Colloids and Surfaces B: Biointerfaces 115 (2014) 212–218

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Generation of nano-sized core–shell particles using a coaxial tri-capillary electrospray-template removal method

Lihua Cao [a], Jun Luo [a], Kehua Tu [a,b], Li-Qun Wang [a,b,**], Hongliang Jiang [a,b,*]

[a] Department of Polymer Science and Engineering, Zhejiang University, Hangzhou 310027, China
[b] MOE Key Laboratory of Macromolecular Synthesis and Functionalization, Zhejiang University, Hangzhou 310027, China

ARTICLE INFO

Article history:
Received 29 October 2013
Received in revised form
23 November 2013
Accepted 25 November 2013
Available online 1 December 2013

Keywords:
Polymeric nanoparticle
Electrospray
Core–shell–corona structure
Size modulation
Drug delivery

ABSTRACT

This study proposed a new strategy based on a coaxial tri-capillary electrospray-template removal process for producing nanosized polylactide-*b*-polyethylene glycol (PLA-PEG) particles with a core–shell structure. Microparticles with core–shell–corona structures were first fabricated by coaxial tri-capillary electrospray, and core–shell nanoparticles less than 200 nm in size were subsequently obtained by removing the PEG template from the core–shell–corona microparticles. The nanoparticle size could be modulated by adjusting the flow rate of corona fluid, and nanoparticles with an average diameter of 106 ± 5 nm were obtained. The nanoparticles displayed excellent dispersion stability in aqueous media and very low cytotoxicity. Paclitaxel was used as a model drug to be incorporated into the core section of the nanoparticles. A drug loading content in the nanoparticles as high as 50.7 ± 1.5 wt% with an encapsulation efficiency of greater than 70% could be achieved by simply increasing the feed rate of the drug solution. Paclitaxel exhibited sustained release from the nanoparticles for more than 40 days. The location of the paclitaxel in the nanoparticles, i.e., in the core or shell layer, did not have a significant effect on its release.

1. Introduction

Drug delivery carriers can be fabricated using several techniques, such as emulsion-solvent evaporation, microfluidic systems, self-assembly and electrospray [1–4]. Of these techniques, the electrospray method has several unique advantages. For example, monodispersed particles can be produced using the cone-jet mode of the electrospray technique, and the particle size can be easily controlled by adjusting the preparation parameters [5–7]. In addition, either hydrophobic or water-soluble drugs can be facilely loaded into electrosprayed particles with high entrapment efficiency [8–12]. Furthermore, core–shell structured particles can be conveniently obtained through coaxial electrospray [4,13]. Compared with the emulsion-solvent evaporation method, which has frequently been used for encapsulating protein drugs into biodegradable microparticles, coaxial electrospray can preserve the structural integrity and bioactivity of protein drugs because there is no direct contact between the proteins and organic solvent during the electrospray process [14,15]. Multidrug encapsulation and multishell encapsulation can also be achieved using tri-capillary coaxial electrospray [10,16,17]. The release profiles of encapsulated drugs can be independently modulated by changing the internal structure and compositions of the particles [11].

Despite the aforementioned advantages, the size of electrosprayed particles was always within micron to submicron range. Many strategies have been developed in an attempt to decrease the particle size. By delicately varying the polymer concentration, feed rate and applied voltage, polymethylsilsesquioxane particles with sizes ranging from 275 to 860 nm were obtained [18]. Drug-loaded polylactide particles with average diameters of approximately 200 nm were produced by reducing the feed rate of the polymer solution and increasing the electrical conductivity of the electrospray solution [12]. By reducing the polymer concentration to as low as 0.01 g/ml and decreasing the fluid flow rate to as slow as 0.036 ml/h, chitosan particles with an average diameter of 124 nm were successfully prepared [19]. However, decreasing the particle size by decreasing either the solution concentration or flow rate would lead to a low particle production capacity. In addition, there have been few reports on the production of polymeric particles with sizes of less than 100 nm. It has been confirmed that the size of drug delivery carriers plays an important role in their in vivo transport behaviors [20]. For example, nanoparticles

* Corresponding author at: Department of Polymer Science and Engineering, Zhejiang University, Zheda Road No. 38, Hangzhou 310027, China.
Tel.: +86 571 87952046.
** Corresponding author at: Department of Polymer Science and Engineering, Zhejiang University, Zheda Road No. 38, Hangzhou 310027, China.
Tel.: +86 571 87952596; fax: +86 571 87952596.
E-mail addresses: caocao2008@zju.edu.cn (L. Cao), m05luojun@zju.edu.cn (J. Luo), tukh@zju.edu.cn (K. Tu), lqwang@zju.edu.cn (L.-Q. Wang), hljiang@zju.edu.cn (H. Jiang).

0927-7765/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.colsurfb.2013.11.046

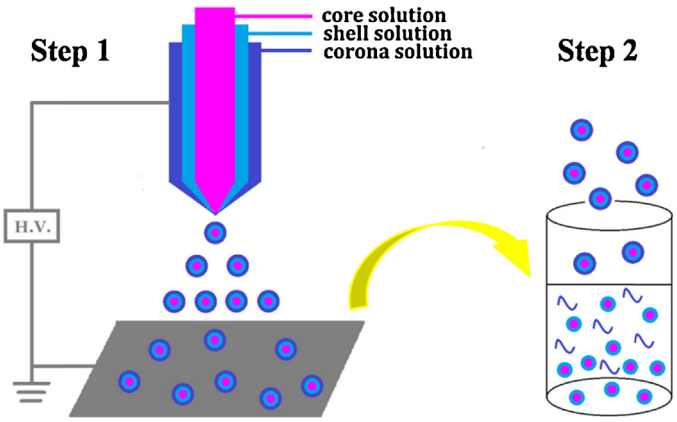

Fig. 1. Schematic diagram of coaxial tri-capillary electrospray-template removal method. Step 1: coaxial tri-capillary electrospray, Step 2: template removal.

presented enhanced colon bioadhesion and increased oral bioavailability compared with microparticles [21,22]. Particles smaller than 500 nm may have the opportunity to escape phagocyte attack in the bloodstream [23]. In addition, particles smaller than 100 nm could be extravasated into tumor tissues through enhanced permeation and retention effects [24]. Thus, it is essential to finely modulate the size of drug delivery carriers to improve therapeutic efficacy, reduce drug dose and minimize unwanted side effects.

In this work, a new strategy was proposed based on a coaxial tri-capillary electrospray-template removal process to control the size of electrosprayed particles. Briefly, microparticles with a core–shell–corona structure were first fabricated through coaxial tri-capillary electrospray. The corona layer was subsequently removed to expose the inner core–shell particles. It was found that core–shell particles with sizes close to 100 nm could be obtained and that the particle size could be conveniently modulated by adjusting flow rate of the corona fluid. The particle properties and cellular uptake were also evaluated. Paclitaxel (PTX) was used as a model drug to be encapsulated into the particles. The drug loading and in vitro release behaviors were investigated. In addition, the effect of the PTX location in the nanoparticles, either in the core or shell section, on its release was investigated.

2. Materials and methods

2.1. Materials

Polyethylene glycol (PEG, Mn = 20,000) was purchased from Sinopharm Chemical Reagent Co. Ltd. Polylactide-*b*-polyethylene glycol (PLA-PEG, PEG10000) was synthesized in our lab, and the number average molecular weight was 46 kDa determined by gel permeation chromatography. PTX was supplied by Hangzhou Haida Pharmaceutical Chemical Co. Ltd. Fluoresceine isothiocyanate (FITC), Nile red and Hochest 33342 were purchased from Sigma–Aldrich. Trifluoroethanol (TFE) was obtained from Weihai Newera Chemical Co. Ltd. RPMI 1640 medium was purchased from Genom biomedical technology Co. Ltd and fetal bovine serum (FBS) was obtained from Zhejiang Tianhang Biomedical Technology Co. Ltd. All other chemicals and solvents were of analytical grade and used as received.

2.2. Fabrication of core–shell–corona microparticles by coaxial tri-capillary electrospray

The schematic diagram of coaxial tri-capillary electrospray system is shown in Fig. 1. The set-up is composed of a tri-capillary spray head, a high-voltage power supply (DW-P503-1ACCC, Tianjin Dongwen High-Voltage Power Supply Co. Ltd, China), three syringe pumps (KDS100, kdScientific, USA) and a collecting plate. The spray head consists of three coaxially arranged stainless needles. The outer diameters (OD) of the three needles are 2.86, 1.70 and 0.80 mm, respectively, and the inner diameters (ID) are 2.06, 1.10 and 0.70 mm, respectively. A foil paper on the platform was used as a collecting plate. The distance between the spray head and the collecting plate was fixed at 16 cm in all experiments. 10% (w/v) PEG solution in TFE was used as corona fluid, 3% (w/v) PLA-PEG solution in chloroform as shell fluid and 3% (w/v) PEG solution in chloroform as core fluid. All three fluids were fed through syringe pumps independently with different feed rate. To visualize the structure of the resultant microparticles, fluorescent dye FITC was contained in core and corona solutions before electrospraying.

2.3. Preparation of nanoparticles from core–shell–corona microparticles

The foil papers for collecting electrosprayed particles were cut into pieces and soaked in deionization water for several hours at room temperature. Afterwards, the suspension was filtered with a low speed quantitative filter paper (retention size 1–3 μm) to remove foil papers and possible aggregates. The filtrate was stored in refrigerator at 4 °C till characterization.

2.4. Characterization of micro- and nanoparticles

The morphologies of the particles were characterized by scanning electron microscope (SEM, Hitachi S-4800, Hitachi, Japan) and transmission electron microscopy (TEM, JEM-1230, JEOL, Japan). The average particle diameters and distributions of microparticles were analyzed with the software Image-Pro Plus (Media Cybernetics, Inc.) ($n = 100$). The particle sizes and Z-potentials of the nanoparticles were determined by nano particle potential analyzer (Malvern ZCEC, Malvern, UK). The structures of the microparticles was observed by laser scanning confocal microscope (LSCM, SP5II, Leica, Germany).

2.5. Dispersion stability of PLA-PEG nanoparticles in water

PLA-PEG nanoparticle solutions prepared according to the procedures described above were stored at 4 °C. The change in the particle size was monitored by nano particle potential analyzer at predetermined time points.

2.6. Cytotoxicity of PLA-PEG nanoparticles

The adenocarcinomic human alveolar basal epithelial cell line A549, which was used for evaluating the cytotoxicity of the PLA-PEG nanoparticles was kindly provided by Professor Guping Tang (Zhejiang University, Hangzhou, China). A549 cells were cultured in RPMI 1640 medium containing 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin at 37 °C in 5% CO_2 in a humidified atmosphere. Hydrophobic fluorescent dye Nile red was added into PEG solution (core fluid) before electrospraying in order to visualize the nanoparticles. The particle size and zeta potential of PLA-PEG particles used in cell experiments were 115 ± 10 nm and -8.0 ± 0.5 mV, respectively.

200 μl of the cells were seeded into a 96-well plate (Costar, Corning Corp., NY) at a density of 1×10^4 cells per well. After incubating for 18 h, the culture medium was replaced with nanoparticle solutions, which were serially diluted by serum free culture medium, and incubated for 4 h. Then, the particle solutions were removed and replaced by MTT reagent diluted with serum free culture medium (0.5 mg/ml). After incubating for another