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Microparticle templating as a route to nanoscale polymer vesicles with controlled size distribution for anticancer drug delivery

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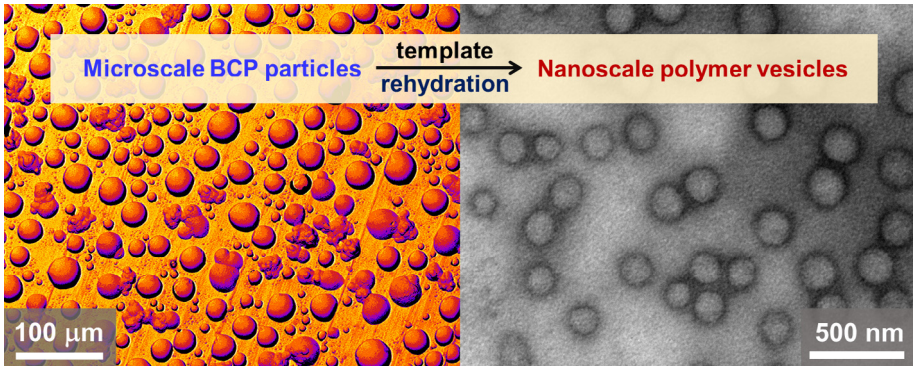
GRAPHICAL ABSTRACT

We report a simple and efficient strategy to produce nanoscale polymer vesicles with controllable size based on electrospraying particle and subsequent rehydration. Significantly, benefiting from the intrinsic advantages of BCP self-assembly, the polymer vesicles formed from the electrosprayed microscale particles showed uniformity in size with nanoscale dimensions. Subsequently, a drug delivery system was demonstrated using the aforementioned polymer vesicles as a carrier of DOX to achieve anticancer synergism.



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ABSTRACT

Polymer vesicles are self-assembled shells of amphiphilic block copolymers (BCPs) that have attracted tremendous interest due to their encapsulation ability and intracellular delivery of therapeutic agents. However, typical processes for the formation of polymer vesicles lead to ensembles of structures with a broad size distribution (from nanometer to micrometer scale) which result in a limitation for efficient cellular uptake. In this study, we present a simple and efficient approach for the fabrication of polymer vesicles with uniform nanoscale dimensions from template formation of electrosprayed particles in a high throughput manner. First, electrospraying was applied to produce micrometer-sized templates of a block copolymer before polymer vesicles were formed from the pre-prepared microparticles *via* rehydration. Four different biocompatible diblock and triblock copolymers were used to successfully fabricate polymer vesicles with uniform size around 150 nm using this approach. Furthermore, we encapsulate anticancer drug doxorubicin (DOX) within the polymer vesicles *via* this method. The kinetics of cellular uptake (HeLa cell) and intracellular distribution of DOX-loaded polymer vesicles have been quntified and monitored by flow cytometry and confocal microscopy, respectively. The results show that our new

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method provides a promising way to fabricate drug-loaded polymer vesicles with controllable nanoscale size for intracellular anticancer drug delivery.

1. Introduction

One of the most challenging and important topics in the pharmaceutical and medical fields is the intracellular delivery of hydrophilic drugs [1]. Generally, small hydrophobic entities can penetrate cell membranes with relative ease, whereas hydrophilic species (small drugs and biomacromolecules such as nucleic acids, peptides and proteins), however, require a carrier to assist their transport across the cell membrane [2]. This carrier or vector must be designed so as to ensure intracellular delivery without compromising cell viability. One effective approach is the use of polymer vesicles as a new generation of targeted drug delivery vehicles [3–5]. Polymer vesicles (or polymersomes) are prepared by the self-assembly of amphiphilic block copolymers (BCPs) in aqueous solution [6,7]. Polymer vesicles, like other vesicular structures, combine the unique ability to encapsulate hydrophilic entities within their lumen (similar to viral capsids) as well as hydrophobic species within the membrane [8–10]. Compared to low molecular weight counterparts (*i.e.* liposomes), polymer vesicles have superior stability and load-carrying ability due to their macromolecular nature [5,11,12]. Due to advanced chemical functionalization and design of BCPs, polymer vesicles can be modified to various "smart" vesicles, which can respond to an external stimuli, such as temperature [13–15], light [16–18], pH [9,19,20–25]. However, one of the limitations in developing commercial applications for polymer vesicles is that their size and size distribution are crucial for drug delivery [26]. Typical processes for the formation of polymer vesicles (such as solvent switch/exchange, electroformation and film rehydration) lead to ensembles of kinetically-trapped structures with broad size distributions (tens of nanometers to tens of micrometers in diameter) [27]. Meanwhile, it has been found that, size governs the fate of the drug carrier or nanoparticles *in vitro* and *in vivo* [28–31]. For example, cellular internalization of polymer vesicles can drop by three orders of magnitude, when the diameter is increased from 100 to 400 nm [32]. Generally, micrometer-sized polymer vesicles have relatively low cellular uptake when compared with nanometer-sized ones, and therefore drugs loaded within the big vesicles result in a waste due to drug delivery with low efficiency. Since gain control over polymer vesicle size distribution and isolate nanoscale size vesicles are vital for efficient intracellular delivery, several approaches have been developed, such as purifying polymer vesicles using size exclusion chromatography (*SEC*) columns [33], hollow fiber filtration systems [34], or modifying the vesicle self-assembly process through thermal cycling [35], patterned templated [36], ultrasound [37], microfluidic devices [38], membrane extrusion under high shear [39,40,41], etc. However, the strategies of latter are either difficult to reproduce or often not compatible with the encapsulation of sensitive agents (e.g. drugs, nucleic acids, peptides or proteins).

Herein we present a simple and efficient approach for the fabrication of polymer vesicles with uniform nanoscale size as a direct product *via* the formation of a template of electrosprayed particles. Four different biocompatible diblock and triblock copolymers, comprising poly(ethylene glycol) (PEG), poly(ε-caprolactone) (PCL) and poly(D,L-lactide) (PLA) blocks, were utilized to prepare vesicles *via* this method (the schematic diagram is shown in Fig. 1 and Fig. S1). Firstly, microscale-sized particles (beads and discs or platelet-like features) were created by electrospraying BCPs solutions, before the templates were used to form polymer vesicles with uniform size (approximately 150 nm diameter) *via* self-assembly (rehydration). The PEG-*b*-PLA polymer vesicles were used as a model to encapsulate anticancer drug doxorubicin (DOX) for efficient intracellular delivery, where DOX was mixed with the polymer during the rehydration process. The kinetics of endocytosis of the DOX-loaded polymer vesicles into HeLa cancer cells and healthy normal oral cells were investigated *via* confocal laser scanning microscopy (CLSM) and fluorescence flow cytometry.

2. Experimental

2.1. Materials

Poly(ethylene glycol) monomethyl ether (mPEG, M_n = 2000 Da and 5000 Da) was purchased from Jinan Daigang Biomaterial Co. Ltd. and dried by azeotropic distillation with dry toluene (purchased from Sigma-Aldrich and stored in a drier with 4 Å molecular sieve) before use. D,L-lactide was purchased from Aladdin Industrial Corporation and recrystallized twice from ethyl acetate to enhance purity before use. Stannous octoate ($Sn(Oct)_2$, 95%), doxorubicin hydrochloride (DOX·HCl), and ε-caprolactone were purchased from Aladdin Industrial Corporation. All chemicals were used as received unless otherwise stated.

2.2. Fabrication of microscale particles and preparation of empty polymer vesicles

Diblock copolymers of mPEG-*b*-poly(ε-caprolactone) (mPEG-*b*-PCL) and mPEG-*b*-poly(D,L-lactide) (mPEG-*b*-PLA) were synthesized using $Sn(Oct)_2$ as a catalyst and *N*-hydroxyethyl maleimide as the initiator according to the method described elsewhere [42,43]. The PLA-*b*-PEG-*b*-PLA triblock copolymer was synthesized by ring opening polymerization of D,L-lactide with PEG macroinitiator [$Sn(Oct)_2$ as a catalyst] according to the method by Asadi et al. [44]. For electrospraying, solutions of $mPEG_{45}$-*b*-PCL_{43}, $mPEG_{110}$-*b*-PCL_{60}, $mPEG_{110}$-*b*-PLA_{110} and PLA_{35}-*b*-PEG_{45}-*b*-PLA_{35} with various concentrations (30–65, w/v, respectively) were prepared by dissolving the appropriate amount of BCP in tetrahydrofuran (THF).

The procedure for the fabrication of polymer vesicles with controllable size distribution by particle rehydration (a combination of electrospraying microparticles and subsequent rehydration) is described below [45]. As shown in Fig. 1 and Fig. S1 (in the Electronic Supporting Information, ESI), in Step 1, microscale templates (particles) were fabricated using electrospraying [46]. Each BCP solution was loaded into a syringe (1 mL) fitted with a blunt-tip needle and fed at 1 mL h^{-1} using a syringe pump (LSP01-1A, Baoding Longer Precision Pump Co., Ltd.). A voltage of 20 kV was applied to the sample solution (via connection to the 0.62 mm inner diameter flat-ended metallic needle) and the distance between the needle and the aluminum flat sheet (earthed and used as the collector) was fixed at 15 cm. During the electrospraying process, ambient humidity and temperature were maintained at RH 50% and 25 °C, respectively, by air-conditioning. In Step 2, polymer vesicles were self-assembled from the pre-prepared microparticles *via* rehydration. The electrosprayed product was removed from the aluminum sheet and placed into deionized water before the mixture was gently agitated and ultrasonicated for 10 min. Subsequently, the solution (the BCP content in the solution was adjusted to approxi-