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Fabrication of double-walled microspheres for the sustained release of doxorubicin

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Abstract

Double-walled microspheres with poly(*L*-lactic acid) shells and poly(*DL*-lactic–co-glycolic acid) cores were fabricated using solvent evaporation technique which involves the phase separation phenomenon of a binary composite of these two polymers. Doxorubicin, a hydrophilic drug, was entrapped within the core of these double-walled microspheres with different core–shell thicknesses and compositions to investigate the in vitro release on this class of microspheres. Microspheres of different size ranging from 50 to 300 μm were also fabricated to investigate whether this method is suitable for fabricating small particles for intramuscular injection applications, and their phase separation and surface morphology were examined by differential scanning calorimetry, scanning electron microscopy, and optical microscopy.

Keywords: Double-walled; Microsphere; Doxorubicin; Controlled release

1. Introduction

Doxorubicin (DOX), one of the anthracycline ring antibiotics with great antitumor activity against solid tumors and leukemia, has the ability to interact with DNA and consequently inhibit important cellular functions [1]. However, its therapeutic potential has been restricted by its dose-limited cardiotoxicity and by the resistance developed by the tumor cells to this molecule after some time of treatment [2]. In view of that, traditional single-polymer microspheres with a high initial burst caused by drug trapped on the surface have their limitations in clinical applications [3]. To eliminate the initial burst and better control the release of this highly water-soluble cardiotoxic drug, double-walled microspheres with drug encapsulated in the inner core are fabricated using several methods. One method is solvent evaporation, which involves a single-step phase separation of composite binary polymer solutions [4–7]. This method has proven advantageous over other early attempts to simply coat existing microspheres using pan coating, fluidized beds or spray drying in eliminating initial bursts and obtaining sustained controlled release [8,9]. The uniform shell layer prevents diffusion of the hydrophilic drug located in the inner core. By careful selection of the mass ratio of the two polymers used, the thickness of shell of the double-walled configuration can be controlled. That, in turn, gives us more degrees of freedom in designing the desired release profile of drugs suitable for various treatments.

Double-walled microspheres have also been investigated extensively as drug carriers for sustained and controlled release. Mathiowitz et al. reported on a double-walled microspheres system consisting of a core of poly(1,3-bis-(*p*-carboxyphenoxypropane)–co-(sebacic anhydride)) 20:80 coated with layer of poly(*L*-lactide) (PLLA) [11–14]. Five process variables were studied to optimize manufacturing conditions: polymer solution concentration, polymer weight ratio, polymer solution volume ratios, encapsulation temperature, and airflow rate across the top of the encapsulation vessel [11].

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Lee et al. [7] demonstrated the fabrication of composite double-walled microspheres with PLLA shells and poly(*DL*-lactic–co-glycolic acid) (PLGA) cores, within which highly water soluble etanidazole was entrapped using solvent evaporation. Rahman et al. [15] also successfully encapsulated fluorescein isothiocyanate (FITC)-labeled bovine serum albumin (FITC-BSA) using this method. A technique for identifying the composition of the core and shell polymer has been devised by Lee et al. [7]. With this dissolution technique, we investigated the critical PLLA:PLGA mass ratio that results in core–shell inversion. Henceforth, based on knowledge of the solubility parameter, which predicts the polymer phase into which the drug will preferentially partition, we developed a formulation to help localize different drugs in the core of such a double-walled system. In our study, in vitro release of doxorubicin was also performed to confirm the double-walled configuration with the hydrophilic drug encapsulated within the core.

2. Materials and methods

2.1. Materials

Poly(*L*-lactic acid) (PLLA, MW (weight-average molecular weight), 85,000–160,000), poly(*DL*-lactic–co-glycolic acid 50:50) (PLGA, MW 40,000–75,000), doxorubicin (MW 214), and poly(vinyl alcohol) (PVA) (MW 31,000–50,000) were all purchased from Sigma–Aldrich, USA, and used without modification. HPLC-grade acetonitrile was purchased from Fisher (Fair Lawn, NJ, USA). Phosphate-buffered saline (PBS) used for in vitro release was obtained from Pierce (Rockford, IL, USA) containing 0.1 M sodium phosphate, 0.15 M sodium chloride, pH 7.2. All other materials or solvents used were of analytical grade.

2.2. Fabrication of double-walled microspheres

Doxorubicin-loaded double-walled microspheres were prepared using a modified oil–oil–water (o/o/w) emulsion solvent evaporation technique developed by Mathiowitz et al. [4] and using the polymer incompatibility between PLLA and PLGA 50:50 (the ratio 50:50 refers to the lactide/glycolide ratio) which results in their complete phase separation.

PLLA and PLGA 50:50 were separately dissolved in 1 ml of dichloromethane (DCM) (overall concentration 20%, w/v). The drug containing particles, doxorubicin (5%, w/w), was added to PLGA 50:50 polymeric solution only and sonicated using an ultrasonic probe (Model XL2000, Misonix, NY, USA) at 2-W output for 30 s to break down the drug crystals. Then the two polymeric solutions were mixed and sonicated for 20 s to form a homogeneous solution. The mixture was injected dropwise into the nonsolvent bath containing 200 ml of polyvinyl alcohol (PVA) solution (0.5%, w/v) which was dissolved with 0.25 ml DCM, stirring continuously at 500 rpm to create the o/o/w emulsion. Continuous stirring for 4 h allows for the extraction and evaporation of DCM which allowed phase separation of PLLA and PLGA before hardening of the microspheres. Next, the microspheres were filtered and rinsed with distilled water to remove residual PVA, and then dried under vacuum in the desiccator. Fabricated microspheres were stored in a desiccator to prevent hydrolytic degradation of the biodegradable polymer under humidity.

Microspheres having different core–shell configurations of PLLA/PLGA and varying shell thickness and core diameter were prepared in the same manner by altering the polymer mass ratio (w/w) of PLLA to PLGA ranging from 1:1 to 1:3. Single-polymer (PLLA and PLGA) microspheres without drug loading intended for characterization and reference comparison were also prepared using the well-established single emulsion method [3,17].

2.3. Characterization

2.3.1. Morphology and composition studies

The morphology of microspheres in in vitro and degradation studies was observed by scanning electron microscopy (SEM, JSM-5600LV, Jeol, Tokyo, Japan) at an accelerating voltage of 15 kV. Microspheres were cross sectioned using a razor blade, mounted on metal stubs, and coated with a layer of platinum using an auto fine coater (JFC-1300, Joel), which allowed us to view the surface morphology and internal structure of the microspheres. Studies were carried out to analyze changes in surface morphology and internal structure of microspheres at various stages of degradation.

For identification of the composition of the core and shell polymer, we used the dissolution method devised by Lee et al. [7] based on the different solubility of the polymer pair PLLA and PLGA in ethyl acetate. Briefly, a cross-sectioned double-walled particle was first immersed in ethyl acetate for 10 min. The remnant was then collected for SEM observation. Knowing that although PLGA 50:50 is soluble in ethyl acetate while PLLA is not, we can determine the core–shell composition by examining whether the remnant has a solid core or hollow shell structure. (Note: the solubility of PLGA and PLLA in ethyl acetate at room temperature (25 °C) is 580 and 0.1 mg/ml, respectively.) The former would mean a double-walled microsphere with PLGA shell, and the latter would be derived from a microsphere with a PLGA core.

The drug distribution was identified by observing the distinct red crystalline drug filaments within the loaded microspheres with an optical microscope (Olympus Vanox Model BX60, Japan). Different polymer layers in double-walled microspheres can be identified based on the difference in crystalline structures as well. In preparation for optical microscopy, the microspheres were sectioned using a microtome blade and mounted onto glass slides for viewing under cross Polaroid. Further, doxorubicin is slightly fluorescent, unlike the polymeric materials used (PLLA and