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Designing multilayered particulate systems for tunable drug release profiles

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ABSTRACT

Triple-layered microparticles comprising poly(D,L-lactide-co-glycolide, 50:50) (PLGA), poly(L-lactide) (PLLA) and poly(ethylene-co-vinyl acetate, 40 wt.% vinyl acetate) (EVA) were fabricated using a one-step solvent evaporation technique, with ibuprofen drug localized in the EVA core. The aim of this study was to investigate the drug release profiles of these triple-layered microparticles in comparison to double-layered (PLLA/EVA and PLGA/EVA) (shell/core) and single-layered EVA microparticles. Double- and triple-layered microparticles were shown to eliminate burst release otherwise observed for single-layered microparticles. For triple-layered microparticles, the migration of acidic PGA oligomers from the PLGA shell accelerated the degradation of the PLLA mid-layer and subsequently enhanced drug release in comparison to double-layered PLLA/EVA microparticles. Further studies showed that drug release rates can be altered by changing the layer thicknesses of the triple-layered microparticles, and through specific tailoring of layer thicknesses, a zero-order release can be achieved. This study therefore provides important mechanistic insights into how the distinctive structural attributes of triple-layered microparticles can be tuned to control the drug release profiles.

1. Introduction

Biocompatible and biodegradable polymeric microparticles have received significant interest in the drug delivery field for the past few decades [1,2]. This is because they have the potential to optimize drug concentration at the site of action over prolonged periods. However, there are several inherent limitations on the use of conventional single-layered microparticles in drug delivery, which include burst release caused by the presence of entrapped drug particles on the surface [3], the inability to provide zero-order release and limitations in controlling drug release profiles and kinetics [4–7].

To better control the drug release rates of polymeric microparticles, the tailoring of the structural composition (e.g. multilayers, multicompartmental particles) of the polymeric matrix was thus introduced [4,8–12]. The use of multilayered or multicompartmentalized microcapsules also allows for simultaneous multiple drug delivery in biomedicine [11,13,14]. The release of encapsulated substance from polyelectrolyte-multilayer and multicompartmental capsules had been demonstrated by using various triggering mechanisms, such as laser light and ultrasound [15,16]. On the other hand, it had been reported that drugs localized within the inner cores of double-layered microparticles prepared by the solvent evaporation technique showed a reduction in burst release, while providing a sustained drug release profile [17,18]. Though studies of drug release from double-layered microparticles have been reported, achieving a desirable drug release profile with optimal release kinetics still remains very much a challenge, and no such studies have been conducted using triple-layered microparticles. The development of triple-layered or even multilayered microparticles can be an important step towards a versatile and robust approach to control the drug release rates, while having the capabilities of delivering multiple drugs (polypharmacies) from the same microparticle, where some layers contain drug substances; others are rate-limiting layers. Multilayered microparticles could be envisioned to provide pulsatile or time-delayed drug release kinetics, such as in the case of delivering vaccines. In addition, drug delivery to the small intestine or colon can be achieved using multilayered microparticles with an enteric shell, while housing multiple drugs in other layers for controlled release. It would also appear that delivery of radiosensitizers and/or anticancer drugs simultaneously through the use of multilayered microparticles would be attractive for cancer therapy. Furthermore, multilayered particulate devices could also be used as scaffold materials to deliver bioactive molecules to cells for tissue engineering. Nevertheless, the large size of the multilayered microparticles is a hindrance for intravenous injection, and surgical operations become a necessity for implantation.

Previous studies have shown that the degradation behavior of multilayered systems differed from that of a monolithic system [19–21]. It was thus postulated that triple-layered microparticles would possess uniquely different hydrolytic degradation

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characteristics, in comparison to single-layered and double-layered microparticles, which could potentially be tapped to alter and fine-tune the drug release profiles. The first objective of this paper was therefore to study the difference in drug release profiles of single-layered (EVA), double-layered (PLGA/EVA and PLLA/EVA) and triple-layered (PLGA/PLLA/EVA) microparticles. Particle sizes were kept similar for unbiased comparisons across these sample groups (single- to triple-layered). Hydrophobic ibuprofen, as a model drug, was selectively localized within the EVA polymer core for all microparticles.

Having established the difference in drug release profiles between single- to triple-layered microparticles, the second objective was to understand how different particle parameters, such as layer thickness, of triple-layered microparticles would have further effects on the drug release profiles. From the knowledge gained from these studies, triple-layered, and essentially multilayered, microparticles can be designed to achieve well-controlled drug release kinetics suiting a wide variety of biomedical applications.

2. Materials and methods

2.1. Materials

Poly(L-lactide) (PLLA) (intrinsic viscosity (IV): 2.38, Bio Invigor), poly(D,L-lactide-co-glycolide, 50:50) (PLGA) (IV: 1.18, Bio Invigor), poly(ethylene-co-vinyl acetate, 40 wt.% vinyl acetate) (EVA) (molecular weight (MW): 42 kDa, Aldrich) and poly(vinyl alcohol) (PVA) (MW: 30–70 kDa, Sigma–Aldrich) were used without further purification. Ibuprofen was purchased from Sigma–Aldrich and used as received. Dichloromethane (DCM), tetrahydrofuran (THF) and chloroform from Tedia Company Inc. were of high-performance liquid chromatography (HPLC) grade and used as received. Phosphate buffered saline (PBS, pH 7.4) solution was purchased from OHME, Singapore.

2.2. Fabrication of microparticles

Triple-layered PLGA/PLLA/EVA microparticles were prepared using the oil-in-water (o/w) emulsion solvent evaporation method [22,23]. Briefly, the three polymers (0.2 g of PLGA, 0.1 g of PLLA, 0.05 g of EVA, PLGA/PLLA/EVA 4:2:1) and ibuprofen (5% w/w) were dissolved in DCM to form a polymer solution (7.5% w/v). Afterwards, the resulting solution was first ultrasonicated for 1 min using an ultrasonic processor (Sonic Vibra-Cell VC 130) to achieve complete homogenization. This solution was then poured into the aqueous solution containing PVA (0.5% w/v), with an oil-to-water ratio (0.013). The emulsion was stirred at 400 rpm using an overhead stirrer (Calframo BDC1850-220) at room temperature (25 °C). The evaporation of DCM gave rise to phase separation of PLGA, PLLA and EVA, yielding triple-layered microparticles. Finally, the particles were centrifuged, rinsed with deionized water, lyophilized and stored in a desiccator for further characterization. The method to obtain triple-layered PLGA/PLLA/EVA 4:2:1 microparticles was employed to produce triple-layered microparticles with different mass ratios (PLGA/PLLA/EVA 10:2:1, 10:5:1 and 2:4:1), double-layered PLGA/EVA 6:1 microparticles, double-layered PLLA/EVA 6:1 microparticles and single-layered EVA microparticles.

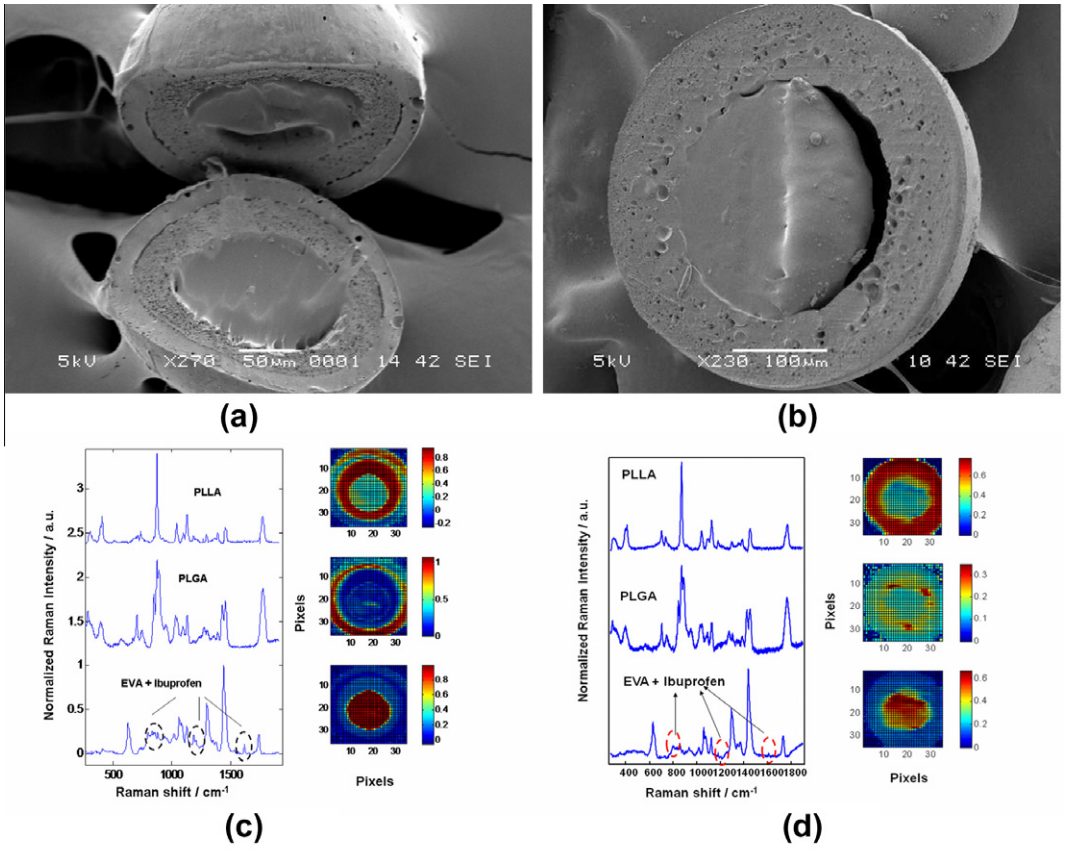

Fig. 1. SEM images of cross-sectional views of triple-layered PLGA/PLLA/EVA microparticles and their corresponding pure component Raman spectra estimates and associated score images obtained via BTEM. (a, c) PLGA/PLLA/EVA 4:2:1 and (b, d) PLGA/PLLA/EVA 2:4:1.

2.3. Characterization

2.3.1. Morphological analysis

The surface and internal morphologies of the microparticles were observed using scanning electron microscopy (SEM, JSM-6360) at an operating voltage of 5 kV. Before analysis, the samples that had been cross-sectioned approximately at the centerline with a razor blade were first mounted onto metal stubs. Samples were then gold-coated using a sputter coater model SPI-Module. At least three independent batches for each particle type were fabricated, and ten microparticles from each independent batch were randomly chosen for SEM analysis. Only one representative SEM image is shown due to consistent particle internal morphologies within each batch for a particle group type. Particle size and layer thickness measurements were conducted using the ImageJ software from the SEM images.

2.3.2. Determination of particle configuration and drug distribution

Raman mapping was utilized to verify the particle configuration (i.e. polymer distribution) and drug distribution within the microparticles. Pre-sectioned microparticles were placed under the microscope objective with laser power of up to ~20 mW. Raman point-by-point mapping measurements were performed on an area of 350 × 340 μm with a step size of 10 μm in both the *x* and *y* directions using a Raman microscope (In-via Reflex, Renishaw) equipped with a near infrared enhanced deep depleted hermoelectrically Peltier-cooled CCD array detector (576 × 384 pixels) and a high grade Leica microscope. The sample was irradiated with a 785 nm near infrared diode laser and a 20× objective lens was used to collect the backscattered light. Measurement scans were collected using a static 1800 groove per mm dispersive grating in a spectral window from 300 to 1900 cm^{-1}. The acquisition time for each spectrum was ~35 s. Spectral pre-processing, including removal of spikes due to cosmic rays, were carried out first and the Raman mapping data were subsequently analyzed using the band target entropy minimization (BTEM) algorithm [24,25]. The BTEM algorithm was developed in order to reconstruct pure component spectral estimates. When all normalized pure component spectra of all underlying constituents have been reconstructed, the relative contributions of each measured point of these signals were calculated by projecting them back onto the baseline-corrected and normalized data set. The spatial distribution of each underlying constituent (i.e. PLGA, PLLA, EVA, and ibuprofen) was then generated. The color-coded scale represents the intensities of score image of each component, while the summation of the intensities (color-coded scale) of all components at each particular pixel is equal to unity. These score images can be used to show the spatial distribution and the semi-quantitative content of all constituents in a microparticle.

2.3.3. Determination of actual ibuprofen loading

The determination of actual loading of ibuprofen was conducted through thermogravimetric analysis (TGA Q500 V6.5 Build 196). Ibuprofen-loaded microparticles (~10 mg) were placed in a platinum pan. Ibuprofen is known to decompose at ~160 °C, which is lower than that of the polymers (~300 °C). Thus, the measurements were performed at 10 °C min^{-1} from room temperature to 160 °C under nitrogen flow of 60 ml min^{-1}, followed by isothermal heating at 160 °C for 1 h, then ramping to 500 °C. Each sample was measured in triplicate.

2.4. Hydrolytic degradation

Microparticles (50 mg) were weighed in triplicate and placed in vials containing PBS solution (pH 7.4, 30 ml) and incubated at 37 °C under moderate shaking. The pH of the PBS solution was monitored and maintained at 7.4 at all times. All the microparticles in a vial were removed at pre-determined time points.

2.4.1. Water uptake study

The microparticles after rinsing with distilled water were weighed, and then vacuum-dried to a constant mass and the dry mass was measured. Water uptake was calculated at each time point according to the difference between the mass of the wet and dry particles, measured at time *t*, and taken as a percentage of the dry mass. Values obtained for triplicate samples were averaged.

2.4.2. Mass loss study

Polymer mass loss was taken as the difference between the initial mass of the microparticles and dry mass at time *t*, and normalized by dividing by the initial mass and reported in terms of percentage. Three vials for each sample were used and the results were averaged.

2.4.3. Molecular weight analysis

The molecular weight of the polymer at each time point was determined using size exclusion chromatography (SEC) (Agilent 1100 Series LC System) with relative to the calibration curve using polystyrene standards (165–5000 kDa). PLLA was separated from PLGA and EVA by the dissolution method, based on the solubility

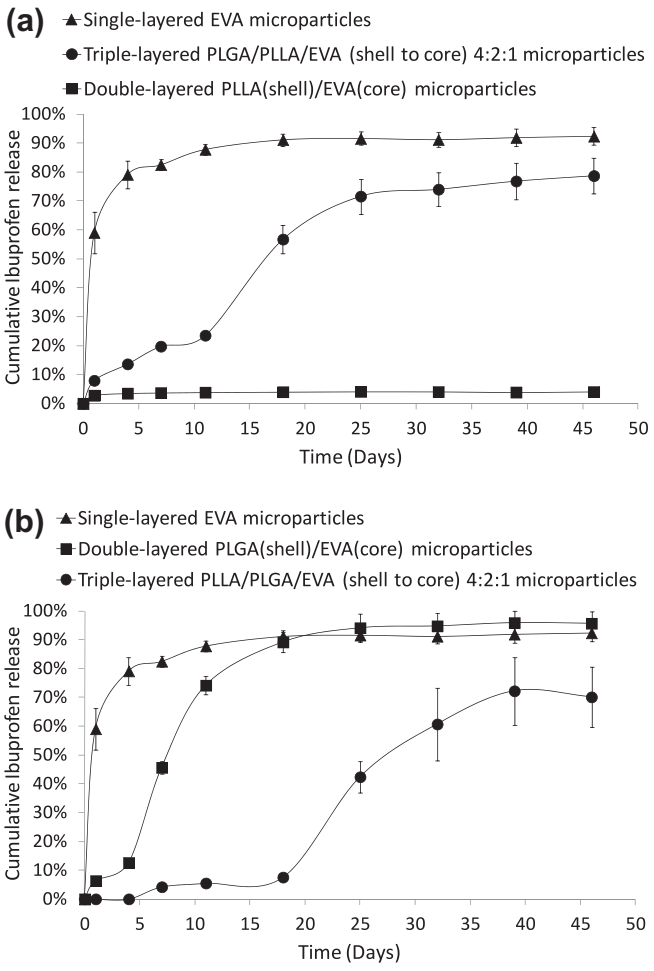

Fig. 2. Release profiles of ibuprofen from (a) the single-layered EVA, double-layered PLLA/EVA and triple-layered PLGA/PLLA/EVA 4:2:1 microparticles, (b) the single-layered EVA, double-layered PLGA/EVA and triple-layered PLLA/PLGA/EVA 4:2:1 microparticles.

differences of the polymers in THF (i.e. PLGA and EVA are soluble in THF while PLLA is not). Microparticles (8 mg) were first immersed in THF (1 ml) to dissolve the PLGA and EVA contents. PLLA remnant and PLGA/EVA solution were then separated by centrifugation. The solvent in the polymer solution containing PLGA and EVA was evaporated slowly in air at room temperature for 48 h. PLGA, PLLA and EVA were then further dried in an oven at 40 °C for a week. Afterwards, PLGA/EVA mixture and PLLA were dissolved separately in chloroform (1 ml) and tested using SEC.

2.4.4. Differential scanning calorimetry (DSC)

The glass transition temperatures (T_g) of the polymers in microparticles were determined using modulated differential scanning calorimetry (MDSC™) (DSC 2920, TA Instruments, USA). The sample and reference pans were purged with nitrogen at a constant flow rate of 48 ml min^{-1} to avoid oxidative degradation. Each sample (~5 mg) was sealed in an aluminium pan and heated from −40 °C to 200 °C for the first heating ramp, cooled to −40 °C, and finally reheated with the second ramp to 200 °C, all at a rate of 5 °C min^{-1}. Thermograms were analyzed using TA universal analyzer software for the determination of T_g.

2.5. Drug release study

Ibuprofen-loaded microparticles (5 mg) in triplicate were placed in vials containing PBS solution (pH 7.4, 5 ml) and incubated in a shaking incubator (37 °C). At pre-determined time intervals, in vitro medium (1 ml) from each sample was drawn out and the amount of ibuprofen released was measured using the Shimadzu UV-2501 UV–Vis spectrophotometer at 220 nm, before replenishing with fresh PBS solution.

2.6. Statistical analysis

Drug release, mass loss and water uptake data from different particle groups were evaluated by unpaired Student's *t*-test and the one-way ANOVA analysis coupled with the Tukey test. Differences were considered statistically significant when $p \leqslant 0.05$.

3. Results and discussion

3.1. Single-, double- and triple-layered microparticles

3.1.1. Morphological properties of single-, double- and triple-layered microparticles

Single-, double- and triple-layered microparticles were fabricated and carefully characterized prior to assessing their drug release profiles. Fig. 1 shows the scanning electron microscopy (SEM) images of the triple-layered PLGA/PLLA/EVA microparticles with different polymer mass ratios (4:2:1 and 2:4:1) (refer to Supplementary information for SEM images of single-layered and double-layered microparticles). The microparticles had a smooth surface, uniform layer thickness and were spherical in shape. Particle sizes of ibuprofen-loaded triple-layered PLGA/PLLA/EVA 4:2:1 microparticles (Fig. 1a) measured from SEM were 270.4 ± 81.1 μm. The particle size distribution is dependant on the process parameters such as stirring speed and surfactant concentration [17]. Since fabrication conditions were kept constant, comparable particle size

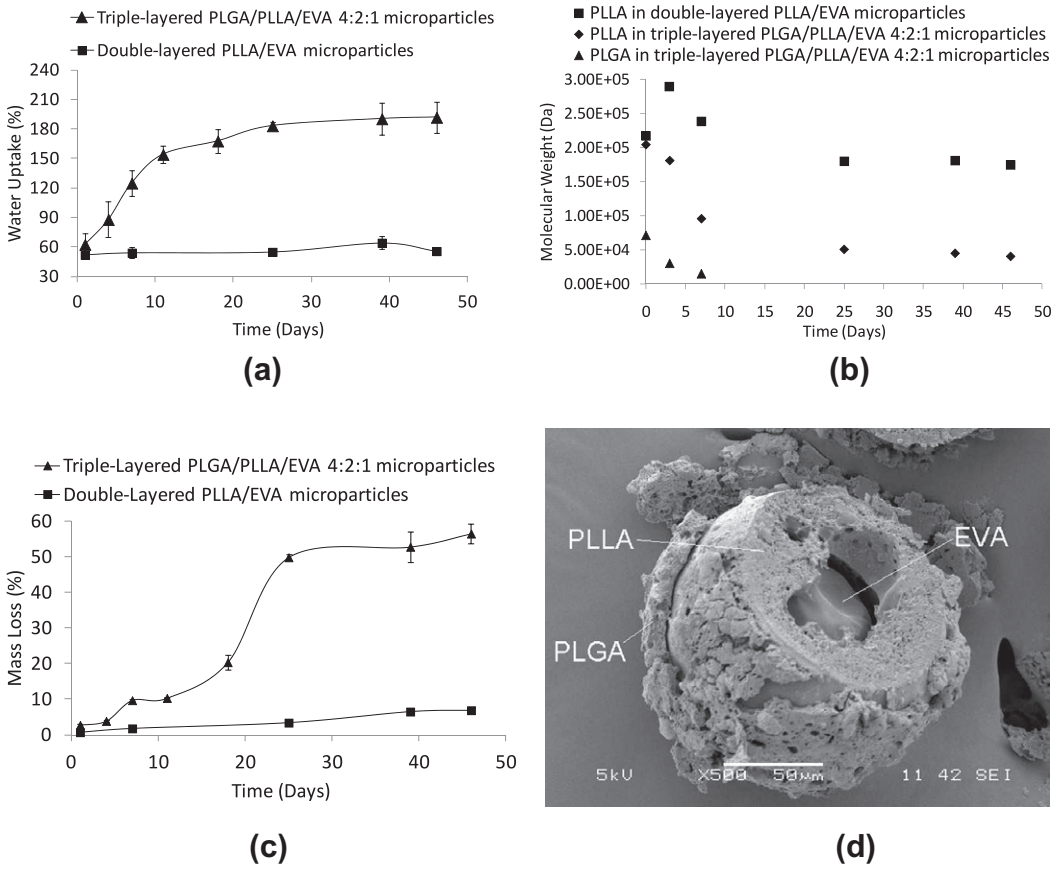

Fig. 3. (a) Water uptake, (b) change in molecular weights of PLGA and PLLA and (c) mass loss of the degrading double-layered PLLA(shell)/EVA(core) microparticles and triple-layered PLGA/PLLA/EVA (shell to core) 4:2:1 microparticles as a function of incubation time. (d) SEM image of the degrading triple-layered PLGA/PLLA/EVA 4:2:1 microparticles after 18 d in vitro.

distributions were also observed for microparticles produced from other polymer pairs. It had been shown that this solvent evaporation technique can be used to prepare triple-layered microparticles with smaller sizes (down to ~20 μm) by increasing the stirring speed in the final emulsification step [23]. As it is difficult to study the internal morphology of small microparticles, larger microparticles were fabricated for study in this work.

From the Raman mapping results (Fig. 1c), it was evident that the triple-layered PLGA/PLLA/EVA 4:2:1 microparticles had an outermost shell, middle layer, and a core, composed of PLGA, PLLA, and EVA, as the abundant regions, respectively. Raman mapping also confirmed that ibuprofen was localized in the EVA core. The reason for this preferential localization of ibuprofen in the EVA core was the strong affinity between the hydrophobic long ethylene chains of EVA and hydrophobic ibuprofen. When the polymer mass ratio of PLGA/PLLA/EVA was changed from 4:2:1 to 2:4:1, a layer inversion between PLGA and PLLA was observed (Fig. 1d), whereby PLLA now formed the outermost shell, and PLGA as the middle layer, with ibuprofen still localized in the EVA core. Drug localization within the microparticles is therefore driven by polymer-drug affinity, and is not affected by changes to the polymer content or microparticle structure configuration [17,18,23,26].

3.1.2. Release of ibuprofen from microparticles

The ibuprofen release profiles of triple-layered PLGA/PLLA/EVA microparticles were compared with single- and double-layered microparticles. The actual drug contents were kept at 4 ± 0.5% w/w, and ibuprofen was localized in the EVA polymer core, in all cases.

Fig. 2 plots the release profiles of ibuprofen from the various microparticles. It was observed that each microparticle system yielded a different release pattern. The single-layered EVA microparticles had a very rapid release due to its highly flexible rubbery state [27]. For the double-layered and triple-layered microparticles, the shell provided additional barriers to the diffusion of ibuprofen encapsulated in the EVA core, thus limiting initial bursts and reducing any rapid drug loss. As observed from Fig. 2a, the PLLA shell of the double-layered PLLA/EVA microparticles substantially retarded the diffusion of ibuprofen localized in the EVA core due to the slow degradation of PLLA. However, interestingly, with the addition of PLGA shell as the third layer to form the PLGA(-shell)/PLLA(middle layer)/EVA(core) triple-layered structure (PLGA/PLLA/EVA 4:2:1), the release of ibuprofen was enhanced and a nearly zero-order release was achieved.

On the other hand, for double-layered PLGA(shell)/EVA(core) microparticles, the release of ibuprofen was faster than that from the triple-layered PLLA/PLGA/EVA 4:2:1 microparticles, as shown in Fig. 2b. With the introduction of a PLLA shell to the double-layered PLGA/EVA microparticles, it was observed that the triple-layered PLLA/PLGA/EVA 4:2:1 microparticles exhibited a lag in the release of ibuprofen up till day 18, before the drug was subsequently released. This release profile can therefore be described as time-delayed release.

The release profiles of the different microparticles can be explained from both water uptake and hydrolytic degradation rates of these microparticles. As shown, double-layered PLLA/EVA microparticles were observed to exhibit a rather suppressed release of ibuprofen when compared to the triple-layered PLGA/PLLA/EVA 4:2:1 microparticles. The semi-crystalline morphology and relatively hydrophobic nature of the PLLA shell retards any appreciable water influx (Fig. 3a) [28], thereby explaining for the suppressed release from the PLLA/EVA microparticles. Water influx was observed, however, to increase drastically with time for the triple-layered PLGA/PLLA/EVA microparticles. The higher water uptake of the triple-layered microparticles was due to the presence of the relatively more hydrophilic PLGA shell, arising from its fully amorphous nature and lower glass transition temperature (T_g) (see Supplementary information). This higher water uptake accelerated the degradation of microparticles, as shown in Fig. 3b (molecular weight of EVA was not plotted because it was found to be consistent throughout the degradation study). As the PLGA shell degraded, some soluble oligomers were leached out (resulting in mass loss), while the remaining PGA oligomers diffused towards the PLLA layer [29], as observed from the Raman mapping results shown from Fig. 4. These migrating oligomers resulted in an increased hydrophilicity and acidity of the PLLA layer, which accelerated the hydrolysis of PLLA. In summary, as compared to the double-layered PLLA/EVA microparticles, the higher water uptake of triple-layered microparticles caused the degradation of PLGA, and subsequently accelerated the PLLA degradation, thus achieving a faster release of ibuprofen (Fig. 2a).

The release of ibuprofen from triple-layered PLGA/PLLA/EVA 4:2:1 microparticles was observed to go through two stages; an

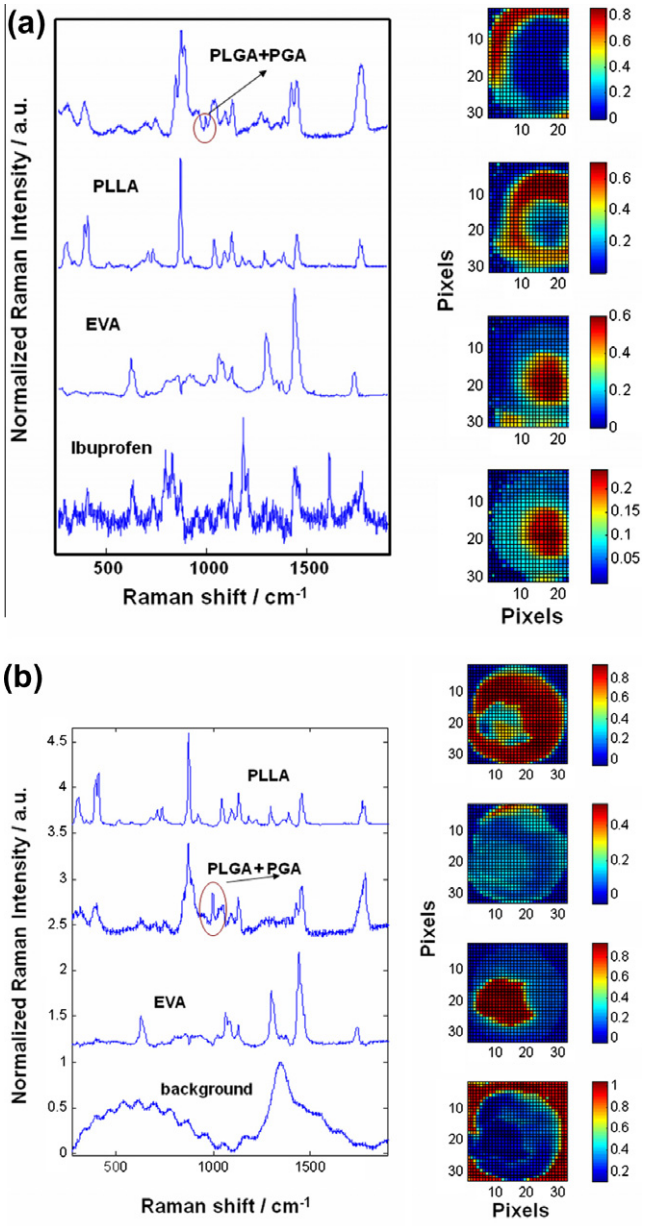

Fig. 4. Pure component Raman spectra estimates and their associated score images obtained via BTEM from a ibuprofen-loaded triple-layered PLGA/PLLA/EVA 4:2:1 microparticles (a) after 4 d and (b) after 25 d in vitro.

initial release of ~20% up till day 10, followed by a relatively rapid release up to 32 d, where it eventually plateaus off. According to Raghuvanshi et al. [30], the initial stage of degradation involves random chain scission, where the molecular weights of polymers decreased significantly (Fig. 3b) but with no appreciable mass loss, as observed for the triple-layered microparticles up to 10 d (Fig. 3c). Drug release was mainly dominated by diffusion during this initial stage of degradation. As evident from Raman mapping (Fig. 4a), some ibuprofen was observed to have diffused into the PLLA and PLGA layers during the early period of release.

After 10 d, as the polymer degradation entered into the second stage of degradation process, the rapid mass loss caused the formation of pores in the PLGA layer (Fig. 3c and d). During this time, the PLLA middle layer was also observed to undergo massive degradation (with significant decrease in its molecular weight), as shown from Fig. 3b. The increased formation of pores in the PLGA layer and the enhanced degradation of the outer layers (PLLA and PLGA) allowed for faster rate of release of ibuprofen. After 25 d in vitro, with substantial mass loss, the PLGA shell was observed to have almost completely degraded (Fig. 4b), and was no longer detectable on the size exclusion chromatography (SEC) results (Fig. 3b). In addition, no Raman signals of ibuprofen were detected, which could be attributed to the very low ibuprofen concentration in the microparticles, with ~70% ibuprofen already released at this stage (Fig. 4b).

From Fig. 2b, triple-layered PLLA/PLGA/EVA 4:2:1 microparticles, with PLLA as the outermost shell, were observed to have a slower release rate than that from the double-layered PLGA/EVA

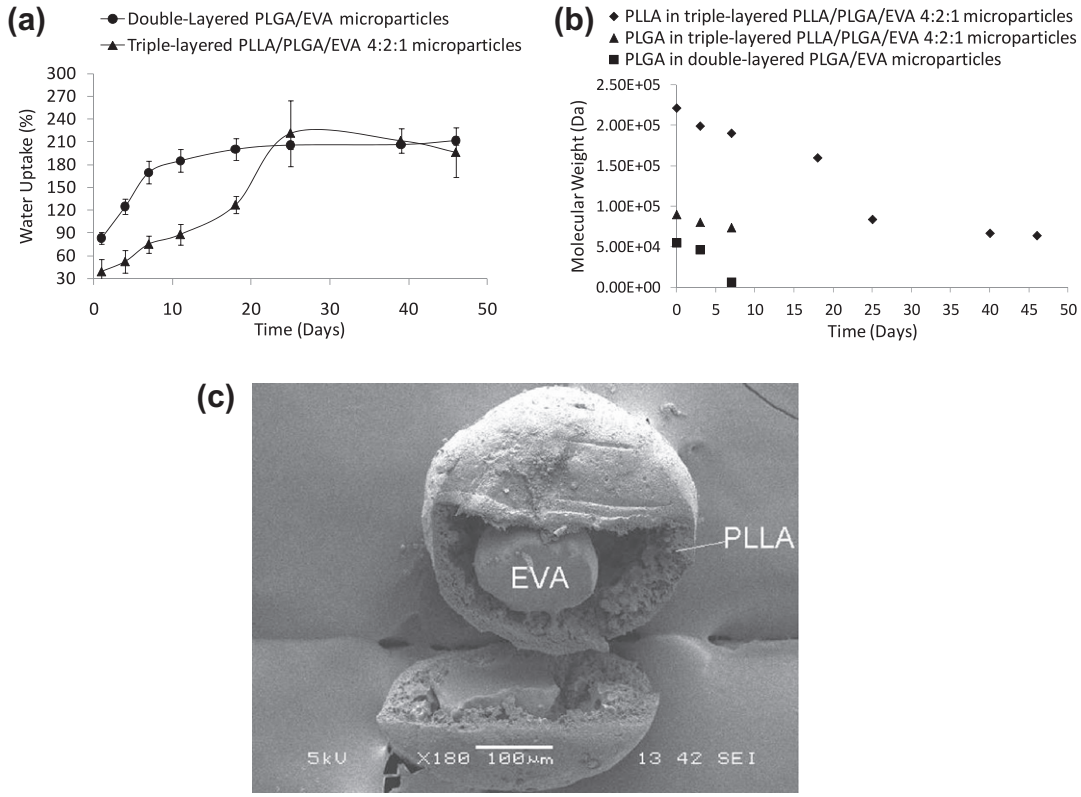

Fig. 5. (a) Water uptake and (b) change in molecular weights of PLGA and PLLA of the degrading double-layered PLGA(shell)/EVA(core) microparticles and triple-layered PLLA/PLGA/EVA (shell to core) 4:2:1 microparticles as a function of incubation time. (c) SEM image of the degrading triple-layered PLLA/PLGA/EVA 4:2:1 microparticles after 25 d in vitro.

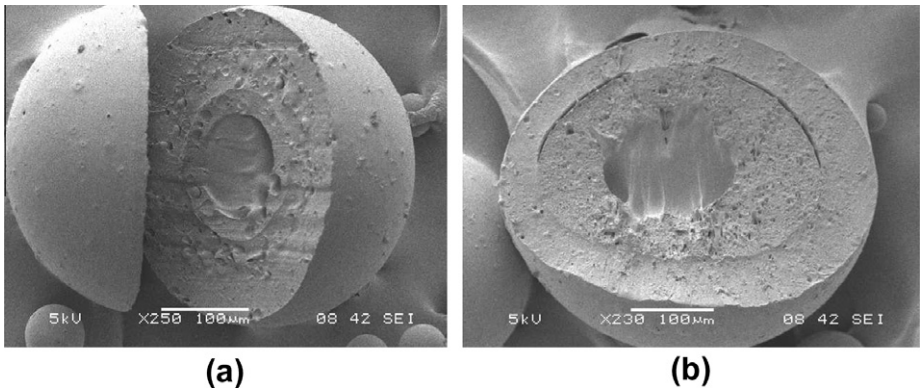

Fig. 6. SEM images of cross-sectional views of triple-layered PLGA/PLLA/EVA (shell to core) microparticles. (a) PLGA/PLLA/EVA 10:2:1 and (b) PLGA/PLLA/EVA 10:5:1.

microparticles. The latter had a faster release of ibuprofen due to the higher water uptake (Fig. 5a), during the initial periods, arising from the fully amorphous and rapidly degrading PLGA shell. When a layer of PLLA (as shell) was added onto the double-layered microparticles, this semi-crystalline and relatively more hydrophobic PLLA shell retards water influx [26,28], thus slowing the degradation of the polymers (Fig. 5b). This result explains the lag phase observed for the triple-layered microparticles. After ~20 d, drug was observed to be released over time. During this period, it was noted that a significant decrease in the molecular weight of PLLA was observed (Fig. 5b). This change in molecular weight of PLLA coincided with the results from the SEM micrograph showing that the PLGA middle layer had significantly eroded, leaving behind gaps between the PLLA shell and EVA core (Fig. 5c). These concurrent events therefore gave rise to an accelerated release of ibuprofen, after the initial time-delayed release.

3.2. Triple-layered microparticles of different layer thicknesses

3.2.1. Morphological properties

Triple-layered microparticles of different layer thicknesses were fabricated to study the effect of these on the drug release profiles. All triple-layered microparticles described here possessed the same polymer distribution (results not shown), whereby the shell, middle layer and inner core were PLGA, PLLA and EVA, respectively [23]. For the triple-layered PLGA/PLLA/EVA 4:2:1 microparticles, the average layer thicknesses of the PLGA shell and PLLA mid-layer were 21.7 ± 4.9 μm and 36.5 ± 6.2 μm, respectively. When the polymer mass ratio of PLGA/PLLA/EVA 4:2:1 was changed to 10:2:1 (Fig. 6a), the PLGA shell thickness increased to 57.9 ± 9.1 μm, while the thickness of the mid-layer was 25.7 ± 5.8 μm. On the other hand, microparticles with mass ratio of PLGA/PLLA/EVA 10:5:1 (Fig. 6b) had thicker layers (i.e. PLGA 32.7 ± 7.34 μm and PLLA 51.2 ± 10.5 μm) in comparison to PLGA/PLLA/EVA 4:2:1.

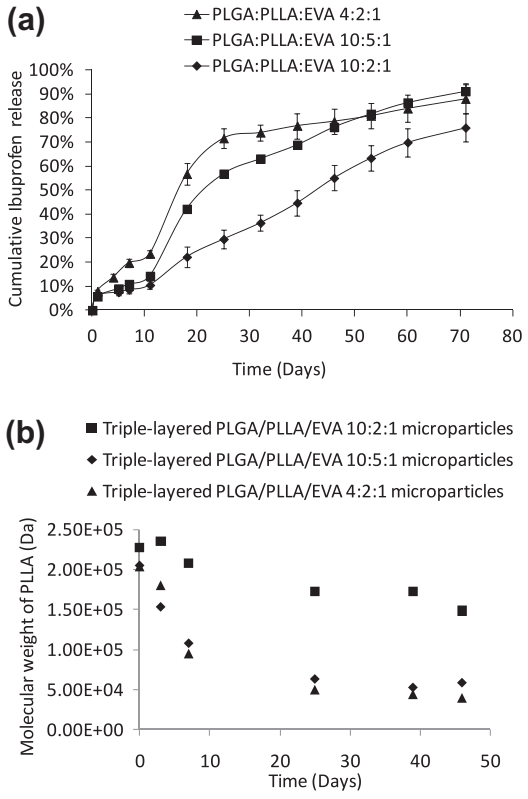

Fig. 7. (a) Release profiles of ibuprofen from the triple-layered microparticles of different layer thicknesses. (b) Change in molecular weight of PLLA in the triple-layered microparticles of different layer thicknesses as a function of incubation time.

3.2.2. Effect of layer thicknesses on ibuprofen release

The effects of layer thicknesses on ibuprofen release from the triple-layered microparticles (drug loading of 4 ± 0.5% w/w and drug located in the EVA core in all cases) were investigated. From Fig. 7a, it was observed that increasing the shell thickness of PLGA (PLGA/PLLA/EVA 4:2:1 vs. 10:2:1) resulted in a reduction in drug release rate. This could be attributed to a longer traveling distance for ibuprofen to diffuse through the thicker outer layer. When both PLGA and PLLA layer thicknesses are increased (i.e. 10:5:1 microparticles), it was also observed that drug release rates were reduced, as compared to 4:2:1 microparticles.

When comparing PLGA/PLLA/EVA 10:5:1 microparticles to 10:2:1 microparticles, one would expect that the latter microparticles would have a faster rate of drug release, but interestingly this was not so. On the contrary, it was observed that a thicker PLGA shell had a more pronounced effect in retarding drug release than a thicker PLLA middle layer. Changes in molecular weight of PLLA were plotted in Fig. 7b. It was observed that the decrease in molecular weight of PLLA in 10:2:1 microparticles was slower than that of 4:2:1 and 10:5:1 microparticles. It is believed that a thicker (57.9 ± 9.1 μm), and thus a higher content of fully amorphous PLGA (77 wt.%), would provide for a more open structure for basic ions (from the phosphate buffer) to diffuse into the PLLA layer of 10:2:1 microparticles, thus providing a buffering effect in comparison to lower PLGA content of 62.5 wt.% for 10:5:1 microparticles. Therefore, these basic ions would help neutralize the acids generated during degradation, resulting in a slower decrease in molecular weight of PLLA [31,32]. In addition, a higher PLLA content (thus a thicker PLLA layer of 51.2 ± 10.5 μm) in 10:5:1 microparticles would result in the occurrence of autocatalytic effect in PLLA; whereby the concentration gradients and mass transport of acidic degradation products would decrease with increasing diffusion pathway lengths [31], as compared to thinner PLLA layer (25.7 ± 5.8 μm) for 10:2:1 microparticles. This degradation behavior was further confirmed from the T_g of PLLA in 10:2:1 microparticles (Supplementary information), which was observed to decrease to a much lesser extent, suggesting lesser free volume in PLLA for this microparticle, even though the T_g of PLGA in different particles decreased at a similar rate. With these factors in play, a zero-order sustained release of ibuprofen from 10:2:1 microparticles was achieved. Therefore, by manipulating the outer layer thicknesses, different release kinetics and even zero-order release rates can be achieved through these triple-layered microparticles for the delivery of ibuprofen.

4. Conclusions

The triple-layered PLGA/PLLA/EVA microparticles with ibuprofen localized in the EVA core were fabricated using a one-step solvent evaporation method. The drug release properties of the triple-layered microparticles were compared with those of the single-layered and double-layered microparticles. The triple-layered microparticles with the PLGA shell yielded an intermediate release rate, between that of single-layered EVA and double-layered PLLA/EVA microparticles. It was observed that the migration of the PGA oligomers to the PLLA layer accelerated the hydrolysis of PLLA in the triple-layered microparticles. In contrast, the presence of PLLA shell resulted in lower water uptake and degradation rate of the triple-layered microparticles, thus retarding the initial drug release. Drug release profiles of triple-layered microparticles can be altered by changing the layer thicknesses. From this study, the triple-layered microparticle system proved to offer unique drug release profiles,

thus providing a robust approach to fine-tune the drug release kinetics by designing appropriate microparticle structures.

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Appendix A. Figures with essential color discrimination

Certain figures in this article, particularly Figures 1 and 4, are difficult to interpret in black and white. The full colour images can be found in the on-line version, at doi:10.1016/j.actbio.2012.02.007

Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.actbio.2012.02.007.

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