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Controlled release properties and final macroporosity of a pectin microspheres–calcium phosphate composite bone cement

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\textbf{A B S T R A C T}

The use of calcium phosphate cements (CPC) is restricted by their lack of macroporosity and poor drug release properties. To overcome these two limitations, incorporating degradable polymer microparticles into CPC is an attractive option, as polymer microparticles could help to control drug release and induce macroporosity after degradation. Although few authors have yet tested synthetic polymers, the potentiality of polysaccharides assuming this role has never been explored. Low-methoxy amidated pectins (LMAP) constitute valuable candidates because of their biocompatibility and ionic and pH sensitivity. In this study, the potentiality of a LMAP with a degree of esterification (DE) of 30 and a degree of amidation (DA) of 19 was explored. The aim of this study was to explore the influence of LMAP microspheres within the composite on the cement properties, drug release ability and final macroporosity after microspheres degradation. Three LMAP incorporation ratios, 2\%, 4\% and 6\% w/w were tested, and ibuprofen was chosen as the model drug. In comparison with the CPC reference, the resulting composites presented reduced setting times and lowered the mechanical properties, which remained acceptable for an implantation in moderate-stress-bearing locations. Sustained release of ibuprofen was obtained on at least 45 days, and release rates were found to be controlled by the LMAP ratio, which modulated drug diffusion. After 4 months of degradation study, the resulting CPC appeared macroporous, with a maximum macroporosity of nearly 30\% for the highest LMAP incorporation ratio, and interconnectivity between pores could be observed. In conclusion, LMAP appear as interesting candidates to generate macroporous bone cements with tailored release properties and macroporosity by adjusting the pectin content within the composites.

1. Introduction

Calcium phosphate materials have gained clinical acceptance for bone substitution because of their similarity to the mineral part of bone and their recognized biocompatibility. Calcium phosphate cements (CPC) are of particular interest for treating low-load- or non-load-bearing bone defects because of their moldability and osteoconductivity. Many compositions have been reported since the first CPC was developed in 1985 \cite{1}. They all consist in a CP powder mixed with an aqueous liquid to form a paste which can conform to osseous defects with complex shapes and set \textit{in vivo}. In most of them, the final crystalline phase is hydroxyapatite \cite{2,3}.

However, two major drawbacks restrict CPC use. The first is their slow resorption rate \cite{4,5}, attributed to their microporosity, with pore sizes from submicrometer to a few micrometers \cite{6}. Several investigators have studied bone ingrowth into porous mate\-rials with different pore sizes, and the consensus seems to be that the optimal pore size is from 100 \textmu m to more than 300 \textmu m \cite{7}. The second is their poor ability as drug delivery systems: inclusion of active compounds into CPC fails to obtain controlled release in more than 1 week in the absence of specific binding affinity between the drug and calcium phosphate (see Ref. \cite{8} for a review). To overcome these two limitations, incorporating degradable polymer microparticles into CPC is an attractive option, as polymer microparticles could help to control drug release and induce macroporosity after degradation.

A few authors have already explored this way, using polymer microspheres either as porogens \cite{9–16} or as drug delivery systems \cite{17–21}. In most cases, synthetic polymers, mainly poly(lactic-co-glycolic) acid (PLGA), were tested. To the authors’ knowledge, although polysaccharides are frequently added to CPC as rheological modifiers or cohesion promoters, their ability to form microparticles has never been exploited to generate microspheres–calcium phosphate composite bone cements.

Pectin is a naturally occurring heterogeneous water-soluble polysaccharide which is found in the cell walls of most plants. It consists mainly of linearly connected $\alpha$-(1,4)-d-galacturonic acid

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monosaccharide units which may be methyl-esterified or amidated to varying extents. Low-methoxy (with a degree of esterification DE <50%) pectins (LMP) and low-methoxy amidated pectins (LMAP) can gel with an “egg-box” configuration in the presence of many divalent cations [22], allowing the formation of microspheres by ionotropic gelation, without use of organic solvents and harsh ingredients. Pectin is biocompatible, biodegradable [23] and has recently demonstrated its potential to be used in the surface modification of medical devices and materials [24,25] and, in particular, bone implant nanocoatings [26–28].

The authors previously studied the feasibility of introducing LMP or LMAP microspheres into an apatitic CPC [29]. LMP presenting various degrees of esterification (DE) and amidation (DA) were tested in terms of ability to form microspheres, resistance to ionic conditions and pH variations occurring in the cement while setting and hardening, and influence the cement chemical evolution. Pectin DE and DA appeared as key formulation parameters in composite formulation, as they controlled microsphere swelling and degradation with time and pH. Some of the LMP tested appeared suitable for the elaboration of organic–mineral composites whose drug release and degradation properties remained to be explored.

In this study, it was decided to explore the potentiality of one of these pectins: a LMAP with DE 30 and DA 19. The aim was to study the influence of pectin microspheres within the composite on the cement properties, drug release ability and final macroporosity after microsphere degradation. To do so, three LMAP incorporation ratios, 2%, 4% and 6% w/w were tested. The resulting composites were characterized in terms of setting times and mechanical properties in order to ensure their suitability for implantation. To study the composite’s release properties, ibuprofen, a non-steroidal anti-inflammatory drug, was chosen as the model drug. Drug release from the composites was followed for 45 days and compared with releases from the microspheres and CPC reference. Then the degrad-ation study was pursued for 2.5 months, and the resulting CPC studied in term of macroporosity.

2. Materials and methods

2.1. Materials

LMAP with DE 30, DA 19, and average molecular weight 228,000 was supplied by CP Kelco (Denmark). Extemporaneous CPC, Cementek™, was kindly supplied by Teknimed (France). The CPC powder consisted of tetracalcium phosphate (TTCP: Ca$_4$(PO$_4$)$_2$; 49%), α-tricalcium phosphate (α-TCP: Ca$_3$(PO$_4$)$_2$; 38%) and sodium glycerophosphate (NaGP: Na$_2$C$_3$H$_5$(OH)$_2$PO$_4$; 13%). The acidic liquid phase was prepared from lime (Ca(OH)$_2$; 38%) and sodium glycerophosphate (NaGP: Na$_2$C$_3$H$_5$(OH)$_2$PO$_4$; 13%). After mixing the two phases, with a liquid to powder weight ratio of 0.43, a succession following equations, respectively:

\[\text{drug loading} = \frac{\text{AQ}}{\text{total weight of microspheres by batch} - \text{AQ}} \times 100\]  \hspace{1cm} (1)

\[\text{encapsulation efficiency} = \left(\frac{\text{AQ}}{\text{TQ}}\right) \times 100\]  \hspace{1cm} (2)

where AQ is the actual quantity of drug present in the matrices (drug content), and TQ is the theoretical quantity of drug (initial ibuprofen loading dose during preparation of the microspheres).

2.2. Preparation of LMAP microspheres

Solutions of 3% w/v LMAP were prepared by dispersing pectin into phosphate buffer, pH 8. LMAP microspheres were produced by ionotropic gelation using an electrostatic bead generator (Inotech encapsulator IE 50 R, Switzerland) equipped with a syringe pump and a 300 μm nozzle. The pectin solutions were dropped into a solution of CaCl$_2$ 500 mM (cross-linking solution) under continuous agitation. The gelled microspheres, instantaneously formed, were allowed to cure in the cross-linking solution for 24 h. Then they were separated by filtration, washed with de-ionized water, dehydrated in a graded series of ethanol, and dried for 48 h at 37 °C. In order to test the release properties of LMAP microspheres, ibuprofen LMAP (IBU–LMAP) microspheres were also prepared according to the same protocol, with ibuprofen added within the pectin solutions at the beginning of preparation.

2.3. Characterization of LMAP microspheres

Particle size distributions were measured using a laser particle sizer (Mastersizer 2000; Malvern, UK) based on a laser light-scattering technique. Each sample was measured in triplicate. The weight average of volume distribution (D[4;3]) was used to describe the particle size.

The morphology (shape and surface) of the microspheres was analyzed by scanning electron microscopy (SEM) using a JEOL scanning electron microscope (JSM-6400F) at 15 kV. Samples were coated with silver under vacuum by a SPI sputter coating unit.

2.4. Preparation of CPC and CP–LMAP microspheres composites

The powder and liquid phase of the cement were mixed for 2 min, then the required amount of dry microspheres (ranging from 0% to 6% w/w of the final mass of the composites) was added and mixed until homogenous repartition of the microparticles within the paste was obtained. The paste was then filled into cylindrical molds and treated further according to the assays.

2.5. Characterization of CPC and CP–LMAP microspheres composites

Setting times of CPC and composites were determined into of 1.25 cm$^2$ with a TA-XT2 Texture analyzer equipped with a needle (0.7 mm in diameter). It was considered that the cement had set when the paste developed a resistance to needle penetration of over 600 g mm$^{-2}$.

The compressive strength of the composites was evaluated with a computed controlled Hounsfield series S apparatus. Tests were conducted in air, on wet specimens (10 mm in diameter and 20 mm high) after 7 days in a saturated atmosphere at 37 °C.

2.6. In vitro drug release studies

2.6.1. Drug loading and encapsulation efficiency

The drug loading and encapsulation efficiency were determined after complete degradation of LMAP microspheres in pH 8 phosphate buffer. The ibuprofen content of the microspheres was assayed by UV spectrophotometry (Perkin-Elmer, USA) at 222 nm. The determinations were performed in triplicate. The drug loading and encapsulation efficiency were calculated according to the following equations, respectively:

The ibuprofen release from microspheres, CPC and composites under in vitro conditions

Ibuprofen release was performed in SBF, pH 7.25, prepared according to Kokubo and Takadama [30]. Standards methods of release experiments in pharmacopeias are hardly suitable for multi-particulate dosage forms, due to the large volumes of the vessels; a modified alternative method, proposed by research groups work-
ing on multi-particulate dosage forms for colon delivery, was used [31]. Briefly, composites (containing IBU–LMAP microspheres at a weight ratio of 2%, 4% and 6%, leading to composites with an increasing drug loading of 2, 4 and 6 mg, respectively) or CPC specimens (accurate weight ~2.5 g) or IBU–LMAP microspheres (accurate weight ~100 mg corresponding to the weight of microspheres incorporated into CP–LMAP 4% composites) were placed in test tubes containing 15 ml of SBF, pH 7.25, at 37 °C under agitation at 100 rpm. Five milliliters of dissolution medium were collected at various time intervals up to 45 days, and the ibuprofen released was assayed spectrophotometrically (Perkin-Elmer, USA) at 222 nm. All UV measurements were performed with a double beam apparatus against a blank of SBF. The release study was continued after replacement of 5 ml of fresh buffer. Cumulated amounts released (in percentages of the initial amounts) were plotted vs time. Each in vitro release study was performed four times. All the studies were performed under sink conditions for ibuprofen.

2.7. Porosity measurements

Samples that had been immersed for 4 months in SBF, pH 7.25, were used to measure the final density and porosity of the composites. Results were obtained using the method previously described by Xu et al. [32]. Briefly, the specimens were polished and dried until their mass remained stable. The density of the materials was measured using the specimen mass divided by its volume, which was calculated by the specimen dimensions measured with a micrometer. Xu showed that this method yielded a density which closely matched values measured by a mercury intrusion method. Four specimens were measured for each material. Composites consisted of intrinsic microporosity and additional macroporosity from microsphere degradation. The total porosity \( P_{\text{total}} \) of the specimen can be obtained by

\[
P_{\text{total}} = \frac{d_{\text{HA}} - d_{\text{measured}}}{d_{\text{HA}}} \tag{3}
\]

where \( d_{\text{HA}} \) is the density of a fully dense hydroxyapatite which is \( 3.14 \text{ g cm}^{-3} \), and \( d_{\text{measured}} \) is the measured density at a specific LMAP mass fraction. The macro pore volume fraction from LMAP microsphere degradation \( P_{\text{pectin microspheres}} \) can be obtained by

\[
P_{\text{pectin microspheres}} = 1 - \frac{d_{\text{measured}}}{d_{\text{measured}}-0\%} \tag{4}
\]

where \( d_{\text{measured}} \) is the measured density of a specimen with a specific LMAP content and \( d_{\text{measured}-0\%} \) is the measured density of CPC reference with 0% LMAP.

2.8. Statistical analysis

Results are expressed as means and standard deviations of at least five experiments. ANOVA one-way analysis was performed to detect significant effects of material composition on the setting time or mechanical properties of the resulting composites. Tukey’s comparison test was used to compare the data. Probability values \( p \leq 0.05 \) were considered significant.

3. Results and discussion

3.1. LMAP microspheres preparation and characterization

Based on previous results, a LMAP with a DE of 30 and a DA of 19 was chosen because its microspheres maintained their multiparticulate structure under pH variations mimicking those occurring in the cement while setting and hardening, and showed moderate swelling ability, considered of good prognostic in obtaining further sustained drug delivery and controlled erosion/degradation with time and pH [29].

As a first step, LMAP microspheres alone or loaded with ibuprofen (IBU–LMAP) were prepared by ionotropic gelation in the presence of calcium ions. Gelled microspheres were obtained instantaneously when LMAP solutions were dropped into a calcium bath. Intermolecular cross-links were formed between the negatively charged carboxyl groups of LMAP and the positively charged counter-ions, as previously described by Grant et al. [22] in the “egg-box model”. After drying, the resulting microspheres were characterized in terms of size distribution and morphology, with the aim of investigating the potential influence of the addition of ibuprofen. Loaded and unloaded LMAP microspheres with a weight average of volume distribution \( D(4;3) \) of 280 μm and 265 μm, respectively, were obtained, with monomodal and narrow particle size distribution (polydispersity index 0.69 and 0.68, respectively). The drug presence did not show a significant influence on the particle size distributions. The microspheres appeared very similar, spherical to ovoid with a smooth surface, when observed by SEM (Fig. 1). In the case of IBU–LMAP microspheres, no crystals were observed at the particles’ periphery, showing that ibuprofen was completely encapsulated within the microsphere structure and not only adsorbed on their surface.

3.2. Physico-chemical characterization of CP–LMAP microspheres composites

LMAP microspheres from 2% to 6% w/w were incorporated into extraporous CPC. Previous formulation tests have shown that those incorporation ratios allowed composites with maintained malleability and cohesion to be obtained. The influence of LMAP weight ratio on the setting and mechanical properties of the composites was studied.

Fig. 2 displays the setting times of the composites according to their LMAP microsphere weight ratio. One-way ANOVA identified significant effects of LMAP incorporation ratios of 4% w/w or higher.
Fig. 2. Setting time of CP–LMAP composites as a function of LMAP incorporation ratio; comparison with CPC reference. The same number of asterisks above the histograms indicates values that are not significantly different (Tukey’s multiple comparison test at \(p = 0.05\)). The setting times of composites containing 4% and 6% LMAP (CP–LMAP4% and CP–LMAP6%) were significantly lower than that of the CPC control, while composites with 2% LMAP microspheres (CP–LMAP2%) showed no statistical difference with CPC control (22.3 ± 0.8 min). The setting times of CP–LMAP4% and CP–LMAP6% (11.7 ± 2.9 min and 10.9 ± 4.1 min, respectively) were not significantly different from each other, but CP–LMAP6% composites presented higher standard deviations due to their higher heterogeneity. However, whatever their LMAP content, the setting times of the composites remained compatible with a surgical implantation. The shortening of composite setting times for LMAP ratios >2% w/w can be attributed to LMAP microspheres’ swelling properties, as they were added in a dry state to the extemporeous CPC. It can be assumed that part of the liquid phase of the cement was absorbed by the microspheres, lowering the amount of liquid available for CPC setting, and hence its setting time. Nonetheless, SEM observations of the composites 1 h after preparation evidenced the presence of leaflet-shaped crystals characteristic of the formation of brushite (dicalcium phosphate dihydrate) in all of them, clearly showing that LMAP microspheres did not hamper the entanglement of calcium phosphate crystals and the setting reaction. This was confirmed by X-ray diffraction analysis (data not shown).

Fig. 3 plots the compressive strength vs composites’ LMAP content. One-way ANOVA identified the significant effects of LMAP ratios (\(p < 0.01\)). For each LMAP ratio, the strength of the CP–LMAP composite was significantly lower than that of the CPC control (5.78 ± 0.12 MPa; \(p < 0.05\)). The strength of CP–LMAP4% and CP–LMAP6% (3.53 ± 0.47 MPa and 2.40 ± 0.90 MPa, respectively) were not significantly different from each other, but significantly lower than the strength of CP–LMAP2%. These results are not surprising, as previous studies on the mechanical strength of CP–polymer (PLGA) microsphere composite scaffolds in vitro showed that the initial strengths of the composite scaffolds were significantly lower than for CPC alone [20]. This is not problematic, as CPC use is limited to low-stress-bearing locations [2]. Nonetheless, the CP–LMAP composites’ strength values still overlap the compressive strength of 2–12 MPa reported for cancellous bone [33]. Moreover, the initial decrease in mechanical strength of CP–polymer microspheres composites should be compensated with implantation time in vivo by bone ingrowth into the composites [11].

The results showed a significant influence of pectin incorporation ratio on the composites properties. However, in the range of LMAP ratios tested, the composites obtained presented setting times, chemical evolutions and mechanical properties compatible with surgical implantation. No significant differences were observed between ibuprofen-loaded and unloaded CP–LMAP composites.

3.3. Ibuprofen release from CP–LMAP microspheres composites under in vitro conditions; comparison with LMAP microspheres and CPC control

The composites’ in vitro drug release properties were evaluated for 45 days in SBF, pH 7.25, at 37 °C, in order to mimic the ionic conditions encountered when implanted in vivo [30]. Ibuprofen was chosen as the model drug because of its hydrophobicity and higher solubility at basic pH (it is a weak acid with \(pK_a\) 4.5), allowing study of the release of a drug able to diffuse freely out of the composites without presenting specific interactions with CP or LMAP. In the operating conditions, an encapsulation efficiency of 77% and a drug loading of 4% of ibuprofen into LMAP microspheres were obtained. Obtaining better entrapment efficiency was not the purpose of the study, but it could be optimized by playing with the counter-ion type and concentration, with the pH of the cross-linking baths and with the composition of the rinsing solutions before drying [34]. Dry IBU–LMAP microspheres were incorporated into CPC at weight ratios of 2%, 4% and 6%, leading to composites with an increasing drug loading. Release patterns were presented by plotting the cumulated percentage of ibuprofen released vs time.

Fig. 4 displays the release profiles of ibuprofen according to the LMAP microspheres ratio in the composites. They were almost identical in shape, presenting a very limited initial burst in the initial 24 h (see Table 1), followed by slow and sustained drug release. The release data were simulated using Higuchi theory, which investigated whether the ibuprofen cumulative release percentages from composites were proportional to the square root of time. They correlated well with this model, as shown in Table 1 by the coefficient of determination of the linear plots obtained, demonstrating that ibuprofen was released by Fickian diffusion from all the composites. But surprisingly, different release rates were obtained according to the LMAP microspheres ratio into the composites, in the order CP/IBU–LMAP2% > CP/IBU–LMAP4% > CP/IBU–LMAP6%. Differences in cumulated ibuprofen release became more apparent as time progressed towards the end of the in vitro study,
clearly showing that the IBU–LMAP content significantly affected drug release.

Two hypotheses can be proposed to explain this phenomenon. First, even if perfect sink conditions were maintained throughout the experiments, the confinement of the drug within the composites must be taken in account. Locally, the drug concentration might have exceeded its solubility and slowed its diffusion out of the cement matrix. But another possible explanation is that LMAP controlled drug release within the composites. Such phenomenon can be related to what was observed by Weir and Takechi when they directly added polysaccharides, namely alginate [35] or chitosan [36,37], into CPC. They established that the release rate of the drug was slowed proportionally to the amount of polysaccharide present in the cement, at least at the beginning of their release studies. They attributed this slowing down to the gel-forming abilities of the polysaccharides which could block some of the intrinsic pores in CPC, thus reducing the porosity responsible for drug release. LMAP microspheres could have operated according to the same mechanism and regulated the diffusion of ibuprofen out of the composites.

In order to confirm this, releases of the same amount of ibuprofen from a CP–LMAP composite, a CPC and LMAP microspheres were compared under the same experimental conditions. The results are presented in Fig. 5. While CPC released 100% ibuprofen within 48 h, clearly showing that there is no interaction between the cement and the drug, composite and microsphere release patterns presented the same shape, demonstrating that LMAP microspheres controlled ibuprofen release from composites. Such sustained release can be explained by LMAP microspheres’ sensitivity to ionic conditions. They behave as hydrophilic matrices whose release ability is currently related to their swelling ability in dissolution media [34]. The presence of calcium in the LMAP surrounding (in SBF medium and CPC in this case) has been shown to enhance cross-linking and aggregation of the pectin chains [38], thus limiting swelling patterns and, subsequently, drug release from microspheres and composites, reducing the release rate.

3.4. Porosity measurements of composites

Specimens that had been immersed into SBF, pH 7.25, were used to measure the porosity of CP–LMAP composites using Eqs. (3) and (4). The values obtained are reported in Table 2. All formulations maintained their integrity during the study. LMAP microspheres composites, the release rate of ibuprofen was even more slowed. This can be attributed to additional drug diffusion through the cement pores partially blocked by LMAP microspheres.

<table>
<thead>
<tr>
<th>LMAP weight fraction (%)</th>
<th>Density (g cm⁻³)</th>
<th>Total pore volume fraction (%)</th>
<th>Macropore volume fraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.57 ± 0.04</td>
<td>50.1 ± 1.4</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1.39 ± 0.03</td>
<td>55.8 ± 0.9</td>
<td>11.5 ± 1.9</td>
</tr>
<tr>
<td>4</td>
<td>1.20 ± 0.04</td>
<td>61.6 ± 1.3</td>
<td>23.1 ± 2.7</td>
</tr>
<tr>
<td>6</td>
<td>1.11 ± 0.06</td>
<td>64.7 ± 1.8</td>
<td>29.2 ± 3.6</td>
</tr>
</tbody>
</table>

Table 1

Kinetic analysis of ibuprofen release from CP–LMAP microspheres composites and LMAP microspheres.

<table>
<thead>
<tr>
<th></th>
<th>Cumulated ibuprofen released within the first 24 h (%)</th>
<th>Cumulated ibuprofen released after 45 days (%)</th>
<th>Higuchi model coefficient of determination r² (%)</th>
<th>Higuchi dissolution constant k₀ (10⁻² h⁻¹/²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP/IBU–LMAP2%</td>
<td>11.9 ± 0.2</td>
<td>69.1 ± 5.5</td>
<td>99.0</td>
<td>2.2</td>
</tr>
<tr>
<td>CP/IBU–LMAP4%</td>
<td>9.1 ± 0.5</td>
<td>47.4 ± 3.0</td>
<td>99.5</td>
<td>1.5</td>
</tr>
<tr>
<td>CP/IBU–LMAP6%</td>
<td>6.7 ± 0.9</td>
<td>31.8 ± 2.9</td>
<td>98.9</td>
<td>1.0</td>
</tr>
<tr>
<td>IBU–LMAP microspheres</td>
<td>14.6 ± 1.1</td>
<td>61.9 ± 1.9</td>
<td>99.4</td>
<td>1.8</td>
</tr>
</tbody>
</table>
non-negligible parameters which can affect the threshold for interconnectivity and hence cement colonization [15]. Moreover, CP–pectin composite formulation could easily be optimized by modifying the pectin type. Choosing a pectin presenting lower swelling properties, i.e. with decreased DE and/or DA, would permit to enhance the pectin mass fraction in the composites, and thus final macroporosity, but would also result in reduced drug release ability and longer microsphere degradation time. A compromise must be found to adjust on-demand composite properties in terms of delivery and final macroporosity.

Fig. 6 displays SEM micrographs of the inner surface of broken composites (Fig. 6B–D) to compare with the CPC control (Fig. 6A). Cross sections suggest a homogeneous distribution of microparticles throughout the scaffolds in all formulations. Whereas on the CPC control only micropores can be seen, well-formed macropores with the shape of the entrapped microspheres can be observed within the composites. The macropores created present sizes suitable for promoting cell infiltration and bone ingrowth [7]. In CP–LMAP2% composites, these macropores appear neighbouring but separated, whereas for CP–LMAP4% and CP–LMAP6%, some interconnections can be observed between the macropores. Fig. 6C is a micrograph of a typical CP–LMAP4% composite; an arrow indicates an open connection at the bottom of a macropore. Moreover, as can be seen in Fig. 6D (CP–LMAP6% composites), pectin microsphere swelling induced microcracks within the CP cement structure, generating additional interconnections between macropores, indicated by arrows.

Although the macropores formed by degradation of the pectin microspheres did not form a fully interconnected network in the case of the lowest LMAP ratios, tissue ingrowth throughout the scaffold should remain possible, as the microporosity and even nanoporosity of CPC also play an important role over small distances, as shown by Ruhe et al. [12] during his study on PLGA microspheres–CPC composites. As a consequence, the macropores induced by LMAP microspheres degradation are expected to enhance the composite’s resorption rate in comparison with the control CPC. The exact gain has to be evaluated by an in vivo test, but the first results presented in this study appear promising.

4. Conclusion

To our knowledge, no previous studies concerning CP–pectin microspheres composite materials have been published. The incorporation of LMA pectin microspheres within a CPC led to organic–mineral composites with original properties, suitable for implantation in moderate-stress-bearing locations. In term of drug delivery, sustained release of ibuprofen was obtained on more than 45 days, and the LMAP ratio appeared to be a key parameter regulating drug diffusion. By adjusting their pectin microsphere content it should be possible to design tailorable composites whose release rates could be controlled upon demand. With time, the microspheres progressively degraded, creating a macroporous CPC scaffold implant expected to present an enhanced resorption rate and faster cell colonization. The results of this first in vitro evaluation warrant further investigation of the material’s in vivo behavior. Taking in account the potentials of these composites for sustained drug delivery and induced macroporosity, CP–LMAP composites appear to be a promising scaffolding material for bone regeneration and bone tissue engineering, with the advantage over CP–PLGA composites that no acidic degradation products were generated.

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