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Bioactive and degradable organic–inorganic hybrids

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Abstract

$CaO–SiO_2$–poly(vinyl alcohol) (PVAL) and $CaO–P_2O_5–SiO_2$–PVAL organic–inorganic hybrids were obtained as monoliths and characterized before and after be soaked in a solution mimicking human plasma. The hybrids were obtained by adding PVAL (0.9, 1.8 and 3.6 wt.%) to three $CaO–(P_2O_5)–SiO_2$ gel glasses with 25 mol% of CaO and 0, 2.5 and 5 mol%, respectively of P_2O_5. The influence of PVAL and P_2O_5 on the monoliths obtaining and on their textural properties and in vitro behavior was analyzed. Additions of PVAL favored the synthesis of cracked-free monoliths able to be coated with bone-like apatite after be soaked in Kokubo's simulated body fluid (SBF), i.e. to present in vitro bioactivity. Increasing P_2O_5 contents made the hybrids syntheses difficult and decreased their in vitro bioactivity. In addition, the in vitro degradation of hybrids increased with the increasing of PVAL and P_2O_5. Thus, hybrids with the highest amounts of both components showed so high degradation in SBF that the apatite layer formation was impeded. Organic–inorganic hybrids in these systems could be clinically used as bone defect fillers in non load bearing applications or as matrices in controlled release systems.

Keywords: Organic–inorganic hybrids; $CaO–P_2O_5–SiO_2$–PVAL system; Bioactivity; Degradable; Monoliths

1. Introduction

In the search of new materials to be used in implants, together with biocompatibility, other two important properties are usually investigated: bioactivity and degradability. Bioactive materials bond to living tissues, whereas biodegradable ones substitute damaged ones while healing takes place.[1] Hench and Polack proposed a major future clinical application for both kind of materials as cells scaffolds in tissue engineering.[2] The in vitro assays, soaking materials in solutions mimicking the features of physiological fluids, play an important role for the evaluation of new bioactive or degradable materials.[3–5] One of the most commonly used solutions for the in vitro assays of bioactivity is the simulated body fluid (SBF) proposed by Kokubo et al.[3] In these assays, the formation of a hydroxycarbonate apatite (HCA) layer on the material surface is monitored. This layer is considered as biologically active, and a relationship between the in vitro HCA formation on a material and its bone bonding ability was established.[1–2] On the other hand, the in vitro degradation tests usually evaluate the weight loss of samples with the immersion time in the assay solution.[4]

The highest in vitro bioactivity was reported for certain glass compositions obtained by the sol–gel method.[5–6] The main clinical applications proposed for gel glasses are as particulates, for bone grafting or tissue augmentation, and as matrices in controlled release systems.[6] Gel glasses are surface reactive materials, therefore their degradation in bulk, that depends on the particle size, is rather small. The synthesis of bioactive materials with tailored degradability in aqueous media would expand their clinical applications. With that purpose, one approach is synthesizing organic–inorganic hybrids based in bioactive gel glasses and a biocompatible hydrophilic organic polymer, such as poly(vinyl alcohol) (PVAL) that would tailor the hybrid degradation. Actually, PVAL has been widely proposed for controlled release systems due to its biodegradablity.[7–8] As far as we know, previous to our studies only one article reported the synthesis of bioactive hybrids based on PVAL-modified gel glasses, but

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the goal was to obtain bioactive coatings.[9] The study of this family of hybrids as monolithic pieces, seemed promising for novel clinical applications. Hence, some preliminary results concerning these bioactive PVAL-containing hybrids were already presented.[10]

The goal of this work was the synthesis and characterization of cracked-free organic–inorganic hybrids as monoliths, based on bioactive and biocompatible $CaO–SiO_2$ and $CaO–P_2O_5–SiO_2$ gel glasses,[11–12] modified with different amounts of PVAL. The effect of P_2O_5 and PVAL on the synthesis, the textural properties (surface area and porosity) and the in vitro behavior in SBF of these hybrids, regarding the bioactivity and degradation, will also be reported.

2. Experimental

2.1. Synthesis of $CaO–SiO_2$–PVAL and $CaO–SiO_2–P_2O_5$–PVAL hybrids

The reactants were tetraethyl orthosilicate (TEOS, Aldrich), triethyl phosphate (TEP, Aldrich) and calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$, Aldrich), precursors of SiO_2, P_2O_5 and CaO, respectively, and PVAL (Aldrich, 99% hydrolyzed, average M_w: 89,000–98,000 g/mol). The alkoxide hydrolysis was carried out with distilled water and catalyzed with HNO_3.

$CaO–SiO_2$–PVAL and $CaO–SiO_2–P_2O_5$–PVAL hybrids were obtained by adding amounts of PVAL (0.9, 1.8 and 3.6 wt.%) during the syntheses of $CaO–(P_2O_5)–SiO_2$ gel glasses with 25 mol% of CaO and 0, 2.5 and 5 mol%, respectively of P_2O_5. The hybrids obtained were termed as H_{x-y}, "x" referring to the P_2O_5 mol% in the parent sol–gel glass and "y", to the nominal wt.% content of PVAL in the hybrids (see Table 1).

In Fig. 1, a scheme of the sequence of processes for the sol–gel synthesis of hybrids is shown. As observed, different amounts of PVAL were solved in water at 80 °C under reflux for 30 min before the reactants addition. Like for the synthesis of the parent gel glasses,[11] the amounts of water and HNO_3 for the hybrids production were those calculated to fulfill a $HNO_3 + H_2O/TEOS + TEP$ molar ratio of 8 and an initial pH of 0.5. Reactants were consecutively added in the order: TEOS, TEP (if required) and calcium nitrate with an hour of stirring after each addition. At the end, aliquot parts of 1.5 ml of this solution were poured into polystyrene multiwells containers, sealing with Teflon® tape. Gelation took place in 3 days at room temperature. Aging was carried out at 60 °C for 2 days and drying at 60 °C for 1 day after removing the Teflon® tape allowing the gases formed during the drying to leak.

Table 1
Codes for hybrids (H_{x-y}). "x" is the P_2O_5 content in the parent glass and "y" the nominal PVAL content

	P_2O_5 (mol%)		
	0	2.5	5
PVAL (wt.%)			
0.9	$H_{0–0.9}$	$H_{2.5–0.9}$	–[a]
1.8	$H_{0–1.8}$	$H_{2.5–1.8}$	$H_{5–1.8}$
3.6	$H_{0–3.6}$	$H_{2.5–3.6}$	$H_{5–3.6}$

[a] $H_{5–0.9}$ sol spontaneously precipitated before gelation.

2.2. Hybrids characterization

The hybrids were characterized through CHN elemental analysis, in a LECO CHNS-932 elemental microanalyser; X-ray diffraction (XRD), in a Philips X'Pert diffractometer, using Cu K_α; thermogravimetric analysis (TGA), in a Seiko Pyris Dyamond Thermo-balance, heating samples in air from 30 °C up to 800 °C at 10 °C/min; differential scanning calorimetry (DSC), in a Seiko SSC 5200 calorimeter, heating samples from 30 to 550 °C at 1 °C/min; Fourier transform infrared spectroscopy (FTIR), in a Nicolet Nexus spectrometer, using a Golden Gate® attenuated total reflectance (ATR) device; scanning electron microscopy (SEM), and

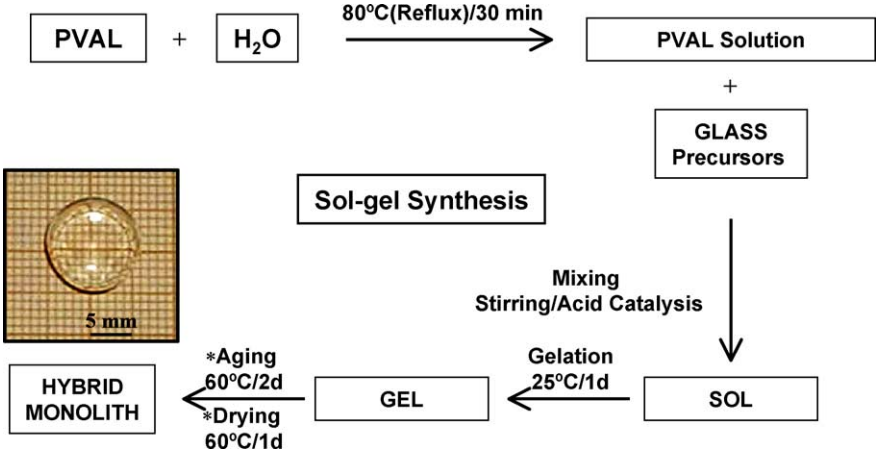

Fig. 1. Schematic illustration of the process followed for preparation of $CaO–SiO_2–(P_2O_5)$–PVAL hybrids. (*) Denotes changes regarding the synthesis conditions of parent PVAL-free gel glasses. All the hybrids showed similar appearance. As an example, a photograph of a piece of $H_{0–0.9}$ is included.

energy-dispersed X-ray spectroscopy (EDS), in a JEOL 6400 microscope coupled with a LINK AN 10000 device; and N_2 adsorption in a Micromeritics ASAP 2010, previously degassing the samples by heating 20 h at 60 °C.

2.3. In vitro studies

The in vitro assays were carried out soaking the cylindrical pieces of hybrids ($\phi = 10$ mm, $h = 4$ mm) in 40 ml of SBF at 37 °C for intervals from 6 h to 7 days. SBF is an aqueous acellular solution with ionic composition almost equal to human plasma: Na^+, 142; K^+, 5.0; Mg^{2+}, 1.5; Ca^{2+}, 2.5; Cl^-, 147.8; HCO_3^-, 4.2; HPO_4^{2-}, 1.0 and SO_4^{2-}, 0.5 mM, buffered at pH 7.30 with tris(hydroxymethyl)aminomethane/HCl.[3] After that, the pieces were removed from SBF, slightly rinsed in water and air-dried. To avoid microorganism contamination, all manipulations were performed inside a laminar flux cabinet (Telstar® AV-100), and SBF was filtered with a 0.22-μm Millipore® system before the in vitro assays.

For the degradation study, each specimen was weighed before and after being soaked in SBF. This way, the dissolution behavior of the samples was monitored.

Ca^{2+} concentration and pH variations of solution were measured by electrode ion selective measurements, using an ILyte Na^+, K^+, Ca^{2+}, pH system. The concentration of phosphorous and silica were determined by complexes formation and UV–vis spectroscopy in a Unicam UV 500 spectrophotometer.

To investigate the in vitro bioactivity, the changes in hybrid surfaces with soaking time were followed through FTIR and SEM–EDS.

3. Results

3.1. Hybrid characterization

The CHN analysis of P_2O_5-containing hybrids showed experimental contents in C higher than theoretical in $H_{2.5}$ (average, 3.5 ± 0.4 wt.%) and H_5 series (6.1 ± 0.1 wt.%) in comparison with H_0 ones (0.4 ± 0.2 wt.%). As it is observed, the C percentage in H_5 hybrids was approximately two-fold $H_{2.5}$ one. However, the N content was analogous in all samples (5.7 ± 0.3 wt.%).

Fig. 2 shows the XRD patterns of $H_{0–0.9}$, $H_{0–1.8}$ and $H_{0–3.6}$ hybrids and PVAL. The absence of XRD maxima in the hybrids patterns reflects their amorphous character although the PVAL used for the syntheses is a semicrystalline polymer. The XRD maxima in the polymer diagram were indexed from data in ref.[13]

PVAL and $H_{0–0.9}$, $H_{0–1.8}$ and $H_{0–3.6}$ hybrids thermograms are shown in Fig. 3. As it can be observed, the total decomposition of PVAL in CO_2 and H_2O took place between 225 and 450 °C. Regarding the hybrids, the three compositions presented a similar behavior with weight losses in two temperature regions. The first loss, of around 17%, occurred from

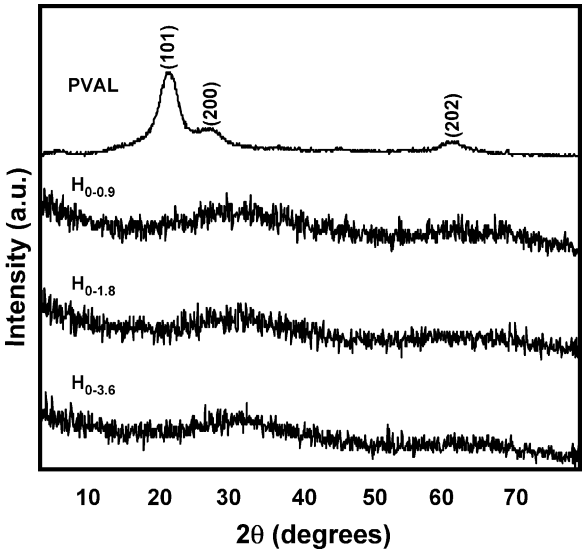

Fig. 2. XRD patterns of PVAL and $H_{0–0.9}$, $H_{0–1.8}$ and $H_{0–3.6}$ hybrids. The diffraction maxima were indexed from data on ref.[13].

room temperature to 140 °C and could be assigned to the elimination of water. The second weight loss, of about 24%, can be attributed to the simultaneous decomposition of nitrates and PVAL.

The DSC study of PVAL, showed an endothermic band at 225 °C, corresponding to the polymer melting point. In all the hybrids DSC curves, an intense band at 100 °C is observed, attributed to moisture evaporation from the samples, together with several bands of lower intensity, above 400 °C, assigned to nitrates removal.

FTIR spectrum of $H_{0–0.9}$ before the in vitro tests ($t = 0$) is shown in Fig. 4. The broad band at 3340 cm^{-1}, characteristic of O–H, was assigned to the overlapping of these bands from PVAL and H_2O. The band at 1638 cm^{-1} was also attributed to the samples moisture. The bands at 1416, 1337 cm^{-1} were attributed to nitrate groups. Finally, the bands at

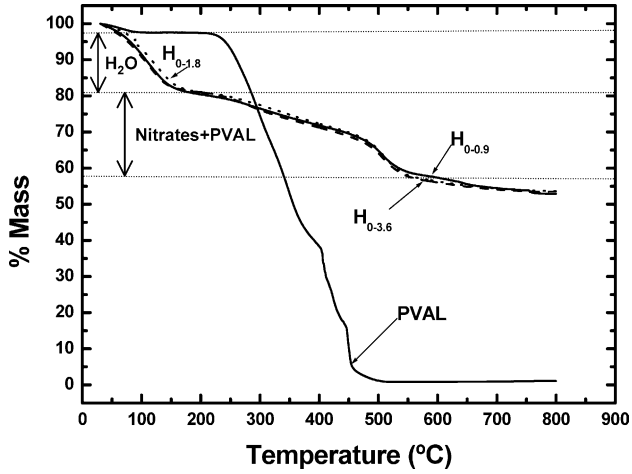

Fig. 3. TGA curves of PVAL and $H_{0–0.9}$, $H_{0–1.8}$ and $H_{0–3.6}$ hybrids. PVAL was totally decomposed at 500 °C. The three hybrids present analogous TGA thermograms.

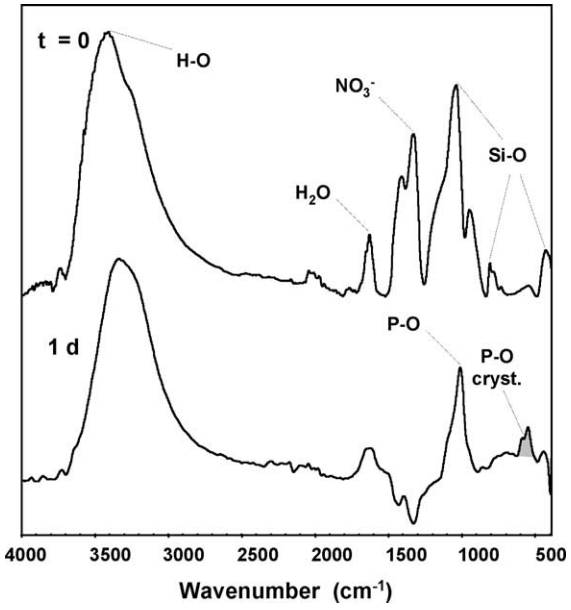

Fig. 4. FTIR spectra of $H_{0–0.9}$ before and after soaking 1 day in SBF.

1048, 817 and 438 cm^{-1} can be assigned to Si–O normal modes.[14]

SEM analysis of hybrids showed even surfaces for all the studied compositions. As an example, a SEM micrograph of $H_{0–0.9}$ before be soaked in SBF ($t = 0$) is shown in Fig. 5.

Textural parameters of hybrids obtained from N_2 adsorption are displayed in Table 2. A general observed tendency is a decrease in the BET surface area and porosity with the increase of PVAL in the hybrids. Effectively, materials with identical composition, but without PVAL, i.e. the parent gel glasses, presented surface areas and porosities higher than those in Table 2.[11]

3.2. In vitro studies

For the degradation studies, the percentage mass variation of hybrids versus the soaking time in SBF was plotted. As an example, in Fig. 6 the results corresponding to the P_2O_5-free hybrids, i.e. those of H_0 series are presented. As it is observed, in the first 6 h the specimens solved quickly up to 50% of their initial mass. An additional loss of mass around 10% was detected between the first 6 and 24 h. Then, the mass remained almost constant till the end of the study. For the other hybrids series the results were analogous. Thus, hybrids $H_{2.5}$ and H_5 lost up to 60 and 70% of their initial mass, respectively.

Table 2
Textural properties of hybrids obtained from N_2 adsorption

Hybrid	S_{BET} (m^2/g)	Vp (ml/g)	Dp (nm)
$H_{0–0.9}$	86	0.064	3.3
$H_{0–1.8}$	13	0.013	3.2
$H_{0–3.6}$	16	0.023	3.4
$H_{2.5–0.9}$	82	0.083	3.6
$H_{2.5–1.8}$	53	0.049	3.6
$H_{2.5–3.6}$	58	0.060	3.6
$H_{5–1.8}$	1.3	0.004	–
$H_{5–3.6}$	1.6	0.005	–

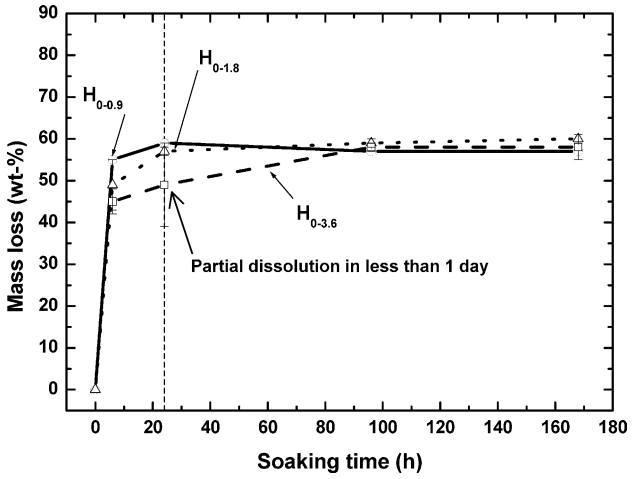

Fig. 6. Degradation of H_0 hybrids with the soaking time in SBF.

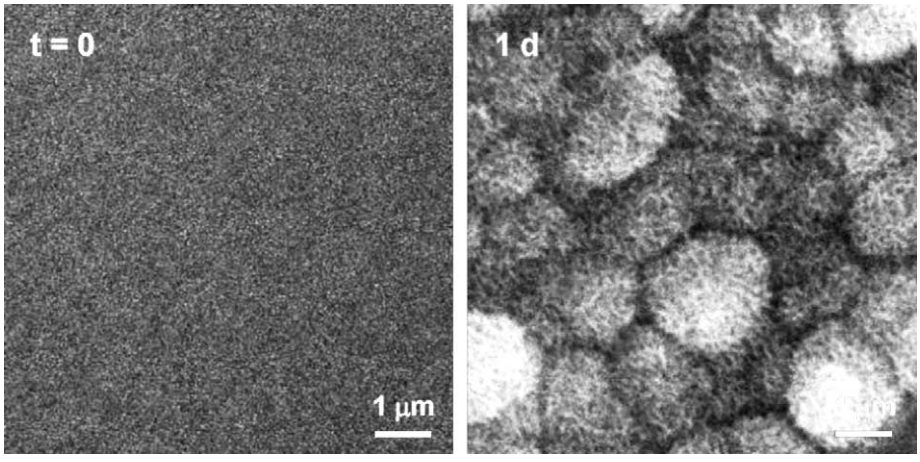

Fig. 5. SEM micrographs of $H_{0–0.9}$ before and after 1 day of in vitro assay.

Ca^{2+} ion concentration in solution drastically decayed in all cases, from the beginning of the assay. Thus, it changed from the initial 2.55 mM of SBF to 0.25 mM at 6 h. This may be due to the precipitation of a calcium salt. The pH also decreased in the 6 first hours, in all cases, from 7.34 to 7.10, and after that, it remained almost constant. Phosphorous concentration also decreased with time. Therefore, it would form part of the HCA layer on the hybrid whereas the rest would precipitate in the solution. On the contrary, the silica concentration in the medium increased for the first 24 h of test as a result of the partial samples dissolution. That fact is in agreement with what was observed in the degradation studies (Fig. 6), where the samples underwent a substantial weight loss during the first day and then, the mass remained constant.

Regarding the in vitro bioactivity, Fig. 4 shows the FTIR spectra of $H_{0–0.9}$ before and after being soaked in SBF for 1 day. The disappearance of the bands at 1415, 1334 cm^{-1} attributed to the nitrate can be noticed. In addition, a band at 1020 cm^{-1}, characteristic of phosphate groups, thinner than the silicate band appearing in this region is present. From the presence of the doublet at 598, 561 cm^{-1}, typical of a crystalline phosphate, the presence of an apatite-like layer was elicited. Furthermore, this layer can be observed after 1 day of assay, covering hybrid $H_{0–0.9}$ surface, in the right micrograph shown in Fig. 5. Therefore, the hybrids of the $CaO–SiO_2–(P_2O_5)$–PVAL systems are bioactive.

4. Discussion

$CaO–SiO_2$–PVAL and $CaO–SiO_2–P_2O_5$–PVAL monolithic pieces of hybrids were obtained by a procedure based on that used for synthesis of bioactive gel glasses.[11] In this case, the desired amounts of PVAL (0.9, 1.8, 3.6 wt.%) were previously dissolved in calculated quantities of water for the material synthesis. The addition of PVAL made possible to obtain the hybrids as monoliths under conditions that made the parent glasses crack after drying.

Due to the presence of the organic component, the maximum heating temperature for the hybrid processing was 60 °C (Fig. 1), considerably lower than for gel glasses synthesis.[11] As a consequence of that, H_2O and NO_3^- groups, arising from the CaO precursor, were detected by FTIR (Fig. 4) in as prepared hybrids. CHN elemental analysis confirmed the presence of 5.7 wt.% of N in hybrids. However, by FTIR, it can be proved that these NO_3^- groups were quickly removed during the first steps of the in vitro tests (Fig. 4).

TG analysis allowed to determine a water content in the hybrids of 17 wt.% (Fig. 3). This technique also let to quantify the NO_3^- and PVAL in hybrids, both simultaneously decomposed in the same temperature interval. Because, the hybrids prepared are based on glasses with 25 mol% of CaO, introduced as calcium nitrate, all must contain the same NO_3^- amount, whereas the PVAL percentage is different for each composition (see Table 1). Therefore, the second weight loss observed by TGA, 24%, for all the hybrid compositions, must be mainly assigned to the nitrates removal. This result agrees with the nominal CaO and PVAL contents (see Table 1) as well as with the CHN elemental analysis data.

On the other hand, the CHN analysis revealed a C content higher than expected for the $CaO–SiO_2–P_2O_5$–PVAL quaternary hybrids. That could be attributed to an incomplete hydrolysis of TEP, P_2O_5 source, making that part of the ethoxy groups (OC_2H_5) remained in the hybrids.

The presence of PVAL modified the textural properties of materials, making S_{BET} and the porosity of the hybrids decrease with regard to the parent gel glasses with identical composition but without PVAL. Actually, unlike the parent gel glasses all the hybrids showed even surfaces before soaking in SBF (Fig. 5).

Table 3 shows the studied hybrids able to be coated by apatite after soaking in SBF, i.e. to present in vitro bioactivity. A relation between the hybrid compositions and their bioactivity was found. For P_2O_5-free hybrids, PVAL additions made the bioactivity hybrids increase with regard to the parent glasses. Thus, the apatite layer was detected after 4–7 days of assay for the parent gel glasses[11] and just after 1 day for hybrids (Figs. 4 and 5). As the P_2O_5 content increased, and subsequently the complexity of the system, it became more difficult to obtain hybrids. In fact, it was not possible to obtain the hybrid with 0.9 wt.% of PVAL and based on a 5 mol% P_2O_5 gel glass, $H_{5–0.9}$. In addition, the bioactivity decreased as P_2O_5 in hybrids increased.

Table 3
In vitro bioactivity of hybrids

Hybrid	Apatite formation	Time (days)
$H_{0–0.9}$	Yes	1
$H_{0–1.8}$	Yes	1
$H_{0–3.6}$	Yes	1
$H_{2.5–0.9}$	Yes	4
$H_{2.5–1.8}$	Yes	7
$H_{2.5–3.6}$	No	–
$H_{5–1.8}$	No	–
$H_{5–3.6}$	No	–

The time in SBF at which the apatite layer was detected, is indicated.

Likewise, materials degradation is related to both their composition and degradation. Thus, if the hybrid solves too quickly, it will not be possible for the HCA layer to form on its surface.

Since these materials are simultaneously degradable and bioactive, their in vitro behavior becomes really complex. On one hand, they began solving while the apatite layer started forming as a result of their bioactivity. For those reasons, for the first instants, the composition of the hybrids surfaces, as well as the ionic composition of the solution underwent marked fluctuations. Since the first day of assay, the

hybrids stopped solving and therefore, their surface became more suitable for the formation of the calcium phosphate layer, which crystallized later, giving rise to nanocrystals with apatite-like structure.

Putting all the results of bioactivity and degradability together, we found that there is a relation between the hybrids composition and their bioactive and degradation behavior. Whereas, both degradability and bioactivity are interdependent as well.

5. Conclusions

- Experimental conditions to obtain $CaO–SiO_2$–PVAL and $CaO–SiO_2–P_2O_5$–PVAL hybrids as cracked-free monoliths were set up. PVAL additions facilitated the obtaining of monoliths, whereas P_2O_5 made difficult the hybrids synthesis.
- The increase of PVAL and P_2O_5 contents in hybrids decreased their surface area and porosity and increased their degradation in SBF.
- PVAL increased the bioactivity in SBF of P_2O_5-free hybrids with respect to the parent gel glass. However, high amounts of PVAL in P_2O_5-containing hybrids, implied an in vitro degradation of hybrids too high for the apatite layer to form.

Acknowledgements

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