Shell-Sheddable Micelles Based on Dextran-SS-Poly(ε-caprolactone) Diblock Copolymer for Efficient Intracellular Release of Doxorubicin

Huanli Sun,[†] Bingnan Guo,[‡] Xiaoqing Li,[†] Ru Cheng,[†] Fenghua Meng,*[,†] Haiyan Liu,[‡] and Zhiyuan Zhong*[,†]

Biomedical Polymers Laboratory and Key Laboratory of Organic Synthesis of Jiangsu Province, College of Chemistry, Chemical Engineering and Materials Science, Soochow University, Suzhou, 215123, People's Republic of China, and Laboratory of Cellular and Molecular Tumor Immunology, Tang Chung Ying Blood Research Center, Medical College, Soochow University, Suzhou, 215123, People's Republic of China

Received August 28, 2009; Revised Manuscript Received February 27, 2010

Reduction-responsive biodegradable micelles were developed from disulfide-linked dextran-*b*-poly(ε-caprolactone) diblock copolymer (Dex-SS-PCL) and applied for triggered release of doxorubicin (DOX) in vitro and inside cells. Dex-SS-PCL was readily synthesized by thiol-disulfide exchange reaction between dextran orthopyridyl disulfide (Dex-SS-py, 6000 Da) and mercapto PCL (PCL-SH, 3100 Da). Dynamic light scattering (DLS) measurements showed that Dex-SS-PCL yielded micelles with an average size of about 60 nm and a low polydispersity index (PDI 0.1−0.2) in PB (50 mM, pH 7.4). Interestingly, these micelles formed large aggregates rapidly in response to 10 mM dithiothreitol (DTT), most likely due to shedding of the dextran shells through reductive cleavage of the intermediate disulfide bonds. DOX could be efficiently loaded into the micelles with a drug loading efficiency of about 70%. Notably, the in vitro release studies revealed that Dex-SS-PCL micelles released DOX quantitatively in 10 h under a reductive environment, mimicking that of the intracellular compartments such as cytosol and the cell nucleus, whereas only about 27% DOX was released from reduction insensitive Dex-PCL micelles in 11.5 h under otherwise the same conditions and about 20% DOX released from Dex-SS-PCL micelles in 20 h under the nonreductive conditions. The cell experiments using fluorescence microscopy and confocal laser scanning microscopy (CLSM) showed clearly that DOX was rapidly released to the cytoplasm as well as to the cell nucleus. MTT studies revealed a markedly enhanced drug efficacy of DOX-loaded Dex-SS-PCL micelles as compared to DOX-loaded reduction-insensitive Dex-PCL micelles. These reduction-responsive biodegradable micelles have appeared highly promising for the targeted intracellular delivery of hydrophobic chemotherapeutics in cancer therapy.

Introduction

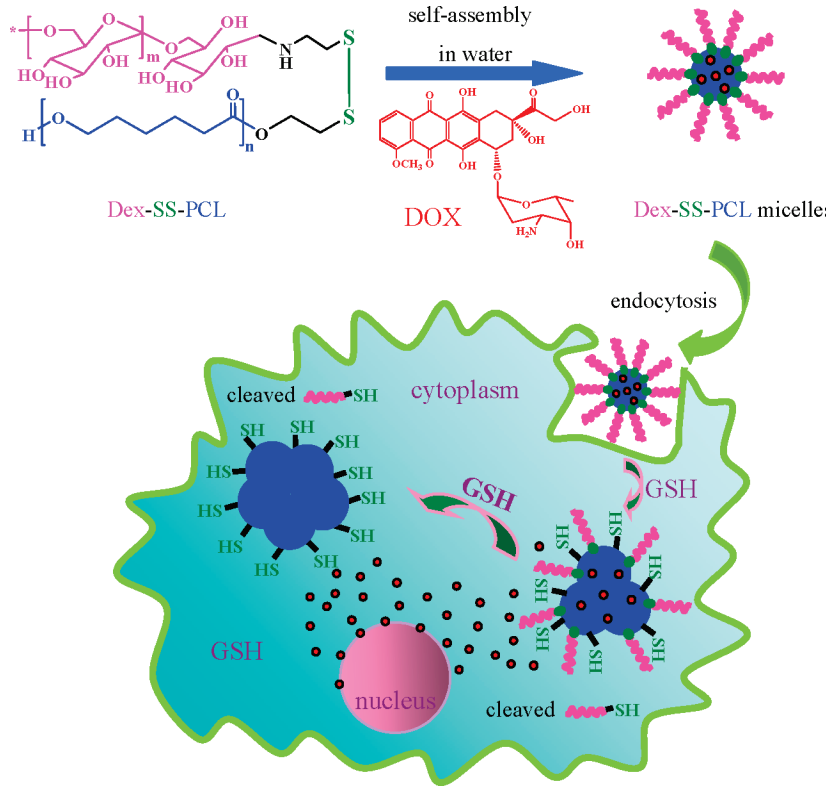
Micelles self-assembled from biocompatible and biodegradable amphiphilic block copolymers have been extensively investigated for the controlled drug delivery applications.[1–6] In particular, block copolymers of poly(ethylene glycol) (PEG) and aliphatic polyesters such as poly(ε-caprolactone) (PCL) and poly(lactic acid) (PLA) have been most frequently employed. These micelles, however, usually show inefficient drug release inside cells due to their gradual biodegradation behavior, which often results in decreased therapeutic efficacy. Interestingly, shedding of hydrophilic shells in response to the intracellular reductive environment has recently reported to achieve efficient release of DOX from micelles inside cells.[7,8] The existence of a large difference in redox potential between the extracellular and the intracellular environments has previously been exploited for a variety of biomedical applications[9] including triggered intracellular release of DNA,[10] siRNA,[11–13] macromolecules,[14] and drugs.[15–18]

Dextran is a natural analog to PEG and has been applied for a range of biomedical applications due to their excellent aqueous solubility, biocompatibility, and nonfouling property.[19–21] For instance, dextran-coated magnetic nanoparticles are conventional magnetic resonance imaging (MRI) contrast agents used in the clinics.[22] Diverse dextran hydrogels and nanoparticles have also been developed for the controlled release of proteins and drugs, respectively.[23–25] In contrast to PEG, dextran contains about 5% branching structure and abundant functional hydroxyl groups along the chain that on one hand warrant superb aqueous solubility and on the other hand facilitate chemical modifications. Dai et al. reported that dextran-coated nanomaterials show superior colloidal stability and improved photophysical properties compared with PEG-coated counterparts.[26] Furthermore, the hydroxyl groups of the dextran shell could be exploited for versatile conjugation of targeting ligands such as RGD peptide,[27] mannose,[28] and galactose[29] to yield targeted drug delivery systems. To our surprise, despite its demonstrated advantages, dextran-based block copolymers and their self-assembling behaviors have not been studied much. Bosker[30] and Gnanou[31] prepared dextran-*b*-polystyrene block copolymers via coupling and atom transfer radical polymerization (ATRP), respectively. Depending on compositions, micelles, and polymersomes could be obtained from dextran-*b*-polystyrene block copolymers in water.[32] Zhang and Liu reported formation of spherical micelles from biodegradable Dex-PCL diblock copolymer.[33] Very recently, Schatz and co-workers prepared dextran-poly(γ-benzyl

* To whom correspondence should be addressed. Tel./Fax: +86-512-65880098. E-mail: zyzhong@suda.edu.cn (Z.Z.); fhmeng@suda.edu.cn (F.M.).
[†] College of Chemistry, Soochow University.
[‡] Medical College, Soochow University.

10.1021/bm1001069
Published on Web 03/08/2010

Scheme 1. Reduction-Responsive Biocompatible and Biodegradable Micelles of Dex-SS-PCL Block Copolymer for Efficient Triggered Intracellular Release of DOX[a]



[a] DOX-loaded Dex-SS-PCL micelles are readily prepared with high drug loading efficiency; Following endocytosis, dextran shells are shed off due to cleavage of the intermediate disulfide bond triggered by glutathione (GSH) tripeptide, which results in fast destabilization of micelles and quantitative release of DOX in the cytosol and into the cell nucleus.

L-glutamate) block copolymer by click chemistry and studied its self-assembly into polymersomes.[34]

In this paper, we report on shell-sheddable biodegradable micelles based on Dex-SS-PCL for efficient intracellular release of DOX (Scheme 1). The effective shedding of the dextran shells and triggered release of DOX in response to 10 mM DTT, analogous to the intracellular redox potential, were demonstrated. The intracellular release of DOX was studied with mouse leukemic monocyte macrophage cell line (RAW 264.7) using fluorescence microscope as well as confocal laser scanning microscope (CLSM). This novel type of biodegradable micelles is highly promising for the targeted intracellular delivery of anticancer drugs.

Experimental Section

Materials. Stannous octoate (95%, Sigma), phosphotungstic acid sodium salt (97%, Sigma), 2,2′-dithiodiethanol (98%, ABCR), sodium cyanoborohydride ($NaCNBH_3$, 99.5%, Alfa Aesar), 2,2′-dithiodipyridine (99%, Fluka), cysteamine (95%, J&K), 1,4-dithio-DL-threitol (DTT, 99%, Merck), 5,5′-dithiobis(2-nitrobenzonic acid) (DTNB, 99%, Alfa Aesar), and doxorubicin hydrochloride (99%, Beijing ZhongShuo Pharmaceutical Technology Development Co.,Ltd., PRC) were used as received. The dextran purchased from Fluka had an M_n of 6000, which was confirmed by Ellman test of dextran-SH derivative. ε-Caprolactone (ε-CL, 99%, Alfa Aesar), dimethyl sulfoxide (DMSO), and *N*,*N*-dimethyl formamide (DMF) were dried over CaH_2 and distilled prior to use. Toluene and tetrahydrofuran (THF) were dried by refluxing over sodium wire for 24 h and distilled prior to use. PCL-SH (M_n = 3100 as determined by ^{1}H NMR end group analysis, PDI = 1.30) was synthesized as previously reported.[7]

Synthesis of Dex-SH. Under an argon atmosphere, to a stirred solution of dextran (6.015 g, 1 mmol) and cysteamine (0.773 g, 1.04 mmol) in 100 mL of DMSO/H_2O (3/1 v/v) was added $NaCNBH_3$ (55 mg, 0.88 mmol). The reaction proceeded at 60 °C for 2 d and 40 °C for 2 d. The solution color changed from orange to brown. After removing water by rotary evaporator, polymer was isolated by precipitation in 20-fold cold diethyl ether, filtered, and dried in vacuo. Yield: 87%. Under an argon atmosphere, to the aqueous solution (60 mL) of above product (Dex-SS-Dex, 5.878 g) was added DTT (1.234 g, 8.0 mmol). After a 2 d reaction, the product was recovered by ultrafiltration (MWCO 1000 MW) under a nitrogen atmosphere for 12 h (water was changed three times), followed by freeze-drying. The thiol functionality in Dex-SH was quantified using Ellman test.

Synthesis of Dex-SS-Py. Under an argon atmosphere, Dex-SH (1.238 g, 0.21 mmol), 2,2′-dithiodipyridine (Py-SS-Py, 0.091 g, 0.42 mmol), and 80 mL of Milli Q water were charged into a three-necked bottle. The solution pH was adjusted to 2.0 using 1.0 M HCl to render Py-SS-Py soluble in water and the reaction was allowed to proceed at rt for 24 h. Then, the solution pH was adjusted to 7.0 using 1.0 M NaOH, and the solution was filtered to remove excess Py-SS-Py. The product was isolated by ultrafiltration (MWCO 1000 MW) for 12 h (water was changed three times), followed by lyophilization. Yield: 52%. ^{1}H NMR (D_2O): δ 3.22−4.06 and 4.99 (dex), 7.36, 7.89, and 8.46 (pyridine aromatic protons). ^{1}H NMR revealed a functionality of about 85%.

Synthesis of Dex-SS-PCL. Under an argon atmosphere at 40 °C, to a DMF solution (30 mL, 10% LiCl) of Dex-SS-Py (0.6871 g, 0.11 mmol) was added acetic acid (0.4 mL), followed by addition of PCL-SH (0.332 g, 0.12 mmol) in DMF. The reaction was allowed to proceed under stirring for 24 h. The resulting Dex-SS-PCL conjugate was isolated by precipitation in cold ethanol, filtration, extensive washing with water and acetone, and drying in vacuo. Yield: 49%. ^{1}H NMR (D_2O/DMSO-d_6 1/1): δ 3.30−3.78 and 4.73 (dex), 3.84, 2.11, 1.42, and 1.17 (PCL).

Characterization. The ^{1}H NMR spectra were recorded on a Unity Inova 400 spectrometer operating at 400 MHz using $CDCl_3$, D_2O or D_2O/DMSO-d_6 (v/v 1/1) as solvents. The chemical shifts were calibrated

against residual solvent signals of $CDCl_3$ or D_2O. The molecular weight and polydispersity of the copolymers were determined by a Waters 1515 gel permeation chromatograph (GPC) instrument equipped with two linear PLgel columns (500 Å and Mixed-C) following a guard column and a differential refractive-index detector. The measurements were performed using THF as the eluent at a flow rate of 1.0 mL/min at 30 °C and a series of narrow polystyrene standards for the calibration of the columns. The size of micelles was determined using dynamic light scattering (DLS). Measurements were carried out at 25 °C using Zetasizer Nano-ZS from Malvern Instruments equipped with a 633 nm He−Ne laser using backscattering detection. Transmission electron microscopy (TEM) was performed using a Tecnai G220 TEM operated at an accelerating voltage of 200 kV. The samples were prepared by dropping 10 μL of 0.1 mg/mL micellar solution on the copper grid followed by staining with phosphotungstic acid.

Micelle Formation and Critical Micelle Concentration (CMC). Typically, micelles of Dex-SS-PCL were prepared under stirring by dropwise addition of 1.0 mL PB (50 mM, pH 7.4) to 1.0 mL of block copolymer solution (0.1 wt %) in DMF/LiCl at 45 °C followed by extensive dialysis against PB for 24 h.

The critical micelle concentration (CMC) was determined using pyrene as a fluorescence probe. The concentration of block copolymer was varied from 6.0×10^{-4} to 0.15 mg/mL and the concentration of pyrene was fixed at 0.6 μM. The fluorescence spectra were recorded using FLS920 fluorescence spectrometer with the excitation wavelength of 330 nm. The emission fluorescence at 372 and 383 nm were monitored. The CMC was estimated as the cross-point when extrapolating the intensity ratio I_{372}/I_{383} at low and high concentration regions.

Reduction-Triggered Destabilization of Dex-SS-PCL Micelles. The size change of micelles in response to 10 mM DTT in PB buffer (50 mM, pH 7.4) was followed by DLS measurement. Briefly, to 1.5 mL of solution of Dex-SS-PCL micelles in PB (50 mM, pH 7.4), previously degassed with nitrogen for 20 min, was added 10 mM DTT. The solution was placed in a shaking bed at 37 °C with a rotation speed of 200 rpm. At different time intervals, the size was determined using DLS.

Loading and Reduction-Triggered Release of DOX from Dex-SS-PCL Micelles. DOX was loaded into micelles by dropwise addition of 0.8 mL PB (50 mM, pH 7.4) to a mixture of 0.8 mL Dex-SS-PCL or Dex-PCL copolymer solution in DMF (5 mg/mL) and 80 μL DOX solution in DMSO (5 mg/mL) under stirring at 45 °C, followed by dialysis against PB (50 mM, pH 7.4) for 24 h at rt (MWCO 3500). The dialysis medium was changed five times. The whole procedure was performed in the dark. The amount of DOX was determined using fluorescence (FLS920) measurement (excitation at 480 nm and emission at 555 nm). For determination of drug loading content, lyophilized DOX-loaded micelles were dissolved in DMSO and analyzed with fluorescence spectroscopy, wherein calibration curve was obtained with DOX/DMSO solutions with different DOX concentrations.

Drug loading content (DLC) and drug loading efficiency (DLE) were calculated according to the following formula:

$$\text{DLC(wt\%)} = \text{(weight of loaded drug/weight of polymer)} \times 100\%$$

$$\text{DLE(\%)} = \text{(weight of loaded drug/weight in feed)} \times 100\%$$

The release profiles of DOX from Dex-SS-PCL micelles were studied using a dialysis tube (MWCO 12000) at 37 °C in two different media, that is, PB (50 mM, pH 7.4) with 10 mM DTT or PB (50 mM, pH 7.4) only. Release of DOX from Dex-PCL micelles (reduction insensitive control) was carried out in PB (50 mM, pH 7.4) with 10 mM DTT. To acquire sink conditions, drug release studies were performed at low drug loading contents (ca. 1.0 wt %) and with 0.7 mL of micelle solution dialysis against 20 mL of the same medium. At desired time intervals, 6 mL of release media was taken out and replenished with an equal volume of fresh media. The amount of DOX released was determined by using fluorescence (FLS920) measurement (excitation at 480 nm). The release experiments were conducted in triplicate. The results presented are the average data with standard deviations.

Intracellular Release of DOX. The cellular uptake and intracellular release behaviors of DOX-loaded Dex-SS-PCL micelles were followed with fluorescence microscopy and CLSM using mouse leukemic monocyte macrophage cell line (RAW 264.7). RAW 264.7 cells were cultured in a disk containing 2 mL of DMEM media for 2 d to ~70% confluency (~2×10^5 cells/disk), 100 μL of PB (50 mM, pH 7.4) solution of DOX-loaded Dex-SS-PCL micelles or free DOX (25 μg) was added. The cells were incubated with DOX-loaded micelles or free DOX for 2, 4, or 24 h at 37 °C in a humidified 5% CO_2-containing atmosphere. The culture media were removed and the cells were rinsed two times with PBS prior to the fluorescence observation.

For CLSM observations, the cells were fixed and the cell nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI, blue). CLSM images of cells were obtained using confocal microscope (TCS SP2).

Cell Viability Assay. RAW 264.7 cells were plated in a 96-well plate (1×10^4 cells/well) using DMEM medium. The cells were incubated with DOX-loaded Dex-SS-PCL micelles, DOX-loaded Dex-PCL micelles, free DOX (25 μg/mL), or blank Dex-SS-PCL micelles for 48 h at 37 °C in a humidified 5% CO_2-containing atmosphere. The media was aspirated, and then 150 μL of a stock solution containing 0.1 mg of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) in PBS was added and incubated for another 4 h. The PBS was aspirated, the MTT-formazan generated by live cells was dissolved in 150 μL of DMSO, and the absorbance at a wavelength of 490 nm of each well was measured using a microplate reader. The relative cell viability (%) was determined by comparing the absorbance at 490 nm with control wells containing only cell culture medium. Data are presented as average ± SD ($n = 6$).

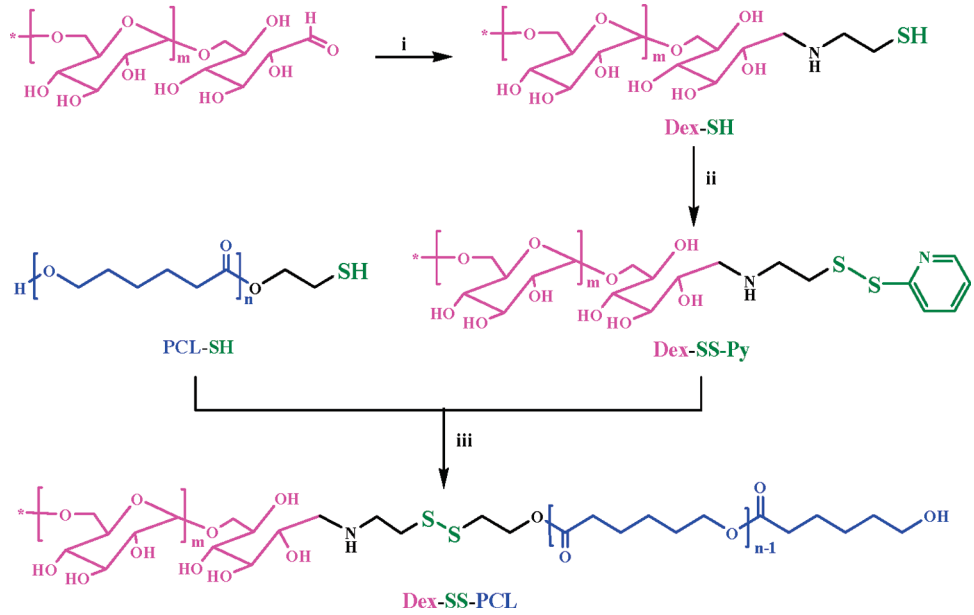
Results and Discussion

Synthesis of Dex-SS-PCL Diblock Copolymer. Dex-SS-PCL was prepared via exchange reaction between dextran orthopyridyl disulfide (Dex-SS-Py) and mercapto PCL (PCL-SH; Scheme 2). Dex-SS-Py was conveniently obtained in two steps: (i) the reductive amination reaction between the terminal aldehyde of dextran (6000 Da) and cysteamine in the presence of $NaCNBH_3$ yielded, after reduction with DTT, Dex-SH with nearly 100% thiol functionality, as revealed by Ellman test; and (ii) the exchange of Dex-SH with 2,2′-dithiodipyridine at pH 2 in water gave rise to Dex-SS-Py. ^{1}H NMR showed a dithiopyridine functionality of about 85% (Supporting Information, Figure S2).

The exchange reaction between Dex-SS-Py and PCL-SH ($M_n = 3100$, PDI = 1.30) was performed in DMF using a PCL-SH/Dex-SS-Py molar ratio of 1.1/1. The resulting Dex-SS-PCL block copolymer was purified by washing once with water and three times with acetone to remove free dextran and PCL if present. In a control experiment on a mixture of dextran and PCL, this washing procedure was found sufficient to remove the nonconjugated dextran and PCL. As shown in Figure 1, peaks assignable to both dextran (δ 3.30−3.78 and 4.73) and PCL (δ 3.84, 2.11, 1.42, and 1.17) were present in the ^{1}H NMR spectrum. Importantly, comparing the integrals of signals at δ 4.73 and 2.11 pointed to an equivalent coupling of dextran and PCL. These results supported successful synthesis of Dex-SS-PCL diblock copolymer. For comparison, reduction insensitive Dex-PCL block copolymer with a similar composition was also prepared (Supporting Information, Figure S3). We were not able to characterize Dex-SS-PCL copolymer using GPC due to lack of good solvents.

Micelle Formation and Reduction-Responsive Destabilization. Micelles of Dex-SS-PCL block copolymer were prepared by solvent exchange method. DLS measurements showed an

Scheme 2. Synthetic Pathway for Dex-SS-PCL. [a]



[a] Reagents and conditions: (i) cysteamine, $NaCNBH_3$, DMSO/H_2O (v/v 3/1), 60 °C for 2 d and 40 °C for 2 d; treatment with 130 mM DTT in water for 2 d; (ii) Py-SS-Py, pH 2.0, H_2O, rt, 24 h; (iii) DMF/LiCl, acetic acid, 40 °C, 24 h.

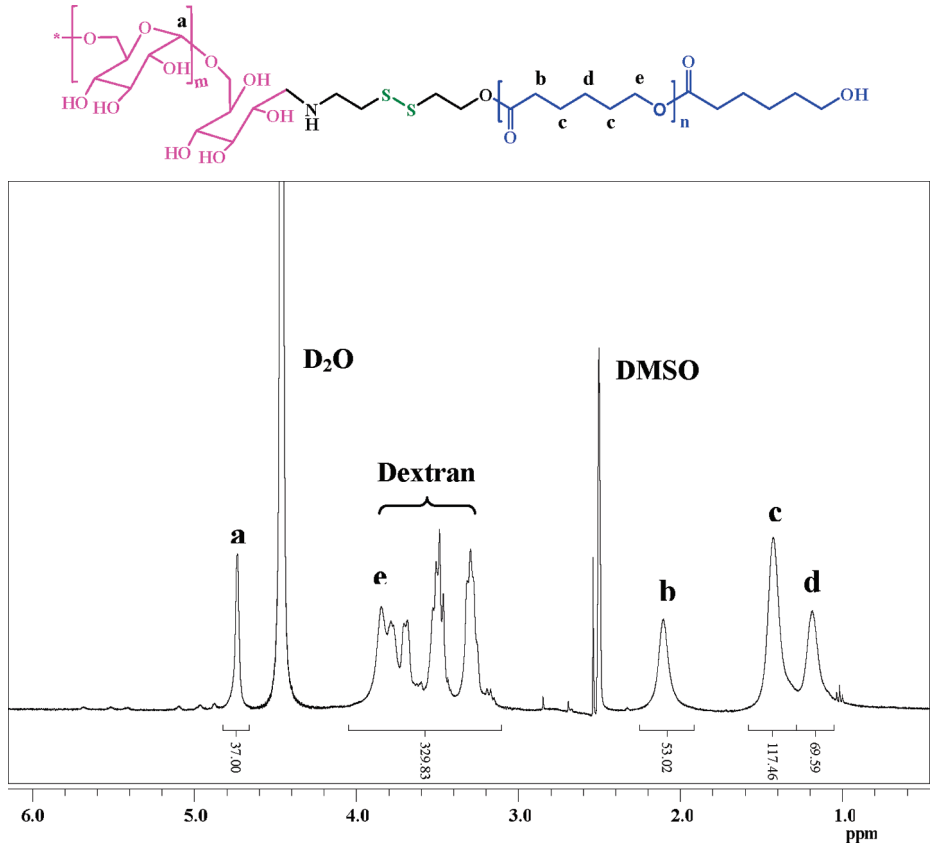


Figure 1. ^1H NMR spectrum (400 MHz, DMSO-d_6/D_2O (1/1 v/v) of Dex-SS-PCL block copolymer.

average micelle size of 60 nm with a narrow size distribution (PDI = 0.12; Figure 2A). The critical micelle concentration (CMC), determined using pyrene as a probe, was estimated to be approximately 9.3 mg/L (Figure 2B).

The size change of micelles in response to 10 mM DTT in PB (50 mM, pH 7.4) was studied using DLS. Notably, at 0.5 h following addition of 10 mM DTT, average micelle size increased from 60 nm to about 200 nm with concomitant increase of PDI from 0.15 to 0.6 (Figure 3). Large aggregates with sizes of over 1000 nm were also observed. The aggregates were formed due to most probably reductive cleavage of the intermediate disulfide bonds, which results in shedding of the dextran shells (Scheme 1). In contrast, little change in micelle sizes was discerned after 24 h in the absence of DTT under otherwise the same conditions.

Loading and Triggered Release of DOX. DOX is one of the most potent anticancer drugs used widely in the treatment of different types of solid malignant tumors.[35–37] DOX is known

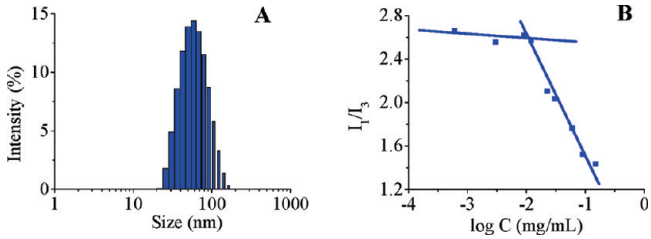


Figure 2. Size distribution of Dex-SS-PCL micelles (1.0 mg/mL) in PB (50 mM, pH 7.4) determined by DLS (A) and the fluorescence intensity ratio I_1/I_3 of pyrene as a function of Dex-SS-PCL concentration (B). The critical micelle concentration (CMC) in aqueous medium is determined to be about 9.3×10^{-3} mg/mL.

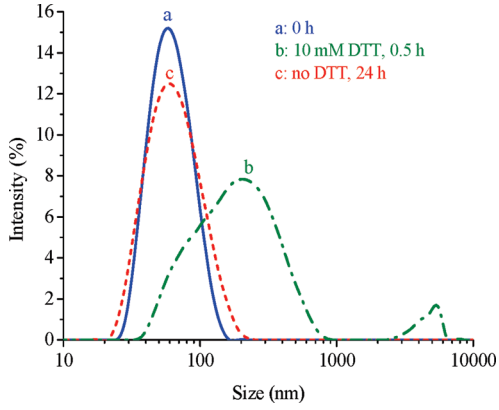


Figure 3. Size change of Dex-SS-PCL micelles in response to 10 mM DTT in PB buffer (50 mM, pH 7.4) determined by DLS measurements.

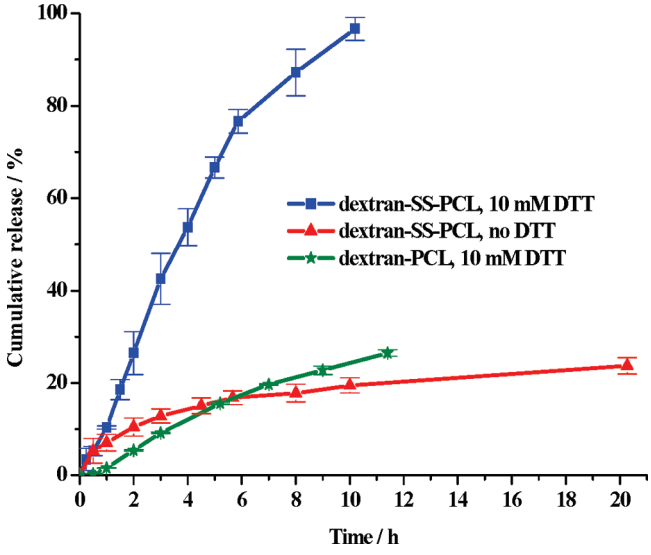


Figure 4. Reduction-triggered drug release from DOX-loaded Dex-SS-PCL micelles in PB (50 mM, pH 7.4). Dex-PCL micelles were used as a reduction insensitive control.

to interact with DNA by intercalation and inhibition of macromolecular biosynthesis.[38] It is crucial, therefore, to deliver and release DOX in the cytoplasm and right into the cell nucleus. The aim of this study was to develop triggered intracellular delivery systems for DOX, which may lead to enhanced cancer chemotherapy.

DOX was loaded into micelles by dialysis of a polymer/DOX solution in DMF against PB buffer. The theoretical drug loading content was set at 10 wt %. The results showed that Dex-SS-PCL micelles encapsulated DOX efficiently, affording a drug loading efficiency of about 70%. Notably, after loading of DOX,

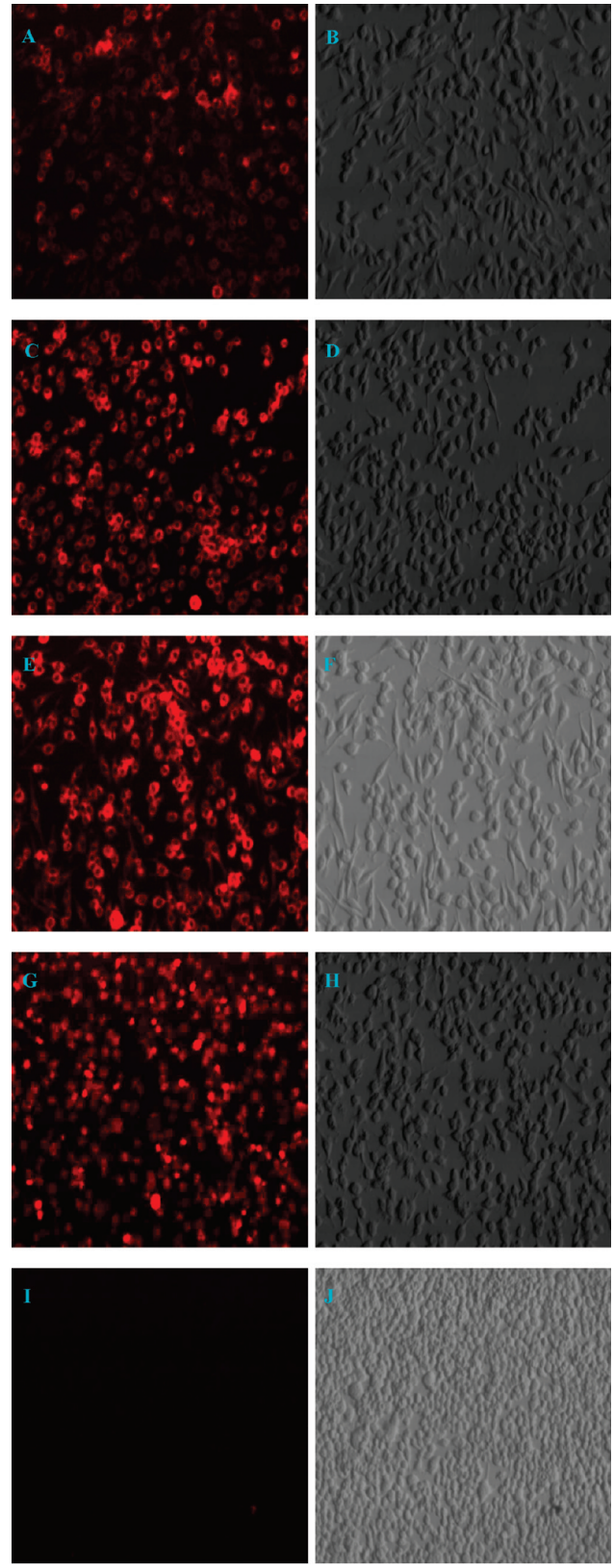


Figure 5. Cell uptake and intracellular DOX release from the reduction-sensitive shell-sheddable DOX-loaded Dex-SS-PCL micelles (12.5 μg/mL DOX) followed by fluorescence microscopy using macrophage RAW 264.7 cells. For each panel, left and right images show DOX fluorescence in cells (red) and white light image of cells, respectively. (A,B) DOX-loaded Dex-SS-PCL micelles, 2 h incubation; (C,D) DOX-loaded Dex-SS-PCL micelles, 4 h incubation; (E,F) DOX-loaded Dex-SS-PCL micelles, 24 h incubation; (G,H) free DOX, 4 h incubation; and (I,J) cells cultured for 24 h without DOX-loaded micelles (blank control). All pictures are in $\times$20 magnification.

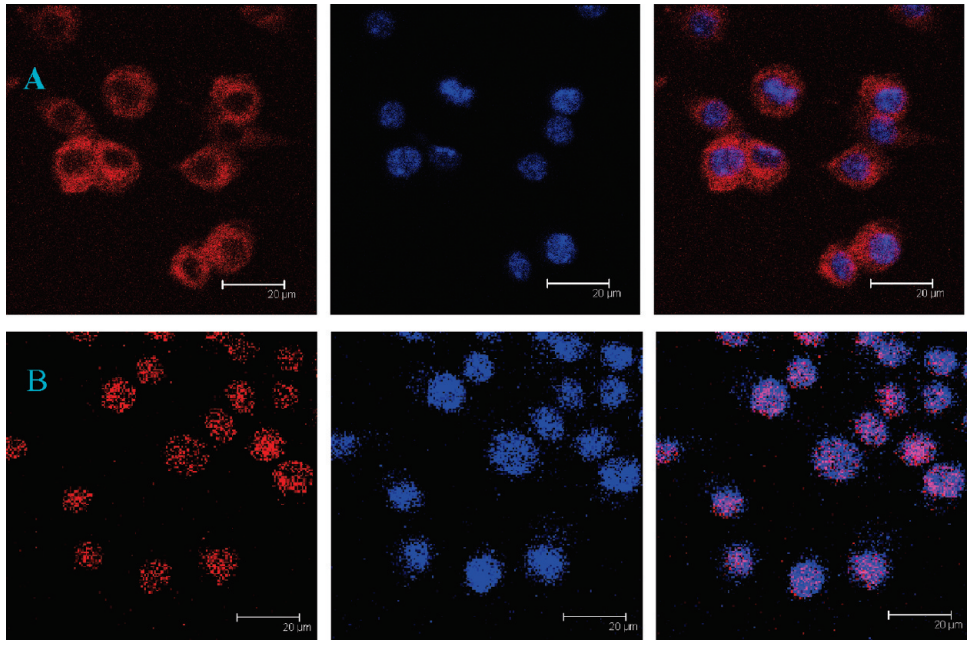


Figure 6. CLSM images of intracellular DOX release from the reduction-sensitive DOX-loaded Dex-SS-PCL micelles using macrophage RAW 264.7 cells after a 4 h incubation. For each panel, images from left to right show DOX fluorescence in cells (red), cell nuclei stained by DAPI (blue), and overlays of two images. The bar represents 20 μm. (A) DOX-loaded Dex-SS-PCL micelles; (B) free DOX control (12.5 μg/mL DOX).

micelle size increased slightly (~10 nm) to an average size of about 80 nm, while PDI remained low. Under the same conditions, a drug loading efficiency of about 55% was obtained for Dex-PCL micelles.

The release studies showed that in the absence of DTT, minimal drug release (<20%) was observed within 20 h for Dex-SS-PCL micelles (Figure 4). However, in the presence of 10 mM DTT, a reductive environment analogous to that of the intracellular compartments, such as cytosol and the cell nucleus, Dex-SS-PCL micelles released DOX rapidly and quantitatively, in which 55% DOX was released in 4 h and virtually quantitative drug release was observed in 10 h. In contrast, only about 27% DOX was released from reduction insensitive Dex-PCL micelles in 11.5 h under otherwise the same conditions (Figure 4). It has been shown that Dex-SS-PCL micelles are destabilized and form aggregates quickly in response to 10 mM DTT (Figure 3). The fast drug release from Dex-SS-PCL micelles under a reductive condition is most likely due to extrusion of drugs during restructuring of micelles. It should be further noted that no burst release was observed and DOX was released from Dex-SS-PCL micelles in a zero order manner up to 75% release. This constant release rate indicates that release of DOX is controlled most likely by a combination of diffusion and degradation. We are convinced that shedding of dextran shells from biodegradable micelles is a highly promising approach to achieve fast yet controlled drug release.

Intracellular Release of DOX. The cellular uptake and intracellular release behaviors of DOX-loaded Dex-SS-PCL micelles were followed with fluorescence microscopy and CLSM using mouse leukemic monocyte macrophage cell line (RAW 264.7). After RAW cells were cultured in a disk to ~70% confluency (~2×10^5 cells/disk), 100 μL of PB solution of DOX-loaded micelles or free DOX (12.5 μg/mL DOX) was added. After 2, 4, and 24 h incubation, the culture medium was removed and the cells were rinsed two times with PBS prior to the fluorescence assessment. Remarkably, results showed strong DOX fluorescence in the cells after just 2 h incubation with DOX-loaded Dex-SS-PCL micelles (Figure 5A), indicating fast internalization of micelles and rapid release of DOX inside cells. This is in accordance with our expectation that disulfide bonds are cleaved in the intracellular compartments such as the cytosol and the cell nucleus due to presence of comparatively high concentrations of reducing glutathione tripeptides (2−10 mM).[9,39,40] It has been reported that that micelles are able to escape from endosomes and transport to cytoplasmic organelles.[41] The fluorescence intensity of DOX inside cells increased further when increasing the incubation time to 4 and 24 h (Figure 5C,E). In contrast, very weak fluorescence of DOX was observed for cells treated with DOX-loaded Dex-PCL micelles for 4 h (Supporting Information, Figure S4). We and others have shown that due to the self-quenching effect of DOX in nanoparticles fluorescence is observed only when DOX is released.[42,43] It is clear from these fluorescence images that DOX has been efficiently released from Dex-SS-PCL micelles to cytosol. Notably, CLSM studies showed that after 4 h incubation, part of DOX has also been transported into the cell nucleus (Figure 6A). In comparison, free DOX was mainly accumulated in cell nucleus (Figure 6B). It should be further noted that after a 24 h incubation, a much smaller number of viable cells (ca. 27%) were observed for DOX-loaded Dex-SS-PCL micelles as compared to the control wells (Figure 5F vs J), indicating a high drug efficacy for shell-sheddable micelles. MTT assays using HepG2 and RAW 264.7 cells revealed that these Dex-SS-PCL micelles were practically nontoxic up to a tested concentration of 3.0 mg/mL. Interestingly, DOX-loaded Dex-SS-PCL micelles showed markedly enhanced drug efficacy as compared to DOX-loaded Dex-PCL micelles, in which cell viabilities of about 20 and 70% were observed for cells treated after 2 d with DOX-loaded Dex-SS-PCL micelles and DOX-loaded Dex-PCL micelles, respectively (Figure 7).

Conclusions

We have demonstrated that shell-sheddable biodegradable micelles based on Dex-SS-PCL diblock copolymer are nontoxic, display a high drug loading efficiency for DOX, are stable, and

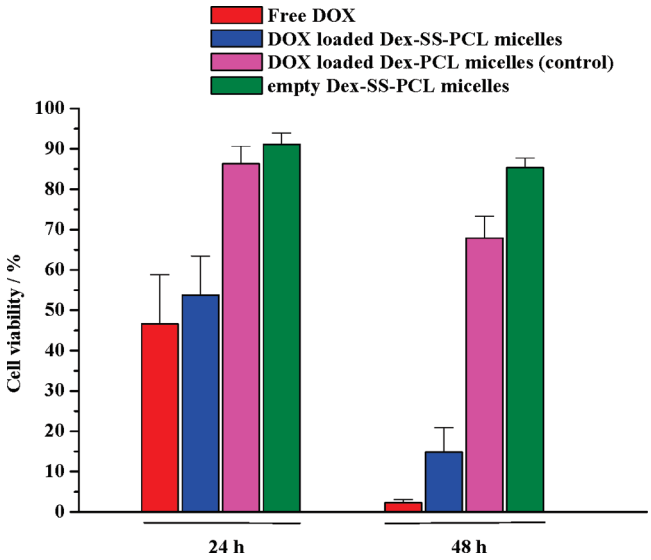


Figure 7. Toxicity of DOX-loaded Dex-SS-PCL micelles vs DOX-loaded Dex-PCL micelles, free DOX, and empty Dex-SS-PCL micelles in RAW 264.7 cells. DOX dosage was 25 μg/mL. The cells were incubated for 24 or 48 h. Data are presented as the average $\pm$ standard deviation ($n = 6$).

show minimal drug release under a nonreductive environment (e.g., extracellular settings), while release drugs rapidly and quantitatively in response to the intracellular level of reducing potential. It is evident from cell experiments that DOX is delivered and released to the cytoplasm as well as to the cell nucleus, achieving high drug efficacy. We are convinced that reduction-responsive shell-sheddable biodegradable micelles hold great promise for efficient cytoplasmic delivery and release of potent hydrophobic anticancer drugs, affording enhanced cancer chemotherapy.

Acknowledgment. This work was financially supported by research grants from the National Natural Science Foundation of China (NSFC 50703028, 20874070, 50973078, and 20974073), the Natural Science Foundation of the Jiangsu Higher Education Institutions of China (Grant No. 08KJB150016), and the Program of Innovative Research Team of Soochow University.

Supporting Information Available. Synthesis and characterization of Dex-PCL copolymer, ^{1}H NMR spectra of PCL-SH and PCL-SS-Py, and fluorescence images of macrophage RAW 264.7 cells incubated for 4 h with DOX-loaded Dex-SS-PCL micelles and DOX-loaded Dex-PCL micelles. This material is available free of charge via the Internet at http://pubs.acs.org.

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BM1001069