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Preparation, drug release and cellular uptake of doxorubicin-loaded dextran-b-poly(ε-caprolactone) nanoparticles

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ABSTRACT

Amphiphilic dextran-b-poly(ε-caprolactone) diblock copolymers were synthesized with the purpose of preparing nanocarriers for doxorubicin (DOX), an anticancer drug. The Dex-b-PCL diblock copolymers were synthesized by end-to-end coupling of amino-terminated dextran and aldehyde-terminated poly(ε-caprolactone) and characterized by ^{1}H NMR spectra and gel permeation chromatography. The DOX-loaded Dex-b-PCL nanoparticles were prepared by a modified nanoprecipitation method and characterized by transmission electron microscopy and dynamic light scattering. *In vitro* release of DOX from DOX-Dex-b-PCL nanoparticles showed a sustained release manner with certain amount of burst release in the first 9 h. *In vitro* cytotoxicity test of DOX-Dex-b-PCL nanoparticles against SH-SY5Y cells showed that DOX is still pharmacologically active after drug loading. The fluorescence imaging results showed that DOX-Dex-b-PCL nanoparticles could be easily uptaken by SH-SY5Y cells. These results indicate that DOX-Dex-b-PCL nanoparticles may be a promising nanocarrier for DOX.

1. Introduction

Polymeric micelles self-assembled from biocompatible and biodegradable block copolymers have attracted much attention due to their potential application in drug delivery (Allen & Cullis, 2004; Mikhail & Allen, 2009). Block copolymer micelles usually have a core-shell structure with hydrophobic segments as the internal core and hydrophilic segments as a surrounding corona. The internal hydrophobic core provides a storeroom for loading hydrophobic drug while the hydrophilic shell allows to retain the stability of the micelle in aqueous environment. As the drug carriers for intravenous therapy, these polymeric micelles are able to preferentially accumulate in tumor tissues by passive targeting such as the enhanced permeability and retention (EPR) effect or active targeting by the antigen–antibody or ligand–receptor interactions (Liu, Wiradharma, Gao, Tong, & Yang, 2007; Shahin, Ahmed, Kaur, & Lavasanifar, 2011; Tanaka, Shiramoto, Miyashita, Fujishima, & Kaneo, 2004).

To prepare biocompatible and biodegradable block copolymers, aliphatic polyesters (Shahin & Lavasanifar, 2010; Vassiliou, Papadimitriou, Bikiaris, Mattheolabakis, & Avgoustakis, 2010), polypeptides (Cho et al., 1999; Izunobi & Higginbotham, 2010) and poly(propylene oxide) (Zhang et al., 2011) have been most frequently employed as hydrophobic blocks. Compared to the versatility of hydrophobic blocks, hydrophilic block is often undertook by poly(ethylene glycol) (PEG). This is because PEG has a number of advantages such as biocompatibility, lack of toxicity, very low immunogenicity and antigenicity. Moreover, PEGylated nanoparticles have long blood circulation time (Zhu et al., 2010). However, PEG has some drawbacks such as the absence of reactive groups at the molecular chain for further modification or ligand coupling, and PEG is not biodegradable. Recently, it was found that plasma concentration and residue time of repeatedly injected PEGylated micelles dramatically reduced once administrating the formulation with several days interval, known as accelerated blood clearance (ABC) phenomenon (Ishihara et al., 2010; Wang, Ishida, & Kiwada, 2007). Thus, the development of appropriate hydrophilic block which has good biocompatible and biodegradable as well as stealthy properties is highly desirable.

Dextran is a natural analog to PEG and has been applied for a range of biomedical applications due to their excellent hydrophilicity, biocompatibility, biodegradability, and non-immunogenicity property (Sun et al., 2010). Clinically, dextran, in particular of low molecular weight (40 and 70 kDa), has been widely used for plasma volume expansion and thrombosis prophylaxis (Almeida, Ferreira, Lopes, & Gil, 2011). Furthermore, the hydroxyl groups at the dextran chain could be exploited for further chemical modification or ligand coupling. Therefore, dextran seems to be an attractive alternative to PEG hydrophilic segments for designing amphiphilic block copolymers. However, up to now, dextran-based block

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copolymers have not been studied much. Bosker et al. (2003) prepared dextran-polystyrene diblock copolymers by directly coupling dextran to amino-terminated polystyrene block. Houga, Meins, Borsali, Taton, and Gnanou (2007) and Houga et al. (2009) prepared dextran-polystyrene diblock copolymers by ATRP method, and micelles and polymersomes were obtained by self-assembly of the dextran-polystyrene diblock copolymers. Hernandez, Soliman, and Winnik (2007) first prepared dextran-poly(ethylene glycol) block copolymers by amidation of dextranelactone with amino-terminated methoxy poly(ethylene glycol), then conversed the neutral diblock copolymers into polyanions by carboxymethylation of the dextran block. Liu and Zhang (2007) prepared a biodegradable dextran-poly(ε-caprolactone) diblock copolymer by coupling reaction between poly(ε-caprolactone) end-capped with the acryloyl group and amino-terminated dextran, and the micellar behavior was investigated. Schatz, Louguet, Le Meins, and Lecommandoux (2009) prepared a dextran-poly(γ-benzyl L-glutamate) diblock copolymer by click chemistry, which was self-assembled in water into polymersomes. Sun et al. (2010) reported a dextran-SS-poly(ε-caprolactone) diblock copolymer synthesized through coupling reaction, and drug release behavior from this block copolymer micelles.

In this paper, dextran-b-poly(ε-caprolactone) (Dex-b-PCL) diblock copolymers was prepared through a new synthetic route, *i.e.* the end-to-end coupling of amino-terminated dextran and aldehyde-terminated poly(ε-caprolactone). The obtained Dex-b-PCL diblock copolymers were further employed to assembly micelles for doxorubicin (DOX) delivery. The DOX-loaded Dex-b-PCL nanoparticles were characterized by transmission electron microscopy (TEM) and dynamic light scattering (DLS). *In vitro* drug release and cytotoxicity of the DOX-loaded Dex-b-PCL micelles were examined. The cellular uptake of the micelles was observed by confocal laser scanning microscopy (CLSM).

2. Materials and methods

2.1. Materials

Dextran (M_n = 6000 Da), ε-caprolactone (ε-CL), Stannous octotate ($Sn(Oct)_2$) and 4-formyl-benzoic acid were purchased from Sigma Co. Sodium cyanoborohydride ($NaCNBH_3$) was bought from Acros Organics. Doxorubicin hydrochloride (DOX·HCl) was obtained as a gift from Shenzhen Main Luck Pharmaceuticals Inc. (Shenzhen, China) in powder. ε-CL was dried over CaH_2 for 24 h and distilled before use. Other reagents and solvents were analytical grade and used as received.

2.2. Synthesis and characterization of Dex-b-PCL diblock copolymers

2.2.1. Synthesis of amino-terminated dextran (Dex-NH_2)

Briefly, 1 g of dextran and 0.1 g of 1,2-ethylenediamine were dissolved in 30 mL of dimethylsulfoxide (DMSO). The solution was stirred for 6 days at 60 °C, and 20 mg of $NaBH_3CN$ as reducing agent was added every day. After the reaction, the product was poured into methanol to form a precipitate. The precipitate was filtered and washed with methanol to remove the excess 1,2-ethylenediamine, then dried in vacuum to obtain Dex-NH_2; yield: 93%.

2.2.2. Synthesis of aldehyde-terminated poly(ε-caprolactone) (PCL-CHO)

First, hydroxyl-terminated poly(ε-caprolactone) (PCL-OH) was obtained by the ring-opening polymerization of ε-CL using benzyl alcohol as initiator and $Sn(Oct)_2$ as catalyst. Typically, ε-CL, $Sn(Oct)_2$ (*ca.* 0.01% of ε-CL in a molar amount) and benzyl alcohol were weighed into a ampule flask, sealed in a vacuum, and immersed in an oil bath at 130 °C for 12 h. The rude product was dissolved in tetrafuran (THF) and then precipitated into cold ether twice. Finally, PCL-OH was dried in vacuum; yield: 91–92%. Second, PCL-CHO was produced by hydroxyl esterification of PCL-OH with 4-formyl-benzoic acid. Typically, 0.2 mmol of PCL-OH and 0.4 mmol of 4-formyl-benzoic acid were dissolved in 20 mL of THF. To this, 0.4 mmol of dicyclohexyl carbodiimide (DCC) and 20 mg of 4-(dimethylamino)pyridine (DMAP) dissolved in 10 mL of THF were added in one portion. The mixture was stirred at room temperature for 2 days. After the reaction, it was filtered to remove dicyclohexylurea (DCU). The remaining clear solution was concentrated and poured into cold ether to form a precipitate. The precipitate was filtered and washed with methanol to remove the excess 4-formyl-benzoic acid and the residual DCU, then dried in vacuum to obtain PCL-CHO; yield: 90–92%.

2.2.3. Coupling of Dex-NH_2 and PCL-CHO

Typically, 1 g of Dex-NH_2 and certain amount of PCL-CHO (200% of Dex-NH_2 in a molar amount) were dissolved in 50 mL of DMSO. The mixture was stirred for 4 days at 60 °C, and 30 mg of $NaCNBH_3$ as reducing agent was added every day. After the reaction, 150 mL of THF was added dropwise to the reaction mixture under stirring, which precipitated out the unreacted Dex-NH_2.The supernatant was separated and evaporated to remove THF. Then 150 mL of water was added dropwise to the remaining solution under stirring, which precipitated out the unreacted PCL-CHO. The supernatant was separated and evaporated to remove water, and poured into methanol to precipitate out Dex-b-PCL diblock copolymer. The precipitated Dex-b-PCL diblock copolymer was further collected and dried in vacuum; yield: 60–68%.

2.2.4. Characterization

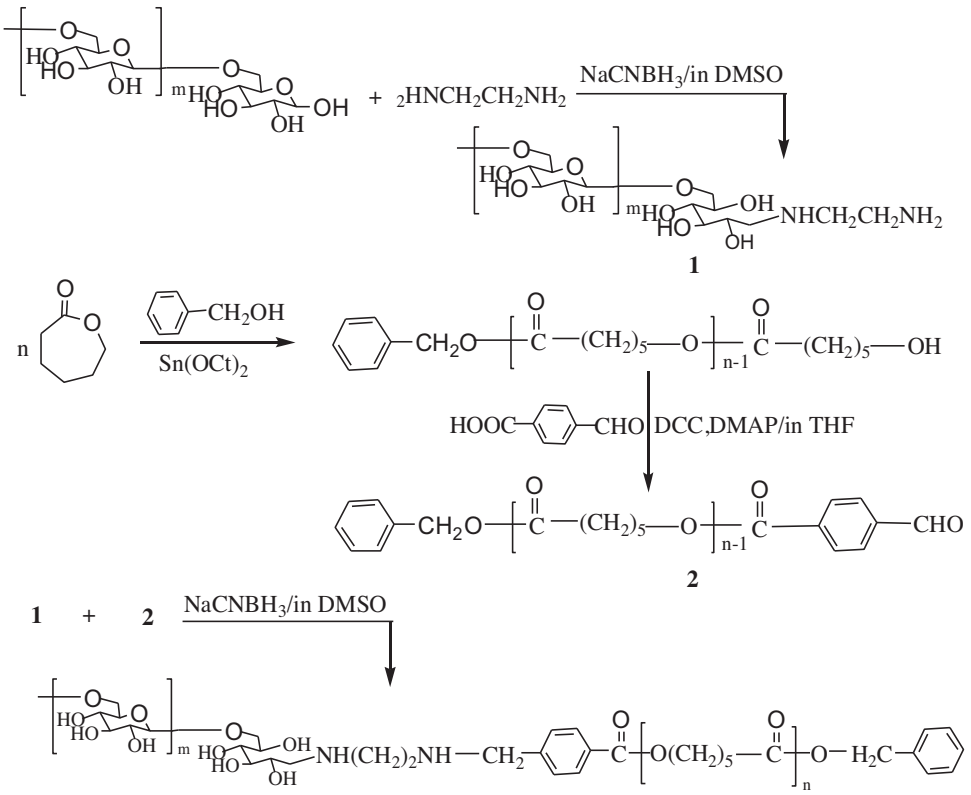
Dex-NH_2, PCL-OH, PCL-CHO and Dex-b-PCL diblock copolymer were characterized using NMR. ^{1}H NMR spectra were recorded on Mercury-Plus 300 (Varian, USA) spectrometer, using $CDCl_3$ or d_6-DMSO as solvent.

Gel permeation chromatography (GPC) measurements of Dex-b-PCL diblock copolymers were conducted at 40 °C with a PerkinElmer-200 GPC instrument equipped with a TSKgel SuperAWM-H*2 column and a differential refractometer detector. DMF + 10 mM LiBr was used as the eluent at a flow rate of 0.6 ml/min.

2.3. Preparation of Dex-b-PCL nanoparticles and drug loading

Blank and DOX-loaded Dex-b-PCL nanoparticles were prepared by a modified nanoprecipitation method. For DOX-loaded Dex-b-PCL nanoparticles, 80 mg of Dex-b-PCL diblock copolymer was dissolved in 3 mL of DMSO at 60 °C, while 20 mg of DOX·HCl was neutralized with triethylamine in 1 mL of DMSO. The two solutions were mixed by stirring for 2 h at 60 °C. To this solution was added 36 mL of water dropwise under stirring. Then, the mixture was placed into a dialysis bag (12 kDa cutoff) and dialyzed against water at room temperature for 24 h to separate free DOX. After dialysis, the solution in the dialysis bag was filtered through 0.45 μm syringe filter. Blank Dex-b-PCL nanoparticles were prepared *via* the same method without the addition of DOX.

To determine the drug loading content and encapsulation efficiency, a certain amount of lyophilized DOX-Dex-b-PCL nanoparticles was dissolved in DMSO. The amount of DOX loaded was determined by UV–vis measurement at the wavelength of 481 nm (Zhou et al., 2010). The concentration of DOX was calculated from the standard curve obtained from DOX in DMSO.



Scheme 1. Synthetic route to Dex-b-PCL diblock copolymers.

2.4. Size measurement and morphology observation of the nanoparticles

Mean hydrodynamic diameter and size distribution of the blank and DOX-loaded nanoparticles were determined by DLS using a Brookhaven BI-9000AT system (Brookhaven Instruments Corporation, USA). The result was the average of triplicate measurements for each sample.

Morphology observation of the blank and DOX-loaded nanoparticles was conducted on TEM (JEM-100s, JEOL, Japan). A drop of sample solution (2 mg/mL) was placed on the copper grids coated with carbon and dried at room temperature. The sample was negatively stained with phosphotungstic sodium solution (1%, w/v) before observation.

2.5. In vitro release of DOX from Dex-b-PCL nanoparticles

Predetermined amount of DOX-loaded Dex-b-PCL nanoparticles were dispersed in 3 mL of PBS (0.01 M, pH 7.4) and put into a dialysis bag. The dialysis bag (12 kDa cutoff) was placed into 30 mL of PBS and gently shaken at 37 °C. At predetermined time intervals, 3 mL of the release medium was withdrawn and equivalent fresh PBS was added. The amount of DOX released was determined by UV–vis measurement at the absorption wavelength of 481 nm. The concentration of DOX was calculated from the standard curve obtained from DOX·HCl in PBS.

2.6. Cytotoxicity assay

Cytotoxicity of Dex-b-PCL nanoparticles and DOX-loaded Dex-b-PCL nanoparticles were assessed by MTT assay. SH-SY5Y cells were seeded in 96-well plate at a density of 5000 cells/well and grown for 24 h prior to the assay. Then, the cells were exposed to a series of doses of DOX·HCl and DOX-loaded Dex-b-PCL nanoparticles as well as different concentrations of empty Dex-b-PCL nanoparticles at 37 °C, respectively. After coincubation for 24 h, 50 μL of MTT indicator dye (5 mg/mL in PBS, pH 7.4) was added to each well and cells were incubated for another 2 h at 37 °C in the dark. The medium was withdrawn and 150 μL of DMSO was added in each well to dissolve the crystals of dye. Absorption of the solution in each well was immediately determined on a microplate reader (Bio-Rad, USA) at a wavelength of 490 nm and 620 nm as reference wavelength. Value obtained was expressed as a percentage of the control cells to which no drug was added.

2.7. Cellular uptake observation

Rhodamine B isothiocyanate was labeled into Dex-b-PCL diblock copolymer for the preparation of Rho-labeled Dex-b-PCL nanoparticles. Briefly, 4 mg of Rhodamine B isothiocyanate and 0.2 g of Dex-b-PCL diblock copolymer were dissolved in 5 mL of dry DMSO and reacted at room temperature for 12 h. After the reaction, the product was precipitated into methanol, then filtered and dried. The obtained Rho-labeled Dex-b-PCL diblock copolymer was self-assembled to Rho-labeled Dex-b-PCL nanoparticles. Unconjugated Rhodamine B was removed by dialysis against water for 48 h.

SH-SY5Y cells were seeded in a plate at a density of 1×10^5 cells/well and grown for 24 h. Then the incubation medium was changed with fresh incubation medium containing test materials (16 μg/mL DOX·HCl, 400 μg/mL Rho-labeled Dex-b-PCL and DOX-loaded Dex-b-PCL with 16 μg/mL DOX), and the cells were incubated for 4 h. After that, the cells were washed with PBS three times and observed by confocal laser scanning microscopy (CLSM,

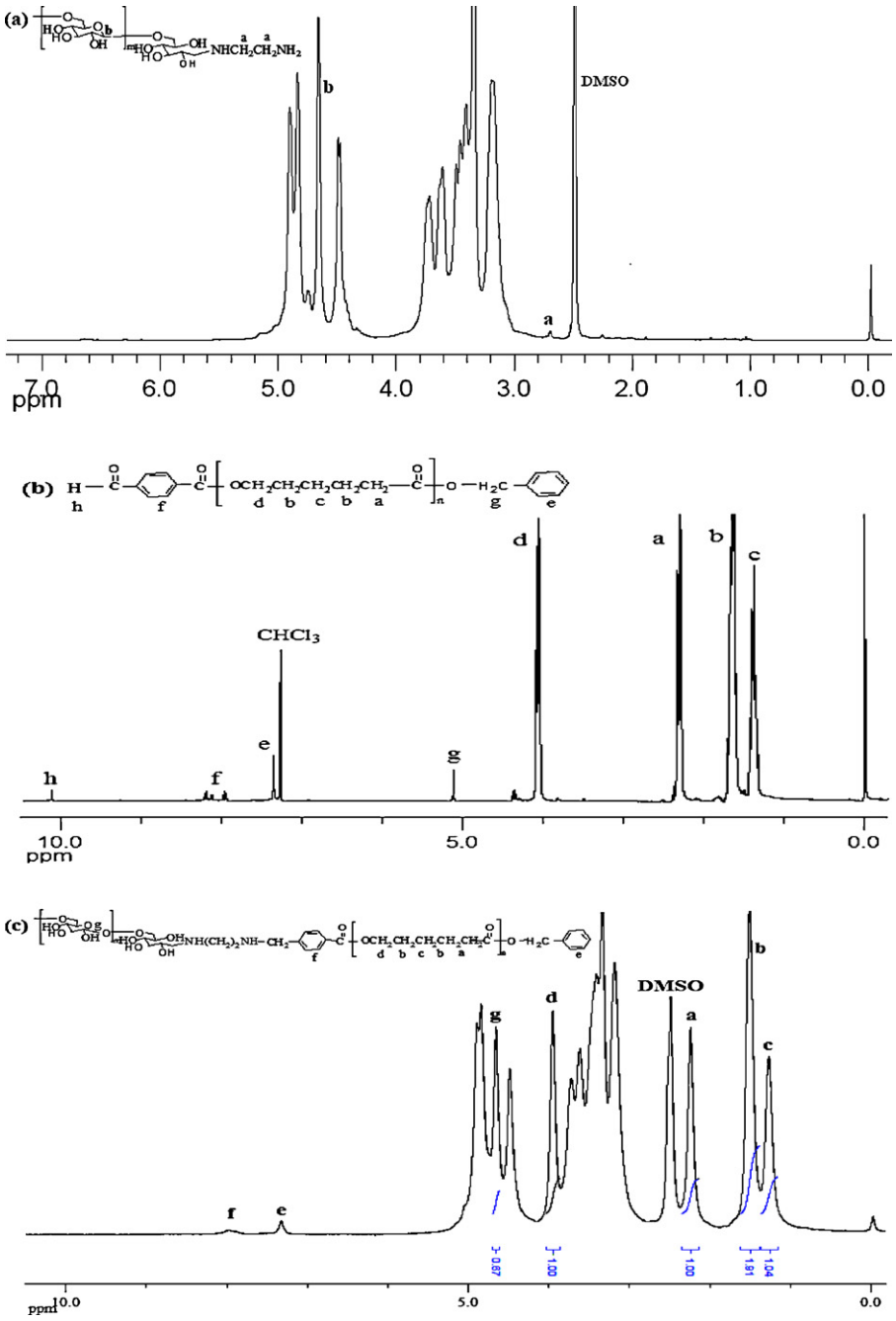


Fig. 1. ^{1}H NMR spectra of Dex-NH_2 (a, in d_6-DMSO), PCL1-CHO (b, in $CDCl_3$) and Dex-b-PCL1 (c, in d_6-DMSO).

LSM 710, Zeiss, Germany) with excitation and emission wavelength of 485 and 595 nm, respectively.

3. Results and discussion

3.1. Synthesis of Dex-b-PCL diblock copolymers

The synthesis procedure of Dex-b-PCL diblock copolymers was illustrated in Scheme 1. First, amino-terminated dextran (Dex-NH_2) was prepared by the amination of the reducing end of dextran using 1,2-ethylenediamine in DMSO. As seen in Fig. 1(a), the signal for C—H proton of N—CH_2CH_2—N at the end of Dex-NH_2 appears at ~2.8 ppm, indicating the successful preparation of Dex-NH_2. The degree of amination of the reducing end of dextran was further determined by ^{1}H NMR analysis. A degree of about 76% was obtained by comparing the integrals of peak **a** and peak **b** (4.65 ppm, anomeric proton) for dextran with M_n of 6000 Da. Using the same method, Yalpani and Brooks (Yalpani & Brooks, 1985) attained a degree of amination of the reducing end of about 60% for dextran with M_n of 10–500 kDa. Here, a higher amination degree should be ascribed to excess 1,2-ethylenediamine used and longer reaction time.

Second, hydroxyl-terminated poly(ε-caprolactone) (PCL-OH) with two different molecular weight was prepared by the ring-opening polymerization of ε-CL and controlling the molar ratio of ε-CL to initiator (Storey & Sherman, 2002). The molecular weight of PCL-OH determined by ^{1}H NMR analysis is 3300 Da and 6316 Da, respectively. The two PCL-OHs were further reacted with 4-formyl-Benzoic acid in the presence of DCC/DMAP (Lele & Leroux, 2002) to produce aldehyde-terminated poly(ε-caprolactone)s (PCL-CHOs).

As seen in Fig. 1(b), signals at 8.2 and 10.1 ppm (**f**, **h**) attributed to the protons of 4-formyl-Benzoic moieties indicate the successful preparation of PCL-CHO. The esterification percentage of the terminal hydroxyl was determined to be 94% by comparing the integrals of peak **g** and peak **h** in Fig. 1(b).

At last, two Dex-b-PCL diblock copolymers were obtained by end-to-end coupling of Dex-NH_2 with the two PCL-CHOs, respectively. That is, Dex-NH_2 reacted with PCL-CHO with molecular weight of 3300 Da and 6310 Da is named as Dex-b-PCL1 and Dex-b-PCL2, respectively. Fig. 1(c) gives the ^{1}H NMR spectrum of Dex-b-PCL1 diblock copolymer. As seen, the signals of both dextran segment and PCL segment appear in the spectrum. Furthermore, by comparing the integrals of peak **a** and peak **g** (4.65 ppm, anomeric proton), an equivalent coupling of Dex-NH_2 and PCL1-CHO to give Dex-b-PCL1 diblock copolymer is confirmed. From the GPC curves shown in Fig. 2, the single peak was observed for two Dex-b-PCL diblock copolymers. These results indicate the successful preparation of Dex-b-PCL diblock copolymer.

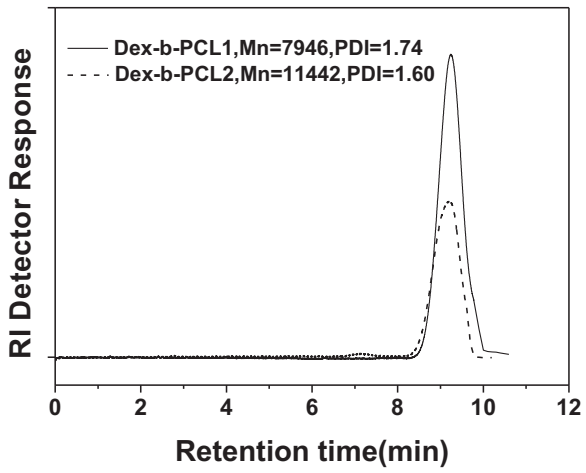


Fig. 2. Gel permeation chromatograms of Dex-b-PCL diblock copolymers.

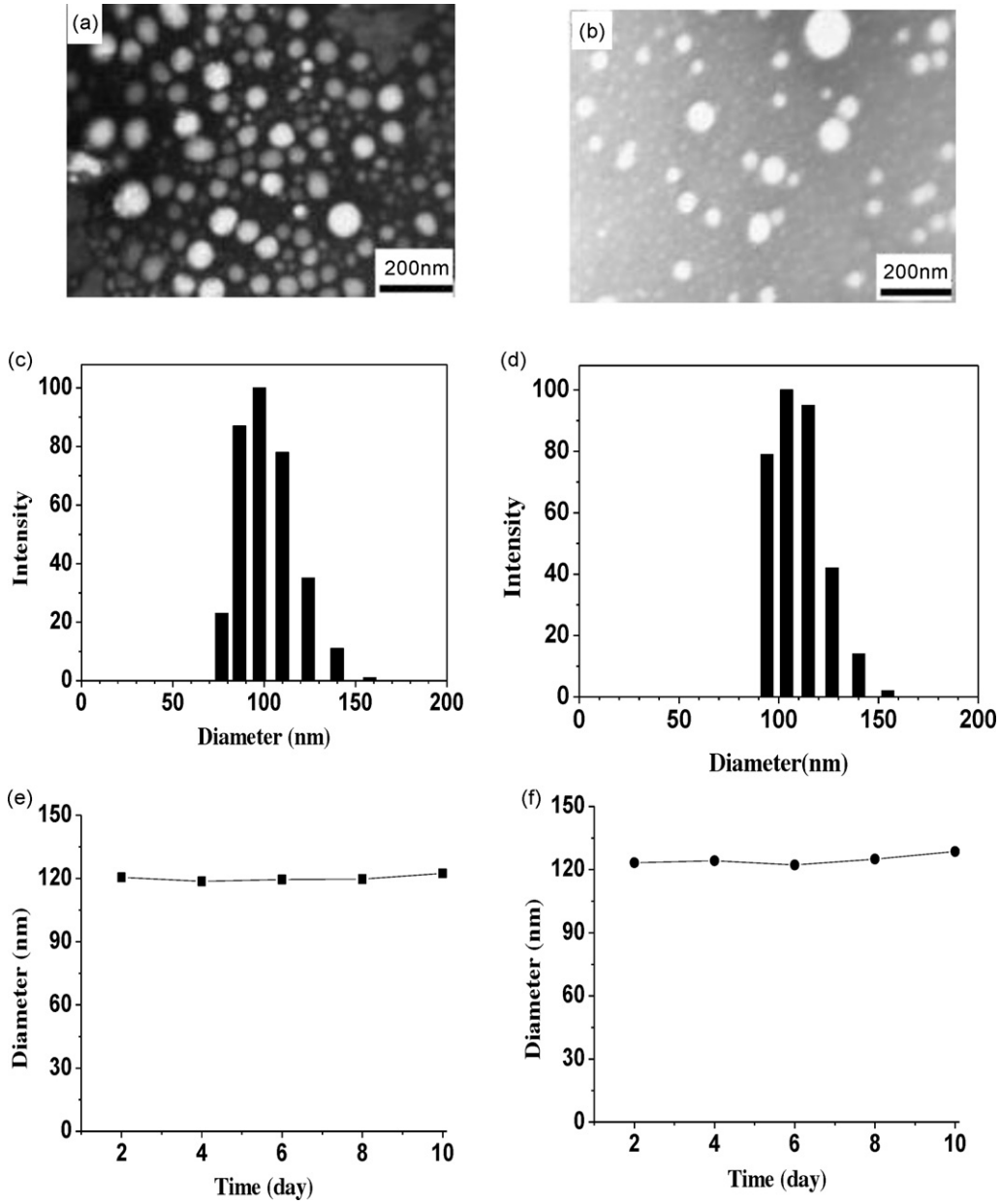


Fig. 3. TEM photos, size distribution graphs measured by DLS and diameter *vs* storage time plots of Dex-b-PCL2 nanoparticles (a, c and e) and DOX-Dex-b-PCL2 nanoparticles (b, d and f).

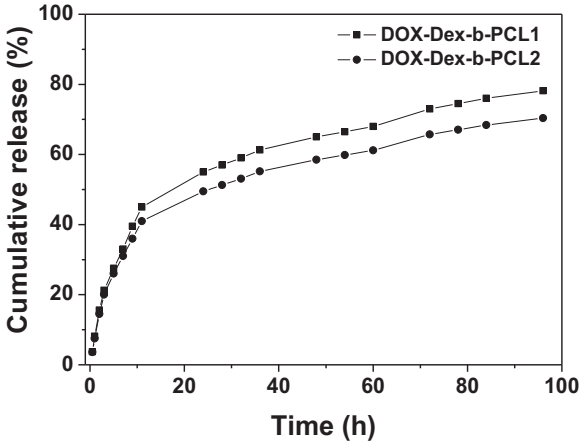


Fig. 4. *In vitro* release profiles of DOX from DOX-Dex-b-PCL nanoparticles at 37 °C.

3.2. Size, morphology and stability of DOX-loaded Dex-b-PCL nanoparticles

DOX-loaded Dex-b-PCL nanoparticles were prepared by a modified nanoprecipitation method. First, DMSO was chosen as a good solvent to dissolve Dex-b-PCL diblock copolymer and DOX. Upon the addition of water to the DMSO solution, rapid precipitation of hydrophobic PCL block and water-insoluble DOX occurs to induce spontaneous formation of DOX-loading Dex-b-PCL nanoparticles. Finally, the DOX-loaded Dex-b-PCL nanoparticle aqueous solution was obtained after dialysis against water and filtration through 0.45 μm syringe filter. Meanwhile, blank Dex-b-PCL nanoparticles were prepared *via* the same method without the use of DOX.

Fig. 3(a)–(d) shows the morphology and size distribution of Dex-b-PCL2 nanoparticles and DOX-loaded Dex-b-PCL2 nanoparticles determined by TEM and DLS. It can be seen that both nanoparticles have a spherical shape and a unimodal size distribution. Moreover, the size distribution does not change much before and after drug loading.

Table 1 summarizes the size and polydispersity index of empty and DOX-loaded Dex-b-PCL nanoparticles. The drug loading content (D.L.) and encapsulation efficiency (E.E.) of DOX-loaded Dex-b-PCL nanoparticles are also listed in Table 1. It is found that the nanoparticles assembled from Dex-b-PCL diblock copolymer with a longer PCL block have a larger hydrodynamic diameter. After drug loading, the particle size does not change much but the polydispersity index increases. The DOX loading content of the two Dex-b-PCL nanoparticles is 8.47% and 10.43%, respectively. Obviously, Dex-b-PCL nanoparticles prepared from the diblock copolymer with a longer PCL block have a higher capability to load drug.

To evaluate the stability of drug loaded and unloaded nanoparticles, the size of the nanoparticles was monitored by DLS as a function of time. As seen in Fig. 3(e)–(f), the particle sizes of Dex-b-PCL2 nanoparticles and DOX-loaded Dex-b-PCL2 nanoparticles keep constant when stored at 25 °C for 10 days, suggesting a good stability of them.

3.3. In vitro release of DOX from Dex-b-PCL nanoparticles

Fig. 4 shows the cumulative *in vitro* release profiles of two DOX-loaded Dex-b-PCL nanoparticles, *i.e.* Dex-b-PCL1 and Dex-b-PCL2 nanoparticles. As seen, the DOX release from two nanoparticles is fast in the first 9 h, with about 40% and 36% DOX release from DOX-Dex-b-PCL1 nanoparticles and DOX-Dex-b-PCL2 nanoparticles, respectively. Then a sustained DOX release is observed up to 96 h for the two DOX-loaded Dex-b-PCL nanoparticles. Moreover,

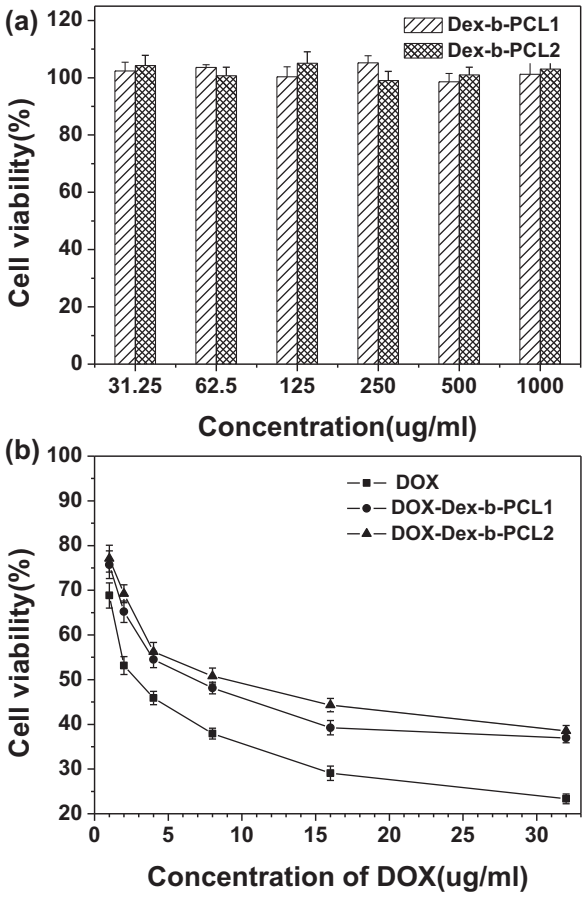


Fig. 5. Viability of SH-SY5Y cells after incubation with Dex-b-PCL nanoparticles (a), DOX·HCl and DOX-Dex-b-PCL nanoparticles (b) for 24 h at different concentration. The results represent the means ± SD ($n = 3$).

it was found that the longer the PCL block in Dex-b-PCL, the slower the drug release rate from DOX-Dex-b-PCL nanoparticles. This may be attributed to a strong hydrophobic interaction between a long PCL block and DOX.

3.4. Cytotoxicity assay

To evaluate the cytotoxicity of DOX loaded Dex-b-PCL nanoparticles, *in vitro* cytotoxicity tests of DOX-loaded Dex-b-PCL nanoparticles and free DOX against human neuroblastoma SH-SY5Y cell line were conducted. To ensure that the cytotoxicity was caused by DOX itself and not by empty Dex-b-PCL nanoparticles, *in vitro* cytotoxicity test of Dex-b-PCL nanoparticles against SH-SY5Y cells was also conducted. Fig. 5(a) reveals the cytotoxicity of empty Dex-b-PCL nanoparticles. It can be seen that empty Dex-b-PCL nanoparticles do not show any cytotoxicity even at higher concentrations, proving that the Dex-b-PCL nanoparticles have good biocompatibility. Fig. 5(b) presents the viability of SH-SY5Y cells after incubation with free DOX, DOX-loaded Dex-b-PCL1 and Dex-b-PCL2 nanoparticles for 24 h at different concentration. When DOX is loaded in Dex-b-PCL nanoparticles, the cell viability decreases from ~80% to ~35% as DOX concentration increases from 1 to 32 μg/mL, indicating that DOX is still pharmacologically active after loading into nanoparticles. On the other hand, the cell viability drops from ~70% to ~25% for free DOX as DOX concentration changes from 1 to 32 μg/mL. Considering that only about 55% of the loaded DOX is released in 24 h as shown in Fig. 4, the real cytotoxicity of DOX-loaded Dex-b-PCL nanoparticles should be higher than that of free DOX. From Fig. 5(b), it also noted that

Table 1
Characteristics of Dex-b-PCL nanoparticles and DOX-Dex-b-PCL nanoparticles.

Nanoparticles	M_n of PCL (Da)	Diameter (nm)	PDI[a]	D.L.[b] (%)	E.E.[c] (%)
Dex-b-PCL1 NPs	3300	89.2 ± 1.4	0.15 ± 0.02	–	–
Dex-b-PCL2 NPs	6316	120.6 ± 0.3	0.12 ± 0.02	–	–
DOX-Dex-b-PCL1 NPs	3300	95.0 ± 3.0	0.27 ± 0.03	8.47	42.4
DOX-Dex-b-PCL2 NPs	6316	123.3 ± 1.5	0.17 ± 0.02	10.43	52.2

[a] PDI = polydispersity index.
[b] D.L. = drug loading content.
[c] E.E. = encapsulation efficiency.

DOX-loaded Dex-b-PCL1 nanoparticles with shorter PCL block show a higher cytotoxicity than DOX-loaded Dex-b-PCL2 nanoparticles with longer PCL block. This may be attributed to a slightly higher drug release speed of DOX-loaded Dex-b-PCL1 nanoparticles.

3.5. Cellular uptake of Dex-b-PCL nanoparticles

Since both Rhodamine B and DOX are fluorescent, cellular uptake of DOX, Rho-labeled Dex-b-PCL nanoparticles and DOX-loaded Dex-b-PCL nanoparticles can be visualized by fluorescence imaging using CLSM. As shown in Fig. 6(a), fluorescence was observed only in nucleus when the cells were incubated with free DOX for 4 h, indicating that free DOX can be rapidly delivered into the nucleus. On the other hand, as shown in Fig. 6(b), the red fluorescence is observed only in the cytoplasm when cells were incubated with Rho-labeled Dex-b-PCL nanoparticles for 4 h, suggesting that Dex-b-PCL nanoparticles can be easily internalized by the cells. When the cells were incubated with DOX-loaded Dex-b-PCL nanoparticles for 4 h, a stronger fluorescence is observed in

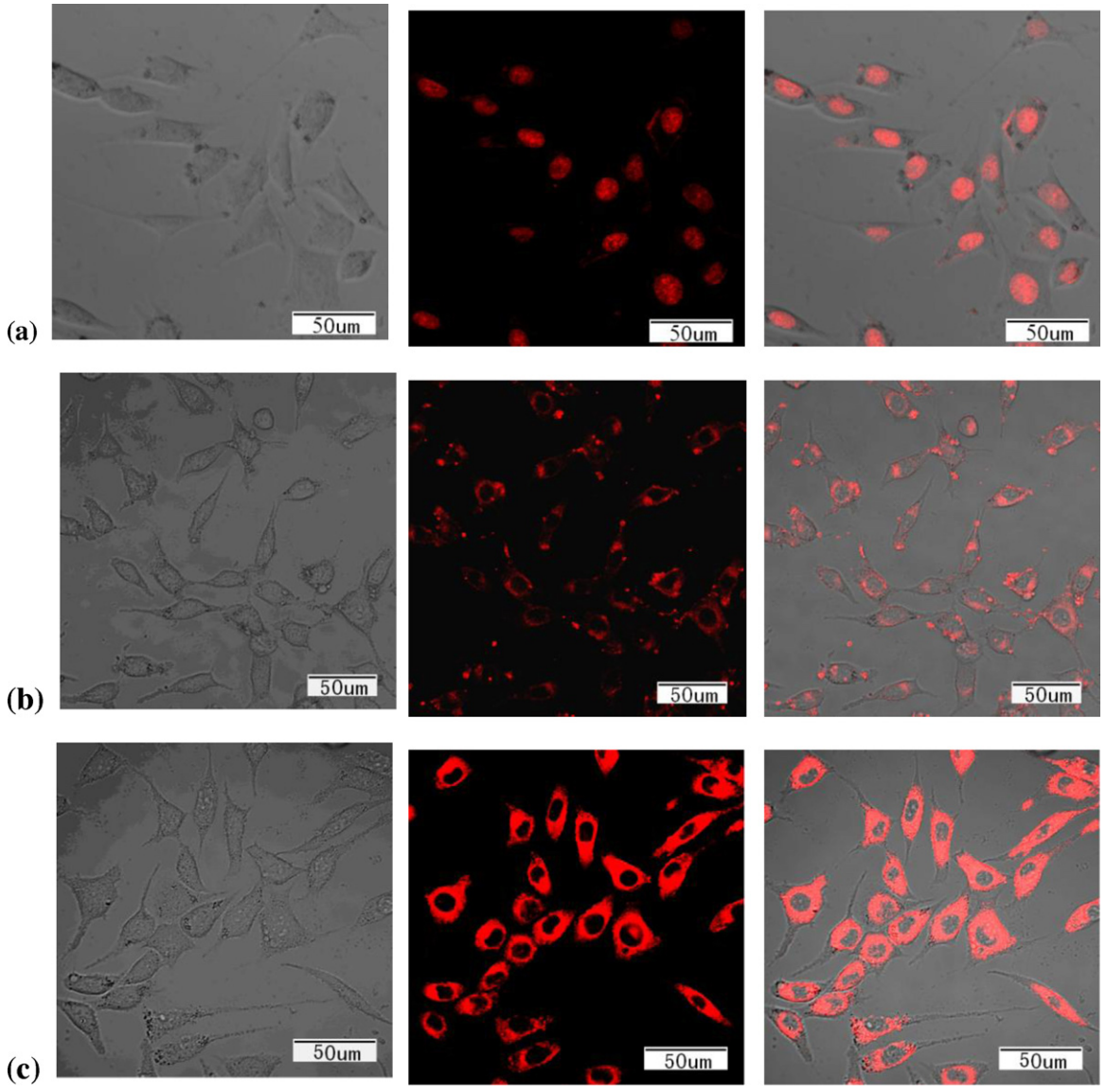


Fig. 6. CLSM images of SH-SY5Y cells incubated with DOX·HCl (a), Rho-labled Dex-b-PCL2 nanoparticles (b) and DOX-Dex-b-PCL2 nanoparticles (c) and cultured for 4 h. For each panel, the images from left to right show bright, fluorescent and merged image, respectively.

cytoplasm as shown in Fig. 6(c). Inside the nucleus, the fluorescence intensity is significantly weaker. This indicates that most of the loaded DOX is not released from Dex-b-PCL nanoparticles in the incubation period and they still stay inside the nanoparticles which are localizes in the cytoplasm.

4. Conclusion

Biocompatible amphiphilic Dex-b-PCL diblock copolymers were successfully synthesized and used to fabricate nanocarriers for DOX, an anticancer drug. The DOX-Dex-b-PCL nanoparticles have a near-spherical shape and a narrow size distribution, with the mean hydrodynamic diameter ranging from 90 to 125 nm. The drug loading content is about 8.47–10.43% and the encapsulation efficiency is about 40–50%. The release of DOX from DOX-Dex-b-PCL nanoparticles showed a sustained release manner with certain amount of burst release in the first 9 h. DOX is still pharmacologically active after drug loading, which was confirmed by *in vitro* cytotoxicity test of DOX-Dex-b-PCL nanoparticles against SH-SY5Y cells. Moreover, DOX-Dex-b-PCL nanoparticles could be easily uptaken by SH-SY5Y cells. The DOX-Dex-b-PCL nanoparticles may be a promising nanocarrier of DOX for intravenous therapy.

Acknowledgments

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