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Controlled drug release system based on cyclodextrin-conjugated poly(lactic acid)-b-poly(ethylene glycol) micelles

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\textbf{A B S T R A C T}

Cyclodextrin-conjugated poly(lactic acid)-b-poly(ethylene glycol) (\(\beta\)-CD-PLA-mPEG), a well-defined amphiphilic copolymer, was synthesized by controlled ring-open copolymerization and click coupling reaction, in order to obtain a biocompatible drug delivery system with controlled release profiles. The \(\beta\)-CD-PLA-mPEG copolymer could self-assemble in aqueous solution to form micelles with a mean particle size of 173.4 nm, which will decrease to 159.2 nm after loaded with a kind of hydrophobic drug (indomethacin, IND). The IND-loaded \(\beta\)-CD-PLA-mPEG micelles show spherical shape within the nano-size scale under TEM imaging. Compared with that formed by PLA-mPEG, the micelles formed by \(\beta\)-CD-PLA-mPEG copolymer present higher drug loading efficiency and controlled release profile of IND, especially in the control of its initial burst release. Meanwhile, \(\beta\)-CD-PLA-mPEG copolymer exhibits low toxicity to cells. The micelles formed by \(\beta\)-CD-PLA-mPEG copolymer could be a promising controlled release system for various hydrophobic drugs.

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1. Introduction

In recent years, polymeric micelles with core–shell architecture have attracted great attention due to their wide application in drug delivery systems (Li et al., 2011; Kim et al., 2012; Savič et al., 2012; Song et al., 2010; Xiong et al., 2012; Zhao et al., 2012a). Generally, polymeric micelles with hydrophobic cores are assembled by macromolecular amphiphiles due to the hydrophobic interaction between core-forming segments in an aqueous solution (Hehir et al., 2012; Licciardi et al., 2010; Liu et al., 2011; Zhou et al., 2003). More recently, multifunctional micelles loaded with various drugs are among the most intensively studied systems in order to construct controlled release systems with better properties, such as high drug loading efficiency or stimuli-responsive capability (Cai et al., 2011; Cajot et al., 2012; Chen et al., 2012; Zhao et al., 2012b). It could be an effective strategy to introduce a second functional structure to the core of micelles to obtain remarkable properties (Hu et al., 2012; Sun et al., 2011; Xu et al., 2008). Kataoka’s group reported that platinum anti-tumor drug (1,2-diaminocyclohexane)platinum (II) (DACHPt) could interact with poly(ethylene glycol)-b-poly(glutamic acid) [PEG-P(Glu)], and form an \(\alpha\)-helix secondary structure between DACHPt and P(Glu) in the core of DACHPt-loaded micelles. After being injected in vivo, the DACHPt-loaded micelles exhibited a longer retention time in

plasma, higher avoidance from the uptake by liver and spleen and the enhanced accumulation of DACHPt in tumor, resulting in the higher anti-tumor efficacy with higher selectivity, all of which were due to the regulation of the \(\alpha\)-helix secondary structure in the micelle core (Mochida et al., 2012; Rafi et al., 2012). Another example is that, the micelles formed by poly(ethylene glycol)-b-poly(\(\gamma\)-aspartic acid)-b-poly(\(\gamma\)-phenylalanine) (PEG-PAsp-PPh) could be reinforced by a mineral (calcium phosphate, CaP) layer as a secondary structure induced by PAsp segment, and results in a largely enhance serum stability of the micelles (Min et al., 2012).

Cyclodextrins (CDs) are natural cyclic oligosaccharides which can be divided into \(\alpha\), \(\beta\) and \(\gamma\)-cyclodextrin according to the number of \(\alpha\)-1,4-D-glucopyranosyl rings. It has a hydrophobic internal cavity, which could include guest molecules, such as various hydrophobic drugs. Therefore, cyclodextrin, especially \(\beta\)-cyclodextrin has been widely used in the pharmaceutical field as drug delivery systems (Li et al., 2004; Wu et al., 2011; Xin et al., 2010; Zhang et al., 2010a). Also, cyclodextrin has been incorporated into various micellar systems, in which the function of cyclodextrin could be divided into three families. In the first situation, cyclodextrin acts as a chemical responsive substance that could cause conformation switch by the host–guest interaction between the CDs unit and the hydrophobic group, which might be the driving force of the formation of micelles through the self-assembly (Zhang et al., 2010b; Zhang and Ma, 2011). Second, the cone-shaped cavities of CDs can act as a host of macromolecular guests via multiple interaction forces to form polyrotaxanes (Rusa et al., 2003; Wu and Li, 2009). Driven by this inclusion interaction,
supramolecular polymer micelles (SMPMs) could be constructed from CD and poly(ε-caprolactone) (Dong et al., 2008). The third one is that the cyclodextrin is utilized as the core to synthesize star-shaped amphiphilic polymers to form unimolecular micelles (UIMs) (Gou et al., 2010) or block copolymeric micelles (BCMs) (Qiu et al., 2010, 2011) as drug delivery systems, in which the amphiphilic segments were bound to cyclodextrins as multi-arms. However, in all the above systems, the cavity of cyclodextrins has been occupied and could not be used to load hydrophobic drugs through the inclusion interaction. Therefore, a new strategy is necessary to conjugate cyclodextrin unit into the hydrophobic core of a micelle as a secondary functional unit, which might provide additional stabilization force to the loaded drugs, and also may encapsulate a second kind of drug in addition to one drug incorporated in the hydrophobic core for multi-drug delivery purpose.

In this work, biodegradable monomethoxy poly(ethylene glycol) (mPEG), poly(ε,l-lactic acid) (PLA) and β-cyclodextrin (β-CD) were utilized to synthesize a well-defined CD-conjugated copolymer by controlled ring-open copolymerization and click coupling reaction (Scheme 1). Herein, the Huisgen 1,3-dipolar cycloaddition reaction of organic azides and alkynes (click coupling reaction) could improve the rate and regioselectivity of the reaction (Meldal and Tornøe, 2008). And it has been widely used in the synthesis of linear polymers (Srinivasachari et al., 2006), cyclic polymers

![Scheme 1](image-url)
2.2. 

2.2.1. Synthesis of alkyne-terminated PLA-mPEG 

The PLA-mPEG copolymer was synthesized by ring-opening polymerization as following: a predetermined amount of mPEG2000, d,l-lactide (d,l-LA) and 1.0% (w/v) stannous octoate (Sn(Oc)2) toluene solution were added to a round bottom flask. The flask was evacuated under vacuum to remove the oxygen and toluene in it. Then the flask was sealed and maintained at 120 °C for 24 h to complete the reaction. The synthesized copolymer was purified by re-dissolving in dichloromethane and precipitating in cold diethyl ether twice.

Alkyne-terminated PLA-mPEG was prepared via the reaction between propargyl bromide and PLA-mPEG in the presence of sodium hydride (NaH). Prior to the reaction, the PLA-mPEG sample was dried under vacuum at 60 °C for overnight. In a typical experiment, NaH (0.036 g, 1.5 mmol) and anhydrous THF (5 mL) were added to a 100 mL flask. PLA-mPEG (1 g, 0.25 mmol) dissolved in 10 mL anhydrous THF was dropwisely added within 30 min. The mixture was vigorously stirred at the temperature for 3 h and then propargyl bromide (0.119 g, 1 mmol) dissolved in 5 mL anhydrous THF was added dropwisely. Further 24 h was needed to complete the reaction at room temperature. The NaBr and the unreacted NaH were removed by filtration. After concentrating by evaporation, the filtrate was added into a large amount of petroleum ether. To purify the products, this procedure was repeated for three times.

2.2.2. Synthesis of mono-(6-azido-6-deoxy)-β-cyclodextrin (β-CD-N3) 

To obtain the sample of β-CD-N3, mono-(6-O-(p-tolysufomyl))-β-cyclodextrin (β-CD-OTS) was first synthesized according to the procedure III and IV shown in Scheme 1. In a typical experiment, p-toluenesulfonyl chloride (10 g, 52.5 mmol) and dry CH2Cl2 were added to a 250 mL flask. After cooling the flask to 0 °C in ice-water bath, imidazole (8 g, 118 mmol) dissolved in 50 mL dry CH2Cl2 was added dropwisely within 1.5 h at the same temperature. The reaction was carried out for a further 2 h with vigorous stirring at room temperature. After filtering off the insoluble solid, the filtrate was collected, concentrated and poured into 150 mL hexane to obtain a white solid, which was dried in vacuum for overnight. Then the dried white solid (8.9 g, 40.0 mmol), β-CD (35.0 g, 30.84 mmol) and 350 mL deionized water were mixed in a conical flask. Under vigorous stirring, the mixture was reacted at room temperature for 4 h. Then NaOH solution (20% (w/v), 50 mL) was added dropwisely to the mixture and continued stirring for an additional 10 min. The insoluble solid was removed by filtering and the filtrate was mixed with NH4Cl which could neutralize the solution to pH 7 and induce precipitation. The crude product was purified by repeated precipitating cold water and acetone. And the final β-CD-OTS was dried under vacuum for overnight.

Then, β-CD-OTS (5.2 g, 4 mmol) and NaN3 (1.3 g, 20 mmol) were dissolved in 40 mL DMF and stirred at 75 °C for 24 h. After completing the reaction, the mixture was precipitated in a large amount of acetone and filtered. Prior to repeating this processing, the crude product was recrystallized in 50 mL of boiling water. The product was dried under vacuum at 60 °C for 24 h.

2.2.3. Synthesis of β-CD-PLA-mPEG 

A mixture of alkyne-terminated-PLA-mPEG (3.448 g, 0.862 mmol), β-CD-N3 (2 g, 1.724 mmol), PMDETA (0.719 mL, 3.448 mmol) and DMF (10 mL) were placed in a flask (50 mL) equipped with a magnetic stir bar. The flask was evacuated and back-filled with nitrogen for several times. CuBr (494.8 mg, 3.448 mmol) was then added. After stirring at 60 °C for 48 h under N2 atmosphere, the mixture was further stirred at room temperature for overnight under open air and dialyzed against distilled water for 3 days to remove DMF and the excess unreacted β-CD-N3. The final solid-state product was obtained by lyophilization.

2.3. Structural identification 

After dissolving in CDC13, D2O or DMSO-d6, the chemical structures of PLA-mPEG, alkyne-terminated PLA-mPEG, β-CD-N3 and β-CD-PLA-mPEG were characterized by 1H NMR spectroscopy recorded on a Varian Unity Inova-400 spectrometer operated at 400 MHz. The molecular weight (Mn) and polydispersity index (PDI) of PLA-mPEG were acquired from an Agilent 1100 gel permeation chromatography (GPC) using tetrahydrofuran (THF) as eluent at a flow rate of 1 mL/min. In addition, FT-IR spectra were acquired with KBr tablets from 32 scans at 2 cm−1 resolution using a Nicolet 6700 FT-IR spectrometer.
2.4. Fluorescence measurement

To prove the potential of micelle formation, critical micelle concentrations (CMC) of the β-CD-PLA-mPEG were determined by fluorescence measurements using pyrene as a probe. An aliquot of 1 mL pyrene dissolved in acetone was transferred into a series of 10 mL vials and evaporated to dryness. Then 10 mL of polymeric micelles (1 × 10⁻⁵–1.0 mg/mL) were added to each vial and shook at 40 °C for overnight to equilibrate pyrene and micelles. The final concentration of pyrene was 6 × 10⁻⁷ M. Steady-state fluorescent spectra were obtained with a Shimadzu fluorescence spectrophotometer (RF-5310PC) at room temperature. The measured emission wavelength was set at 395 nm with a 0.2 nm slit width for excitation.

2.5. Dynamic light scattering

The particle size of the micelles was determined by dynamic light scattering (DLS) with a He–Ne laser at 532 nm on a Brookhaven BI-200 SM system. The scattering angle was kept at 90° and the temperature was set to 25 °C. All micellar solutions had a final concentration of 1.0 mg/mL and were filtered through a 0.45 μm syringe filter before measurement.

2.6. Loading of IND into micelles

A typical process is described as: β-CD-PLA-mPEG and indomethacin (IND) powder was dissolved in 5 mL dimethylacetamide (DMAC), and then distilled water (15 mL) was added dropwise within 30 min to the solution with vigorous stirring. After stirring for 30 min, the mixture was dialyzed against distilled water over 2 days to remove DMAC. The loading efficiency (LE) and entrapment efficiency (EE) of IND in micelles were calculated as LE (%) = [M_{BIND}/(M_{IND} + M_{polymer})] × 100 and EE (%) = ([IND]_{solubilized}/[IND]_{added}) × 100, respectively.

2.7. In vitro release of IND-loaded micelles

In vitro release of IND from micelles was conducted with dialysis method. 5 mL of 0.5 mg/mL IND-loaded micelles were transferred into a dialysis pocket (molecular weight cut-off 3500 Da, MWCO 3500). The pocket was then placed in 50 mL phosphate buffered saline (PBS) of pH 7.4. The release study was carried out at 37 °C in a water bath shaker. 5 mL of the samples was collected and the equal volume of fresh PBS was refilled at predetermined intervals. IND concentration of all collected solutions was recorded based on absorbance intensity at 319 nm on a MAPADA UV-vis analysis (PC1800, China).

2.8. Morphology study of IND-loaded micelles

The morphology study of micelles was carried out using a transmission electron microscope (TEM, FEI Tecnai G² F20 S-TWIN, USA) operating at an acceleration voltage of 75 kV. Samples were prepared by dropping micelle solution (5.0 mg/mL) containing 2 wt.% phosphotungstic acid onto a 200 mesh–copper grid coated with carbon and dried at room temperature after blotting the extra solution with filter paper.

2.9. In vitro cytotoxicity study

The cytotoxicity of the blank and IND-loaded micelles was evaluated via MTT assay in the COS7 cell line. The cells were cultured in DMEM, supported with 10% heat-inactivated FBS, 1% of penicillin and streptomycin at 37 °C in a humidified 5% CO₂–95% air atmosphere. The COS7 cells were seeded in a 96-well microtiter plate (Nunc Co., Wiesbaden, Germany) at a density of 1 × 10⁴ cells per well and incubated in 100 μL of DMEM/well for 16 h. Then the culture media were replaced with fresh culture media containing various concentrations of micelles. After incubation for 4 h, 10 μL of MTT stock solution in PBS (5 mg/mL) was added to each well and the cells were incubated for another 4 h. Finally, the formazan crystals dissolved in DMSO were added at a quantity of 100 μL/well. The absorbance was recorded using a microplate reader at 570 nm.

3. Results and discussion

3.1. Synthesis and characterization

β-CD-PLA-mPEG was synthesized by click coupling reaction of β-CD-N₂ and alkyn-terminated-PLA-mPEG as shown in Scheme 1. First, PLA-mPEG was synthesized by ring-opening copolymerization of d,l-LA monomers using mPEG as an initiator catalyzed by Sn(Oct)₂ at 120 °C for 24 h. Propargyl bromide derivative is an effective method to introduce alkyn terminal in PLA-mPEG. On the other hand, β-CD has three kinds of hydroxyl groups, among which 6-OH is the most active one. According to previous studies, tosylated CD was a very effective intermediate to synthesize β-CD derivatives. In this work, β-CD-OTs was obtained by two-step reaction between p-toluensulfonyl, imidazole and β-CD. Then NaN₃ was added to synthesize β-CD-N₃ by a substitution reaction. Finally, β-CD-PLA-mPEG was obtained by click coupling reaction, in which the most important factor was maintaining the [Cu(I)] from CuBr at a high level at all times during reaction (Meldal and Tornøe, 2008). During the reaction, the color of reaction solution did not turn green, which indicated that [Cu(I)] did not oxidize and keep at a high concentration. The synthesis route and polymerization process are shown in Scheme 1. As can be seen from the FT-IR spectra shown in Fig. 1, β-CD-PLA-mPEG sample presented a strong signal at 1754 cm⁻¹ which is attributed to the carbonyl group (C=O) in PLA segment. The signal at 1099 cm⁻¹ could be assigned to the ether bond in both PLA and mPEG chain. The stretching frequency at 1040 cm⁻¹ indicated the ether bond (C-O-C) existed in β-CD. All these signals suggested the successful linking of mPEG, PLA and β-CD.

The 1H NMR spectra of the β-CD-N₃, alkyn-terminated-PLA-mPEG and β-CD-PLA-mPEG are illustrated in Fig. 2a–c. Fig. 2a exhibits a representative spectrum of β-CD-N₃ in DMSO-d₆. It clearly shows the major proton signals as follows: 5.80–5.63

![Fig. 1. FT-IR spectra recorded from KBr tablets of β-CD-N₃ (a), alkyn-terminated-PLA-mPEG (b), β-CD-PLA-mPEG (c) and IND-loaded β-CD-PLA-mPEG micelles (d).](image-url)
Fig. 2. ¹H NMR spectra of β-CD-N₃ in DMSO-d₆ (a), alkyne-terminated-PLA-mPEG in CDCl₃ (b), β-CD-PLA-mPEG in DMSO-d₆ (c) and β-CD-PLA-mPEG in D₂O (d).

(OH-2,3, 14H), 4.87 (H-1, 7H), 4.47 (OH-6, 6H), 3.76–3.30 (H-2,3,4,5,6, 42H). In addition, the change of H-6′ chemical shift confirmed the successful single-substitute of β-CD. Also, the peak corresponding to mPEG, PLA and alkyne terminat were clearly observed in Fig. 2b, and could be assigned as follows: 5.26–5.16 (O=C–CH=O, 1H, PLA segment), 3.76–3.54 (O–CH₂–CH₂, 4H, mPEG segment), 3.38 (O–CH₃, 3H), 1.87 (CH=CH, 1H), 1.59–1.42 (O=C–CH–CH₃, 3H, PLA segment). Compared to the spectrum of β-CD-N₃ and alkyne-terminated-PLA-mPEG, the chemical shift of β-CD-PLA-mPEG (Fig. 2c) do not change greatly except for the down-field shift of CH=CH signal of 1.87 ppm. The conjugation ratio of β-CD to PLA-mPEG in β-CD-PLA-mPEG could be calculated as 0.95 according to the peak areas obtained from the numbers of the H proton of C1 of β-CD and the (O=C–CH=O, 1H) of PLA-mPEG. In addition, the area of the (O=C–CH=O) and (O=C–CH–CH₃) peaks were used to calculate the number of d,l-LA units and then
the molecular weight of PLA-mPEG. Table 1 shows the molecular weights measured by \(^{1}H\) NMR and GPC. The well-defined copolymer was confirmed with a narrow distribution of 1.32, which indicated the high purity of the copolymer. The molecular weight measured either from \(^{1}H\) NMR or GPC was very close to theoretical value, indicating the controlled synthesis process can be obtained.

3.2. Micelle formation

The synthesized β-CD-PLA-mPEG copolymer could undergo self-assembly in aqueous solution due to the hydrophobic interaction of PLA segment. Fig. 2d shows the \(^{1}H\) NMR spectrum of β-CD-PLA-mPEG in D₂O. The specific signals of CH₂ (δ = 1.59–1.42) and CH (δ = 5.26–5.16) in PLA segment were both weakened as compared with its \(^{1}H\) NMR spectrum in DMSO-d₆ (Fig. 2c). Meanwhile, the peaks of [OH-2.3 (δ = 5.80–5.63), H-1 (δ = 4.87), OH-6 (δ = 4.47) and H-2.3,4,5,6 (δ = 3.76–3.30)] in β-CD were all disappeared even though there was hydrophilic outer shell in its surface. This result indicates that the cyclodextrin units are not at the peripheral, but in the hydrophobic center after the formation of micelles, which might be owing to the strong chemical bonding between PLA and β-CD. Therefore, the β-CD-PLA-mPEG copolymer could form micelles with mPEG shell and a specific core constituted by PLA chain and β-CD.

The CMCs of PLA-mPEG and β-CD-PLA-mPEG were obtained by fluorescent determination using pyrene as a probe. Pyrene is preferentially incorporate into hydrophobic microenvironment exhibiting strong fluorescence intensity while weak fluorescence intensity in a polar solution. With the increase of polymer concentration mixed with pyrene, there was a red-shift of excitation peak in aqueous solution (Astaﬁeva et al., 1993). And, when the polymeric concentration increases to its CMC, an abrupt change in the fluorescence depolarization occurs. Therefore the sharp rise in intensity ratio of peaks at 333.6 and 335.8 nm of pyrene in excitation spectra indicated the formation of micelles, thereby determine the CMC of polymer. Fig. 3 shows the relationship of the intensity ratios (I₂₃₅/₈/I₁₃₂₃₂₆) as a function of polymer concentration at 25°C. It can be calculated that the CMC values of β-CD-PLA-mPEG and PLA-mPEG were relatively low (0.0106 mg/mL and 0.0027 mg/mL, respectively), as showed in Table 2. Furthermore, the CMC value of β-CD-PLA-mPEG was higher than that of PLA-mPEG. The higher CMC value of β-CD-PLA-mPEG, might be attributed to the hydrophobic outer shell of β-CD in the core. It reveals that the introduction of β-CD into this system might enhance the core fluidity of micelles (Zhao et al., 2012b).

### Table 1

<table>
<thead>
<tr>
<th>Polymers</th>
<th>Mn(^a)</th>
<th>Mn(^b)</th>
<th>Mn(^c)</th>
<th>Mw(^d)</th>
<th>Mw/Mn</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA-mPEG</td>
<td>4000</td>
<td>3927</td>
<td>3031</td>
<td>3923</td>
<td>1.29</td>
</tr>
<tr>
<td>β-CD-PLA-mPEG</td>
<td>5135</td>
<td>5078</td>
<td>3792</td>
<td>5005</td>
<td>1.32</td>
</tr>
</tbody>
</table>

\(^a\) Theoretical value calculated from feed ratio.
\(^b\) Calculated from the \(^{1}H\) NMR data.
\(^c\) Measured via gel permeation chromatography (GPC).

### Table 2

<table>
<thead>
<tr>
<th>Polymers</th>
<th>Critical micelle concentration (mg/mL)(^a)</th>
<th>Particle size (nm)(^b)</th>
<th>Poly index(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA-mPEG</td>
<td>0.0027</td>
<td>132.6 ± 15.3</td>
<td>0.101</td>
</tr>
<tr>
<td>β-CD-PLA-mPEG</td>
<td>0.0106</td>
<td>173.4 ± 16.7</td>
<td>0.193</td>
</tr>
</tbody>
</table>

\(^a\) Critical micelle concentration determined in deionized water by fluorescence measurements.
\(^b\) Particle size and poly index were measured by dynamic light scattering (DLS) at a concentration of 1.0 mg/mL (n = 5).

A further evidence of micelle formation was validated by determining the particle size of the micelles. The sizes of blank PLA-mPEG and β-CD-PLA-mPEG micelles measured by DLS are exhibited a monomodal distribution with mean diameters of 132.6 and 173.4 nm, respectively (Table 2). PDIs of them were below 0.2, indicating a very narrow size distribution. Furthermore, larger size particles were formed for β-CD-PLA-mPEG (173.4 nm), compared with that of PLA-mPEG (132.6 nm), probably due to the specific stereoscopic cone structure and hydrophilic outer surface of β-CD in the hydrophobic core of PLA segment.

3.3. Preparation and characterization of IND-loaded micelles

IND-loaded polymer micelles has been prepared using dialysis method with DMAC as the cosolvent of drug and polymer, and the volume ratio of DMAC to water was fixed at 1:3 for all samples. The enhanced solubilization of IND by β-CD-PLA-mPEG copolymer could be caused by both the hydrophobic PLA segment and the inclusion complexation of β-CD functional unit (as illustrated in Scheme 2). To confirm the presence of the inclusion between IND and β-CD, the \(^{1}H\) NMR spectra of IND and their complex are shown in Fig. 4. As compared to the spectrum of β-CD-PLA-mPEG copolymer (Fig. 2c), the peak corresponding to IND was clearly observed in Fig. 4b, and could be assigned as follows: 7.65 (H-a, p-chlorobenzoyl ring), 7.04 (H-b, indole ring), 6.92 (H-c, indole ring), 7.34 (H-d, indole ring), 6.89 (H-e, indole ring), 6.72 (H-f, indole ring), 7.68 (H-g, indole ring), 3.95 (H-i, 1-proline), 2.90 (H-j, 2-proline), 2.79 (H-k, 3-proline), 1.79 (H-l, 4-proline).

### Scheme 2

Self-assembly of IND loaded micelles formed by PLA-mPEG and β-CD-PLA-mPEG.
Since this measurement was carried out by dissolving IND-loaded β-CD-PLA-mPEG micelles into DMSO-d$_6$, thus it could prove that IND was incorporated into the core of micelles. Moreover, proportion of integral area of protons assigned to different parts of IND has been changed. The integral area proportions of H-e to H-a are changed from 3:4 (Fig. 4a) to 3:2.72. This result suggests that the p-chlorobenzoyl ring is to some extent included by the β-CD of micelles, which is consistent with previous reports that parent CDs could form inclusion complexes with the p-chlorobenzoyl ring of IND (Fronza et al., 1996; Xin et al., 2010). FT-IR spectrums provide further information on the incorporation of IND into the core of micelle and the formation of IND/β-CD inclusion complexes. As shown in Fig. 1d, the band at 1324 cm$^{-1}$ is due to the flexural vibration of –CH$_3$ linking on the indole ring of IND, and the band at 755 cm$^{-1}$ is contributed by the C–H stretching vibration of the p-chlorobenzoyl ring. Both of them confirm the incorporation of IND into the core of micelles. Meanwhile, shifts of the β-CD-PLA-mPEG characteristic peak from 1040 and 2915 cm$^{-1}$ to 1038 and 2920 cm$^{-1}$ is observed in Fig. 1d, which also confirms the formation of the IND/β-CD inclusion complexes in the core of micelles.

The characteristics of the IND-loaded micelles are presented in Table 3. As can be seen, the LE (%) increased with the increase of the feed ratio of IND to polymer from 1:10 to 3:10. In addition, to
evaluate the influence of CD content in micelles on loading properties, the loading efficiency (LE) and entrapment efficiency (EE) of β-CD-PLA-mPEG/PLA-mPEG mixed micelles (molar ratio: 3:7 and 8:2, named as MM-30 and MM-80, respectively) were also analyzed. At a fixed feed ratio, the LE (%) and EE (%) of PLA-mPEG, MM-30, MM-80 and β-CD-PLA-mPEG micelles exhibited an obvious trend that the introduction of β-CD into the core could facilitate the loading of IND into micelles. This was because of the cavity of β-CD could effectively increase the hydrophobic space to encapsulate more IND besides the hydrophobic PLA segment.

The particle sizes of IND-loaded micelles at the concentration of 1.0 mg/mL are shown in Fig. 5. As can be seen, the size of the IND-loaded micelles formed by β-CD-PLA-mPEG is 159.2 nm, which is smaller than that of the corresponding blank micelles (Table 2). It might reveal that the IND molecules trapped into the core could enhance the hydrophobic interaction in the core of micelles, which is consistent with previous report that a larger amount of hydrophobic portion could result in the formation of smaller particles (Qiu et al., 2010, 2011). Moreover, previous report has revealed that the host–guest interaction between β-CD and guest molecule could enhance the hydrophobic property of β-CD to provide the driving force of self-assembly (Zhang et al., 2010b). Thus the slightly smaller diameters of IND-loaded micelles are caused by the hydrophobic interaction between PLA segment and IND molecules and also the formation of the IND/β-CD inclusion

![Fig. 5. Particle size and distribution of IND-loaded micelles measured by dynamic light scattering (DLS).](image1)

![Fig. 6. Transmission electron microscopic (TEM) image of blank β-CD-PLA-mPEG micelles (a), IND-loaded PLA-mPEG micelles (b) and IND-loaded β-CD-PLA-mPEG micelles (c).](image2)

![Fig. 7. In vitro release profile of IND from β-CD-PLA-mPEG micelles prepared with different drug feed ratio in PBS (pH 7.4) at 37°C. Data represent as mean ± S.D. (n = 3).](image3)

![Fig. 8. In vitro release profile of IND from micelles formed by PLA-mPEG, MM-30, MM-80 and β-CD-PLA-mPEG with drug feed ratio of 10:1 in PBS (pH 7.4) at 37°C. Data represent as mean ± S.D. (n = 3).](image4)
complexes. The morphology of the blank and IND-loaded micelles assembled by PLA-mPEG and β-CD-PLA-mPEG were measured by TEM (Fig. 6). The mean micelle diameter in the micrograph is evidently smaller than that determined by DLS measure. This is owing to the shrinkage of the micelles during the drying process and water evaporation under high vacuum during TEM imaging (Giacomelli et al., 2006; Luo et al., 2009; Sun et al., 2009; Yu et al., 2011). Therefore, an increase in the micelle diameter obtained from DLS is probably caused by the hydration of the PEG shell of micelles (Prabaharan et al., 2009).

3.4. In vitro release of IND from micelles

The release profiles of IND-loaded β-CD-PLA-mPEG micelles were measured and illustrated in Fig. 7 and Fig. 8. Fig. 7 shows the influence of drug feed ratio on the release behavior. Compared with the release profile of IND from β-CD-PLA-mPEG micelles prepared at low drug feed ratio (10:1), micelles prepared with 10:2 and 10:3 drug feed ratio exhibited higher burst release, which might be due to two reasons. First, generally, a higher drug feed ratio leads to a higher LE (%), then there might have some dissociated drug adsorbed at other place except the core of micelles, which could cause the burst release. Second, there should have an equilibrium value of the function of β-CD, i.e., after all the cavities of β-CD units have been occupied by IND, the β-CD-PLA-mPEG micelles could not provide the stabilization function on further added IND, which could be trapped in the PLGA segment as that in PLA-mPEG micelles. Thus, according to the finding in Fig. 7, the feed ratio of 10:1 was chosen in following investigations.

The release profiles of IND from PLA-mPEG, MM-30, MM-80 and β-CD-PLA-mPEG in PBS (pH 7.4) are presented in Fig. 8. All these micelles were prepared in a drug feed ratio of 10:1 as discussed above. As can be seen, the mixed micelles of MM-30 exhibited the fastest release of IND, which might be attributed to the heterogeneous micelles formed by the two kinds of copolymers. As for the MM-80 sample, a slower release of IND after 20 h could be observed, it could be due to the enhanced physical stability of mixed micelles, which could keep the physical integrity of micelles and extend drug circulation time (Attia et al., 2011). Among all the samples, β-CD-PLA-mPEG micellar system exhibited the best controlled release profile and efficiently decreased the burst release of IND, which should due to the inclusion interaction between IND and β-CD in the core of micelles. The β-CD in the core could form a 1:1 complex with IND molecule (Xin et al., 2010), which provides a relatively strong inclusion complexation to drug molecules. This result indicates that the introduction of β-CD into the core of micelles provides an effective secondary functional unit to normal micelles, which might be help to obtain controlled drug release profiles with decreased initial burst release of drug.

3.5. In vitro cytotoxicity studies

The cytotoxicity of both blank and IND-loaded micelles of β-CD-PLA-mPEG was examined by MTT assay. Meanwhile, we have also examined the cytotoxicity of PLA-mPEG, in order to evaluate the cytotoxic effect of the introduction of β-CD. The purpose of this biological test is to evaluate the biocompatibility of the micelles and whether IND, once loaded in the micelles, could change the biocompatibility of the materials or not. Therefore, we have determined their cytotoxicity based on a series of concentrations of 0.1, 1, 5, 10, 20, 50 and 100 μg/mL. Cytotoxicity was estimated by incubation with COS7 cells. The results (Fig. 9) demonstrated that both the parent PLA-mPEG micelles and the β-CD introduced ones exert low cytotoxicity at the concentrations set from 0.1 to 100 μg/mL. Up to 80% cell viability was obtained. The toxicity of the two IND-loaded micelles was similar. Generally, drugs usually exert a relatively higher toxicity in biological cells. Then the low toxicity indicated that the IND-loaded micelle systems could remain a low cytotoxicity even IND was loaded in the core.

4. Conclusion

In this study, β-CD-PLA-mPEG copolymer has been synthesized by click reaction of β-CD-N3 and alkyne-terminated-PLA-mPEG. The β-CD-PLA-mPEG copolymer could self-assemble in aqueous solution to form micelles with a mean particle size of 173.4 nm, which will decrease to 159.2 nm after loaded with the hydrophobic drug (IND). Compared with that formed by PLA-mPEG copolymer, the micelles formed by β-CD-PLA-mPEG copolymer present higher drug loading efficiency and controlled release profile of IND, especially in the control of its initial burst release. It suggests that the introduction of β-CD unit can enhance the stability of IND in the core of micelles. Cytotoxicity results demonstrated that β-CD-PLA-mPEG copolymer has low toxicity to cells. Thus the micelles formed by β-CD-PLA-mPEG copolymer could be a promising controlled release system for various hydrophobic drugs.

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