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The use of cationic MPEG-PCL-g-PEI micelles for co-delivery of Msurvivin T34A gene and doxorubicin

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ABSTRACT

In our previous study, a series of triblock copolymers based on MPEG-PCL-g-PEI were successfully synthesized, and the physicochemical properties of their self-assembled micelles were also investigated. Here, a further evaluation of these micelles was carried out, including *in vitro* drug release behavior, body distribution as well as blood compatibility. The developed MPEG-PCL-g-PEI micelles was labeled with ^{99}Tc for tracing the body distribution of micelles after *i.v.* injection, and the results showed that the MPEG-PCL-g-PEI micelles mainly concentrated in the tumor tissue. Meanwhile, the anti-tumor activity on both B16F10 subcutaneous tumor model and lung metastasis model was tested and the results indicated that DOX-loaded micelles could significantly inhibit tumor growth as compared with free doxorubicin, which was accompanied by significantly increased apoptosis of tumor cells. By introduction of gene Msurvivin T34A in combination with chemotherapies in the treatment of lung metastasis tumor, it could greatly reduce systemic toxicity as well as improved the anti-tumor efficiency. These results demonstrated that it is possible to use cationic MPEG-PCL-g-PEI micelles for effectively co-delivering functional gene and chemotherapeutic agent, and thus improving anti-tumor effect and systemic toxicity.

1. Introduction

Nanomedicine based on polymers has received great interest due to their flexible and diverse chemical composition. It is easy to construct a suitable structure with physical and chemical properties to meet several requirements for effective drug delivery [1]. Meanwhile, drug delivery systems obtain a role in active targeting due to the incorporation of targeting ligands for recognition of organ-specific receptor [2]. However, diseases such as tumors, with these complex heterogeneous microenvironments, the actual effect and potential use of these target-labeled nanoparticles had been challenged [3,4].

The blood vessel in tumor is different from the one in normal tissue. Due to the gaps between adjacent endothelial cells of the angiogenic blood vessels in tumor tissues, macromolecules could be benefit from the enhanced permeability and retention (EPR) effect [5,6]. The used nanoparticles are widely modified with hydrophilic groups on the surface in order to prolong the blood circulation time of nanocarriers and prevent the removal of liver cells [7]. As a hydrophilic agent, poly (ethylene glycol) (PEG) linked with hydrophobic groups can improve bioavailability and biocompatibility. Furthermore, amphiphilic block copolymers can self-assemble into micelles in aqueous medium with a hydrophobic "core" and a hydrophilic "shell". Encapsulation of therapeutic agents in self-assembled block copolymers micelles have many prominent advantages, such as improved stability and solubility of hydrophobic drugs, the enhancement of drug utilization and prolonged circulation time with lower toxicity [8]. Thus, a series of block copolymers based on poly (ethylene glycol)-poly (ε-caprolactone) (PEG-PCL) had been widely studied as drug delivery systems [9–11].

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Nowadays, for therapeutic purposes, the combination using genetic and chemotherapeutic drugs had been proved a feasible strategy in tumor therapy [12,13]. Polyethyleneimine (PEI) is one of the most extensively investigated non-viral carriers for gene delivery. The commercially available branched polyethyleneimine with high molecular weight (25 kDa) is an effective vector to deliver genes *in vitro*. However, the cytotoxicity limits its further applications *in vivo*. In a prior study, we linked the PEI (MW = 2000Da) to MPEG-PCL copolymer and prepared triblock copolymers MPEG-PCL-g-PEI with various compositions [14,15]. These previous results demonstrated that the developed MPEG-PCL-g-PEI micelles could have great ability to co-encapsulation of chemical drugs and genes together [16,17].

Doxorubicin (DOX) is an anthracycline antibiotic, and it is one of the most effective chemotherapeutics for many cancers treatment, such as ovarian, breast cancer and pediatric solid tumor [18]. However, the use of doxorubicin is associated with severe hematological and cardiac toxicity [19]. In the last two decades, many DOX-vectors were developed to enhance its effect on chemotherapy and reduce systemic toxicity [18,20,21]. The gene Msurvivin T34A is a dominant negative mutant, which can reduce the proliferation of tumor cells and lead to the caspase-mediated apoptosis [22–24]. The co-delivery of gene and drug might not be released at the same tumor region due to the complexity of physiological environment, which would induce the synergistic antitumor effect, and it had achieved an improved antitumor effect in prior report [25]. As drug carriers with variable chemical structure and controllable characters, cationic micelles had the same purpose and application with liposomes in co-delivery genes and chemotherapeutics.

In the present study, we attempted to investigate the potential application using cationic block copolymer micelles to co-deliver doxorubicin and functional gene *in vivo* for tumor therapy.

2. Materials and methods

2.1. Materials

Materials included monomethoxy poly(ethylene glycol) (MPEG, Mn = 5000, 2000, Aldrich, USA), ε-caprolactone (ε-CL, Mw = 114, Alfa Aesar, USA), polyethyleneimine (PEI, Mw = 2000, Sigma, USA), atannous octoate ($Sn(Oct)_2$, Sigma, USA), 97% glycidyl methacrylate (GMA, Aldrich, USA), ethanol, methanol, and petroleum ether were purchased from Chengdu KeLong Chemicals (Chengdu, China). All reagents and solvents were analytical grade. Doxorubicin chloride (Doxorubicin, DOX) was purchased from Zhejiang Hisun Pharmaceutical Company (Zhejiang, China). The functional plasmid encoding the phosphorylation-defective mouse surviving threonine 34 → alanine mutant (Msurvivin T34A plasmid) was prepared by our lab. ^{99}Tc (Technetium) was obtained from the radiology department of West China Hospital.

2.2. Cell lines and culture

The B16F10 murine melanoma cell line, human breast tumors (MCF-7) and CT26 colon carcinoma cell line were purchased from American Type Culture Collection (ATCC, Reckville, MD). C57 and BALB/c female mice at 6–8 weeks were purchased from the Laboratory Animal Center of Sichuan Univercity (Chengdu, China). The animal care and all animal experimental procedures were conducted according to Institutional Animal Care and Use Committee of Sichuan University.

2.3. Synthesis and characterization of MPEG-PCL-g-PEI triblock copolymer

The block copolymer MPEG-PCL-g-PEI was synthesized according to our previous report [15]. First of all, MPEG-PCL was synthesized through ring-opening polymerization of ε-CL, which was initiated by MPEG and catalyzed by $Sn(Oct)_2$. The crude polymer MEPG-PCL was dissolved in dichloromethane and precipitated with petroleum ether. Residual petroleum ether was removed in vacuo at 45 °C, and then an excess amount of acryloyl chloride was added to the MPEG-PCL anhydrous dichloromethane solution drop by drop. After stirring for 6 h at 40 °C, the copolymer MPEG-PCL containing −C═C was obtained. Finally, a certain amount of PEI was dissolved in chloroform, and then the above copolymer chloroform solution was added dropwise and stirred for 24 h at 50 °C to complete the reaction. All these products were collected by petroleum ether coprecipitation and purification through dialysis and lyophilization. The chemical composition and molecular weight were confirmed by ^1H-NMR spectra.

2.4. Aggregate of erythrocytes test in vitro

In a preliminary experiment, the mice showed different maximal tolerance dose to these micelles formulated with various compositions, thus the erythrocyte aggregate test was used to evaluate the biocompatibility of these self-assembled micelles. The erythrocyte suspension (2%) was prepared using rabbit blood and saline solution. After adding different samples with the concentration of 0.5 mg/mL, the mixture solutions were incubated for 30 min at 37 °C before observing the erythrocyte aggregation by an optical microscope.

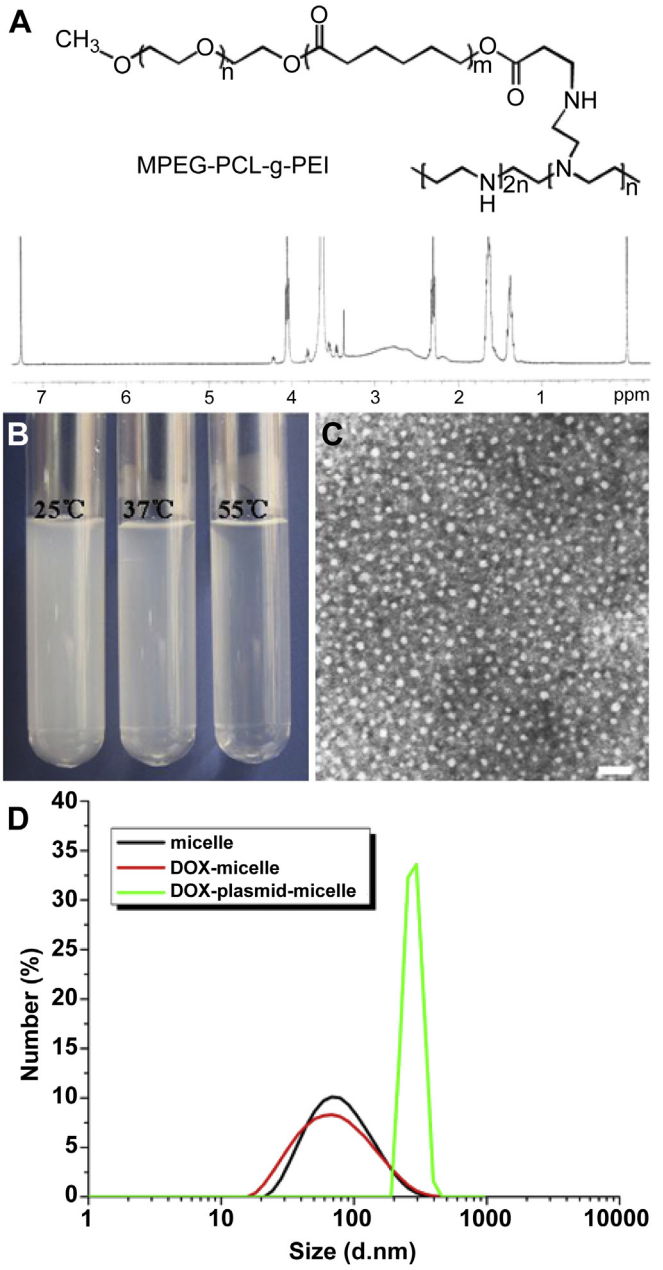

Fig. 1. (A) The chemical formula and the ^1H-NMR spectra (400 MHz, $CDCl_3$) of MPEG-PCL-g-PEI; (B) A photo of the copolymer (5 mg/mL) in various temperatures and (C) A transmission electron microscopy (TEM) image of the self-assembled micelles. The scale bar was 100 nm. (D) Size distribution spectrum of blank micelles, DOX-micelles and DOX/DNA micelles.