Electronic Supplementary Information

Polymeric micelles stabilized by polyethylenimine-copper (C_2H_5N-Cu) coordination for sustained drug release

Yu Dai,[a] Xiaojin Zhang[b,*] and Renxi Zhuo[b]

Experimental Section

1. Synthesis of PEG-PEI-PCL

ABC triblock copolymer PEG-PEI-PCL was synthesized as described in our previous report.[1] PEG-PEI-PCL is an amphiphilic linear copolymer with the molecular weight of 2000-900-2700 for each block and can form polymeric micelles by self-assembly in water.

2. Micelle Formation and Stabilization

PEG-PEI-PCL (5 mg) was dissolved in 2 mL of DMF and 5 mL of deionized water was added dropwise. The solution was dialyzed in a dialysis bag (MWCO 3500 Da) against deionized water for 2 days and the micelles were obtained by lyophilization for 3 days. The obtained micelles were re-dissolved in 5 mL of deionized water and passed through a membrane filter with a pore size of 0.22 μm.

Stabilized micelles were prepared by adding the designed amount of copper(II) sulfate pentahydrate to the micelle solution, followed by stirring at room temperature for 24 h. The solution was passed through a membrane filter with a pore size of 0.22 μm.

3. Methotrexate Encapsulation and Determination

PEG-PEI-PCL (10 mg) and methotrexate (2.0 mg) were dissolved in 4 mL of DMF. Methotrexate encapsulated micelles and stabilized micelles in deionized water were produced using the same procedure described above. 1 mL aliquot of methotrexate-loaded micelles and stabilized micelles were dried by lyophilization and then dissolved in DMF (chromatographic grade). UV absorbance at 303 nm was measured to determine the amount of loaded methotrexate with a Perkin-Elmer Lambda Bio 40 UV-Vis spectrophotometer. The drug loading content (DLC) and entrapment efficiency (EE) were calculated as follows:

DLC (%) = weight of loaded drug/weight of polymer and loaded drug × 100

EE (%) = weight of loaded drug/weight of drug in feed × 100.

4. Micelle Characterization

A JEOL-3011 HREM transmission electron microscope (TEM) was used to characterize the morphology of micelles. The samples for TEM analysis were prepared as follows: One drop of micelle solution was added onto a carbon-coated copper grid. After 3-5 min, most of the solution was removed by touching edge of filter paper until the grid surface is nearly dry. A drop of 1% phosphotungstic acid solution (pH 7.2 adjusted with NaOH) was

added onto the copper grid for negative staining. 1-2 min later, the staining solution was removed by touching on a piece of filter paper. The grid was allowed to dry under ambient conditions. The average diameter of polymeric micelles was calculated by measuring the diameters of 100 nanoparticles from the TEM images and using Gaussian fitting to fit the data. The micelle size and size distribution were determined by dynamic light scattering (DLS). Measurements were carried out and repeated three times at 25 °C with a scattering angle (θ) of 90° in optically homogeneous quartz cylinder cuvette by using a Beckman Coulter N4 Plus submicron particle sizer.

5. In Vitro Cytotoxicity Assay

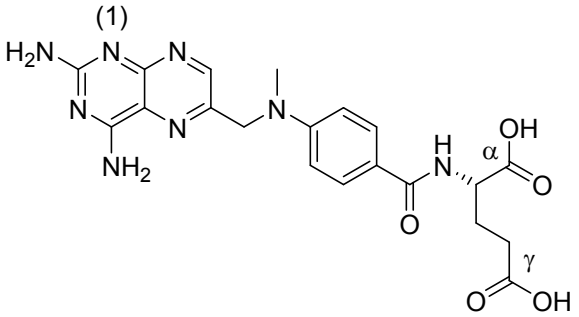
The $CuSO_4$ extract, non-stabilized micelle extract, and stabilized micelle extract were prepared by dissolving freeze-dried powder of sample into 1 ml of cell culture medium (Dubelcco's Modified Eagle's Medium, DMEM) containing 2×10^{-3} M glutamine, 10% FBS, and 50 units P/S. The extracts were filtered through a 0.22 μm membrane filter and diluted in DMEM to different concentrations. COS7 cells were seeded into a 96-well plate, 5000 cells/well, in 100 μL complete DMEM. After the cells have grown at 37 ºC for 24 h in an atmosphere containing 5% CO_2 and 95% air, 100 μL of extract was added to the wells containing cells and complete DMEM (100 μL). Cells were cultured with extracts for 24 h at 37 ºC and then 20 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) solution (5 mg mL^{-1}) in PBS was added to each well and cultured at 37 ºC for a further 4 h. 150 μL of DMSO was added to each well to dissolve the formed purple crystals derived from MTT. The absorbance of the solution was measured using microplate reader (Bio-Rad 550, USA) at 570 nm. The percent relative viability related to control well containing complete DMEM without extract was calculated by the following equation:

Cell Viability (%) = $(OD_{sample} - OD_{blank})/(OD_{control} - OD_{blank}) \times 100$

where OD_{sample} is the absorbance of the solution containing cells cultured with sample; OD_{blank} is the absorbance of the medium; and $OD_{control}$ is the absorbance of the cells cultured with the medium only.

6. In Vitro Drug Release Behaviour

The methotrexate-loaded micelle solution was placed in a dialysis bag (MWCO 3500 Da). The dialysis bag was sealed and immersed in 40 mL of phosphate-buffered saline (PBS) (pH 7.4, 0.1 M). The initial polymer concentration of the micelle solution in the drug release experiment is 0.1 mg/mL. *In vitro* drug release study of methotrexate-loaded micelles was carried out in a shaking water bath at 37 ºC. 3 mL of solution was taken out and the same volume of PBS solution was added after each sampling at predetermined time intervals. The drug concentration was determined by measuring the absorbance of methotrexate at 303 nm. The rate of drug release was measured by the released concentration of methotrexate at predetermined time intervals according to the calibration curve of methotrexate.



Scheme 1 Structural formula of methotrexate.

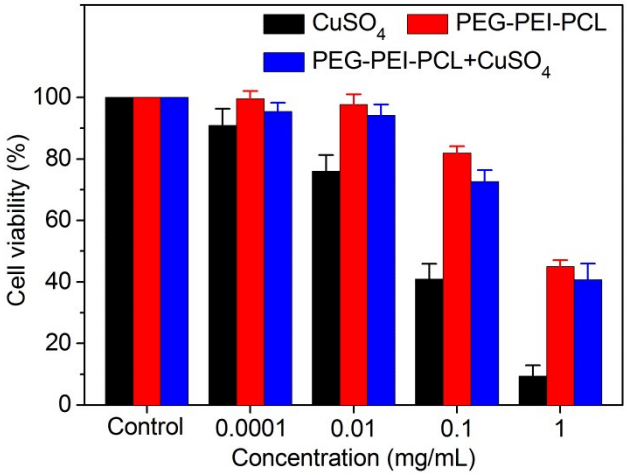


Fig. S1 Relative cell viability at 24 h on COS7 cells in the presence of $CuSO_4$, non-stabilized micelles, and stabilized micelles as demonstrated by MTT assay (average of four measurements).

Refenrences:

1. Y. Dai, Y. Li and S. P. Wang, *J. Cata.*, 2015, **329**, 425-430.