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Intracellular pH-Sensitive PEG-*block*-Acetalated-Dextrans as Efficient Drug Delivery Platforms

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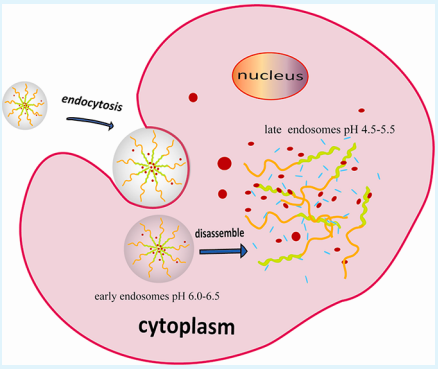
Supporting Information

ABSTRACT: Intracellular pH-sensitive micelles of PEG-*block*-acetalated-dextran (PEG-*b*-AC-Dex) were prepared and used for acid-triggered intracellular release of anticancer drug. The hydrodynamic radii (R_h) of PEG-*b*-AC-Dex micelles could increase after incubation in PBS solution at pH 5.5. Based on the pH-responsive R_h variation behavior, it was expected that the PEG-*b*-AC-Dex micelles should be interesting for intracellular drug delivery. Thus, doxorubicin (DOX), a wide-spectrum anticancer drug, was loaded into the micelles and the pH-dependent release of the payload DOX was tested *in vitro*. The *in vitro* drug release profiles showed that only a small amount of the loaded DOX was released in PBS solution at pH 7.4, while up to about 90% of the loaded DOX could be quickly released in PBS solution at pH 5.5. Compared to pH-insensitive PEG-PLA micelles, the PEG-*b*-AC-Dex micelles displayed a faster drug release behavior in tumor cells. Moreover, higher cellular proliferation inhibition efficacy was achieved toward tumor cells. These features suggested that DOX could be efficiently loaded and delivered into tumor cells *in vitro* by the intracelluar pH-sensitive micelles, leading to enhanced inhibition of tumor cell proliferation. Therefore, the pH-sensitive micelles may provide a promising carrier for acid-triggered drug release for cancer therapy.



KEYWORDS: *intracellular pH-sensitive, acetalated-dextran, doxorubicin, tumor therapy*

1. INTRODUCTION

Nowadays, the continual development of new drugs for the treatment of cancer has led to an appreciable reduction in both mortality and morbidity.[1] However, conventional chemotherapy has only proven partially successful in treating and prolonging the lives of patients because most small molecule drugs still carry a substantial risk of systemic toxicity for normal tissue.[2] Moreover, only small fraction of the drugs can be delivered to and act at the cancer site.[3]

To resolve these problems, various studies focus on enhancing the cytotoxic effect of anticancer drugs and reducing the side effects have been reported.[4] Polymeric micelles, especially based on amphiphilic copolymers self-assembly, provide an promising to alter the pharmacokinetic profile of drugs, reduce off-target toxicity and side effects, and enhance the therapeutic efficiency because of the accumulation in tumor sites *via* the passive "EPR effect".[5−7] However, it is often insufficient for polymer micelles to carry the drug into the intracellular compartments of the cancer cell due to the slow drug release from micelles.[8] Thus, extensive studies have focused on developing polymeric micelles that can rapidly release the drugs triggered by intracellular stimuli such as acidic pH,[9−11] reductive agents,[12−14] and enzymes.[15] Drug delivery systems that can release therapeutic agents in response to weakly acidic environments may have advantages in many applications. Weakly acidic conditions may be found in some pathological sites, such as inflammation, tumor tissues, and endosomal and lysosomal compartments.[16] For example, both the pH in late endosomes (pH 4.5−5.5) and lysosomes (pH 5.0), in which the drug deliveries are entrapped by endocytosis, are remarkably lower than extracellular pH.[17] Hence, pH-sensitive delivery may be the best strategy and confer advantages over others. So far, extensive studies focused on intracellular pH-sensitive covalent bonds such as hydrazone,[18,19] acetal,[20,21] and orthoester[22] have been reported. These pH-sensitive covalent bonds are selectively cleaved in the endosomal compartment (pH ≈ 5), making these covalent bonds interesting for intracellular pH-sensitive drug delivery systems.

For any drug delivery to be useful practically, the nevertheless fundamental consideration is to tailor a safe biocompatible and biodegradable material. Dextran, a homopolysaccharide of glucose, is a natural analog to PEG and has attracted considerable interest for use as polymeric carriers based on its unique properties, such as biodegradability, wide availability, and nonfouling property.[23−25] Dextran has already

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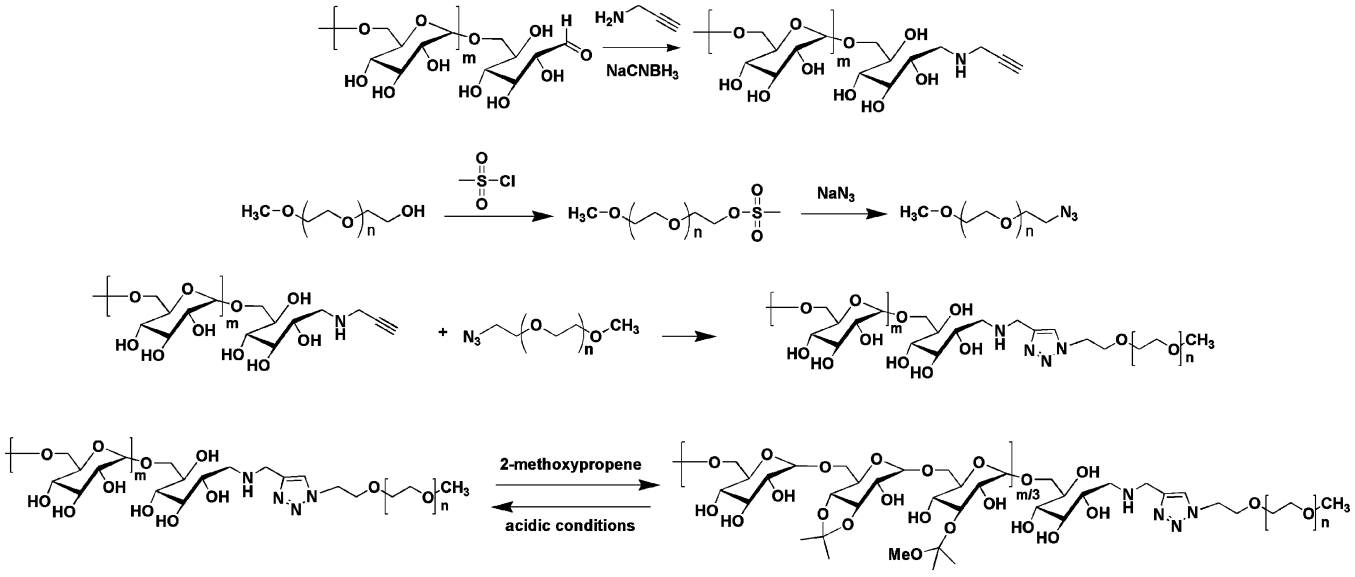
Scheme 1. Synthetic Routes of PEG-*b*-AC-Dex Copolymers



be chosen as plasma volume expansion and plasma substitution in clinical applications.[26] Moreover, attributed to the 5% branching structure as well as plentiful of hydroxyl groups on the chain, dextran can easily be chemically modified, rendering the capability of versatile functionalization.[27] For example, various targeting ligands including RGD peptide,[28] mannose,[29] and galactose[30] may be conjugated with dextran *via* hydroxyl groups to construct targeted drug delivery systems. Acetals have been used to modify dextran to build pH-sensitive nanoparticles due to their facile preparation processes. Fréchet and co-workers designed a series of acid-responsive biodegradable acetal-derivatized dextran nanoparticles for therapeutic applications. Acetal-derivatized dextran provided not only a hydrophobic material that is easily processable by emulsion techniques but also an approach for introducing pH-sensitivity under weakly acidic environment.[16,31,32] However, the blood biocompatibility and slow release rate of the hydrophobic nanoparticles may be limitations for application. Preparing amphiphilic copolymers micelles may be a useful way to improve the biocompatibility and enhance the release rate by introducing PEG block.

To achieve the above objective, we have exploited new biodegradable polymer micelles based on the amphiphilic block copolymers of PEG and acid-labile acetalated-dextran. The effective loading and triggered release of DOX in response to different pH values were demonstrated. The intracellular release of DOX was investigated with HeLa cells using CLSM as well as flow cytometry. The micelles exhibited great potential to control the DOX's release in a pH-dependent way. Obviously accelerated drug release was detected at mildly acidic pH 5.5, analogous to the acid environment of late endosome. These intracellular pH-sensitive micelles may be promising candidates as platforms for targeted intracellular drug delivery.

2. EXPERIMENTAL SECTION

2.1. Materials. Monomethoxy poly(ethylene glycol) (mPEG, M_n = 5000) was obtained from Sigma-Aldrich and used as received. Dextran (Dex, M_n = 6 kDa, Sigma), propargylamine (98%, Sigma), sodium cyanoborohydride (95%, Sigma), sodium azide (Sigma), methylsufonyl chloride (Sigma), *N,N,N′,N′,N″*-pentamethyldiethylenetriamine (PMDETA, 99%, Sigma), polyethyleneimide (PEI, M_w = 25 kDa, Sigma), and 2-methoxypropene (Sigma) were used directly. Doxorubicin hydrochloride (DOX·HCl) was bought from Zhejiang Hisun Pharmaceutical Co., Ltd. 3-(4,5-Dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was obtained from Sigma-Aldrich.

2.2. Characterizations. ^{1}H NMR spectra were collected on a Bruker AV 400 NMR spectrometer. Fourier transform infrared (FT-IR) measurements were performed on a Bio-Rad Win-IR spectrometer. Transmission electron microscopy (TEM) images were taken by a JEOL JEM-1011 transmission electron microscope. The size and distribution of particles were tested by a WyattQELS dynamic laser scattering (DLS) instrument.

2.3. Synthesis of *α*-Alkyne Dextran. The modification of dextran was conducted as shown in Scheme 1. Typically, Dex (2.7 g, 0.454 mmol) was dissolved in 2% (w/v) acetate buffer (pH 5.0) in a flask at 50 °C. Propargylamine (2.5 g, 45.4 mmol) and sodium cyanoborohydride (2.85 g, 45.4 mmol) were added under stirring. The reaction was allowed to proceed at 50 °C for 96 h. The solution was concentrated by rotavapor and then dialyzed (MWCO 3.5 kDa) for 4 days, and the product was collected by lyophilization (1.9 g, yield: 72%).

2.4. Synthesis of Azido-mPEG (PEG-N_3). PEG-N_3 was synthesized according to the following steps. First, mPEG (4.0 g, 0.8 mmol) was dissolved in methylene dichloride (40 mL) under N_2 with strong stirring, and then 2.3 mL of triethylamine (2-fold molar excess relative to that of methylsufonyl chloride) was added. After degassing, 0.62 mL of methylsufonyl chloride (8.0 mmol, 10-fold molar excess compared to mPEG) was added dropwise. After reaction at 0 °C for 12 h, the reactive solution was then precipitated in 10-fold ethyl ether to yield crude product. After redissolving the crude product into deionized water, dialyzing for 4 days (MWCO 3.5 kDa) and lyophilizing, the resultant methylsufonyl-mPEG was obtained (yield: 74%).

Second, NaN_3 (0.252 g, 4 mmol) was added to a DMF solution (10.0 mL) of the obtained methylsufonyl-mPEG (2 g, 0.4 mmol) at 80 °C and the reaction proceeded at 80 °C for 18 h. Then, the reactive mixture was cooled to room temperature and precipitated in 80.0 mL of diethyl ether. The resulting product was redissolved in trichloromethane and washed at least twice with saturated NaCl. After drying the solution with $MgSO_4$, precipitating in diethyl ether and drying in vacuum for 24 h, azido-mPEG (PEG-N_3) was obtained with 78% yield.

2.4. Synthesis of mPEG-*block*-Dex Copolymer. mPEG-*block*-Dex was perpared by an alkyne−azide click reaction, as shown in Scheme 1. Briefly, PEG-N_3 (1 g, 0.2 mmol), *α*-alkyne Dex (1.2 g, 0.2 mmol), and pentamethyldiethylenetrimine (PMDETA) (40 *μ*L, 0.2

Table 1. Characterizations of PEG-*b*-AC-Dex

micelles	PEG-*b*-AC-Dex (mg)	2-methoxypropene (mL)	pyridiniump-toluenesulfonate (mg)	acetalated ratios[a] (%)	CMC[b] (μg/mL)	R_h[b] (nm)	DLC (wt %)	DLE (wt %)
PEG-*b*-AC-Dex 1	400	0.37	40	30.2		124 ± 8.8	2.46	14.76
PEG-*b*-AC-Dex 2	400	0.75	40	53.7	80.2	96 ± 5.7	4.25	25.5
PEG-*b*-AC-Dex 3	400	1.50	40	71.3	7.2	72 ± 6.3	7.42	44.52

[a]Determined by ^{1}HNMR. [b]Determined at pH 7.4.

mmol) were dissolved in 30.0 mL of dried DMSO with stirring for 10 min. After degassing by three freeze−thaw cycles, the mixture was transferred to another flask containing CuBr (30 mg, 0.2 mmol) via N_2-purged syringe, and the reaction was allowed to proceed at 60 °C for 72 h. After the reaction finished, the reaction medium was dialyzed (MWCO 7 kDa) for 4 days and the product was collected by lyophilization (yield: 77%).

2.5. Synthesis of mPEG-*block*-Acetalated-Dextran Copolymer (PEG-*b*-AC-Dex). To an anhydrous DMSO solution of mPEG-*block*-Dex, 2-methoxypropene and *p*-toluenesulfonate were added at 20 °C. After the reaction proceeded for 2 h, the reaction was quenched by addition of triethylamine and the reaction medium was dialyzed (MWCO 7 kDa) for 4 days. The product was then collected by lyophilization. The feeding ratios of mPEG-*block*-Dex, 2-methoxypropene, *p*-toluenesulfonate, and triethylamine are listed in Table 1.

2.6. Synthesis of mPEG-*block*-Polylactide (PEG-PLA) Copolymer. PEG-PLA was synthesized as a control sample without pH-sensitive property. First, 10.0 g of lactide (0.07 mol), 10 g of mPEG (M_n = 5000) (0.002 mol) and 0.028 g $Sn(Oct)_2$ as catalyst (0.1 mol % with respect to lactide) were added into a flask with a magnetic bar. The reaction was allowed to proceed in an oil bath at 110 °C for 72 h. The crude product was collected by precipitation in 10-fold ethyl ether. After redissolving the crude product into trichloromethane, precipitating at least twice in ethyl ether and drying in vacuum, PEG-*b*-PLA was obtained with 73% yield.

2.7. *In Vitro* Drug Loading and Release. Doxorubicin (DOX) was used as a model drug for *in vitro* drug loading and release. DOX-loaded PEG-*b*-AC-Dex and PEG-PLA micelles were obtained by a simple dialysis technique. PEG-*b*-AC-Dex (20.0 mg) or PEG-PLA (20.0 mg) and drug (4.0 mg) were dissolved in 2.0 mL of DMSO. The solution was stirred for 24 h and then added dropwise into 20.0 mL of PBS at pH 7.4. DMSO was then dialyzed against water at pH 8.0 for 24 h. The dialysis procedure was carried out in dark and the dialysis medium was changed five times. After filtering and lyophilizing the solution, the drug loading content (DLC) and drug loading efficiency (DLE) were quantified via fluorescence measurement against a standard calibration curve (λ_{ex} = 480 nm). The DLC and DLE of drug-loaded micelles were obtained according to eqs 1 and 2:

$$\text{DLC (wt \%)} = \frac{\text{weight of drug in micelle}}{\text{weight of drug loaded micelle}} \times 100 \quad (1)$$

$$\text{DLE (wt \%)} = \frac{\text{weight of drug in micelle}}{\text{weight of total drug}} \times 100 \quad (2)$$

The *in vitro* drug release profiles were studied in PBS at pH 5.5 and 7.4, respectively. The freeze-dried DOX-loaded micelles (2 mg) were dissolved in 5.0 mL of PBS and enclosed with a dialysis bag (MWCO 3500 Da). The dialysis bag was placed in 50.0 mL of PBS at 37 °C under constant shaking at 100 rpm. 2.0 mL of external release medium was withdrawn at chosen time intervals, and then 2.0 mL of fresh release medium was added. The amount of released DOX was quantified using fluorescence measurement (λ_{ex} = 480 nm) against a standard curve. The measurements were carried out in triplicate.

2.8. Intracellular Drug Release. The intracellular drug release behaviors of DOX-loaded micelles were monitored by CLSM and flow cytometric analyses.

CLSM. HeLa cells were placed into 6-well plates (2×10^5 cells/well) and cultured in 2.0 mL of complete Dulbecco's modified Eagle's medium (DMEM). After incubation for 24 h, the culture media were withdrawn and culture media containing DOX-loaded micelles were supplemented (final DOX concentration: 10.0 mg L^{-1}). The cells were incubated for another 3 h and washed with PBS three times. The cells were then fixed in 4% paraformaldehyde for 30 min and washed with PBS thrice. For staining the nuclei, the cells were incubated with 4′,6-diamidino-2-phenylindole (DAPI, blue) for 20 min. The images of cells were observed using a laser scanning confocal microscope (Olympus FluoView 1000).

Flow Cytometric Analyses. HeLa cells were placed into 6-well plates (2×10^5 cells/well) and cultured in 2.0 mL of complete DMEM for 24 h. The culture media were then withdrawn and culture media with DOX-loaded micelles were supplemented at a final DOX concentration of 10.0 mg L^{-1}. The cells were incubated for additional 3 h, followed by washing with PBS three times and trypsinized. Then, 1.0 mL of PBS was added, and the solutions were centrifuged for 4 min at 3000 rpm and the cells were resuspended in 0.3 mL of PBS. The analysis was performed by flow cytometer (Beckman, California, U.S.A.) for 1×10^4 cells.

2.8. Cell Viability Assays. The cytotoxicities of DOX-free micelles against HeLa and HepG2 cells were tested using a standard MTT assay. The cells were placed in 96-well plates (1×10^4 cells/well) in 200 μL of complete DMEM, followed by incubation at 37 °C for 24 h. The culture medium was then withdrawn. Micelles solutions with different concentrations (0−10.0 g L^{-1}) in complete DMEM were added. The MTT assay was performed after incubation for another 72 h. The absorbance at 490 nm was measured (Bio-Rad 680 microplate reader). Cell viability (%) was determined by the following eq 3:

$$\text{Cell viability (\%)} = \frac{A_{\text{sample}}}{A_{\text{control}}} \times 100 \quad (3)$$

where A_{sample} and A_{control} are the absorbences of the sample and control wells, respectively.

The cytotoxicities of DOX-loaded micelles against HeLa and HepG2 cells were also measured by using a standard MTT assay. The cells were placed in 96-well plates (1×10^4 cells/well) in 200 μL of complete DMEM, followed by incubation at 37 °C for 24 h. The cells were then washed with PBS and 180 μL of complete DMEM was added. DOX-loaded micelle solutions (20 μL) in PBS were added, leading to different DOX concentrations in the well (0−10.0 mg L^{-1} DOX). The MTT assay was conducted after incubation for 24, 48, and 72 h. The absorbance was tested at 490 nm. Cell viability (%) was also calculated by eq 3.

3. RESULTS AND DISCUSSION

3.1. Synthesis of α-Alkyne Dextran. The chemical structure of α-alkyne dextran was confirmed by ^{1}H NMR and FT-IR. As shown in Figure 1, the complete disappearance of the anomeric proton peaks of the reducing end group at 6.7 ppm and 6.3 ppm was a strong indication of the successful synthesis of the desired α-alkyne dextran.[33] Fourier transform infrared spectroscopy (FT-IR) analysis of α-alkyne dextran was also performed (Figure 2). The appearance of α-alkyne peak at 2100 cm^{-1} further suggested the successful synthesis of α-alkyne dextran.

3.2. Synthesis of Azido-mPEG. The chemical structure of azido-mPEG was confirmed by FT-IR. As shown in Figure 2, the disappearance of sulfonyl peak at about 1300 cm^{-1} and the appearance of α-alkyne peak at 2100 cm^{-1} suggested the successful synthesis of azido-mPEG.

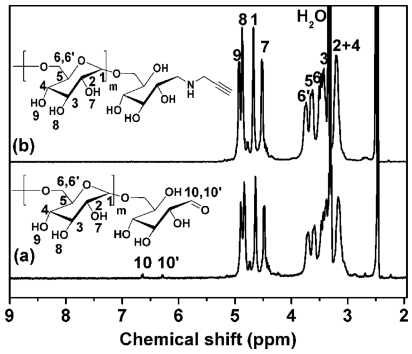


Figure 1. ^{1}H NMR spectra of Dex (a) and α-alkyne Dex (b) in DMSO-d_6.

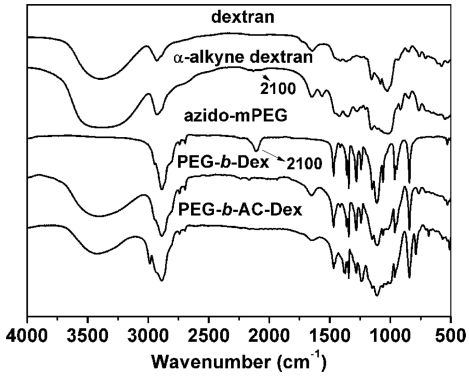


Figure 2. FT-IR spectra of dextran, α-alkyne dextran, azido-mPEG, PEG-*b*-Dex, and PEG-*b*-AC-Dex.

3.3. Synthesis of mPEG-*block*-Dex Copolymer. In this study, mPEG-*block*-Dex copolymer was synthesized by click reaction. The structures of copolymers were demonstrated by ^{1}H NMR, FT-IR, and GPC analyses.[34,35] FT-IR analysis of mPEG-*block*-Dex was performed to confirm the structure of mPEG-*block*-Dex (Figure 2). The disappearance of the azide peak of azido-mPEG and α-alkyne peak of α-alkyne dextran at about 2100 cm^{-1} demonstrated that azido-mPEG had been completely consumed during the reaction with alkyne-terminated dextran, suggesting the successful synthesis of mPEG-*block*-Dex. In addition, GPC profile of mPEG-*block*-Dex was exhibited in Supporting Information (SI) Figure S1, the single peak of the elution time also confirmed the successful synthesis of mPEG-*block*-Dex. The molecular weight is about 12600, and PDI is 1.21.

3.4. Synthesis of mPEG-*block*-Acetalated-Dextran (PEG-b-AC-Dex) Copolymer. mPEG-*block*-acetalated-dextran was synthesized according to previous literature with some modification.[27−29] The structures of the copolymers were demonstrated by ^{1}H NMR analyses (Figure 3). The peak appeared at 1.5 ppm assigned to methyl groups of cyclic isopropylidene acetals and marked reduction of the hydroxyl groups of dextran all indicated the successful synthesis of mPEG-*block*-acetalated-dextran. The acetalated ratios were calculated by the integration ratio between the protons of hydroxyl groups of dextran appearing at 5.5 ppm and methyne appearing at 5.1 ppm, by the following equation:

$$\text{acetalated ratios of hydroxyl groups (mol \%)} = [1 - (I_{8,9} + I_7)/I_1/3] \times 100 \quad (4)$$

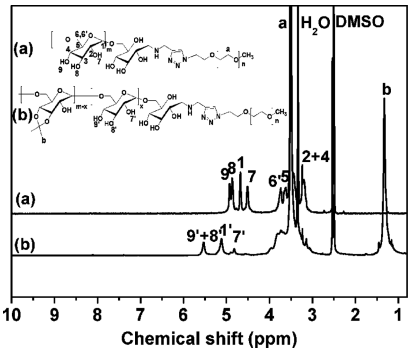


Figure 3. ^{1}H NMR spectra of PEG-*b*-Dex (a) and PEG-*b*-AC-Dex 2 (b) in DMSO-d_6.

3.5. Micellization of Block Copolymers. In this study, the PEG-*b*-AC-Dex copolymers formed micelles in water with changing R_h values determined by the compositions of copolymers (Table 1, SI Figure S2). To demonstrate the formation of micelles and the influence of the composition of block copolymers on the properties of micelles, the CMC value was investigated by a fluorescence method using pyrene as a probe. The excitation spectra of pyrene with increased concentration of block copolymers were measured to confirm the self-assembly of block copolymers. Typically, a red shift of absorption band was observed with the increasing concentration of the copolymer as depicted in SI Figure S3. The red shift indicated the formation of the micelles, attributed to the move of pyrene molecules from a water environment to a hydrophobic micellar core. Moreover, the CMC values were collected by the plot of fluorescence intensity ratio of I_{338}/I_{335} versus $\log_{10} C$ of the copolymer. The CMC values (listed in Table 1) were found to be influenced by the acetalated ratio of the block copolymers. In this study, the CMC values of block copolymers were determined by the hydrophobicity of acetalated-dextran blocks. The excitation spectra of pyrene did not exhibited obvious red shift with increasing the concentration of PEG-*b*-AC-Dex 1 copolymer, and no CMC date was obtained. When the acetalated ratios of the Dex segments enhanced, the CMC value was detected and became lower, indicating the increased hydrophobicity of the block copolymer.

To demonstrate the pH-sensitivity of PEG-*b*-AC-Dex micelle, the change in micelle size in PBS at pH 5.5 was monitored over time by DLS measurement (Figure 4). Notably, the R_h values of PEG-*b*- AC-Dex 3 micelle gradually

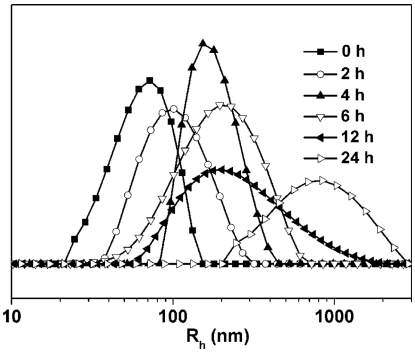
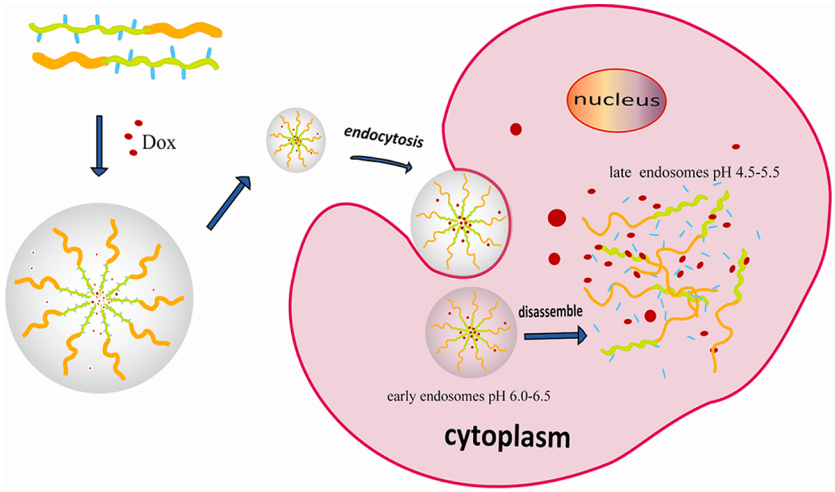


Figure 4. Change in the particle size (R_h) of PEG-*b*-AC-Dex 3 in PBS at pH 5.5.

Scheme 2. Schematic Illustration of DOX Loading and Intracellular Microenvironment Triggered Release from DOX-Loaded PEG-*b*-AC-Dex Micelles



increased from 72 ± 6.3 to 820 ± 102 nm in 24 h. It should be attributed to the decrease of hydrophobicity of the core resulted from the cleavage of acetal bond.

3.6. *In Vitro* DOX Loading and Triggered Release. Doxorubicin (DOX) is a widely used antineoplastic drug in the treatment of several adult and pediatric cancers, such as leukemia, lymphomas, breast cancer, and many other solid tumors. In the current study, to verify the feasibility of using the pH-sensitive micelle for intracellular drug delivery in cancer chemotherapy, DOX was loaded into the micelles as a model drug (Scheme 2), and a pH-insensitive poly (ethylene glycol)-*b*-polylactide (PEG-PLA) copolymer was synthesized for comparison (SI Figures S10 and S11 and Table S1). As shown in Table 1, the DLC of PEG-*b*- AC-Dex micelles were in the range 2.46−7.46%, while the DLE of PEG-*b*-AC-Dex were 14.76−44.52%. These data indicated that higher hydrophobicity of the core improve the drug loading capacity of PEG-*b*-AC-Dex copolymer micelles. Moreover, the DLC and DLE of pH-insensitive PEG-PLA micelle were 5.75% and 34.5%, respectively. The *in vitro* release behaviors were investigated at pH 5.5 and 7.4 respectively. The cumulative release percentages of DOX loaded in PEG-*b*-AC-Dex micelles and PEG-PLA micelles versus time are plotted in Figure 5. Up

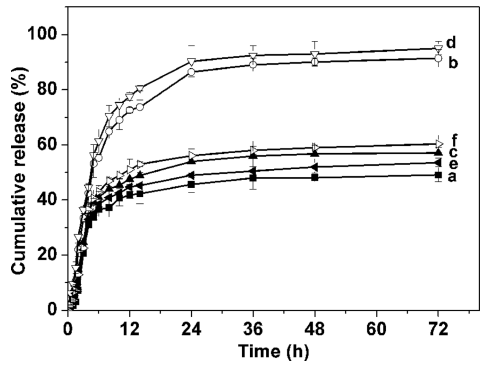


Figure 5. *In vitro* DOX release from DOX-loaded PEG-*b*-AC-Dex 3 in PBS at 37 °C at pH 7.4 (a); PEG-*b*-AC-Dex 3 in PBS at 37 °C at pH 5.5 (b); PEG-*b*-AC-Dex 2 in PBS at 37 °C at pH 7.4 (c); and PEG-*b*-AC-Dex 2 in PBS at 37 °C at pH 5.5 (d); PEG-PLA in PBS at 37 °C at pH 7.4 (e); and PEG-PLA in PBS at 37 °C at pH 5.5 (f).

to about 90% of DOX was released from DOX-loaded PEG-*b*-AC-Dex micelles in PBS at pH 5.5 in 24 h. On the other hand, the DOX release rate was much lower at pH 7.4. But DOX-loaded PEG-PLA micelles exhibited not obviously different release rates at pH 5.5 and 7.4. The different release behaviors were likely resulted from the acid-triggered disassembly of the PEG-*b*-AC-Dex micelle. The results suggest that the PEG-*b*-AC-Dex micelles could effectively hinder the release of the encapsulated drug in normal physiological conditions, while accelerate the drug release in response to intracellular lower pH value. These properties make the pH-sensitive micelles have tremendous potential for cancer chemotherapy.

3.7. Intracellular DOX Release and Cellular Proliferation Inhibition. The cellular uptake and intracellular drug release behaviors of DOX-loaded PEG-*b*-AC-Dex in HeLa cells were monitored with CLSM and flow cytometry. Then, the DOX-loaded PEG-*b*-AC-Dex micelle was incubated with HeLa cells for 3 h. As expected, stronger intracellular DOX fluorescence was detected in the cells after incubation with DOX-loaded PEG-*b*-AC-Dex micelles for 3 h compared to those incubated with DOX-loaded pH-insensitive PEG-PLA micelles (Figure 6A and B). The drug release triggered in intracellular environment was also observed by flow cytometric analyses. As shown in Figure 6C, the flow cytometric histogram for the cells incubated with DOX-loaded PEG-*b*-AC-Dex micelle shifted to the obviously higher fluorescence intensity region in contrast to that for the cells incubated with DOX-loaded PEG-PLA micelle. Thus, the higher fluorescence intensity in the HeLa cells incubated with DOX-loaded PEG-*b*-AC-Dex micelles should result from the faster intracellular DOX release induced by acid-trigged disassociation of the micelle.

The *in vitro* cytotoxicity of the PEG-*b*-AC-Dex and PEG-PLA to HeLa and HepG2 cells was evaluated using a MTT assay. As demonstrated in Figure 7, the viabilities of HeLa and HepG2 cells treated with PEG-*b*-AC-Dex and PEG-PLA for 72 h were over 90% at all test concentrations. The results suggested that PEG-*b*-AC-Dex has low cytotoxicity and can be safely used as biocompatible carriers for drug delivery.

The *in vitro* cellular proliferation inhibitions of DOX-loaded PEG-*b*-AC-Dex and PEG-PLA micelles against HeLa (Figure 8), HepG2 (SI Figure S4−S6), and A549 (SI Figure S7−S9)

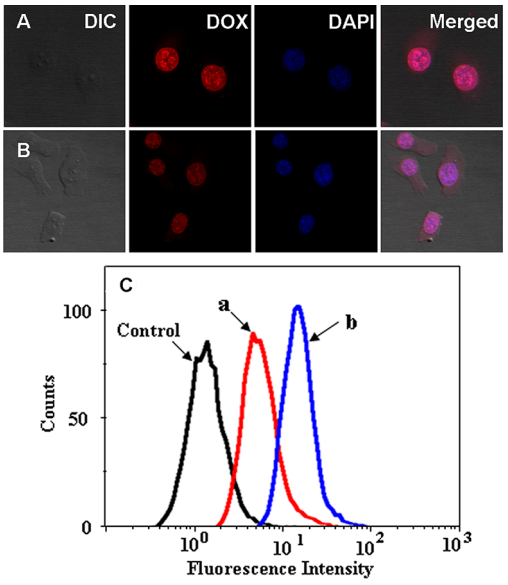


Figure 6. Representative CLSM images of HeLa cells incubated with DOX-loaded PEG-*b*-AC-Dex 3 micelles (A) and DOX-loaded PEG-PLA micelles (B) for 3 h. For each panel, the images from left to right show differential interference contrast (DIC) image, cell nuclei stained by DAPI (blue), DOX fluorescence in cells (red), and overlays of the three images. Flow cytometric profiles of HeLa cells incubated with DOX-loaded PEG-*b*-AC-Dex 3 (b) and PEG-PLA (a) micelles for 3 h (C).

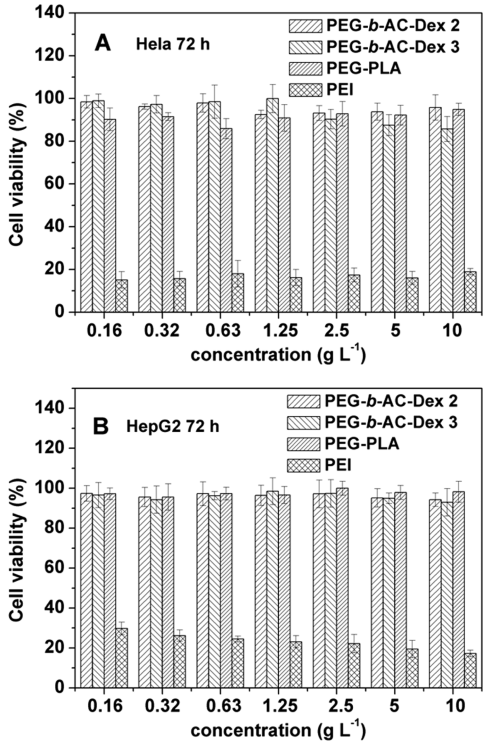


Figure 7. Cytotoxicities of PEG-*b*-AC-Dex 2, PEG-*b*-AC-Dex 3, PEG-PLA and PEI toward HeLa (A) and HepG2 (B) cells after incubation for 72 h.

cells were also estimated using a MTT assay. As shown in Figure 8, in contrast to DOX-loaded PEG-PLA, DOX-loaded PEG-*b*-AC-Dex exhibited significantly higher growth inhibition efficiency to HeLa cells at 24 h, 48 h, and 72 h. Moreover,

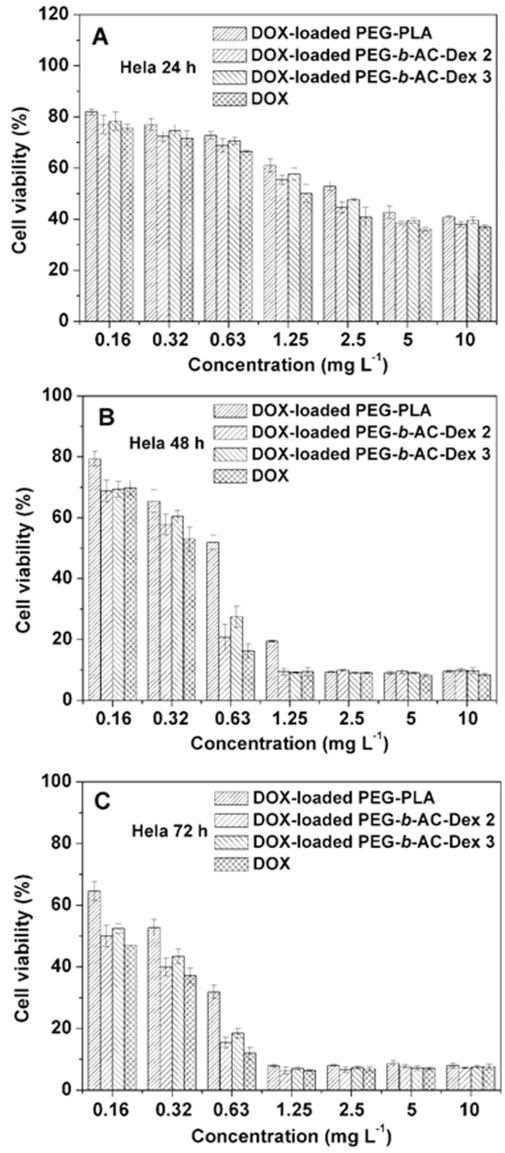


Figure 8. Cytotoxicities of DOX-loaded PEG-PLA, PEG-*b*-AC-Dex 2, PEG-*b*-AC-Dex 3 and free DOX toward HeLa cells after incubation for 24 h (A), 48 h (B), and 72 h (C).

DOX-loaded PEG-*b*-AC-Dex also exhibited significantly higher growth inhibition efficiency to HepG2 and A549 cells (SI Figures S4−S9). The results revealed that the faster DOX release from DOX-loaded PEG-*b*-AC-Dex micelles was triggered by the endosomal pH, leading to enhanced inhibition of cell proliferation as compared with the pH-insensitive DOX-loaded PEG-PLA micelles. The PEG-*b*-AC-Dex provided an efficient drug delivery platform for inhibition of different cancer cells.

4. CONLUSIONS

In this study, a series of pH-responsive micelles based on diblock copolymers of PEG and an acid-labile acetalated-dextran were prepared and used for intracellular pH-sensitive drug delivery. The acid-degradable micelles were studied for rapid intracellular release of doxorubicin (DOX). The DOX release from all DOX-loaded pH-sensitive micelles was accelerated in acid conditions mimicking the endosomal/lysosomal compartments. The enhanced intracellular DOX

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release was observed in HeLa cells. DOX-loaded intracelluar pH-sensitive micelles showed higher cellular proliferation inhibition toward HepG2, HeLa, and A549 cells than pH-insensitive micelles. Therefore, with the good biocompatibility and accelerated intracellular drug release, the PEG-*b*-AC-Dex micelles provide an efficient platform to build intelligent drug delivery systems for cancer therapy.

■ ASSOCIATED CONTENT

Supporting Information

Additional characterization data including GPC trace, TEM micrographs, and fluorescence plots for the copolymers, cytotoxicities toward HepG2 and A549 cells, ^{1}H NMR spectrum and characterizations of PEG-PLA. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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