JOURNAL OF TISSUE ENGINEERING AND REGENERATIVE MEDICINE
J Tissue Eng Regen Med (2015)
Published online in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/term.2066

RESEARCH ARTICLE

Development and dissolution studies of bisphosphonate (clodronate)-containing hydroxyapatite–polylactic acid biocomposites for slow drug delivery

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Abstract

An increase in clinical demand on the controlled release of bisphosphonates (BPs) due to complications associated with systemic administration, has been the current driving force on the development of BP drug-release systems. Bisphosphonates have the ability to bind to divalent metal ions, such as Ca^{2+}, in bone mineral and prevent bone resorption by influencing the apoptosis of osteoclasts. Localized delivery using biodegradable materials, such as polylactic acid (PLA) and hydroxyapatite (HAp), which are ideal in this approach, have been used in this study to investigate the dissolution of clodronate (non-nitrogen-containing bisphosphonate) in a new release system. The effects of coral structure-derived HAp and the release kinetics of the composites were evaluated. The release kinetics of clodronate from PLA–BP and PLA–HAp–BP systems seemed to follow the power law model described by Korsmeyer–Peppas. Drug release was quantified by ^{31}P-NMR with detection and quantification limits of 9.2 and 30.7 mM, respectively. The results suggest that these biocomposite systems could be tuned to release clodronate for both relatively short and prolonged period of time. In addition to drug delivery, the degradation of HAp supplies both Ca^{2+} and phosphate ions that can help in bone mineralization. Copyright © 2015 John Wiley & Sons, Ltd.

Received 16 January 2015; Revised 12 May 2015; Accepted 12 June 2015

Keywords **drug release; thin film composites; coral; hydroxyapatite; ^{31}P-NMR; quantification**

1. Introduction

Bisphosphonates (BPs) have the ability to bind to divalent metal ions in bone mineral and prevent bone resorption by influencing apoptosis in osteoclasts (Weinstein *et al*., 2009). BPs are characterized by the structure P–C–P, in which germinal bisphosphonates share the same carbon atom; they are analogues of pyrophosphates (P–O–P), in which the central oxygen atom is replaced by a carbon atom, to render them resistant to enzymatic hydrolysis. In addition, two side-groups or chains normally attach to the central carbon atom, resulting in a number of bisphosphonate derivatives with varying potencies in terms of their antiresorptive activity (Shinoda *et al*., 1983). The clinical uses of bisphosphonates include the prevention and treatment of diseases related to osteoclast-mediated bone resorption, including tumour-associated osteolysis, bone metastasis, primary and secondary hyperparathyroidism and osteoporosis (Kanis *et al*., 1996). Nitrogen-containing bisphosphonates (NBPs) have been first-choice drugs for the clinical treatment of diseases involving increased bone resorption, because of their greater effect than non-nitrogen-containing bisphosphonates (non-NBPs). It has been reported that NBPs, among many side-effects, might also cause osteonecrosis of the jaw in people with a history of oral and/or maxillofacial surgery, periodontal surgery or endodontic therapy (Capsoni *et al*., 2006; Heng *et al*., 2012;

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Ruggiero *et al*., 2004). Kim *et al*. (2013) suggested that etidronate and clodronate, which are non-NBPs, apart from their anti-bone resorptive effect may also exert an analgesic effect. It has been shown that clodronate does not impair the mineralization of bone even at high doses, making it a safe drug for long-term use in osteoporosis treatment (Kanis *et al*., 1996).

Oral and parenteral are routes conventionally used for the administration of BPs. Adverse events have been reported to be associated with both oral and intravenous NBPs (alendronate, risedronate, ibandronate, pamidronate, ibandronate and zoledronic acid), including in the upper gastrointestinal tract, acute phase response, hypocalcaemia and secondary hyperparathyroidism, musculoskeletal pain and ocular events (Papapetrou, 2009). The molecular mechanism of action of BPs has been reviewed by Roelofs *et al*. (2006).

The effect of oral clodronate (non-NBP) on bone mass, bone turnover and subsequent metastases was studied by McCloskey *et al*. (2010). It has been stated that oral administration of clodronate is associated with poor drug bioavailability (<5%) and poor gastrointestinal tolerability, due to its high water solubility and extensive ionization. In addition, it is known that it penetrates biological membranes poorly, which leads to poor absorption from the gastrointestinal tract (Lin, 1996). One way to overcome this problem is to increase drug availability through the use of high doses. However, high bisphosphonate doses (e.g. 1500 mg) can cause severe renal toxicity unless infused slowly over many hours (Conte and Guarneri, 2004).

Many researchers have developed different strategies to administer BPs locally, in order to avoid the complications associated with systemic methods. Chou *et al*. (2009) reported a β-TCP microsphere and bisphosphonate system for drug delivery. BP showed high affinity and bonding to the calcium phosphate drug-delivery microspheres. Oliveira *et al*. (2010) successfully incorporated clodronate at different doses in calcium phosphate (CaP) coatings produced on starch-based degradable composites. Other researchers have developed BP delivery devices based on CaP compounds of various compositions (Cattalini *et al*., 2012; Denissen *et al*., 1994; Josse *et al*., 2005). The devices have been shown to have the ability to release BPs, resulting in the inhibition of osteoclastic activity and consequent bone resorption. Recently it has been proposed that BPs are adsorbed on an apatitic layer via an ion-exchange reaction between phosphate species on the apatitic surface and BP molecules in solution (Pascaud *et al*., 2014).

In the past, a number of delivery systems using clodronate have been attempted. Nanoparticles loaded with clodronate have been shown to mitigate murine experimental colitis (Niebel *et al*., 2012). Monkkonen *et al*. (1994) encapsulated clodronate with liposomes and used them to inhibit the growth of macrophages. Jordan *et al*. (2003) suggested that liposomal clodronate therapy may act like a temporary, medicinal splenectomy in situations where rapid response to therapy is critical or other medical therapies are inadequate.

Currently, there is little or no information on the delivery of clodronate from biodegradable thin film composites. Biodegradable thin films have the potential of easy applicability and conformity to use on internal and external surfaces, which makes them clinically attractive medical materials. In addition, the release rate can be easily controlled with relatively small changes in terminal acidic functional groups or molecular weight to produce a wide range of drug-release rates (Steele *et al*., 2013). Ease of formation of biocomposites aids in control of the dissolution of particles and allows us to form and apply a range of release rates. Researchers have attempted to develop different assays for BP detection and quantification. Kosonen (1992) determined disodium clodronate by ion chromatography with post-column derivatization. Xie *et al*. (2006) successfully analysed four bisphosphonates by reverse liquid chromatography with evaporative light-scattering detection. Other techniques, such as capillary electrophoresis for alendronate (Su *et al*., 2012; Tsai *et al*., 1992) and the use of an inductively coupled plasma (ICP) for specific phosphorus detection for etidronate disodium (Reed *et al*., 1995), have also been reported and suggested to have results comparable to those obtained by more complex derivatization procedures. The need for a quick and simple method to determine BP amounts is required to address the complications associated with most techniques developed so far. Direct UV detection of clodronate and other non-nitrogen-containing bisphosphonates is not possible, due to the absence of chromophores in their structures. Alternatively, derivatization of non-chromophoric BPs, either pre- or postcolumn, is normally used for UV detection. However, these techniques require extensive sample preparation and/or specialized equipment.

This research is directed to the development of polylactic acid (PLA) thin film composites containing calcium phosphate (hydroxyapatite) microspheres converted from calcium carbonate (coral) as clodronate delivery devices. The drug-release kinetics of these thin film composites were inverstigated using ^{31}P-NMR as the detection and quantification technique.

2. Materials and methods

Chemicals used in this research were obtained from Sigma Aldrich (Castle Hill, Australia). Corals and marine structures were obtained from Queensland and Western Australia.

2.1. Drug loading and film composites

Thin film composites were prepared by the solvent casting technique described in previous publications (Buzarovska and Grozdanov, 2012; Macha *et al*., 2014). Briefly, plain

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PLA films were prepared by dissolving 0.50 g polymer in 30 ml chloroform at room temperature under magnetic stirrign until it was completely dissolved. Then the solution was cast into a Petri dish and chloroform was allowed to evaporate in a vacuum desiccator for 48 h. The films were then stored in desiccators for further analyses. The coral samples were crushed then cleaned with 2% v/v NaClO, and then ground within an aluminium oxide ball mill (46 rpm, 2 h), sieved with a 100 μm sieve, cleaned with 2% v/v NaClO and then dried at 100°C for 2 h before use. Hydrothermal conversion was carried out following Ben-Nissan procedures (Ben-Nissan, 2003; Ben-Nissan and Green, 2014). Briefly, coral powder was mixed with the required amount of diammonium hydrogen phosphate $[(NH_4)_2HPO_4]$ to obtain HAp and then converted in a Parr reactor at 250°C and 8.0 MPa pressure. Coral has been characterized previously (Ben-Nissan *et al*., 2004; Cegla *et al*., 2014; Chou *et al*., 2011; McCutcheon *et al*., 2004). Hydrothermally converted coralline hydroxyapatite was loaded with 25% w/w clodronate by dissolving the required amount of clodronate in purified 18 MilliQ water (Millipore), mixed with HAp, sonicated and then allowed to dry in a Rotavapor R-210 coupled with a vacuum controller (V-850, BUCHI, In Vitro Technologies, Australia) at 60°C. Clodronate-loaded HAp was then dried in vacuumed desiccators and then used to produce thin film composites. Drug loading to the PLA films was performed by adding the required amount of clodronate or clodronate-loaded HAp into PLA–chloroform solution to give 10% drug in the composites, under magnetic stirring, followed by sonication to break down the agglomerations of particles, then cast into Petri dishes and dried in a desiccator under vacuum. The experimental samples used were designated as follows: PLA, polylactic acid film; PLABP, PLA film loaded with bisphosphonate (throughout this paper, clodronate is designated as BP); PLAHAp, PLA–hydroxyapatite composite film; and PLAHApBP, composite film of bisphosphonate-loaded hydroxyapatite with PLA. Confirmation of drug purity after loading into a PLA matrix was checked by running Fourier transform infrared spectroscopy (FTIR) of BP and PLABP films; a spectrum in the range 400–40000 cm^{-1} was obtained using FTIR (Nicolet Magna 6700 FTIR spectrometer) for the product samples. BP was ground in an agate mortar and thoroughly mixed with KBr (FTIR grade). For the analyses, 3 mg was ground with 300 mg of KBr and pressed into a pellet (Carver press).

2.2. Specific surface area and porosity

Specific surface areas were measured in a Tristar II apparatus from Micromeritics. Samples were initially degassed under vacuum (16 MPa) at 60°C for 15 h before analysis. Analysis was performed using the static method at –196.15°C, with nitrogen as the adsorbent. The Brunauer–Emmett–Teller (BET) method was applied to calculate the total surface area. Porosity measurements were carried out using a mercury intrusion porosimeter (Autopore III, Micromeritics Instruments Inc., Norcross, GA, USA) with a 5 cm^3 powder penetrometer.

2.3. Drug encapsulation efficiency

Drug encapsulation efficiency (DEE) was determined by soaking 2 mg samples in 2 ml distilled water at 37°C for 5 min, and then the drug concentration of the solution was measured using UV-Vis. This process dissolves drugs that are on the outer surface of the films as the adsorbed drug (Weinstein *et al*., 2009). The efficiency was determined using equation (1):

$$DDE = \frac{W_{df} - W_{ds}}{W_{df}} \quad (1)$$

where W_{df} is the weight of drug in the film and W_{ds} is the weight of drug dissolved in solution.

2.4. Drug efficiency (DE)

The dissolution efficiency was evaluated and used to compare the thin film composites. According to Khan (1975), the DE of a pharmaceutical dosage form is defined as the area under the dissolution curve up to a certain time, *t*, expressed as a percentage of the area of the rectangle described by 100% dissolution at the same time. DE can be calculated using equation (2):

$$D.E. = \frac{\int_0^t y.dt}{y100^{.t}}.100\% \quad (2)$$

where y is the drug percentage dissolved at time t and y_{100}, the amount corresponding to the 100% dissolution.

2.5. Drug release and quantification

A drug-release study of PLA thin film composites was conducted in the volume of dissolution medium enough to provide complete dissolution of the expected amount of drug present in the samples (SINK) conditions in Tris–HCl buffer (0.1 M, pH 7.4) at 37 ± 0.1°C in a temperature-controlled waterbath shaker running at a constant speed of 100 rpm. Sampling was conducted every week and each prescribed sampling time had its own independent sample in which the experiment was terminated afterwards. The quantification of released clodronate was performed using ^{31}P-NMR. ^{31}P-NMR spectra were recorded on an Agilent 500 MHz NMR magnetic apparatus (Agilent Technologies, Australia) integrated with a Varian NMR system with a broadband probe, ø 5 mm, equipped with manual tuning and matching. All spectra were recorded using the standard phosphorus parameters: decoupling with 90° pulses, number of scans 1024 and acquisition time 0.668 s, with relaxation delay of 1 s, no rotation, and temperature 24°C. The measuring time for one spectrum was 28 min.

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Tuning, locking of magnetic field to prevent drifting during NMR experiment and shimming to achieve optimal and homogeneous magnetic field for high resolution NMR spectrum on aqueous dispersions were performed on triphenylphosphate in deuterochloroform $CDCl_3$. Data were processed using VnmrJ software v. 4.0. In order to compare dissolution kinetics of the two thin films, the difference (f1) and similarity (f2) factors were used (Costa and Sousa Lobo, 2001). Two dissolution profiles are considered as similar when f1 is not > 15 and f2 varies from 50 to 100. In order to determine the release mechanism, dissolution data obtained for PLABP release were fitted to zero-order, first-order, Higuchi, Hixson, Hixson–Crowell and Korsmeyer–Peppas models.

A standard curve of five points of clodronate, with concentrations in the range 50–300 ppm, was prepared using deuterated aqueous solution of 2 mg/ml KH_2PO_4 in 100% Deuterium oxide (D_2O). A standard curve was constructed using the ratio of signal integrals BP:KH_2PO_4 as a function of clodronate concentration, which revealed a linear relationship. Samples from the release study were collected every week, the films were separated and the solutions were concentrated at 45°C for 48 h. The resulting solids were dissolved in 1 ml deuterated aqueous solution containing 2 mg/ml KH_2PO_4 in 100% D_2O, and then a volume of 0.6 ml was transferred into economy NMR tubes for analysis. Integrals were obtained using the automatic integral function in the NMR software.

2.6. Morphology

The morphology of samples used in the drug-release study were analysed by scanning electron microscopy (SEM; ZEISS Supra55VP, Zeiss, Germany). All samples were fixed by mutually conductive adhesive tape on aluminium stubs and coated with a thin layer of carbon, using a sputter-coater. Images were taken at various magnifications at an acceleration voltage of 20 kV.

2.7. Validation of the procedure

The guidelines on the validation of analytical procedures [Q2(R1)], developed by the International Conference on Harmonization (ICH), was used to determine the limits of quantification and detection, LQ and LD, respectively (ICH, 2005). Briefly, standard samples containing concentrations in the range 25–100 ppm were subjected to ^{31}P-NMR quantification and the standard deviation (SD) of y intercepts of regression lines was used as σ in equations (3) and (4) to determine DL and QL, respectively:

$$DL = \frac{3.3\sigma}{S} \quad (3)$$

$$QL = \frac{10\sigma}{S} \quad (4)$$

where σ is the SD of the response and S is the slope of the calibration curve.

Repeatability was determined by subjecting the same sample with 250 ppm clodronate in 2 mg/ml KH_2PO_4 solution to NMR determination six times within 2 months.

3. Results

3.1. Detection and quantification limit, repeatability, encapsulation efficiency, BET surface area, porosity and dissolution efficiency

In this study it was found that the detection (DL) and quantification (QL) limits for clodronate were 9.2 and 30.7 mM, respectively. Repeatability was measured in terms of SD and relative SD and was found to be within ± 12.9 mM and ± 5%, respectively. Encapsulation efficiency was found to be 73.3% for polylactic acid–clodronate (PLABP) and 94.8% for polylactic acid–clodronate containing hydroxyapatite (PLAHApBP), respectively. The BET surface area of the HAp particles was determined to be 3.23 m^2/g. The porosity of HAp particles was determined to be 58.15%. Dissolution efficiencies for PLABP and PLAHApBP were found to be 40.4% and 19.8%, respectively.

Controlled release of clodronate from PLA thin films and composites was performed for 11 weeks under SINK conditions. Figure 1 shows the detection of clodronate in deuterated aqueous solution containing 2 mg/ml KH_2PO_4 in 100% D_2O. The result shows good separation between the clodronate and standard (KH_2PO_4) peaks. The ratios of the clodronate integral to that of standard were used to construct a standard curve (Figure 2), which was then used to determine the concentrations of clodronate released from the films. The cumulative drug release was quantified by ^{31}P-NMR and presented in Figure 3.

The release study of BP from thin films shows kinetic profiles characteristic of a sustained release. However, the profiles show a substantial difference, since the whole quantity of BP was totally dissolved from the PLA film in 4 weeks, whereas only 44% of the drug was released in the same time period when BP was loaded onto HA particles before being introduced into a PLA matrix.

This difference was confirmed by the (f1) and similarity (f2) factors obtained, 49 and 18 respectively, indicating that adsorbing BP onto the surface of HA before introduction to the PLA film induces a modification of the drug-release behaviour. Thus, when BP is loaded onto HA particles, it allows a significant decrease of the initial burst effect and a decrease of the release rate, therefore prolonging the release period and permitting the extension of the therapeutic efficacy of the material.

To enable a better understanding of the phenomena occurring during drug release, we used mathematical models to allow a quantitative interpretation of the dissolution profiles and thus describe the mechanisms that govern the dissolution. In the case of PLABP, we chose some mathematical models adapted to film forms (zero-order, first-order, Higuchi, Hixson–Crowell and Korsmeyer–Peppas) to describe the drug dissolution. Only the first

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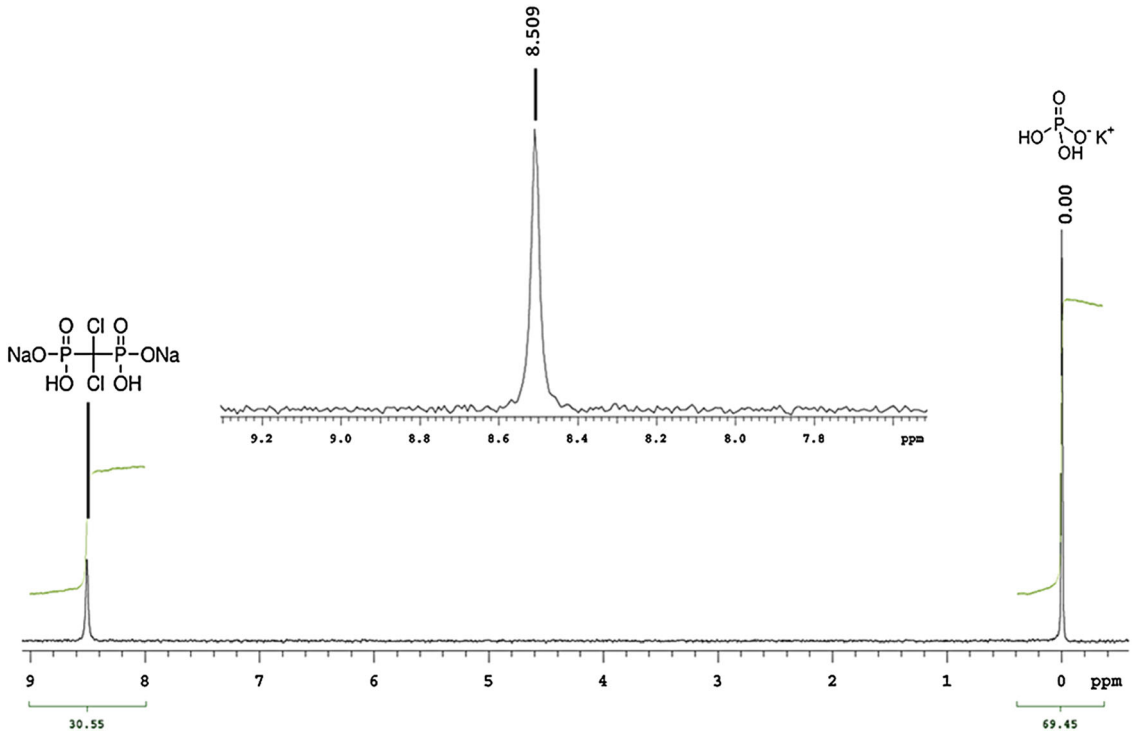


Figure 1. ^{31}P-NMR results of clodronate and KH_2PO_4 in D_2O; (inset) magnified area, 8–9 ppm

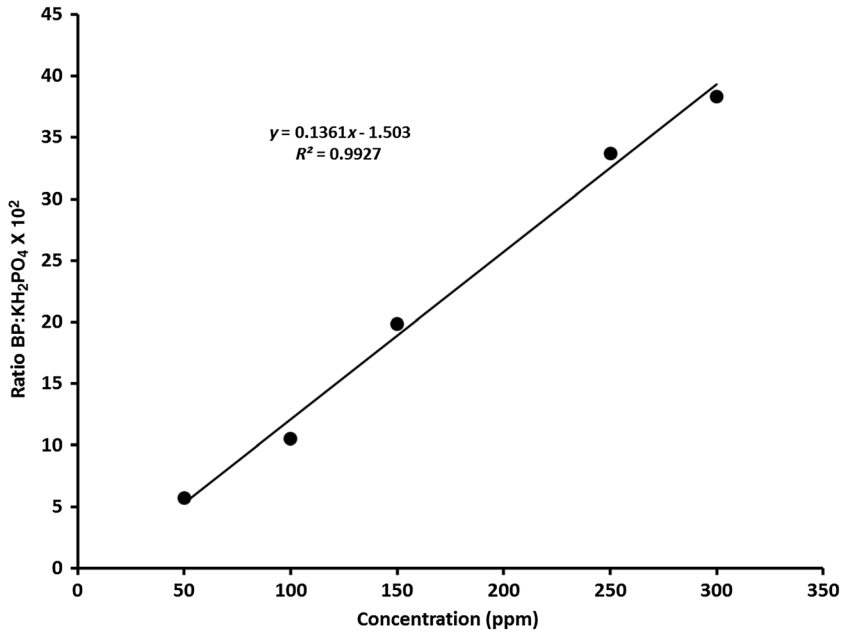


Figure 2. Standard curve of integral ratios of clodronate/KH_2PO_4 against clodronate concentration, revealing linearity of $R^2 = 0.99$

weeks were considered, as the rest of the curve corresponded to the plateau. Dissolution data closely fitted to the zero-order equation ($r^2 = 0.997$), which is usually used to describe the drug dissolution from transdermal systems and corresponds to a kinetic with the same amount of drug dissolved/unit of time (in this case, 24.3 μg BP/day).

In the case of PLAHApBP, drug is previously adsorbed on mineral particles embedded in the PLA thin matrix film. Pascaud *et al*. (2012) have highlighted the strong affinity of BP molecules for the nanocrystalline apatites and have described the adsorption phenomena that occur at the surfaces of apatite crystals. Thus, BP molecules in contact with apatite crystals are exchanged with phosphate ions located in the reactive labile apatitic layer, and cannot be spontaneously released without another anionic exchange or without dissolution of crystals. Unlike the kinetic release of BP dispersed into PLA (PLABP), which is constituted by two clearly defined portions (the first

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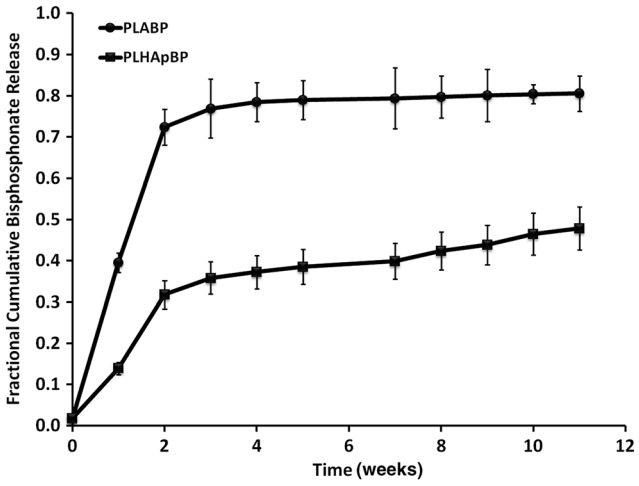


Figure 3. Fractional cumulative release of clodronate from PLA thin film composite in Tris–HCl buffer solution, pH 7.4, at 37°C and 100 rpm for 11 weeks. Error bars are mean ± SD of replicate experimental data

constituting zero-order drug release and the other corresponding to the plateau, i.e. when the drug is totally dissolved).

On the other hand, the release kinetics of BP contained in the composite film (PLAHApBP) seems to present three separate linear parts. Considering the fact that each portion is linear, we were able to determine the daily dose released for each part of the kinetics. The first part corresponds to the first 2 weeks; during this period, the daily drug released is quite important and is equal to 10.59 μg/day ($r^2 = 0.995$). The second part of the curve corresponds to the release that occurs during weeks 3–5; the slope of the curve is greatly reduced and the daily dose then released is 0.91 μg/day ($r^2 = 0.999$). The third part of the release kinetics occurs during weeks 7–11; there was a noticeable increase of the release rate, with the daily dose released equal to 1.33 μg/day ($r^2 = 0.990$). Finally, 60% of the drug contained into the composite is released after 11 weeks, which allows considering a release, and therefore a local therapeutic action, over several months.

3.2. Morphological study of drug release samples

The morphologies of samples after 7 weeks of release were compared with samples before release, as shown in Figure 4. It is shown that, for both drug–PLA and drug-loaded HAp-PLA matrix, an agglomeration of the drug particles exists as 100 nm–2 μm sizes at time 0. The results show the evidence of PLA matrix degradation as a function of time. The presence of clodronate in the films seems to affect the degradation rate of the matrix. Normally, degradation depends on the rate of water absorption into the polymeric matrix. Clodronate has extensive ionization, which makes PLABP more hydrophilic than PLAHApBP and PLAHAp. Water diffused into the polymeric matrix affects both polymer degradation, as it is simultaneously consumed through hydrolysis of the matrix, and the drug-release rate, as it dissolves the drug within the matrix.

Clodronate loaded in HAp is located on the surface, and also in nano- and mesopores of the particles (McCutcheon *et al.*, 2004). There is also some clodronate dispersed in the matrix alone, which degrades as in PLABP. As the amount of clodronate not bound to HAp is much less than for PLABP, PLAHApBP is relatively less hydrophilic and is observed to degrade least. Based on these results, it is observed that

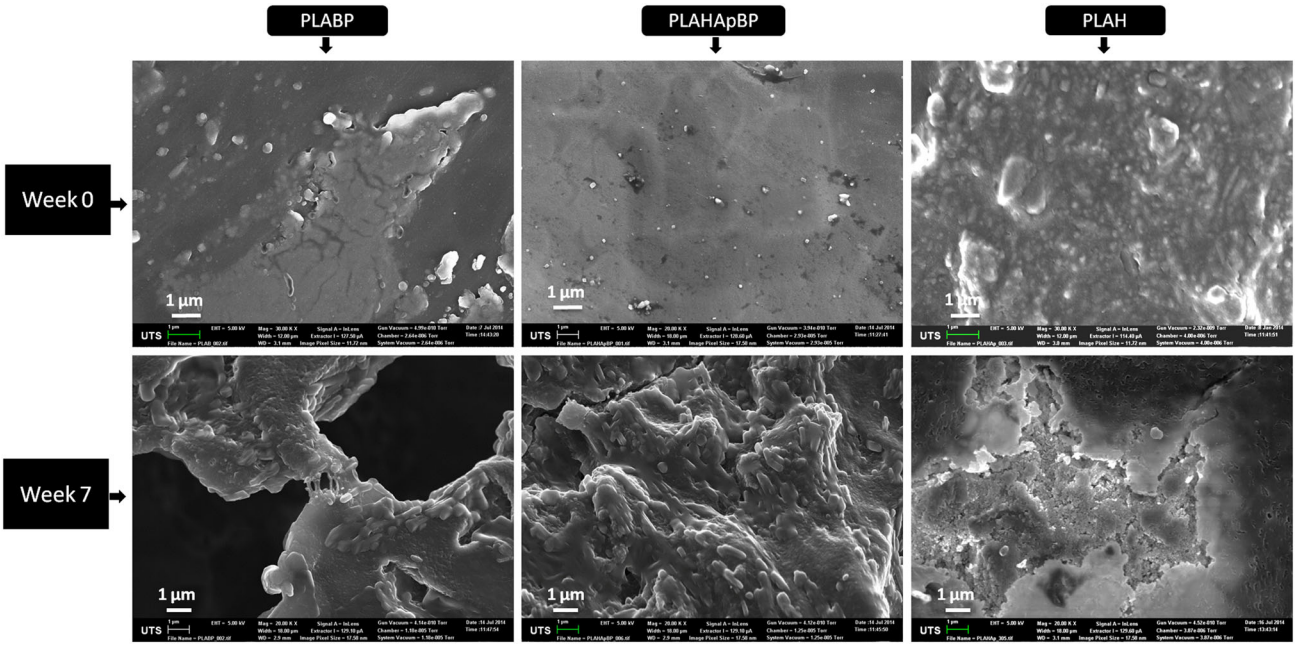


Figure 4. Morphology of PLA drug-release devices in SINK conditions before and after 7 weeks of drug release

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DOI: 10.1002/term

clodronate release will be faster in PLABP than in PLAHApBP composite (Figure 3).

4. Discussion

Dissolution and release of active substances or drugs from pharmaceutical dosages within a biocomposite is governed by number of factors. For a hydrolysable polymer matrix such as PLA, water diffusion to the matrix, size of the matrix, drug dissolution kinetics, matrix hydrolysis rate and matrix charge are among the factors reported to limit drug-release rates (Costa and Sousa Lobo, 2001; Ford Versypt *et al*., 2013; Liechty *et al*., 2010). These factors seem, on the one hand, to limit the release of drug, while on the other hand they could be positively tuned to control the release for different clinical purposes.

Clodronate is a hydrophilic drug with relatively high water solubility. There are a number of techniques to control its dissolution rate, including encapsulation with relatively less hydrophilic materials, or preferably with hydrophobic materials, or strongly attaching one of its molecules to the drug carrier. Both techniques could delay drug dissolution, which could then occur over longer time periods, consequently affecting the release profiles. It is recognized that the dissolution and release of the drug depends partly on its solubility and availability, which is closely linked with transport phenomena that occur within the matrix system. But it is also important not to overlook the possible interactions that may exist between the drug and the carrier, which may hinder its ability to be released and influence its drug efficiency.

FTIR results obtained for PLABP (Figure 5) showed that there is no interaction between BP and polymer. Thus, drug release is mainly due to diffusion phenomena of fluids within the PLA matrix, which are limited by the hydrolysis rate and the barrier effect (due to its hydrophobicity) of PLA.

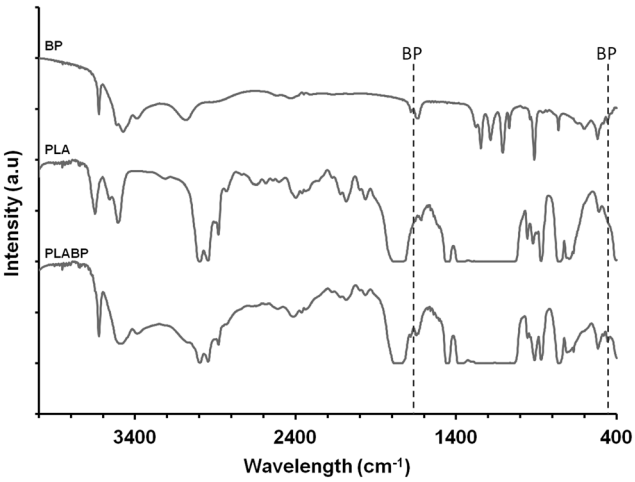


Figure 5. FTIR spectra of BP, PLA and PLABP, showing no denaturing of BP after embedding in PLA matrix: other BP peaks overlap with PLA; region 1400–1000 is flattened due to the PLA matrix

This is the reason why the drug dispersed into PLA polymer matrix (PLABP) is not immediately dissolved and why complete release occurs after only 3–4 weeks. The burst effect is due to the quick dissolution of surface-attached free BP particles. When the drug is previously adsorbed onto HAp particles before being embedded within the PLA thin matrix (PLAHApBP), interactions due to the strong affinity of BP with calcium phosphate, which exist between BP and HAp, have to be considered. The release rate observed for this composite shows a much slower release rate for the first 3–4 weeks for that reason (Figure 3). Moreover, considering that the dissolution kinetics of the drug depend on its availability, we must consider the different locations where the BP is deposited and its long-term accessibility.

First, during the HAp drug-loading process, BP molecules are adsorbed to the HAp particles, as previously described (Oliveira *et al*., 2010; Pascaud *et al*., 2014), onto the HAp surface by anionic exchange. The rest of the drug coats the particle surfaces and can also be located within their micro-, meso- and nanoporous interconnected structure (ICH, 2005). Their availability depends on accessibility to fluids, which decreases considerably when pore size or pore accessibility decreases.

During the loading process to the PLA film, part of the drug that covers the particles detaches and comes to be dispersed in the polymer matrix. The BP availability depends first on the location into (and onto) the matrix and also on solution penetration and diffusion into the polymer network. In this way, drug loosely bound to the surface of the film will be dissolved very easily and quickly, and the rest, which is embedded with the particles into the polymer film, will be dissolved relatively more slowly.

Taking into account the factors mentioned above, it is plausible that the dissolution kinetics of BP in the composite follows the following four-step scenario, thus explaining the two different parts which constitute the curve of PLA–HABP in Figure 3:

1. During the first 2 weeks, BP located at the surface of the PLA film dissolves rapidly and some of this dissolved drug might interact with the polymer matrix and dissolve more progressively (as confirmed by PLABP dissolution kinetics). During this period, only 30% of the BP was observed to dissolve.
2. During the next 2 weeks, the polymer releases the whole dispersed BP contained therein, thereby creating a secondary surface porosity favourable for fluid transport phenomena and a further pathway for dissociation inside the composite.
3. Then the drug that covers the surface of the hydroxyapatite particles depending on its accessibility starts to dissolve. The drug is gradually dissolved by the fluid on the surface of the particles and then diffuses through interconnected pores within the

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hydroxyapatite and the polymer network before being released.
4. During weeks 7–11, the degradation of the polymer induces relaxation of the thin films. This accelerates the diffusion of fluids into the composite and further drug release. At the end of this period, 44% of BP has been dissolved. With further degradation of HAp particles and the polymeric matrix, more pores and surfaces will be available for further drug release.

The low rate of drug released after 11 weeks is of course related to the characteristics of the composite, but also to the amount of BP remaining within the particles, which are not directly accessible by simple release, and further degradation of the HAp particles is required. It is conceivable that this part of the drug release may be more sustained and possibly will be controlled by *in vivo* factors in clinical applications.

5. Conclusion

Clodronate is prescribed to combat osteoporosis and to ease bone pain in people with bone cancer and to reduce the hypercalcaemia associated with some cancers, and is also used in postmenopausal osteoporosis therapy. Clodronate was introduced in PLA and PLA thin film composites as a local drug delivery device to control its release rate.

The loading of drug onto HAp drug carrier particles induces a significant decrease of the release period, permitting the therapeutic efficacy of composite biomaterial locally to be extended. The release study further showed the ability of these devices to control clodronate release for a prolonged period of time. Depending on the degree of clinical dosage required, the devices developed could be tuned to release the drug in both short and prolonged periods of time. In addition to the therapeutic effect of the drugs, the use of HAp with clodronate has the advantage of improving the bone-regenerative effect, due to local Ca^{2+} and PO_4^{2-} ion release. The suggested ^{31}P-NMR method, using triphenyl phosphate as an internal standard, is a simple and reliable method for the determination of clodronate release in Tris–HCl buffer solution referenced to KH_2PO_4. ^{31}P-NMR signals were linear in heavy water, with detection and quantification limits below the minimal release concentration. Quantitative ^{31}P-NMR method can be applied in clodronate detection. Marine structures based on calcium carbonate can be easily converted to calcium phosphates for controlled-release devices in medical applications.

Conflict of interest

The authors declare no conflict of interest.

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DOI: 10.1002/term