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Controlled protein release from monodisperse biodegradable double-wall microspheres of controllable shell thickness

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Abstract

Biodegradable polymer microparticles are promising delivery depots for protein therapeutics due to their relatively simple fabrication and facile administration. Double-wall microspheres (DWMS) comprising a core and shell made of two distinct polymers may provide enhanced control of the drug release profiles. Using precision particle fabrication (PPF) technology, monodisperse DWMS were fabricated with model protein bovine serum albumin (BSA)-loaded poly(lactide-*co*-glycolide) (PLG) core and drug-free poly(D,L-lactic acid) (PDLL) shell of uniform thickness. Monolithic single-wall microspheres were also fabricated to mimic the BSA-loaded PLG core. Using ethyl acetate and dichloromethane as shell- and core-phase solvents, respectively, BSA was encapsulated selectively in the core region within DWMS with higher loading and encapsulation efficiency compared to using dichloromethane as core and shell solvents. BSA *in vitro* release rates were retarded by the presence of the drug-free PDLL shell. Moreover, increasing PDLL shell thickness resulted in decreasing BSA release rate. With a 14- μ m thick PDLL shell, an extended period of constant-rate release was achieved.

Keywords

Monodisperse double-wall microspheres; Poly(lactide-*co*-glycolide); Poly(lactic acid); Bovine serum albumin; Controlled release

1. Introduction

Advances in biotechnology have led to an explosion of therapeutic proteins as potential drug candidates [1]. However, due to their macromolecular size and short half lives *in vivo*, the administration routes of protein drugs are limited. In order to maintain effective drug concentration, frequent injections are typically needed, which may lead to poor patient compliance. Thus, well-controlled and sustained delivery methods are crucial for the application of protein therapeutics, and the development of effective delivery systems is required to turn biological potential into medical reality.

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Biodegradable polymer-based delivery systems administered by oral, pulmonary or parenteral injection bear advantages such as reduced dosing frequency, high local drug concentrations at the site of administration, protection of fragile therapeutics, minimized side effects, simple production process and no requirement for surgical resection. In particular, biodegradable polymer microparticles have received much attention, and several products been commercialized.

Drug release rates from monolithic single-wall microspheres (SWMS) may be controlled by choosing polymers with appropriate degradation kinetics [2], varying physical characteristics of particles [3–7], conjugating drugs to polymers [8] or controlling the particle size distribution [9–15]. However, the control is limited due to the relatively simple structures of such particles. Adding a second drug-free polymer layer of variable thickness as a rate-controlling membrane provides more flexibility in acquiring desired release rates as well as improves encapsulation of the protein drugs. Such core-shell particles have been fabricated previously [16–21]. Most have relied on immiscibility of the core and shell materials, and control of shell thickness was poor.

In this study, we report the first example of monodisperse double-wall microspheres (DWMS) comprising a protein-encapsulating poly(lactide-co-glycolide) (PLG) core and highly uniform, drug-free poly(D,L-lactic acid) (PDLL) shell as the release rate-controlling layer by using precision particle fabrication technique (PPF) [22–26]. Bovine serum albumin (BSA) was used as a model protein. In particular, monodisperse DWMS were produced with constant-sized BSA-PLG cores and PDLL shell with varying thickness. Also, monodisperse PLG SWMS loaded with BSA, mimicking the PLG core in DWMS, were fabricated for comparison. Most importantly, *in vitro* BSA release was quantified over a period of five months to determine the effect of uniform shell thickness on protein release rates. In addition, observation of DWMS erosion provides insight into the mechanism of BSA release.

2. Materials and methods

2.1 Materials

Poly(lactide-co-glycolide) (PLG; 50/50 lactide/glycolide ratio, Mw 4.2 kDa) and poly(D,L-lactic acid) (PDLL, Mw 43 kDa) were purchased from LACTEL Absorbable Polymers. Bovine serum albumin (BSA, Mw 66,700 Da) and reagent grade sodium bicarbonate were purchased from Fisher Scientific. Chromatography grade of dimethyl sulfoxide (DMSO), ethyl acetate (EtAc) and dichloromethane (DCM) were obtained from Sigma-Aldrich. Fluorescent dye 5-(and-6)-carboxytetramethylrhodamine succinimidyl ester (TAMRA) was obtained from Molecular Probes. Poly(vinyl alcohol) (PVA, Mw 25,000 Da, 88% hydrolyzed) was purchased from Polysciences. Tween 80 was purchased from Acros Organics.

2.2 Fabrication of double-wall microspheres with controlled shell thickness

Triple nozzle and double nozzle systems were employed for producing DWMS and SWMS, respectively (Supplementary Information Fig. S1). For the triple nozzle system, a steel hypodermic needle (PrecisionGlide, Becton Dickinson Co.) with flat tip was used as the innermost nozzle, which was surrounded coaxially by the inner glass nozzle made from a glass capillary (World Precision Instrument, Inc.). The outer glass nozzle surrounded the inner glass nozzle and was made of Pyrex glass (Kimax). For the double nozzle system, no outer glass nozzle was used.

The core phase containing BSA (100 mg/mL in water) was emulsified with PLG/DCM solution (30% w/v) at a volumetric ratio of 1:10 using Ultrasonic tip (CE Converter 102 C, Branson) at 60% amplitude for 1 minute. By keeping flow rates the same and varying the shell phase PDLL concentration (3% w/v, 6% w/v, 9% w/v) while keeping the core phase PLG concentration constant (30% w/v), DWMS with different shell thickness were fabricated with different PDLL/PLG mass ratio. PVA water solution (0.5% w/v) was used as non-solvent carrier stream.

For DWMS fabrication, two syringe pumps (Pump 11, Harvard Apparatus) were employed for carrying the core and shell phase. One gear pump (IP65, ISMATEC) was used for PVA carrier stream. The core phase PLG/BSA emulsion stream passed through the innermost metal nozzle (0.05–0.10 mL/min) and the shell phase PDLL stream passed through the inner glass nozzle (0.5–1.0 mL/min). The outer glass nozzle was for PVA non-solvent carrier stream (5–10 mL/min), which was used to improve the round shape of particles as well as providing “drag force” in order to produce particles smaller than the nozzle opening [13]. For SWMS fabrication, only one syringe pump was used for the PLG/BSA emulsion stream. This core phase passed through the innermost metal nozzle (0.05–0.10 mL/min) and the inner glass nozzle was used for carrier stream (2.5–10 mL/min) which was pumped by the gear pump. The fabrication of SWMS was based on the results of DWMS fabrication. The outer diameters of SWMS were equal to the calculated core diameters of DWMS. The frequency generator (Agilent 33220A) and piezoelectric transducer (CV33, Sonic & Materials Inc.) of PPF system generated an acoustic wave on the nozzle to break the polymer-based stream into uniform droplets with desired outer diameter. Nascent DWMS and SWMS (wet particles before lyophilizing) were collected in a beaker with 500 mL of 0.5% (w/v) PVA solution and were stirred for another 3 h for organic solvent extraction/evaporation. The particles were filtered (Filter Paper #4, Whatman), washed three times by deionized water and lyophilized for 48 h. Samples were stored until use in a –20 °C freezer with desiccant.

2.3 Particle size distribution

The size distributions of nascent DWMS and SWMS were determined using a Coulter Multisizer III (Beckman Coulter Inc.) with a 200 micron aperture. More than 10,000 particles were measured for each sample.

2.4 Protein loading and encapsulation efficiency

To measure the BSA loading of each batch of microparticles, a sample of approximately 5 mg microparticles was dissolved in 100 μ L DMSO. After complete dissolution, the solution was added dropwise into 1 mL of phosphate-buffered saline (PBS, pH 7.4 $\pm$ 0.05) and then incubated for 1 h at 37 °C with shaking (240 rpm). The mixture was centrifuged for 10 minutes at 10,000 rpm to settle the precipitate. BSA concentration in the supernatant was determined using BCA assay (Pierce). All absorbance measurements were taken on a SpectraMax 340 PC reader equipped with SoFTMax Pro software. The loading of each batch equaled the mass of BSA measured by absorbance per mass of particles. The encapsulation efficiency of each batch of microparticles equaled the actual loading divided by theoretical BSA loading multiplied by 100.

2.5 Particle DSC study

Differential Scanning Calorimetry (DSC) was performed on each batch of microparticles as well as pure polymers to study their thermal properties such as glass transition temperatures (T_g) and melting temperature (T_m). Perkin Elmer Pyris Diamond DSC was used, and all samples were heated from –10 °C to 200 °C at 10 °C/min.

2.6 Scanning electron microscopy (SEM)

DWMS and SWMS were prepared for imaging by placing a droplet of an aqueous particle suspension on a silicon stub. The samples were dried overnight and were sputter coated with gold and platinum prior to imaging. In order to image the particle cross-sections, microparticles were frozen in a 1.7 mL micro-centrifuge tube immersed in liquid nitrogen and then fractured with a blade. The JEOL 6060 LV Scanning Electron Microscope was used at an acceleration voltage of 10–20 kV.

2.7 Confocal fluorescence microscopy

Twenty milligrams of BSA were dissolved in 2 mL of sodium bicarbonate (Fisher) at pH 8.3 ± 0.05 . A solution of 1 mg TAMRA in 100 μ L DMSO (Fisher) was then pipetted into a foil-wrapped vial containing the BSA solution. The solution was stirred for 60–120 minutes at room temperature, and then separated using PD-10 desalting column (GE Healthcare). The labeled protein was collected from the column, frozen, and lyophilized. The degree of labeling (DoL, the number of TAMRA molecules attached to each protein molecule) as determined from the relative absorbances of TAMRA and BSA was 3.40. Particles were loaded with 5% of TAMRA-labeled BSA and 95% unlabeled BSA.

Fluorescent and transmitted light images of the protein-loaded DWMS and SWMS were taken with a Leica SP2 visible laser confocal microscope. Images were obtained with a 63 $\times$ oil immersion lens. Fluorescence was excited using a HeNe laser (543 nm) and emission collected with a 575–640 nm band-pass filter.

2.8 *In vitro* BSA release

For each batch of DWMS or SWMS, a sample of approximately 30 mg was suspended in 1.25 mL release buffer consisting of PBS and 0.05% (v/v) Tween 80. These samples were incubated for three to five months at 37 °C with shaking (240 rpm). At various time points, 1.0 mL supernatant was removed and replaced with fresh media in order to maintain constant pH sink condition. The release study was performed in triplicate, and BSA concentrations in the collected supernatants were measured using BCA assay (Pierce).

2.9 Particle degradation study

For each batch of DWMS or SWMS, approximately 5 mg was suspended in 1.25 mL release buffer consisting of PBS and 0.05% (v/v) Tween 80. These samples were incubated at 37 °C with shaking (240 rpm). At various time points, supernatant was discarded and the samples were frozen and lyophilized for at least 48 h. The samples were prepared for SEM as described above.

2.10 SDS-PAGE

BSA in supernatants during *in vitro* release was subjected to non-reduced sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis using precast gradient gels (4–20% Tris-HCl/glycine) and Mini-PROTEAN II system (Bio-Rad Laboratories, Inc.). Running buffer (25 mM Tris, 192 mM glycine and 0.1% (w/v) SDS, pH 8.3) were diluted from 10 $\times$ Tris/Glycine/SDS buffer. Samples were diluted 1:1 in Laemmli sample buffer (62.5 mM Tris-HCl, pH 6.8, 25% glycerol, 2% SDS, 0.01% Bromophenol blue) under non-reduced conditions (without β -mercaptoethanol or DTT), and heated for 1 min at 95 °C for prior to loading. Gels were electrophoresed for 40 min at 200 V and then stained with Coomassie blue to visualize the protein bands.

3. Results

3.1 Production of monodisperse BSA-loaded DWMS with varying shell thickness

Organic solvents DCM and EtAc are commonly used for dissolving biodegradable polymers [27–30]. For DWMS, we can choose different configurations of DCM and EtAc to dissolve the core polymer (PLG) and shell polymer (PDLL). DWMS fabricated with PPF using DCM as shell solvent and EtAc as core solvent (designated as DCM(EtAc)) or EtAc as both shell and core solvents (EtAc(EtAc)) exhibited poor uniformity. EtAc(DCM) and DCM(DCM) configurations could generate DWMS with good uniformity [31].

We produced DCM(DCM) and EtAc(DCM) DWMS with varying shell thickness by keeping PDLL shell and PLG core flow rates constant and increasing PDLL concentration in the shell phase from 3% to 9% (w/v), thus increasing the PDLL/PLG mass ratio. Assuming no polymer mass loss, volume additivity and ideal concentric core-shell structure of DWMS, the PLG core diameters and PDLL shell thickness can be calculated from the measured overall particle diameter (Table 1 and Supplementary Data).

DWMS fabricated using both DCM(DCM) and EtAc(DCM) solvent configurations had good uniformity (Fig. 1). For a given PDLL/PLG mass ratio, particles exhibited similar outer diameter, within 2 μm , regardless of solvent selection (Table 1). The calculated diameter of PLG core and the PDLL shell thickness are also close. The calculated PLG core diameter was constant at 48–50 μm , and the shell thickness increased from ~6 μm to ~14 μm upon increasing the PDLL/PLG mass ratio from 1.09 to 3.04 (Table 1). When increasing PDLL concentration in DCM or EtAc to 12% (w/v) or higher, stable DWMS production could not be achieved.

We fabricated PLG SWMS (sample O) in DCM to mimic the PLG core of DWMS using a double nozzle PPF system. The measured diameter of SWMS, 48.5 μm diameter, was similar to the calculated core diameter of DWMS (Table 1). These SWMS were used for comparison to the DWMS in BSA *in vitro* release as well as microscopic study.

Initial protein loading and encapsulation efficiency were measured after extracting BSA from the particles. The BSA loadings in DWMS of both DCM(DCM) and EtAc(DCM) decreased as PDLL/PLG mass ratio increased, due to addition of BSA-free PDLL layer. The loadings of BSA in DCM(DCM) DWMS were lower than those in EtAc(DCM) DWMS (Fig. 2A). This may be due to the longer particle hardening time of DCM(DCM) DWMS than EtAc(DCM) DWMS, allowing more BSA to escape. PLG SWMS exhibited an encapsulation efficiency of 30%, which was close to those of DCM(DCM) DWMS of different shell thickness. This meant the drug-free PDLL shells of DCM(DCM) DWMS had no effect on encapsulation of BSA in the PLG cores. However, the encapsulation efficiencies of EtAc(DCM) DWMS with different PDLL/PLG mass ratio were higher (~50%) than those of SWMS and DCM(DCM) DWMS (~30%). Surprisingly, the encapsulation efficiency showed no increase with increasing PDLL/PLG mass ratio for both DCM(DCM) and EtAc(DCM) DWMS (Fig. 2B).

3.2 DWMS thermal properties

DWMS as well as pure PDLL and PLG were examined using DSC to determine the glass transition temperature T_g and T_m . For pure PDLL and PLG, no T_m were observed within the temperature range of 0–200 $^{\circ}\text{C}$, proving these two polymers were amorphous at these specific molecular weights. The T_g peaks of PDLL and PLG centered at 50 and 35 $^{\circ}\text{C}$ respectively (Fig. 3). For DCM(DCM) DWMS A1–A3, the DSC graphs showed combinations of PDLL and PLG. The PLG peaks were centered within 30–35 $^{\circ}\text{C}$ while the PDLL peaks were located in the 50–55 $^{\circ}\text{C}$ range with even stronger signal than pure PDLL

(Fig. 3A). These may be caused by the partial mixing of PLG and PDLL in these DCM(DCM) DWMS. For EtAc(DCM) DWMS B1-B3, the DSC graphs were also combinations of pure PLG and PDLL, although there were plateaus from 35–50 °C and no strong single peaks (Fig. 3B).

3.3 Surface and interior morphology of DWMS and SWMS

Scanning electron microscopy was employed to study the initial morphology of the microparticle surface and interior (Fig. 4). Micrographs of SWMS mimicking the BSA loaded PLG cores showed coarse surface and porous interior structures (Fig. 4O). For DCM(DCM) DWMS, the surfaces were porous and the porosity increased with PDLL/PLG mass ratio (Fig. 4A1, A2, A3). Cross-sections showed that porous structures concentrated near the particle peripheries especially for high PDLL/PLG mass ratio. This may be due to the relatively slow removal rate of DCM and the relatively longer particle hardening time which causes the BSA-water droplets of the primary emulsion to migrate towards the particle surface or even escape the particles. The relatively low BSA loadings and encapsulation efficiencies of DCM(DCM) DWMS agreed with this observation. The surfaces of EtAc(DCM) DWMS were smooth, and cross-section micrographs revealed that the porous structures were relatively confined near the core for all three PDLL/PLG mass ratios (Fig. 4B1, B2, B3). This was probably due to the faster extraction rate of EtAc from the shell phase and faster particle hardening process that could better encapsulate the BSA-loaded PLG core. Higher loading and encapsulation efficiency of BSA within EtAc(DCM) DWMS agreed with this result.

3.4 BSA distribution in DWMS and SWMS

Confocal fluorescence microscopy allowed visualization of spatial distribution of TAMRA-labeled BSA within SWMS and DWMS (Supplementary Data Fig. S2), and image analysis of the micrographs provided average radial fluorescence intensities of the particles (Fig. 5). TAMRA-BSA distribution within SWMS (sample O) was relatively uniform across the particles. For DCM(DCM) DWMS A1-A3, the TAMRA-BSA fluorescence was concentrated in the periphery. As PDLL/PLG mass ratio and overall particle size increased, the distance between peaks increased accordingly. For the EtAc(DCM) solvent configuration, B1-B3, TAMRA-BSA concentrated in the core area and there were clearly drug-free PDLL layers, likely due to the fast extraction rate of EtAc from the shell. The intensity plateau areas are similar for all three shell thicknesses within 25–30 μm of the center, which suggests relatively constant BSA-loaded cores and increasing shell thickness.

3.5 BSA *In vitro* release

BSA release from DWMS with different PDLL shell thicknesses were compared with the release profile of BSA-loaded PLG SWMS mimicking the PLG cores of DWMS. For DCM(DCM) DWMS A1-A3, the profiles of cumulative release of BSA with regard to initial particle BSA loadings showed a fast initial release in the first 10 days, a lag phase exhibiting slower BSA release from ~10–20 days, and a final faster release phase (Fig. 6A). This was also the case for BSA release profile of SWMS (O). The initial BSA release rate increased with PDLL shell thickness because a significant amount of BSA concentrated near the surface of DWMS (Fig. 5). The steady release rates of A1-A3 after the lag phase were slower than that of O, likely due to the effect of slowly degrading PDLL shell hindering BSA release from the core, as expected.

EtAc(DCM) DWMS B1-B3 exhibited a similar initial release phase, with ~35% of encapsulated BSA released in the first 10 days (Fig. 6B). The lag phase almost merged with steady release phase for DWMS when PDLL/PLG mass ratio increased and there was clearly evidence of prolonged BSA release compared to SWMS. For EtAc(DCM) DWMS

B1 (6.28 μm PDLL shell), the BSA release profile showed a longer lag phase from 10 to ~40 days, then an increasing release rate achieving complete BSA release at ~90 days. For B2 (10.64 μm PDLL shell), the lag phase could not be distinguished from the final steady release phase, and a dramatically slower release rate compared to SWMS was observed. Complete BSA release was reached at ~120 days. B3 (13.93 μm PDLL shell) exhibited a similar profile with nearly constant release rate from 10 to 140 days. Thus, using EtAc(DCM) solvent configuration, BSA release rates showed clearly slower and prolonged release compared to that of SWMS or DCM(DMC) DWMS. Upon increasing drug-free PDLL shell thickness, BSA *in vitro* release rates were decreased accordingly, as the PDLL shells serve as rate-controlling diffusion barrier.

3.6 Particle morphology during *in vitro* DWMS degradation/erosion

To elucidate relationships between initial BSA distribution, release rate profiles and changes in porosity and morphology, particles were imaged by SEM at various times during the release experiment. For DCM(DCM) DWMS A1-A3 (Fig. 7), most of the surface and interior pores (c.f. Fig. 4) were not observed at 21 days. The surfaces became smooth and the cross-sections were dense compared to the initial state. After 42 days, the diameters of A1-A3 particles increased, and particle surfaces and interiors became porous again, likely due to polymer degradation and erosion. By 63 days, hollow cores began to develop, and A1 and A2 (6.37 μm and 10.85 μm PDLL shells, respectively) broke into pieces while A3 (14.01 μm PDLL shell) remained spherical. For (DCM) SWMS O, particles deformed and agglomerated by 21 days and quickly broke into pieces after day 42.

For EtAc(DCM) DWMS B1-B3 (Fig. 8), at 21 days, most porous structures near the center disappeared, similar to A1-A3. By 42 days, the sizes of B1-B3 DWMS had increased, but not as much as A1-A3. From the cross-section micrographs, hollow cores began to appear for all three PDLL/PLG mass ratios after 42 days. The initial porosity of cores in B1-B3 (c.f. Fig. 4) perhaps contributed to their faster erosion. At 63 days, the surfaces became coarser and the hollow core areas expanded. However, B1-B3 retained a spherical shape. After 90 days, B1 (6.28 μm PDLL shell) broke into pieces while B2 (10.64 μm PDLL shell) and B3 (13.94 μm PDLL shell) remained spherical.

3.7 BSA stability during *in vitro* release

BSA in supernatants during *in vitro* release was subjected to SDS-PAGE to study the protein stability. Reducing agents β -mercaptoethanol or DTT in reduced SDS-PAGE can eliminate any protein aggregates, thus, no-reduced condition was chosen for this study. From literature, BSA may undergo covalent/non-covalent aggregation as well as degradation during matrix degradation/erosion, since BSA may attach to the matrix and the microclimate of inner matrix may turn acidic. Non-reduced SDS-PAGE study was performed at day 7, 42 and 73 during BSA *in vitro* release (Supplementary Information Fig. S3). From the gels, at day 7, both fresh BSA solution and particle supernatants show strong bands of BSA monomer (around 60 kDa) and dimer (around 120 kDa). This proves some BSA undergoes aggregation at the initial stage of *in vitro* release. At Day 42, BSA release from SWMS and DWMS showed single bands of BSA monomer, proving most BSA retain integrity during release. At Day 73, there were no bands observed from SWMS sample O as well as DCM(DCM) DWMS sample A1-A3, which was in accordance with previous *in vitro* release study that BSA release was almost finished in these samples (Fig. 6). For EtAc(DCM) DWMS sample B1-B3, BSA monomers were still observed.

4. Discussion

The size distribution and structure control of biodegradable microparticles are crucial factors for encapsulated protein delivery. Microspheres and double-wall microspheres fabricated using conventional methods such as emulsion-solvent evaporation/extraction usually have broad size distributions. Thus the factors influencing protein encapsulation and release cannot be easily elucidated. By using a precision particle fabrication technique, we are able to finely control the diameter as well as the shell thickness of microparticles. This allows us to explore the interconnections between particle structures, initial drug distribution and protein release rate.

By carefully selecting fabrication parameters such as polymer solution flow rate, polymer concentration, and organic solvent configuration, we were able to fabricate DWMS with constant core diameter and varying uniform shell thickness. Because PLG and PDLL are very similar polymers, it is likely that some mixing of the core and shell materials occurs. Thus, one expects that the DWMS in fact comprise a PLG-rich core and a PDLL-rich shell, as well as a region containing both polymers near the core-shell boundary. Unfortunately, the core and shell materials cannot be distinguished by SEM (Figure 4). Regardless, confocal fluorescence microscopy revealed that BSA was localized preferentially to the core of the EtAc(DCM) DWMS (Figure 5).

The outer diameters of DWMS using either DCM(DCM) or EtAc(DCM) solvent configuration at the same flow rates and same PDLL/PLG mass ratio were close (Table 1). However, the surface and cross-section SEM images of DCM(DCM) and EtAc(DCM) DWMS showed large differences. Although increasing PDLL/PLG mass ratio produced an increase of outer diameters in both solvent configurations, the DCM(DCM) DWMS A1-A3 had porous surfaces while EtAc(DCM) DWMS B1-B3 had relatively smooth surfaces. In addition, the inner pores of DCM(DCM) DWMS were concentrated near the periphery while those of EtAc(DCM) DWMS were concentrated in the center (Fig. 3). These differences were probably caused by the difference in extraction/evaporation rate of DCM and EtAc in the shell phase. The relatively fast extraction rate of EtAc in the shell phase better encapsulated BSA-loaded core for all three PDLL/PLG mass ratios, while for DCM the slower removal rate and longer particle hardening time allowed the BSA-water droplets to migrate to the particle surfaces.

Combining the data of microparticle morphology, initial drug distribution, and *in vitro* release, we found that by using EtAc(DCM), BSA-loaded PLG cores were better encapsulated, and the release of BSA was dramatically slower than SWMS without PDLL shell. Increasing the thickness of PDLL drug-free shells postponed BSA release to some extent and ultimately constant-rate release was achieved. However, for DCM(DCM) DWMS, increasing shell thickness did not slow down BSA release rate due to the redistribution of BSA toward the particle surface during fabrication.

The *in vitro* erosion study of DCM(DCM) DWMS showed the relatively even distribution of porous structures at earlier periods of degradation and hollow core areas began to develop after 63 days (Fig. 6). The porous structure might be caused by BSA-water droplet aggregation, and because of the slow hardening process of DCM(DCM) DWMS, the aqueous phase tended to move to the periphery. Since the degradation and erosion rates of PLG are much faster than PDLL [32], hollow regions developed in the particle cores. For EtAc(DCM), BSA-water droplets were confined near the cores due to the fast EtAc extraction rate, and hollow cores were observed at an earlier stage (Fig. 8).

Non-reduced SDS-PAGE was carried out to study the stability of BSA during *in vitro* release. From the gels, BSA undergoes aggregation at the initial stage of *in vitro* release and retains most of its integrity during release.

5. Conclusions

Monodisperse DWMS with varying shell thickness as well as uniform PLG SWMS were successfully fabricated using precision particle fabrication. By using EtAc(DCM) solvent configuration, DWMS with constant BSA-loaded PLG core and BSA-free PDLG shell were fabricated with higher BSA loading and encapsulation efficiency. The *in vitro* release profiles of BSA confirmed the hypothesis that increasing shell thickness while keeping the protein-loaded core diameter constant postponed the release rate of encapsulated protein and constant release rate was achieved when PDLG/PLG mass ratio increased from 1.09 to 3.04.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Size distributions of DCM(DCM) (A) and EtAc(DCM) (B) DWMS/SWMS: Sample O, (DCM), PDLL/PLG 0; Sample A1, DCM(DCM), PDLL/PLG 1.09; Sample A2, DCM(DCM), PDLL/PLG 2.14; Sample A3, DCM(DCM), PDLL/PLG 3.04; Sample B1, EtAc(DCM), PDLL/PLG 1.09; Sample B2, EtAc(DCM), PDLL/PLG 2.14; Sample B3, EtAc(DCM), PDLL/PLG 3.04.

Figure 2.

Loading (A) and encapsulation efficiencies (B) of DWMS with different PDLL/PLG mass ratio: Sample O, (DCM), PDLL/PLG 0; Sample A1, DCM(DCM), PDLL/PLG 1.09; Sample A2, DCM(DCM), PDLL/PLG 2.14; Sample A3, DCM(DCM), PDLL/PLG 3.04; Sample B1, EtAc(DCM), PDLL/PLG 1.09; Sample B2, EtAc(DCM), PDLL/PLG 2.14; Sample B3, EtAc(DCM), PDLL/PLG 3.04.

Figure 3.

DSC graphs of DCM(DCM) DWMS (A), EtAc(DCM) DWMS (B) and pure PDLL (43 kDa), PLG (4.2 kDa): Sample A1, DCM(DCM), PDLL/PLG 1.09; Sample A2, DCM(DCM), PDLL/PLG 2.14; Sample A3, DCM(DCM), PDLL/PLG 3.04; Sample B1, EtAc(DCM), PDLL/PLG 1.09; Sample B2, EtAc(DCM), PDLL/PLG 2.14; Sample B3, EtAc(DCM), PDLL/PLG 3.04.

Figure 4.

SEM images of DCM/SWMS: Sample O, (DCM), PDLL/PLG 0; Sample A1, DCM(DCM), PDLL/PLG 1.09; Sample A2, DCM(DCM), PDLL/PLG 2.14; Sample A3, DCM(DCM), PDLL/PLG 3.04; Sample B1, EtAc(DCM), PDLL/PLG 1.09; Sample B2, EtAc(DCM), PDLL/PLG 2.14; Sample B3, EtAc(DCM), PDLL/PLG 3.04.

Figure 5.

Average fluorescence intensities of TAMRA-labeled BSA along radial direction of microparticles: Sample O, (DCM), PDLL/PLG 0; Sample A1, DCM(DCM), PDLL/PLG 1.09; Sample A2, DCM(DCM), PDLL/PLG 2.14; Sample A3, DCM(DCM), PDLL/PLG 3.04; Sample B1, EtAc(DCM), PDLL/PLG 1.09; Sample B2, EtAc(DCM), PDLL/PLG 2.14; Sample B3, EtAc(DCM), PDLL/PLG 3.04.

Figure 6.

In vitro release profiles of DCM(DCM) DWMS (A) and EtAc(DCM) DWMS (B) with different PDLL/PLG mass ratio: Sample O, (DCM), PDLL/PLG 0; Sample A1, DCM(DCM), PDLL/PLG 1.09; Sample A2, DCM(DCM), PDLL/PLG 2.14; Sample A3, DCM(DCM), PDLL/PLG 3.04; Sample B1, EtAc(DCM), PDLL/PLG 1.09; Sample B2, EtAc(DCM), PDLL/PLG 2.14; Sample B3, EtAc(DCM), PDLL/PLG 3.04.

Figure 7. SEM images of DCM(DCM) DWMS degradation/erosion study: Sample O, (DCM), PDLL/PLG 0; Sample A1, DCM(DCM), PDLL/PLG 1.09; Sample A2, DCM(DCM), PDLL/PLG 2.14; Sample A3, DCM(DCM), PDLL/PLG 3.04.

Figure 8.
SEM images of EtAc(DCM) DWMS degradation/erosion study: Sample B1, EtAc(DCM), PDLL/PLG 1.09; Sample B2, EtAc(DCM), PDLL/PLG 2.14; Sample B3, EtAc(DCM), PDLL/PLG 3.04.

Table 1

Dimensions of DWMS/SWMS with different shell thickness

Sample	Solvent Selection Shell (Core)	PDLL (PLG) conc. w/v	PDLL/PLG mass ratio	Outer Diameter Measured (μm)	Core Diameter Calculated (μm)	Shell Thickness Calculated (μm)
A1	DCM (DCM)	3% (30%)	1.09	61.3 $\pm$ 1.4	48.6	6.4
A2	DCM (DCM)	6% (30%)	2.14	71.1 $\pm$ 1.3	49.4	10.8
A3	DCM (DCM)	9% (30%)	3.04	76.8 $\pm$ 1.2	48.7	14.0
B1	EtAc (DCM)	3% (30%)	1.09	60.5 $\pm$ 2.2	47.9	6.3
B2	EtAc (DCM)	6% (30%)	2.14	69.8 $\pm$ 1.6	48.5	10.6
B3	EtAc (DCM)	9% (30%)	3.04	76.3 $\pm$ 1.9	48.4	13.9
O	(DCM)	(30%)	0	48.5 $\pm$ 1.0	N/A ^a	N/A ^a

^aNot applicable