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Amino–Polysiloxane Hybrid Materials for Bone Reconstruction

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Organic–inorganic hybrid materials containing amino functional groups were prepared as monoliths and their in vitro bioactivity and degradation were investigated using a simulated body fluid (SBF). The amino–polyorganosiloxane hybrids were obtained via sol–gel starting from *N*-(2-aminoethyl)-3-aminopropyltrimethoxysilane (DAMO). With the addition of γ -methacryloxypropyl-trimethoxysilane (MPS), hydrolytically stable and flexible polyorganosiloxane networks were obtained. To induce bioactivity, we added calcium to the hybrids as a calcium salt during the synthesis. A nanocrystalline apatite-like phase was formed on the hybrid surfaces after being soaked in SBF. The bioactivity and degradability of calcium-containing hybrids increased as the DAMO:MPS molar ratio increased, because the molar ratio affects both the functional groups and the charge of the hybrid surfaces, as well as the rate of Ca^{2+} release to the SBF solution. Zeta-potential (ζ) measurements revealed that at physiological pH, the net surface charge of the bioactive hybrids was negative because of a $\text{Si}-\text{O}^-$ group density higher than that of protonated amino groups (NH_3^+ and NH_2^+). These new materials are a promising alternative in bone tissue regeneration because of their tailored in vitro bioactivity and degradability and the presence of amino groups that are able to interact with biological entities.

Introduction

In recent years, the development of new bioactive and biocompatible materials has received great attention. It has been previously reported that bioactive materials have the ability to induce direct bonding to living bone, which is related to the formation of a carbonate hydroxyapatite (CHA) layer on their surfaces when in contact with body fluids or solutions mimicking properties of human plasma (simulated body fluid, SBF).¹

In this context, bioactive organic–inorganic hybrids are a wide and very interesting alternative for bone tissue regeneration,² as they combine the properties of traditional materials, such as ceramics and organic polymers, on the nanometric scale.^{3,4} In addition, sol–gel technology allows us to synthesize hybrid materials at low temperature and permits a very good control of their composition.

The incorporation of amino functionality into bioactive hybrids is an attractive goal, as amino functions play a relevant role in many biological applications, such as attaching peptides to promote cell adhesion⁵ or the covalent coupling of proteins.^{6,7} Regarding this topic, several authors have developed self-assembled monolayers functionalized

with amino groups and performed in vitro assays.⁸ They reported that the protonation of amino groups impeded the formation of an apatite-like layer. These results confirmed that negatively charged surfaces are always favorable for the heterogeneous nucleation of CHA in SBF, whereas the nucleation is inhibited on positive surfaces.^{9–11} The accepted interpretation is that the mechanism of nucleation and crystallization of apatite in SBF is induced by a catalytic effect of silanol groups ($\text{Si}-\text{OH}$) formed on the material surface and accelerated by the release of calcium ion (Ca^{2+}) from the material into the solution.^{12,13}

Aminopolysiloxane matrices seem to be promising and interesting materials for the development of functionalized bioactive hybrids, as they contain both silanol and amino groups. In the presence of water, the proton transfer from silanols to amino groups is promoted, allowing the formation of zwitterion-like species ($\text{Si}-\text{O}^-$, $^+\text{H}_3\text{N}$).^{14,15} However, aminopolysiloxane hybrids present a great limitation due to their high solubility in aqueous medium, because of the hydrolysis of siloxane bonds.¹⁵

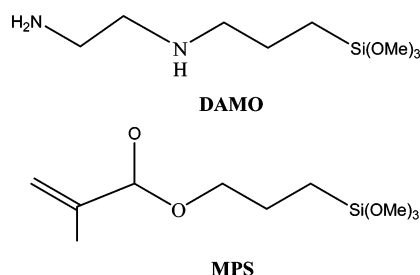
To overcome the problem of chemical instability in aqueous media, we attempt here the development of new bioactive polysiloxane matrices, starting from an aminosilane

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Scheme 1. Molecular Structures of the *N*-(2-Aminoethyl)-3-aminopropyl-trimethoxysilane (DAMO) and γ -Methacryloxypropyltrimethoxysilane (MPS) Used in This Work



together with another alkoxy silane containing a more hydrophobic functional group. For this purpose, we have chosen *N*-(2-aminoethyl)-aminopropyl-trimethoxysilane (DAMO) and γ -methacryloxypropyl-trimethoxysilane (MPS) as precursors. On one hand, DAMO is a source of primary and secondary amino groups, providing the resulting hybrids of the desired functionality. On the other hand, MPS, which has previously been reported in the literature to be appropriate for application as a biomaterial,^{16,17} generates stable and flexible monolithic polysiloxane networks.¹⁸ The DAMO:MPS molar ratio was varied in order to tailor the hydrophilicity degree and in vitro degradability of the resulting hybrid materials. A calcium salt was added during the hybrid synthesis to promote bioactivity. The effect of both functional groups and the charge of the surface was investigated to try to explain its effect on the formation of an apatite-like layer and the gradual degradability of the polysiloxane matrices.

Experimental Section

Reactants. *N*-(2-Aminoethyl)-3-aminopropyl-trimethoxysilane (DAMO) and γ -methacryloxypropyltri-methoxysilane (MPS) were supplied by Gelest Inc, PA (Scheme 1) and used as received. Calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, Fluka) and methanol (Aldrich) were used without further purification.

Preparation of the Organic–Inorganic Hybrid Materials. DAMO–MPS hybrids were synthesized via sol–gel by dissolving appropriate amounts of DAMO and MPS (alkoxy silane total amount = 6 mmol) in 487 μL of methanol. The corresponding amount of CaCl_2 was then added, and the mixture was stirred for 5 min at room temperature. After that, a mixture of 324 μL of H_2O and 243 μL of methanol was added dropwise to facilitate their compatibility and to produce alkoxy silane hydrolysis. The final molar composition of the system was 1:3:3 total-silanes:methanol: H_2O . The sol was stirred for another 10 min, deposited in appropriate polyethylene molds, and left to stand overnight at room temperature to reach the sol–gel transition. The resulting gel was dried at room temperature in air for 24 h to obtain stable disc-shaped monolithic xerogels (7 mm diameter \times 2 mm height). Table 1 shows the nominal composition (in molar ratios) of the hybrids synthesized in this work.

Characterization of Hybrid Materials. The hybrids were characterized by chemical microanalysis with a Perkin–Elmer 2400

Table 1. Nominal Composition in Molar Ratios of MPS–DAMO Hybrids

code ^a	DAMO	MPS	CaCl_2 ^b
H _{0.40–1}	0.40	1	
C _{0.40–1}	0.40	1	0.10
H _{0.67–1}	0.67	1	
C _{0.67–1}	0.67	1	0.10
H _{1–1}	1	1	
C _{1–1}	1	1	0.10
H _{1.33–1}	1.33	1	
H _{1–0}	1		

^a H, only MPS and DAMO; C, contains calcium. Subindexes indicate the DAMO:MPS molar ratio. ^b Molar ratio with respect to the total molar amount of alkoxy silanes.

CHN analyzer. Thermal analyses (TG and DTA) were carried out in a dynamic nitrogen atmosphere between 30 and 950 $^{\circ}\text{C}$ (flow rate of 50 mL/min with a heating rate of 10 $^{\circ}\text{C}/\text{min}$) using a Perkin–Elmer Diamond analyzer. Fourier transform infrared spectroscopy was performed with a Nicolet Nexus spectrometer using the KBr pellet method. Scanning electron microscopy (SEM) and energy dispersive X-ray spectrometry (EDS) were carried out with a JEOL 6400 microscope coupled to a LINK AN 1000 device. Transmission electron microscopy (TEM), electron diffraction (ED), and EDS analysis were performed in a JEOL 2000 FX electron microscope working at 200 kV coupled with an Oxford Pentafet Super A/W (EDS) analyzer microscope. The zeta-potential (ζ) measurements were performed in a Zetasizer Nano Series instrument coupled to a MPT-2 multipurpose titrator from Malvern. ζ -potential can be described by Smoluchowski's equation:¹⁹ $\zeta = 4\pi\eta U/\epsilon$, where U is the electrophoretic mobility, η is the viscosity of water, and ϵ is the dielectric constant of water. Fifty milligrams of each hybrid ground to a powder was added to 10 mL of water, and an aliquot of 50 μL was then added to 10 mL of a solution containing 1 mM KNO_3 (used as the supporting electrolyte); the pH was adjusted by adding appropriate volumes of 0.025 M HNO_3 or 0.025 M KOH solutions.

In vitro Bioactivity. The assessment of in vitro bioactivity assays of the samples was carried out by soaking the monoliths, vertically held over platinum holders, in 30 mL of SBF at pH 7.4 and 37 $^{\circ}\text{C}$. SBF is an acellular aqueous solution proposed by Kokubo et al.¹ with inorganic ion composition almost equal to human plasma. To avoid microorganism contamination, we previously filtered the SBF solution with a 0.22 μm Millipore System, and all operation/manipulations of the pieces and SBF were done in a laminar flux cabinet Telstar AV-100. After 7 and 14 days in SBF, the samples were removed from the fluid, rinsed with water and ethanol, and dried in air at room temperature. The variations of Ca^{2+} concentration and pH in solution were determined with an ILyte Na^+ , K^+ , Ca^{2+} , pH analyzer, and the variations in the sample surfaces were characterized by FTIR, SEM–EDS, and TEM–ED–EDS.

Results and Discussion

Characterization of DAMO–MPS Hybrid Materials.

The hydrolysis and polycondensation reactions of DAMO and MPS produce a polyorganosiloxane matrix via sol–gel. The synthesis process takes place at room temperature in the presence of water molecules, without needing thermal activation and with the amino groups of DAMO acting as basic catalysts of the sol–gel process.^{15,20} Almost complete

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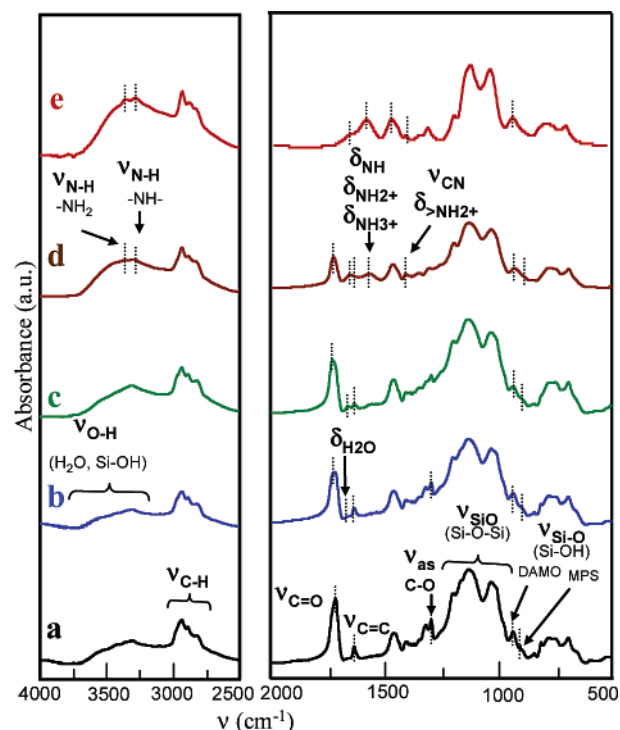
Table 2. Theoretical and Experimental C, H, and N Contents (wt %) for $H_{0.40-1}$ and $C_{0.40-1}$ Hybrids

hybrid	type	wt %		
		C	H	N
$H_{0.40-1}$	theor.	39.4	5.9	4.1
	exp.	41.0	6.6	4.3
$C_{0.40-1}$	theor.	37.3	5.6	3.9
	exp.	39.0	6.3	4.2

hydrolysis and polycondensation of the alkoxy silanes are achieved within 24 h. The addition of $CaCl_2 \cdot 2H_2O$ during the step synthesis allows the incorporation of calcium in the resulting hybrids, as confirmed by EDS analysis. Independently of the compositions, all the obtained materials were colorless and transparent and showed certain flexibility.

CHN elemental microanalysis was used to determine the carbon, hydrogen, and nitrogen contents in the hybrid materials. Theoretical and experimental C, H, and N contents for $H_{0.40-1}$ and $C_{0.40-1}$ hybrids, chosen as a representative example, are summarized in Table 2. There is a good concordance between theoretical and experimental values for nitrogen content. On the other hand, when comparing the obtained carbon contents with theoretical ones, the experimental data are slightly higher, which could probably be due to the presence of methoxy groups remaining nonhydrolyzed after the hydrolysis and condensation processes and/or residual methanol molecules entrapped in the polysiloxane matrix. Finally, the experimental hydrogen contents are slightly higher than the theoretical ones. This disagreement can be explained by the presence of methoxy groups remaining nonhydrolyzed and also by the presence of water molecules entrapped in the polyorganosiloxane matrix.

The main regions in FTIR spectra of DAMO–MPS calcium-free hybrids are shown in Figure 1 (curves a–d). For comparison, FTIR spectrum of a pure DAMO xerogel H_{1-0} (curve e) is also displayed. DAMO–MPS hybrids show bands at 1720 cm^{-1} ($\nu_{C=O}$), 1640 cm^{-1} ($\nu_{C=C}$), and 1300 cm^{-1} (ν_{asC-O}) attributed to the methacryloxy group of MPS. As the DAMO:MPS ratio increases (curves d and e), characteristic vibration bands of amino groups appear, at 3368 and 3283 cm^{-1} , that are assigned to ν_{N-H} of primary (NH_2) and secondary (NH) amine groups, respectively. The strong absorption in the $3600\text{--}3400\text{ cm}^{-1}$ range appearing in all FTIR spectra is attributable to the presence of water molecules in the solid. This fact agrees with the appearance of a band in the $1654\text{--}1600\text{ cm}^{-1}$ region, ascribed to the δ_{HOH} vibration of adsorbed water molecules (labeled in curve b). The intensity of the bands corresponding to adsorbed water molecules increases as the DAMO content increases, suggesting higher water content, according to the more hydrophilic character of such hybrids.²¹ This fact will be corroborated by the thermogravimetric analysis. In $H_{1.33-1}$ (Figure 1d) and pure DAMO hybrid H_{1-0} (Figure 1e) spectra, the appearance of a new band around 1580 cm^{-1} , which can be attributed to δ_{NH} , $\delta_{NH_2^+}$, and $\delta_{NH_3^+}$, should be highlighted. Although δ_{CH_2} bands of Si–CH₂ groups of MPS and DAMO could be also present in the same interval, they are

**Figure 1.** FTIR spectra ($4000\text{--}2500$ and $2000\text{--}500\text{ cm}^{-1}$ regions) of (a) $H_{0.40-1}$, (b) $H_{0.67-1}$, (c) H_{1-1} , (d) $H_{1.33-1}$, and (e) H_{1-0} hybrids.

not distinguished in hybrids with the lower DAMO content. Moreover, at 1409 cm^{-1} , there is a characteristic band of $\delta_{NH_2^+}$ within the ν_{CN} band whose relative intensity increases as the DAMO content increases. These two bands suggest that hybrids with higher DAMO:MPS ratios contain protonated amino groups, forming zwitterions species previously reported in the literature for aminopolysiloxane matrices.^{14,15}

Finally, in the $1250\text{--}1000\text{ cm}^{-1}$ range, high-intensity bands attributed to ν_{Si-O} of siloxane groups (Si–O–Si) are observed. Besides, in mixed DAMO–MPS xerogels, two weak bands at 930 and 900 cm^{-1} , which can be assigned to ν_{Si-O} of silanol groups (Si–OH) of DAMO and MPS, respectively, also appear. The high intensity of siloxane bands compared to silanol bands suggests a high polymerization degree and the formation of a three-dimensional network.²² On the other hand, the intensity of the band at 930 cm^{-1} attributed to Si–OH groups of DAMO is stronger than that corresponding to Si–OH groups of MPS, appearing at 900 cm^{-1} . In addition, as the DAMO:MPS ratio increases, there is also an increase in the relative intensity of siloxane/silanol absorption bands. These results suggest that higher DAMO content in mixed hybrids generates more open networks with a higher number of silanol groups.

Figure 2 shows TG curves ($25\text{--}950\text{ }^{\circ}\text{C}$ temperature range) of several calcium-free hybrids. The DTA trace of the H_{1-1} hybrid, chosen as a representative example, is also displayed. Weight loss processes of such hybrids can be classified in three different regions, denoted as I, II and III. All processes are associated with endothermic peaks in DTA curves. Region I ($25\text{--}130\text{ }^{\circ}\text{C}$ range) is assignable to the loss of water molecules entrapped in the polyorganosiloxane network. As can be seen in Figure 2, this weight loss increases with the

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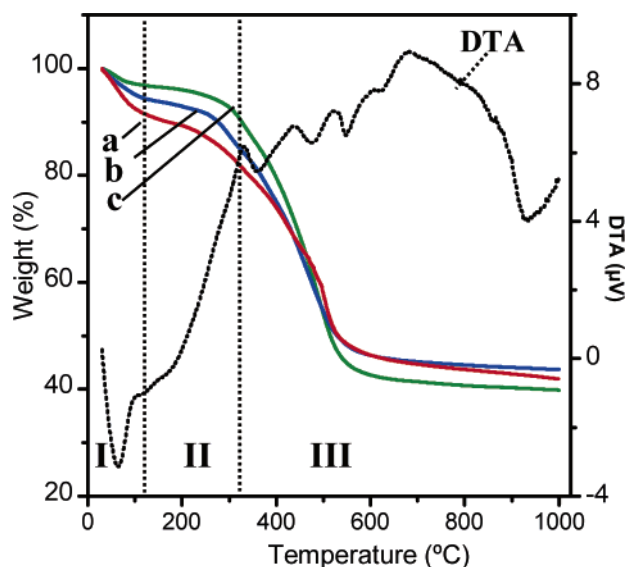


Figure 2. TG traces of (a) $H_{1.33-1}$, (b) H_{1-1} , and (c) $H_{0.40-1}$ hybrids. DTA curve of H_{1-1} is also displayed.

amount of DAMO present in the hybrid composition. This fact agrees with the more hydrophilic character of these materials. On the other hand, the addition of calcium does not seem to affect water retention, as TG traces of such hybrids (curves not shown) are similar to calcium-free ones. The elimination of organic matter in hybrids obtained from pure DAMO (H_{1-0}) occurs mainly between 400 and 500 °C,¹⁵ whereas in the DAMO-MPS hybrids, this loss starts at a lower temperature, 310 °C. This last process (region III) occurs in several stages (total weight loss between 40 and 50%), each one ascribed to several endothermic phenomena. These processes are associated with the pyrolysis of organic groups present in the hybrid in different steps probably induced by a sequential thermal degradation of methacryloxy group of MPS, as previously described for several propylmethacrylate functions.^{21,23} On the other hand, the condensation of silanol groups would start at about 130 °C and a progressive weight loss could be expected to take place until the temperature surpassed 950 °C. Hence, the weight loss in region II (130–310 °C temperature range) could give certain information about the number of silanol groups present in the hybrids. According to this assumption, a higher number of silanol groups are obtained in hybrids containing higher DAMO content, corroborating the previously discussed FTIR results. The content of silanol groups is expected to play a key role in the *in vitro* bioactivity behavior of hybrid materials.

In vitro Bioactivity Studies. The *in vitro* bioactivity of the DAMO-MPS hybrids was evaluated by soaking the monoliths in SBF for 7 and 14 days. The variations of Ca^{2+} and pH in SBF were measured and changes in the sample surfaces were characterized by FTIR, SEM-EDS, and TEM-ED-EDS.

Figure 3 shows SEM micrographs of calcium-free hybrid surfaces before and after being soaked in SBF. Hybrids with DAMO:MPS > 1 ($H_{1.33-1}$) completely dissolve after 10 h

in SBF, increasing the pH of the solution to 8.0. As can be seen in Figure 3a, the $H_{1.33-1}$ hybrid surface cracks after 2 h in SBF. A detail at higher magnification and the corresponding EDS are also displayed. The deposition of a new compound in the grain boundaries, whose EDS spectrum reveals a Ca:P ratio close to 1, can be distinguished. It could consist of an amorphous calcium phosphate phase. However, the formation of an apatite-like layer at higher soaking times is impeded, as the hybrid solubilizes completely. When immersed in SBF, hybrids with MPS:DAMO = 1 (H_{1-1}) form hydrogels that partially dissolve after 14 days in SBF and crack after drying. In Figure 3b, a change in the appearance of the hybrid surface after being soaked in SBF, probably due to the partial solubilization of the polysiloxane matrix, can be observed. The EDS spectrum reveals the deposition of NaCl over the hybrid surface after 14 days in SBF. Hybrids with DAMO:MPS < 1 ($H_{0.40-1}$ and $H_{0.67-1}$) are stable and crack-free and maintain their colorless and transparent aspect after 7 and 14 days in SBF. Figure 3b shows the SEM micrographs of the $H_{0.40-1}$ hybrid surface before and after the *in vitro* assays. As can be observed, the hybrid surface maintains its initial appearance, and the EDS spectrum reveals a composition based on Si and O. In light of these results, it can be confirmed that increasing the MPS content in mixed xerogels results in less-hydrophilic compounds, which are more stable in aqueous media than pure DAMO hybrids or mixed hybrids with higher DAMO amounts. The stability of polyorganosiloxane networks containing amino groups has been successfully achieved by the addition of appropriate amount of MPS. However, these hybrids do not present *in vitro* bioactivity. To induce bioactivity in such mixed xerogels, we added a calcium salt ($CaCl_2 \cdot 2H_2O$) during the synthesis step.

Figure 4 shows SEM micrographs of calcium-containing hybrid surfaces before and after the *in vitro* assays. After 7 days in SBF, all hybrid surfaces are covered by spherical agglomerates consisting of numerous corn-shaped particles. EDS spectra reveal the formation of Ca-P-rich layers with a Ca:P ratio close to 1.40 in all cases. These values are lower than that corresponding to a stoichiometric apatite (Ca:P = 1.67).²⁴ As the DAMO:MPS ratio increases, a denser layer is observed in SEM micrographs. The density of such a layer increases with soaking time (14d) in hybrids with higher DAMO amounts ($C_{0.40-1}$ and $C_{0.67-1}$), maintaining the Ca:P ratio close to 1.40. The *in vitro* behavior of C_{1-1} hybrid should be mentioned. The corresponding SEM micrograph, after 14 days in SBF, shows a spherical agglomerate of particles that seem to be formed over the initial Ca-P-rich layer that would be dissolving at the same time as the hybrid substrate (Ca:P \approx 1.40). The more hydrophilic character of C_{1-1} hybrids, containing higher DAMO amounts that would degrade in contact with SBF, would support this phenomenon.

With the aim of further characterizing the nature of the Ca-P-rich layer formed after the *in vitro* assays, we performed FTIR and TEM-EDS-ED measurements. Figure 5 shows the results of such characterization for $C_{0.67-1}$,

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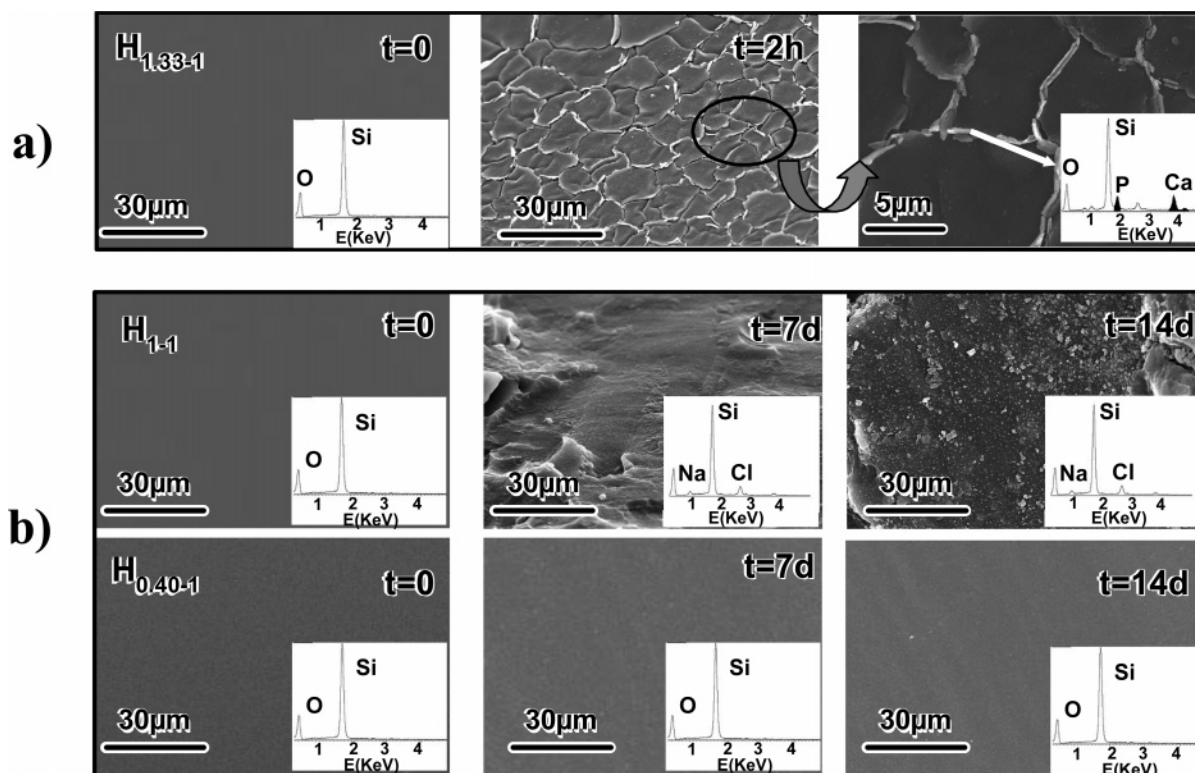


Figure 3. SEM micrographs of Ca-free DAMO–MPS hybrid surfaces: (a) $H_{1.33-1}$, before and after 2 h in SBF (a detail at higher magnification is also shown); and (b) H_{1-1} and $H_{0.40-1}$ before and after 7 and 14 days in SBF. EDS spectra are also displayed as insets.

chosen as a representative example, after 14 days in SBF. The FTIR spectrum (Figure 5a) is quite similar to that of bone CHA,^{25,26} showing bands at 1035 and 955 that can be assigned to the phosphate group, two bands at 601 and 568 cm^{-1} that are typical of the antisymmetric stretching vibration of P–O bonds attributed to crystalline calcium phosphate, and bands at 1489, 1419, and 871 cm^{-1} that can be assigned to the carbonate group.

In order to get information about the crystalline nature of the layer formed in the hybrid surfaces, we also carried out TEM–EDS–ED studies. The image obtained by TEM (Figure 5c) clearly shows the needle-like shape of the crystallites, whose EDS spectrum reveals a Ca:P ratio of 1.39 (Figure 5b), forming the corn-shaped particles observed by SEM. ED diagram (Figure 5d) shows the presence of diffuse diffraction rings with more intense diffraction maxima. The interplanar spacings of the polycrystalline materials agree with those characteristics of an apatite-like structure,²⁷ with a maximum at 2.8 Å, which can be attributed to the more intense reflection (211) of an apatite-like phase overlying (112), (300), and (202) reflections.

The combined application of SEM–EDS, FTIR, and TEM–EDS–ED techniques allowed us to monitor the formation of this layer and identify it as consisting of nanocrystals of carbonate hydroxyapatite similar to biological apatites in bone.^{25,26,28}

Influence of Functional Groups and Charge of Hybrid Surfaces on Hybrid Bioactivity and Degradability. According to the above-described results, the DAMO:MPS ratio determines the rate of growth of an apatite-like layer, i.e., the bioactivity degree. Consequently, an experimental degradability and bioactivity sequence can be established for the investigated hybrids as follows: $C_{0.40-1} < C_{0.67-1} < C_{1-1}$. Now, it is necessary to establish the influence of the surface properties of hybrids on the bioactivity and degradability of hybrid materials under in vitro conditions. For this purpose, FTIR spectroscopy and electrochemical measurements were performed. One of the most important parameter that defines surface properties of solids in aqueous solutions is ζ -potential.^{29,30}

Therefore, considering the acid–base properties of aminopolysiloxane matrices, we can propose three possible protolytic equilibria:

- (1) $\text{NH}_2 + \text{H}^+ \rightleftharpoons \text{NH}_3^+$, protonation of primary amino groups
- (2) $\text{NH} + \text{H}^+ \rightleftharpoons \text{NH}_2^+$, protonation of secondary amino groups
- (3) $\text{Si-OH} \rightleftharpoons \text{Si-O}^- + \text{H}^+$, deprotonation of silanol groups

On one hand, primary and secondary amino groups present in the hybrid matrices are susceptible to protonation by deprotonating an adjacent silanol group and forming a

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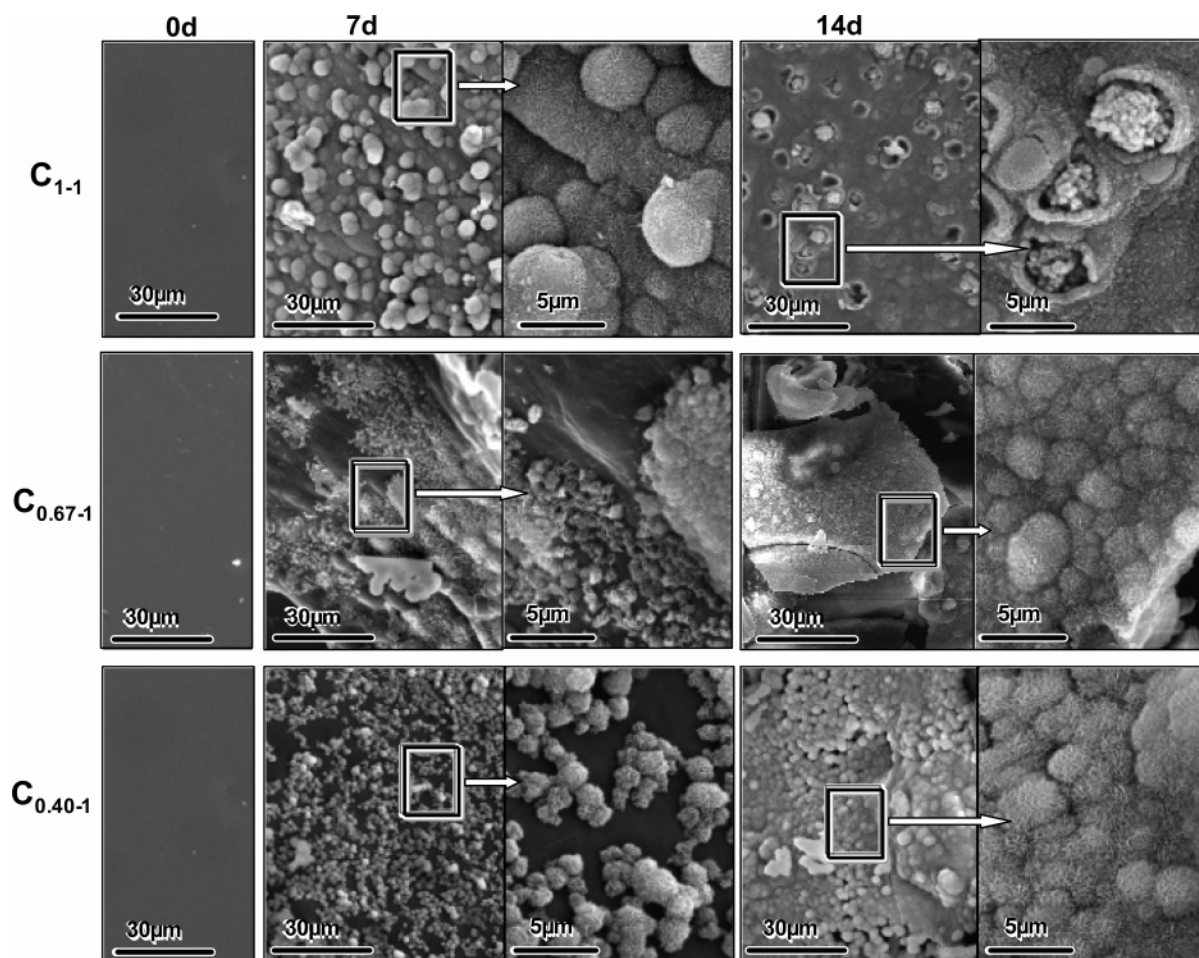


Figure 4. SEM micrographs of C_{1-1} , $C_{0.67-1}$, and $C_{1-0.40}$ hybrids before (0d) and after 7 (7d) and 14 (14d) days in SBF.

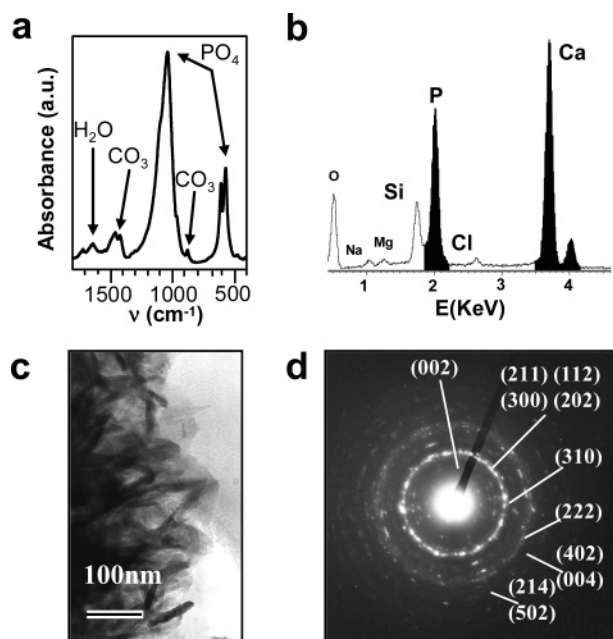


Figure 5. (a) FTIR spectrum, (b) EDS, and (c) TEM micrograph and the corresponding ED pattern of the $C_{0.67-1}$ hybrid surface after 14 days in SBF.

zwitterion species^{14,15} or by protonation in contact with water molecules.³¹ In fact, such amino groups appear to be completely protonated over a wide pH range.^{15,18,32} With the aim of investigating whether amino groups are protonated

at the physiological pH of 7.4, we performed FTIR spectra of a calcium-free hybrid ($H_{0.67-1}$) before and after being soaked in SBF (Figure 6). After 7 and 14 days in SBF, significant differences compared to the above-discussed initial FTIR spectrum ($t = 0$ days) are observed. A vibration band near 1580 cm^{-1} assignable to $\delta_{\text{NH}_2+}/\delta_{\text{NH}_3+}$ appears.^{21,33} Moreover, there is an increase in the relative intensity of the band at 1410 cm^{-1} , which is characteristic of δ_{NH_2+} within the $\nu_{\text{C-N}}$ band. These bands show that protonation of amino functional groups of hybrids takes place in aqueous medium, corroborating previous results reported in the literature.^{34,35} On the other hand, it is known that in the presence of water, the silanol groups ionize, producing mobile protons that associate/dissociate with the surface to impart an electrical conductivity to the surface.

Results of electrokinetic studies reflect the acid–base alterations taking place that are due to the variation in the number of silanol and amino groups present in the hybrid surfaces. ζ -potential curves of three bioactive hybrid suspensions versus the pH of the aqueous phase (adjusted using

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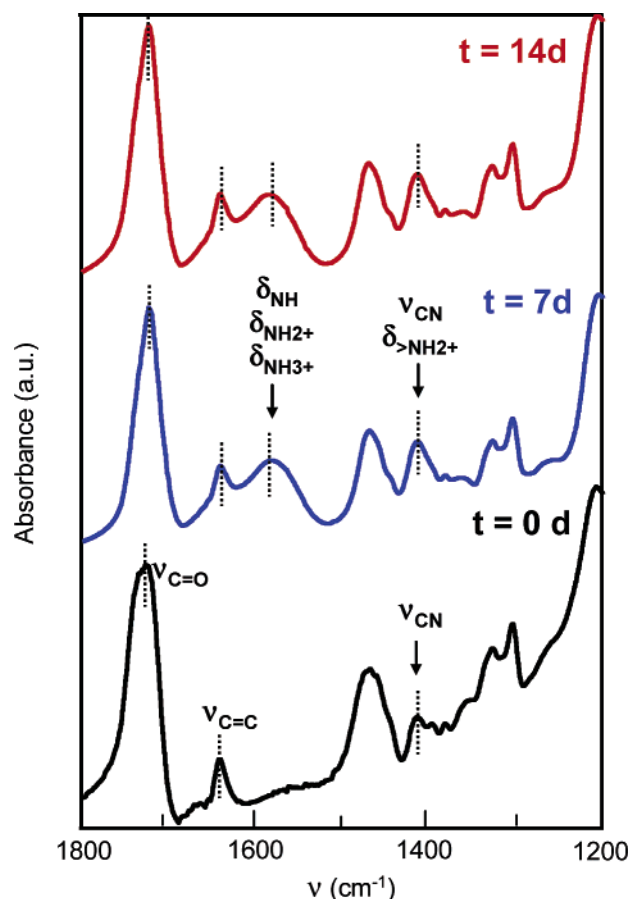


Figure 6. FTIR spectra (1800–1200 cm^{-1} region) of the $\text{H}_{0.67-1}$ hybrid before and after 7 and 14 days in SBF.

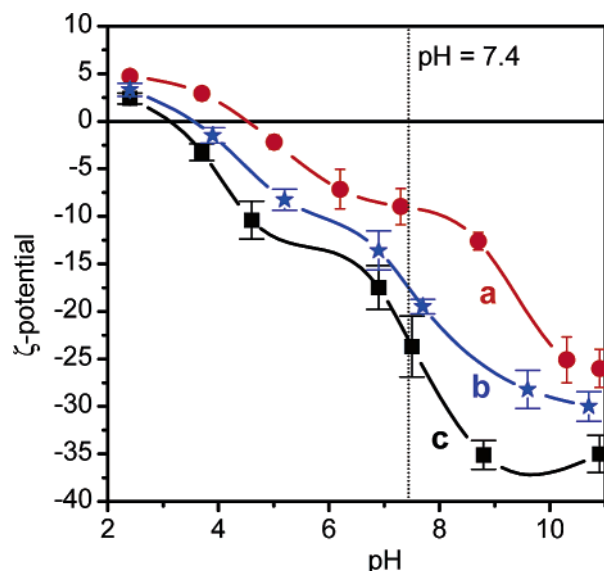


Figure 7. ζ -Potential of (a) C_{1-1} , (b) $\text{C}_{1-0.67}$, and (c) $\text{C}_{1-0.40}$ hybrids vs pH at $I = 0.001$ mol/L of KNO_3 .

HNO_3 or KOH) were recorded between pH 2 and 11 using 1 mM KNO_3 as the supporting electrolyte (Figure 7). The morphology of these curves is similar to that of previously reported aminosilane-modified surfaces.³⁶ ζ -potential values can be explained by the coexistence of $\text{NH}_3^+/\text{NH}_2^+$ and Si-O^- species and the variation in the surface composition as a

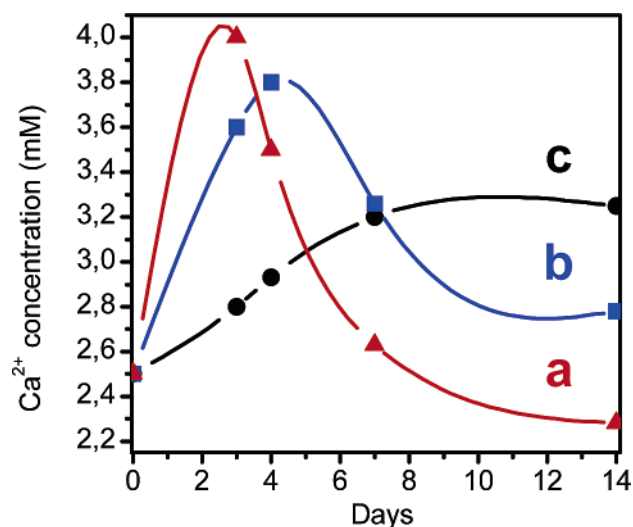


Figure 8. Variation of calcium content (mmol/L) with soaking time in SBF for (a) C_{1-1} , (b) $\text{C}_{0.67-1}$, and (c) $\text{C}_{0.40-1}$ hybrids.

function of pH. At pH values lower than the isoelectric point (IEP), there is predominance in Si-OH and protonated amino groups, leading to positive ζ -potential values. At pH values higher than the IEP, there are still protonated amino groups, but there is a major concentration of Si-O^- groups, which leads to a negatively charged surface. When ζ -potential versus pH curves of different hybrids (curves a–c) are compared, the IEP, which is a quantitative reflection of surface acidity or alkalinity, is shifted toward a higher value as the DAMO:MPS ratio increases, according to the increase in the number of amino groups. On the other hand, at $\text{pH} > \text{pH}_{\text{IEP}}$, the negative net ζ -potential is higher when the DAMO:MPS ratio is decreased. As the amount of DAMO increases, the number of amino groups that protonate is higher; however, there is also an increase in the number of silanol groups, as deduced from the results of hybrid characterization, and thus there is a negative net charge on the surface. An immediate consequence of this study is that the protonation of amino groups is not a barrier for the in vitro formation of an apatite-like layer on the hybrid surface (at pH 7.4) if there is a large enough number of silanol groups to originate a net negative charge on the surface.

On the other hand, in view of these results and assuming that negative surfaces are always favorable for the nucleation of CHA ,^{9,10} hybrids with a higher net negative density charge would be expected to be more bioactive, and the ζ -potential study would thus suggest a theoretical bioactivity sequence of $\text{C}_{0.40-1} > \text{C}_{0.67-1} > \text{C}_{1-1}$. There is a disagreement between theoretical predictions (on the basis of surface charge properties) and experimental results. This fact suggests that another important factor has to be considered: the release of Ca^{2+} from the hybrids to the SBF solution depends on the hydrophilic degree of the polysiloxane matrices. The evolution of Ca^{2+} concentration in SBF with time for these bioactive hybrids is shown in Figure 8. The maximum amount of Ca^{2+} released from the less hydrophilic and hydrolytically stable hybrid ($\text{C}_{0.40-1}$) is reached after 7 days in SBF. On the contrary, the maximum Ca^{2+} is reached earlier during the first days for more hydrophilic and degradable hybrids (C_{1-1}); thus, after 7 days, the Ca^{2+}

dramatically decreases as a consequence of faster CHA layer formation. It seems clear that by modulating both functional groups and the charge of the surface, and consequently the hydrophilic character of DAMO–MPS hybrids, we can tailor the in vitro degradation and bioactivity of these materials.

Conclusions

Organic–inorganic hybrid materials containing amino functional groups were successfully obtained as monoliths. They were prepared from DAMO, and MPS was added to overcome the problem of hydrolytic instability. The presence of calcium in the polyorganosiloxane networks seems to be an essential requirement for the in vitro formation of an apatite-like layer.

The DAMO:MPS molar ratio determined both functional groups and the charge of the surface, resulting in a wide range of performances in SBF, ranging from highly bioactive materials susceptible of degradation (DAMO:MPS \approx 1) to moderately bioactive and stable hybrids (DAMO:MPS <

0.67). ζ -Potential measurements showed that the surface charge of bioactive hybrids was negative at physiological pH, which was explained considering a density of Si–O[−] groups higher than that of protonated amino groups. In addition, this value became more negative as the DAMO:MPS ratio decreased, following the opposite trend of that in the in vitro bioactivity. However, the maximum amount of Ca²⁺ released from hybrid to SBF solution was reached earlier in hybrids with a higher DAMO:MPS ratio. This fact suggests that the release rate of calcium predominates over the net negative surface charge in the apatite formation on these hybrid materials.

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