Bioactive Carbonate−Hydroxyapatite Coatings Deposited onto Ti6Al4V Substrate

N. Hijón, M. V. Cabañas, I. Izquierdo-Barba, and M. Vallet-Regí*

Departamento de Química Inorgánica y Bioinorgánica, Facultad de Farmacia, Universidad Complutense, 28040-Madrid, Spain

Calcium hydroxyapatite coatings showing bioactive properties have been deposited by a sol−gel dipping method onto Ti6Al4V alloy substrates from aqueous precursor sols. To obtain homogeneous and monophasic hydroxyapatite coatings, the aging time and temperature of the sol were, fundamentally, the variables studied. The pH measurement was a good tool to evaluate the best conditions of the sols to deposit these coatings. SEM and SFM techniques show that the coatings deposited are dense and homogeneous with a low roughness, which depends on the sol viscosity and the film thickness.

Introduction

In orthopedic surgery, metals and their alloys are the most widely used implant materials due to their good mechanical properties, although in contact with body fluids or tissues they corrode.[1,2] An interesting alternative for protection of metal surfaces against corrosion is to coat the metal surface with a ceramic, which can act as an interface between the substrate and the bone, favoring the bone bonding. In this sense the calcium phosphates, such as hydroxyapatite (OHAp) and β-tricalcium phosphate (β-TCP), are the best examples of such coatings.

Although different deposition methods have been applied in the last years,[3−7] the sol−gel method offers a good alternative, since the synthesis temperatures are low and it can be applied to a great number of substrates, including those that can be oxidized at high temperatures. Several authors have prepared OHAp via sol−gel technique using different precursors,[8−13] showing that the temperature required to form OHAp depends on the chemical reactivity of the precursors. Livage et al.[14] investigated the sol−gel synthesis of phosphates and found that alkyl phosphates esters or phosphoric acid were unsuitable precursors, either because the hydrolysis is too slow or because it reacts so fast that a precipitate is formed, as in the case of phosphoric acid. In the same way, different works show the necessity of controlling the aging effect of the sol in the synthesis of powders of single phase OHAp by the sol−gel method,[15−17] but there is not a similar systematic study for the optimization of the films' deposition.

The aim of the present work is the preparation of bioactive and single phase OHAp coatings deposited on Ti6Al4V by the sol−gel dipping technique from aqueous solutions containing triethyl phosphite and calcium nitrate, focusing on processing parameters, such as aging temperature and age of the precursor solution.

Experimental Section

Apatite coatings were deposited from aqueous sols containing phosphorus and calcium, in a Ca/P ratio corresponding to OHAp of 1.67. For the preparation of sols, triethyl phosphite, $P(OCH_2CH_3)_3$, was hydrolyzed in water, in a 1:4 ratio, for 24 h; then a 4 M aqueous calcium nitrate solution, $Ca(NO_3)_2 \cdot 4H_2O$, was added. The mixed sol solution was stirred for 15 min and aged at different temperatures (30, 40, 60, and 80 °C) for different times before being used to make coatings. The pH variation of the sols with the time was measured by using a pH-meter (Model Metrohm 744), the sol viscosity was measured with a Haaker ReoStress RS75 rheometer, at a shear rate range from 1 to 200 s^{-1} at 20 °C.

For the preparation of the films, disk substrates of Ti6Al4V were dip-coated with the precursor-sol solution aged at different times, with a withdrawal speed varying from 200 to 2500 μm/s.[18] To obtain coatings with higher thickness, this operation can be repeated several times. The coatings were dried at 100 °C (1 h) and annealed in air at temperatures from

* To whom the correspondence should be addressed. Phone: 34-913941861. Fax: 34-913941786. E-mail:vallet@farm.ucm.es.

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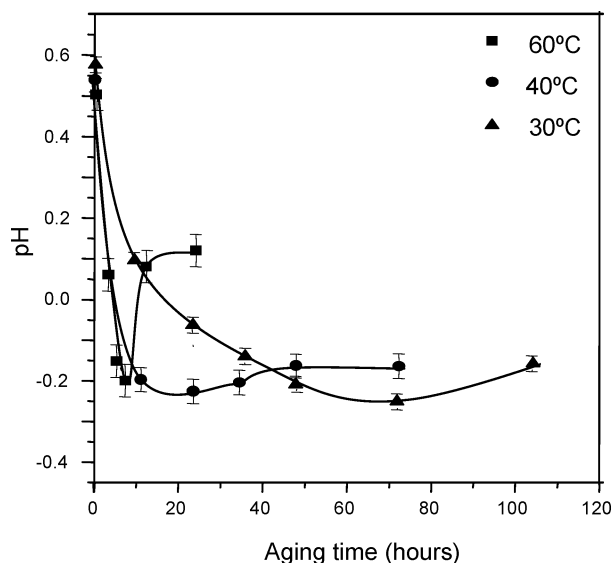
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Figure 1. pH variation of the different sols aged at different temperatures versus aging time.

Figure 2. XRD patterns (a) and FTIR spectra (b) corresponding to coatings deposited using a sol aged at 30 °C over different aging times and annealed at 550 °C.

200 to 700 °C in order to obtain a crystalline OHAp. Finally, the coatings were washed with ethanol in an ultrasonic bath for 2 min. Before coating deposition, metal substrates were polished with SiC of grit 320 and 9-3-1 μm diamond paste and washed for 5 min in an ultrasound bath with distilled water, alcohol, and acetone. The substrate so polished present a roughness value of 8 nm.

A dissolution/bioactivity test of synthesized coatings was performed. For this purpose, the samples were soaked in a simulated body fluid[19] (SBF) at 37 °C and buffered at pH = 7.4 up to 14 days. The variation of Ca^{2+} concentration and pH versus soaking time were determined with an Ilyte Na^+, K^+, Ca^{2+}, pH system. After soaking, the coatings were removed from the fluid and washed with water and ethanol.

The coatings were characterized by X-ray diffraction (XRD) in a Philips X-Pert MPD diffractometer equipped with a thin-film (grazing incidence) attachment and using Cu Kα radiation and by Fourier transform infrared spectroscopy (FTIR) in a Nicolet Nexus spectrometer using an ATR Golden Gate. The microstructure of the films was examined by scanning electron microscopy (SEM) on a JEOL 6400 instrument and by scanning force microscopy (SFM) on a Autoprobe-cp (Park Scientific Instruments) working in contact mode. The Ca/P ratio of the coatings was determined by energy-dispersive X-ray spectrometry (EDS) with a Oxford Pentafet Super A/W analyzer microscope system.

Results and Discussion

All the aqueous sols used for the dip-coating preparation were similar in appearance, with a pale yellow color for all of them. These sols have allowed the preparation of coatings only at speeds below 800 μm/s. It was not possible to prepare coatings with higher speed, because, from visual observations, either no adherence between the sol and substrate was observed or the apparent film homogeneity was not adequate. Then, the results present in this work refer to the results obtained using a withdrawal speed of 800 μm/s.

The pH measurement of the sols during the aging shows a continuous decreasing of pH values as the aging time increases for all assayed temperatures (Figure 1). This pH variation reaches a minimum that shifts toward higher times as the sol temperature decreases.
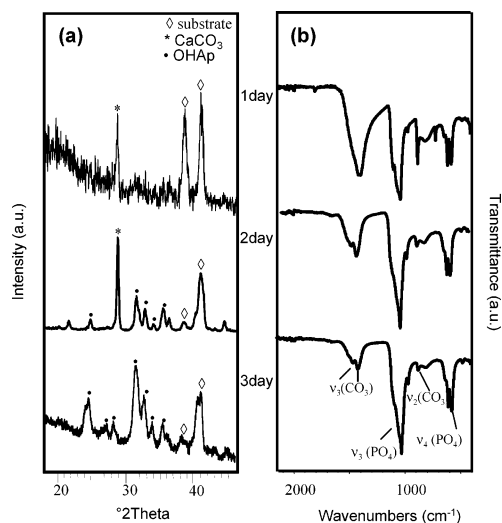
Sols aged at different times and temperatures were used for the coating preparations. Figure 2a shows the XRD patterns of the coatings deposited by using a sol aged at 30 °C at different times. These coatings showed a calcium carbonate [calcite, (c)] crystalline phase for 1 day of aging, a mixture of $CaCO_3$(c) and OHAp after 2 days, and only OHAp when the aging time increased to 3 days. Moreover, short-time aging lower than 1 day leads to poor substrate coverage, whereas aging times higher than 3 days lead to the formation of powder on the substrate after the thermal treatment.

Infrared spectra performed over these coatings (Figure 2b) show the presence of absorption bands belonging to PO_4^{3-} and CO_3^{2-} groups. As the aging time increases, the absorption bands corresponding to carbonate groups decrease. In the FTIR spectra corresponding to the coatings deposited at 3 days (where the XRD pattern shows the presence of crystalline apatite only), the most intense bands, in the 560–605 and 950–1100 cm^{-1} ranges, can be assigned to the major absorption modes of the phosphate groups of apatite,[20] and the bands at 873, 1412, and 1457 cm^{-1} correspond to the CO_3^{2-} groups substituting for phosphate groups in the apatite structure.[21] The FTIR study determines that the coatings correspond to a B-type carbonate–hydroxyapatite, similar to biological apatites.[22]

The sol temperature also exerts an important effect on the time of appearance of the crystalline phases. When the sol temperature was increased, the diffraction maxima corresponding to the apatite phase become more apparent at lower aging times (Table 1). The results collected in Table 1 show that by heating the precursor sol its reactivity increases, that is, lower times are necessary to deposit single OHAp phase onto the substrate; but simultaneously, the stability of the dip-coating solution decreases, i.e., the formation of powder on the substrate is also observed at lower time periods. No differences were observed by XRD and FTIR between

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OHAp Coatings Deposited on Ti6Al4V Substrate

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Table 1. Crystalline Phases Detected by XRD on the Coatings Deposited from Sols Aged at Different Times and Temperatures[a]

aging time (h)	30 °C	40 °C	60 °C	80 °C
1	no film	no film	no film	OHAp + CaCO$_3$(c)
2	no film	no film	no film	OHAp
6	no film	no film	OHAp	powder
24	CaCO$_3$(c)[b]	OHAp	powder	
48	OHAp + CaCO$_3$(c)	powder		
72	OHAp			
96	powder			

[a] All the films were annealed at 550 °C, 10 min. [b] Calcite.

OHAp coatings deposited by using sols aged at different temperatures.

On the other hand, the viscosity measurements of the sols indicate Newtonian behavior. The viscosity of the sols used to deposit single phase OHAp takes values of 3, 4, and 5 mPas, for sols aging at 30, 40, and 60 °C, respectively. The results obtained as a function of the aging time and temperature can be related with the variation of pH observed as the sol ages (Figure 1). In all cases, the sol becomes more and more acidic with time. According to Liu et al.,[17,23] the hydrolyzed phosphite interacts with Ca ions in aqueous solution through a polymerization reaction to form a Ca−P intermediate, releasing protons during the reaction:

$$HPO(OC_2H_5)_{2-x}(OH)_x + Ca^{2+} + NO_3^- \rightarrow$$
$$Ca-P \text{ intermediate} + H^+ \quad (1)$$
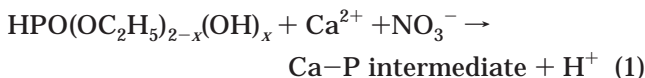
In the same way, a study by ^{31}P NMR[12,24] shows that, during the sol aging, the hydrolysis of the thiethyl phosphite yields diethyl phosphite, which undergoes reaction with calcium precursors, and the coordination number of phosphorus changes from III to V. The liberation of protons during the reaction justifies the pH decrease observed. The time at which the pH minimum is reached coincides with the aging time necessary to obtain monophasic OHAp films (Table 1), whereas when the pH increases, powder is formed on the substrate. These results are in agreement with the kinetic acceleration of reaction 1 as the sol temperature increases, since higher times are needed to reach the pH minimum when the sol temperature is lower. Besides, the region of minimum pH is narrower when the temperature increases, which justifies the shorter time allowed to made films. Therefore, the pH measurement in these sol−gel aqueous systems is a good tool to evaluate the aging time needed to obtain single OHAp phase coatings.

The above results indicate that the polymerization reaction between calcium and phosphorus precursors is improved when the aging time or temperature of the sol increase, allowing the different species present in the precursor sol to mix thoroughly. In this sense, the use of ultrasonic irradiation has been used by other authors to enhance the reactivity between the precursors in the synthesis of the materials by sol−gel.[18,25]

Hence, we prepared the OHAp precursor sol by applying 20 MHz ultrasound to the mixture of the components, supplied by a sonicator device (Sonic &Materials Vibracell) during different times. However, no improvement on the results shown above were obtained, since it was not possible to prepare films by using the ultrasonic sols.

The thermal treatment is another important parameter in the coating preparation, being necessary to reach an agreement between the temperature needed to deposit OHAp of high degree of crystallinity and that to avoid the substrate oxidation. All results discussed up to now correspond to the data obtained for coatings dried at 100 °C (1 h) and annealed at 550 °C for 10 min. This temperature was chosen according to the results obtained by XRD and FTIR. Apatite phase begins to crystallize at 300 °C, although the FTIR spectra shows a large amount of carbonate groups. As the annealing temperature increases, the OHAp phase is more crystalline, decreasing the carbonate content. A thermal treatment at 700 °C leads to the substrate oxidation [diffraction maxima corresponding to TiO$_2$ (rutile) and Al$_2$O$_3$ (corundum) are observed in the XRD patterns]. Although crystalline calcium phosphate coatings have been obtained using other sol−gel systems,[26,27] the reached temperatures were high; this is not possible when the substrate is titanium or its alloy, because their oxidation could degrade their mechanical properties. On the other hand, it is interesting to remark that the thermal treatment of appropriate coatings leads to a single OHAp phase, i.e., no additional Ca−P phases have been observed, even at 700 °C. Therefore, although our aqueous system is very acidic, which promotes the formation of HPO$_4^{2-}$ groups, the growth of a Ca-deficient phase, similar to that observed in the powder preparation,[16,26] is not favored in the coating deposition.

Single phase OHAp coatings have been analyzed by SEM. Figure 3a shows a SEM micrograph of the coating deposited using a dipping solution aged at 60 °C for 6 h and annealed at 550 °C. Similar SEM micrographs were obtained for the OHAp coatings deposited from sols aged at different temperatures. For an adequate aging time, the films deposited are homogeneous with a smooth surface; EDS analysis shows the presence of calcium and phosphorus on the coated substrate, in a Ca/P ratio close to that of stoichiometric OHAp. However, when the aging time of the sol increases, films exhibit degraded surfaces, as can be seen in Figure 3b, corresponding to a coating deposited from a sol aged at 60 °C for 12 h. The annealing temperature also influences the coatings microstructure, as can be seen in Figure 3c, which corresponds to a coating deposited under similar conditions to those used for the film shown in Figure 3a, but annealed at 700 °C. The formation of microcracks on the coating surface is observed. Therefore, it is necessary to use an appropriate aging time and low annealing temperatures in order to prevent the formation of cracks and degraded surfaces.
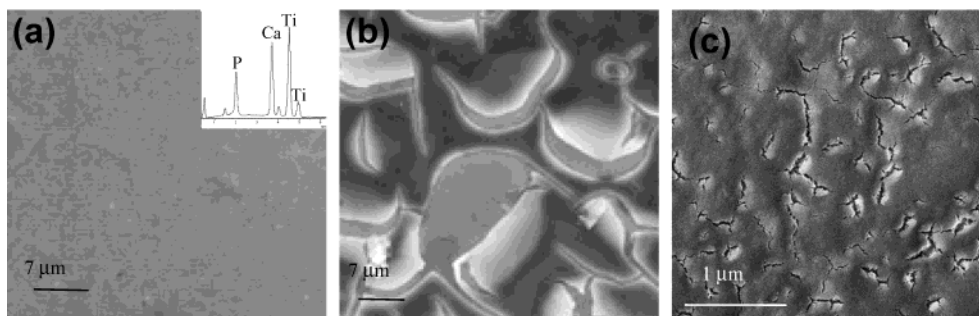
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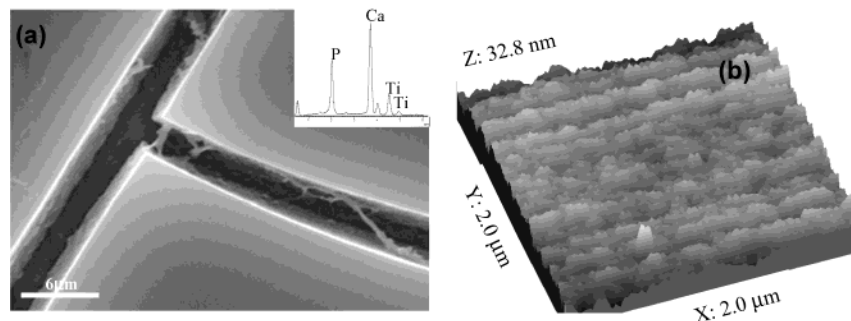
Figure 3. SEM micrographs corresponding to OHAp coatings deposited (a) using a sol (a) aged at 60 °C for 6 h and annealed at 550 °C, (b) aged for 12 h and annealed at 550 °C, and (c) aged at 60 °C for 6 h but annealed at 700 °C.
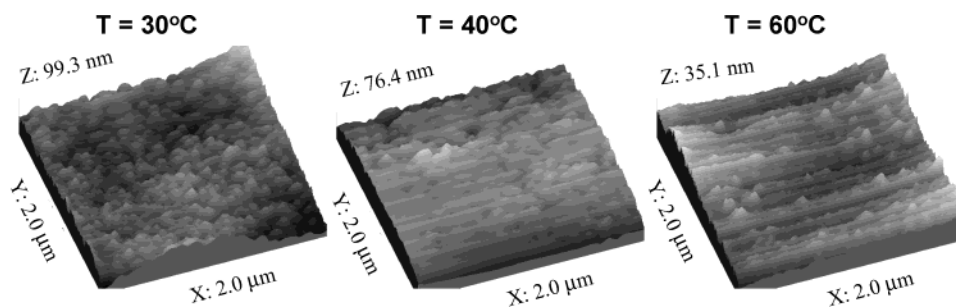
Figure 4. SFM three-dimensional view corresponding to OHAp coatings deposited from sols aged at different temperatures and annealed at 550 °C.

Figure 5. OHAp coating, constituted by six layers, deposited using a sol aged at 60 °C for 6 h: (a) SEM micrograph and (b) SFM three-dimensional view.

The SFM study has been performed over those coatings deposited in the optimum conditions of aging time (Table 1) and annealed at 550 °C. Figure 4 shows SFM images of the films deposited by using sols aged at different temperatures (30, 40, and 60 °C). SEM analysis showed similar coating surfaces, independent of the sol temperature used for the coatings. The advantages in the use of SFM lay in the real 3D information offered, which allows us to study the evolution of the surface roughness versus aging temperature. The roughness (rms) calculated shows values of 11, 8, and 5 nm for the films deposited from sols aged at 30, 40, and 60 °C, respectively. A decrease of the rms value is observed when the precursor aging temperature increases. The roughness can be related to the sol viscosity measured, since higher sol viscosity corresponds with higher film roughness.

All the data showed above correspond to films constituted by only one layer, presumably very thin, close to 0.2 μm, according to other authors.[23] To obtain OHAp coatings with higher thickness, the dip-coating method is repeated several times (up to 10 times). An SEM study shows that these coatings constituted by multiple layers are very dense and homogeneous, with a smooth surface. In coatings of six or more layers, the formation of cracks on the coating is observed (Figure 5a), which must be due to the larger thickness of these films. Therefore, it is necessary to reach a compromise between the film thickness, around 1 μm for six layers, and crack formation. The topography of these coatings was also studied by SFM. This study shows that the higher roughness corresponds to one layer with 8 nm (Figure 4), and a decrease of the rms value is observed when the number of depositions onto Ti6Al4V increases, i.e., 4 nm for six layers (Figure 5b). So, the rms value decreased when the thickness of the film increased. In addition, SFM images indicate that the grain size of the coatings is about 75 nm, being apparently the same, independent of the number of layers deposited and of the sol aging temperature of the dipping solution.

In the dissolution/bioactivity test, coatings constituted by 1, 3, 6, and 10 layers were soaked in SBF. During the 14 days of essay, no variations of the pH were observed in the SBF solution. However, the Ca^{2+} concentration decreased during this period, with a higher and earlier decrease in thicker films.

The SEM pictures and the XRD patterns obtained from coatings soaked in SBF (Figure 6) show that the
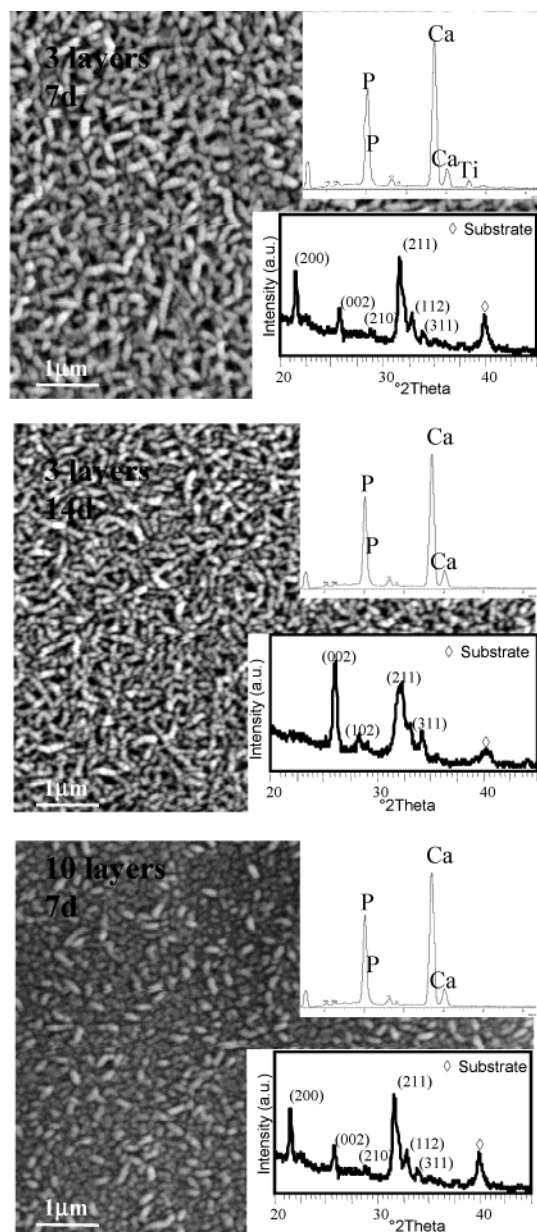
Figure 6. SEM microgaphs and XRD patterns of an OHAp dip-coating constituted by three layers soaked for 7 days, three layers soaked for 14 days, and 10 layers soaked for 7 days in SBF.

surface of the films is now covered by a new layer of material that appears to be constituted by numerous needlelike crystallites. The size of these crystallites decreases with the soaking time and the thickness (three and 10 layers). All the diffraction maxima can be assigned to an apatite structure, but slight differences can be observed when compared with nonimmersed films (Figure 2a). After 7 days, a new diffraction maximum at $2\theta = 21.6°$ is observed, which is not observed in the initial coating. This diffraction maximum can be assigned to the (200) reflection of apatite structure (Although not included, the (100) maximum at $2\theta = 10.7°$ was also observed in these soaked

coatings). After 14 days of soaking, these (h00) reflections do not appear, but a change in the relative intensity of the (002) and (211) reflections with respect to the initial coating was observed. On the other hand, the FTIR spectra of coatings before and after soaking were very similar, showing bands corresponding to phosphate and carbonate groups.

Consequently, the in vitro bioactivity study shows that the soaking of these sol−gel coatings in a SBF solution leads to the formation of a new layer over the coating surface. This new layer corresponds to an apatite-like structure, and it is constituted by small crystals. According to the XRD data, these crystallites seem to present a preferred orientation (h00) after 7 days of soaking, whereas it changes to a (00l) orientation, similar to that observed in glass surfaces,[29,30] after 14 days of soaking.

The results obtained indicate that, independent of the coating thickness, the OHAp sol−gel coatings deposited show bioactive properties under in vitro conditions. Therefore, these sol−gel coatings may promote the implant bonding with living tissues and increases the longevity of prosthesis during in vivo implanting.

Conclusions

Single phase OHAp coatings that are stable and have the ability to induce bonelike apatite formation on their surface when immersed in simulated body fluid have been deposited by a sol−gel dipping method with control of the aging time, which depends on the temperature of sol precursor; the higher the sol temperature, the shorter the aging time needed to obtain pure OHAp after coating annealing. When the aging parameters or the annealing temperature are not adequately controlled, additional phases or poor surfaces are obtained.

The conditions to obtain the best coatings have been related with the pH decrease on the aqueous sols observed during the aging, according to the polymerization reaction between calcium and phosphorus.

To obtain homogeneous, crack-free coatings, the annealing temperature and thickness of the coatings must be controlled. Films roughness is related with the viscosity of the sol precursor used to do the deposition, as well as with the number of coating layers.

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