Journal of Controlled Release 172 (2013) 707–714

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

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

Yujie Xia, Pedro F. Ribeiro, Daniel W. Pack *

Department of Chemical and Biomolecular Engineering, University of Illinois, 600 S. Mathews Avenue, Urbana, IL 61801, USA

ARTICLE INFO

Article history:
Received 7 May 2013
Accepted 8 August 2013
Available online 15 August 2013

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

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-μm thick PDLL shell, an extended period of constant-rate release was achieved.

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.

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 have 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

* Corresponding author at: Department of Pharmaceutical Sciences, University of Kentucky, 467 Biological Pharmaceutical Bldg., 789 S. Limestone, Lexington, KY 40536-0596, USA. Tel.: +1 859 218 0907; fax: +1 859 257 7585.
E-mail address: dan.pack@uky.edu (D.W. Pack).

0168-3659/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jconrel.2013.08.009

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 min. 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 μm 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 μL DMSO. After complete dissolution, the solution was added dropwise into 1 mL of phosphate-buffered saline (PBS, pH 7.4 ± 0.05) and then incubated for 1 h at 37 °C with shaking (240 rpm). The mixture was centrifuged for 10 min 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 340PC 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). PerkinElmer 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 6060LV 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 μL DMSO (Fisher) was then pipetted into a foil-wrapped vial containing the BSA solution. The solution was stirred for 60–120 min 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× oil immersion lens. Fluorescence was excited using a HeNe laser (543 nm) and emission was 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.