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Preparation of Copolymer Paclitaxel Covalently Linked via a Disulfide Bond and Its Application on Controlled Drug Delivery

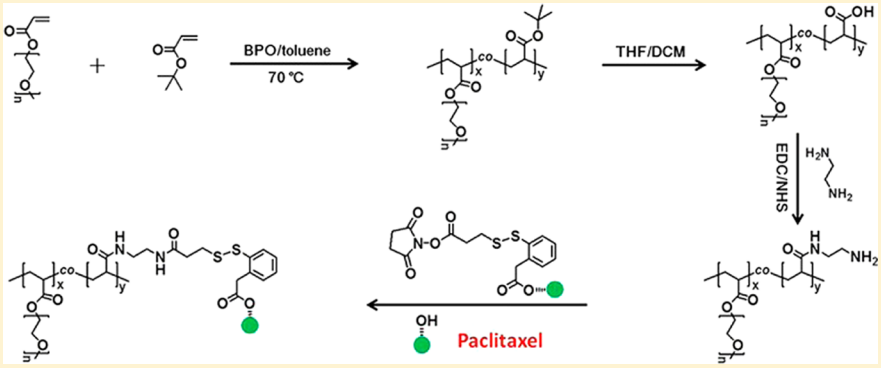
Wulian Chen,[†] Yuanlin Shi,[†] Hua Feng,[‡] Ming Du,[‡,*] Jin Zhong Zhang,[§] Jianhua Hu,[†] and Dong Yang[†,*]

[†]State Key Laboratory of Molecular Engineering of Polymers, Department of Macromolecular Science, Fudan University, Shanghai 200433, P. R. China

[‡]Obstetrics & Gynecology Hospital, Shanghai Medical College, Fudan University, Shanghai 200011, P. R. China

[§]Department of Chemistry and Biochemistry, University of California, Santa Cruz, California 95064, United States

Supporting Information



ABSTRACT: A novel controlled drug delivery system based on copolymer covalently linked paclitaxel via a disulfide bond was constructed. Copolymer with poly(ethylene glycol) (PEG) side chains and carboxyl groups on the backbone was prepared by radical copolymerization of *tert*-butyl acrylate and poly(ethylene glycol) methyl ether acrylate, followed by selectively hydrolyzing *tert*-butyl groups to carboxyl groups. Utilizing the carboxyl group as an active reaction site, paclitaxel, a well-known chemotherapeutic drug, could be covalently linked to the backbone of a copolymer via a disulfide bond, and the loading content of paclitaxel could reach up to 32 wt %. In aqueous solution, this drug-loaded copolymer could self-assemble into a spherical micelle, with the hydrophobic drug as the core and hydrophilic PEG as the shell. The mean diameter of the micelles evaluated by transmission electron microscopy (TEM) and dynamic light scattering (DLS) was approximately 60 nm. The in vitro cytotoxicity experiments showed that the copolymer was biocompatible and suitable to use as a drug carrier. After covalently loading the drug, the copolymer showed apparent cytotoxicity to OS-RC-2 cells (kidney tumor cells) and low cytotoxicity to macrophage cells (human normal cells), indicating that the disulfide bond was stable in human normal cells, but would be broken in tumor cells. This selective bond scission behavior is potentially favorable for reducing the toxic and side effects of chemotherapeutic drugs.

1. INTRODUCTION

Chemotherapy is an indispensable and important strategy in the comprehensive treatment of cancer.[1] Anticancer drugs, such as paclitaxel (PTX) and doxorubicin (DOX), are widely used for the chemotherapy of lung, ovarian, and breast cancers.[2,3] However, the poor water-solubility and nonselective cytotoxicity to cancer cells and normal cells of anticancer drugs have greatly limited their administration dosage and application scope.[4,5] It is highly desirable to develop a controlled drug delivery system (CDDS) that can load a drug with high efficiency to resolve the poor water-solubility of the drug, and release the drug at a specific lesion location to reduce the toxic and side effects of the drug.

Copolymeric micelles aimed to deliver various anticancer drugs have been extensively developed.[6−12] They are composed of two distinct domains: a drug-loading core and a hydrophilic shell. Thus, hydrophobic drugs can be entrapped in the core via noncovalent interactions or covalent bondings.[13] Up to date, most of the CDDSs employing copolymers as drug carriers were loaded drugs via noncovalent interactions.[14−22] For example, Kataoka et al. prepared amphiphilic block copolymers comprising poly(ethylene glycol)−poly(β-benzyl L-aspartate) (PEG-PBLA) forming a core−shell micelle structure.[23] DOX was then incorporated into the PEG-PBLA polymeric micelles via physical entrapment. However, as similar with most noncovalent CDDSs, low drug entrapment and micelle stability are inevitable due to the weak affinity of copolymers with anticancer drugs.[24] These CDDSs usually have a default of drug

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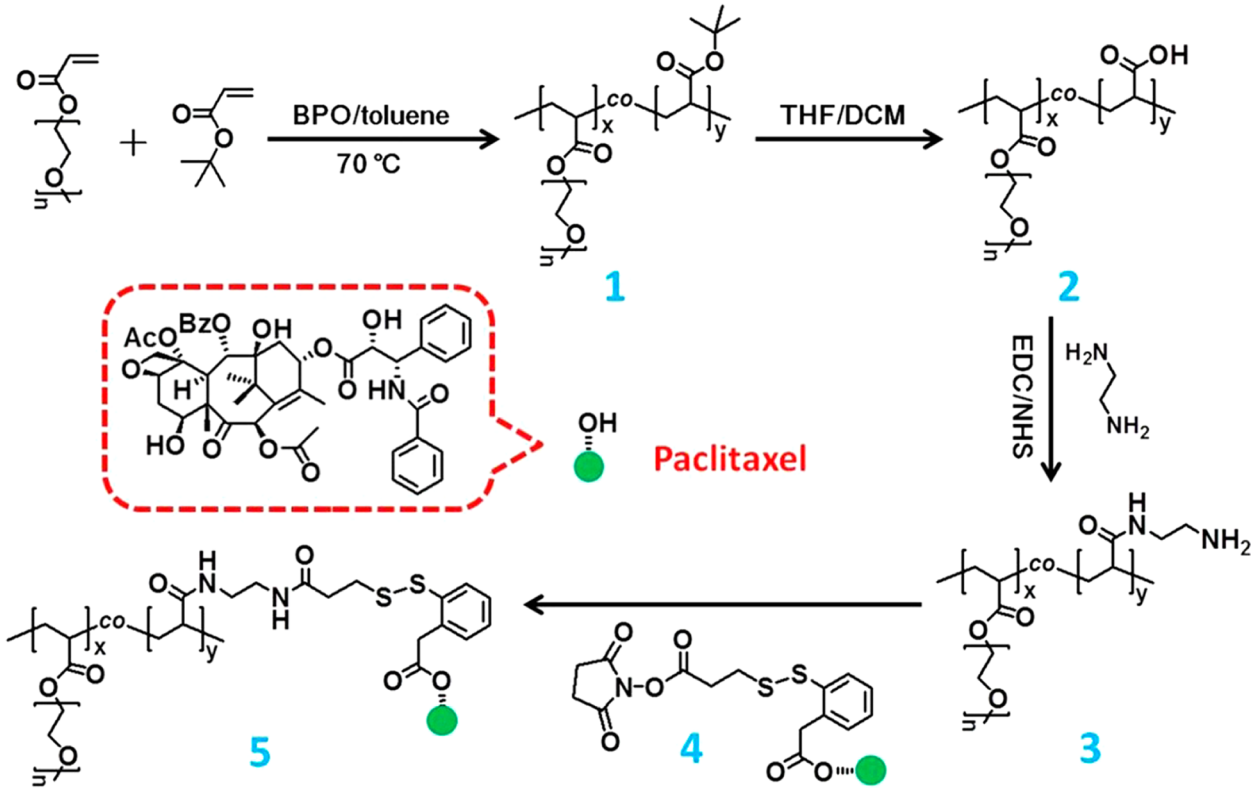

Figure 1. Synthesis of PTX covalently loaded copolymer via a disulfide bond.

burst release,[25−28] which was the main reason for the induced toxic and side effects to patients.

To improve their performance, a variety of stimuli-responsive copolymers have been used as drug carriers in CDDSs. For instance, Frechet et al. used a pH-sensitive PEO-dendritic polyester copolymer as a drug carrier to load DOX, and found that the release rate of DOX increased with the decrease of pH.[29] Hedrick et al. prepared a thermosensitive block copolymer, and found that a PTX-loaded copolymer could kill HepG2 cancer cells more efficiently at body temperature than at the temperature below the lower critical solution temperature (LCST) of the copolymer.[30] Li et al. prepared multistimuli-responsive copolymer nanogels, and found that the release rate of the drug-loaded nanogels could be greatly accelerated by a cooperative effect of both acid-triggered hydrolysis and dithiothreitol (DTT)-induced degradation.[31] Although the aforementioned CDDSs could control the drug release behaviors to a certain degree, the loaded drug would be unavoidably released before it was transferred to the lesion location.[32−35] By contrast, drug covalently loaded CDDSs appear to be more stable than drug noncovalently loaded CDDSs. Their drug release pattern can be modified according to the chemical stability of drug-binding linkers, which could prevent the prerelease of drug before reaching the lesion location.[36,37]

A disulfide bond is reduction sensitive and readily cleavable by thiols, such as glutathione (GSH),[38] and the concentration of GSH in tumor cells (2−8 mM) is much higher than that in blood plasma (1−2 μM).[39] This provides a new and intriguing way to construct CDDSs utilizing the disulfide bond as a switcher to release drug, which would not release the drug in blood plasma and normal cells, but release the drug in cancer cells. Mortera et al. prepared cysteine covalently loaded mesoporous silica nanoparticles via a disulfide bond (Cy-S-MSN), and found that the cysteine would not be released except after the addition of reducing agent.[40] The in vitro cell assay results also demonstrated that the Cy-S-MSN could kill HeLa cells more efficiently than cysteine noncovalently loaded MSN. Ojima et al. reported that taxoid was conjugated to the monoclonal antibody, and this conjugate showed remarkable target-specific antitumor activity.[41,42] However, the existing CDDSs based on disulfide bonds to covalently load drug have two main drawbacks: the low drug loading content and the changed chemical structure of released drug, which might be a disadvantage for the drug function.[43]

Recently, Ojima et al. reported a novel disulfide linker that could react with reducing agents to release a drug without changing the chemical structure of the drug.[44,45] Herein, we utilized this disulfide linker to construct a novel CDDS based on copolymer covalently linked to PTX via a disulfide bond. A copolymer with poly(ethylene glycol) (PEG) side chains and carboxyl groups on the backbone was prepared by radical copolymerization of *tert*-butyl acrylate (*t*BA) and poly(ethylene glycol) methyl ether acrylate (PEGMEA), followed by selectively hydrolyzing *tert*-butyl groups to carboxyl groups. Utilizing the carboxyl group as an active reaction site, PTX could be covalently linked to the backbone of the copolymer via a disulfide bond (Figure 1), and the loading content of PTX could reach up to 32 wt %. In aqueous solution, this drug-loaded copolymer could self-assemble into a spherical micelle, with hydrophobic drug as the core and hydrophilic PEG as the shell. The mean diameter of the micelles evaluated by transmission electron microscopy (TEM) and dynamic light scattering (DLS) was approximately 60 nm. In vitro cellular cytotoxicity experiments were performed to evaluate the biocompatibility of the copolymer, and the bond scission

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behavior of the CDDS in human normal cells (macrophage) and kidney cancer cells (OS-RC-2).

2. EXPERIMENTAL SECTION

2.1. Chemicals. PTX was purchased from Beijing Huafeng United Technology Co., Ltd. PEGMEA (M_n = 480, 99%), *t*BA (98%), benzoyl peroxide (BPO, 98%) and 1,2-ethanediamine were purchased from Aldrich. 1-Ethyl-3-(3-dimethyllaminopropyl) carbodiimide hydrochloride (EDC), 1-hydroxy-2,5-pyrrolidinedione (NHS), dimethylformamide (DMF), tetrahydrofuran (THF), hexane, and trifluoracetic acid (TFA) were purchased from Shanghai Medpep Co., Ltd. A dialysis bag with a cutoff molecular weight of 3.5 kDa was purchased from Shanghai Greed Bird Science & Technology Development Co., Ltd. PEGMEA was passed through a neutral alumina column to remove inhibitor. *t*BA was washed successively with 5% NaOH aqueous solution and deionized water, and then dried over $CaCl_2$, and distilled from CaH_2 under reduced pressure. BPO was purified by dissolving in chloroform and precipitating in methanol. All the other chemicals were used without further treatment.

2.2. Synthesis of Copolymer 1 by Radical Copolymerization. In a typical synthesis, BPO (11.1 mg, 0.046 mmol), *t*BA (0.4415 g, 3.45 mmol), PEGMEA (0.5450 g, 1.20 mmol), and toluene (1.2 mL) were added into a 50 mL of Schlenk flask. The mixture was heated to 70 °C. After reaction for 1 h, the polymerization was terminated by immersing the flask into liquid N_2. Then, the solution was precipitated by dropwise addition into cold hexane. After repeated purification by dissolving in THF and precipitating in cold hexane three times, 0.63 g (64.2%) of colorless viscous liquid, P*t*BA-*co*-PPEGMEA, was obtained by drying in vacuo overnight. GPC: M_n = 31 kDa, polydispersity index (PDI) = 2.3. ^{1}H NMR δ (ppm): 4.10 ($COOCH_2$), 3.50 (OCH_2CH_2), 3.24 (OCH_3), 1.36 ($C(CH_3)_3$). ^{13}C NMR δ (ppm): 25.7 (CH_2CH), 27.8 ($C(CH_3)_3$), 40.1 (CH_2CHCO_2), 58.6 (OCH_3), 68.6−71.7 (OCH_2CH_2), 80.3 ($C(CH_3)_3$), 173.6 ($C{=}O$).

2.3. Preparation of Copolymer 2 by Selective Acidic Hydrolysis. PtBA-*co*-PPEGMEMA (0.6 g, containing 3.5 mmol of *tert*-butyl groups), CH_2Cl_2 (50 mL), and TFA (2.6 mL, 35 mmol) were added into a 100 mL of flask. The mixture was stirred at room temperature for 48 h, followed by concentrating and precipitating in cold hexane. After repeated purification by dissolving in THF and precipitating in cold hexane three times, 0.52 g (83%) of a colorless viscous liquid, PAA-*co*-PPEGMEA, was obtained by drying in vacuo overnight. ^{1}H NMR δ (ppm): 4.10 ($-COOCH_2-$), 3.50 ($-OCH_2CH_2-$), 3.24 ($-OCH_3$). ^{13}C NMR δ (ppm): 25.7 (CH_2CH), 40.1 (CH_2CHCO_2), 58.6 (OCH_3), 68.6−71.7 (OCH_2CH_2), 176.3 ($C{=}O$).

2.4. Preparation of Copolymer 3 by Amidation Reaction. PAA-*co*-PPEGMEA (0.50 g, containing 5.2 mmol of carboxyl group), EDC (1.0 g, 5.2 mmol), NHS (0.60 g, 5.2 mmol) and dry DMF (30 mL) were added into a 100 mL of three-neck flask. The mixture was stirred at room temperature for 6 h. Then, 1,2-ethanediamine (3.5 mL, 0.10 mol) was added via a gastight syringe. The mixture was stirred at room temperature for another 24 h, followed by precipitating in ether. The sediment was dissolved in deionized water and purified by dialysis in deionized water using a dialysis bag with a cutoff molecular weight of 3.5 kDa. A white solid powder (0.36 g; 50%), PAM-*co*-PPEGMEMA, was obtained by freeze-drying. ^{1}H NMR δ (ppm): 4.16 ($COOCH_2$), 3.63 (OCH_2CH_2), 3.31 (OCH_3), 3.07 ($CONHCH_2$), 2.48 (CH_2NH_2). ^{13}C NMR δ (ppm): 24.6 (CH_2CH), 37.0 (CH_2NH_2), 38.9 (CH_2CHCO_2), 42.8 ($CONHCH_2$), 58.0 (OCH_3), 68.4−71.0 (OCH_2CH_2), 177.7−182.6 ($C{=}O$). Elemental analysis: C, 46.02%; H, 6.40%; N, 9.68%.

2.5. Preparation of PTX Covalently Loaded Copolymer 5. PAM-*co*-PPEGMEMA (0.15 g, 0.52 mmol amino group), PTX-Linker 4 (78 mg, 0.067 mmol) prepared according to refs 37 and 38 (please see Supporting Information), and dry DMF (5 mL) were added into a 20 mL of flask. The solution was stirred at room temperature for 24 h, followed by precipitating in ether. After repeated purification by dissolving in DMF and precipitating in ether, the crude product was dissolved in deionized water and dialyzed in deionized water using a dialysis bag with a cutoff molecular weight of 3.5 kDa. A slightly orange solid (0.12 g), PAM-*co*-PPEGMEA-Linker-PTX, was obtained by freeze-drying. ^{1}H NMR δ (ppm):[31] 1.13 (s, 3 *H*), 1.14 (s, 3 *H*), 1.25 (s, 4 *H*), 1.34 (s, 9 *H*), 1.66 (s, 3 *H*), 1.71 (s, 1 *H*), 1.75 (s, 6 *H*), 1.82 (s, 1 *H*), 1.86(m, 1 *H*), 1.91 (s, 3 *H*), 2.31 (s, 1 *H*), 2.33 (s, 1 *H*), 2.37 (s, 3 *H*), 2.60 (m, 2 *H*), 2.87 (d, *J* = 6 Hz, 2 *H*), 2.97 (d, 2 *H*), 3.80 (d, 1 *H*), 4.17 (s, 2 *H*), 4.19 (d, 1 *H*), 4.30 (d, 1 *H*), 4.43 (dd, 6.6 Hz, 1 *H*), 4.9−5.0 (m, 4 *H*), 5.19 (s, 1 *H*), 5.66 (d, 1 *H*), 6.17 (t, 1 *H*), 6.29 (s, 1 *H*), 7.34 (m, 3 *H*), 7.47 (t, 2 *H*), 7.60 (t, 1 *H*), 7.80 (d, 1 *H*), 8.10 (d, 2 *H*), 3.50 (OCH_2CH_2). Elemental analysis: C, 46.87%; H, 7.21%; N, 8.98%; S, 2.44%.

2.6. In Vitro Cell Assay. The cytotoxicity of PAM-*co*-PPEGMEA and PAM- *co*-PPEGMEA-linker-PTX against macrophage and OS-RC-2 cells was evaluated by MTT assay. All samples were dissolved in deionized water. The cells were seeded in 96-well plates with a density of 2×10^4 cell/well. Then, the cells were exposed to a series of doses of PAM-*co*-PPEGMEA or PAM-*co*-PPEGMEA-linker-PTX at 37 °C. After incubation for 24 h, 20 μL of MTT solution in PBS (5 mL/mg) was added to each well, and the cells were incubated for another 4 h at 37 °C in dark. Afterward, the MTT-containing medium was aspirated, and 150 μL of dimethyl sulfoxide (DMSO) was added to each well and agitated thoroughly to dissolve the formazan crystals. Absorption was measured at a wavelength of 490 nm in a BioTek Elx800 apparatus. The cell viability was calculated as follows:

$$\text{Cell viability (\%)} = I_{\text{sample}}/I_{\text{control}} \times 100$$

where I_{sample} was the absorbance at the presence of sample solutions, and I_{control} was the absorbance without treatment. All the tests were performed in triplicate.

2.7. Characterization. Fourier transformed infrared (FT-IR) measurements were performed on a NEXUX-470 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Philips DMX500 300 MHz spectrometer using DMSO-*d6* or D_2O as solvents. The molecular weight and molecular weight distribution were determined by gel permeation chromatography (GPC). The GPC was performed in THF at 35 °C with an elution rate of 1.0 mL/min on Aginent 1100 equipped with a G1310A pump, a G1362A refractive index detector, and a G1314A variable wavelength detector. Polystyrene standard samples were employed for the GPC calibration. TEM images were obtained on a JEOL JEM 2100 F transmission electron microscope, and samples for TEM measurements were made by casting one drop of the sample's solution on carbon copper grids. The size distribution of the micelles was measured by

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DLS using a Malvern autosizer 4700. Elemental analysis was carried out on a Carlo-Erba 1106 system.

3. RESULTS AND DISCUSSION

3.1. Preparation of Copolymer Covalently Linked PTX via a Disulfide Bond. Because that the reactivity ratios of *t*BA and PEGMEA are similar,[46,47] they could be facilely copolymerized to P*t*BA-*co*-PPEGMEA by radical copolymerization. The number average molecular weight (M_n) and the PDI of P*t*BA-*co*-PPEGMEA determined by GPC were 31 000 and 2.3, respectively. The chemical structure of P*t*BA-*co*-PPEGMEA copolymer was characterized by ^{1}H and ^{13}C NMR. As can be seen from Figure 2A, the ^{1}H NMR spectra of P*t*BA-*co*-PPEGMEA showed two strong peaks at 3.24 (OCH_3) and 3.50 ppm (OCH_2CH_2), characteristic of the protons of PEG, and a strong peak at 1.36 ppm originating from the nine protons of *tert*-butyl groups. The ratio of *t*BA to PEGMEA in the copolymer was approximately 3:1, calculated by comparing the integral areas of peaks at 3.24 and 1.36 ppm. In addition, the ^{13}C NMR spectra of P*t*BA-*co*-PPEGMEA (Figure 3A) showed two obvious signals at 68.6−71.7 and 27.8 ppm, assigned to the carbon atoms derived from PEG and the *tert*-butyl group, respectively. All of these results suggested the successful preparation of P*t*BA-*co*-PPEGMEA.

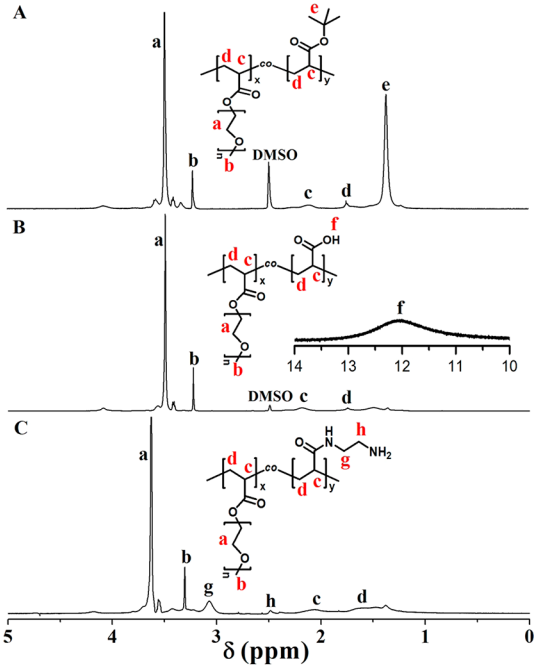

Figure 2. ^{1}H NMR spectra of (A) P*t*BA-*co*-PPEGMEA, (B) PAA-*co*-PPEGMEA in DMSO-d_6, and (C) PAM-*co*-PPEGMEA in D_2O.

The *tert*-butyl group of P*t*BA could be readily hydrolyzed to carboxyl group by TFA in CH_2Cl_2.[48−50] The hydrolyzed product was characterized by NMR and FT-IR, to confirm the selective hydrolysis of *tert*-butyl group without destroying the ester bond between the PEG side chain and the backbone. As seen from Figure 2B, the peak of the nine protons of the *tert*-butyl group at 1.36 ppm completely disappeared after hydrolysis, and a new and broad peak, assigned to the proton of the carboxyl group, appeared at 12.3 ppm. In the ^{13}C NMR spectrum of Figure 3B, the characteristic signals of the carbons of *tert*-butyl group at 27.8 and 80.3 ppm also completely disappeared after hydrolysis, which also demonstrated the successful hydrolysis of the *tert*-butyl group. The FT-IR spectra of P*t*BA-*co*-PPEGMEA and PAA-*co*-PPEGMEA were shown in Figure 4. After hydrolysis, a new broad peak appeared at 3448 cm^{-1}, indicating the presence of hydroxyl groups. Furthermore, all the typical signals of PEG side chains remained in the ^{1}H and ^{13}C NMR spectra, and the characteristic C−O−C stretching vibrations of PEG side chains at 1110 cm^{-1} also remained in the FT-IR spectrum. All the evidence above indicated that the P*t*BA-*co*-PPEGMEA was selectively hydrolyzed to PAA-*co*-PPEGMEA.

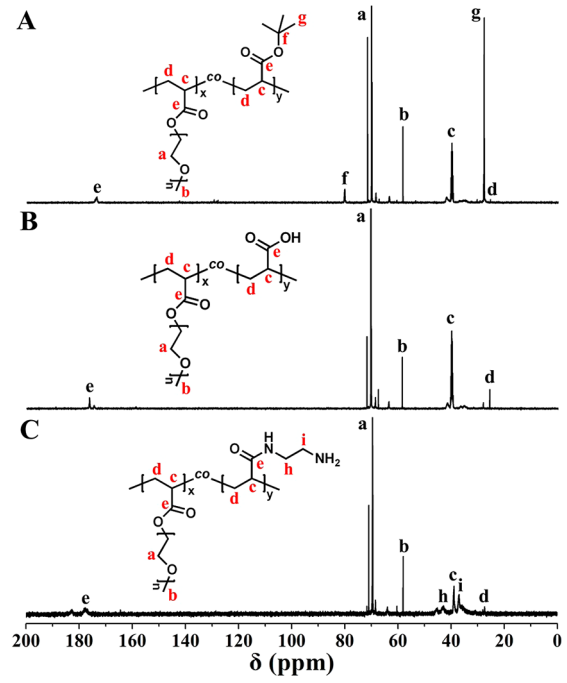

Figure 3. ^{13}C NMR spectra of (A) P*t*BA-*co*-PPEGMEA, (B) PAA-*co*-PPEGMEA in DMSO-d_6, and (C) PAM-*co*-PPEGMEA in D_2O.

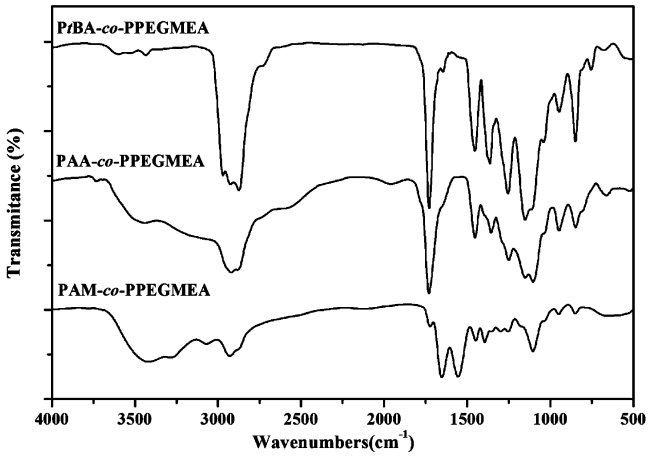

Figure 4. FT-IR spectra of PtBA-*co*-PPEGMEA, PAA-*co*-PPEGMEA, and PAM-*co*-PPEGMEA.

To covalently load the drug, the carboxyl groups of PAA-*co*-PPEGMEA were transferred to amino groups by reacting with 1,2-ethanediamine, using EDC/NHS as coupling reagents in DMF at room temperature. As seen from Figure 2C, after amidation reaction, two new peaks appeared at 2.48 and 3.07

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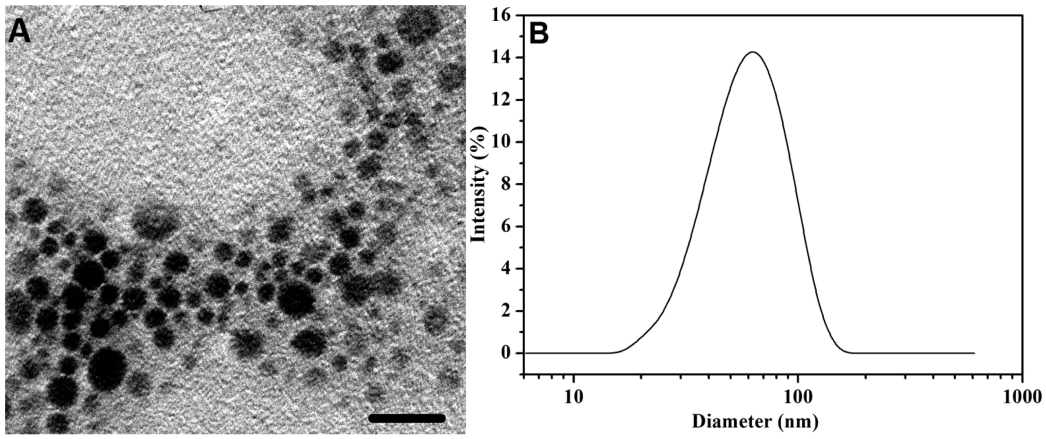

Figure 5. (A) TEM images of self-assembled micelles and (B) DLS curves of the micelle aqueous solution, scale bar =100 nm.

ppm in the ^{1}H NMR spectrum of PAM-*co*-PPEGMEA, assigned to the protons of CH_2NH_2 and $CONHCH_2$, respectively. The signals of $CONHCH_2$ and CH_2NH_2 were also observed at 37.0 and 42.8 ppm in the ^{13}C NMR of Figure 3C. In the FTIR spectrum of Figure 4, the peak at 1720 cm^{-1} disappeared, and two new peaks appeared at 1665 and 1552 cm^{-1}, indicating that the carboxyl groups successfully reacted with amino groups to form amide bonds. The content of nitrogen in PAM-*co*-PPEGMEA evaluated by elemental analysis was 9.68%, which suggested that about 80% of the carboxyl groups reacted with 1,2-ethanediamine. This result was consistent with the result calculated by NMR.

The PTX-Linker containing the disulfide bond was synthesized according to refs 44 and 45, and the detailed synthetic route and characterization data are supplied in the Supporting Information. The carboxyl groups of the PTX-Linker were activated with EDC/NHS as to easily react with the amino groups of PAM-*co*-PPEGMEA. Thus, PTX was covalently loaded onto PAM-*co*-PPEGMEA via a disulfide bond. The resulting PAM-*co*-PPEGMEA-Linker-PTX was amphiphilic, and could self-assemble into micelles with hydrophobic PTX as the core and hydrophilic PEG side chain as the shell in an aqueous solution. As seen from the TEM image of Figure 5A, the micelles were spherical with an average diameter of approximately 60 nm, which was also confirmed by the DLS measurement (Figure 5B). The PDI of the micelles evaluated by DLS was 0.32.

To ascertain the core−shell structure, ^{1}H NMR characterizations of PAM-*co*-PPEGMEA-Linker-PTX using D_2O and DMSO-d_6 as solvents were performed. As can be seen from Figure 6, the ^{1}H NMR spectrum of PAM-*co*-PPEGMEA-Linker-PTX using DMSO-d_6 as a solvent (Figure 6B) showed distinguished characteristic PTX peaks at 1.2 and 1.0 ppm, and aromatic signals at 7 to 8 ppm. While using D_2O as a solvent (Figure 6A), these characteristic signals disappeared, indicating that the hydrophobic PTX was in the inner core of the micelles. This is because the amphiphilic drug loaded copolymer chain could well extend in DMSO-d_6, yet form a spherical micelle with PTX as the core and PEG chains as the shell. The element analysis results showed that the sulfur content in PAM-*co*-PPEGMEA-Linker-PTX was 2.44%, thus the loading content of PTX on the copolymer was about 32%.

3.2. In Vitro Cell Assay. To evaluate the potential cytotoxicity of PAM-*co*-PPEGMEA and the selective cytotoxicity of PAM-*co*-PPEGMEA-Linker-PTX to cancer cells and

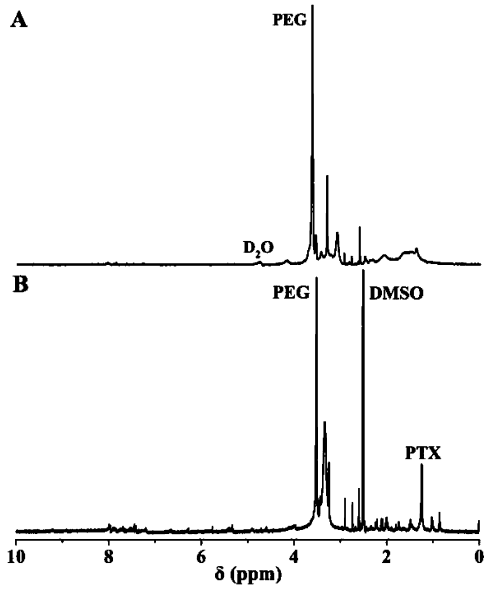

Figure 6. ^{1}H NMR spectra of PAM-*co*-PPEGMEA-Linker-PTX (A) in D_2O and (B) in DMSO-d_6.

normal cells, macrophage cells (human normal cells) and OS-RC-2 cells (kidney tumor cells) were chosen to conduct the in vitro cell cytotoxicity tests by MTT assays. It is very important to evaluate the potential toxicity of PAM-*co*-PPEGMEA used as a drug carrier. The *in vitro* cellular cytotoxicity of PAM-*co*-PPEGMEA to macrophage cells (human normal cells) and OS-RC-2 cells (kidney tumor cells) at different concentrations was showed in Figure 7A. As seen from Figure 7A, after incubation for 24 h, the PAM-*co*-PPEGMEA showed no obvious cytotoxic effect on macrophage cells and OS-RC-2 cells at a concentration range of 5−320 μg/mL. Even at the concentration of PAM-*co*-PPEGMEA as high as 320 μg/mL, the cell viability was above 90% after incubation for 24 h, suggesting that the cytotoxicity of PAM-*co*-PPEGMEA is pretty low and suitable to use as a drug carrier in CDDSs.

Figure 7B shows the in vitro cellular cytotoxicity of PAM-*co*-PPEGMEA-Linker-PTX to macrophage cells (human normal cells) and OS-RC-2 cells (kidney tumor cells). As shown in Figure 7B, PAM-*co*-PPEGMEA-Linker-PTX did not show obvious cytotoxicity to macrophage cells, and the cell viability was kept above 90% within the PTX concentration range from 3 to 50 μg/mL after incubation for 24 h, indicating that PTX

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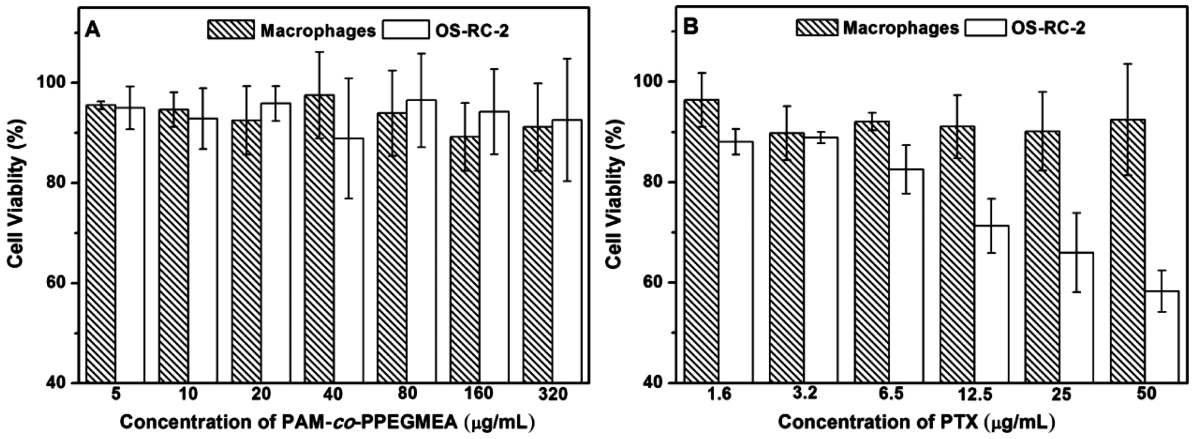

Figure 7. Cell cytotoxicity of (A) PAM-*co*-PPEGMEA against macrophages and OS-RC-2; (B) PAM-*co*-PPEGMEA-Linker-PTX against macrophages and OS-RC-2 at different concentrations.

was almost not released. However, in contrast with macrophage cell viability, the OS-RC-2 cell viability declined along with the increase of PTX concentration. During incubation with 50 μg/mL of PTX for 24 h, the cell viability of OS-RC-2 was decreased to 58%, suggesting that the disulfide bond was broken, and PTX was released. After covalently loading PTX via a disulfide bond, PAM-*co*-PPEGMEA-Linker-PTX showed distinct cytotoxicity to macrophage cells and OS-RC-2 cells, because the concentration of GSH in OS-RC-2 cells was much higher than that in macrophage cells. This selective bond scission and drug release behavior is favorable to decrease the toxic and side effects of PTX.

4. CONCLUSIONS

A novel CDDS was constructed by using PAM-*co*-PPEGMEA to covalently load PTX via a disulfide bond, and the PTX loading content could reach up to 32%. The TEM, DLS, and NMR results demonstrated that the PAM-*co*-PPEGMEA-Linker-PTX could self-assemble into spherical micelles in aqueous solution, with hydrophobic PTX as the core and hydrophilic PEG chains as the shell. In vitro cell assays demonstrated that the PAM-*co*-PPEGMEA was biocompatible and suitable to use as a drug carrier. The PTX-loaded copolymer showed apparent cytotoxicity to OS-RC-2 cells and low cytotoxicity to macrophage cells, indicating that the disulfide bond was stable in human normal cells, but would be broken in tumor cells. This selective bond scission behavior is favorable to reduce the toxic and side effects of chemotherapeutic drug. The strategy of using copolymers as drug carriers and disulfide linker as linkage is a versatile approach to construct promising CDDSs for biomedicine.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthetic processes and ^{1}H NMR characterizations of PTX-Linker. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(D.Y.) Fax: +86-21-65640293; Tel: +86-21-65643575; e-mail: yangdong@fudan.edu.cn. (M.D.) Fax: +86-21-65640293; Tel: +86-21-65643575; E-mail: mutudu@gmail.com.

Notes

The authors declare no competing financial interest.

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