

## Supporting Information Available

# Shell-sheddable Micelles Based on Star-shaped Poly( $\epsilon$ -caprolactone)-SS-poly(ethyl glycol) Copolymer for Intracellular Drug Release

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### Materials and Methods

Tin 2-ethylhexanoate ( $\text{Sn}(\text{Oct})_2$ ; Aldrich) was distilled under reduced pressure before use. Dipentaerythritol (Aldrich) and poly(ethylene glycol) methyl ether (mPEG,  $M_n=1.3$  K) were dried in vacuum for 24 h before use. Tetrahydrofuran (THF) was dried by refluxing over sodium wire and distilled prior to use.  $\epsilon$ -Caprolactone ( $\epsilon$ -CL; Acros Organic) and dichloromethane (DCM) were dried by refluxing over  $\text{CaH}_2$  and distilled before use. 3,3-Dithiodipropionic acid (DTDP; Aldrich), Dicyclohexyl-carbodiimide (DCC; GL Biochem, Shanghai), 4-dimethyl-aminopyridine (DMAP; GL Biochem, Shanghai), dithiothreitol (DTT; 99%, Merck) and doxorubicin hydrochloride (Zhejiang Hisun Pharmaceutical Co., Ltd, China) were used as received. All other chemicals obtained from Sinopharm Chemical Reagent Company (SCRC) were of analytical grade and were used as received. MCF-7 cancer cells were a gift from Medical College of Tongji University, 4',6-diamidino-2-phenylindole (DAPI) and medium were purchased from Shanghai Pufei Bio-Technology Co.

### Synthesis of hexa-armed PCL (6sPCL)

The hydroxyl-terminated 6sPCL was synthesized by ring-opening polymerization of  $\epsilon$ -CL using  $\text{Sn}(\text{Oct})_2$  as catalyst. Typically, CL (2.01 g, 17.63 mmol), dipenta-

erythritol (49.9 mg, 0.195 mmol) and a catalytic amount of Sn(Oct)<sub>2</sub> were added to a flame-dried polymerization tube quickly. The tube was then connected to a Schlenk line, where exhausting-refilling processes were repeated for three times. Then the polymerization was carried out at 120 °C for 24 h under nitrogen atmosphere with stirring. The resulting product was dissolved in 5 mL of CHCl<sub>3</sub> and poured dropwise into 50 mL of cold methanol under vigorous stirring at room temperature. The precipitate was harvested and dried in vacuum at 40 °C.

### **Synthesis of PEG-SS-COOH**

3,3-Dithiodipropionic acid (DTDP) (2.10 g, 10 mmol) and thionyl chloride (10.0 mL) were added into a 50 mL round-bottom flask, then the mixture was heated to 85 °C for 4 h. DTDP was reacted completely as the white powder was turned into yellow liquid. The mixture was distilled at 85 °C under vacuum to remove unreacted thionyl chloride to obtain dithiodipropionic chloride. Then together with PEG (2.6 g, 2.0 mmol) and triethyl amine (0.24 g, 2.0 mmol), dithiodipropionic chloride (2.46 g, 10.0 mmol) were dissolved into THF (60.0 mL) and heated to 75 °C for 6 h under magnetic stirring. After centrifugation to remove triethylamine-hydrochloride, the supernatant was dropped into 210 mL of ethyl ether to obtain dithiodipropionic chloride modified PEG (PEG-SS-COCl). Finally, PEG carboxyl disulfide was prepared by hydrolysis of PEG-SS-COCl. The product was purified three times by repeated precipitations.

### **Synthesis of 6sPCL-SS-PEG**

6sPCL-SS-PEG was synthesized via a condensation reaction of mPEG-SS-COOH and 6sPCL-OH. Typically, 6sPCL-OH (100 mg, 0.01 mmol), mPEG-SS-COOH (91 mg, 0.07 mmol), DCC (34.7 mg, 0.168 mmol), and DMAP (10.2 mg, 0.084 mmol) were dissolved in 5.0 mL of anhydrous methylene chloride and reacted at room temperature for 24 h under nitrogen. The reaction byproduct dicyclohexylcarbodiurea was removed by filtration and then precipitated in ethyl ether. The purified 6sPCL-SS-PEG was dried at ambient temperature under vacuum for 24 h.

## Characterization

$^1\text{H}$  NMR data were obtained by a Bruker DMX-500 NMR spectrometer with  $\text{CDCl}_3$  or  $\text{D}_2\text{O}$  as the solvent. The chemical shifts were relative to tetramethylsilane at  $\delta = 0$  ppm for protons. Gel permeation chromatography (GPC) measurements were conducted on a gel permeation chromatographic system, equipped with a Waters 150C separations module and a Waters differential refractometer. The molecular weight and molecular weight distributions were calibrated against polystyrene standards, with THF as the eluent at a flow rate of 1 ml/min. Fluorescence spectra were performed on a Hitachi F2500 luminescence spectrometer (Hitachi, Ltd, Hong Kong). The hydrodynamic diameter and the particle size distribution of the micelles were determined using a dynamic light scattering spectrophotometer (DLS, Malvern Instruments Ltd., Worcestershire, UK). All samples for dynamic light scattering (DLS) were dispersed in deionized water (1 mg/mL), and filtered through a 450 nm filter. Transmission electron microscopy (TEM) was taken on a Tecnai-12 Bio-Twin transmission electron microscope (FEI, Netherlands) operating at 120 kV. The samples were prepared by depositing a small drop of the aqueous copolymers solution onto carbon-coated copper TEM grid. The excess of copolymer solution was wiped off with a filter paper, and the grid was dried under ambient atmosphere for 1 h.

## Micelle formation and critical micelle concentration

Micelles of 6sPCL-SS-PEG were prepared by 2.0 ml of 6sPCL-SS-PEG solution (1 mg/mL) in THF extensive dialysis against deionized water for 24 h.

The critical micelle concentration (CMC) was determined using pyrene as a fluorescence probe. The concentration of star-armed copolymer was varied from 1 mg/ml to  $4.88 \times 10^{-4}$  mg/ml and the concentration of pyrene was fixed at  $6.16 \times 10^{-7}$  M. The fluorescence spectra were recorded using Hitachi F2500 luminescence spectrometer (Hitachi, Ltd, Hong Kong) with the excitation wavelength of 310 nm. The emission fluorescence at 383 was monitored. The CMC was taken as the

intersection of the tangents to the horizontal line of intensity with relatively constant values and the diagonal line with rapid increased intensity.

### **Reduction-responsive destabilization of 6sPCL-SS-PEG micelles**

The size change of micelles in response to 10 mM DTT was followed by DLS measurement. Briefly, 2 ml solution of 6sPCL-SS-PEG micelles in deionized water with 10 mM DTT was placed in a shaking bed at 37 °C with a rotation speed of 200 rpm. The size was determined using DLS at different time intervals.

### **Encapsulation of DOX**

DOX was loaded into micelles by dialysis method, a solution of 2 mg 6sPCL-SS-PEG and 0.2 mg DOX in DMSO was dialysis against deionized water for 24 h at 37 °C (MWCO of 3500). The dialysis medium was changed five times. The whole procedure was performed in the dark. The contents inside the dialysis tube were lyophilized. The amount of DOX was determined using Hitachi F2500 luminescence spectrometer (Hitachi, Ltd, Hong Kong). For determination of drug loading content, 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:

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

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

### **Reduction-triggered release of DOX from 6sPCL-SS-PEG micelles**

The release profiles of DOX from 6sPCL-SS-PEG micelles were studied using a dialysis tube (MWCO 12 000) at 37 °C in two different media, i.e. PBS (50 mM, pH 7.4) with 10 mM DTT or PBS (50 mM, pH 7.4) only. At desired time intervals, 2 mL release media was taken out and replenished with an equal volume of fresh media.

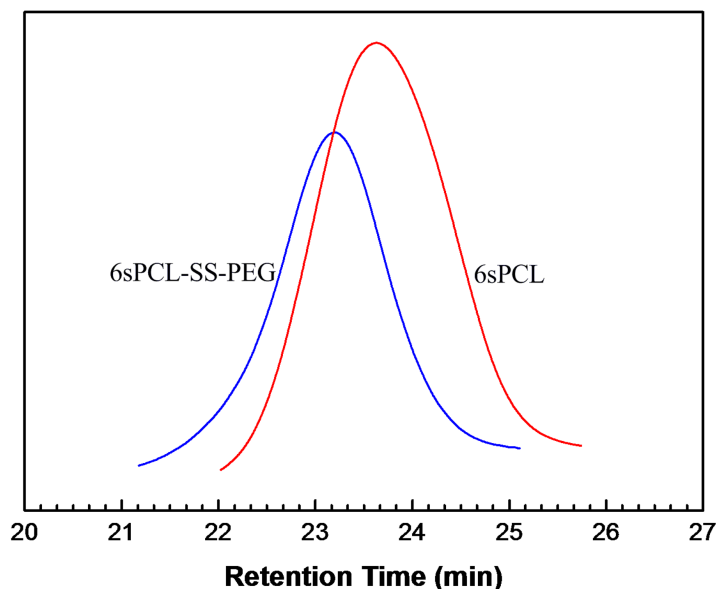
The amount of DOX released was determined by using fluorescence measurement (excitation at 470 nm).

### **Cell growth inhibition measurements**

MCF-7 cells were seeded in 96-well plate at a density of 5000 cells/well and further incubated for 24 h. The cells were then treated with 5mM/10mM GSH for 2h. After washing off GSH with PBS, DOX-loaded 6sPCL-SS-PEG micelles diluted in complete medium (DMEM-11965, Shanghai Pufei Bio-Technology Co., 100  $\mu$ L) was added to cells. As a control group, DOX-loaded 6sPCL-SS-PEG micelles DMEM solutions with the same concentrate were added to cells without GSH treatment. The cytotoxicity was evaluated by MTT assay. The cytotoxicity of blank 6sPCL-SS-PEG micelles and 5mM/10mM GSH were evaluated by the same method.

### **Confocal laser scanning microscopy observation of DOX-loaded 6sPCL-SS-PEG micelle treated MCF-7 cells**

MCF-7 cells were seeded in a 6-well plate at a density of  $1 \times 10^5$  cells/well with complete medium. The cells were incubated for 24 h in a humidified atmosphere with 5 % CO<sub>2</sub> at 37 °C. Cells were washed by PBS and incubated at 37 °C for 24 h with DOX-loaded 6sPCL-SS-PEG micelles in complete medium. The cells were washed with PBS twice and fixed with 4 % formaldehyde. The slides were mounted and observed with a laser scanning confocal microscope (Olympus, FV300, IX71, Tokyo, Japan) equipment.

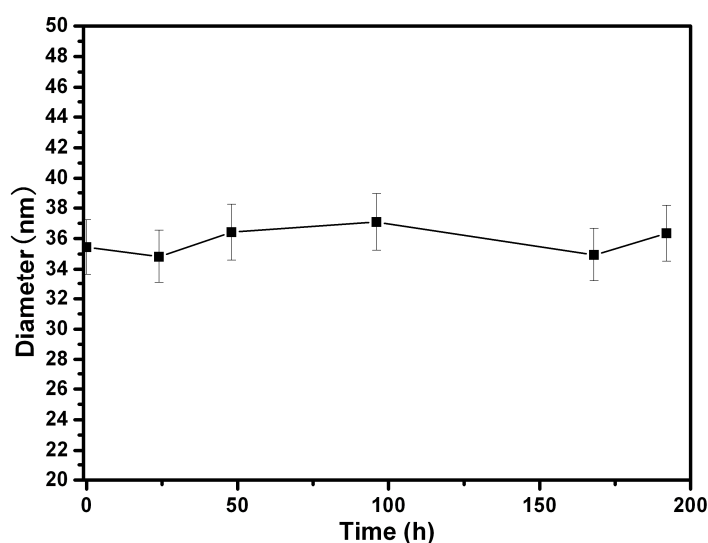


**Figure S1.** GPC trace of 6sPCL and 6sPCL-SS-PEG

The synthesis route was shown in Scheme III, which includes three main steps. Firstly, the six armed PCL was successfully synthesized via the controlled ring-opening polymerization with dipentaerythritol as initiator and  $\text{Sn}(\text{Oct})_2$  as catalyst. Secondly, mPEG-SS-COOH was prepared via hydrolytic reaction of mPEG-SS-COCl derived from reaction of PEG and thionyl chloride. Finally, the star-shaped copolymer 6sPCL-SS-PEG was obtained through the condensation reaction of star-shaped 6sPCL-OH with a slight excess of mPEG-SS-COOH in the presence of DCC and DMAP.

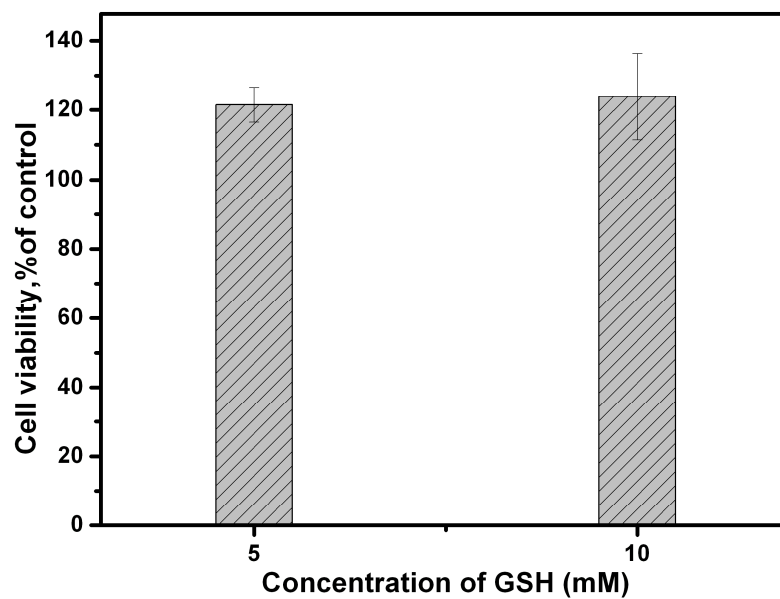
The molecular weight of the 6sPCL has been measured by gel permeation chromatography (GPC) (Figure S1,  $M_{n,\text{GPC}}=14740$ ), which is consistent with the calculation by  $^1\text{H}$  NMR spectra (Figure 1b,  $M_{n,\text{NMR}}=14600$ ). In addition, from Figure S1, GPC trace showed a monomodal shape, indicating the single composition of the synthesized polymer. The molecular weight of the 6sPCL-SS-PEG has been measured by GPC (Figure S1,  $M_{n,\text{GPC}}=26500$ ) and calculated by  $^1\text{H}$  NMR spectra (Figure 1c,  $M_{n,\text{NMR}}=28390$ ), which agrees with the theoretical value. Furthermore, the GPC traces of the copolymers (Figure S1) showed that there was a shift between two curves, which indicated the change of molecule weight from 6sPCL to 6sPCL-SS-PEG.

From Figure 1b, the  $^1\text{H}$  NMR spectrum of 6sPCL indicated the typical signals of the methylene (4) protons of the PCL at 4.05 ppm and the terminal methylene (4') protons at 3.65 ppm which was consistent with the structure of hydroxyl-terminated PCL. The weak peak detected at 4.20 ppm was attributed to the methylene protons of the dipentaerythriol initiator. Combining the molecular weight data from GPC (Figure S1) and the  $^1\text{H}$  NMR spectrum of 6sPCL, it can be inferred that the hexa-armed star-shaped PCL was successfully synthesized.



**Figure S2.** Size change of 6sPCL-SS-PEG micelles under 37 °C in 200 h determined by DLS

We can observe from Figure S2 that the diameters kept at about 36 nm without obvious difference from original diameters of the micelles, indicating that the 6sPCL-SS-PEG micelles may keep stable during circulation in vivo for a long time.



**Figure S3.** Viability of pretreated MCF-7 cells with either 5 or 10 mM of GSH for 2h