Novel sol-gel organic-inorganic hybrid materials for drug delivery

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ABSTRACT: Purpose: The aim of the present study was to synthetize and characterize novel sol-gel organic-inorganic hybrid materials to be used for controlled drug delivery application. Materials and Methods: Organic-inorganic hybrid class I materials based on poly(ε-caprolactone) (PCL 6, 12, 24 and 50 wt%) and zirconia-yttria (ZrO₂-5%Y₂O₃) were synthesized by a sol-gel method, from a multicomponent solution containing zirconium propoxide [Zr(OC₂H₇)₄], yttrium chloride (YCl₃), PCL, water and chloroform (CHCl₃). The structure of the hybrids was obtained by means of hydrogen bonds between the Zr-OH group (H-donor) in the sol-gel intermediate species and the carboxylic group (H-acceptor) in the repeating units of the polymer. Results: The presence of hydrogen bonds between organic-inorganic components of the hybrid materials was suggested by Fourier transform infrared (FTIR) analysis, and strongly supported by solid-state NMR. A single-step, sol-gel process was then used to precipitate microspheres containing ketoprofen or indomethacin for controlled drug delivery applications. Release kinetics in a simulated body fluid (SBF) were subsequently investigated. The amount of drug released was detected by UV-VIS spectroscopy. Pure anti-inflammatory agents exhibited linear release with time, in contrast drugs entrapped in the organic-inorganic hybrids were released with a logarithmic time dependence, starting with an initial burst effect followed by a gradual decrease. Conclusions: The synthesis of amorphous materials containing drugs, obtained by sol-gel methods, helps to devise new strategies for controlled drug delivery system design. (Journal of Applied Biomaterials & Biomechanics 2010; 8: 42-51)

Key words: Sol-gel, Organic-inorganic hybrid, Solid-state NMR

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INTRODUCTION

ε-Polycaprolactone (PCL) is a biodegradable, synthetic, aliphatic polyester that has attracted widespread interest due to its possible applications as a biomaterial. PCL-based three-dimensional (3-D) scaffolds for orthopedic surgery (1), and film substrates for tissue engineering (2) were recently proposed. Micelles of PCL-PEO were used as drug delivery vehicles (3). Organic-inorganic nanocomposites were prepared with montmorillonite (4), silicates (5), SiO₂ (6), and TiO₂ (7). Class I and class II hybrids were prepared by sol-gel (8).

We recently prepared, by sol-gel methods, several PCL-based hybrids for drug delivery using different oxides, including CaO and/or SiO₂ (9-11), TiO₂ (12), and ZrO₂ (13). In all cases, our Fourier transform infrared (FTIR) measurements gave circumstantial evidence of the formation of class I hybrids, characterized by the presence of hydrogen bonds among the carboxyl groups of the polymer, and the OH- groups of the inorganic phase.

Solid-state NMR is a well-suited technique to study inter- and intra-chain interactions, as well as both short and long range dynamics in polymers (14). This technique has been recently applied to PCL. 1-D and 2-D ¹³C NMR analyses on PCL crystallized from the melt were reported by Kaji and Horii (15). The formation of hydrogen bonds between the hydroxyl groups of poly(4-vinylphenol) and the PCL carbonyl was characterized by ¹³C NMR (16). The structure and dynamics of PCL-clay nanocomposites were investigated by ³¹P and ¹³C NMR (17). Finally, both static and CP-MAS ¹³C NMR were used to investigate PCL-CaCO₃ composites (18).

Controlled, localized drug release offers several advantages over other delivery options. Plasma concentrations of drugs administered via injection, inhalation or ingestion, methods which can require repeated and relatively greater dosing and patient compliance, reflect the typical kinetics of an inefficient bolus delivery. Controlled local release systems can provide the desired constant drug concentrations at the delivery site, lower systemic drug levels, and a reduced potential for deleterious side effects.

The controlled release of pharmaceuticals such as anti-inflammatory agents and antibiotics from strong and
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biocompatible hosts has important applications (19, 20). They include implantable therapeutic systems, filling materials for bone or tooth repair, and show a reduction in inflammatory or infectious side effects of implant materials by applying coatings of biocompatible materials containing anti-inflammatory or antibiotic drugs (21, 22).

The use of ceramic materials as carriers for drug release in many biomedical applications has been extensively reported (23-25). However, only a few studies on bone filling materials exist, showing simultaneously controlled drug release and bioactive behavior (26). Currently, it seems to be a very attractive idea to search for materials, showing bioactive properties that could release anti-inflammatory agents in a local and controlled way (27-29).

In this study (ZrO$_2$-5%Y$_2$O$_3$)/PCL (PCL=6, 12, 24 and 50 wt%), materials were tested as support matrices for the controlled release of drugs. The nature of the hybrid materials was tested by several techniques, including FTIR and solid-state $^{13}$C NMR. Using a sol-gel method we encapsulated ketoprofen or indomethacin, as model drugs. The drug-loaded bioactive materials were studied in terms of their drug release kinetics.

MATERIALS AND METHODS

SOL-GEL SYNTHESIS

The hybrid inorganic-organic materials (ZrO$_2$-5 wt%Y$_2$O$_3$)/PCL (PCL=6, 12, 24 and 50 wt%) were prepared by a sol-gel process, starting from analytical reagent grade zirconium propoxide Zr(OC$_3$H$_7$)$_4$, yttrium chloride (YCl$_3$) in ethanol-acetylacetone-water mixture, and poly-caprolactone (PCL, Mw=65000) in chloroform as the solvent. The anti-inflammatory drugs ketoprofen and indomethacin (Sigma-Aldrich) were selected. Acetylacetone was added to control the hydrolytic activity of zirconium alkoxide. The alcohol solution of YCl$_3$ was slowly added to the Zr(OC$_3$H$_7$)$_4$ solution and then stirred with a magnetic stirrer. Figure 1 shows the scheme of the sol-gel reaction, including the molar ratios of the starting materials.

(ZrO$_2$-5%Y$_2$O$_3$)/PCL (PCL=6, 12, 24 and 50 wt%) hybrids were then mixed with a solution of H$_2$O/EtOH/drugs (5 wt% and 10 wt%) (Sigma-Aldrich). After the addition of each reactant the solutions were carefully stirred to give uniform and homogeneous sols. The time of gelification was about 7 days. After gelation the gels were air-dried at 50 °C for 24 hr to remove the residual solvent. This treatment did not modify the stability of drugs, and small glassy pieces were obtained (Fig. 2). Discs with a diameter of 13 mm and a thickness of 2 mm were obtained by pressing the gel powders (average diameter <125 µm) into a cylindrical holder.

STRUCTURAL AND MICROSTRUCTURAL CHARACTERIZATION

The structure of ZrO$_2$-5 wt% Y$_2$O$_3$ gel, polycaprolactone (PCL) and (ZrO$_2$-5 wt%Y$_2$O$_3$)/PCL hybrid materials was investigated by X-ray diffraction (XRD) using a Philips diffractometer. Powder samples were scanned from 2θ = 5° to 60° using CuKα radiation.

The presence of hydrogen bonds between the organic and the inorganic components of the hybrid materials was ascertained by Fourier transform infrared (FTIR) analysis. FTIR transmittance spectra were recorded in the 400-4000 cm$^{-1}$ region using a Prestige 21 system, equipped with a DTGS KBr (Deuterated Tryglycine Sulphate with potas-
sium bromide windows) detector, with a resolution of 2 cm\(^{-1}\) (20 scans). KBr pelletized disks containing 2 mg of sample and 200 mg KBr were produced. The FTIR spectra were elaborated by Prestige software (IRsolution).

The local order was also investigated by means of solid-state NMR. The NMR spectra were acquired on an AMX-400 Spectrometer (Bruker, Karlsruhe) equipped with a 4 mm MAS probe (Bruker). All the spectra were obtained by averaging over ~1500 acquisitions. The measurements were performed at a sample spinning frequency in the range 4-6 kHz, with a \(^1H\) 90\(^\circ\) pulse of 5.5 \(\mu\)s, recycle time of 5 sec, and values of the contact time from 1 to 5 ms. The spectra were referenced to TMS, using adamantane as a secondary shift standard.

The microstructure of the gels was studied by scanning electron microscopy (SEM) (Cambridge model S-240) on samples previously coated with a thin Au film, and by atomic force microscopy (AFM) (Digital Instruments Multimode) in contact mode in air.

**STUDY OF IN VITRO RELEASE**

For the drug release study, the discs of the investigated materials were soaked in 15 ml simulated body fluid (SBF) at 37 °C under continuous stirring. The SBF was previously filtered with a Millipore\(^\circ\) (nominal pore size: 0.22 µm) system, avoiding bacterial contamination. Drug release measurements were carried out by means of UV-VIS spectroscopy with a Shimadzu UV mini-1240. The absorbance values were taken at the wavelength at which each drug showed an absorbance maximum in SBF (Tab. I). The calibration curve was determined by taking absorbance vs. drugs concentration between 0 and 30 µM as the parameter. For this interval, the calibration curve fits the Lambert and Beers’ law (30) of molar specific absorbance.

Chromatographic experiments were carried out with a Shimadzu HPLC system, equipped with Class-VP 5.0 software, a UV spectrophotometric detector SPD-10AVvp and two pumps LC-10ADvp, with low-pressure gradient systems. Samples of the solutions were injected by syringe via a Rheodyne loop injector. The loop volume was 20 µl. The analytical column was a Phenomenex C18 (150x4.60mm; 5µ). The flow rate of the mobile phase A (water) was set at 0.8 ml/min and that of the mobile phase B (methanol) was set at 0.2 ml/min. The total run time was 10 min. A standard solution of anti-inflammatory 3mM in SBF was prepared and the samples were taken at the end of the release from the materials.

**RESULTS AND DISCUSSION**

Gelation of samples is the result of hydrolysis and condensation reactions according to the following scheme:

\[
\begin{align*}
Zr(OC\text{C}_3\text{H}_7)_4 + n\text{H}_2\text{O} & \Rightarrow Zr(OC\text{C}_3\text{H}_7)_4-n(O\text{H})_n + n\text{C}_3\text{H}_7\text{OH} \quad [1] \\
-Zr\text{OH} + C_3\text{H}_7\text{O}-Zr- & \Rightarrow \equiv Zr-O-Zr\equiv + C_3\text{H}_7\text{OH} \quad [2] \\
≡ Zr-O\text{H} + OH-Zr≡ & \Rightarrow \equiv Zr-O-Zr\equiv + \text{H}_2\text{O} \quad [3] \\
Y^3+ + 3\text{H-OZr≡} & \Rightarrow [Y(-OZr≡)]_3 + 3\text{H}^+ \quad [4] \\
\end{align*}
\]

The reaction mechanism is generally accepted to proceed through a second order nucleophilic substitution (31).

Reaction 5 shows the formation of a hydrogen bond between the carboxylic group of the polymer and the hydroxyl group of the inorganic matrix.

**STRUCTURAL AND MICROSTRUCTURAL CHARACTERIZATION**

**Infrared spectroscopy**

Figure 3 shows the FTIR spectra of PCL, ZrO\(_2\)-5wt%Y\(_2\)O\(_3\) (a) and ZrO\(_2\)-5wt%Y\(_2\)O\(_3\)/PCL (6, 12, 24 and 50 wt%) gels (b, c, d, e). The peak assignment of PCL is...
made according to Young et al (31). The peaks in the range 2840-2928 cm\(^{-1}\) are attributed to the symmetric stretching of \(-\text{CH}_2\)\(\text{−}\). The peaks at 1730 cm\(^{-1}\) and 1250 cm\(^{-1}\) are attributed to C=O and C\((=\text{O})\text−\text{O}\)\(−\) stretching, respectively. The spectra of ZrO\(_2\)-5wt%Y\(_2\)O\(_3\) and of the composites (b, c, d, e) also show a broad O-H stretching absorption in the region 3200-3600 cm\(^{-1}\), which is absent in pure PCL. The intensity of this absorption band seems to be almost independent on the PCL content, which may suggest the formation of H-bonds among the carbonyl groups of the polymer chains and the inorganic part, as described in reaction 5. We now focus our attention on the C=O stretching absorption at 1730 cm\(^{-1}\). By decreasing the amount of polymer in the hybrids (from curve e to curve b) the intensity of this band progressively decreases, but its shape remains nearly unchanged. This seems to exclude the formation of strong bonds among the inorganic and the organic parts of the composites, but not the presence of H-bonds, so pointing to the formation of class I hybrids. Further information will be given by solid-state NMR.

**Solid-state NMR**

Figure 4 shows the \(^{13}\)C CPMAS-NMR spectrum of pure PCL, together with the peaks assignment on the basis of Kaji et al (15). The authors (15) found two resonances for each carbon positions that were assigned to crystalline and amorphous phases, respectively. Our sample, in contrast, shows only one resonance for each carbon. However, the full widths at half height (FWHH) of the peaks in Figure 4 are \(~\)30% larger than those deduced from Kaji (15), which means that our PCL is more disordered than that obtained by Kaji and Horii by isothermal crystallization.

Figure 5 shows the spectra of the ZrO\(_2\)-5wt%Y\(_2\)O\(_3\)/PCL hybrids (PCL 6, 12, 24 and 50 wt%). Compared to the pure PCL, the addition of the ceramic phase determines some relevant changes that are common to all the compositions: i) a \(~\)8 ppm shift to higher frequencies of the C=O resonance; ii) the splitting of the C=O resonance with the formation of a second peak around 190 ppm; iii) a 3-4 ppm shift to higher frequencies of the 2,5 aliphatic carbons, accompanied by a significant depression of their intensity, which is particularly evident at low PCL contents; iv) a \(~\)2 ppm shift towards lower frequencies of the ether carbon 6. These changes are ac-

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**Fig. 4** - \(^{13}\)C MAS NMR spectrum of pure PCL.

**Fig. 5** - \(^{13}\)C MAS NMR spectra of the ZrO\(_2\)-5wt%Y\(_2\)O\(_3\)/PCL hybrids (PCL 6, 12, 24 and 50 wt%). The red lines represent the resonances of the pure PCL, as reported in Figure 1. The spinning sidebands are marked with asterisks.
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companied by a generalized increase of the peaks FWHHs, which reflects the higher degree of disorder of the hybrids with respect to the pure PCL. Generally speaking, the modifications of the polymer spectra induced by the inorganic phase are compatible with the formation of hydrogen bonds among the –OH groups of the hydrolyzed metals and the carbonyl, as suggested above and also in previous papers based on IR spectroscopy results (12, 13). Downfield shifts of several ppm of the 13C carbonyl resonances are associated with the hydrogen bond formation in polymer blends (32). Therefore, the shift of the carbonyl peak is strong support for the formation of H-bonds and, therefore, of class I hybrids.

An interesting question arises concerning the splitting of the carbonyl resonance, which can be due to: i) different chemical shifts due to the presence of both yttrium and zirconium metals connected to the polymer chains through the hydrogen bonds, or ii) the presence of end groups due to a relevant depolymerization of the bulk polymer giving a telechelic product. A choice between these two possibilities can be made by considering the relative areas of the two carbonyl resonances that can be obtained by a standard Gaussian best-fitting of the spectra. Figure 6a shows the percentage areas of the high frequency (HF) carbonyl peak, normalized to the total C=O intensity, vs. the PCL content, together with the behavior expected in considering different models of hydrogen bonding in the hybrids. In particular, the continuous line represents the behavior in the case of no preferential bonding among the carbonyls and the OH functionalities of yttrium and zirconium (Model A: HF% ~ Y/(Y+Zr)). The dashed line, in turn, is the expected behavior in the case of preferential bonding to Y-OH (Model B: HF% ~ Y/PCL). The last case of preferential bonding to Zr-OH gives HF% = 0 at least up to PCL 24 wt%. None of these
at 180 and 190 ppm are due to the ester group $-\text{CO}_2^{-}\text{CO}_2^{-}$, and to the carboxyl functionality, respectively. Similar results were recently reported about telechelic polylactones (33). Under this assumption, the relative areas of the two peaks allow the determination of the polymerization degree (DP) of the PCL in the hybrids, which is reported in Figure 6b.

X-ray diffraction and microscopy

The diffractograms in Figure 7a show that ZrO$_2$-5 wt%Y$_2$O$_3$ gel exhibits broad humps characteristic of amorphous materials, while sharp peaks, typical of a crystalline material, can be detected in the diffractogram of polycaprolactone (Fig. 7b). The XRD spectrum of ZrO$_2$ 5wt% Y$_2$O$_3$/PCL (PCL 6, 12, 24 and 50wt%) (Fig. 7c), again exhibits broad humps characteristic of amorphous materials, as observed for the ceramic part, without any evidence of the peaks typical of PCL alone. We can conclude that our class I hybrids are completely amorphous.

The degree of mixing of the organic-inorganic components, ie the samples homogeneity, was ascertained by applying AFM. The AFM contact mode image can be measured in the height mode or in the force mode. Force images have the models, however, is in quantitative or at least qualitative agreement with the NMR results. We stress that all the models rest on two reasonable assumptions: 1) each metal atom has one -OH functionality free to make hydrogen bonds, and 2) no selective metal clustering is allowed. On the other hand, regular deviations from assumption 1), above, do not alter the expected monotonous trend of the models, and complex clustering phenomena are needed to justify the non-linear behavior of the NMR data. Moreover, simple considerations on the samples’ stoichiometry show that, at least in the case of the sample PCL 50 wt%, a significant fraction of the carbonyls should be unaffected by hydrogen bonds. For these reasons, we can rule out that our hypothesis i) is at the basis of the carbonyl peak splitting reported in Figure 5.

A more reasonable possibility is that PCL undergoes depolymerization by cleavage of the ether bonds during the preparation of the hybrids, and that the peaks at 180 and 190 ppm are due to the ester group $-\text{CO}_2^{-}\text{CO}_2^{-}$, and to the carboxyl functionality, respectively. Similar results were recently reported about telechelic polylactones (33). Under this assumption, the relative areas of the two peaks allow the determination of the polymerization degree (DP) of the PCL in the hybrids, which is reported in Figure 6b.
advantage that they appear sharper and richer in contrast and that the contours of the nanostructure elements are clearer. In contrast, height images give a more exact reproduction of the height itself (34). In this work, the height mode was adopted to evaluate the homogeneity degree of the hybrid materials. Table II reports the AFM topographic data of ZrO$_2$ and ZrO$_2$-5wt%Y$_2$O$_3$/PCL gels samples. The average domain sizes are 21 nm for ZrO$_2$-5wt%Y$_2$O$_3$ and 19-21 nm for PCL/

<table>
<thead>
<tr>
<th>Hybrid materials</th>
<th>Surface distance (nm)</th>
<th>Horizontal distance (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZrO$_2$-5wt%Y$_2$O$_3$</td>
<td>21.0</td>
<td>20.5</td>
</tr>
<tr>
<td>ZrO$_2$-5wt%Y$_2$O$_3$/PCL6wt%</td>
<td>19.8</td>
<td>19.5</td>
</tr>
<tr>
<td>ZrO$_2$-5wt%Y$_2$O$_3$/PCL12wt%</td>
<td>21.0</td>
<td>19.4</td>
</tr>
<tr>
<td>ZrO$_2$-5wt%Y$_2$O$_3$/PCL24wt%</td>
<td>19.9</td>
<td>19.5</td>
</tr>
<tr>
<td>ZrO$_2$-5wt%Y$_2$O$_3$/PCL50wt%</td>
<td>20.6</td>
<td>20.2</td>
</tr>
</tbody>
</table>

ZrO$_2$-5wt%Y$_2$O$_3$ (PCL 6, 12, 24 and 50 wt%), respectively. This result confirms that the PCL/ZrO$_2$-5wt%Y$_2$O$_3$ gels can be considered an organic/inorganic hybrid material as suggested by the literature (35).

A chromatographic analysis was carried out to ensure the integrity of both the anti-inflammatory agents after the synthetic treatment. The conditions described in the experimental section allow the separation of the two isomers of the anti-inflammatory agents with retention times of 1.6 min and 2.2 min. All the materials, ZrO$_2$-5wt%Y$_2$O$_3$/PCL (6, 12, 24 and 50 wt%) release anti-inflammatory agents without any sign of decomposition, as demonstrated by the perfect superimposition of the sample chromatograms with that of a standard solution of anti-inflammatory agent (data not shown).

**DRUGS RELEASE KINETICS**

Kinetic measurements of drug release from the materials studied were carried out in 15.0 ml of SBF incubated at 37 ± 0.1 °C under continuous magnetic stirring at 150 rpm. Sink condit-
tions were maintained throughout all studies. The discs used were obtained from particle sizes in the range 63-125 μm compressed at 3 tons, and aliquots of 600 μl and withdrawn at 1-hr intervals and then replaced with an equal volume of release medium pre-equilibrated to room temperature. In order to establish the relationship between the UV absorbance and the concentration of the drug solutions, calibration curves ($r^2 = 0.9903$) (Fig. 9), were drawn for standard solutions of drugs with four levels of concentration: 0.0 μM, 10 μM, 20 μM and 30 μM. The solutions were prepared in SBF.

Figures 10a, 11a, 12a and 13a show the cumulative percentage of drugs (5 and 10% ) released over time from ZrO$_2$ 5wt% Y$_2$O$_3$/PCL (0, 6, 12, 24 and 50 wt%) gels. As far as the pure inorganic gel (PCL 0%) is concerned both the anti-inflammatory agents were released in a relatively rapid way during the initial phase, 20 hr for ketoprofen, 25 hr for indomethacin and then more slowly during the rest of the experimental period.

The amount of drug released and the speed of the release depends on the drug concentration (5,10 wt%).

The reasons for this behavior could be varied. One possibility is that initially the drug on the surface of the material dissolves into solution. This process increases the number of pores existing in the matrix, allowing a greater amount of solvent to diffuse into the network. This phenomenon, more evident for materials with higher drug concentrations increases the speed and the amount of the drug released. This is due to the fact that the ZrO$_2$ gel interacts more efficiently with drugs at lower concentrations; and therefore, the drug is released more slowly.

**TABLE III - MOLECULAR WEIGHT OF DRUGS**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Molecular weight (g/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketoprofen</td>
<td>254.28</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>372.82</td>
</tr>
</tbody>
</table>
The differences observed in the release behavior of the different drugs might be due to the different interaction networks of the material with different drugs. It can be observed that the speed of release is faster as the molecular weight increase (36) (Tab. III).

The two-stage release observed in all cases suggests that the initial stage of release occurs mainly by dissolution and diffusion of the drug entrapped close to or at the surface of the samples. The second and slower release stage was thought to involve the diffusion of the drug entrapped within the inner part of clusters. An interesting observation is the general presence of a lag period, which is indicative of the need for solvent penetration in the structure.

Figures 10b, 11b, 12b and 13b, show these particular kinetics and illustrate the changes of the release speed during the two stages. For all materials, no appreciable differences were found when they were air dried at room temperature or at 60 °C.

CONCLUSION

In this study, the synthesis of amorphous materials containing drugs, obtained by sol-gel methods, was performed to devise new strategies for controlled release dosage forms.

The structure and the microstructure of the host hybrid materials have been investigated by spectroscopy (IR, solid-state NMR), XRD and microscopy. We showed that our composites are class I hybrids, and that the sol-gel reaction leads to a depolymerization of the organic part.

The release kinetics demonstrate that the investigated materials supply high doses of the anti-inflammatory agents during the first hours when soaked in SBF and then a slower drug release until the end of the experiment. The increase in the percentage of drugs (anti-inflammatory agents) increases the speed of release.

Conflict of interest statement: None to declare.

REFERENCES

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