Powder Technology 275 (2015) 77–84

Contents lists available at ScienceDirect

Powder Technology

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

Polymer hydrophobicity regulates paclitaxel distribution in microspheres, release profile and cytotoxicity in vitro

Lianyan Wang [a,*,1], Mingming Cai [a,b,1], Yuan Liu [a,c], Tingyuan Yang [a], Yejing Zeng [a], Yueling Zhang [a], Qiang Li [a], Baocheng Zhu [b], Guanghui Ma [a,**]

[a] National Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing, 100190, PR China
[b] College of Life Sciences, Agricultural University of Hebei, Baoding, Hebei 071001, PR China
[c] Graduated University of Chinese Academy of Sciences, Beijing, 100049, PR China

ARTICLE INFO

Article history:
Received 20 February 2014
Received in revised form 14 October 2014
Accepted 21 January 2015
Available online 28 January 2015

Keywords:
Hydrophobicity
Paclitaxel
Polymer microspheres
Distribution
Crystal morphology
Antitumor activity

ABSTRACT

Water-insoluble drugs such as paclitaxel (PTX) face a huge challenge for clinical applications due to their low aqueous solubility. In this study, uniform-sized poly (DL-lactic acid) (PLA), poly(DL-lactic-co-glycolic acid) (PLGA) and poly(ethylene glycol-co-lactide acid) (PELA) microspheres containing PTX were prepared by premix membrane emulsification technique integrated with solvent evaporation method. The different hydrophobicity of polymer led to different distribution and crystal morphology of PTX in microspheres, which further resulted in different loading and encapsulation efficiency, release profile in vitro and cytotoxicity. The PLGA microspheres showed the highest loading and encapsulation efficiency of PTX (5.15%, 70.46%), followed by PLA microspheres (4.33%, 64.33%), together with PELA microspheres (3.35%, 56.68%). The results of PTX release profile in vitro suggested that PLGA and PELA microspheres achieved a more rapid release rate than PLA microspheres due to their hydrophilic property. The antitumor activity in vitro was also evaluated by Lewis Lung Cells (LLC). The PTX-PLGA microspheres exhibited excellent antitumor activity compared with PLA and PELA microspheres, even much better than Taxol®. Therefore, the PLGA microspheres show great potential in application as delivery carriers for water-insoluble drugs, PTX and others.

1. Introduction

Poor water-solubility of therapeutic drugs has often caused significant delivery and formulation problems for clinical applications. Paclitaxel (PTX), as a potent anti-cancer drug, is poorly soluble in water, which has become a major limitation in developing patient friendly formulations [1–3]. To enhance its delivery and allow for parenteral administration, a co-solvent system as Cremophor EL which is made of (a polyoxyethylene castor oil)/ethanol (50/50, V/V) has been reported to solubilize PTX [4]. However, Cremophor EL usually brings about serious side effects e.g., hypersensitivity, nephrotoxicity and neurotoxicity as well as effects on endothelial and vascular muscles causing vasodilatation, labored breathing, lethargy and hypotension [5–7]. To eliminate the side effects of Cremophor EL and improve PTX solubility, a number of optional formulations are investigated, including liposomes, microspheres, nanoparticles and polymeric micelles [8–14]. Among them, polymeric microspheres have become attractive formulations for insoluble drugs due to many advantages, such as increasing the solubility, controlling release profile in vivo, improving absorption, and reducing side effects [15,16]. As delivery carriers of PTX, the distribution and crystal morphology of drug in microspheres have significant influence on solubility, encapsulation efficiency and release profile of drug in vitro [17,18], which further affects antitumor activity.

In order to obtain PTX-loaded microspheres with different distribution and crystal morphology, several polymers with different hydrophobicity as PLA, PLGA and PELA were chosen to prepare microspheres. Although biodegradable microspheres formulated from PLA, PLGA and PELA polymers were being extensively investigated for various drug delivery applications [19–22], there was little literature to report the effects of polymer hydrophobicity on distribution and crystal morphology of drug loaded in microspheres, which played an important role in final drug efficacy. Furthermore, the distribution and crystal morphology of drug in microspheres were also affected by the size of the microspheres. In order to eliminate the impact of size, it was necessary to prepare microspheres with narrow size distribution and size controllability.

* Correspondence to: L. Wang, National Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, P.O. Box 353, Beijing 100190, PR China. Tel./fax: +86 10 82544931.
** Correspondence to: G. Ma, National Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, P.O. Box 353, Beijing 100190, PR China. Tel./fax: +86 10 82627072.
E-mail addresses: wanglianyan@home.ipe.ac.cn (L. Wang), ghma@home.ipe.ac.cn (G. Ma).
[1] The authors have the same contributions.

http://dx.doi.org/10.1016/j.powtec.2015.01.041
0032-5910/

Table 1
The optimal conditions for preparation of PLA, PLGA and PELA microspheres containing PTX by premix membrane emulsification.

Polymer	Concentration of polymer (g/L)	Concentration of PTX (g/L)	Concentration of PVA (g/L)	Volume ratio (oil:water)	Pressure (MPa)	Passes (batch)
PLA	40	2	20	1:10	0.60	3
PLGA	40	2	20	1:5	0.75	3
PELA	50	2	10	1:5	0.50	3

The premix membrane emulsification developed in our previous study was a potential technique for the preparation of uniform-sized and size-controllable microspheres [23–28].

In this study, uniform-sized PLA, PLGA and PELA microspheres containing PTX were prepared by premix membrane emulsification combined with solvent evaporation method. The distribution and crystal morphology of PTX in microspheres were deeply analyzed, and the corresponding effects on loading and encapsulation efficiency, release profile and antitumor activity in vitro were systematically investigated.

2. Materials and methods

Poly (DL-lactic acid) (PLA, M_W 10 kDa) and poly (DL-lactic acid-co-glycolic acid) (PLGA, L/G ratio = 75:25, M_W 15 kDa) were purchased from Shandong Institute of Medical instrument (Shandong, China), and poly(ethylene glycol-co-lactide acid) (PELA, mPEG(2000)-PLA, 1:19, M_W 40 kDa) was obtained from Daigang BIO Engineer Limited Co. (Shandong, China). Dichloromethane (DCM) was purchased from Beijing Chemical Reagents Company (China). Poly(vinyl alcohol) (PVA-217, degree of polymerization 1700, degree of hydrolysis 88.5%) was provided by Kuraray (Japan). SPG membrane (Shirasu Porous Glass) was purchased from SPG Technology Co. Ltd. (Japan). All other reagents were of reagent grade and used as received.

Lewis lung cells (LLC) were obtained from Peking University Healthy Science Center (China). Cell culture medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco (UK). Culture flasks and dishes were products of Corning (USA). Cell Counting Kit-8 (CCK-8) was from Beyotime (China). The Live/dead® Viability Kit, Alex Fluor 635 phalloidin, DAPI (4, 6-diamidino-2-phenylindole), and Nile red were provided by Invitrogen (USA). Paclitaxel (PTX, M_W 853.9), penicillin, streptomycin, and Cremophor EL were purchased from Sigma-Aldrich (UK). Taxol® was obtained from a hospital pharmacy (China).

2.1. Measurement of hydrophobicity

The hydrophobicity of PTX, PLA, PLGA and PELA was analyzed by using contact angle measurement (Dataphysics OCA 20, Germany). A certain amounts of PLA, PLGA and PELA were dissolved into DCM, and then the solutions were coated on a glass slide with a SPIN wafer spinner under 3000 rpm, which were further employed for the measurement of the contact angle. Certain volume of the deionized water was slowly injected on the coated glass slide to measure the contact angle via the sessile drop method. The average values of contact angle were calculated from the video images after three measurements. PTX as powder was ground and pressed into a thin flake for contact angle measurement.

2.2. Preparation of PLA, PLGA and PELA microspheres containing PTX

Uniform-sized PLA, PLGA and PELA microspheres containing PTX were prepared by premix membrane emulsification technique

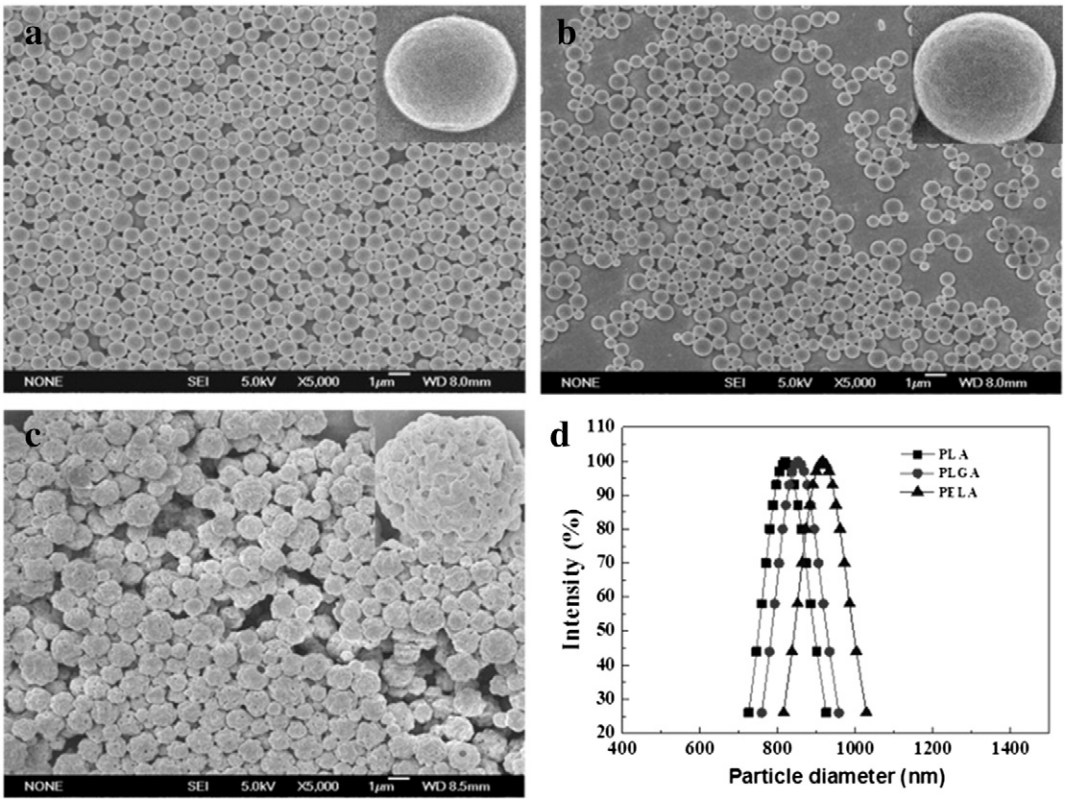

Fig. 1. SEM micrographs (a, PLA; b, PLGA; c, PELA) and size distributions of PTX-loaded microspheres.