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ORIGINAL PAPER

Biological properties and enzymatic degradation studies of clindamycin-loaded PLA/HAp microspheres prepared from crocodile bones

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Abstract Polylactic acid (PLA)/hydroxyapatite (HAp) biocomposite microspheres with a specific core–shell structure for application as drug carriers were synthesised using an ultrasound field. In addition, the loading efficiency of clindamycin phosphate increased when the HAp content was increased to 30%. The effect of HAp content on enzymatic degradation of PLA/HAp microspheres loaded with clindamycin phosphate was that the degradation rate increased with increasing HAp content. Apatite formed on the surfaces of the PLA and PLA/HAp microsphere loading of clindamycin phosphate showed Ca and P peaks in energy-dispersive X-ray spectroscopy (EDX) data. In addition, the PLA and PLA/HAp microspheres loaded with clindamycin phosphate did not show any cytotoxicity against the human lung fibroblast MRC-5.

Keywords Hydroxyapatite (HAp) · Polylactic acid (PLA) · Biological properties · Enzymatic degradation · Crocodile bones · Clindamycin phosphate

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Introduction

Recently, drug delivery systems have been used as carriers of protein drugs, anticancer drugs, anti-inflammatory drugs such as ibuprofen and antibiotics [1–6]. Research in many countries has been aimed to discover the cause of failure of artificial knees. Seventy to eighty percent of patients had problems with wear or loosening, but 10–15% of failures had been caused by infection. Thus, infection is a devastating complication of any surgical procedure. In total knee replacement, the large foreign metal and plastic implants can serve as a surface for the bacteria to latch onto, inaccessible to antibiotics. Even if the implants remain well fixed, the pain, swelling, and drainage from the infection make revision surgery necessary. Any surgery can have potential complications. The complexity of revision joint surgery increases the chance of surgical complications. Possible complications include infection, bleeding, damage to nerves or blood vessels and intra-operative fractures. The materials are mostly imported from foreign countries resulting in high cost of treatment [7]. One of the most widely used antibiotic drugs is clindamycin [8, 9]. It has been extensively studied as a drug model for sustained and controlled drug delivery because of its good pharmacological activity.

Thailand has the largest crocodile farming industry in the world and famous international fashion brands source their exotic skins from the kingdom. Thailand exports crocodile hides, boned and boneless alligator meat, as well as processed foods including crocodile sausage and ground meat. Crocodile blood is also in demand in many Asian markets for its perceived medicinal properties. Crocodile bone is high in calcium and phosphorus, so can be used for the prevention and treatment of osteoporosis in the elderly, for calcium deficiency in infants, and is efficacious for improving rheumatism. It is thus of interest in this study to convert crocodile bones into HAp. In addition, the hydroxyapatite from a natural source (crocodile bone) was reported to enhance significantly the bioactivity compared to other chemical sources. Its chemical stability and the biocompatibility of natural products in the body have greatly accounted for its uses in recent times [10]. Hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] (HAp) is a calcium phosphate ceramic having similar biocompatibility, bioactivity, and osteoconductivity as bone. Its unit cell contains a complete representation of the apatite crystal, consisting of Ca^{2+}, PO_4^{3-}, and OH^- groups. It is one of the inorganic components of the hard tissues of living bodies such as bones, teeth, etc. It has also been extensively investigated as a possible drug delivery vehicle. Its compatibility with the composition of natural bone, its capacity to promote and stimulate the regeneration of bone tissue due to its bioactivity, and its ability to be resorbed at the implantation site during the hard tissue repair make it a natural candidate for a carrier of drugs against bone infections [11, 12]. However, pure HAp is suitable only for the repair of non-load-bearing bones because of its fragility, low mechanical strength, easy rupture, and weak fatigue resistance; it cannot withstand the normal operating loads of bone or joints. The addition of biodegradable polymers, such as polylactic acid (PLA), can improve the degradability of HAp and alter its mechanical properties.

Polylactic acid (PLA) is a biodegradable plastic produced from fermentation of plant carbohydrates (corn), which makes it an alternative to petroleum-based plastics. The advantages of PLA are its eco-friendliness, biocompatibility, better thermal processibility, and energy savings [13, 14]. It can be put into body tissue and can be absorbed by the biological systems in the body. Thus, PLA is one of the original and most frequently used groups of materials in the field of bone tissue engineering and one of the most widely used materials in the application to bone scaffold, which can effectively control the rate and duration of drug release.

The aim of this work was to develop further the PLA/HAp biocomposite microsphere with a specific core–shell structure to be applied as a drug carrier. Clindamycin phosphate was chosen as the drug in this study. Clindamycin phosphate is a lincosamid antibiotic, with a broad spectrum of activity against both Gram-positive cocci and Gram-positive or -negative anaerobes, and is used as an antibacterial to treat severe problems in the bones or joints, and exhibits consistently high penetration in bone [15–17]. It is thus of interest to study the effect of HAp content converted from crocodile bones in PLA/HAp microspheres on the efficiency of drug (clindamycin phosphate) loading process and enzymatic degradation rate. The apatite formation ability of elements in PLA/HAp microspheres after immersion in SBF (simulated body fluid) for 30 days was also investigated. Moreover, the acute toxicity of PLA/HAp microsphere was evaluated by means of in vitro biocompatibility tests.

Experiment

Materials and methods

Crocodile bones were obtained from a local Thai crocodile farm. Polylactic acid (PLA), product code of 2003D, was purchased from Nature Works Company (Thailand). Clindamycin phosphate (ProspPharma Co., Ltd, Thailand) was used as antibacterial drug applied in the loading process. Acetonitrile, polyoxyethylene (20) sorbitanmonooleate (Tween 80), and sodium chloride (NaCl) were purchased from Quality Lab Co., Ltd (Thailand). Sodium hydrogen carbonate ($NaHCO_3$), potassium chloride (KCl), di-potassium hydrogen phosphate (K_2HPO_4), sodium sulphate (Na_2SO_4), hydrochloric acid (HCl), and calcium chloride dihydrate ($CaCl_2.2H_2O$) were purchased from Ajax Finechem Pty. Ltd (Thailand). Magnesium chloride hexahydrate ($MgCl_2{\cdot}6H_2O$) was purchased from LobaChemie Pvt. Ltd (Thailand). Tris-hydroxymethylaminomethane [$(CH_2OH)_3CNH_2$] was purchased from Fisher Scientific UK. Proteinase K (Bang Trading 1992 Co., Ltd, Thailand) was used in the enzymatic degradation. In addition, the preparation procedure of simulated body fluid (SBF) for enzymatic degradation study followed that given by Kokubo and Takadama [18]. Minimum Essential Medium (MEM) culture media, phosphate-buffered saline (PBS), trypsin EDTA, foetal bovine serum (FBS), penicillin–streptomycin, and MRC-5 human normal lung cell line were purchased from American Type Culture Collection (ATCC) located in Manassas, VA, USA.

The concentration of drug in filtrates obtained after the PLA/HAp microspheres loading of clindamycin phosphate and the drug release study of PLA/HAp/drug microspheres were measured by HPLC using an Agilent 1100 series. The separation was carried out on a reversed-phase column C18 (length 250 mm diameter 4.6 mm (VARIAN)). The elution solvent consisted of acetonitrile and water (60:40 v/v, flow rate 0.7 ml min^{-1}, and the injection volume 20 μL). The monitor wavelength was set at λ_{max} (ε) = 210 nm.

The surface morphology and size of the microspheres were estimated by Scanning Electron Microscopy (SEM) and Energy-dispersive X-ray spectroscopy (EDX) with a Quanta 3D FEG Environmental Scanning Electron Microscope and Focus Ion Beam. The microsphere sample was spread on an SEM stub and sputtered with gold.

PLA/HAp clindamycin phosphate loaded microsphere preparation

Hydroxyapatite and PLA/HAp clindamycin phosphate loaded microspheres were both synthesised according to the previous literature procedure [19]. In the first step, PLA was dissolved in acetonitrile and the solution of clindamycin phosphate was added to the PLA solution drop-wise in an ultrasonic field. The concentration of clindamycin phosphate loaded was fixed at 13.5% by weight. Then, the PLA/HAp composite material was processed according to the modified procedure described in the literature of Jevtić et al. [20] with the application of different PLA/HAp (wt%) ratios: 100:0, 90:10, 80:20, and 70:30. After precipitation, the obtained colloid mixture was mixed with Tween 80 (100 ml, 2×10^{-2}% solution) as a surfactant solution. Finally, the microspheres were washed with distilled water and dried in air.

Enzymatic degradation

To study the influence of enzymatic degradation, the sample was placed in a vial filled with 5 ml of SBF buffer solution (pH 7.40) containing 1 mg proteinase K (0.2 mg/ml). The vial was incubated at 25 rpm in water bath shaker at 37 °C. The buffer-enzyme system was changed every 24 h to reactivate the original level of enzymatic activity. For the present experiment, three replicate samples were withdrawn from the degradation medium, washed with distilled water, and weighed.

In vitro bioactivity testing

The bone-bonding ability of the material was evaluated by examining apatite formation on its surface in simulated body fluid (SBF). The ionic concentration was similar to that of human blood plasma. Fifty mg of PLA and PLA/HAp microspheres was immersed in 10 ml of SBF solution maintained at body temperature (37 °C) and incubated for 30 days in a water bath shaker. After that, the samples were removed from the SBF solution and dried at room temperature.

Toxicity test

An MTT (3-[4,5-dimethylthiazol-2-yl]-2,5diphenyltetrazolium bromide) assay was employed to assess the in vitro cytotoxicity of the polymer. MRC-5 cells were seeded onto a 24-well plate at 25,000 cells/1000 μL/well and incubated for 24 h. All cells were plated in quadruplicate. After the medium was removed, 45 mg of PLA/HAp microspheres was added to the wells. Growth medium containing Minimum Essential Medium (MEM) was used as a control for 100% viability (non-sample cells). Twenty percent of DMSO-containing medium was used as a negative control. Following 72 h of incubation, a 10-μL solution of 5 mg/mL freshly prepared MTT was added to each well and allowed to incubate for an additional 2 h for human lung cell lines. Then, the medium/sample/MTT solution was removed and spectrophotometric grade DMSO was added at 1000 μL/well. Plates were then shaken on an orbital shaker to facilitate formazan crystal solubilisation. The absorbance was measured at 570 nm using a microplate reader.

Results and discussion

The characterisations of hydroxyapatite (HAp) have been shown in previous literature [19]. PLA/HAp microspheres loaded with clindamycin phosphate fixed at the amount of 13.5 wt% were formed in the ultrasonic field with four different polymer-to-HAp wt% ratios: 100:0, 90:10, 80:20, and 70:30. The use of an ultrasonication technique in the synthesis procedure had a significant influence in helping the dispersion of HAp particles in the PLA matrix. This was due to the small particle size relative to the aggregation of the material. Thus, this ultrasonic irradiation reduced the effect of HAp aggregation in the synthesis of PLA/HAp. The proposed mechanism for the formation of spherical PLA/HAp loaded with clindamycin phosphate is shown in Fig. 1.

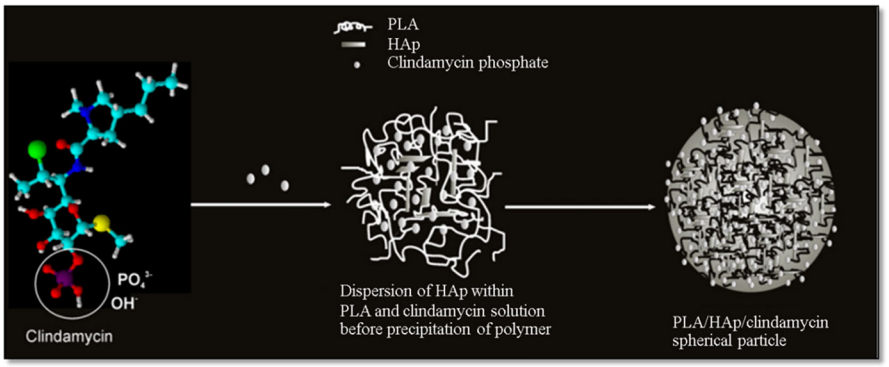

Fig. 1 Proposed mechanism for the formation of spherical PLA/HAp loaded with clindamycin phosphate (Adapted from Vukomanović et al. [9])

The antibiotic drug delivery properties of PLA/HAp microspheres were studied. Standard clindamycin phosphate in aqueous solution of 0.8946 mg/ml was used for the drug loading procedure. The percentages of drug loading of the specimens were calculated from the following equation:

$$\%\ \text{drug loading} = \frac{(x - y)}{x} \times 100$$

where x and y represent the initial and final concentrations of drug (mg/ml), respectively.

After calculation, drug loading was 0.75, 0.79, 0.79, and 0.81 mg/ml for PLA/HAp (100:0), (90:10), (80:20), and (70:30), respectively. The drug loading efficiencies of PLA/HAp biocomposite microspheres with different PLA/HAp wt% ratios (100:0, 90:10, 80:20, and 70:30) are listed in Table 1. It can be seen that the loading efficiency increased by increasing HAp content to 30% by weight. The reason was that clindamycin phosphate dispersed in the PLA matrix and a proportion of the drug was also captured by HAp. The capacity of HAp to adsorb clindamycin phosphate was explained by electrostatic interactions between the Ca^{2+} or PO_4^{3-} groups of HAp with oppositely charged groups of the drug molecule, as discussed in the report of Vukomanović et al. [9]. Thus, the increase in HAp content improved the drug loading efficiencies of PLA/HAp biocomposite microspheres. Moreover, the loading efficiency in this work was higher than those of other works that used HAp in application of drug delivery, such as the work of Jafari et al., Ignjatovic et al., and Guo et al. [21–23].

To investigate drug release, the concentration of drug in the filtrates, obtained after the immersion of PLA/HAp/drug microspheres in SBF solution maintained at body temperature (37 °C) and incubated in a water bath shaker for many days, was measured by HPLC. Unfortunately, the drug release profile of clindamycin phosphate indicated that the concentration of drug release was higher than the drug loading level. The reason could be that the degradation of PLA might interfere the chromatogram of clindamycin phosphate, leading to an increase in drug concentration.

Enzymatic degradation

It is generally considered that the mechanism of degradation of aliphatic polyester microspheres is a hydrolytic mechanism [24]. The differences in the degradation behaviour of the amorphous and crystalline samples could be

Table 1 Loading data obtained according to HPLC results

Sample	Concentration of loaded clindamycin (mg/ml)	Loading efficiency (%)
PLA/Clindamycin phosphate	0.75	84 ± 0.14
PLA(90)/HA(10)/Clindamycin phosphate	0.79	88 ± 0.13
PLA(80)/HA(20)/Clindamycin phosphate	0.79	88 ± 0.10
PLA(70)/HA(30)/Clindamycin phosphate	0.81	90 ± 0.11

explained by assuming a simple hydrolysis as the main degradation mechanism, affecting the whole polymer if in an amorphous state, but only the amorphous domains in a crystalline polymer. To study the influence of enzymatic degradation, the sample was placed in a vial filled with SBF buffer solution (pH 7.40) containing proteinase K. At each specific incubation time, the weights of the composite materials were measured. The weight loss percentage over time could be analysed to represent the degradation rate of the composite with different PLA/HAp (wt%) ratios: 100:0, 90:10, 80:20, and 70:30 [25]. The weight loss data of the PLA samples are shown in Fig. 2. After 5 h in the incubation medium, it was detected that PLA(100)/drug lost only 3.32%, while PLA(70)/HAp(30)/drug lost nearly 8.03%. Thereafter, the percentage of weight loss increased steadily to reach 30.21, 42.94, 46.94, and 48.33% after 67 h for PLA(100)/drug, PLA(90)/HAp(10)/drug, PLA(80)/HAp(20)/drug, and PLA(70)/HAp(30)/drug, respectively. It was assumed that these samples broke down into smaller fragments, which were eventually biodegraded after exposure to the incubation medium for a long time. The result suggested that the enzymatic degradation was enhanced with increased amount of HAp in the microspheres at all periods of exposure. This could be explained by an increase in the irregularity of PLA molecular chains when the amount of HAp was increased, resulting in a weakening of the intermolecular force between PLA molecular chains, and leading to a higher rate of degradation of PLA [26]. Thus, PLA(70)/HAp(30)/drug, which had the highest HAp content, displayed the highest weight loss percentage compared to the other samples.

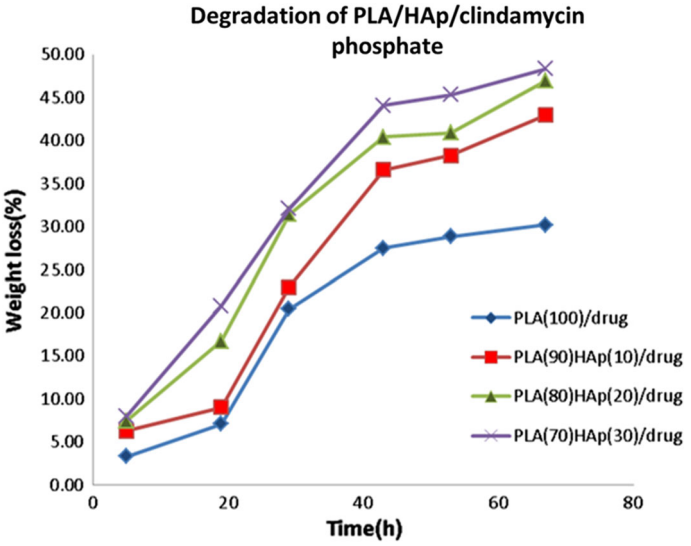

Fig. 2 Weight loss of PLA(100)/drug, PLA(90)/HAp(10)/drug, PLA(80)/HAp(20)/drug, and PLA(70)/HAp(30)/drug during the enzymatic degradation at 37 °C (Clindamycinphosphate was fixed at 13.5 wt%)

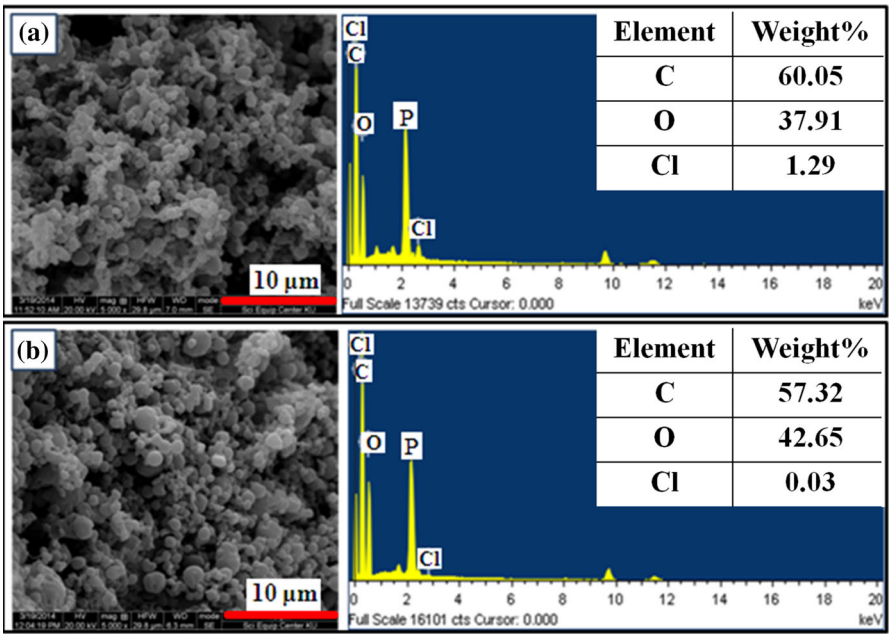

Fig. 3 SEM and EDX analysis of sample of PLA microsphere loaded with clindamycin phosphate after immersion in SBF solution maintained at body temperature (37 °C) and incubated in a water bath shaker for **a** 0 day and **b** 30 days

Apatite formation ability

The prepared PLA microspheres loaded with clindamycin phosphate and PLA/HAp microspheres loaded with clindamycin phosphate were immersed in SBF solution maintained at body temperature (37 °C) and incubated for 30 days in a water bath shaker. This part focused on the apatite formation ability of elements in the sample after immersion in SBF, as shown in Figs. 3 and 4. It was found that there was no apatite formed on the PLA microsphere surface after immersion in SBF for 30 days, as seen in Fig. 3b. It was also shown in the EDX spectra of PLA microsphere loaded with clindamycin phosphate that there were the appearances of C, O, Cl, and P peaks, but without Ca peak, indicating the lack of HAp formation of the surface of PLA microspheres after 30 days of immersion in SBF. In addition, the intensities of C, O, Cl, and P peaks confirmed the presence of PLA (C and O peaks) and clindamycin phosphate (Cl and P peaks) in the microspheres.

Apatite formation in PLA/HAp microsphere samples was observed by SEM and EDX, as shown in Fig. 4a–e. The SEM images indicated that the samples were covered with a layer of crystals with a typical morphology of HAp after 30 days of immersion in SBF (Fig. 4a–e. In addition, the SEM observations demonstrated that the surfaces of the samples were partially covered by a dense apatite layer. The HAp grew in wt% with increasing soaking time, as shown in Fig. 5. The EDX spectra of PLA/HAp microsphere loaded with clindamycin phosphate revealed P, Ca, C, O,

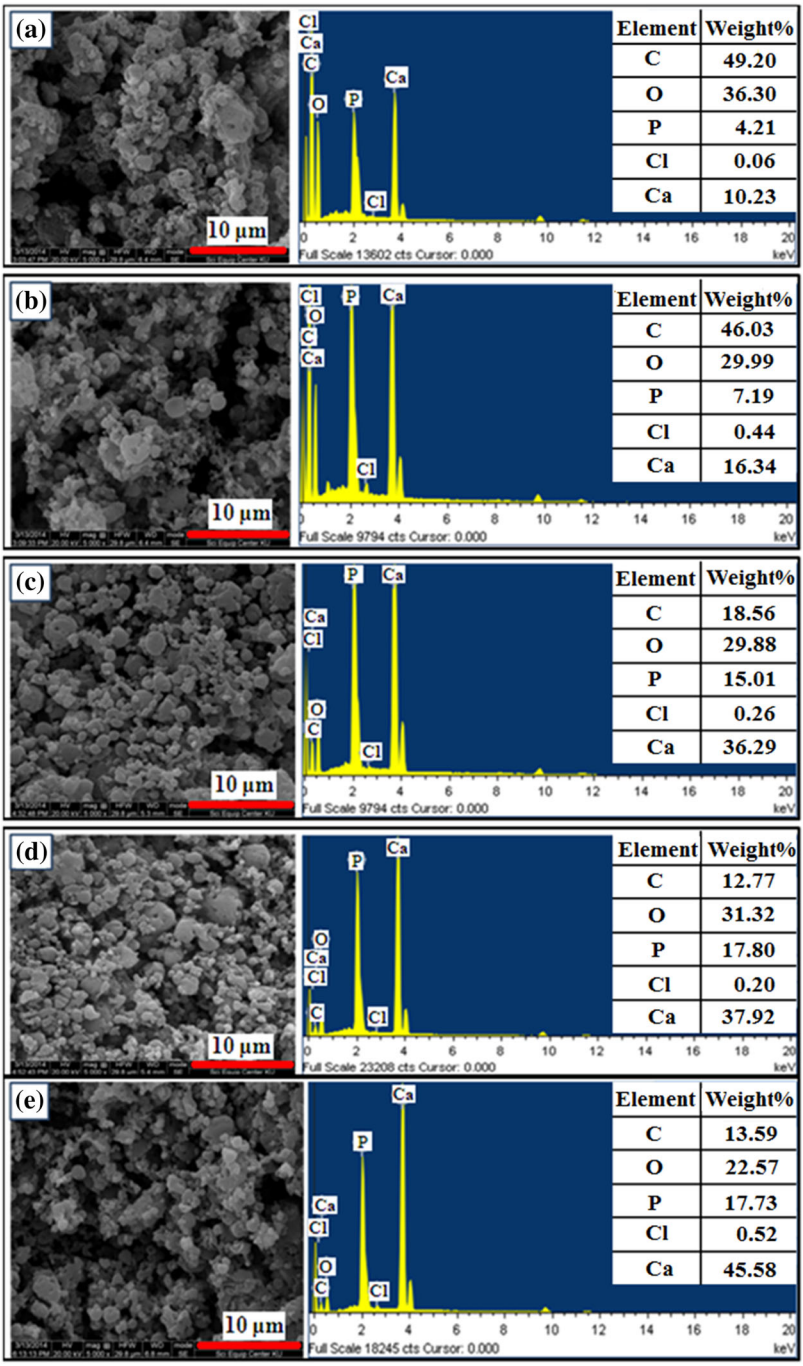

Fig. 4 SEM and EDX analysis of samples of PLA/HAp microspheres loaded with clindamycin phosphate after immersion in SBF solution maintained at body temperature (37 °C) and incubated in a water bath shaker for **a** 0 day, **b** 7 days, **c** 14 days, **d** 21 days, and **e** 30 days

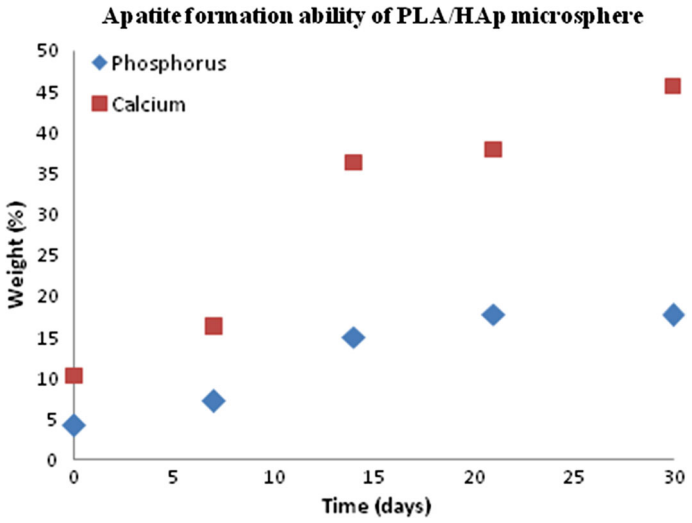

Fig. 5 The weight percent of calcium and phosphorus elements formed after immersion in SBF solution maintained at body temperature (37 °C) and incubated in a water bath shaker

and Cl peaks, indicating the presence of these elements in the microsphere. Apatite formed on the surfaces of the PLA/HAp microspheres showed the increase in wt% of Ca and P peaks in EDX spectra. The Ca/P ratios from the EDX analysis of PLA/HAp microspheres loaded with clindamycin phosphate samples at 0 day, 7, 14, 21, and 30 days were 2.43, 2.27, 2.42, 2.13, and 2.57, respectively. The result confirmed the Ca^{2+}-rich apatite deposition on the surface of the PLA/HAp/drug sample. This indicated that crystalline apatite was formed on the surfaces of the PLA/HAp microspheres when the time of immersion in SBF was increased [27–29]. This might be explained by the effect of the phosphate (PO_4^{3-}) and hydroxide (OH^-) groups on the HAp. These groups cause a negative surface charge. The negative ions on the sample surface then interact with the positive ions in the SBF to develop calcium-rich amorphous calcium phosphate (ACP), leading to the formation of bone-like apatite [30–32].

In vitro toxicity assessment

In vitro toxicological study of materials is a new developing field of current research. Cell cytotoxicity testing (MTT assay) is a very common preliminary step in assessing implants in general. They must not cause abnormal responses in local tissues and should not produce toxic or carcinogenic effects, either locally or systemically. The MTT cytotoxicity assay reveals various cellular metabolic actions by the reduction of the yellow tetrazolium salt MTT to a purple MTT formazan. The absence of cytotoxicity was assayed with cell metabolic activity (MTT) tests on MRC-5 cell lines.

The PLA and PLA/HAp (70/30 wt%) microspheres were exposed to human lung fibroblast MRC-5 cells. Cell viability and cell death were determined by MTT assay

Table 2 The percent viability and percent cell death (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide [MTT] assay) data of human lung fibroblast MRC-5 cells exposed to PLA and PLA/HAp microsphere at 72 h exposure time

Sample	Viability (%)				Cell death (%)			
	I	II	III	Average	I	II	III	Average
PLA	93.53	92.57	92.26	92.78 ± 0.66	6.47	7.43	7.74	7.22 ± 0.66
PLA/HAp (70/30 wt%) microsphere	80.25	81.08	86.75	82.69 ± 3.54	19.75	18.92	13.25	17.31 ± 3.54

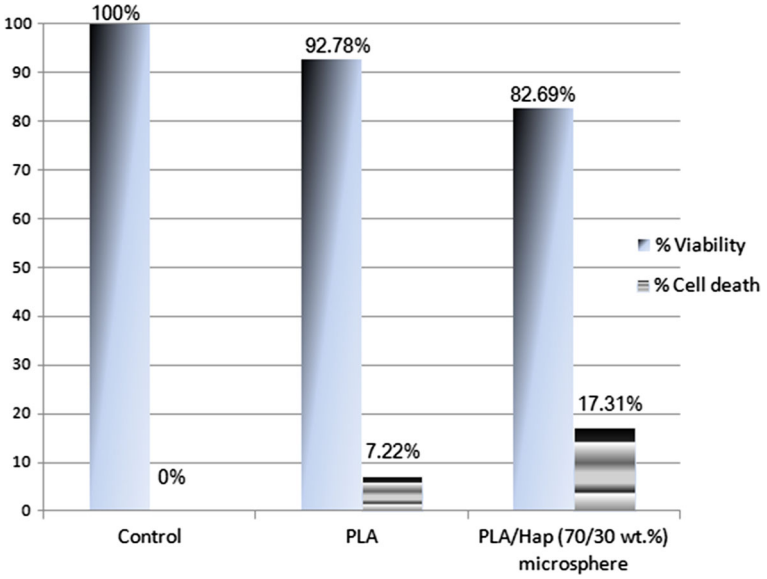

Fig. 6 The percent viability and percent cell death (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide [MTT] assay) graph of human lung fibroblast MRC-5 cells exposed to PLA and PLA/HAp microsphere at 72 h exposure time

(Table 2 and Fig. 6). The average percent viability of the PLA was 92.78 and the average percent cell death was 7.22. The average percent viability of the PLA/HAp (70/30 wt%) microspheres was 82.69 and the average percent cell death was 17.31. Moreover, the benefit of HAp from crocodile bones was the absence of cytotoxicity when used in a drug delivery application. The PLA/HAp microsphere had about 83% viability of human lung fibroblast MRC-5 cells, which was in a similar range as that in the work of Ignjatovic et al. [22]. Therefore, the PLA/HAp microsphere formulations were biocompatible, non-toxic, and without interference with tissue healing as discussed in the report of Athanasiou et al. [33]. This microsphere was appropriate for drug-delivery purposes.

Conclusions

Clindamycin could be successfully incorporated into PLA/HAp microspheres by applying ultrasonic irradiation. This process was a simple, low-cost, and highly efficient method for the processing of materials for controlled drug delivery. In addition, the clindamycin-loaded PLA/HAp microspheres had the added advantage of using hydroxyapatite from a natural source (crocodile bone) that enhanced bioactivity compared with other chemical sources and provided a continuous supply of calcium and phosphate ions, which could assist bone regeneration and repair. The synthesised PLA/HAp loaded with clindamycin phosphate with different polymer-to-HAp (wt%) ratios (100:0, 90:10, 80:20, and 70:30) showed that the loading efficiency increased with increasing HAp content to 30% by weight. The degradation of PLA polymers confirmed the catalytic activity of proteinase K. It could also be noted that the enzymatic degradation was enhanced with increased amount of HAp in the microspheres after all exposure periods. The PLA/HAp microspheres loaded with clindamycin showed apatite formation ability. The results showed that HAp size grew with increased soaking time. The MTT results indicated that the average percent viability of PLA/HAp microspheres was 82.69, and average percent cell death 17.31. Thus, the PLA and PLA/HAp microspheres did not show any cytotoxicity against the human lung fibroblast MRC-5 cells. This showed that PLA/HAp microspheres are good candidates for bone repair applications, due to their biocompatible and non-toxic properties.

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References

1. Yu D, Wong J, Matsuda Y, Fox JL, Higuchi WI, Otsuka M (1992) Self-setting hydroxyapatite cement: a novel skeletal drug-delivery system for antibiotics. J Pharm Sci 81(6):529–532
2. Yang L-X, Yin J-J, Wang L-L, Xing G-X, Ying P, Liu Q-W (2012) Hydrothermal synthesis of hierarchical hydroxyapatite: preparation, growth mechanism and drug release property. Ceram Int 38:495–502
3. Tomoda K, Ariizumi H, Nakaji T, Makino K (2010) Hydroxyapatite particles as drug carriers for proteins. Coll Surf B 76:226–235
4. Palazzo B, Sidoti MC, Roveri N, Tampieri A, Sandri M, Bertolazzi L, Galbusera F, Dubini G, Vena P, Contro R (2005) Controlled drug delivery from porous hydroxyapatite grafts: an experimental and theoretical approach. Mater Sci Eng C 25:207–213
5. Otsuka M, Matsuda Y, Suwa Y, Fox JL, Higuchi WI (1994) A novel skeletal drug delivery system using a self-setting calcium phosphate cement. 5. Drug release behavior from a heterogenous drug-loaded cement containing ananticancer drug. J Pharm Sci 83(11):1565–1568
6. Oner M, Yetiz E, Ay E, Uysal U (2011) Ibuprofen release from porous hydroxyapatite tablets. Ceram Int 37:2117–2125
7. Deirmengian CA, Lonner JH (2012) What's new in adult reconstructive knee surgery. J Bone Joint Surg Inc 94:181–188

8. Vukomanović M, Škapin I, Žagar E, Kralj B, Ignjatović N, Uskoković D (2011) Poly(D, L-lactide-co-glycolide)/hydroxyapatite core–shell nanosphere. Part 2: simultaneous release of a drug and a prodrug (clindamycin and clindamycinphosphate). Coll Surf B 82:414–421
9. Vukomanović M, Skapin SD, Jančar B, Maksin T, Ignjatović N, Uskoković V, Uskoković D (2011) Poly(d, l-lactide-co-glycolide)/hydroxyapatite core-shell nanospheres. Part 1: a multifunctional system for controlled drug delivery. Colloids Surf B: Biointerfaces 82:404–413
10. Ige OO, Umoru LE, Aribo S (2012) Natural products: a minefield of biomaterials. ISRN Mater Sci 2012:1–20
11. Murugan R, Ramakrishna S (2005) Development of nanocomposites for bone grafting. Compos Sci Technol 65:2385–2406
12. Gaharwar KA, Schexnailder PJ, Schmidt G (2011) Nanobiomaterials Handbook Taylor and Francis Group, LLC
13. Rasal RM, Janorkar AV, Hirt DE (2010) Poly(lactic acid) modifications. Prog Polym Sci 35:338–356
14. Spinu M, Jackson C, Keating MY, Gardner KH (1996) Material design in poly(lactic acid) systems: block copolymers, star homo- and copolymers and stereocomplexs. Pure Appl Chem A33(10):1497–1530
15. Smieja M (1998) Current indications for the use of clindamycin: a critical review. Can J Infect Dis 9(1):22–28
16. O'Grady F, Finch RG, Lambert HP, Greenwood D (1997) Antibiotic and chemotherapy: anti-infective agents and their use in therapy. Elsevier, UK
17. Darley ESR (2004) Antibiotic treatment of Gram-positive bone and joint infections. J Antimicrob Chemoth 53(6):928–935
18. Kokubo T, Takadama H (2006) How useful is SBF in predicting in vivo bone bioactivity. Biomaterials 27:2907–2915
19. Pradid JKW, Tangbunsuk S (2014) Study the Effect of HAp content in PLA/HAp microphere on the efficiency of drug (Clindamycin) loading process. Adv Mat Res 834–836:559–562
20. Jevtić M, Radulović A, Ignjatović N, Mitrić M, Uskoković D (2009) Controlled assembly of poly(D, L-lactide-co-glycolide)/hydroxyapatitecore–shell nanospheres under ultrasonic irradiation. Acta Biomater 5:208–218
21. Jafari S, Maleki-Dizaji N, Barar J, Barzegar-Jalali M, Rameshrad M, Adibkia K (2016) Methylprednisolone acetate-loaded hydroxyapatite nanoparticles as a potential drug delivery system for treatment of rheumatoid arthritis: in vitro and in vivo evaluations. Eur J Pharm Sci 91:225–235
22. Ignjatovic NL, Penov-Gasi KM, Wu VM, Ajdukovic JJ, Kojic VV, Vasiljevic-Radovic D, Kuzmanovic M, Uskokovic V, Uskokovic DP (2016) Selective anticancer activity of hydroxyapatite/chitosan-poly(d, l)-lactide-co-glycolide particles loaded with an androstane-based cancer inhibitor. Coll Surf B Biointerfaces 148:629–639
23. Guo Y, Shi X, Fang Q, Zhang J, Fang H, Jia W, Yang G, Yang L (2014) Facile preparation of hydroxyapatite–chondroitin sulfate hybrid mesoporous microrods for controlled and sustained release of antitumor drugs. Mater Lett 125:111–115
24. Pistner H, Bendix DR, Muhling J, Reuther JF (1993) Poly(L-lactide): a long-term degradation study in vivo Part III. Analytical characterization. Biomaterials 14:291–304
25. Li S, Tenon M, Garreau H, Braud C, Vert M (2000) Enzymatic degradation of stereocopolymers derived from L-, DL- and meso-lactides. Polym Degrad Stab 67:85–90
26. Lin PL, Fang HW, Tseng T, Lee WH (2007) Effects of hydroxyapatite dosage on mechanical and biological behaviors of polylactic acid composite materials. Mater Lett 61:3009–3013
27. Wong KL, Wong CT, Liu WC, Pan HB, Fong MK, Lam WM, Cheung WL, Tang WM, Chiu KY, Luk KDK, Lu WW (2009) Mechanical properties andin vitro response of strontium-containinghydroxyapatite/polyetheretherketone composites. Biomaterials 30:3810–3817
28. Kim HM, Himeno T, Kokubo T, Nakamura T (2005) Process and kinetics of bonelike apatite formation on sinteredhydroxyapatite in a simulated body fluid. Biomaterials 26:4366–4373
29. Iqbal N, Kadir MRA, Mahmood NH, Salim N, Froemming GRA, Balaji HR, Kamarul T (2013) Characterization, antibacterial andin vitro compatibilityof zinc–silver doped hydroxyapatite nanoparticles preparedthrough microwave synthesis. Ceram Int 40:4507–4513
30. Ramana Ramya J, Thanigai Arul K, Elayaraja K, Narayana Kalkura S (2014) Physicochemical and biological properties of iron and zinc ions co-doped nanocrystalline hydroxyapatite, synthesized by ultrasonication. Ceram Int 40(10):16707–16717

31. Thanigai Arul K, Ramana Ramya J, Bhalerao GM, Narayana Kalkura S (2014) Physicochemical characterization of the superhydrophilic, magnesium and silver ions co-incorporated nanocrystalline hydroxyapatite, synthesized by microwave processing. Ceram Int 40:13771–13779
32. Thanigaiarul K, Elayaraja K, Magudapathy P, Kamachi MU, Nair KGM, Sudarshan M, Krishna JBM, Chakraborty A, Narayana Kalkura S (2013) Surface modification of nanocrystalline calcium phosphate bioceramic by low energy nitrogen ion implantation. Ceram Int 39(3):3027–3034
33. Athanasiou KA, Gabriele GN, Agrawal CM (1996) Sterilization, toxicity, biocompatibility and clinical applications of polylactic acid/polyglycolic acid copolymers. Biomaterials 17:93–102