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Synthesis and characterization of biodegradable microcapsules for the controlled delivery of calcium hydroxide

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Abstract: This study aimed to synthesize and characterize biodegradable microcapsules based on poly(lactic acid) (PLA) and ethylcellulose (EC) for a controlled delivery of calcium hydroxide. Phase separation technique was adopted to synthesize calcium hydroxide-loaded PLA/EC microcapsules. Four PLA/EC blends (4/1, 1/1, 1/4, pure EC) were used as shell materials and the input ratio of calcium hydroxide to shell polymer was 4:1 for all microcapsules. The morphology and composition were studied using SEM-EDS and TEM. Particle size distribution, glass-transition temperature, drug loading, and encapsulation efficiency were characterized. *In vitro* release of the microcapsules was evaluated using a pH microelectrode and an auto-biochemistry analyzer. SEM images of microcapsules showed uniform spherical structures with smooth surfaces. Core-shell, hetero-structures were confirmed using TEM. The presence of calcium in the microcapsules was verified with EDS. Pure calcium hydroxide was 160 nm in diameter and the particle size of the microcapsules ranged between 500 nm and 4 μm. With an increase of PLA in PLA/EC blend, the size of microcapsules increased accordingly. Encapsulation efficiency of these microcapsules was higher than 57% and drug loading was higher than 80%, which were not significantly different among four microcapsules. Pure calcium hydroxide powder was used as a control and 90% was released within 48 h, while release of calcium hydroxide from microcapsules took between 168 and 456 h, depending on the PLA/EC ratio. Compared with calcium hydroxide powder, the calcium hydroxide-loaded microcapsules showed a sustained and prolonged release, which could be controlled via the regulation of the PLA/EC ratio. © 2011 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 99B: 120–126, 2011.

Key Words: microcapsule, polymer, calcium hydroxide, controlled release, dental/craniofacial material

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INTRODUCTION

Calcium hydroxide is used widely in dentistry due to its antimicrobial activity, tissue-dissolving ability, inhibition of tooth resorption, and induction of hard tissue deposition.[1] To improve antibacterial and handling properties,[2,3] calcium hydroxide powder is usually mixed with vehicles and produced in various formulations for clinical use. The dissociation of calcium hydroxide into OH^- and Ca^{2+} is largely affected by the vehicle, whether aqueous, viscous, or oily.[4] The lower the viscosity of the vehicle, the faster the ionic dissociation. An ideal vehicle should allow gradual and slow release of Ca^{2+} and OH^- ions, allowing it to diffuse into the tissue without having an adverse effect on the induction of hard tissue deposition.[5] Various vehicles have been used, such as distilled water, methylcellulose, glycerin, Ringer's solution, anesthetic solution and camphorated para-monochlorophenol (CMCP).

Sustained release of calcium hydroxide is required when it is used as a pulp capping agent and intra-canal dressing, particularly in cases of severe periapical lesions, apexification procedures and root resorption. Normally, aqueous and viscous vehicles do not allow a sustained release of calcium hydroxide.[5] However, higher molecular weight vehicles minimize the dispersion of calcium hydroxide into the tissue and remain *in situ* for longer.[6] Oily vehicles are often used for the purpose of slow calcium hydroxide release, however, cytotoxicity and immunogenicity may limit the application of oily vehicles.[7,8] On the other hand, oily vehicles cannot achieve the high calcium hydroxide loadings possible with aqueous vehicles. For example, the calcium hydroxide content of Vitapex (a widely used calcium hydroxide paste) is only 30.3%.[5] Oily vehicles are also not preferred as they can be difficult to remove, leaving an oily film on canal walls, which can affect the obturation of the root canal system.[6] The development of new vehicles for calcium hydroxide, with sustained release and biodegradable properties, is therefore warranted.

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In medical areas, biodegradable and biocompatible microparticles designed for controlled drug delivery have been widely investigated and successfully applied.[9–12] Particle size is generally less than 250 μm (ideally <125 μm).[13] Microparticle delivery has the advantages of controlled release of drug molecules, targeted drug delivery, reduced side effects, and improved therapeutic effect. Drugs encapsulated in microcapsules are released either by diffusion through the polymer barrier, by erosion of the polymer material, or by a combination of both diffusion and erosion mechanisms.[13] A microcapsule delivery system has been previously studied and used in dentistry for the controlled-release of chlorhexidine, doxycycline, and rhBMP-2.[10–12] So far, no studies on the encapsulation of calcium hydroxide for sustained release have been reported.

Unlike natural polymers, which often suffer from high cost and low purity,[13] synthetic biodegradable polymers such as poly(ε-caprolactone) (PCL), poly(lactic acid) (PLA), poly (glycolic acid) (PGA), and poly(D,L-lactide-*co*-glycolide) (PLGA), have been widely studied for the synthesis of microparticles. PLA, a biocompatible polymer, can be completely degraded into H_2O and CO_2. It has been applied in the fields of bone-fraction inner-fixing, tissue-repair materials, and surgical seam and drug delivery systems. Ethylcellulose (EC) is a water-insoluble and pH-independent polymer. EC-based microcapsules show a slower drug release compared to other polymer-based microcapsules.[14–17] In addition, polymer blends have also been studied for drug release and showed superior properties to single polymer microcapsules.[11,14,16]

This study was designed to develop a new type of biocompatible calcium hydroxide delivery system: calcium hydroxide-loaded microcapsules. Polymers used for microcapsule preparation were PLA and EC blended in different ratios. Various formulations of microcapsules were prepared by the phase separation (coacervation) method and characterized. The aim was to obtain a controlled and sustained release of calcium hydroxide *via* encapsulation.

MATERIALS AND METHODS

PLA (MW = 48 kDa) was kindly provided by the Institute of Chemistry, Chinese Academy of Sciences (Beijing, China). EC and calcium hydroxide were purchased from Sinopharm Chemical Reagent (Beijing, China). Four formulas of PLA/EC blends (W/W ratio) were prepared and used as shell materials: Formula A, pure EC; Formula B, PLA/EC 1:4; Formula C, PLA/EC 1:1; Formula D, PLA/EC 4:1. Calcium hydroxide was ground with a disintegrator and sieved with a mesh.[18]

Preparation of microcapsules

A phase separation technique was adopted to synthesize four formulations of calcium hydroxide-loaded microcapsules. In brief, 2.4 g of PLA/EC blend was dissolved in 50 ml of methylene dichloride (AR, Beijing Chemical Works, Beijing, China) and 9.6 g of calcium hydroxide was dispersed in this solution using a JJ-1 homogenizer (Jintan Medical Instrument Factory, ChangZhou, China) at 500 rpm for 2 h. Petroleum ether (160 mL, AR, Beijing Chemical Works, Beijing, China) was slowly added into the polymer-drug-solvent system at 2 drop s^{-1}, then the mixture was homogenized at 500 rpm for 1.5 h. The resulting microcapsules were isolated by filtration and vacuum dried for 24 h using a DZF-6050 vacuum dryer (Pudong Rong-feng Scientific Instrument, Shanghai, China).

Characterization of microcapsules

The surface morphologies of pure calcium hydroxide and the microcapsules were observed using a model S-4800 SEM (Hitachi, Tokyo, Japan). Samples were platinum sputter-coated and placed on a silicon chip above a copper stub prior to observation.

TEM images were obtained using a JEM-1011 transmission electron microscope (JEOL, Tokyo, Japan). Calcium hydroxide and microcapsule samples were deposited onto carbon coated copper grids and dried before observation.

Particle size distribution was analyzed using dynamic laser light scattering (Nano-ZS, Malvern Instruments, UK). Calcium hydroxide and microcapsules were suspended in de-ionized water prior to detection.

The chemical compositions of microcapsules were determined with energy dispersive, X-ray spectroscopy (S-4300 SEM-EDS).

The glass-transition temperature (T_g) was assessed by performing DSC on PLA, EC, PLA/EC blends with different ratios (1/4, 1/1, 4/1) and the four formulations of microcapsules. DSC measurements were carried out on a differential scanning calorimeter (Q2000, TA Instruments, USA) with a heating rate of 20°C min^{-1} in a nitrogen atmosphere.

To determine calcium hydroxide loading, 0.15 g of microcapsules were dissolved in 5 mL of methylene dichloride and ethanol. After vigorous mixing and centrifugation, the supernatant liquid was discarded. This process was repeated five times in order to completely remove the PLA/EC. The resulting solid materials were isolated and vacuum dried for 24 h. Measurements were conducted in triplicate. Drug loading (%) and encapsulation efficiency (%) of the microcapsules were calculated as follows.

Drug loading (%) = weight of drug in microcapsules/weight of microcapsules × 100%

Encapsulation efficiency (%) = weight of drug in microcapsules/weight of drug put into operation × 100%

In vitro release profile of microcapsules

Microcapsules (50 mg) were suspended in 1 mL of de-ionized water and placed in a dialysis bag. The dialysis bag was then kept in a serum bottle containing 100 mL of de-ionized water as dissolution medium. The serum bottles were maintained at 37°C and shaken at 50 rpm using a THZ-22 constant temperature shaking incubator (Taicang Laboratory Equipment Factory, Taicang, China). Five samples were prepared for each microcapsule formulation. Analytically pure calcium hydroxide was used as a control. At selected times (6, 8, 12, 24, 48, 72, 120, 168, 216, 264, 312, 360, 408, 456, and 504 h), a 200 μL aliquot was taken out, then an equal volume of fresh de-ionized water was added. The solution samples were analyzed for their Ca^{2+} concentration with a Hitachi 7180 chemistry analyzer (Tokyo, Japan) using a calcium kit (Biosino Bio-technology and

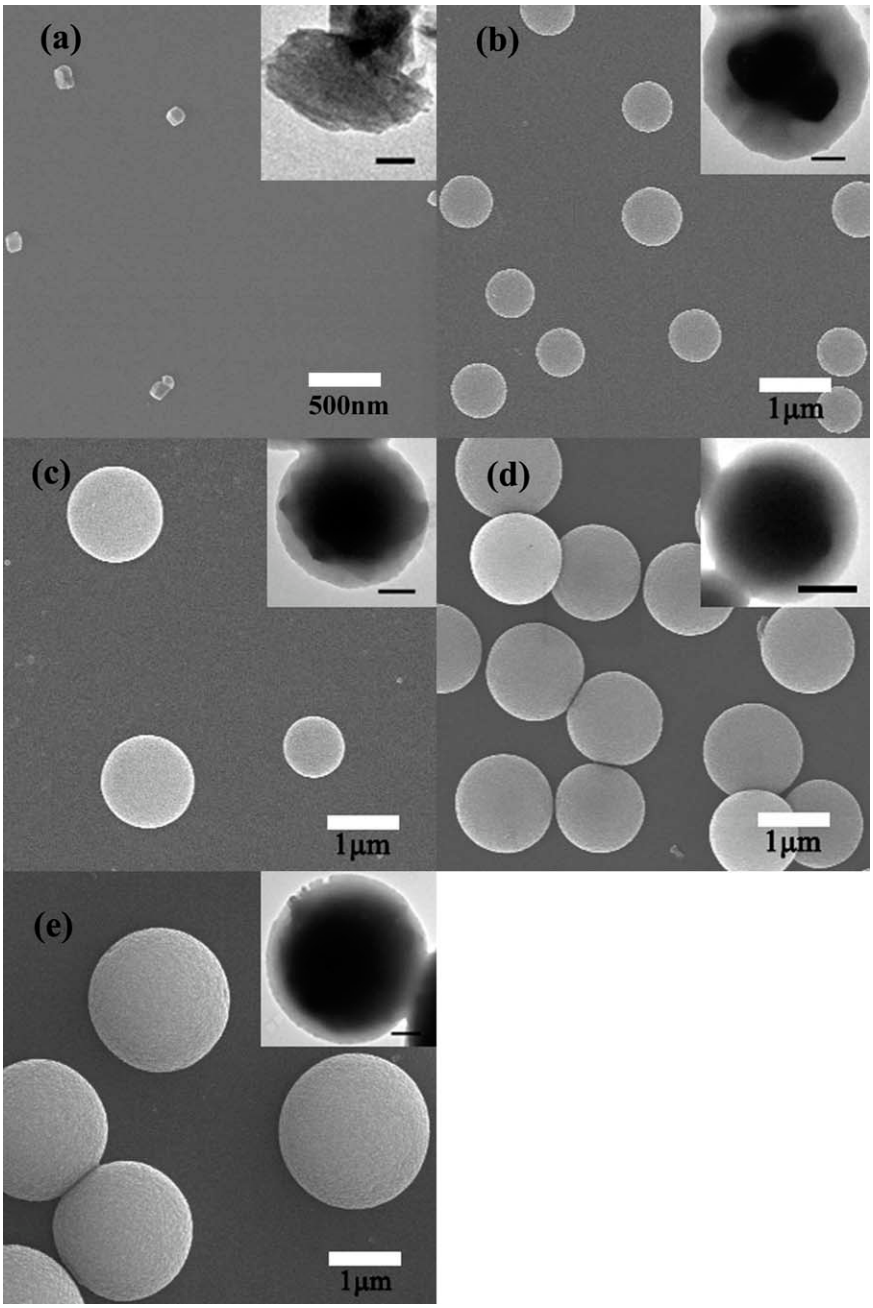

FIGURE 1. SEM and TEM (inset figure) images of microcapsules: (a) Pure calcium hydroxide (the bar in the inset figure represents 50 nm), (b) Formula A (the bar in the inset figure represents 100 nm), (c) Formula B (the bar in the inset figure represents 200 nm), (d) Formula C (the bar in the inset figure represents 500 nm), (e) Formula D (the bar in the inset figure represents 500 nm).

Science, Beijing, China). The pH of the samples was analyzed with a KS701 pH microelectrode (Shindengen electric manufacturing, Tokyo, Japan). The release of pure calcium hydroxide was also studied and used as a control.

RESULTS

The morphology of calcium hydroxide and microcapsules under SEM and TEM (inset figure) are shown in Figure 1. Under SEM, calcium hydroxide presented an irregular shape while all microcapsules presented smooth and uniform surfaces. TEM images revealed the core-shell, hetero-structure of the microcapsules. On the other hand, calcium hydroxide presented as homo-structure particles. Under TEM, inorganic calcium hydroxide and organic polymers appeared black and grey, respectively. These TEM images clearly indicated that calcium hydroxide was encapsulated in PLA/EC polymers.

The particle size distributions of the microcapsules are shown in Figure 2. The mean particle size of calcium hydroxide was 160 nm. The size of the microcapsules ranged from 500 nm to 4 μm depending on the PLA/EC blend. The mean particle sizes of the microcapsules were 905, 1173, 1240, and 2179 nm for formula A, B, C, and D, respectively.

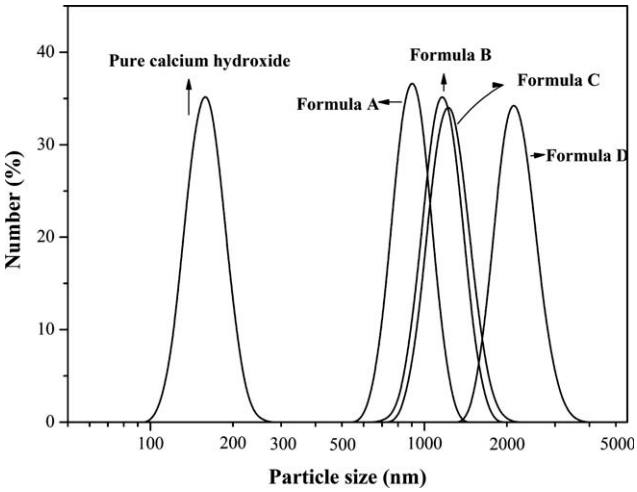

FIGURE 2. Particle size distributions of calcium hydroxide and microcapsules.

An increase in PLA content correlated with increased microcapsule size and an increased size distribution.

As shown in Figure 3, signals for the elements Ca, C, and O were found in the EDS spectrum for all microcapsules. Additionally, there were distinct signals for two other elements, Si and Pt which are not labeled in the EDS spectrum. Signals from these two elements are interference signals from the silicon chip and sputter-coated platinum.

T_g of PLA/EC blends, as shown in Table I, were between the T_g of PLA and EC. Changing the ratio of PLA/EC did not markedly influence the T_g of the polymer blends. The calcium hydroxide microcapsules showed similar T_g values to pure shell polymers.

The mean and standard deviations of drug loading (%) and the encapsulation efficiency (%) of microcapsules are listed in Table II. The encapsulation efficiency of these calcium hydroxide-loaded microcapsules was higher than 57% and drug loadings were higher than 80%. There were no significant differences among the four formulations of microcapsule in both drug loading and encapsulation efficiency ($p > 0.05$).

In vitro release of calcium ions and pH profiles are presented in Figures 4 and 5. For pure calcium hydroxide, 84% of the total calcium hydroxide was released within 24 h, and 91% within 48 h. All microcapsules showed a slow release of calcium hydroxide when compared to the control. The release profile of calcium hydroxide-loaded microcapsules was shell material dependent. When more EC was

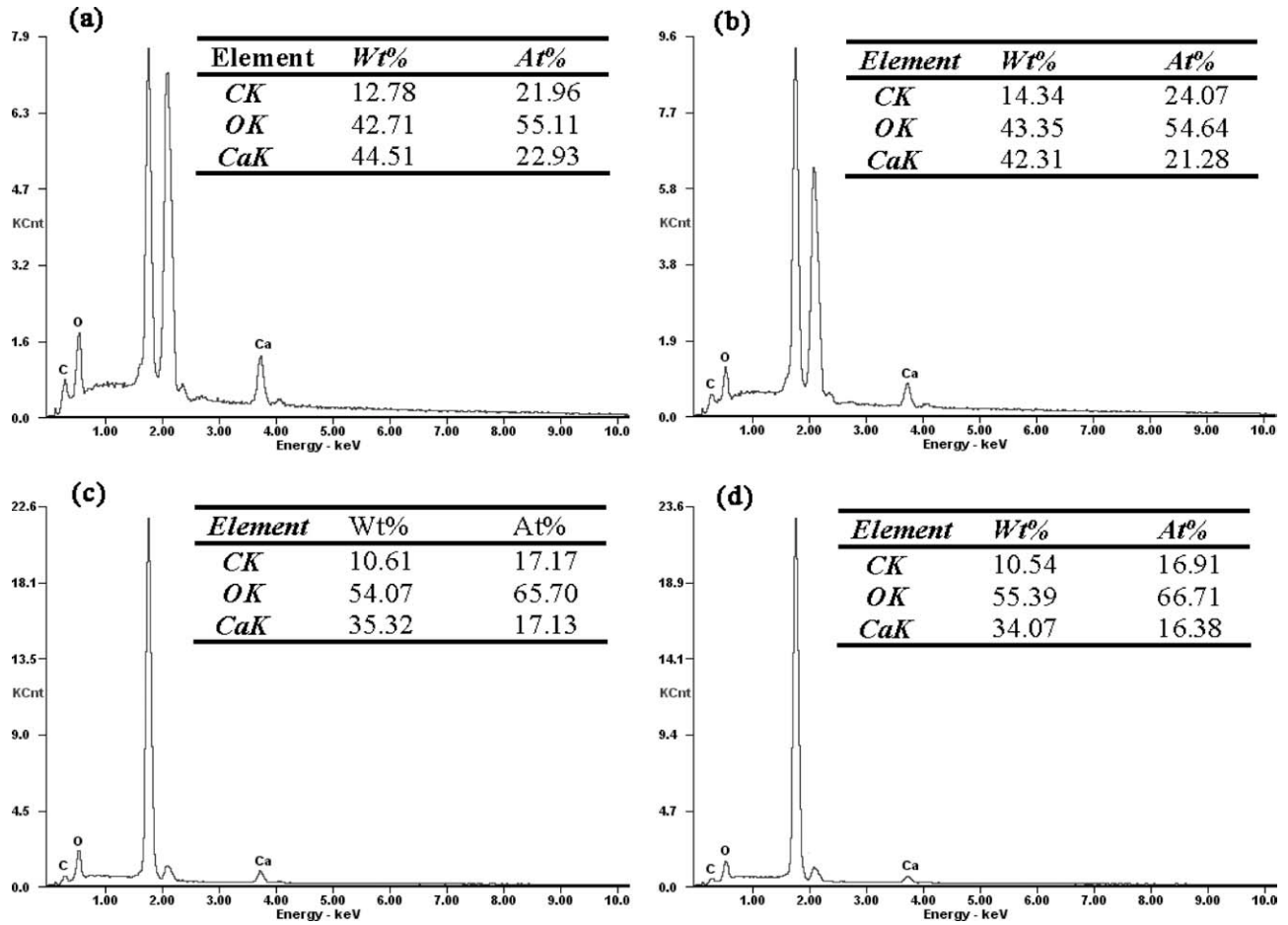

FIGURE 3. EDS spectra of microcapsules: (a) Formula A, (b) Formula B, (c) Formula C, (d) Formula D.

TABLE I. The T_g of PLA, EC, PLA/EC Blends, and Four Formulations of Microcapsules

Samples	T_g (°C)
PLA	57.24
EC	65.79
Polymer blend with PLA/EC ratio of 1/4	59.29
Polymer blend with PLA/EC ratio of 1/1	59.86
Polymer blend with PLA/EC ratio of 4/1	59.33
Microcapsule Formula A	65.90
Microcapsule Formula B	59.05
Microcapsule Formula C	59.65
Microcapsule Formula D	59.58

used as shell material, the release was much slower. It took 168, 264, 312, and 456 h for Formulas D, C, B, and A, respectively, to release 90% of their total calcium hydroxide content. The surrounding medium of all microcapsules and pure calcium hydroxide powder reached high pH values (above pH 11) rapidly, and the pH was maintained at pH 12. The release data of pure calcium hydroxide and the microcapsules were compared at 24, 48, 168, 264, 312, and 456 h using SPSS software (ANOVA/ post hoc Tukey's tests) (Table III). The release rates of the microcapsules were significantly slower than that of pure calcium hydroxide, and were dependent on the shell polymers ($p < 0.05$). As shown in Table IV, the kinetics of the calcium hydroxide release by the microcapsules fitted well to a first order model.

DISCUSSION

The aim of this study was to synthesize calcium hydroxide-loaded microcapsules based on PLA and EC carriers using phase separation. PLA has been approved by the FDA for clinical use as a biodegradable material. The biodegradation of PLA *in vivo* and *in vitro* have been verified in various studies.[19–21] EC is a cellulose-based material and can be degraded by enzymes and bacteria.[22–24] EC has been used widely as a coating material for drugs.[25,26] Using SEM, the microparticles produced were seen to be smooth and spherical. EDS confirmed the presence of calcium hydroxide in all microparticles. The microparticles were micrometer in scale, or smaller, as expected. Using TEM, the microparticles presented a core-shell, hetero-structure. These results indicate that calcium hydroxide was successfully encapsulated in the PLA/EC polymers.

A number of microencapsulation techniques have been developed including phase separation, solvent evaporation and extraction, and spraying drying. In this study, we used the phase separation (coacervation) method to prepare calcium hydroxide microcapsules. The phase separation process consisted of dissolving the polymer in an organic solvent, dispersing drug in the polymer solution and decreasing the solubility of the encapsulating polymer in a non-solvent which is miscible with the polymer solvent. The non-solvent should not dissolve either the polymer or the drug.[13] Calcium hydroxide is slightly soluble in water and insoluble in most organic solvents. Therefore methylene dichloride and petroleum ether were chosen as the organic solvent and non-solvent in this study. These two solvents are volatile and widely used for microencapsulation.[10,11] It has been reported that the stirring rate, addition of

TABLE II. Drug Loading and Encapsulation Efficiency of Calcium Hydroxide-Loaded Microcapsules

Samples	Drug Loading (%)	Encapsulation Efficiency (%)
Formula A	82.9 ± 9.5	62.9 ± 7.2
Formula B	81.8 ± 3.3	57.0 ± 2.3
Formula C	81.3 ± 10.1	68.6 ± 8.5
Formula D	82.2 ± 10.5	60.1 ± 7.7

Note: Both drug loading and encapsulation efficiency showed no significant difference among four formulations ($p > 0.05$).

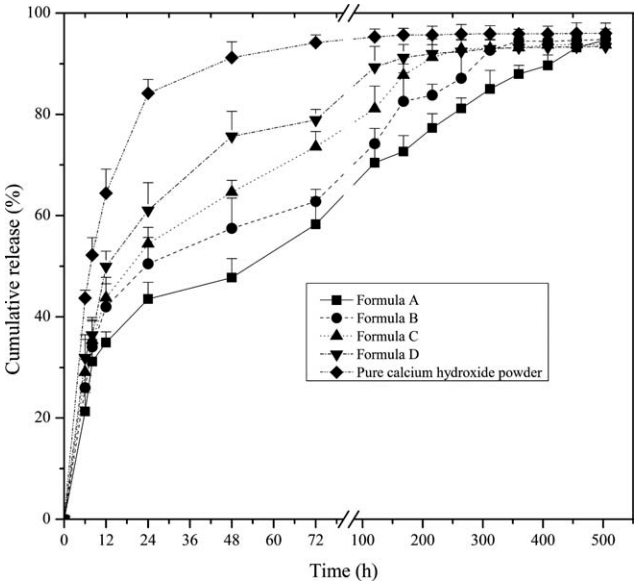

FIGURE 4. *In vitro* release profiles of calcium ion.

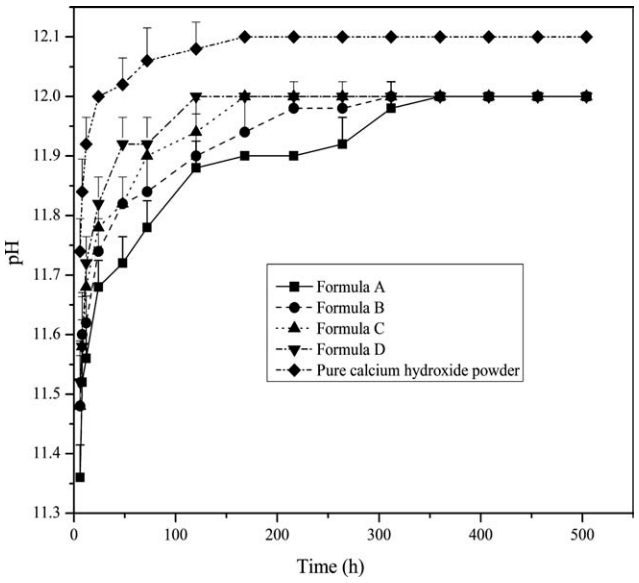

FIGURE 5. The pH profiles of microcapsules and pure calcium hydroxide powder.

TABLE III. Comparison of Calcium Ion Release by Calcium Hydroxide and Microcapsules

Sample	24 h (%)	48 h (%)	168 h (%)	264 h (%)	312 h (%)	456 h (%)
Formula A	43.5 ± 3.3^a	47.7 ± 3.8^a	72.6 ± 3.2^a	81.2 ± 2.1^a	85.0 ± 3.7^a	93.2 ± 2.6^a
Formula B	50.5 ± 5.2^b	57.5 ± 6.0^b	82.6 ± 5.6^b	87.1 ± 5.5^b	92.7 ± 2.0^b	94.6 ± 1.2^a
Formula C	54.4 ± 3.2^b	64.6 ± 2.4^c	87.8 ± 2.2^c	92.9 ± 1.9^c	93.0 ± 1.8^b	93.8 ± 1.5^a
Formula D	61.1 ± 5.5^c	75.6 ± 5.0^d	91.2 ± 2.6^c	92.4 ± 2.2^c	93.0 ± 3.1^b	93.3 ± 2.1^a
Pure calcium hydroxide	84.2 ± 2.7^d	91.2 ± 3.1^e	95.7 ± 1.4^d	95.9 ± 1.9^c	95.9 ± 1.6^b	96.0 ± 2.0^a

Note: Different superscript letters (a,b,c,d,e) indicate significant difference among calcium hydroxide and four formulations of microcapsules ($p < 0.05$).

non-solvent and mixing time (the duration of the hardening stages) affect the size distribution, surface morphology and internal porosity of the final microparticles.[13,27] The addition rate of non-solvent should allow the polymer solvent to extract slowly, so that the polymer has sufficient time to deposit uniformly on the drug particle surface during the coacervation process.[13] During the phase separation process used in this study, therefore, the non-solvent was added in a drop-wise fashion.

This study also investigated the effect of shell material on the properties of the calcium hydroxide microcapsules. Results showed that the particles size of the microcapsules decreased with increasing EC proportion in the shell polymer. This is likely to be due to differences in the viscosity of the PLA and EC solution, as the preparation method parameters, such as stirring rate, addition of non-solvent and mixing time, were well controlled. Compared with PLA, EC has a lower viscosity in the dispersed phase and lower viscous resistance against shear forces during phase separation, leading to the formation of smaller particles when adding the flocculant.[11] The addition of calcium hydroxide did not markedly influence the T_g of PLA/EC blends, which were higher than the environmental temperature *in vivo*. No significant differences in drug loading or encapsulation efficiencies were observed among the four microcapsule formulations. In this study, the input ratio of calcium hydroxide to shell polymer was fixed at 4:1 for all microcapsules. Regardless of the polymer composition, all microcapsules showed higher drug loading than the theoretical drug loading (80%). This may be due to loss of PLA and EC by dissolution in methylene dichloride during the phase separation process.

In vitro calcium hydroxide release was investigated in de-ionized water using a standard dialysis bag model.[11] Pure calcium hydroxide powder exhibited fast diffusion behavior. More than 50% of the calcium hydroxide was released within 8 h and 84% was released within 24 h. When calcium hydroxide was encapsulated, the release was significantly slower, with the release rate depending on the formula of the shell polymers. All microcapsules released calcium hydroxide largely via a diffusion mechanism. The slower release of calcium hydroxide from microcapsules was clearly due to the barrier effect of the shell material. It was observed that the composition of polymers affected microcapsule release behavior. A higher EC content in the polymer blend led to a slower release of encapsulated calcium hydroxide. This can be largely attributed to the lower permeability of EC films.[17] Another possible reason may be the occurrence of complex/non-covalent interactions of Ca^{2+} with the hydroxyl groups of the EC polymer.[28] Such interactions might lead to the slower release of Ca^{2+} from microcapsules with EC. Although calcium hydroxide was encapsulated and released much slower, all microcapsules showed a slight tendency to undergo burst release in the first 24 h, which may be attributed to the higher osmotic pressure in the early release period. This burst release effect would quickly increase the concentration of calcium hydroxide to an effective concentration, and this release profile may be beneficial to the control of infection in the early stages, when used in dentistry.

In conclusion, calcium hydroxide-loaded biodegradable microcapsules, based on PLA and EC polymers, were synthesized using phase separation. These microcapsules successfully prolonged the release of calcium hydroxide *in vitro*.

TABLE IV. Release Kinetics of Calcium Hydroxide and Microcapsules

Samples	First Order	Higuchi Kinetics
Formula A	$\mathrm{Ln}(1 - Q) = -0.005t - 0.453$ $r^2 = 0.987$	$Q = 0.033t^{1/2} + 0.268$ $r^2 = 0.972$
Formula B	$\mathrm{Ln}(1 - Q) = -0.007t - 0.498$ $r^2 = 0.980$	$Q = 0.037t^{1/2} + 0.298$ $r^2 = 0.972$
Formula C	$\mathrm{Ln}(1 - Q) = -0.009t - 0.521$ $r^2 = 0.982$	$Q = 0.046t^{1/2} + 0.293$ $r^2 = 0.956$
Formula D	$\mathrm{Ln}(1 - Q) = -0.012t - 0.606$ $r^2 = 0.941$	$Q = 0.051t^{1/2} + 0.320$ $r^2 = 0.902$
Pure calcium hydroxide	$\mathrm{Ln}(1 - Q) = -0.032t - 0.730$ $r^2 = 0.922$	$Q = 0.071t^{1/2} + 0.395$ $r^2 = 0.857$

Note: Q represents cumulative release of calcium ion (%); t represents release time (h); r^2 represents correlation coefficient.

It is also noteworthy that the release of calcium hydroxide could be controlled by regulating the ratio of PLA/EC used to form the microcapsules.

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