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Supporting Information for

Facile preparation of shell crosslinked micelles for redox-responsive anticancer drug release

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Experimental section

Materials

Oligo(ethylene glycol) (OEG, Fluka, M_n = 400) was dried by azeotropic distillation in the presence of dry toluene. ε-Caprolactone (CL, 99%, Acros) was distilled under reduced pressure in the presence of calcium hydride before use. DL-dithiothreitol (DTT, 99%, Aladdin), paclitaxel (PTX, 99.5%, Haoxuan Bio-technique Co. Ltd) and DL-thiomalic acid (TMA, 98 %, Aladdin) were used without further purification. Scandium triflate was synthesized according to previous report.[1] All other reagents were purchased from Shanghai Chemical Reagent and used as received.

Characterization

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker Avance DMX500 spectrometer in $CDCl_3$ with tetramethylsilane as internal standard. Gel permeation chromatography (GPC) curves were recorded on a Waters Breeze 1525 GPC analysis system with two PL mix-D column and a Waters 2414 RI detector, using DMF with 0.5 M LiBr as eluent and PMMA as calibration sample at the flow rate of 1.0 mL/min at 60 °C. FT-IR spectra were recorded on a Bruker Vector 22 IR spectrometer. The hydrodynamic diameter and size distribution of micelles were determined by dynamic light scattering (DLS) at 90° angle to the incident beam and at 25 °C on a Brookhaven 90 Plus particle size analyzer. All micelle solutions had a final concentration of 0.5 mg/mL and were filtered through 0.45 μm filters. Transmission electron microscopy (TEM) images were obtained using JEM-1230 operating at an acceleration voltage of 60 kV. A drop of 0.5 mg/mL micelle solution was placed on a copper grid with carbon film and dried before measurement. SCL micelles in aqueous solution were negatively stained by 2-wt % phosphotungstic acid and SCL micelles in DMF were positively stained by osmium tetroxide. All the ultraviolet–visible (UV) spectrophotometric measurements were performed on a Shimadzu UV-2550 spectrophotometer.

One-pot synthesis of PCL-*b*-POEGTMA-*b*-PCL by a combination of polycondensation and controlled ring-opening polymerization

Fist, anhydrous OEG (5.27 g, 13.2 mmol), TMA (1.82 g, 11.9 mmol), and $Sc(OTf)_3$ (0.06 g, 0.12 mmol) were added into eggplant-shaped bottle immersed in a 80 °C oil bath under nitrogen atmosphere with vigorous stirring. The esterification was carried out until the reaction system turned into transparent. Then polycondensation was started at 90 °C with a pressure about 30 mmHg. After 2 h, the reduced pressure was gradually increased to 0.3-3 mmHg and maintained for 10 h to complete the polycondensation. Then the bottle was refilled with nitrogen. A small quantity of the first-step product was taken by a syringe for ^{1}H NMR and GPC measurements. Sequentially, 10.0 g CL (9.35 mL, 88.0 mmol) was injected into the bottle at 65 °C and polymerized for 12 h. The crude product

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was purified by repeated precipitation from CH_2Cl_2 solution into diethyl ether three times (14.66 g, yield 85.8 %).

Preparation of micelles and shell crosslinked (SCL) micelles from triblock copolymer

20 mg triblock polymer was dissolved in 10 mL THF and 5 mL of distilled water was added dropwise into the solution. The solution were dialyzed against distilled water for 24 h using a tubular dialysis membrane (MWCO = 3500 Da). The shell cross-linked micelles were obtained by adding diluted H_2O_2 (1 mL, 3 wt-%) into the above micelles solution under vigorously stirring for 24 h and dialyzing against distilled water for another 24 h. The final micellar concentration was adjusted to 0.5 mg/mL. The solid state of SCL micelles for FT-IR measurement was recovered by lyophilization. SCL micelles solution in DMF was prepared by dialyzing the aqueous solution of SCL micelles against DMF. The final concentration in DMF was also adjusted to 0.5 mg/mL

***In vitro* cytotoxicity**

The *in vitro* relative cytotoxicities of PCL-*b*-POEGTMA-*b*-PCL triblock copolymer and its SCL micelles against HeLa cells were evaluated by a methyl thiazolyl tetrazolium (MTT) assay. Appropriate amounts of PCL-*b*-POEGTMA-*b*-PCL triblock copolymer and SCL micelles were separately dissolved in PBS to obtain their extracts with concentration of 1000, 500, 100, 50, 10, 5, 1 and 0 μg/mL, respectively. HeLa cells were seeded into 96-well plates at a density of 10000 cells per well in 180 μL of culture medium. 6 h later, 20 μL extracts of various concentrations were added to the well. Cells were exposed to the gel precursor for 48 h. Before 100 μL fresh medium and 10 μL CCK-8 reagent were added, each well was washed three times with PBS. After a further incubation of 3 h, the optical density (OD) of the wells was measured at 450 nm with a Microplate Spectrophotometer (SpectraMax M5).

Preparation of PTX-loaded SCL micelles and in vitro drug release

20 mg polymer and 2 mg PTX were dissolved in 10 mL THF and 5 mL of distilled water was added dropwise into the solution. The organic solvent and unloaded PTX were removed by dialysis against distilled water for 24 h at ambient temperature and filtration. Diluted H_2O_2 (1 mL, 3 wt-%) was added to the solutions to prepare PTX-loaded shell crosslinked micelles followed by dialyzing against distilled water to remove extra H_2O_2. The final micellar concentration was adjusted to 0.5 mg/mL and the solid-state drug-loaded SCL micelles were recovered by lyophilization. The drug-loading capacity of the SCL micelles was investigated by UV. The drug loading capacity (DLC) and drug loading efficiency (DLE) were calculated using eq. 1 and 2, respectively:

$$\text{DLC (wt-\%)} = (\text{weight of loaded drug/total weight of loaded drug and polymer}) \times 100\ \% \quad (1)$$

$$\text{DLE (\%)} = (\text{weight of loaded drug/weight of drug in feed}) \times 100\ \% \quad (2)$$

4 mL of drug-loaded SCL micelles solution (0.5 mg/mL) was introduced into a tubular dialysis membrane (MWCO = 3500 Da) against 50 mL PBS (pH 7.4) containing 10 mM DTT. Another group was conducted without DTT. At desired time intervals, 4 mL release mediums were taken out and replenished with an equal volume of corresponding fresh media. The amounts of released PTX were determined by UV measurements.

Gelation of concentrated PCL-*b*-POEGTMA-*b*-PCL solution by H_2O_2

100 mg PCL-*b*-POEGTMA-*b*-PCL triblock copolymer was dissolved in 2.5 mL THF, a drop of 30 wt-% H_2O_2 was added into the solution, macroscopic gel was formed and the gel remained intact for 5 min when the tube was inverted by 180° (Fig. S2).

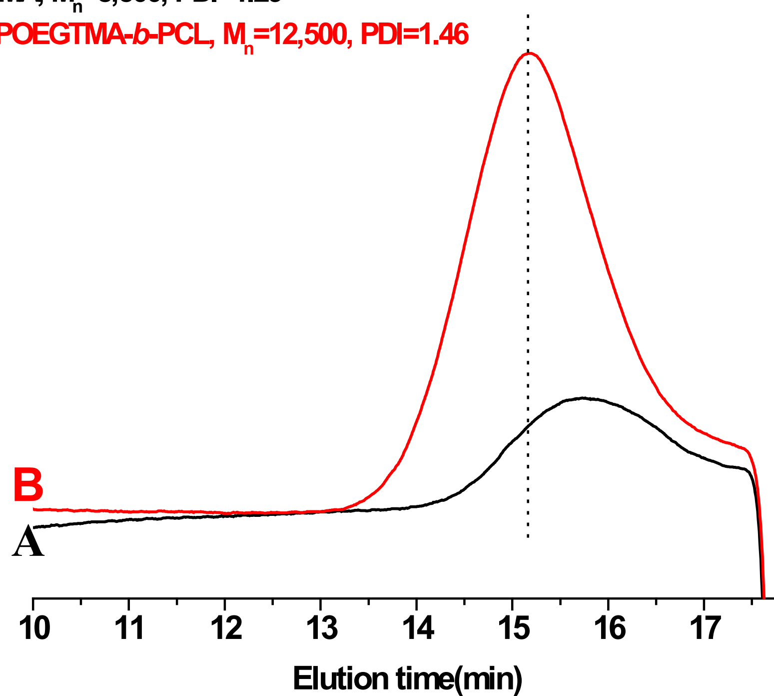

Fig. S1 GPC traces of POEGTMA and PCL-*b*-POEGTMA-*b*-PCL.

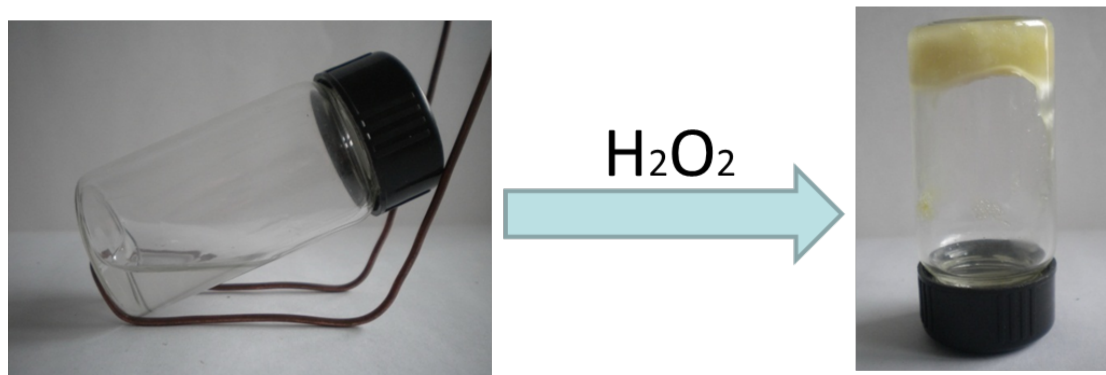

Fig. S2 Gelation of concentrated PCL-*b*-POEGTMA-*b*-PCL solution by H_2O_2.

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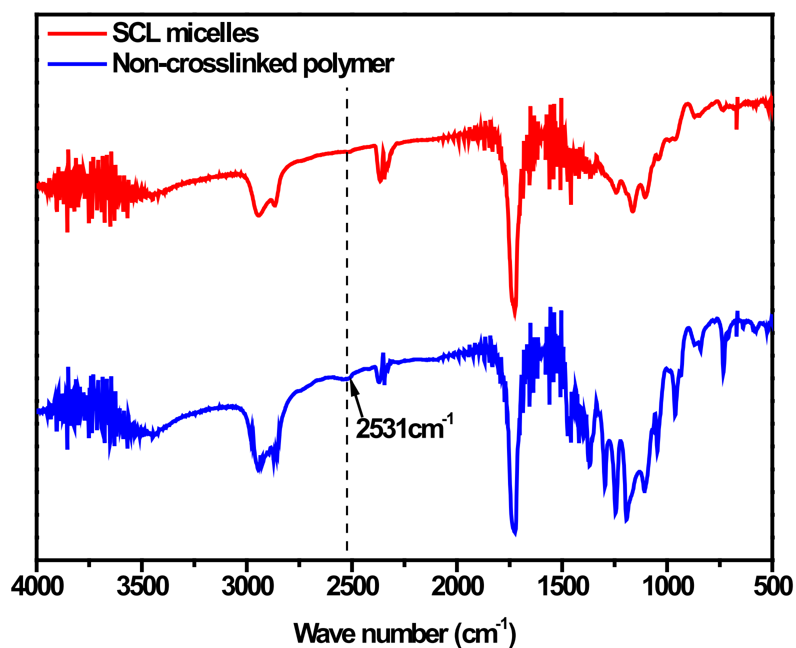
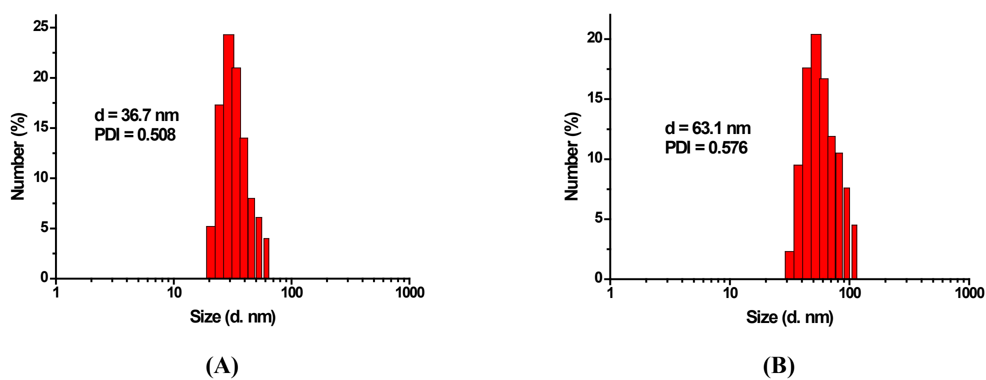


Fig. S3 FT-IR spectra of PCL-*b*-POEGTMA-*b*-PCL triblock copolymer and SCL micelles.



(A) **(B)**

Fig. S4 Particle size, distribution of (A) SCL micelles and (B) PTX loaded SCL micelles, in PBS solutions (pH = 7.4).

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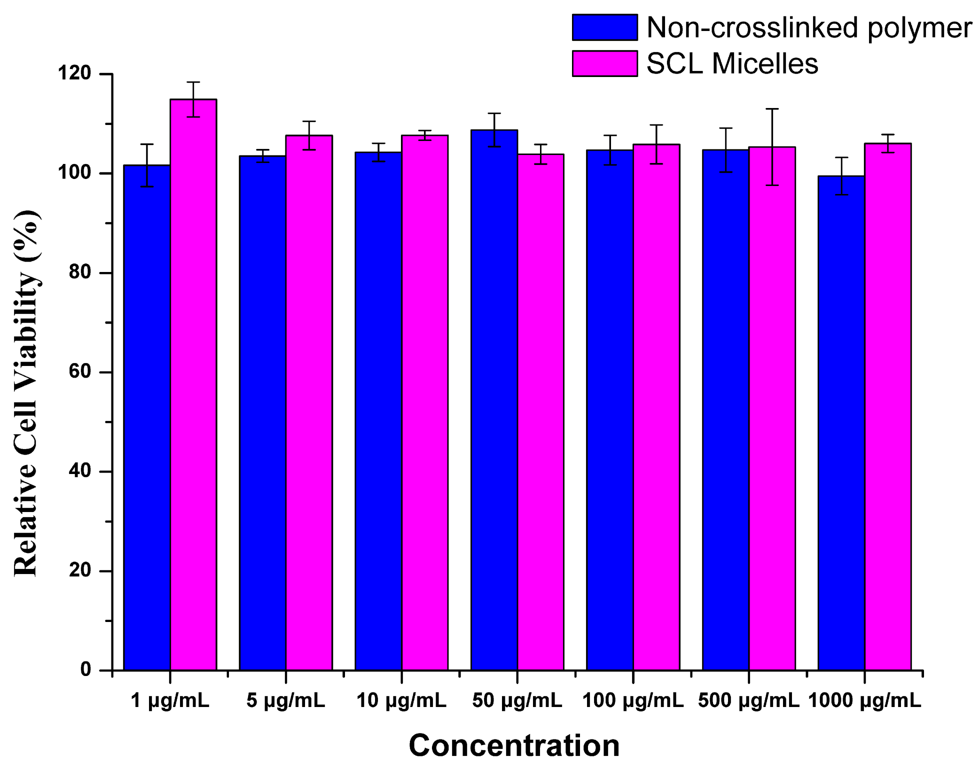

Fig. S5 Relative cell viability of PCL-*b*-POEGTMA-*b*-PCL triblock copolymer and SCL micelles against HeLa cells.

1. S. Kobayashi and I. Hachiya, *J. Org. Chem.*, 1994, **59**, 3590-3596.