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Controlled release of doxorubicin from amphiphilic depsipeptide–PDO–PEG-based copolymer nanosized microspheres

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ABSTRACT

Novel biodegradable amphiphilic ABA triblock copolymers, i.e. poly(3(S)-methyl-morpholine-2,5-dione-*co*-*p*-dioxanone)-block-poly(ethylene glycol)-block-poly(3(S)-methyl-morpholine-2,5-dione-*co*-*p*-dioxanone) [P(MMD-*co*-PDO)-*b*-PEG-*b*-P(MMD-*co*-PDO)], were successfully prepared by ring-opening polymerization of 3(S)-methyl-morpholine-2,5-dione (MMD) and *p*-dioxanone (PDO) in the presence of poly(ethylene glycol) 6000 as an initiator. These triblock copolymers were characterized by ^{1}H NMR, ^{13}C NMR, Fourier transform infrared, gel permeation chromatography and differential scanning calorimetry measurements. P(MMD-*co*-PDO)-*b*-PEG-*b*-P(MMD-*co*-PDO) could self-assemble into stable nanosized microspheres with critical micellar concentrations of 0.41–0.66 μg/mL. The microspheres showed high hydrolytic degradation. In addition, doxorubicin (DOX) was chosen as a model drug and successfully encapsulated into the microspheres by hydrogen-bond interaction and hydrophobic effect. The transmission electron microscopy and dynamic light scattering measurements revealed that these microspheres were ellipsoidal nanoparticles with diameters ranged from 50 to 100 nm. These copolymer microspheres exhibited high loading capacity (LC), encapsulation efficiency (EE) of DOX and sustained drug release behavior in phosphate buffered solution (PBS). Moreover, the release rate of DOX from those microspheres in pH 4.0 PBS was faster than that in pH 7.4 due to pH sensitivity of the polymer–drug systems and the degradation of the matrix polymers. These amphiphilic depsipeptide multiblock copolymers would be potential promising carriers for anti-tumour drug delivery.

1. Introduction

Recently amphiphilic nanosized polymeric particles (NPPs) with core–shell nanostructure have attracted more significant attention and have been widely used in the field of drug delivery because of their good biocompatibility and biodegradability [1–5]. Many amphiphilic block copolymers have been developed for the preparation of NPPs as drug delivery carriers, and among them, synthetic biodegradable NPPs consisting of poly(ethylene glycol) (PEG) and aliphatic polyesters are highly anticipated in biomedical applications [6–9]. The PEG with excellent antigenicity and immunogenicity could form the hydrophilic corona shell via self-assembly [10–14]. This shell could inhibit protein adsorption, secondary aggregation and reticuloendothelial system uptake, thus leading to long circulation time and high stability of the NPPs. On the other hand, aliphatic polyesters, as a kind of synthetic copolymers, are known to be nontoxic, hydrophobic and degradable *in vitro* and *in vivo* [15,16]. The aliphatic polyesters can serve as hydrophobic core to provide appropriate mechanical properties for desired application, such as controlled release of water-insoluble drugs [17].

So far, many block copolymers have been prepared from PEG and polyesters for the application in drug delivery systems, e.g., PEG-*b*-poly(D,L-lactide) (PEG-*b*-PDLLA) [18], PEG-*b*-poly(lactide-*co*-glycolide) (PEG-*b*-PLGA) [19], poly(ε-caprolactone)-*b*-PEG-*b*-poly(ε-caprolactone) (PCL-*b*-PEG-*b*-PCL) [20], poly(lactic acid)-*b*-PEG-*b*-poly(lactic acid) (PLA-*b*-PEG-*b*-PLA) [21,22], PPDO-*co*-PCL-*b*-PEO-*b*-PPDO-*co*-PCL [23] and P[LA-*co*-(GA-*alt*-LGA)]-*b*-PEG-P[LA-*co*-(GA-*alt*-LGA)] [24] have been widely studied by many researchers, but they still have respective drawbacks, e.g. relatively slow degradation and low drug loading content. Therefore, it is necessary to develop the drug delivery systems from novel block copolymers in order to improve their therapeutic efficacy, meanwhile minimizing side effects and abrogating drug resistance [25–27].

Currently, polydepsipeptides have been widely accepted as a very valuable new group of synthetic biodegradable polymers, not only because of the strong intermolecular hydrogen-bond

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interaction between the amide groups, but also because of the presence of easily hydrolysable ester linkages and amido-bonds that ensure high degradability [28–33]. An effective method for the synthesis of polydepsipeptides is ring-opening polymerization (ROP) of the corresponding cyclic mono- or diesters, which has attracted much attention of many researchers. In 1985, Helder et al. [34] for the first time synthesized polydepsipeptides by ring-opening polymerization of morpholine-2,5-dione derivatives. Morpholine-2,5-dione derivatives are a class of monomers of depsipeptides which have been used to synthesize biodegradable copolymers with alternating amido and ester groups [35–37]. Moreover, the degradation products including L-amino acids can be properly metabolized by living tissues [38,39].

3(S)-Methyl-morpholine-2,5-dione (MMD) prepared from L-alanine possesses a relatively simple structure and low steric hindrance for ROP. MMD should have a better performance of polymerization than other morpholine-2,5-dione derivatives with large substitutes. Meanwhile, the copolymers including MMD unit with different crystallization degree can be synthesized owing to the optical activity of the monomer. In addition, these copolymers have ester and amido groups, and perform a faster biodegradation rate *in vivo* than poly(L-lactic acid) (PLLA). Therefore, it is a very promising approach to develop novel drug delivery systems based on the copolymers of MMD with other cyclic monomers.

Poly(*p*-dioxanone) (PPDO), as one of the biodegradable and biocompatible aliphatic polyesters, has high flexibility and good tensile strength [40–45]. PPDO can be utilized not only in medical materials such as surgical sutures and carrier systems for the controlled release of drugs and genes, but also in films, and non-woven material [46]. However, only one intrinsic degradation rate of PPDO has significantly restricted the practical application like drug delivery systems in medical field. In order to overcome this drawback, introducing depsipeptide unit into PPDO chains is presented in this paper. As far as we know, there were only rare reports about introducing depsipeptide unit into PPDO, especially using those block copolymers as drug release systems. Moreover, introducing functional monomers into the block copolymers would adjust the encapsulation and release properties from NPPs via some special interactions between the core-forming blocks and drugs, such as hydrogen bond, electrostatic complexation and $\pi-\pi$ interaction [47–50].

As far as model drug concerned, doxorubicin (DOX), as a kind of hydrophobic anti-tumour drug, contains liposoluble anthraquinone ligand, phenolic hydroxyl and basic amino group [51,52]. Until now, it is considered as one of the most effective anthracycline antibiotics against a wide range of cancers and extensively investigated as advantageous drug [53]. In our previous studies, DOX was combined with ibuprofen as model drug. When DOX was encapsulated with ibuprofen in depsipeptide-based carriers, the release rate of DOX was relatively slow so that it could reduce the side effects caused by the initial rapid release of the two drugs.

In this article, a series of amphiphilic triblock copolymers P(MMD-*co*-PDO)-*b*-PEG-*b*-P(MMD-*co*-PDO) were synthesized by ROP of MMD and PDO using PEG6000 as an initiator in the presence of stannous octoate. Their structural characteristics, thermal properties and molecular weight were identified or determined by ^{1}H NMR, ^{13}C NMR, FT-IR, DSC and GPC analysis. Moreover, the present study for the first time examined the possibility of using P(MMD-*co*-PDO)-*b*-PEG6000-*b*-P(MMD-*co*-PDO) as potential carriers for DOX. It was expected that DOX could blend into the inner P(MMD-*co*-PDO) core through hydrogen bonds between amide groups and terminal hydroxyl group of the block copolymers. In addition, hydrophobic effect also played an important role during drug loading. The properties of those copolymer microspheres including the morphology, size distribution, loading capacity (LC), encapsulation efficiency (EE), pH-responsiveness and release behavior of DOX were explored.

2. Experimental section

2.1. Materials

L-Alanine (food grade), chloroacetyl chloride, Celite 545, stannous octoate [Sn(oct)$_2$] (95%), and PDO (99%, analytical grade) were supplied by Aladdin Reagent Co., Ltd. (Shanghai, China). PDO was purified by vacuum distillation over CaH_2 under nitrogen before use. Toluene and acetonitrile were purchased from Tianjin Guangfu Fine Chemical Research Institute, and they were dried by reflux over CaH_2 and distilled just before use. Tetrahydrofuran (THF) and N, N-dimethylformamide (DMF) were purchased from Jiangtian Chemical Technology Co., Ltd. and dried with anhydrous magnesium sulfate. PEG6000 (Tianjin Guangfu) was purified by dissolution in chloroform and precipitation in diethyl ether, followed by drying through azeotropic distillation of toluene. After evaporation of toluene, PEG6000 was allowed to stand under reduced pressure (10^{-3} mbar) at room temperature for 24 h. All other reagents were of analytical grade and used as received. In addition, DOX was obtained from Zhejiang Hisun Pharmaceutical Co., Ltd.

2.2. Instrumentation and analysis

2.2.1. FT-IR analysis

The chemical structure of synthetic depsipeptide-based copolymers was confirmed by Fourier transform infrared spectrometer (FT-IR) and the measurement was performed as KBr pellets on a Nicolet 60SXR FT-IR-spectrometer.

2.2.2. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy

^{1}H and ^{13}C NMR spectra were measured by an ECA-500 400 MHz spectrometer with deuterochloroform ($CDCl_3$) as deuterated solvent at 25 °C.

2.2.3. Molecular weight determination by gel permeation chromatography (GPC)

The relative molecular weight and PDI of the resulting block copolymers were determined by GPC using a Kontron HPLC-420 instrument. The measurements were performed using THF as eluent at a flow rate of 1.0 mL/min at 30 °C and a series of polystyrene as standards.

2.2.4. Differential scanning calorimetry (DSC) analysis

The DSC measurements were performed on a NETZSCH DSC 204 F1 in aluminum pans under nitrogen. The specimens were heated in sealed aluminum pans and scanned from −50 to 175 °C using the heating rate of 10 K/min.

2.2.5. Determination of critical micelle concentration (CMC)

The CMC of the amphiphilic triblock copolymers was determined using pyrene as an extrinsic probe [54]. An aliquot of 100 μL pyrene solution in chloroform was added to volumetric flasks and evaporated to dryness. 10 mL of polymeric micelle solutions of different concentrations were added into the flasks and the concentration of pyrene in each flask was maintained at 5.0×10^{-7} mol L^{-1}, then, the flasks were incubated at room temperature overnight under stirring. The excitation spectra were recorded on a Fluorolog fluorescene spectrophotometer (Horiba Jobin Yvon, Inc.) with λ_{em} at 390 nm and slit width of 3 nm. The CMC value was calculated from the intersection of two tangent