Altering the drug release profiles of double-layered ternary-phase microparticles

Lee, Wei Li; Loei, Cedric; Widjaja, Effendi; Loo, Say Chye Joachim

Corresponding Author: Asst Prof Joachim Say Chye Loo, Ph.D.

Corresponding Author's Institution:

First Author: Wei Li Lee

Order of Authors: Wei Li Lee; Cedric Loei; Effendi Widjaja; Joachim Say Chye Loo, Ph.D.

Abstract: Double-layered ternary-phase microparticles composed of a poly(D,L-lactide-co-glycolide) (50:50) (PLGA) core and a poly(L-lactide) (PLLA) shell impregnated with poly(caprolactone) (PCL) particulates were loaded with ibuprofen (IBU) and metoclopramide HCl (MCA) through a one-step fabrication process. MCA and IBU were localized in the PLGA core and in the shell, respectively. The aim of this study was to study the drug release profiles of these double-layered ternary-phase microparticles in comparison to binary-phase PLLA(shell)/PLGA(core) microparticles and neat microparticles. The particle morphologies, configurations and drug distributions were determined using scanning electron microscopy (SEM) and Raman mapping. The presence of PCL in the PLLA shell gave rise to an intermediate release rate of MCA between that of neat and binary-phase microparticles. The ternary-phase microparticles were also shown to have better controlled release of IBU than binary-phase microparticles. The drug release rates for MCA and IBU could be altered by changing the polymer mass ratios. Ternary-phase microparticles, therefore, provide more degrees of freedom in preparing microparticles with a variety of release profiles and kinetics.

Letter of Submission

10 February 2011

Prof. K. Park
Editor-in-Chief, Journal of Controlled Release
College of Pharmacy
Purdue University
575 Stadium Mall Drive, West Lafayette, IN 47907-2091, USA.

Through:
Dr. Akihiko Kikuchi
Associate Editor, Journal of Controlled Release

Joachim Loo
Nanyang Technological University
School of Materials Science and Engineering
50 Nanyang Avenue, N4.1-01-4a, Singapore 639798

Re: Submission of Revised Journal Article for Further Review

Dear Prof. Park/ Dr. Kikuchi,

My co-authors and I would like to thank you and the reviewers for taking time to review our submitted manuscript entitled "Altering the Drug Release Profiles of Double-Layered Ternary-Phase Microparticles" (JCR-D-10-01085R2). In this re-submission, we have carefully considered the comments by the reviewer and editor and incorporated the amendments into the manuscript (see attached "Response to Reviewers' Comment-third revision").

We hereby again state that this submission has not been published previously, that it is not under consideration for publication elsewhere, and that if accepted it will not be published elsewhere in the same form, in English or in any other language, without the written consent of the publisher.

Any correspondences with regards to this submitted manuscript can be referred directly to me through email at joachimloo@ntu.edu.sg. Alternatively, I can also be contacted through telephone (+65 67904603) or fax (+65 67909081).

My co-authors and I would greatly appreciate if you could kindly re-review this article for publication in Journal of Controlled Release.

Thank you.

Best Regards,
Joachim Loo

<u>**Response to Reviewers' Comments**</u>

The authors' would like to again thank the Editor and Reviewers for their constructive comments and time in reviewing this manuscript. Our responses to the reviewer's comments are shown below in blue fonts.

Reviewers' comments:

- For supplementary materials, do not separately upload high-resolution figures, but include all figures and tables within a file for supplementary materials, and upload supplementary material as doc, xls, or pdf files.   ONLY THE LAST THREE PAGES IS NECESSARY AS SUPPLEMENTARY MATERIAL.   Please read guideline for authors carefully to produce supplementary material.

R: Figures have been included within a file for supplementary materials and uploaded as doc file. No figure for supplementary materials was uploaded separately.

- High-resolution graphical abstract should be uploaded via EES.

R: Yes, high-resolution graphical abstract (1000dpi) has been uploaded via EES.

- For Figure 1, do not separately upload Figure 1a and Figure 1b via EES, but combined file should be uploaded.   So did Figures 3, 4, 5, 6, and 7.

R: Each combine file for figures 1, 3,4,5,6 and 7 has been uploaded.
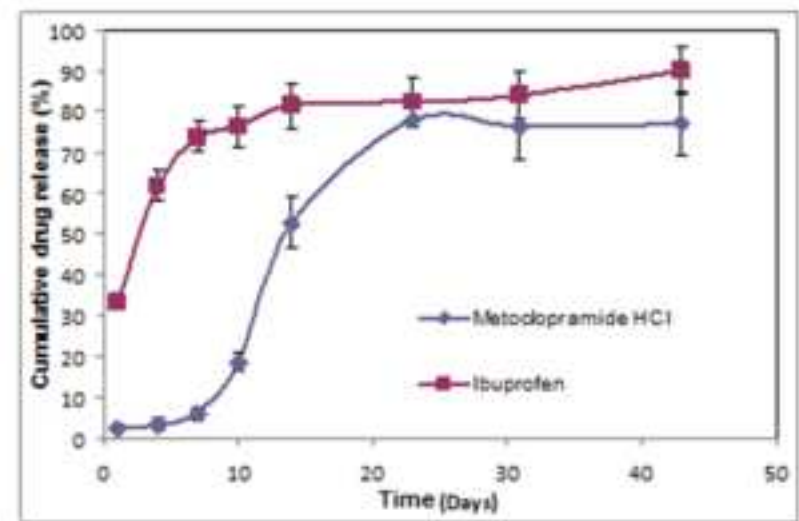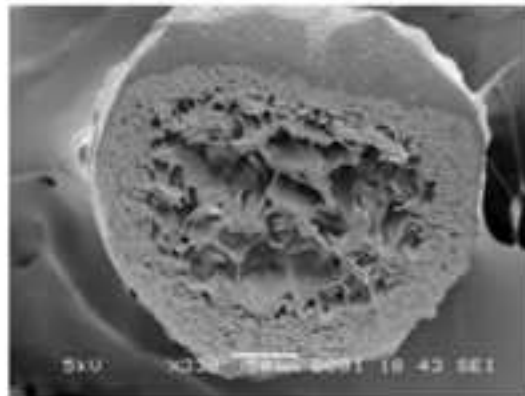
Altering the Drug Release Profiles of Double-Layered Ternary-Phase Microparticles

Wei Li LEE[1], Cedric LOEI[1], Effendi WIDJAJA[2], Say Chye Joachim LOO[1,*]

[1]School of Materials Science and Engineering
Nanyang Technological University
50 Nanyang Avenue, Singapore 639798

[2]Process Science and Modeling
Institute of Chemical and Engineering Sciences
1 Pesek Road, Jurong Island, Singapore 627833

*Corresponding author:
Say Chye Joachim Loo
Phone: +65 6790-4603
Fax: +65 6790-9081
Email: joachimloo@ntu.edu.sg

Abstract
Double-layered ternary-phase microparticles composed of a poly(D,L-lactide–co-glycolide) (50:50) (PLGA) core and a poly(L-lactide) (PLLA) shell impregnated with poly(caprolactone) (PCL) particulates were loaded with ibuprofen (IBU) and metoclopramide HCl (MCA) through a one-step fabrication process. MCA and IBU were localized in the PLGA core and in the shell, respectively. The aim of this study was to study the drug release profiles of these double-layered ternary-phase microparticles in comparison to binary-phase PLLA(shell)/PLGA(core) microparticles and neat microparticles. The particle morphologies, configurations and drug distributions were determined using scanning electron microscopy (SEM) and Raman mapping. The presence of PCL in the PLLA shell gave rise to an intermediate release rate of MCA between that of neat and binary-phase microparticles. The ternary-phase microparticles were also shown to have better controlled release of IBU than binary-phase microparticles. The drug release rates for MCA and IBU could be altered by changing the polymer mass ratios. Ternary-phase microparticles, therefore, provide more degrees of freedom in preparing microparticles with a variety of release profiles and kinetics.

Keywords: drug delivery, double-walled microparticle, degradation, poly(lactic acid), poly(lactide-co-glycolide), poly(caprolactone)

1. Introduction
Biocompatible polymeric microparticles of poly(lactic acid) (PLA) or poly(lactic acid-co-glycolic acid) (PLGA) have garnered much interest in the field of drug delivery in the past decade [1, 2]. These biodegradable particulate systems protect drugs from premature degradation, provide controlled and sustained drug release, and aid in improving therapeutic efficacy. However, as drug carriers, monolithic polymeric microparticles have several inherent problems, such as initial burst release [3], the inability to provide a variety of release profiles [4-10], and the inability to deliver multiple drugs from a single particle [11].

Double-layered particles composed of a polymeric shell surrounding a core of a second polymer have been shown to have better control drug release kinetics [11-14]. The outer layer in this core-shell structure allows drugs localized in the core to be released by diffusion through this "membrane". With the appropriate selection of the core and shell polymers, a variety of release profile and kinetics can be achieved, while eliminating some of the undesirable release characteristics of single-layered particles. Matsumoto et al. [15, 16] demonstrated that an outer non-drug-holding layer of poly(D,L-lactide) can eliminate the initial burst of cisplatin from that localized in the PLGA cores of multi-reservoir type microspheres. Shi et al. [11] have also reported that a near-complete and sustained release of hydrophilic bovine serum albumin and hydrophobic cyclosporin A was achieved using poly(ortho ester)–PLGA double-walled microspheres. Double-layered and even multi-layered and/or multi-phase particles can therefore provide an attractive and robust approach in drug delivery. Such particulate systems can offer greater versatility in controlling drug release through the manipulation of particle parameters, such as layer thicknesses, structural configurations, and polymer types.

Our group previously reported the fabrication of ternary-phase microparticles with a poly(D,L-lactide–co-glycolide) (PLGA) core and a poly(L-lactide) (PLLA) shell impregnated with poly(caprolactone) (PCL) particulates [17]. We also showed that the particle parameters (e.g., PCL particulate size, layer thickness, etc.) can be altered using this one-step fabrication process. For example, the physicochemical properties of the shell can be manipulated by changing the polymer content, whereby a higher PLLA content would yield a denser and thicker shell, while more PCL would result in a more rubbery shell impregnated with larger PCL particulates. Through such alterations, an assortment of "designer" microparticles can be fabricated.

While studies of drug release from double-layered microparticles have been reported, no such studies have been conducted using double-layered ternary-phase microparticles. Therefore, the aim of this study was to understand how the release profiles and kinetics of double-layered PLLA(shell)/PLGA(core) microparticles are altered when particles are transformed from being binary-phase to ternary-phase by adding PCL particulates to the shell. It is also of interest to know how different particle parameters and drugs can affect drug release. The model drugs used in this study were ibuprofen (IBU), which is hydrophobic, and metoclopramide monohydrochloride monohydrate (MCA), which is hydrophilic. In this study, MCA was localized in the core, while the IBU was localized in the shell of the microparticles.

2. Materials and Methods
2.1 Materials
PLLA (intrinsic viscosity (IV): 2.38, Bio Invigor), PLGA (50:50) (IV: 1.18, Bio Invigor), PCL (molecular weight (MW): 80 kDa, Aldrich), and poly(vinyl alcohol) (PVA) (MW: 30–70 kDa, Sigma–Aldrich) were used without further purification. Drugs were purchased from Sigma-Aldrich. Solvents such as dichloromethane (DCM), chloroform, and tetrahydrofuran (THF) were from Tedia Co., Inc. Phosphate-buffered saline (PBS) solution (pH 7.4) was purchased from OHME, Singapore. All drugs and solvents were used as received, unless otherwise noted.

2.2 Fabrication of microparticles
Drug-loaded ternary-phase PLLA/PLGA/PCL microparticles with a mass ratio of 3:2:1 were prepared using the water-in-oil-in-water (w/o/w) double emulsion solvent evaporation method

[17, 18]. To achieve a more uniform distribution of hydrophilic drug, the aqueous drug solution was first prepared and emulsified with the polymer solution, rather than adding solid drug particles to the polymer solution [19]. Briefly, the polymers (0.3 g, 6% w/v) and the hydrophobic drug (20% w/w) were first dissolved in DCM (organic phase). Hydrophilic drug (20% w/w) was then dissolved in 0.1 mL deionized water (internal aqueous phase). Both solutions were mixed and ultra-sonicated for 30 sec using an ultrasonic processor (Sonic Vibra-Cell VC 130) to prepare the first water-in-oil emulsion. The emulsion was then poured into deionized water containing PVA (0.5% w/v) as an emulsifier (external aqueous phase) to produce a water-in-oil-in-water double emulsion with an oil-to-water ratio of 0.02. The emulsion was then stirred at 300 rpm at room temperature (25 ℃) using an overhead stirrer (Calframo BDC1850-220). The evaporation of DCM resulted in the phase separation of PLLA, PLGA and PCL, yielding the ternary-phase microparticles. Finally, the particles were centrifuged, rinsed with deionized water, lyophilized and stored in a desiccator for further characterization.

Other ternary-phase PLLA/PLGA/PCL, binary-phase PLLA/PLGA and neat microparticles were similarly prepared whereby the drug type and polymer mass ratios were correspondingly altered. Deionized water was still used as the internal aqueous phase for the fabrication of the ternary-phase PLLA/PLGA/PCL and binary-phase PLLA/PLGA microparticles without any hydrophilic drug.

2.3 Characterization
2.3.1 Morphological analysis
The surface and internal morphologies of the microparticles were analyzed using scanning electron microscopy (SEM) (JEOL JSM-6360A) at an operating voltage of 5 kV. Before analysis, the samples were first mounted onto metal stubs and cross-sectioned at their center with a razor blade. Samples were then coated with gold using an SPI-Module sputter coater. At least three independent batches for each particle type were fabricated, and ten microparticles from each independent production batch were randomly chosen for SEM analysis. Particle morphologies and configurations were found to be consistent within each independent batch for a particle group type. Hence, only one representative SEM micrograph is shown.

2.3.2 Determination of particle configuration and drug distribution
Raman mapping was utilized to verify the final particle configuration (i.e., polymer distribution) and drug distribution within the microparticles. Microparticles that had been pre-sectioned were placed under a microscope objective with a laser power of up to approximately 20 mW. Raman point-by-point mapping measurements were performed on an area of 400×200 μm with a step size of 5 μm in both the x and y directions using a Raman microscope (In-Via Reflex, Renishaw) equipped with a near-infrared enhanced deep depleted thermoelectrically 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}, and the acquisition time for each spectrum was approximately 35 s. Spectral pre-processing, including the removal of spikes due to cosmic rays, was carried out before the Raman mapping data were further analyzed using the band target entropy minimization (BTEM) algorithm [20, 21]. The BTEM algorithm was developed to reconstruct pure component spectral estimates. When all of the normalized pure

component spectra of all underlying constituents had been reconstructed, the relative contributions of each measured point of these signals could be calculated by projecting them back onto the baseline-corrected and normalized data set. The color-coded scale represents the intensities of score image of each observed component, in which 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 for all observed component in the microparticles [20].

2.3.3 Drug encapsulation efficiency

Encapsulation efficiency is defined as the ratio of actual to theoretical drug loading within the microparticles. For quantification of hydrophilic MCA loading, approximately 10 mg of microparticles were first weighed and dissolved in 1 mL of DCM. MCA was then extracted using 10 mL of deionized water, into which the hydrophilic drug preferentially partitioned. The drug concentration was determined using a UV-Vis spectrophotometer (Shimadzu UV-2501) at 309 nm. For quantification of hydrophobic IBU, thermogravimetric analysis (TGA Q500 V6.5 Build 196) was used. Approximately 10 mg of the drug-loaded particles was placed on a platinum pan, and the sample was heated at 10 $^\circ$C min^{-1} from room temperature to 160 °C under nitrogen at a flow rate of 60 mL min^{-1}, followed by isothermal heating at 160 °C for 60 min before ramping to 500 °C. IBU is known to decompose at 160°C, which is lower than MCA (~280°C) and the polymers (~300°C). The mass loss percentage at 160°C determined in the TGA analysis was taken as the weight percentage (wt%) of IBU in the microparticles. All measurements were done in triplicate.

2.4 Hydrolytic degradation

Microparticles (50 mg) were weighed and placed in vials containing 30 mL of PBS (pH 7.4) (n = 3). Samples were incubated at 37°C with moderate shaking. The pH of the solution was monitored over time to ensure that the pH was maintained at 7.4 throughout the study. Microparticles were removed from the vials at pre-designated times by filtration.

2.4.1 Water uptake

After rinsing with distilled water, the microparticles were weighed and then vacuum-dried to constant weight to determine the difference in weights. The water uptake was calculated at each time point according to the following equation:

$$Water\ Uptake = 100\% \times \frac{W_{wet} - W_{dry}}{W_{dry}}$$

where W_{wet} and W_{dry} are the weights of the wet and dry microparticles, respectively, measured at time t. Values obtained from three samples were averaged and reported.

2.4.2 Molecular weight

The polymer's molecular weight at each time was determined using size-exclusion chromatography (SEC) (Agilent 1100 Series LC System). The molecular weights of the samples were obtained relative to a calibration curve constructed using polystyrene standards (165-5000 kDa). The polymers in the binary-phase and ternary-phase microparticles were separated by the dissolution method, based on the solubility differences of the polymers in THF (PLGA and PCL are soluble in THF, while PLLA is not). Eight milligrams of microparticles was first immersed in 1 mL of THF to dissolve the PLGA and PCL. PLLA remnants and the polymer solution were

later separated by centrifugation. The solvent in the polymer solution containing PLGA and PCL was evaporated slowly in air at room temperature for 48 h. PLLA was further dried in an oven at 40 °C for one week. After which, the PLGA/PCL mixture and PLLA were dissolved separately in 1 mL chloroform and analyzed using SEC.

2.5 Drug Release Study
Drug-loaded microparticles (5 mg) were placed, in triplicate, in vials containing 5 mL PBS and were maintained at 37 °C in a shaking incubator. At pre-determined time intervals, 1 mL of medium from each vial was removed and analyzed using a Shimadzu UV-2501 UV-Vis spectrophotometer (λ_{MCA} = 309 nm, λ_{IBU} = 220 nm) before replacing the removed volume with fresh PBS solution.

2.6 Statistical Analysis
Drug release and water uptake data from different particles were evaluated by unpaired Student's t-test and the one-way ANOVA analysis followed by Tukey-test. Differences were considered statistically significant when $P \leq 0.05$.

3. Results
3.1 Drug-loaded microparticles
A range of drug-loaded microparticles were fabricated. **Fig. 1(a)** shows the Raman mapping of IBU-MCA-loaded double-layered ternary-phase PLLA/PLGA/PCL (3:2:1) microparticles. PLGA and PLLA comprised the core and shell structures, respectively, with PCL uniformly dispersed as particulates within the PLLA shell. Hydrophilic MCA was encapsulated within the relatively more hydrophilic PLGA core, while hydrophobic IBU was localized in the relatively more hydrophobic shell, based on polymer-drug affinity [11-13, 18, 22]. **Fig. 1(b)** shows the Raman mapping of IBU-MCA-loaded binary-phase PLLA/PLGA microparticles. Similarly, MCA was localized in the PLGA core, while IBU was predominantly dispersed at the edge of the PLLA shell.

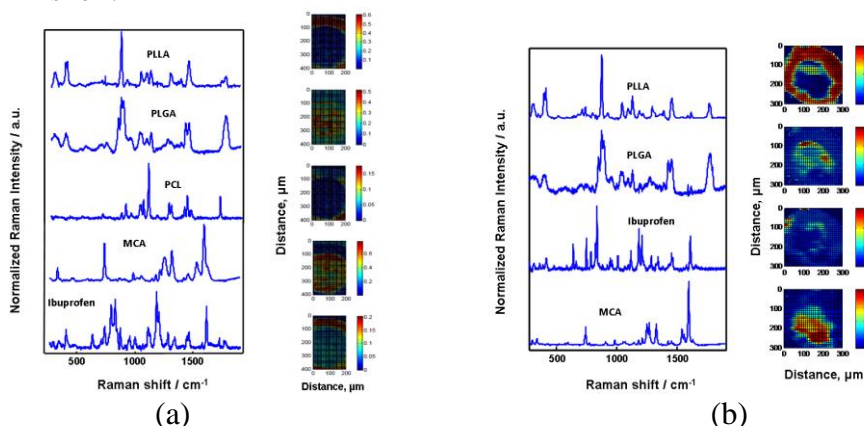(a)   (b)

Figure 1. Pure component Raman spectra estimates and their associated score images obtained via BTEM from (a) a MCA-IBU-loaded PLLA/PLGA/PCL 3:2:1 microparticle, (b) a MCA-IBU-loaded PLLA/PLGA microparticle.

Fig. 2 shows SEM micrographs of microparticles prepared emulsion solvent evaporation (refer to the supplementary information for the SEM images of neat PLGA, PLLA and PCL microparticles). The diameter of the IBU-MCA-loaded double-layered ternary-phase

PLLA/PLGA/PCL (3:2:1) microparticles measured by SEM was 255.4 ± 70.1 µm. Comparable particle sizes were also observed for the other microparticle samples (i.e., binary-phase and neat). The non-drug loaded binary-phase and ternary-phase micropartcles exhibited smooth and non-porous exterior surfaces. Cross-sectioning the microparticles revealed a porous PLGA core due to the repulsion of the internal aqueous droplets by the relatively more hydrophobic PLLA/PCL phase during the fabrication process. It was observed from the SEM micrographs that the presence of drugs does affect the morphological makeup of the microparticles. Microparticles containing MCA only were observed to have porous surfaces, possibly due to the hydration of MCA that drives the external water to diffuse into the nascent emulsion droplets by osmotic pressure. Comparing binary-phase and ternary-phase microparticles, one can tell from the cross-sections that the ternary-phase particles had a less dense shell as the result of the rubbery PCL particulates dispersed within the PLLA [17]. Binary-phase microparticles (with IBU) were observed to have uneven surfaces that are likely due to the drug particles that reside close to the surface [23], as was also evident from the Raman results (**Fig. 1(b)**). Therefore, the presence of hydrophobic and rubbery PCL, as particulates, allows for better dispersion of hydrophobic IBU in the shell of the ternary-phase microparticles (**Fig. 1(a)**), resulting in a smoother surface. The surface morphology of the microparticles is therefore dependent on a combination of effects, such as the drug and polymer types.
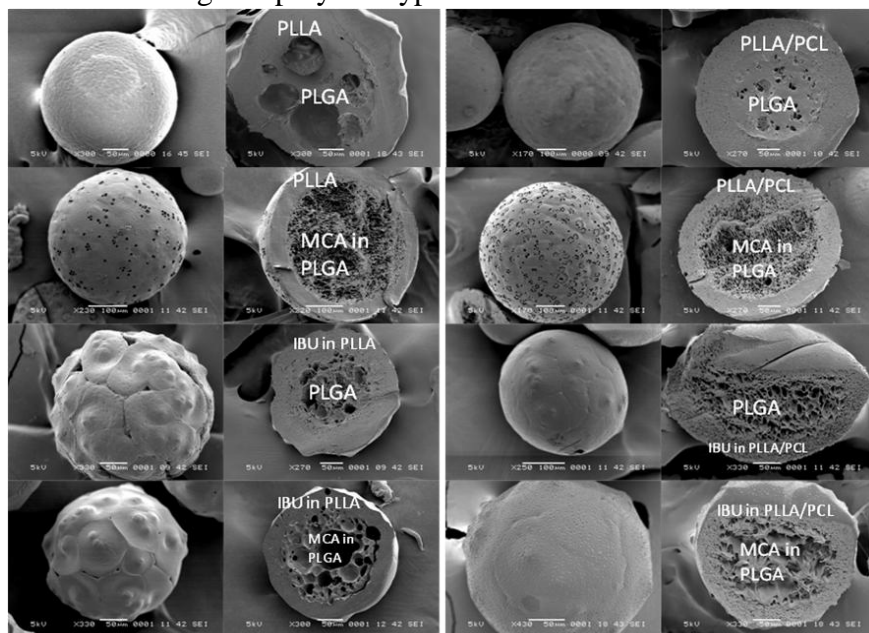

Figure 2. External and interrnal morphologies of the double-layered binary-phase PLLA/PLGA and ternary-phase PLLA/PLGA/PCL microparticles.
(Left column: binary-phase microparticles, Right column: ternary-phase microparticles;
Row 1: Non-drug loaded, Row 2: MCA-loaded, Row 3: IBU-loaded, Row 4: MCA-IBU-loaded)

Table 1 shows the encapsulation efficiency of IBU and MCA in the various microparticles. A higher MCA encapsulation efficiency was achieved for double-layered microparticles relative to that of the single-layered microparticles; this difference highlights the advantages of double-layered microparticles. It is generally believed that the hydrophobic PLLA shell prevents the aqueous MCA droplets from dispersing into the external aqueous medium during fabrication [12,

13], thus entrapping more MCA within the core. There was, however, no significant difference in the drug loading efficiency between binary-phase and ternary-phase microparticles.

Table 1. Encapsulation efficiencies of drugs (%)

Particle configuration	Neat	Neat	Neat	Binary phase double-layered	Ternary phase double-layered		
Polymers	PLGA	PLLA	PCL	PLLA/PLGA	PLLA/PLGA/PCL		
Mass Ratios				2:1	3:2:1	6:2:1	3:2:2
MCA only	30.1 ± 2.4	-	-	52.9 ± 5.4	57.5 ± 4.1	55.4 ± 3.3	54.1 ± 4.2
IBU only	-	54.4 ± 4.7	58.4 ± 7.3	53.4 ± 3.3	54.1 ± 2.5	-	56.4 ± 3.5
MCA + IBU	-	-	-	52.3 ± 4.2 63.2 ± 4.2	51.7 ± 4.9 64.2 ± 3.2	-	54.3 ± 3.8 65.5 ± 2.1

3.2 In vitro Drug Release
3.2.1 Microparticles with MCA only
Figure 3(a) shows the release profiles of MCA from MCA-loaded microparticles. As expected, single-layered PLGA microparticles exhibited a fast release of MCA. The presence of a shell in the binary- and ternary-phase microparticles, however, retarded the rapid release of MCA from the PLGA core, with the ternary-phase microparticles having intermediate release kinetics. The PLLA shell, therefore, acted as a rate-limiting barrier that impedes rapid drug diffusion from the core. For ternary-phase microparticles, the PCL particulates with a very low glass transition temperature (about -60°C) resulted in a less dense and more rubbery shell [24]. Hence, at drug releasing condition of 37°C and surrounded by PBS medium, PCL chains are in a highly mobile and flexible rubbery state with sufficient free volume [25], which increases probabilities for the drug molecules to diffuse from one cavity into another through the shell; thus, a relatively faster release was observed for ternary-phase microparticles compared to binary-phase microparticles. Similar release results for binary-phase PLLA/PLGA particles have also been reported by others [12, 13, 19, 26].
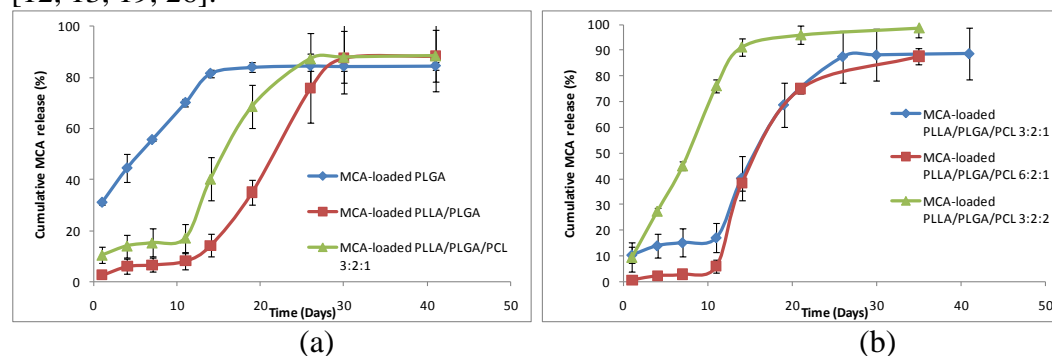
(a)   (b)

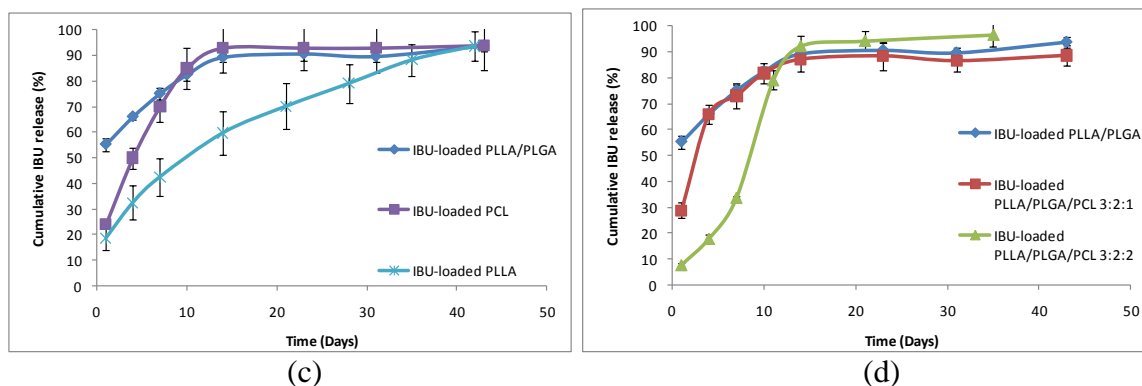| (c) | (d) |

Figure 3. Release profiles of MCA from (a) PLGA, PLLA/PLGA, and PLLA/PLGA/PCL microparticles, (b) ternary-phase PLLA/PLGA/PCL microparticles in the mass ratio of 3:2:1, 3:2:2 and 6:2:1. Release profiles of IBU from (c) neat PLLA, neat PCL and PLLA/PLGA microparticles, (d) PLLA/PLGA, PLLA/PLGA/PCL 3:2:1 and PLLA/PLGA/PCL 3:2:2 microparticles.

Changing the particle parameters also had an effect on drug release kinetics. Based on **Fig. 3(b)**, it is clear that the dense and thicker shell of ternary-phase microparticles (6:2:1) (refer to supplementary information) further suppressed the initial rapid release. When the PCL content was further increased (3:2:2) to form a more rubbery shell, rapid zero-order release kinetics were observed. Altering the properties of the shell by changing the polymer mass ratios can, therefore, result in different release kinetics and provide time-delayed drug release kinetics.

3.2.2 Microparticles with IBU only
Fig. 3(c) plots the release profiles of IBU from binary-phase and neat microparticles. It was observed that IBU release from the neat PCL microparticles proceeded relatively quickly in comparison to neat PLLA microparticles due to a highly flexible rubbery state of PCL chains. On the other hand, it was easier for IBU to be released into the PBS medium from the PLLA shell than neat PLLA microparticles because the diffusion distance for IBU was shorter for the former, resulting in a faster release from binary-phase microparticles. In the case of IBU-loaded multi-phase microparticles **(Fig. 3(d)),** binary-phase microparticles exhibited a very high initial burst release at day 1; this burst was somewhat suppressed in the ternary-phase microparticles. This observation can be attributed to the morphology of the shell. Binary-phase microparticles were observed to have surface irregularities **(Fig. 2)** due to drugs that reside close to the surface, resulting in easy water ingress. This ingress of water accelerates the release of these surface drug particles and thus causes a huge burst release. Ternary-phase microparticles, on the other hand, exhibited a slightly smaller burst, as the hydrophobic IBU was well dispersed within the PLLA-PCL shell **(Fig. 1(a)**). Increasing the PCL content (3:2:2) further reduced the burst release of IBU due to the favorable dispersion of hydrophobic IBU in the PLLA-PCL shell.

3.2.3 Microparticles with both MCA and IBU
Drug release from microparticles with more than one drug was also compared. The cumulative release of IBU from MCA-IBU-loaded microparticles is plotted in **Fig. 4(a)**. The release profile of IBU from dual-drug-loaded microparticles was similar to that of single-drug-loaded microparticles (IBU only). Binary-phase microparticles again showed a higher burst release of

IBU when compared to ternary-phase microparticles. Similarly, a higher PCL content (3:2:2) reduced the initial release of IBU.
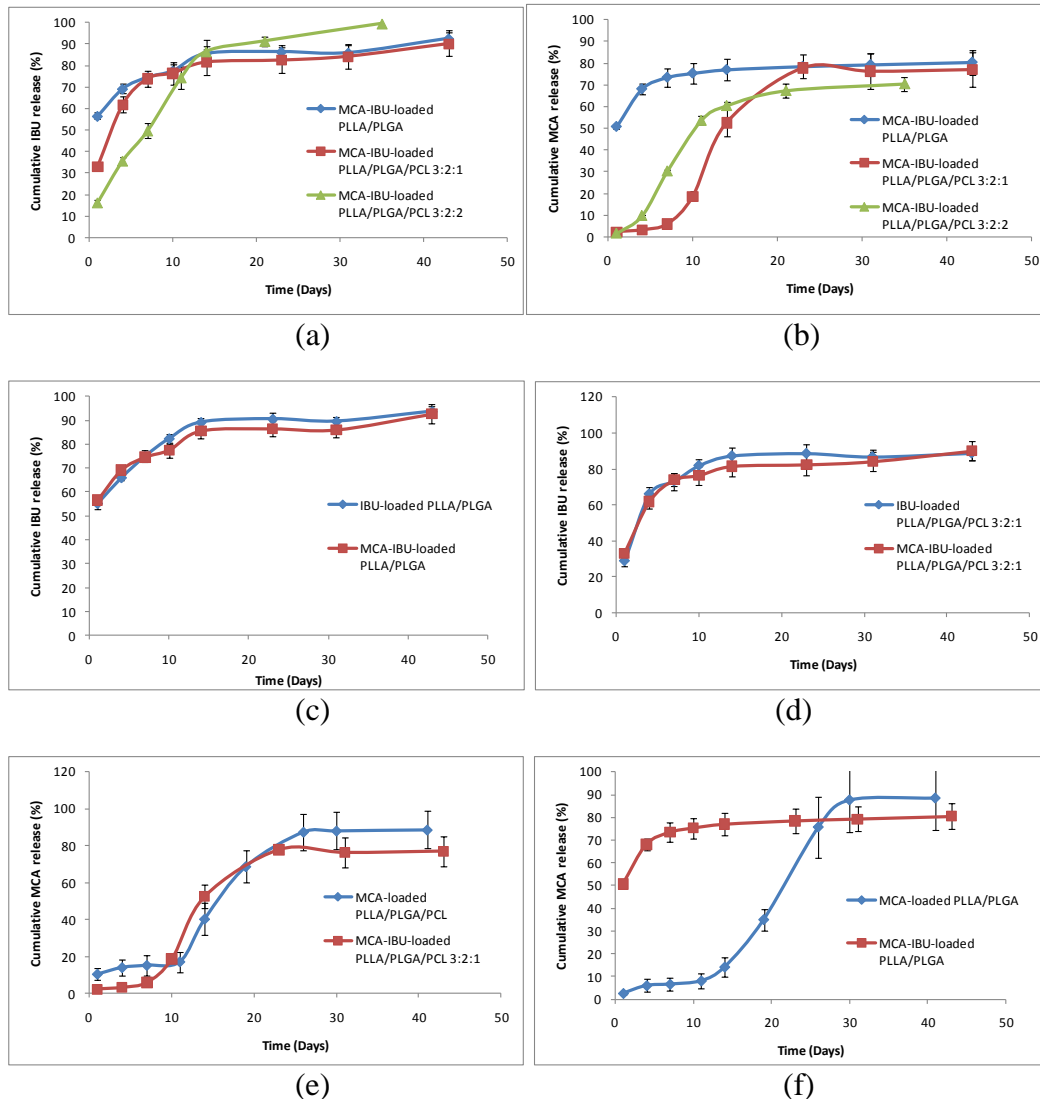

Figure 4. Release profiles of (a) IBU and (b) MCA from MCA-IBU-loaded PLLA/PLGA, PLLA/PLGA/PCL3:2:1, and PLLA/PLGA/PCL 3:2:2 microparticles. Release profiles of IBU from (c) IBU-loaded and MCA-IBU-loaded PLLA/PLGA, (d) IBU-loaded and MCA-IBU-loaded PLLA/PLGA/PCL 3:2:1 microparticles. Release profiles of MCA from (e) MCA-loaded and MCA-IBU-loaded PLLA/PLGA/PCL 3:2:1 microparticles, (f) MCA-loaded and MCA-IBU-loaded PLLA/PLGA microparticles.

Binary-phase microparticles exhibited the fastest release of MCA (**Fig. 4(b)**); ternary-phase microparticles significantly retarded the rapid release of MCA. However, the release of MCA was faster from ternary-phase 3:2:2 microparticles. The more rubbery shell matrix accelerated the diffusion of MCA.

3.2.4 Single-drug-loading vs. dual-drug-loading

It is of interest to understand how multiple drug loading can affect the release of a drug from these microparticles. Interestingly, from the release profiles of IBU and MCA **(Figs. 4(d), 4(e)),** it was evident that the drug release kinetics for both drugs (IBU or MCA only) were not affected by the presence of other drugs in ternary-phase microparticles. In addition, single- and dual-drug-loaded binary-phase microparticles have showed similar drug delivery profiles for IBU **(Fig. 4(c)).** The only exception was for MCA release from binary-phase microparticles. As shown in **Fig. 4(f)**, a lag in the release of MCA was observed for single-MCA-loaded binary-phase microparticles, while MCA-IBU-loaded binary-phase microparticles showed rapid release. This result was likely due to the huge (60%) initial burst release of IBU from MCA-IBU-loaded binary-phase microparticles that subsequently resulted in the rapid release of MCA. The initial suppression of IBU release from ternary-phase microparticles therefore prevented the rapid release of MCA that was observed in binary-phase microparticles, as the rapid release of IBU from the shell would have resulted in a more porous shell. Therefore, it was observed that MCA-IBU-loaded PLLA/PLGA/PCL 3:2:1 particles were able to release multiple drugs in a sequential manner, with the release of IBU within the first 10 days, followed by the release of MCA after 10 days.

3.3 Hydrolytic Degradation of Microparticles
3.3.1 Microparticles with MCA only
The release profile and kinetics of MCA-loaded microparticles can be explained by the hydrolytic degradation of these microparticles. As reported, ternary-phase microparticles were observed to have intermediate release kinetics, between those of neat (fastest) and binary-phase (slowest) microparticles. This result is in agreement with the faster degradation of the PLLA shell of ternary-phase microparticles and correspondingly higher water uptake (refer to the supplementary information). Ternary-phase microparticle shells were less dense due to the PCL particulates, whereas the PLLA shells of binary-phase microparticles were denser. SEM micrographs (**Figs. 5(a)** and **5(c)**) show that the shells were relatively dense at day 4 and retarded the release of MCA. Subsequently, after 10 days, the increased formation of pores and the enhanced degradation of the shell, along with the extensive erosion of the PLGA core (**Figs. 5(b)** and **5(d)**), allowed for faster diffusion of MCA from the core. The degradation of PLGA is clearly evident from the Raman mapping, which shows the presence of poly(glycolic acid) (PGA), a product of PLGA hydrolysis, in the core after 4 days (**Fig. 6(a)**); at this time, MCA was still present within the PLGA core. After 14 days of immersion (**Fig. 6(b)**), the PLGA core had substantially degraded to form PGA, while some MCA was observed to have diffused into the PLLA/PCL shell. Faster MCA release can therefore be attributed to polymer degradation, which is enhanced by the higher water uptake of the ternary-phase microparticles, arising from their porous PLLA/PCL shell. Raman mapping confirmed the absence of MCA after 30 days (**Fig. 6(c)**).
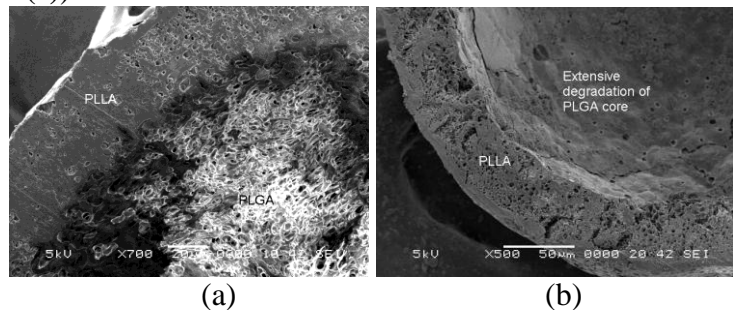
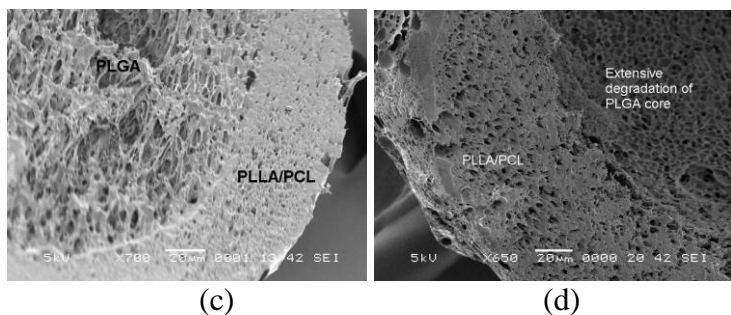(a)   (b)

10

(c)   (d)

Figure 5. SEM images of the degrading MCA-loaded PLLA/PLGA microparticles after (a) 4 days (b) 14 days in vitro. SEM images of the degrading MCA-loaded PLLA/PLGA/PCL 3:2:1 microparticles after (c) 4 days (d) 14 days in vitro.
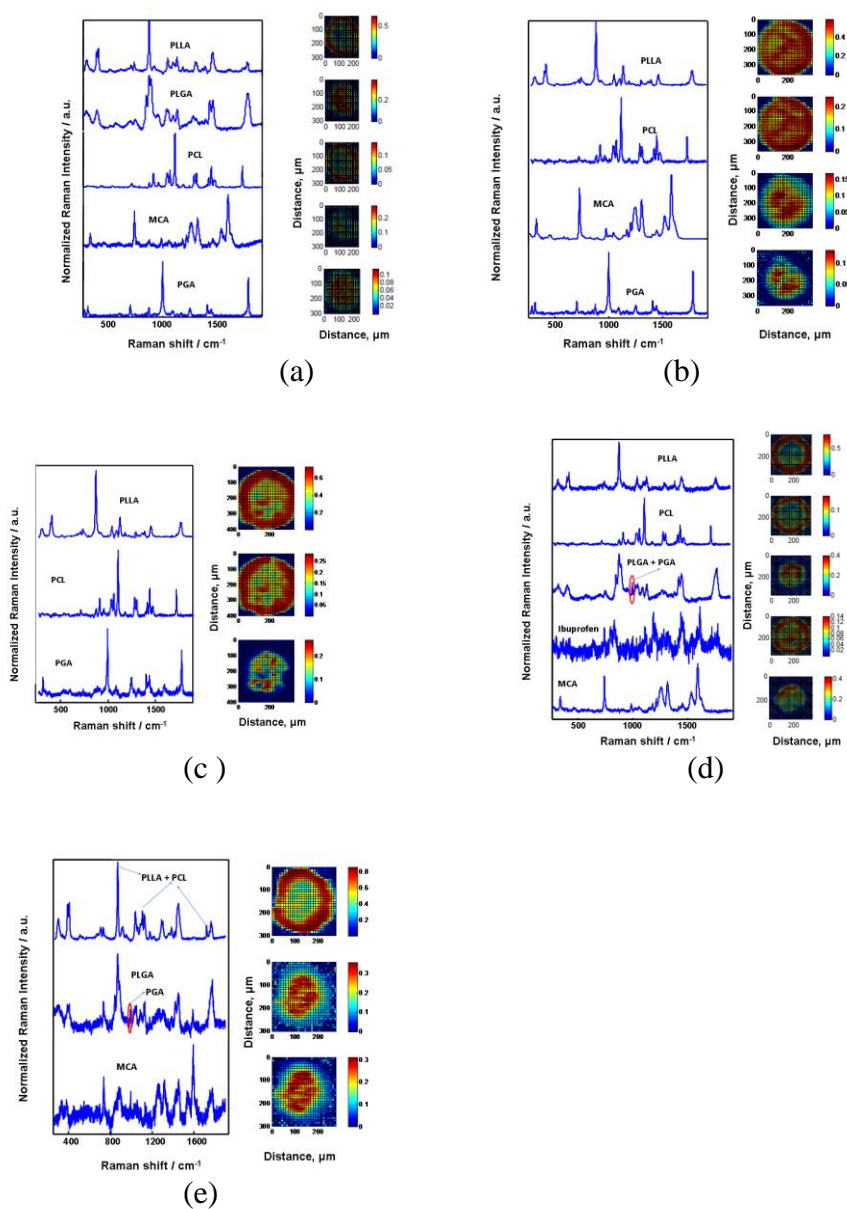

(a)   (b)

(c)   (d)

(e)

Figure 6. Pure component Raman spectra estimates and their associated score images obtained via BTEM from a MCA-loaded PLLA/PLGA/PCL 3:2:1 microparticle (a) after 4 days, (b) after 14 days, and (c) after 30 days in vitro. Pure component Raman spectra estimates and their associated score images obtained via BTEM from a MCA-IBU-loaded PLLA/PLGA/PCL 3:2:1 microparticle (d) after 4 days, and (e) after 11 days in vitro.

3.3.2 Microparticles with IBU only

Unlike MCA-loaded microparticles, there was no distinctive difference in the SEC results for IBU-loaded microparticles (refer to the supplementary information). The results show a similar decreasing IBU release rate for the molecular weights of PLLA in binary- and ternary-phase microparticles. Therefore, there was no significant difference in the rate of hydrolytic degradation of PLLA for both ternary-phase and binary-phase microparticles.

3.3.3 Microparticles with both MCA and IBU

The release kinetics of microparticles loaded with MCA and IBU can be explained using SEM micrographs, SEC results and Raman mapping results. The fast release of IBU from binary-phase microparticles resulted in larger pores in the shell and on the surface to allow for the faster release of MCA, as shown in **Figs. 7(a)** and **7(b)**. On the other hand, the shell matrix of ternary-phase microparticles after 4 days of immersion remained relatively unchanged (**Figs. 7(c)** and **7(d)**). The water uptake and the changes in the molecular weight of PLLA in binary-phase microparticles (refer to the supplementary information) were also found to be more significant than those of ternary-phase microparticles, thus explaining the faster MCA release from binary-phase microparticles. This result could be due to the fact that the microparticles loaded with MCA had a higher osmotic driving force for water ingress through the porous shell of the binary-phase microparticles [27], thus accelerating hydrolytic degradation.
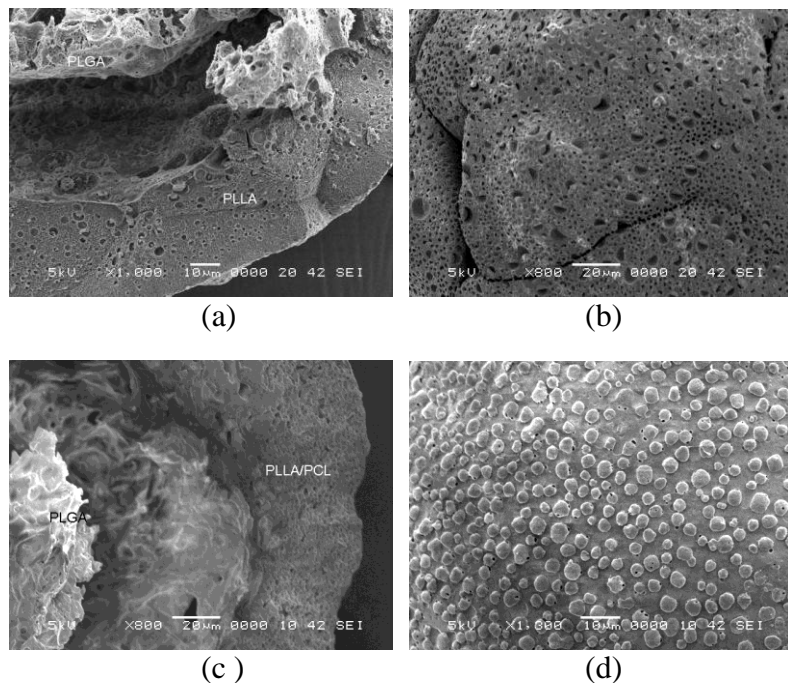

(a)

(b)

(c)

(d)

Figure 7. Internal and external morphologies of (a,b) the degrading MCA-IBU-loaded PLLA/PLGA microparticles and (c,d) the degrading MCA-IBU-loaded PLLA/PLGA/PCL 3:2:1 microparticles after 4 days in vitro.

Raman mapping was again utilized to study the drug release mechanism of MCA-IBU-loaded ternary-phase PLLA/PLGA/PCL 3:2:1 microparticles. Initially, MCA was dispersed in the PLGA core, with IBU localized in the shell (**Fig. 1(a)**). After 4 days of release (**Fig. 6(d)**), the presence of PGA in the microparticles was observed. Some of the PLGA had degraded to form PGA. At this time, most of the MCA was still dispersed in the PLGA core, affirming the slow release of MCA. It was also observed that some IBU had diffused into the PLGA core during this time. After 11 days of release **(Fig. 6e),** some MCA has dispersed into the PLLA/PCL shell and was subsequently released into the medium, resulting in an increased release rate (**Fig. 4(b)**). During this time, no Raman signals of IBU were detected, which could be attributed to the very low IBU concentration in the particles, with about 80 % IBU having been released (**Fig. 4(a)**).

4. Discussion
The drug release kinetics and profiles of binary- and ternary-phase microparticles are clearly distinct. The presence of PCL particulates within the PLLA shell (ternary-phase) played an important role in determining how hydrophilic drugs (present in the core) and hydrophobic drugs (present in the shell) are released. SEM, SEC and Raman mapping results show that two key factors are involved in differentiating the release kinetics of binary- and ternary-phase microparticles: morphological differences and the rate of hydrolytic degradation of the microparticles.

The release of hydrophobic IBU was largely determined by microparticle morphology, whereby the morphological differences between the binary- and ternary-phase microparticles account for the difference in the release kinetics for IBU. The hydrophobic IBU, which was loaded in the shell, was better dispersed in the ternary-phase microparticles than in the binary-phase microparticles. IBU was poorly dispersed in the PLLA shell, resulting in an uneven particle surface that contributed to the rapid release of IBU from binary-phase microparticles. The increase in the PCL content to PLGA/PLLA/PCL 3:2:2 showed a further retardation of the release of IBU from ternary-phase microparticles, confirming that shell properties are crucial in determining the release kinetics of hydrophobic drugs (in the shell). The single- (IBU) and dual- (IBU and MCA) drug-loaded microparticles showed similar drug delivery profiles for IBU, implying that the release of IBU from the shell was not affected by the presence of MCA in the core.

The release of MCA from single MCA-loaded microparticles was also influenced by the presence of PCL. A higher shell porosity, along with the embedding of rubbery PCL particulates in the PLLA shell, enhanced the degradation rate of the shell of the ternary-phase microparticles. Water uptake also increased, resulting in faster MCA release than that from binary-phase microparticles. This faster release resulted in an intermediate release rate of MCA from ternary-phase microparticles, between that of neat (too rapid) and binary-phase microparticles (longer lag phase).

The release of hydrophilic MCA from dual-drug-loaded microparticles (containing both MCA and IBU) was determined by two factors: first, the initial release kinetics of IBU and second, the degradation rate of PLLA. Rapid IBU release from binary-phase microparticles usually results in faster MCA release. This result can be attributed to the formation of surface pores resulting from the fast IBU release, which subsequently increases water uptake and further accelerates PLLA degradation and, thus, MCA release. On the other hand, MCA-IBU-loaded ternary-phase microparticles have a lag in the release phase of MCA, exhibiting a similar drug release rate as MCA-loaded ternary-phase microparticles. This implies that, unlike binary-phase microparticles, the presence of IBU in ternary-phase microparticles does not significantly affect the release of MCA, as IBU is well dispersed in the shell and gives rise to a more controlled release of MCA.

5. Conclusions

Drug-loaded, double-layered ternary-phase PLLA/PLGA/PCL microparticles, with a PLGA core and a PLLA shell impregnated with PCL particulates, were fabricated using the water-in-oil-in-water double emulsion solvent evaporation technique. It was found that MCA and IBU were localized in the PLGA core and in the shell, respectively. The drug release properties of the ternary-phase microparticles were compared with those of binary-phase PLLA/PLGA and neat microparticles. The MCA-loaded ternary-phase microparticles yielded an intermediate rate of release, between that of neat microparticles and binary-phase microparticles. Changing the polymer mass ratios of the ternary-phase microparticles also changed the release kinetics. In contrast, the presence of PCL resulted in the good dispersal of IBU in the ternary-phase microparticles, thus retarding the initial burst release of IBU. Similarly, the rate of IBU released can also be altered by changing polymer mass ratios. From this study, we determined that drug release profiles and kinetics can be altered by "designing" microparticles that suit a particular drug delivery application.

6. Acknowledgments

7. References

[1]   S. Freiberg, X. Zhu, Polymer microspheres for controlled drug release, Int. J. Pharm. 282 (2004) 1-18.
[2]   L. Brannon-Peppas, Recent advances on the use of biodegradable micropraticles and nanoparticles in controlled drug-delivery, Int. J. Pharm. 116 (1995) 1-9.
[3]   R. Jalil, J.R. Nixon, Microencapsulation using poly (L-lactic acid) IV: Release properties of microcapsules containing phenobarbitone, J. Microencapsulation 7 (1990) 53-66.
[4]   K.J. Pekarek, J.S. Jacob, E. Mathiowitz, One-step preparation of double-walled microspheres, Adv. Mater. 6 (1994) 684-687.
[5]   K.J. Pekarek, J.S. Jacob, E. Mathiowitz, Double-walled polymer microspheres for controlled drug-release, Nature 367 (1994) 258-260.
[6]   A. Sanchez, R.K. Gupta, M.J. Alonso, G.R. Siber, R. Langer, Pulsed controlled-release system for potential use in vaccine delivery, J. Pharm. Sci. 85 (1996) 547-552.
[7]   M.L. Lorenzo-Lamosa, C. Remunan-Lopez, J.L. Vila-Jato, M.J. Alonso, Design of microencapsulated chitosan microspheres for colonic drug delivery, J. Controlled Release 52 (1998) 109-118.

[8]   M. Rodriguez, J.L. Vila-Jato, D. Torres, Design of a new multiparticulate system for potential site-specific and controlled drug delivery to the colonic region, J. Controlled Release 55 (1998) 67-77.

[9]   Y. Krishnamachari, P. Madan, S. Lin, Development of pH-and time-dependent oral microparticles to optimize budesonide delivery to ileum and colon, Int. J. Pharm. 338 (2007) 238-247.

[10]   P.C. Naha, V. Kanchan, P.K. Manna, A.K. Panda, Improved bioavailability of orally delivered insulin using Eudragit-L30D coated PLGA microparticles, J. Microencapsulation 25 (2008) 248-256.

[11]   M. Shi, Y.Y. Yang, C.S. Chaw, S.H. Goh, S.M. Moochhala, S. Ng, J. Heller, Double walled POE/PLGA microspheres: encapsulation of water-soluble and water-insoluble proteins and their release properties, J. Controlled Release 89 (2003) 167-177.

[12]   T.H. Lee, J.J. Wang, C.H. Wang, Double-walled microspheres for the sustained release of a highly water soluble drug: characterization and irradiation studies, J. Controlled Release 83 (2002) 437-452.

[13]   E.C. Tan, R.Y. Lin, C.H. Wang, Fabrication of double-walled microspheres for the sustained release of doxorubicin, J. Colloid Interface Sci. 291 (2005) 135-143.

[14]   N.A. Rahman, E. Mathiowitz, Localization of bovine serum albumin in double-walled microspheres, J. Controlled Release 94 (2004) 163-175.

[15]   A. Matsumoto, Y. Matsukawa, T. Suzuki, H. Yoshino, M. Kobayashi, The polymer-alloys method as a new preparation method of biodegradable microspheres: Principle and application to cisplatin-loaded microspheres, J. Controlled Release 48 (1997) 19-27.

[16]   A. Matsumoto, Y. Matsukawa, T. Suzuki, H. Yoshino, Drug release characteristics of multi-reservoir type microspheres with poly(DL-lactide-co-glycolide) and poly(DL-lactide), J. Controlled Release 106 (2005) 172-180.

[17]   W.L. Lee, W.L. Foo, E. Widjaja, S.C.J. Loo, Manipulation of process parameters to achieve different ternary-phase microparticle configurations, Acta Biomater. 6 (2010) 1342-1352.

[18]   W.L. Lee, E. Widjaja, S.C.J. Loo, One-step fabrication of triple-layered polymeric microparticles with layer localization of drugs as a novel drug-delivery system, Small 6 (2010) 1003-1011.

[19]   W. Zheng, A water-in-oil-in-oil-in-water (W/O/O/W) method for producing drug-releasing, double-walled microspheres, Int. J. Pharm. 374 (2009) 90-95.

[20]   E. Widjaja, W.L. Lee, S.C.J. Loo, Application of Raman microscopy to biodegradable double-walled microspheres, Anal. Chem. 82 (2010) 1277-1282.

[21]   E. Widjaja, C.Z. Li, W. Chew, M. Garland, Band target entropy minimization. A general and robust algorithm for pure component spectral recovery, Anal. Chem. 75 (2003) 4499-4507.

[22]   W.L. Lee, M. Hong, E. Widjaja, S.C.J. Loo, Formation and degradation of biodegradable triple-layered microparticles, Macromol. Rapid Commun. 31 (2010) 1193-1200.

[23]   J. Siepmann, N. Faisant, J. Akiki, J. Richard, J.P. Benoit, Effect of the size of biodegradable microparticles on drug release: experiment and theory, J. Controlled Release 96 (2004) 123-134.

[24]   L. Chen, J. Yang, K. Wang, F. Chen, Q. Fu, Largely improved tensile extensibility of poly (L-lactic acid) by adding poly (ε-caprolactone), Polym. Int. 59 (2010) 1154-1161.

[25]   L.L. Lao, S.S. Venkatraman, N.A. Peppas, Modeling of drug release from biodegradable polymer blends, Eur. J. Pharm. Biopharm. 70 (2008) 796-803.

[26]   H.X. Tan, J. Ye, Surface morphology and in vitro release performance of double-walled PLLA/PLGA microspheres entrapping a highly water-soluble drug, Appl. Surf. Sci. 255 (2007) 353-356.

[27]   A. Frank, S.K. Rath, S.S. Venkatraman, Controlled release from bioerodible polymers: effect of drug type and polymer composition, J. Controlled Release 102 (2005) 333-344.

8. Table and Figure Captions

Figure 1. Pure component Raman spectra estimates and their associated score images obtained via BTEM from (a) a MCA-IBU-loaded PLLA/PLGA/PCL 3:2:1 microparticle, (b) a MCA-IBU-loaded PLLA/PLGA microparticle.

Figure 2. External and interrnal morphologies of the double-layered binary-phase PLLA/PLGA and ternary-phase PLLA/PLGA/PCL microparticles.
(Left column: binary-phase microparticles, Right column: ternary-phase microparticles;
Row 1: Non-drug loaded, Row 2: MCA-loaded, Row 3: IBU-loaded, Row 4: MCA-IBU-loaded)

Figure 3. Release profiles of MCA from (a) PLGA, PLLA/PLGA, and PLLA/PLGA/PCL microparticles, (b) ternary-phase PLLA/PLGA/PCL microparticles in the mass ratio of 3:2:1, 3:2:2 and 6:2:1. Release profiles of IBU from (c) neat PLLA, neat PCL and PLLA/PLGA microparticles, (d) PLLA/PLGA, PLLA/PLGA/PCL 3:2:1 and PLLA/PLGA/PCL 3:2:2 microparticles.

Figure 4. Figure 4. Release profiles of (a) IBU and (b) MCA from MCA-IBU-loaded PLLA/PLGA, PLLA/PLGA/PCL3:2:1, and PLLA/PLGA/PCL 3:2:2 microparticles. Release profiles of IBU from (c) IBU-loaded and MCA-IBU-loaded PLLA/PLGA, (d) IBU-loaded and MCA-IBU-loaded PLLA/PLGA/PCL 3:2:1 microparticles. Release profiles of MCA from (e) MCA-loaded and MCA-IBU-loaded PLLA/PLGA/PCL 3:2:1 microparticles, (f) MCA-loaded and MCA-IBU-loaded PLLA/PLGA microparticles.

Figure 5. SEM images of the degrading MCA-loaded PLLA/PLGA microparticles after (a) 4 days (b) 14 days in vitro. SEM images of the degrading MCA-loaded PLLA/PLGA/PCL 3:2:1 microparticles after (c) 4 days (d) 14 days in vitro.

Figure 6. Pure component Raman spectra estimates and their associated score images obtained via BTEM from a MCA-loaded PLLA/PLGA/PCL 3:2:1 microparticle (a) after 4 days, (b) after 14 days, and (c) after 30 days in vitro. Pure component Raman spectra estimates and their associated score images obtained via BTEM from a MCA-IBU-loaded PLLA/PLGA/PCL 3:2:1 microparticle (d) after 4 days, and (e) after 11 days in vitro.

Figure 7. Internal and external morphologies of (a,b) the degrading MCA-IBU-loaded PLLA/PLGA microparticles and (c,d) the degrading MCA-IBU-loaded PLLA/PLGA/PCL 3:2:1 microparticles after 4 days in vitro.

Table 1. Encapsulation efficiencies of drugs (%)

Table 1

Table 1. Encapsulation efficiencies of drugs (%)

Particle configuration	Neat	Neat	Neat	Binary phase double-layered	Ternary phase double-layered		
Polymers	PLGA	PLLA	PCL	PLLA/PLGA	PLLA/PLGA/PCL		
Mass Ratios				2:1	3:2:1	6:2:1	3:2:2
MCA only	30.1 ± 2.4	-	-	52.9 ± 5.4	57.5 ± 4.1	55.4 ± 3.3	54.1 ± 4.2
IBU only	-	54.4 ± 4.7	58.4 ± 7.3	53.4 ± 3.3	54.1 ± 2.5	-	56.4 ± 3.5
MCA + IBU	-	-	-	52.3 ± 4.2 63.2 ± 4.2	51.7 ± 4.9 64.2 ± 3.2	-	54.3 ± 3.8 65.5 ± 2.1

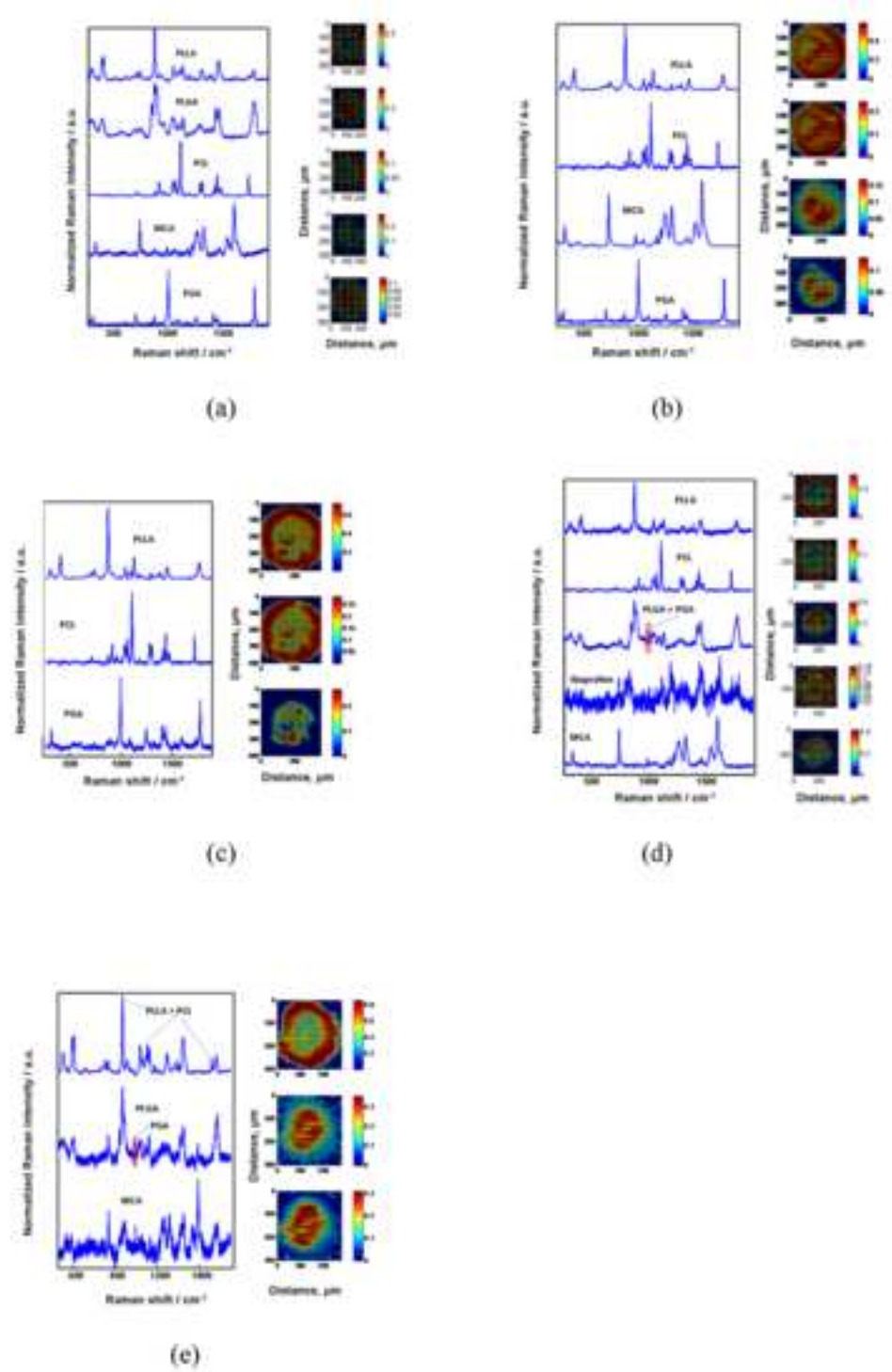**Figure 1**
Click here to download high resolution image

(a)

(b)
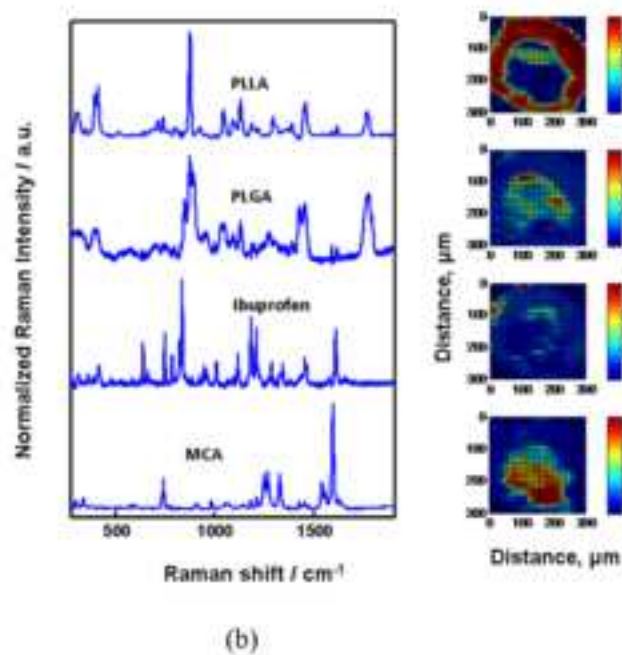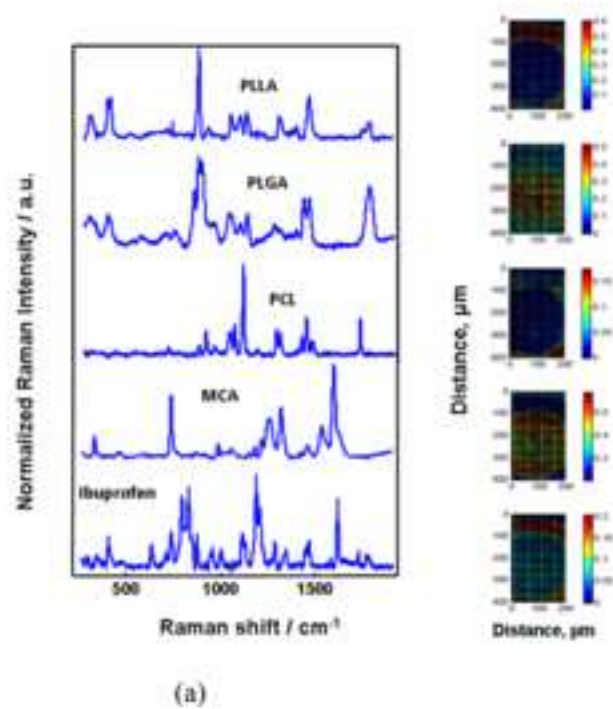
Figure 2
[Click here to download high resolution image](#)
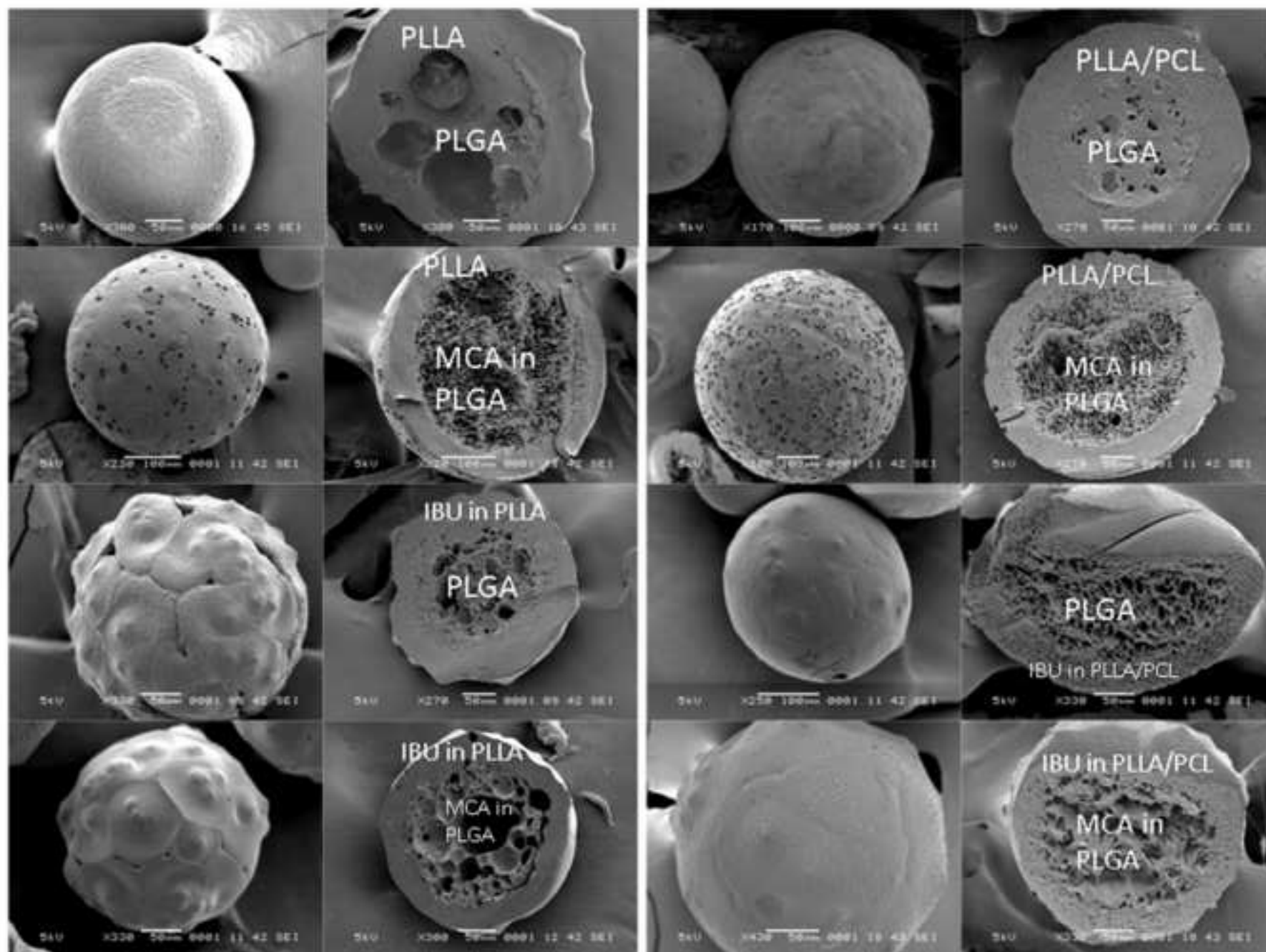
Figure 3
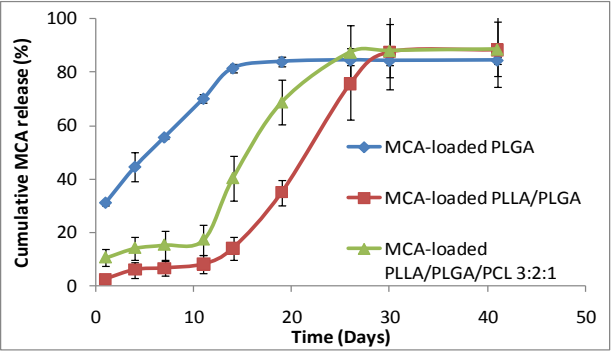
(a)
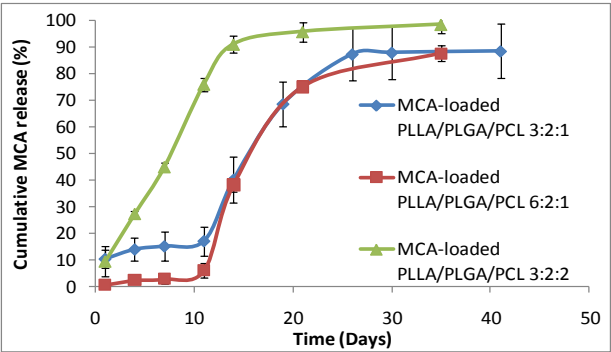
(b)

(c)

(d)

Figure 4

(a)

(b)

(c)

(d)

(e)

(f)
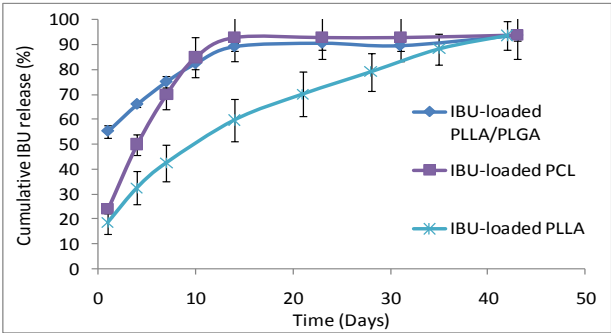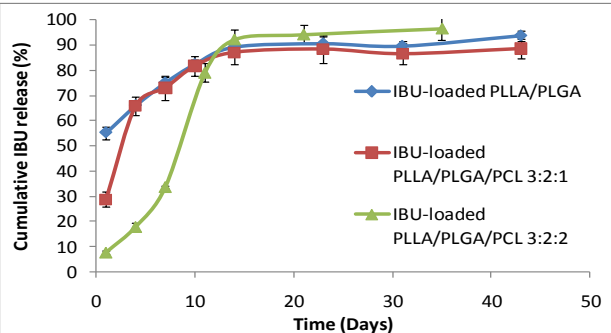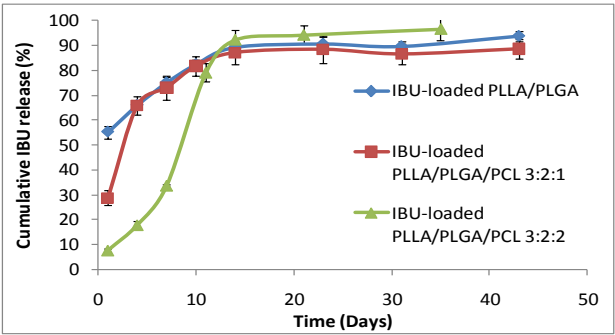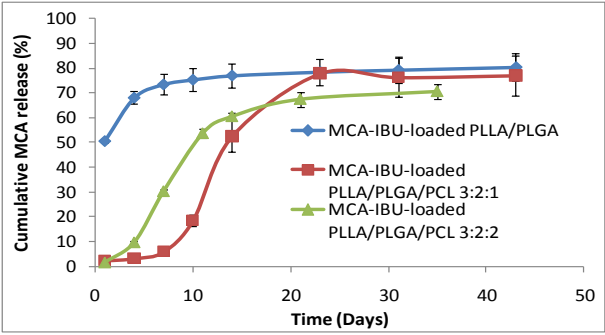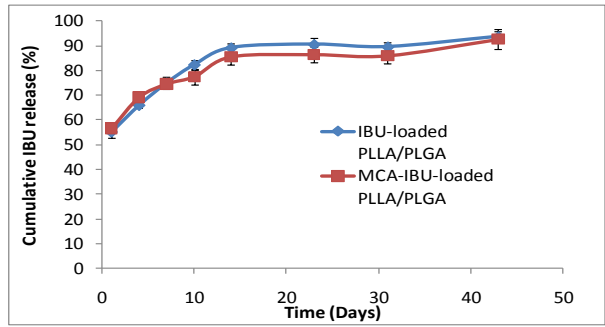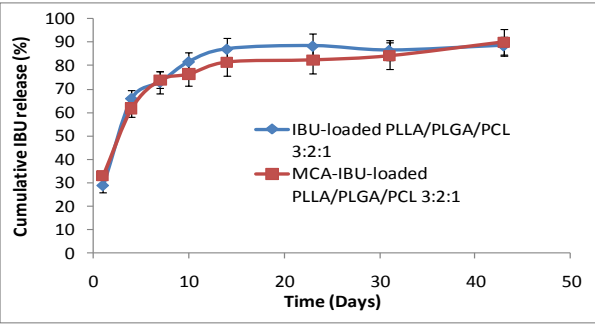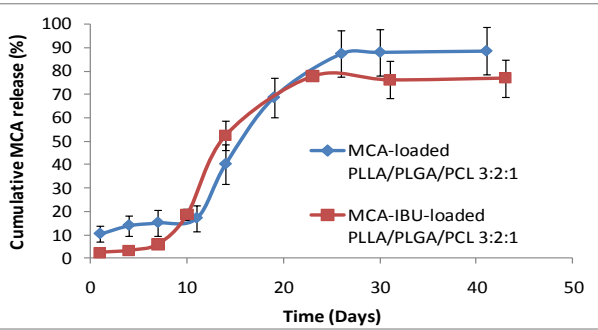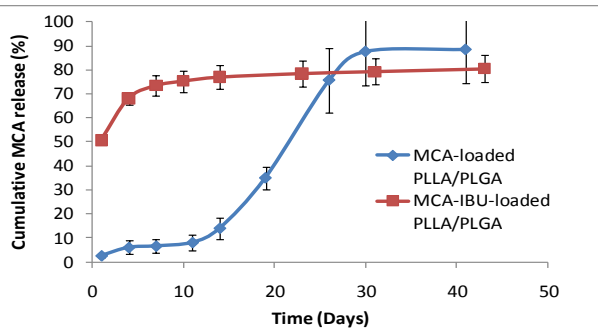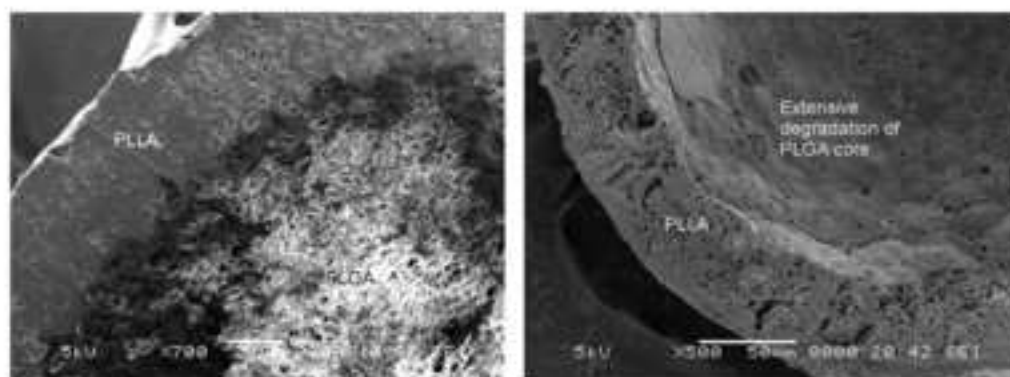
(a)

(b)
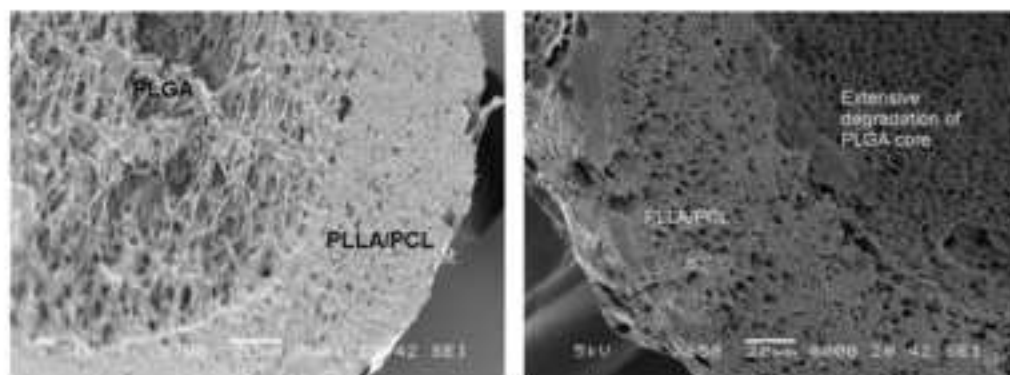
(c)

(d)

Figure 6
Click here to download high resolution image

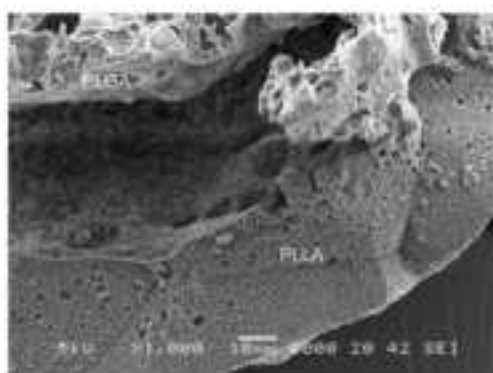(a)

(b)

(c)

(d)

(e)

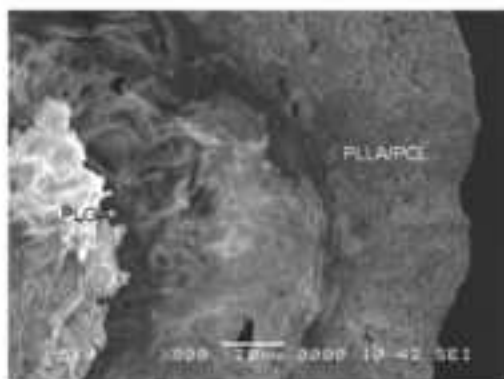**Figure 7**
Click here to download high resolution image

(a)

(b)

(c)

(d)

Supplementary Material