Electronic Supplementary Information (ESI)

Methoxy Poly(ethylene glycol) Conjugated Denatured Bovine Serum Albumin Micelles for Effective Delivery of Camptothecin

Liming Zhang, Zhuoxuan Lu*, Xiaolong Li, Fengqin Zhang, Chao Ma, Nongyue He*

Experimental section

Preparation of Methoxy Poly(ethylene glycol) Benzaldhyde (mPEG-CHO)

mPEG-CHO was synthesized according to previous preport,[1] Typically, mPEG (5000 Da, 8 g, 4 mmol) was dissolved in 150 mL dichloromethane (DCM). p-formylbenzoic acid (6 g, 40 mmol), dicyclohexyl carbodiimide (DCC, 8.2 g, 40 mmol), and 4-(dimethylamino)pyridine (DMAP, 1.2 g, 10 mmol) were added into the mPEG solution, respectively. After being stirred for 24 h, the solution was filtered. The filtrate was concentrated, dissolved in 80 mL isopropanol, and cooled at 0 ^{o}C for 2 h. the crystals were washed with isopropanol and diethyl ether and collected by centrifuging at 8000 rpm. The final product was characterized by ^{1}H-NMR (δ 10.12 (s, 1H), 8.23 (d, 2H), 7.97 (d, 2H), 3.65 (m, CH2 of PEG), 3.39 (s, 3H)), and the data was in line with the previous literature.[1]

Synthesis of mPEG-dBSA

Bovine serum albumin (500 mg) was dissolved in 1% SDS solution (50 mL). mPEG-CHO (500 mg) was added under vigorous stirring and kept at 60 ^{o}C for 2 h. cyanoborohydride sodium hydride (1 g) was then added and kept reaction over night.

The product was filtered via a 50 K ultrafilter, and repeatedly washed with ethyl alcohol and then with distilled water. The final product was dispersed in PBS, and formed mPEG-dBSA micelles.

Characterization of mPEG-dBSA and mPEG-dBSA micelles

^{1}H-NMR data of mPEG-dBSA and BSA were obtained by a Bruker NMR spectrometer (400 M) with D_2O as the solvent. The chemical shifts were relative to tetramethylsilane at $\delta = 0$ ppm for protons. FT-IR spectra were captured by using a Thermo Nicolet 6700 FT-IR spectrometer in KBr pellets. The hydrodynamic diameter and the particle size distribution of the mPEG-dBSA micelles were measured by a dynamic light scattering spectrophotometer (DLS, Malvern Instruments Ltd.,). All samples for DLS were dispersed in ddH_2O, and filtered through a 450 nm filter. Transmission electron microscopy (TEM) was taken on a Techai G2 F20 S-Twin transmission electron microscopy equipped with Energy Dispersive Spectrometry (EDX). The samples were prepared by depositing a small drop of the aqueous solution onto carbon-coated copper TEM grid, and the grid was dried under ambient atmosphere followed by negative staining with 1% phosphotungstic acid.

CMC of the samples were calculated by fluorescence measurements data according to the previous literature[2]. In brief, fluorescence spectra were recorded on a luminescence spectrometer (Hitachi F-4600 fluorescence spectrometer). Pyrene was used as a hydrophobic fluorescent probe and placed in the tubes. Aliqouts of pyrene solutions (6×10^{-6} M in acetone, 1mL) were added to the tubes, and the acetone was

allowed to evaporate at 60 ^{o}C. Ten-millitre aqueous samples solutions with different concentrations were added to the tubes. The solutions were kept at room temperature for 24 h to reach the equilibrated solubilization of pyrene. Emission was carried at 390 nm, and the intensities at 337 nm were recorded and analysized as a function of the samples concentrations.

Encapsulation of CPT

CPT was loaded into micelles by dialysis method. A solution of mPEG-BSA (7 mg/mL, 1 mL) and different concentration of CPT in DMSO (1 mL) were subjected to dialysis against PBS for 24 h at room temperature (MWCO = 8000-14000). The dialysis medium was changed five times to remove the free CPT and DMSO. The amount of CPT was determined by UV absorbance at 365 nm.

Cell lines and cell culture

HeLa cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS). Cells were seeded in tissue culture flasks (about 3×10^5 cells) and incubated in a fully humidified atmosphere at 37 ^{o}C with 5% CO_2. For cellular uptake tracking of mPEG-dBSA, the cells were seeded in 24-well plates at a density of about 1×10^4 cells per well in culture medium (0.5 mL) and maintained for 24 h. For cytotoxicity assays, the cells were seeded in 96-well plates at a density of 3×10^3 cells per well in medium (200 μL) and maintained for 24 h.

Cellular uptake of FITC labeled mPEG-dBSA micelles and BSA

mPEG-dBSA and BSA were labeled by FITC using a FITC Protein Labeling Kit. For cellular uptake assay, same amounts of FITC-mPEG-dBSA and FITC-BSA (calculated by FITC fluorescence intensity) were incubated with HeLa cells in 24-well plates for 2 h, then the cells were washed with PBS buffer for three times. The fluorescence images were captured with a fluorescence microscope**.**

WST assays

WST assays were performed to evaluate the cytotoxicity of mPEG-dBSA, CPT loaded mPEG-dBSA and free CPT at different concentrations. In brief, HeLa cells were seeded in 96-well plates, and incubated with mPEG-dBSA, CPT loaded mPEG-dBSA and free CPT for 24 h, and washed with PBS buffer. The relative cellular viability was checked by the WST assay. The data were present as the mean ± standard deviation.

References

1. J. Gu, W.-P. Cheng, J. Liu, S.-Y. Lo, D. Smith, X. Qu and Z. Yang, *Biomacromolecules*, 2007, **9**, 255-262.
2. Y. Y. Li, X. Z. Zhang, J. L. Zhu, H. Cheng, S. X. Cheng and R. X. Zhuo, *Nanotechnology*, 2007, **18**, 215605.

Supplementary figure

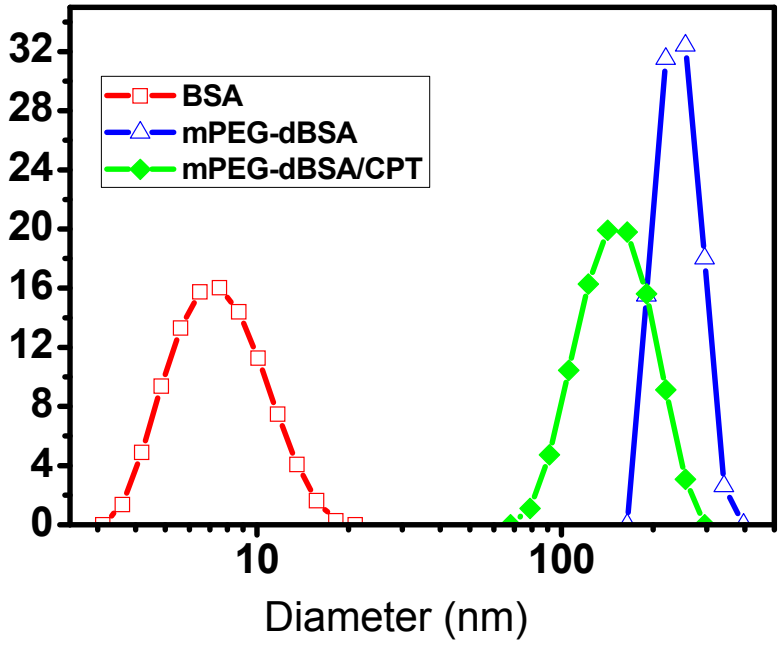

Figure S1. Hydrodynamic size of nanoparticles measured by DLS

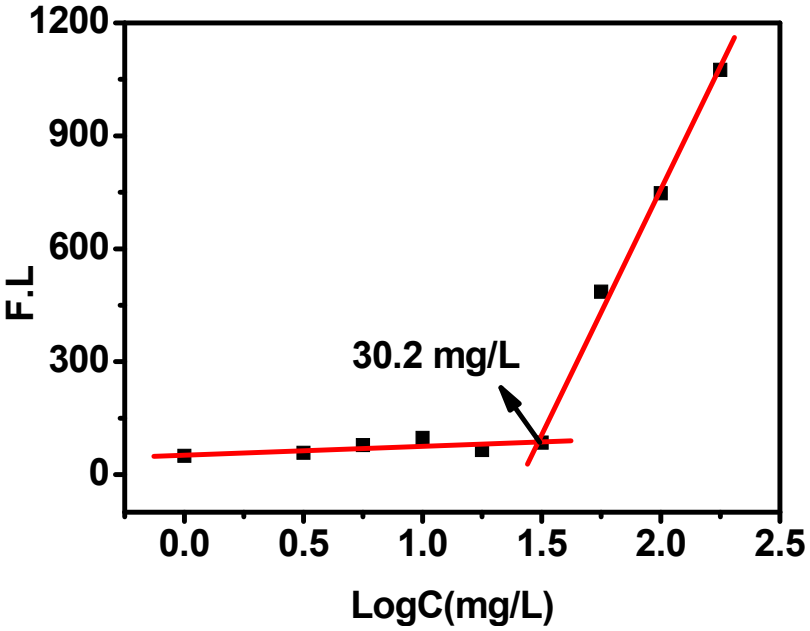

Figure S2. CMC value of mPEG-dBSA micelles measured by fluorescence measurements

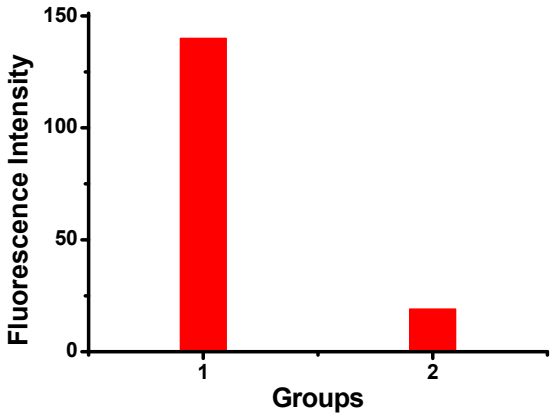

Figure S3. Cellular fluorescence intensity of groups (1) FITC-labeled mPEG-dBSA and (2) FITC-BSA after being incubated with Hela cells (analyzed by the software Image-Pro Plus version 6.0)