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Controlled co-delivery nanocarriers based on mixed micelles formed from cyclodextrin-conjugated and cross-linked copolymers

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ABSTRACT

The combination of multiple drugs within a single nanocarrier can provide significant advantages for disease therapy and it is desirable to introduce a second drug based on host–guest interaction in these co-delivery systems. In this study, a core-stabilized mixed micellar system consisting of β-cyclodextrin-conjugated poly(lactic acid)-*b*-poly(ethylene glycol) (β-CD-PLA-mPEG) and DL-Thioctic acid (TA) terminated PLA-mPEG (TA-PLA-mPEG) was developed for the co-delivery of DOX and fluorescein isothiocyanate labeled adamantane (FA). DOX can be loaded within the hydrophobic segment of PLA and FA may form stable complexation with β-CD in the core. The mixed micelles (MM) are based on well-accepted medical materials and can be easily cross-linked by adding 1,4-dithio-D,L-threitol (DTT), which can enhance the stability of the system. Drug-loaded MM system was characterized in terms of particle size, morphology, drug loading and in vitro release profile. Cytotoxicity test showed that blank MM alone showed negligible cytotoxicity whereas the drug-loaded MM remained relatively high cytotoxicity for HeLa cancer cells. Confocal laser scanning microscopy (CLSM) demonstrated that the MM could efficiently deliver and release DOX and FA in the same tumor cells to effectively improve drugs' bioavailability. These results suggested that the core-stabilized MM are highly promising for intracellular co-delivery of multiple drugs.

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1. Introduction

Drug delivery system has attracted considerable interest because it can significantly improve the solubility of poorly water-soluble drug, carry it into target sites, reach for sustained/controlled release profiles, prolong circulation time and improve biodistribution/bioavailability [1,2]. However, most of existing drug delivery systems are applied to load and delivery a single drug, which does not satisfy the demands of combination therapy, and far from perfect due to their limited bioavailability, toxicity and other side effects. Therefore, it is necessary to develop co-delivery system, which can carry two or more drugs, for superior profiles such as controlled release sequence, timing, doses and duration of each drug, to achieve an effective combination therapy [3,4]. For example, Sengupta et al. reported a new drug carrier named as 'nanocell' for solid tumor treatment, in which doxorubicin (DOX) was conjugated to the poly(lactic-*co*-glycolic) acid (PLGA)-based nanoparticle while an anti-angiogenesis agent (combretastatin-A4) was trapped within the outer lipid envelope. This 'nanocell' enabled a temporal release model of the two drugs: the outer envelope first released the combretastatin-A4 and caused a vascular shutdown; the inner nanoparticle could be trapped inside the solid tumor and then released DOX to kill the tumor cells [5]. Also, we note that Celator Pharmaceuticals has carried out Phase II/III clinical trials with CombiPlex®, which indicates the potential of drug combination therapy.

As a well-studied drug delivery system, polymeric micelle consists of hydrophobic core and hydrophilic shell. The micelles are formed by macromolecular amphiphiles when the concentration is above the critical micelle concentration (CMC). They have been well investigated for drug co-delivery systems with improved properties [6–13]. Shin and coworkers reported the multi-agent loaded micelles based on poly(ethylene glycol)-*block*-poly(D,L-lactic acid) (PEG-*b*-PLA), which were utilized to delivery several drugs, e.g. paclitaxel (PTX), etoposide (ETO), docetaxel (DCTX) and 17-allylamino-17-demethoxygeldanamycin (17-AAG). Combination of PTX/17-AAG, ETO/17-AAG, DCTX/17-AAG and PTX/ETO/17-AAG were all solubilized at the level of mg/mL and stable for 24 h in the micelles. The presence of 17-AAG could help to keep the stability of 2- or 3-drug combination PEG-*b*-PLA

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micelles [7]. Other strategies, such as forming micelle-drug conjugates via complexation or electrostatic interactions [8–12], have also been used to load drugs or bioactive agents in co-delivery systems. For instance, Lee et al. reported that cationic micellar nanoparticle could be employed as carriers to co-delivery PTX and Herceptin through different interaction mechanisms. This system achieved targeted delivery of PTX to human epidermal growth factor receptor-2 (HER2/neu)-overexpressing human breast cancer cells, and enhanced cytotoxicity through synergistic activities as compared with single-drug treatment [12].

Although previous researches have made great progress in this field, there are very few reports about introducing the second drug via the inclusion interaction between host and guest molecules in co-delivery systems. Cyclodextrins (CDs) are well-known host molecules consisting of six to eight glucose units. The internal hydrophobic cavities in the CDs facilitate the inclusion of various guest molecules [14–16], thus CDs could be desirable functional units for co-delivery systems. In our previous work, we synthesized a well-defined amphiphilic copolymer, i.e., β-cyclodextrin-conjugated poly(lactic acid)-*b*-poly(ethylene glycol) (β-CD-PLA-mPEG), which could self-assemble in aqueous solution to form micelles with high drug loading efficiency and controlled release profile, especially in the control of the initial burst release of a single drug (indomethacin, IND) [17]. Since the cavity of β-CD may encapsulate a second drug in addition to one drug incorporated in the hydrophobic core of micelles, the micelles assembled by β-CD-PLA-mPEG copolymer could be a promising co-delivery system to achieve differential release profiles.

Another requirement for micellar delivery system is that it should have enough stability to avoid the drug leakage during circulation. Since micelles self-assembled from single copolymer often lack multiple functionalities due to the limited number of building blocks, mixed micelles (MM) can provide an alternative approach to enhance the stability of micellar system. MM formed from two or more dissimilar block copolymers are efficient to introduce considerable functional units without the need of complicated synthetic steps [18–20], and can also achieve significant improvements, e.g., lower CMC [21], higher drug loading efficiency [22], controlled particle size [23], enhanced stability and more efficient tumor inhibition [24,25]. Recently, several groups have reported the enhanced stabilization of polymeric micelles via forming cross-linked cores [26–31], thus the introduction of cross-linking units into one of the copolymers of mixed micelles could be a facile strategy to stabilize the co-delivery system.

Herein, we design a core-stabilized mixed micellar system, which is formed by β-CD-PLA-mPEG and DL-Thioctic acid (TA) terminated PLA-mPEG (TA-PLA-mPEG), for the co-delivery of DOX and fluorescein isothiocyanate labeled adamantane (FA), as shown in Scheme 1. DOX is loaded within the hydrophobic segment of PLA and FA may form stable complexation with β-CD in the core. TA is produced naturally in the human body and could be widely used as an antioxidant drug for treating diseases such as HIV and diabetes [27,32]. Thus the MM are based on well-accepted medical materials. The MM can be easily cross-linked by adding 1,4-dithio-D,L-threitol (DTT) relative to the lipoyl groups at the end of TA-PLA-mPEG copolymer, which can improve the stability of the system. Drug-loaded MM were characterized in terms of particle size, morphology, drug loading and in vitro release profile. Cytotoxicity test showed that blank MM alone showed negligible cytotoxicity whereas the drug-loaded MM remained relatively high cytotoxicity for HeLa cancer cells. Furthermore, confocal laser scanning microscopy (CLSM) demonstrated that the MM system could efficiently deliver and release DOX and FA in the same tumor cells to effectively improve drugs' bioavailability. These results suggested that the core-stabilized MM system are highly promising for intracellular co-delivery of multiple drugs.

2. Experimental details

2.1. Materials

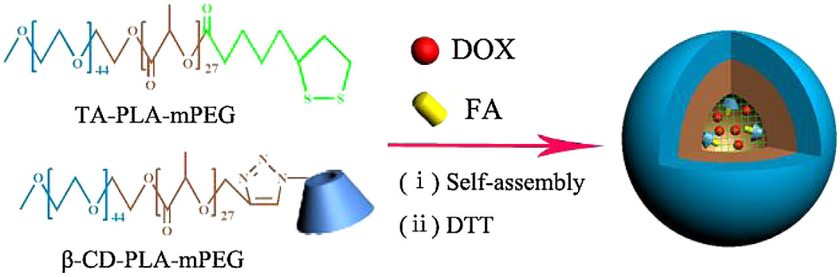
PLA-mPEG and β-CD-PLA-mPEG were synthesized in our previous work [17]. DL-Thioctic acid (TA) was purchased from Acros (Acros Organics, Geel, Belgium). 1,4-dithio-D,L-threitol (DTT) was purchassed from Merck (Darmstadt, Germany). 4-dimethylamino pyridine (DMAP, 99%) was provided by Kelong Chemical Reagent Plant (Chengdu, China). 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and fluorescein isothiocyanate were purchased from Aladdin (Shanghai, China). 1-adamantanamine was purchased from Adamas (Shanghai, china). FA was prepared following reported procedure in literature [33]. Doxorubicin hydrochloride (DOX HCl) was obtained from Melone biotechnology Co., Ltd. (Dalian, China) and desalted with triethylamine and dimethyl sulfoxide. *N,N*-dimethylformamide (DMF) was purchased from Bodi Chemical Holding Co., Ltd. (Tianjin, China) and used after drying.

2.2. Synthesis of TA-PLA-mPEG

PLA-mPEG (Mn = 4000, 2 g, 0.5 mmol) was dissolved in dry DMF (15 mL). Under a nitrogen atmosphere, EDC (0.19 g, 1 mmol), DMAP (0.1125 g, 0.75 mmol), and TA (0.15 g, 0.75 mmol) were added in order. After stirring for 15 min, the reaction bottle was sealed and placed into an oil bath thermostatted at 25 °C for 2 days. The obtained solution was diluted with 15 mL water and dialyzed against water by a dialysis tube (MWCO 3500 Da) for 3 days. The obtained copolymer solution was lyophilized. Products were characterized by ^{1}H NMR (400 MHz) (AVII-400; Bruker, Karlsruche, Germany).

2.3. MM formation and determination of CMC

β-CD-PLA-mPEG and TA-PLA-mPEG were dissolved in DMF at three different mass ratios (8:2, 5:5, 2:8) and stirred overnight. Then a certain amount of water was dropwise added to the solution under stirring. The resultant solution was dialyzed against distilled



Scheme 1. Illustration of mixed micelles based on β-CD-PLA-mPEG and TA-PLA-mPEG for the co-delivery of DOX and FA.

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