Journal of Controlled Release 138 (2009) 235–242

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Fabrication of biodegradable spheroidal microparticles for drug delivery applications

Michael J. Heslinga, Eric M. Mastria, Omolola Eniola-Adefeso *

Department of Chemical Engineering, University of Michigan, Ann Arbor, MI 48109, USA

ARTICLE INFO

Article history:
Received 8 November 2008
Accepted 17 May 2009
Available online 22 May 2009

Keywords:
Poly(lactic-co-glycolide acid) (PLGA)
Ellipsoids
Oil-in-water solvent evaporation
Paclitaxel
Poly(vinyl alcohol)(PVA)

ABSTRACT

Particle shape, in addition to size, is becoming increasingly recognized as important in the design of drug carriers for *in vivo* use. However, few methods exist for fabricating non-spherical particles from biodegradable polymers. This work describes for the first time the fabrication of biodegradable spheroidal microparticles using the simple oil-in-water emulsion solvent evaporation technique (O/W ESE). Unloaded and paclitaxel-loaded spheroids were fabricated from poly(lactic-co-glycolic acid) (PLGA), and the shape and size of fabricated spheroids were manipulated by controlling fabrication process parameters including stir speed, aqueous and oil phase viscosity, aqueous phase pH, and the polymer molecular weight and end group. The presented data show that high aqueous phase viscosity, basic aqueous phase pH and hydrophilic polymer side chains and end groups are all conditions that favor the formation of spheroidal particles. The described technique is advantageous over methods currently described in the literature in its simplicity in setup, high particle yield and adaptability to a wide range of biodegradable polymers and therapeutics.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

Biodegradable spherical particles are typically proposed for use as drug carriers for localized delivery of therapeutics in many human diseases due to their ease of fabrication and well-defined model for degradation and drug release [1–6]. The need for a paradigm shift away from spherical carriers for localized drug delivery, however, has become evident in recent literature suggesting that non-spherical particles may be optimum for *in vivo* drug delivery [7]. In one study, Champion and Mitragotri showed that macrophages can easily internalize spherical particles having diameters up to 15-μm, but the internalization of spheroids (rod-like particles) is unachievable when macrophage attacks the rods on their major axis [8]. Similarly, Desimone et al. in their recent publication showed that HeLa cells internalized rod-like particles at a higher rate than spheres of the same volume [9]. Also, Decuzzi and Ferrari using a theoretical model found that oblate (disk-like) particles were more effective in adhering to cell surface in laminar flow than spherical particles of the same volume [10], and Muzykantov et al. showed that immunotargeted disks displayed longer blood circulation *in vivo* than spheres of similar volume [7]. Finally, Shapiro and Gavze presented a theoretical model for particle motion in shear flow near a wall which suggested that rod-like particles preferentially drift towards the wall (over spherical particles) due to the complex hydrodynamic forces and torques acting on them, thereby suggesting spheroidal-shaped carriers would possess a higher affinity for the vascular wall than their spherical counterparts [11]. Thus, the use of non-spherical carriers for localized drug (or gene) delivery can lead to enhanced *in vivo* efficacy of therapeutics (e.g. higher cell transfection) and hence improve the treatment of many human diseases.

Overall, the increasing interest in non-spherical particles for use as drug carriers highlights the critical need for practical methods of fabricating these particles from biodegradable polymers in a manner that allows for easy loading and release of therapeutics. To date, two methods have been described for fabricating drug-loaded, non-spherical particles from biodegradable polymers for potential application in drug delivery. One method utilizes heat/solvent stretching of monodisperse spherical particles in polymer templates to achieve monodisperse non-spherical shapes [12,13]. While this method can achieve a narrow distribution of particle size, heating of particles can denature/degrade drug cargo and the use of solvent to render particles stretchable can lead to leaching of drug content — thus making this method not particularly suitable for drug delivery applications [12]. A second method utilizes imprint lithographic techniques called PRINT to produce diversely shaped particles [14]. While particles are fabricated under relatively mild conditions in the PRINT method, this process can be expensive to set up and is currently not well proven for use with a wide range of biodegradable polymeric material or therapeutics. Others have also described microfluidic-based methods for fabricating non-spherical particles that have focused on the use of photoactive polymers but have yet to demonstrate the ability to load therapeutics into these particles for drug delivery applications [15,16]. Described herein is the fabrication of biodegradable prolate spheroids for potential drug delivery applications using the simple oil-in-water (O/W) emulsion/solvent evaporation (ESE) technique that has been

* Corresponding author. Department of Chemical Engineering, University of Michigan, 2300 Hayward Street, 3074 H. H. Dow Building, Ann Arbor, MI 48109, USA. Tel.: +1 734 936 0856; fax: +1 734 764 1761.
E-mail address: lolaa@umich.edu (O. Eniola-Adefeso).

0168-3659/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jconrel.2009.05.020

extensively described for fabricating spherical drug carriers loaded with a wide range of therapeutics [17,18]. Specifically, by simple manipulation of the droplet dynamics and the diffusion process during the emulsification and solidification (evaporation) stages of particle formation, spheroidal microparticles can be fabricated from poly(lactic-co-glycolic acid) (PLGA) using the ESE method. Particle minor axis length and aspect ratio were found to be functions of the physical properties of the oil (droplet) and aqueous (continuous) phases, monomer ratio of lactic to glycolic acid, and stir speed. The presented data demonstrate the ability to fabricate spheroidal particles with average minor axes down to 2.5 μm — a size that is significantly smaller than the average luminal diameter in human capillaries and thus these spheroids may easily traverse these vessels. Also, it is shown that spheroids can be fabricated loaded with paclitaxel as proof-of-concept that the described method can allow for the encapsulation of therapeutic agents. Overall, the described ESE method for fabricating spheroidal particles has the advantages of using a proven microparticle fabrication technique that is simple in setup and operation, inexpensive, amendable to a range of biodegradable polymers, and provides favorable surface characteristics that permit the attachment of ligands/antibodies for targeted drug delivery [19].

2. Materials and methods

2.1. Microparticle fabrication

Microparticles were fabricated via the oil-in-water (O/W) solvent evaporation method as previously described [19] with some modifications (high surfactant concentration in the presence of Tris base). Briefly, 50 mg of PLGA polymer of choice was dissolved in 10 ml methylene chloride. The relevant characteristics of the different types of PLGA used in this study are summarized in Table 1. The polymer solution (oil phase) was injected into 100 ml of an aqueous buffer continuously stirred (1800 rpm) using a Lightnin' mixer (model L1U08F) fitted with a glass propeller (Beckman Coulter — shaft diameter = 2.8 cm). Unless otherwise stated, the aqueous buffer consisted of the surfactant polyvinyl alcohol (PVA; M_W 30,000–70,000) at 1% w/v and Tris base (Trizma) at 1.2% w/v in deionized (DI) water and maintained at pH 8.40 via titration with hydrochloric acid (base aqueous formulation). This composition of the aqueous buffer permitted the formation of stretchable oil droplets, and the high concentrations of PVA allowed for slowed solvent evaporation that is necessary for droplets to solidify in the stretched form. The emulsion was stirred for 1 h in order to produce the necessary shear force for droplet stretching and facilitate solvent evaporation upon stretching. The resulting microparticles were collected and washed (DI water) via centrifugation at 750 rpm prior to freeze-drying in a Labconco lyophilizer. The resultant powder was stored at −20 °C until use. For drug-loaded spheroids, 2.5 mg paclitaxel and 47.5 mg PLGA polymer were dissolved in 10 ml methylene chloride to form the oil phase. Similar to unloaded spheroids, the oil phase was injected into the aqueous phase and the emulsion stirred at 1800 rpm.

2.2. Microparticle characterization

Physical/surface characteristics of microparticles were studied using a Nikon TE 2000-S optical microscope and a Philips XL30FEG scanning electron microscope (SEM). Samples were prepared for light microscopy and SEM by suspending microparticles in DI water and pipetting the suspension onto a microscope slide or double-sided carbon tape fastened to an SEM stub. Prior to loading on the SEM for analysis, the particle suspension on carbon tape was air-dried and particles were subsequently gold-coated in a SPI-Module sputter coater. Particle size and aspect ratio were obtained from microscopy and SEM images via Metamorph analysis software. Unless otherwise stated, data is reported as average of at least 3 batches ± standard error between batches. Significance in data between different process variables was assessed using *all* data points obtained over multiple batches via student's *t*-test and one-way Anova with post-test. p value < 0.05 was considered significant.

2.3. Characterization of particle drug loading and encapsulation efficiency

Drug loading and encapsulation efficiency for paclitaxel-loaded microparticles were determined by dissolving dried paclitaxel-loaded microparticles in methylene chloride. The concentration of paclitaxel in the polymer-drug solution was measured via UV absorption at 232 nm, where absorption data was converted into concentration of paclitaxel using a calibration curve generated from solutions with known concentration of the drug. Background absorbance of PLGA was subtracted using a similar calibration curve. Drug loading was calculated to be the mass of entrapped paclitaxel divided by the mass of dry microparticles. Encapsulation efficiency was calculated as the ratio of entrapped drug mass fraction to that of the drug mass fraction present in the oil phase during fabrication.

2.4. In vitro release studies

The *in vitro* release of paclitaxel-loaded microparticles was measured in 10 mM Dulbecco's phosphate-buffered saline (DPBS with Ca^{2+} and Mg^{2+}, Invitrogen) at pH 7.4 and 37 °C. Specifically, 7.5 mg of paclitaxel-loaded (2.7 wt.%) spheroids was suspended in 15 ml of DPBS in a screw capped polypropylene centrifuge tube. Tubes were placed on an orbital shaker bath maintained at 37 °C. At desired time points, tubes were centrifuged at 1000 *g* for 5 min. The supernatants were removed completely and pellets resuspended in fresh DPBS to maintain sink conditions for release as done in previous publications [20–23] and returned to the water bath. 5 ml of dichloromethane was added to collected supernatants and the mixture shaken for 30 s to facilitate drug extraction. After 30 min of allowing the oil and water to phase separate, the drug-rich dichloromethane was collected using a glass pipet and analyzed for drug content using a spectrophotometer as previously described (Section 2.3). Release studies were done in at least triplicate.

Table 1
PLGA polymers used in fabricating spheroidal particles ("–" information not available).

Polymer	Co-monomer ratio (lactic:glycolic acid)	Average molecular weight (M_w in Da)	IV (dl/g)	End group	Manufacturer
A	50:50	66,000	–	–COOH	Birmingham Polymer
B	50:50	47,000	0.36	–COOH	Lakeshore Biomaterials
C	50:50	55,200	0.47	–COOH	
D	50:50	~84,000	0.57	–COOH	
E	65:35	~65,000	0.48	–COOH	
F	75:25	~61,000	0.46	–COOH	
G	85:15	~65,000	0.48	–COOH	
H	50:50	47,000	0.37	$-(-CH_2-CH_2-O)_a-H$	
I	50:50	51,000	0.41	$CH_3(CH_2)_{11}O$	

Download English Version:

https://daneshyari.com/en/article/1426217

Download Persian Version:

https://daneshyari.com/article/1426217

Daneshyari.com