Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Journal of Controlled Release 89 (2003) 167–177
journal of controlled release
www.elsevier.com/locate/jconrel

Double walled POE/PLGA microspheres: encapsulation of water-soluble and water-insoluble proteins and their release properties

Meng Shi[a,b], Yi-Yan Yang[a,*], Cheng-Shu Chaw[a], Suat-Hong Goh[b], Shabbir M. Moochhala[c], Steve Ng[d], Jorge Heller[d]

[a]Institute of Materials Research and Engineering, No. 3 Research Link, Singapore 117602, Singapore
[b]Department of Chemistry, National University of Singapore, 3 Science Drive, Singapore 117543, Singapore
[c]Defence Medical Research Institute, no. 01-06, MD2 National University of Singapore, Singapore 119260, Singapore
[d]A.P. Pharma, Redwood City, CA 94063, USA

Received 28 August 2002; accepted 6 December 2002

Abstract

The poly(orthoester) (POE)–poly(D,L-lactide–co-glycolide) (50:50) (PLGA) double-walled microspheres with 50% POE in weight were loaded with hydrophilic bovine serum albumin (BSA) and hydrophobic cyclosporin A (CyA). Most of the BSA and CyA was entrapped within the shell and core, respectively, because of the difference in their hydrophilicity. The morphologies and release mechanisms of proteins-loaded double-walled POE/PLGA microspheres were investigated. Scanning electron microscope studies revealed that the CyA–BSA-loaded double-walled POE/PLGA microspheres yielded a more porous surface and PLGA shell than those without BSA. The neat POE and PLGA yielded slow and incomplete CyA and BSA release. In contrast, nearly complete BSA and more than 95% CyA were released in a sustained manner from the double-walled POE/PLGA microspheres. Both the BSA- and CyA–BSA-loaded POE/PLGA microspheres yielded a sustained BSA release over 5 days. The CyA release pattern of the CyA-loaded double-walled POE/PLGA microspheres was biphasic, characterized by a slow release over 15 days followed by a sustained release over 27 days. However, the CyA–BSA-loaded double-walled POE/PLGA microspheres provided a more constant and faster CyA release due to their more porous shell. In the CyA–BSA-loaded double-walled POE/PLGA microspheres system, the PLGA layer acted as a carrier for BSA and mild reservoir for CyA. During the first 5 days, most BSA was released from the shell but only 14% CyA was left from the microspheres. Subsequently, more than 80% CyA were released in the next 25 days. The distinct structure of double-walled POE/PLGA microspheres would make an interesting device for controlled delivery of therapeutic agents.
© 2003 Elsevier Science B.V. All rights reserved.

1. Introduction

A previous paper reported the preparation and characterization of the double-walled POE/PLGA microspheres consisting of a core of POE surrounded

*Corresponding author. Tel.: +65-68-748-123; fax: +65-68-727-528.
E-mail address: yy-yang@imre.org.sg (Y.Y. Yang).

0168-3659/03/$ – see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0168-3659(02)00493-5

by a shell of PLGA [1]. The double-walled POE/PLGA microspheres achieved distinct degradation behavior. The POE core underwent rapid and accelerated erosion, resulting in a pronounced hollow structure after 1 week during the in vitro degradation process. However, the PLGA shell remained relatively intact even after 4 weeks in vitro due to its slow degradation. Double walled microspheres systems were addressed to have the potential to alleviate burst effect, realize zero-order release patterns, or deliver two kinds of drugs in controlled manners [2–5]. However, their applications have not been reported yet.

Based on the distinct structure and degradation behavior of the double-walled POE/PLGA microspheres, it was expected that they would deliver two drugs with different water-solubility in a controlled manner. Cyclosporin A (CyA) is a water-insoluble peptide. As a potent immunosuppressive agent, CyA is widely used for the prevention of graft rejection in various organ transplantations [6]. To improve the therapeutic efficacy of CyA, biodegradable polymeric drug delivery systems have been attempted extensively, including microspheres, nanoparticles, liposomes and micelles, which were based on poly(D,L-lactide–co-glycolide) (PLGA), poly(D,L-lactide) (PLA), polycaprolactone or PLA–poly(ethylene glycol) (PLA–PEG) copolymers [7–10]. However, it is difficult to achieve ideal release patterns. An initial burst and incomplete release were the common issues to be overcome for CyA delivery.

In this report, the double-walled POE/PLGA microspheres system was used to deliver two proteins with different water-solubility in a controlled manner. CyA and bovine serum albumin (BSA) employed as hydrophobic and hydrophilic model proteins were encapsulated in the double-walled POE/PLGA microspheres. The distribution of BSA within the microspheres was examined using a confocal laser scanning microscope. The release properties of CyA and BSA from the double-walled POE/PLGA microspheres were investigated.

2. Materials and methods

2.1. Materials

POE was synthesized as previously described [11]. The molecular weight (Mw) of POE is 24.1 kDa. PLGA (50:50) with a Mw of 42.7 kDa was obtained from Sigma–Aldrich. Poly(vinyl alcohol) (PVA) (87–89 mol.% hydrolysed, Mw 31–50 kDa) and BSA (fraction V, 58 kDa) were obtained from Aldrich (USA). CyA (Mw 1202) was kindly provided by National University Hospital of Singapore. Dichloromethane (DCM) and hexanes were obtained from Merck (Germany). Tetrahydrofuran (THF) and acetonitrile from Tedia (USA), were of HPLC grade and used as received.

2.2. Fabrication of microspheres

The CyA–BSA-loaded double-walled POE/PLGA microspheres with 50% POE in weight were prepared by using a water-in-oil-in-water double emulsion solvent evaporation method as described previously [1]. Briefly, 300 mg POE, 300 mg PLGA and 70 mg CyA were dissolved in 12 ml DCM (the organic phase); 70 mg BSA was dissolved in 0.15 ml water containing 0.2% (w/v) PVA (the internal aqueous phase). The two solutions were mixed and sonicated for 15 s using a VC 50T sonicator (Sonics and Materials, CT, USA) to produce the first water-in-oil emulsion. The emulsion was then poured into 250 ml PBS (pH 7.4) containing 0.2% (w/v) PVA as an emulsifier (the external aqueous phase) to produce a water-in-oil-in-water double emulsion, which was stirred at a constant temperature (15 °C) for 3.5 h using a mixer (Cole-Parmer Instrument, IL, USA) controlled by a low temperature circulator (LTD6G, Grant Instruments, Cambridge, UK). The resultant microspheres were filtered, washed, freeze-dried overnight and stored at 4 °C.

The neat POE and PLGA microspheres containing BSA or CyA were prepared by the same method as detailed above. The internal aqueous phase was still used for the fabrication of the CyA-loaded double-walled POE/PLGA microspheres.

2.3. BSA distribution

A Bio-Rad confocal laser scanning microscope (CLSM, MRC 1024, UK) was used to observe BSA distribution within microspheres using BSA fluorescence [12]. An excitation wavelength of 488 nm and

a 522 DF 32 emission filter were used, and a Photo Multiplier Tube 2 (Iris: 6, Gain: 1300, low signal) was selected. The laser power was 100%. Filter blocks were T2A (560 DRLP) and B1 (beam splitter). All observations were conducted using the same resolution.

2.4. Determination of protein encapsulation efficiency

For the determination of BSA encapsulation efficiency, 10 mg microspheres were dissolved in 1 ml DCM and kept at room temperature for about 30 min. After dissolution of microspheres, 10 ml PBS buffer (pH 7.4) was added and the mixture was shaken vigorously for 2 min. The mixture was left to stand at room temperature for 1 h before the aqueous layer was drawn out. The aqueous solution was then filtered. BSA content in the filtered solution was analyzed using high-performance liquid chromatography (HPLC) (Waters 2690D) [13]. The HPLC system consisted of Waters 2690 separation module, a 996PDA detector, an 1050 Quatern Pump, an 1100 autosampler injector and a diode-array UV detector. A Zorbax GF-250 column (25 cm×4.6 mm, Dupont) was used as the separation column. The column temperature was set at 25 °C. The flow-rate of the mobile phase (PBS, pH 7.0) was 1.0 ml/min and the UV detection was at 210 nm.

For the determination of CyA encapsulation efficiency, 5 mg microspheres were dissolved in 1 ml DCM. After dissolution of microspheres, 5 ml hexane were added to precipitate polymers and extract CyA. The mixture was filtered and the filtrate was dried. A 20 ml volume of acetonitrile/water (85:15, v/v) was added to dissolve the solid sample. The CyA content was analyzed by the HPLC system as described above. A Waters SymmetryShield™ RP_8 15.0×4.6 cm column fitted with RP_8 precolumn was employed. The flow rate of the mobile phase consisting of acetonitrile and ultrapure water in the volume ratio of 85:15 was 1.0 ml/min. The UV detection was at 210 nm. The column and sample temperatures were set at 50 and 15 °C, respectively [14].

The drug loading and encapsulation efficiency were calculated as the ratio of drug to polymer contents and of actual to theoretical drug contents, respectively.

2.5. Particle size measurement

The diameter of the microspheres was measured by using a Nikon polarizing microscope (Optiphot 2-pol, Japan) and analyzed by the built-in IMAGEPRO software. The mean diameter of the microspheres was taken to be the number average of the diameters of the particles captured on the computer image.

2.6. Morphological analysis

The surface and internal morphologies of microspheres before and after in vitro degradation in PBS at 37 °C were analysed using a scanning electron microscope (SEM, Model JSM-5600, Jeol Japan). Cross-sectioned samples were prepared using a razor blade for viewing their internal structure. The microspheres and their sectioned samples were mounted on metal stubs using double-sided adhesive tape and vacuum-coated with a platinum layer prior to the examination.

2.7. Water uptake study

The microspheres samples were incubated in PBS (pH 7.4) at 37 °C. The water uptake of the microspheres at predetermined time intervals was measured gravimetrically and calculated as the weight ratio of absorbed water to dried microspheres.

2.8. In vitro protein release

The in vitro protein release analysis of the microspheres were carried out in triplicate at 37 °C in PBS (pH 7.4). A 40 mg amount of freeze-dried microspheres was dispersed in 10 ml PBS (pH 7.4) containing 0.1 (w/v)% Tween 80, which was agitated moderately. At predetermined time intervals, in vitro medium from each sample was removed and replaced with fresh PBS buffer. For the BSA-loaded microspheres, the BSA content in the in vitro medium was directly analyzed using HPLC as stated above. For the CyA-loaded microspheres, an extraction method was employed to separate CyA from the in vitro medium. Briefly, 10 ml hexane was added to the in vitro medium and the mixture was vigorously shaken for 5 min to extract CyA. The mixture was left to stand at room temperature

overnight before the organic layer was drawn off and dried. A 5 ml volume of acetonitrile/water (85:15, v/v) was then added to dissolve the residues for further HPLC analysis. The CyA standard samples were prepared according to the same procedures. However, for the CyA–BSA-loaded microspheres, after the extraction of CyA, the aqueous layer was collected to analyze BSA content. The weight percentage of CyA or BSA cumulative release (%, w/w) was investigated as a function of incubation time.

3. Results and discussion

3.1. Protein distribution

Fig. 1 shows the CLSM images of the BSA-loaded PLGA and the CyA–BSA-loaded double-walled POE/PLGA microspheres. Bright color represents BSA. The neat PLGA microspheres yielded a uniform BSA distribution. No signal was observed from the neat POE microspheres probably because the laser light was absorbed by the dense POE domains. The CLSM picture of the double-walled POE/PLGA microspheres shows a pronounced circular green image, resulting from BSA loaded in the PLGA shell. This was further confirmed by the release pattern of BSA from the double-walled POE/PLGA microspheres, which will be discussed later.

As a double emulsion process was employed to prepare the double-walled POE/PLGA microspheres, the interactions between proteins and polymers might act as the main driving force for the distribution of proteins. The higher the affinity between the protein and polymer, the stronger is the tendency for the protein to be loaded into the polymer. During the fabrication process, PLGA migrated towards the external aqueous phase and readily precipitated as the shell due to its hydrophilicity. POE partially repelled the BSA aqueous droplets. However, the affinity between PLGA and BSA drove them to dwell in the PLGA phase. Likewise, CyA is hydrophobic, and had strong tendency to load in the hydrophobic POE core. The distribution of the two proteins would lead to interesting release patterns.

3.2. Morphologies of the microspheres

Surface and internal SEM scans of the protein-loaded microspheres were carried out to study the effect of proteins. It was observed that the presence of proteins did not affect the morphologies of the neat POE and PLGA microspheres (images not shown). However, they did make differences to the

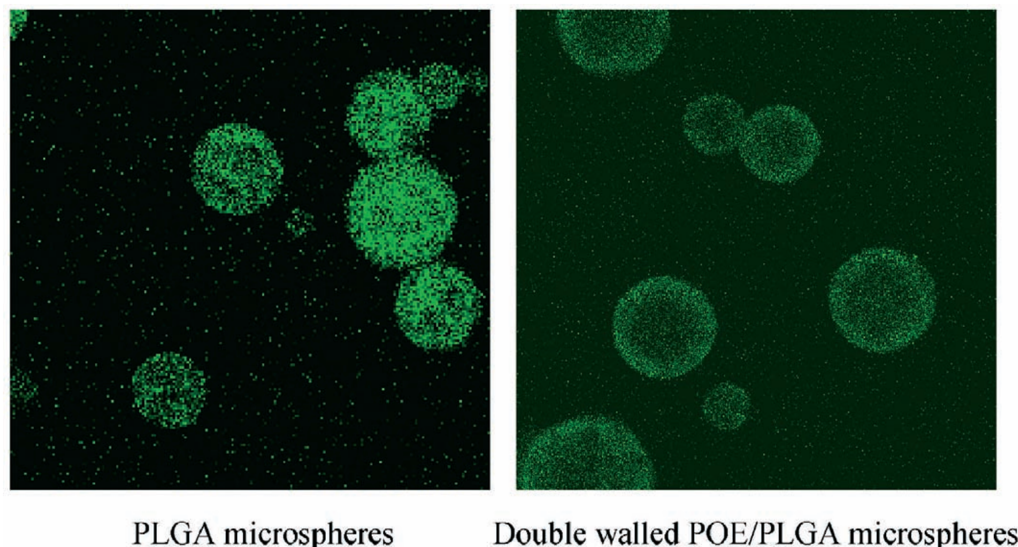

Fig. 1. CLSM images of the BSA-loaded PLGA and double-walled POE/PLGA microspheres.

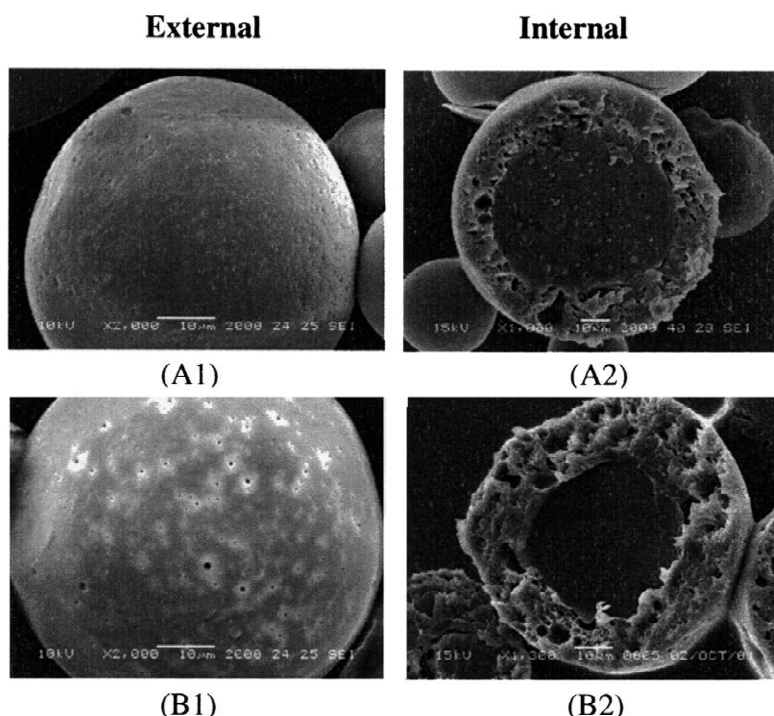

Fig. 2. External and internal morphologies of the CyA- and CyA–BSA-loaded double-walled POE/PLGA microspheres. (A1, A2) CyA-loaded double-walled microspheres; bars = 10 μm. (B1, B2) CyA–BSA-loaded double-walled microspheres; bars = 10 μm.

double-walled POE/PLGA microspheres. Particularly, the presence of BSA resulted in more porous surface and PLGA layer of the CyA-loaded double-walled POE/PLGA microspheres (Fig. 2). As discussed above, the BSA aqueous droplets preferred to stay with the PLGA solution, which migrated towards the external aqueous phase and precipitated as the nascent shell. BSA was highly concentrated in the shell. The hydration of BSA drove the external water molecules to diffuse in, eventually resulting in more porous structure.

3.3. Characteristics of the protein-loaded microspheres

Table 1 lists mean diameter of the neat POE, PLGA and double-walled POE/PLGA microspheres before and after freeze-drying. The double-walled

Table 1
Mean diameters of the microspheres before and after freeze-drying

Microspheres	After freeze-drying		Before freeze-drying		Size decrease (%) $(D_b - D_a)/D_a \times 100$
	Mean diameter D_a (μm)	Standard deviation	Mean diameter D_b (μm)	Standard deviation	
CyA–BSA–POE/PLGA	59.6	14.3	66.4	18.7	11.4
CyA–POE/PLGA	50.9	19.9	55.0	17.6	8.1
BSA–POE/PLGA	58.0	23.7	66.7	18.9	15.0
CyA–POE	48.2	22.7	50.3	20.9	4.4
BSA–POE	51.8	20.3	53.2	20.2	2.7
CyA–PLGA	67.1	24.6	72.8	22.4	8.5
BSA–PLGA	68.3	22.7	73.1	20.3	7.0

POE/PLGA microspheres containing BSA were slightly larger than those without BSA due to the more porous shell created by BSA. In addition, it was observed that the size of the microspheres decreased to various degrees after freeze-drying, depending on polymer nature and porosity of microspheres. Compared to POE, the size decrease of the PLGA microspheres was more obvious. This was due to the fact that hydrophilic PLGA absorbed more water. Thus, the PLGA matrices exhibited greater shrinking after water removal. Interestingly, the double-walled POE/PLGA microspheres containing BSA experienced the largest decrease in size because of the highly porous PLGA shell (Fig. 2).

Table 2 shows the encapsulation efficiency of BSA and CyA in the various kinds of microspheres. Generally, high encapsulation efficiency of BSA and CyA was yielded by all the batches of microspheres. The encapsulation efficiency of proteins depends on their solubility in aqueous medium, affinity with polymers, stability of the first emulsion and preparation conditions. CyA is hydrophobic, and has higher affinity to POE than PLGA. Thus, the neat POE and double-walled POE/PLGA microspheres achieved higher encapsulation efficiency for CyA than the neat PLGA microspheres. However, BSA is a hydrophilic protein. The neat PLGA microspheres yielded greater encapsulation efficiency for BSA than the neat POE microspheres. The encapsulation efficiency of BSA was obviously lower in the double-walled POE/PLGA microspheres than in the neat PLGA microspheres. This was due to the fact that less PLGA was employed in the PLGA–POE–solvent system. On the other hand, during the fabrication process, it was easier for BSA to diffuse into the external water phase from the nascent PLGA shell than the neat PLGA microspheres because the distance for BSA to travel was shorter in the shell.

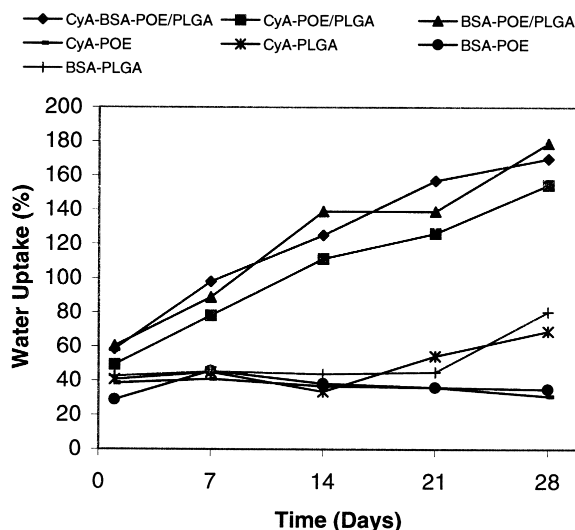

Fig. 3. Water uptake of the degrading microspheres.

3.4. Water uptake

The water uptake of the degrading microspheres is shown in Fig. 3. The water uptake is mainly dependent on the porosity and hydrophilicity of the matrices. The double-walled POE/PLGA microspheres generally yielded higher water uptake than the neat POE and PLGA microspheres. In addition, the water uptake of the double-walled POE/PLGA microspheres containing BSA was greater than that of the double-walled POE/PLGA microspheres with-

Table 2
Encapsulation efficiencies and actual loading levels of CyA and BSA

Microspheres	BSA		CyA	
	Loading level (%)	Encapsulation efficiency (%)	Loading level (%)	Encapsulation efficiency (%)
CyA–BSA–POE/PLGA	7.8	61.4	10.0	82.9
CyA–POE/PLGA	–	–	10.8	79.0
BSA–POE/PLGA	8.1	59.7	–	–
CyA–POE	–	–	11.6	85.8
BSA–POE	10.3	69.8	–	–
CyA–PLGA	–	–	6.5	53.4
BSA–PLGA	11.1	88.6	–	–

out BSA. These findings agree with the morphological observations reported earlier on. On the other hand, the water uptake of the protein-loaded neat POE microspheres remained unchanged during the in vitro tests, indicating a steady degradation of POE and unchanged porosity of the matrices. Similarly, the water uptake for the protein-loaded PLGA microspheres remained relatively constant during the first 3 weeks. However, it increased thereafter. This was probably because significant bulk erosion of the PLGA microspheres occurred after 3 weeks and led to increase in porosity [1]. All the double-walled POE/PLGA microspheres exhibited increasing water uptake with the progression of polymer degradation. For instance, the water content reached about 180% after 4 weeks in vitro for the BSA-loaded double-walled POE/PLGA microspheres. In the previous work [1], we reported that the POE core achieved rapid degradation in the double-walled POE/PLGA microspheres system. The majority of the POE core disappeared after 3 weeks, leaving the PLGA shell. Increase in water uptake was attributed to increased porosity resulting from the formation of the hollow microspheres and PLGA degradation.

3.5. In vitro release kinetics

Fig. 4 shows the release profiles of BSA from the various BSA-loaded microspheres. In the case of neat POE and PLGA microspheres, a nonrelease of the loaded BSA was followed after an initial burst. For example, after 4 weeks in vitro, 25 and 26% BSA was released out from the neat POE and PLGA microspheres, respectively. This finding was consistent with results reported previously [15–17] and has already been attributed to protein aggregation and degradation, nonspecific protein adsorption occurring within the microspheres and slow degradation of polymers. For the neat PLGA microspheres, the nonrelease of BSA was most likely caused by the acidic microenvironment produced within the microspheres [18]. The acidic environment led to the destabilization and noncovalent aggregation of encapsulated acid-labile biomacromolecules. Moreover, another possible reason was that the dense structure made it difficult for BSA to be released out of the matrices before destabilization.

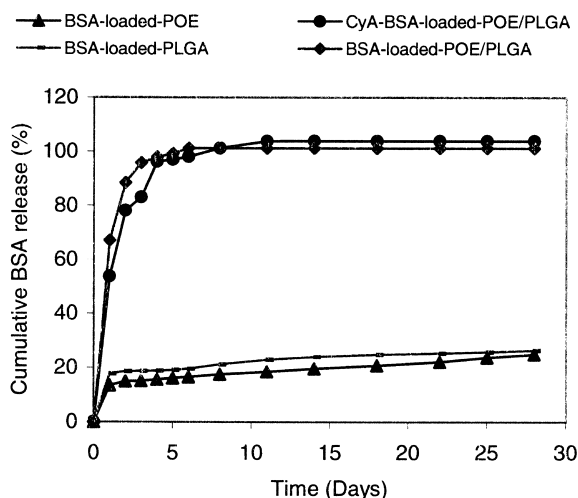

Fig. 4. Release profiles of BSA from the POE, PLGA and double-walled POE/PLGA microspheres.

Compared to POEs reported by Chia et al. [17], the chain of POE applied in the present work is more flexible because it does not contain the rigid diol *trans*-cyclohexanedimethanol. The former POE microspheres were still well dispersed even after 6 weeks in vitro. However, the current POE microspheres experienced pronounced agglomeration after incubated in PBS buffer at 37 °C. As a consequence of the agglomeration, BSA was tightly entrapped in the agglomerations and the surface area accessible to the in vitro medium was tremendously reduced. This definitely led to slow BSA release so that it was not detectable by HPLC.

The BSA- and CyA–BSA-loaded double-walled POE/PLGA microspheres shared a similar release pattern of BSA. Nearly complete BSA was released out in a sustained manner during the first 5 days. The BSA release results support the CLSM observations that BSA molecules were mainly distributed in the PLGA shell. The double-walled POE/PLGA microspheres have two advantages over the neat POE and PLGA microspheres. On one hand, the hydrophilic PLGA shell allowed the microspheres to disperse more easily in the in vitro medium. The good dispersion ensured the complete release of BSA. On the other hand, the BSA-loaded PLGA shell was more porous and the water uptake was much higher during the entire in vitro testing. Thus, it was easier for the acidic degradation products of PLGA and POE to diffuse out from the microspheres, prevent-

ing formation of an acid microenvironment and thus leading to rapid and complete BSA release. The mechanism for BSA release from the double-walled POE/PLGA microspheres was believed to be diffusion-based through a network of water-filled pores and channels.

Fig. 5 shows the release profiles of CyA from the various microspheres over a period of 7 weeks. Each microspheres system exhibited a different CyA release mechanism. The neat POE microspheres yielded very slow CyA release. The cumulative CyA release over 7 weeks was about 37% while the microspheres had already lost more than 90% of their initial weight [1]. A nonrelease of CyA was observed after 7 weeks in vitro. The CyA release was most likely controlled by its solubilization in the in vitro medium. As discussed in the previous paragraph, the agglomeration of the POE microspheres occurred after incubated in PBS at 37 °C, leading to a decreasing surface area with the progression of polymer degradation and subsequent slow CyA release. Particularly, after a great majority of POE was degraded, the CyA release was too slow to be detected. Similarly, Urata et al. reported that the CyA release of the PLA microspheres was faster than that of the PLGA microspheres due to the high porosity of the PLA microspheres [8]. The addition of fatty acid esters promoted CyA release because the acid esters improved the water solubility of CyA.

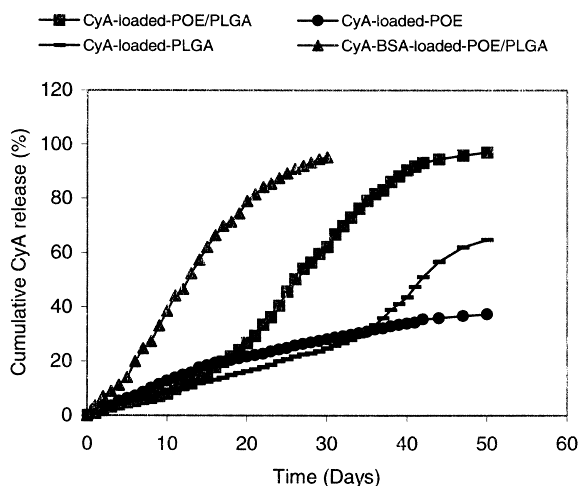

Fig. 5. Release profiles of CyA from the POE, PLGA and double-walled POE/PLGA microspheres.

Two distinct phases of CyA release were observed for the neat PLGA microspheres. The first phase was described as a slow and constant CyA release lasting 5 weeks, which was attributed to the degradation of the microspheres and the subsequent solubilization of CyA in the in vitro medium. Following the slow release phase, there was an accelerated CyA release due to the increased solubilization of CyA caused by the degradation products of PLGA and the bulk erosion of the matrices.

In contrast, the CyA- and CyA–BSA-loaded double-walled POE/PLGA microspheres yielded sustained and complete CyA release over 42 and 30 days, respectively. For the CyA-loaded double-walled POE/PLGA microspheres, a biphasic CyA release pattern was yielded. In the first phase lasting about 15 days, the CyA release rate was similar to that of the neat POE microspheres. Thereafter, an increased CyA release was achieved over 27 days. As reported in the previous work [1], POE underwent more rapid degradation in the double-walled POE/PLGA microspheres than in the neat POE microspheres due to the acidic microenvironment produced by PLGA degradation. Pronounced hollow structure was formed as the polymer degradation proceeded. The acidic degradation products of POE and PLGA as well as greater water uptake promoted the solubilization of CyA, resulting in faster CyA release. The CyA–BSA-loaded double-walled POE/PLGA microspheres yielded even more sustained and faster CyA release. There was no significant slow-release-phase observed. More than 95% CyA was released in a sustained manner over 30 days. The CyA–BSA-loaded double-walled POE/PLGA microspheres were more porous on the surface and in the PLGA shell than the CyA-loaded double-walled POE/PLGA microspheres (Fig. 2). Fig. 6 shows the external and internal morphologies of the CyA- and CyA–BSA-loaded double-walled POE/PLGA microspheres after the in vitro degradation. The POE core of both microspheres formulations experienced rapid degradation during the first 5 days. However, clearly, many pores of as big as 10 μm were created on the surface of the CyA–BSA-loaded double-walled POE/PLGA microspheres. Thus, the area accessible to the in vitro medium for CyA solubiliza-

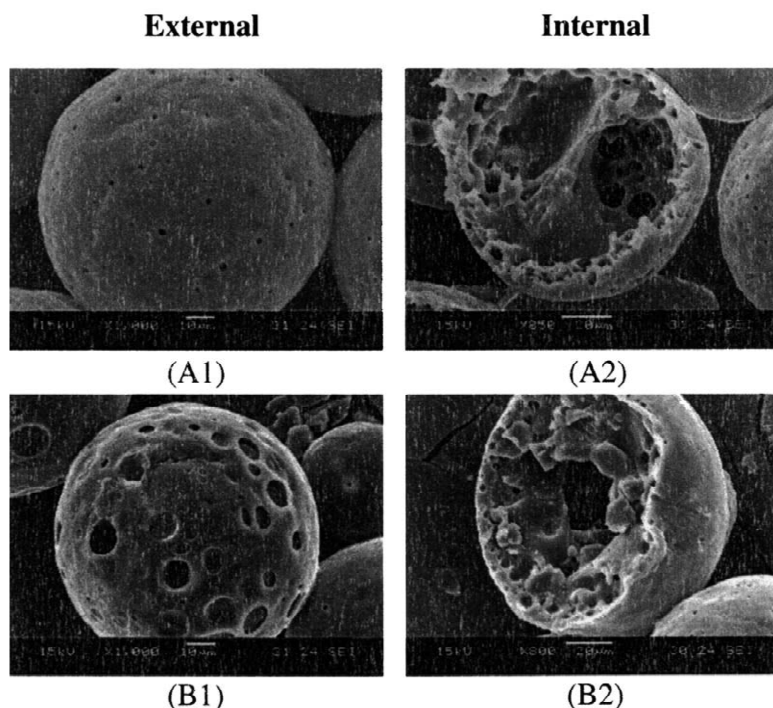

Fig. 6. External and internal morphologies of the CyA- and CyA–BSA-loaded double-walled POE/PLGA microspheres after 5 days in vitro. (A1, A2) CyA-loaded double-walled microspheres; (B1, B2) CyA–BSA-loaded double-walled microspheres; (A1, B1) bars = 10 μm; (A2, B2) bars = 20 μm.

tion was greater for the degrading CyA–BSA-loaded double-walled POE/PLGA microspheres, leading to faster CyA release.

In the double-walled POE/PLGA microsphere system, the PLGA shell acted as a carrier for BSA and a reservoir for CyA especially after the POE core disappeared. Provided that most BSA molecules distributed within the PLGA shell and the majority of CyA was loaded in the POE core, a possible CyA release mechanism of both CyA- and CyA–BSA-loaded double-walled POE/PLGA microspheres was proposed (Fig. 7). In the case of CyA-loaded double-walled POE/PLGA microspheres, CyA release was slow in the initial stage because of the PLGA shell acting as a barrier for water flux. After about 2 weeks, however, the acidic degradation products of POE and PLGA increased the water-solubility of CyA. The bigger pores were created in the shell due to the degradation of PLGA, and the hollow structure was formed after extensive POE degradation. Both accelerated CyA release. Unlike the POE and PLGA microspheres, the PLGA shell held CyA in numerous micro-reservoirs and prevented it from aggregation. In addition, the PLGA shell provided a relatively constant surface area due to the absence of agglomeration, resulting in a sustained CyA release following the initial slow release phase. For the CyA–BSA-loaded double-walled POE/PLGA microspheres having a porous surface and shell, a sustained CyA release was observed from the right beginning. After 5 days in vitro, most BSA molecules were released, leaving bigger pores in the shell (Fig. 7), leading to slightly increased CyA release. On the other hand, the acidic environment produced by the degradation products of POE and PLGA might also be another reason for this slight increase in CyA release. Compared to the CyA-loaded double-walled POE/PLGA microspheres, no obvious biphasic release pattern for the CyA–BSA-loaded double-walled POE/PLGA microspheres was observed because

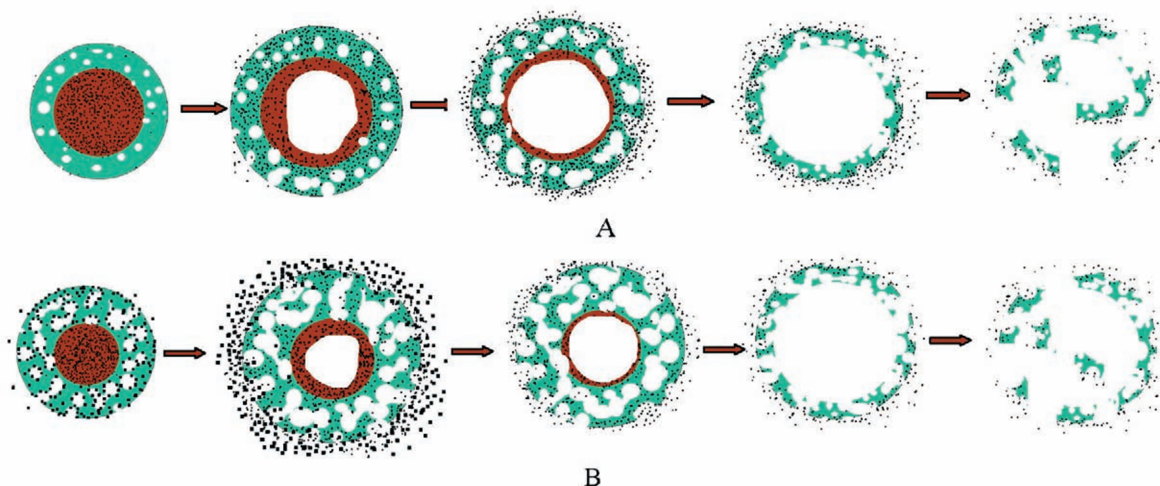

Fig. 7. Schematic representation of the proposed release mechanism for the double-walled POE/PLGA microspheres. (A) CyA-loaded double-walled microspheres; (B) CyA–BSA-loaded double-walled microspheres. ■, BSA; ●, CyA.

they were more porous on the surface and in the shell before and after the in vitro degradation.

4. Conclusions

In this study, hydrophilic BSA and hydrophobic CyA were encapsulated in the double-walled POE/PLGA microspheres by a double emulsion solvent evaporation process. It was believed that most BSA and CyA were loaded in the PLGA shell and POE core, respectively, due to the difference in their hydrophilicities. The BSA and CyA release properties of the double-walled POE/PLGA microspheres were compared with the neat POE and PLGA microspheres. High encapsulation efficiencies of BSA and CyA were achieved. The presence of CyA did not affect the physical properties of, and BSA release from, the double-walled POE/PLGA microspheres. However, the double-walled POE/PLGA microspheres containing BSA possessed a much more porous surface and PLGA shell compared to those without BSA. A complete BSA and CyA release was achieved by the double-walled POE/PLGA microspheres, and the BSA release was sustained over 5 days. The CyA-loaded double-walled POE/PLGA microspheres yielded a biphasic CyA release pattern—14% CyA was released during the initial slow release phase of 15 days, followed by 78% CyA release over 27 days. More importantly, the CyA–BSA-loaded double-walled POE/PLGA microspheres provided a more constant and faster CyA release—95% CyA was released in a relatively constant manner over 30 days. The double-walled POE/PLGA microspheres proved to be more advantageous as a carrier for the delivery of hydrophilic and/or hydrophobic proteins than the neat POE and PLGA microspheres. The double-walled POE/PLGA microspheres may find new applications in drug delivery.

Acknowledgements

We would like to express our gratitude to Mr. Xue-Ming Liu at the Institute of Materials Research and Engineering, Singapore for his technical assistance with the NMR.

References

[1] Y.Y. Yang, M. Shi, S.H. Goh, POE/PLGA composite microspheres: formation and in vitro behaviors of double-walled microspheres, J. Control. Rel. submitted for publication.
[2] K.J. Pekarek, J.S. Jacob, E. Mathiowitz, One-step preparation of double-walled microspheres, Adv. Mater. 6 (1994) 685–687.
[3] K.J. Pekarek, M.J. Dyrud, K. Ferrer, Y.S. Jong, E.

Mathiowitz, In vitro and in vivo degradation of double-walled polymer microspheres, J. Control. Rel. 40 (1996) 169–178.
[4] K.J. Leach, E. Mathiowitz, Degradation of double-walled polymer microspheres of PLLA and P(CPP:SA) 20:80. I. In vitro degradation, Biomaterials 19 (1998) 1973–1980.
[5] K.J. Pekarek, J.S. Jacob, E. Mathiowitz, Double-walled polymer microspheres for controlled drug release, Nature 367 (1994) 258–260.
[6] C. Richardson, P. Emery, Clinical use of cyclosporin in rheumatoid arthritis, Drugs 50 (1995) 26–36.
[7] R. Gref, P. Quellec, A. Sanchez, P. Calvo, E. Dellacherie, M.J. Alonso, Development and characterization of CyA-loaded poly(lactic acid)– poly(ethylene glycol) micro- and nanoparticles. Comparison with conventional PLA particulate carriers, Eur. J. Pharmaceut. Biopharmaceut. 51 (2001) 111–118.
[8] T. Urata, K. Arimori, M. Nakano, Modification of release rates of cyclosporin A from poly(L-lactic acid) microspheres by fatty acid esters and in vivo evaluation of the microspheres, J. Control. Rel. 58 (1999) 133–141.
[9] M.K. Lee, L. Choi, M.H. Kim, C.K. Kim, Pharmacokinetics and organ distribution of cyclosporin A incorporated in liposomes and mixed micelles, Intern. J. Pharmaceut. 191 (1997) 87–93.
[10] J. Molpeceres, M.R. Aberturas, M. Guaman, Biodegradable nanoparticles as a delivery system for cyclosporin: preparation and characterization, J. Microencap. 17 (2000) 599–614.
[11] S.Y. Ng, H.R. Shen, E. Lopez, Y. Zherebin, J. Barr, E. Schacht, J. Heller, Development of a poly(ortho ester) prototype with a latent acid in the polymer backbone for 5-fluorouracil delivery, J. Control. Rel. 65 (2000) 367–374.
[12] T. Peters, All About Albumin: Biochemistry, Genetics, and Medical Applications, Academic Press, San Diego, 1996.
[13] Y.Y. Yang, T.S. Chung, X.L. Bai, W.K. Chan, Effect of preparation conditions on morphology and release profiles of biodegradable polymeric microspheres containing protein fabricated by double-emulsion method, Chem. Eng. Sci. 55 (2000) 2223–2236.
[14] T. Urata, K. Arimori, M. Nakano, Modification of release rates of cyclosporin A from poly(L-lactic acid) microspheres by fatty acid esters and in vivo evaluation of the microspheres, J. Control. Rel. 58 (1999) 133–141.
[15] H.K. Kim, T.G. Park, Microencapsulation of human growth hormone within biodegradable polyester microspheres: protein aggregation, stability and incomplete release mechanism, Biotechnol. Bioeng. 65 (1999) 659–667.
[16] S. Cohen, T. Yoshioka, M. Lycarelli, L.M. Hwang, R. Langer, Controlled delivery systems for proteins based on poly(lactic/glycolic acid) microspheres, Pharm. Res. 8 (1991) 713–720.
[17] H.H. Chia, Y.Y. Yang, T.S. Chung, S.Y. Ng, J. Heller, Autocatalyzed poly(ortho ester) microspheres: a study on their erosion and drug release mechanism, J. Control. Rel. 75 (2001) 11–25.
[18] K. Fu, D.W. Pack, A. Laverdier, R. Son, R. Langer, Visualization of pH in degrading polymer microspheres, Proceed. Intern. Symp. Control. Rel. Bioact. Mater. 25 (1998) 150–151.