

## Supporting information

### **Gemini quaternary ammonium-incorporated biodegradable multiblock polyurethane micelles for brain drug delivery**

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## **1. Synthesis of biodegradable multiblock polyurethanes (BMPUs)**

To obtain BMPUs, GQA chain extender was synthesized in our laboratory according to previous reports<sup>1</sup>. The monomer feed ratios (Table S1) were optimized according to our previous studies<sup>1-4</sup>. BMPUs were prepared *via* a multi-step polymerization. Briefly, poly ( $\epsilon$ -caprolactone) diols (PCL, MW 2000) was dehydrated in three-necked bottle stirring for 1-2 h at 100 °C. After cooling to room temperature, an L-lysine ethyl ester diisocyanate (LDI) solution in N, N-dimethylacetamide (DMAc) was added under a dry nitrogen atmosphere and stirred for 1 h at 60 °C. Thereafter, chain extension was carried out in the presence of stannous octoate and 1, 4-butandiol (BDO) at 65 °C for 1 h and GQA chain extender at room temperature and 60 °C for 1 h and 2 h, respectively. At last, mPEG (MW 1900) was added and the reaction was continued at 60 °C and 85 °C for 1 and 5 h, respectively. After cooling overnight, the resultant solutions were precipitated three times in a mixture of methanol and diethyl ether and dried under vacuum at 60 °C for 72 h. The schematic structure of gemini cationic BMPUs is illustrated in Fig. S1.

## **2. Preparation of drug-free BMPUs micelles**

Amphiphilic multiblock polyurethanes could self-assemble into core-shell nanomicelles in aqueous solution (Fig. S2)<sup>2, 4, 5</sup>. Polyurethane nanomicelles were prepared by dialysis method. Briefly, 10 mL of BMPUs solution in DMAc was added dropwise (1 drop every 30 s) into deionized water. The resulting solution was then transferred to a dialysis tube (MWCO 3500) and dialyzed against deionized water for about 3 days to remove the organic solvent and unloaded free Dox at room temperature.

The micellar solution was centrifugalized at 3000 rpm for 10 min and passed through a 0.45  $\mu\text{m}$  pore-sized syringe filter (Milipore, Carrigtwohill, Co. Cork, Ireland). Before cell experiment, the micellar solution was passed through 0.22  $\mu\text{m}$  bacteria filter and the osmotic pressure was adjusted to physiological condition.

### 3. General measurements

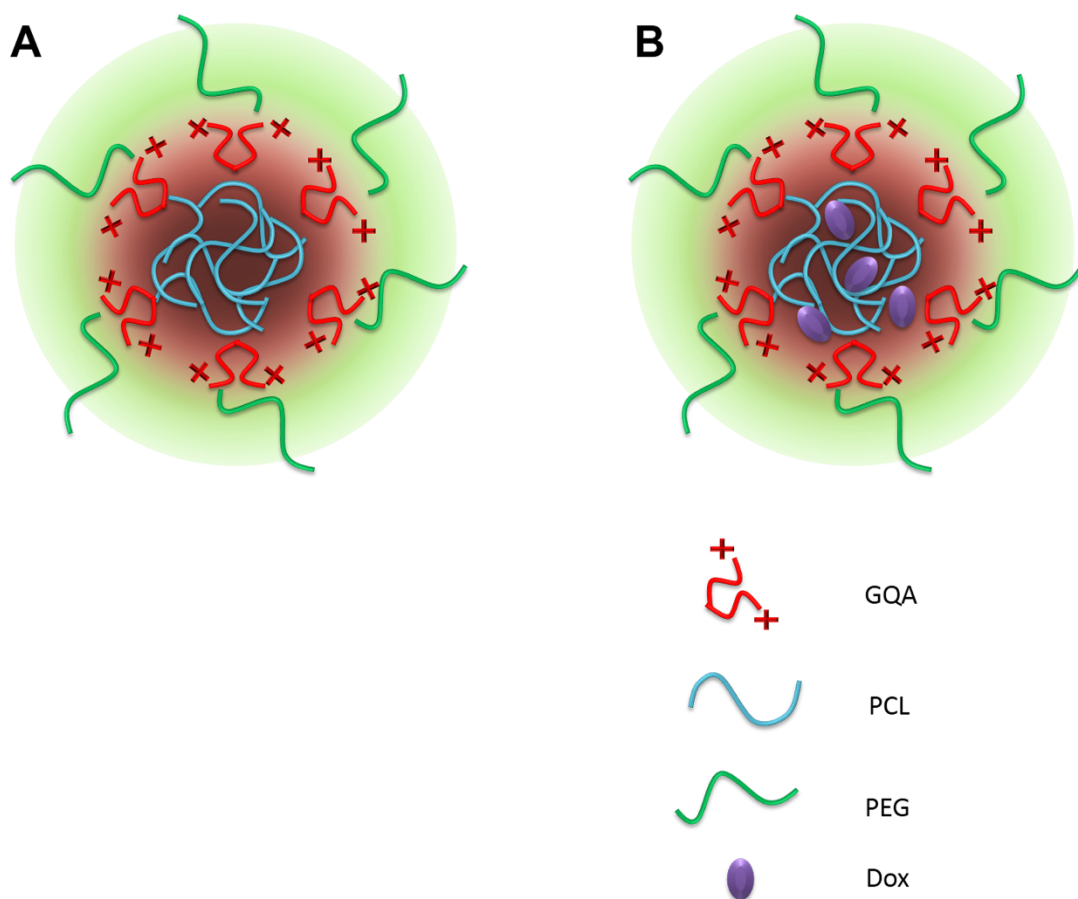
**3.1. Gel permeation chromatography (GPC).** To determine the molecular weights and molecular weight distributions of BMPUs, GPC was performed on a PL-GPC 220 (Polymer Laboratory Ltd., England) using N,N-dimethylformamide (DMF)/LiBr as eluent and polymethyl methacrylate (PMMA) as reference. The sample concentration was 1.000 mg/mL, and the flow rate was 1.000 mL/min.

**3.2. Critical Micelle Concentration (CMC).** CMC measurements were carried out using pyrene as a hydrophobic probe <sup>6</sup>. Certain amounts of pyrene in acetone were added into a series of vials. After acetone was evaporated, to each vial was added micellar solution with different concentrations. The final concentration of pyrene was  $5.0 \times 10^{-7}$  M. All the samples were equilibrated upon shaking for 2 h at 40-50 °C and incubated overnight at room temperature. Steady-state fluorescence spectra were recorded using an F-7000 FL spectrophotometer with bandwidths of 10.0 nm for excitation and 2.5 nm for emission, respectively. For fluorescence emission spectra,  $\lambda_{\text{ex}}$  was 334.0 nm, and for excitation spectra,  $\lambda_{\text{em}}$  was 373.0 nm. The intensity ratio of the peak at 337.2 nm to that at 334.0 nm ( $I_{337.2}/I_{334.0}$ ) from excitation spectra is plotted against the log of the BMPU concentration. CMCs were calculated according to the plot

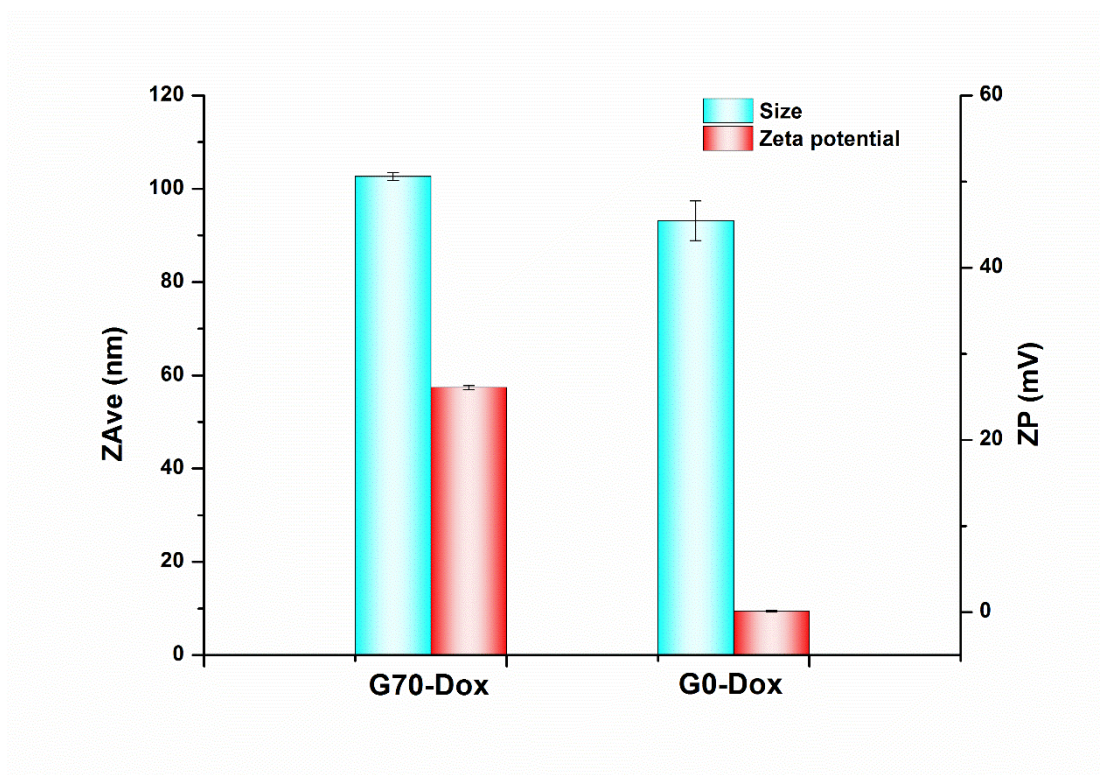
## Reference

1. M. Ding, J. Li, X. Fu, J. Zhou, H. Tan, Q. Gu and Q. Fu, *Biomacromolecules*, 2009, **10**, 2857-2865.
2. M. Ding, L. Zhou, X. Fu, H. Tan, J. Li and Q. Fu, *Soft matter*, 2010, **6**, 2087.
3. D. Mingming, Q. Zongzheng, W. Jin, L. Jiehua, T. Hong, G. Qun and F. Qiang, *Polymer Chemistry*, 2011, **2**, 885-891.
4. M. Ding, X. He, Z. Wang, J. Li, H. Tan, H. Deng, Q. Fu and Q. Gu, *Biomaterials*, 2011, **32**, 9515-9524.
5. M. Ding, X. He, L. Zhou, J. Li, H. Tan, X. Fu and Q. Fu, *J. Controlled Release*, 2011, **152**, E87-E89.
6. I. Astafieva, X. F. Zhong and A. Eisenberg, *Macromolecules*, 1993, **26**, 7339-7352.

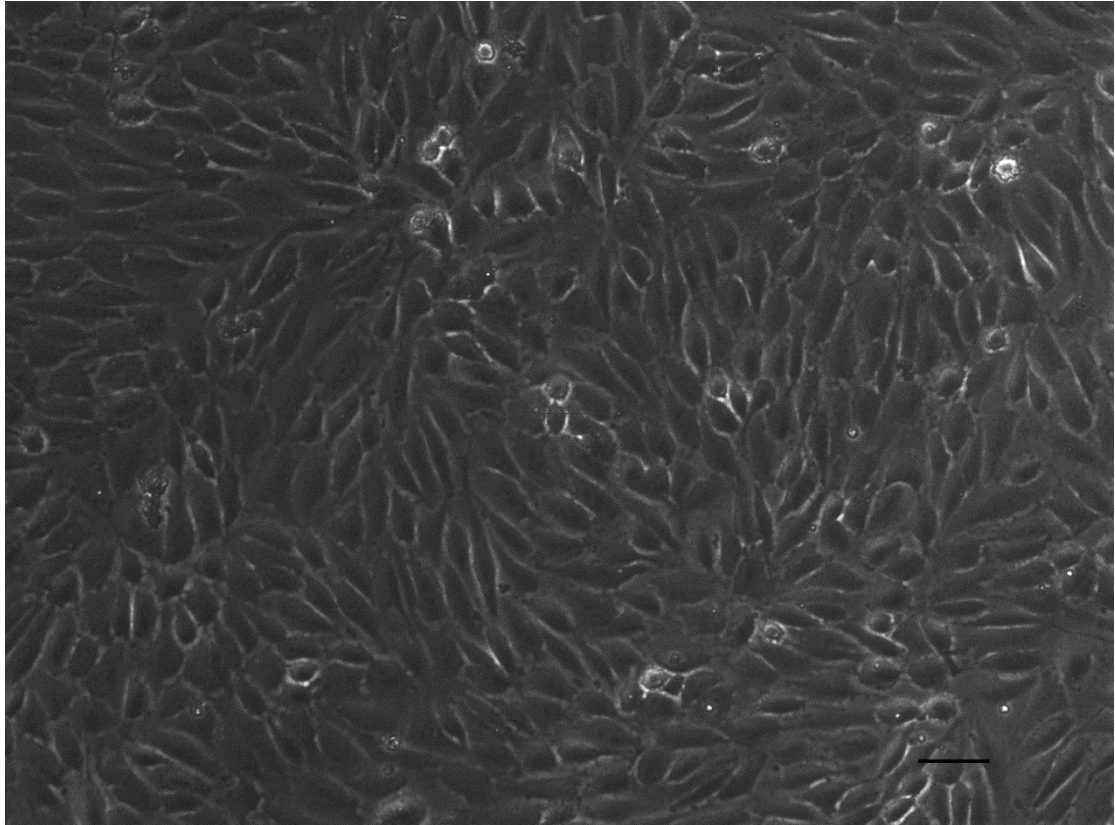




**Fig. S2.** Schematic of drug free (A) and Dox-loaded (B) gemini cationic BMPUs micelle.



**Fig. S3.** Sizes and zeta potentials of Dox-loaded BMPUs micelles.



**Fig. S4.** Bright field of human brain microvascular endothelial cells (HBMECs) 3 days after passage. Scale bar: 40  $\mu$ m.



**Table S1.** monomer feed ratios of gemini cationic BMPUs

| Samples <sup>a</sup> | Feed ratio(mol) |                  |                    |                  |     |
|----------------------|-----------------|------------------|--------------------|------------------|-----|
|                      | LDI             | PCL <sup>b</sup> | m-PEG <sup>c</sup> | Chain extends    |     |
|                      |                 |                  |                    | GA8 <sup>d</sup> | BDO |
| G0                   | 2               | 0.8              | 0.4                | 0                | 1   |
| G70                  | 2               | 0.8              | 0.4                | 0.7              | 0.3 |

<sup>a</sup> Genimi cationic multiblock polyurethanes are denoted as GX, where G is for GA8, X are for the GA8 molar content in chain extend.

<sup>b</sup> The molecular weight of PCL is 2000g/mol.

<sup>c</sup> The molecular weight of m-PEG is 1900g/mol.

<sup>d</sup> GA8 represents quaternary chain extender.

**Table S2.** Molecular weight, size, size distribution (PDI), zeta potential and critical micelle concentration (CMC) of gemini cationic BMPUs.

| Sample | M <sub>n</sub> (g/mol) <sup>a</sup> | Size (nm) <sup>b</sup> | Zeta potential (mV) <sup>b</sup> | CMC (10 <sup>-3</sup> mg mL <sup>-1</sup> ) <sup>c</sup> |
|--------|-------------------------------------|------------------------|----------------------------------|--|
| G0     | 8500                                | 118.7                  | 3.63                             | 9.42 x10 <sup>-4</sup>                                   |
| G70    | 12400                               | 24.66                  | 27.78                            | 21.59x10 <sup>-4</sup>                                   |

<sup>a</sup> Molecular weights and molecular weight distributions were determined by GPC.

<sup>b</sup> Sizes, PDI and zeta potentials of micelles were detected by a Zetasizer Nano ZS dynamic light-scattering (DLS) instrument (Malvern, UK) at 25 °C at an angle of 90°.

<sup>c</sup> CMC values were determined according to a pyrene probe fluorescence method.