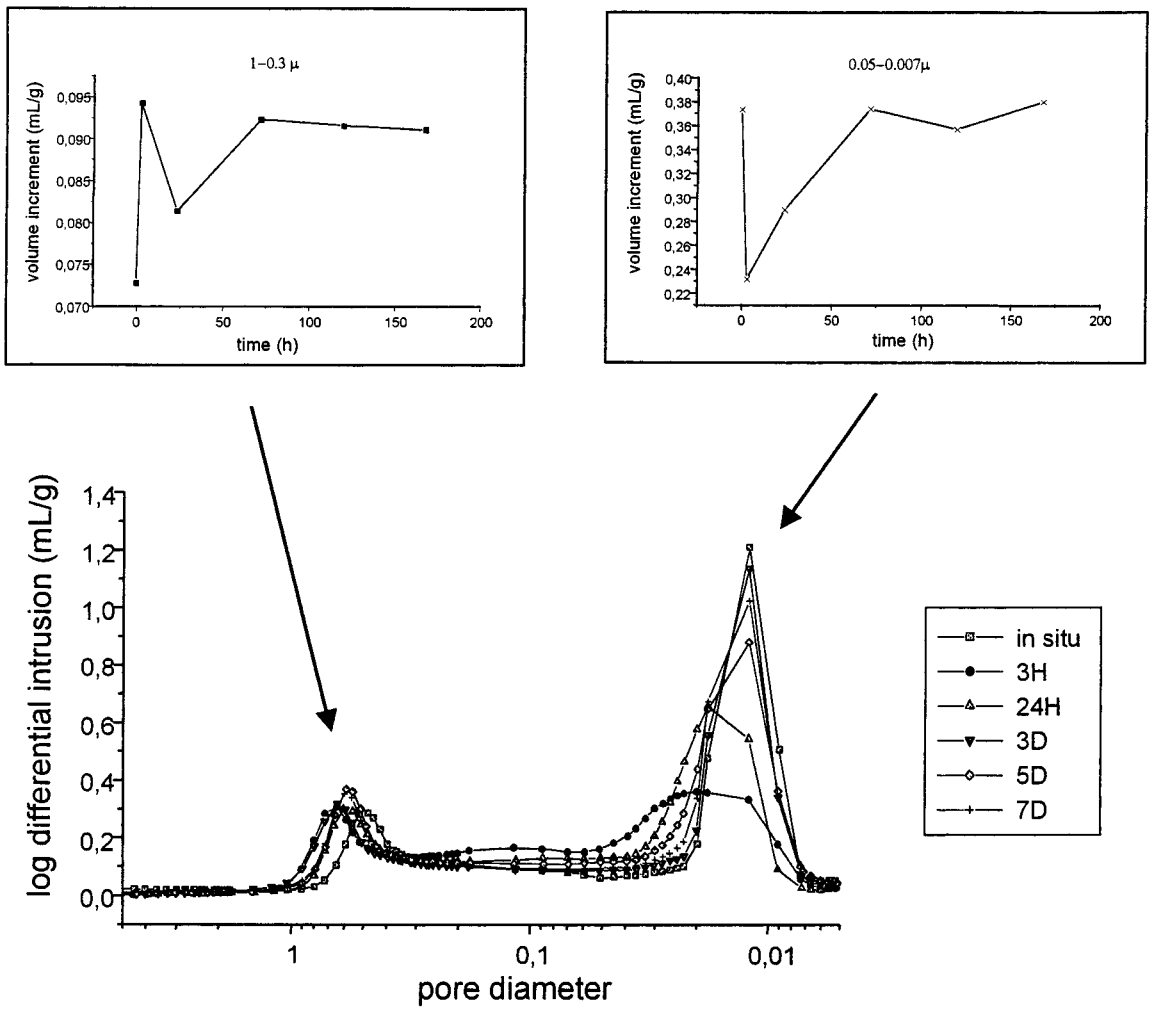

Figure 8. Variation of intrusion volume as a function of pore diameter, and variation of relative intrusion volume with time for macro and mesopores.

observed that the number of macropores increases during the first 3 h of soaking, due to Ca dilution, while mesopores decrease as a consequence of the layer formation. After the first day, in which macropore volume decreases (the formation of the apatite-like layer partially covers those pores), the number of both macro- and mesopores increases. This can be attributed to hydroxyapatite layer porosity itself; as can be observed in both graphs, pore volumes do not seem to change between 3 and 7 days, coinciding with the apparition of aggregates in the newly formed layer.

These results agree with SEM micrographs as long as both macro- and mesopores increase in number with the layer growth, but the size of mesopores decreases from 3 h of soaking sample to the seventh day of assay. This is consistent with Figure 2, in which the progressive covering of pores was noticed, and was especially significant when the difference between 24 and 30 h samples is observed, or when the covering of pores at 39 h is detected.

Conclusions

A bioactive glass has been synthesized as proven by the in vitro study carried out. This study combined different techniques to determine the composition and properties of materials that are formed on glass surfaces after soaking in SBF solution. The combined application of SEM-EDS, FTIR, TEM, and XRD techniques allowed the monitoring of the formation of this layer and the identification of the layer as consisting of crystallites of carbonate hydroxyapatite, similar to biological apatites in bone.

In addition, this glass, with a high calcium content, has a high porosity due to $Ca(NO_3)_2$ decomposition during the stabilization process. Also, the massive release of Ca^{2+} leads to an additional porosity increase.

This high porosity promotes the layer growth not only on the glass surface, but also inside the pores, leading to a decrease in their volume and size.

It must be pointed out that the study of the samples porosity at different stages of the CHA formation provides a useful tool for the understanding of the bioactivity in these glasses. The presence of macropores on glass surface allows an increase of total surface and, too, an increase of HCA layer growth. Therefore, we can conclude that the higher porosity, the higher amount of new formed bone.

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