Supporting information

PEGylated Poly(amine-*co*-ester) Micelles as Biodegradable Non-viral Gene Vectors with Enhanced Stability, Reduced Toxicity and Higher *In Vivo* Transfection Efficacy

Xiaofang Zhang[a,†], Wenxin Tang[b,†], Zhe Yang[a], Xingen Luo[a], Huiyan Luo[a, c], Di Gao[a], Ya Chen[a], Qing Jiang[a], Jie Liu[a,*], Zhaozhong Jiang[d,*]

[a] *Department of Biomedical Engineering, School of Engineering, Sun Yat-sen University, Guangzhou, Guangdong 510006, China. E-mail: bmeliu@gmail.com*

[b] *Research Institute of Cellular and Molecular Biology, College of Medicine, Wuhan University of Science and Technology, Wuhan, Hubei 430065, China. E-mail: tangwenxin@wust.edu.cn*

[c] *Department of Medical Oncology, Sun Yat-sen University Cancer Center, State Key Laboratory of Oncology in Southern China, Guangzhou, Guangdong 510060, China. E-mail: luohy@sysucc.org.cn*

[d] *Molecular Innovations Center, Yale University, 600 West Campus Drive, West Haven, Connecticut 06516, United States. E-mail: zhaozhong.jiang@yale.edu*

[†] These authors contribute equally to this work.

* Corresponding authors

Contents of Supporting Information

Critical micelle concentration (CMC) measurement

The CMC values of the PEG-PPMS block copolymers were determined by fluorescence spectroscopy using pyrene as a probe molecule. Briefly, pyrene solution in THF was transferred to empty containers, and the solvent was allowed to evaporate in the dark. Subsequently, a series of PEG-PPMS solutions in sodium acetate buffer (25 mM, pH = 5.2) with different polymer concentrations (1.0×10^{-4} to 1.0 mg/mL) were added to each container, and the solutions were kept at room temperature for 24 h to ensure that solubilization of pyrene in the aqueous phase reached equilibrium. Emission spectra of the solutions were recorded from 350 to 420 nm using a fluorescence steady-state system with an excitation wavelength of 334 nm. The intensity ratios (I_3/I_1) of the third peak at 391 nm vs. the first peak at 371 nm in the pyrene emission spectra were plotted as a function of logarithm of copolymer concentration. The CMC value was determined from the intersection of the best-fit lines, which corresponded to the minimum polymer concentration required for formation of stable micelles in the aqueous medium.

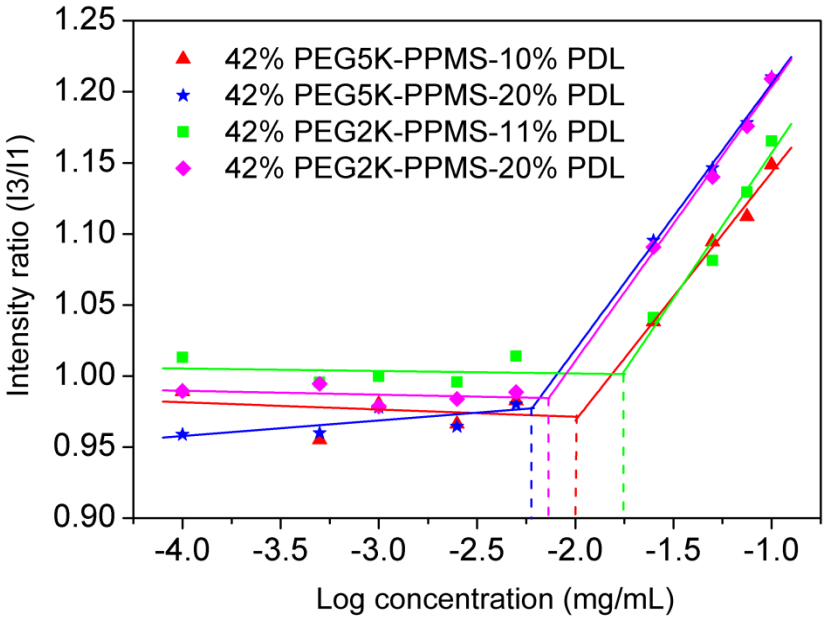

Figure S1. Variations of pyrene I_3/I_1 intensity ratio as a function of logarithm of polymer concentration (mg/mL) for PEG-PPMS micelle samples in sodium acetate buffer solution (25 mM, pH = 5.2).

The CMC values of four representative copolymer samples (42%PEG5K-PPMS-10%PDL, 42%PEG5K-PPMS-20%PDL, 42%PEG2K-PPMS-11%PDL and 42%PEG2K-PPMS-

20%PDL) were measured according to the above procedures. At concentrations below the CMC, the PEG-PPMS copolymers do not form micelles in NaAc solution and the pyrene concentration in the medium remains constant at the saturated concentration. At concentrations above the CMC, the copolymers form stable micelles and the amount of pyrene molecules entrapped in the micelle cores increases with increasing copolymer concentration. Thus, CMC is a turning point where pyrene concentration in aqueous medium starts to increase with higher polymer concentration. Figure S1 shows the pyrene fluorescence intensity ratio (I_3/I_1) vs. the logarithm of copolymer concentration for the above four polymer samples. From these curves, the CMC values were calculated to be 10.2 μg/mL, 6.0 μg/mL, 15.5 μg/mL and 8.0 μg/mL for 42%PEG5K-PPMS-10%PDL, 42%PEG5K-PPMS-20%PDL, 42%PEG2K-PPMS-11%PDL and 42%PEG2K-PPMS-20%PDL, respectively.

Analysis of fixed aqueous layer thickness (FALT)

In this study, FALT was measured using the Gouy-Chapmann theory as reported previously.[1] According to the theory, zeta potential ψ_L decreases with increasing distance (L) from the charged surface following the equation below.

$$\psi_L = \psi_0 \mathrm{e}^{-\mathrm{k}L}$$

where ψ_L is the potential at a distance L from the surface; ψ_0 is the surface charge; and k^{-1} is the Debye length. In addition, k equals to $3.33\sqrt{C}$ (C is the molar concentration of electrolyte). Since ξ is the potential at the slipping plane of micelles, the thickness (L) of the fixed aqueous layer in micelles can be determined from the slope of $\ln\xi - k$ plot. During the zeta potential measurement, micelle solution was diluted with a series of NaCl solutions. The resultant micelle solutions contained 0.2 mg/mL polymer and varied amount of sodium chloride electrolyte. The zeta potential values of the solutions were measured at least three times and reported as the mean values.

Fixed aqueous layer thickness (FALT) or the thickness of the outer PEG shell in PEG-PPMS micelles was determined from the plots of the micelle zeta potential as a function of electrolyte concentration. In ultrapure water, the zeta potential of the micelles decreases with increasing PEG molecular weight, reflecting the increased shielding of positive charges in the

micelle cores by the outer PEG shell. The hydrophilic PEG chain blocks shift the shear plane away from the surface of the hydrophobic micelle cores, thus reducing the zeta potential.

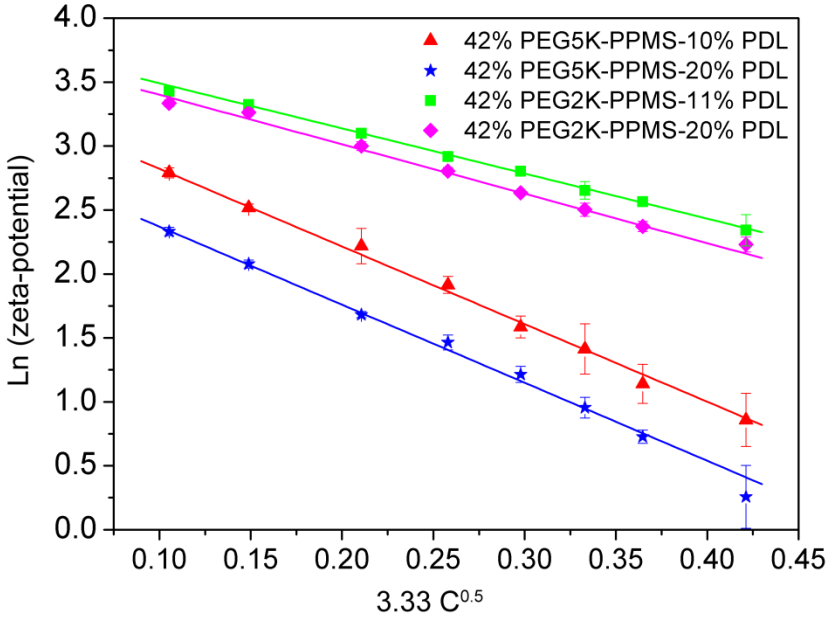

Figure S2. Plots of Ln(zeta-potential) against $3.33C^{0.5}$ (k) at various NaCl molar concentrations (C) for different PEG-PPMS micelles. The values are presented as means from at least three measurements ± SD.

As shown in Figure S2, the zeta potential decreased with increasing ionic strength in NaCl aqueous solution for all micelle samples formed from 42%PEG5K-PPMS-10%PDL, 42%PEG5K-PPMS-20%PDL, 42%PEG2K-PPMS-11%PDL and 42%PEG2K-PPMS-20%PDL. According to Gouy-Chapmann theory, the PEG shell thickness (d_{shell} in nm) of each micelle sample was obtained as the slope of its linear $\ln\xi - k$ plot. The d_{shell} values were correspondingly 6.2, 6.3, 3.5 and 3.6 nm for 42%PEG5K-PPMS-10%PDL, 42%PEG5K-PPMS-20%PDL, 42%PEG2K-PPMS-11%PDL and 42%PEG2K-PPMS-20%PDL.

Compatibility of DNA/PEG-PPMS micelles and DNA/PPMS polyplex particles with erythrocytes

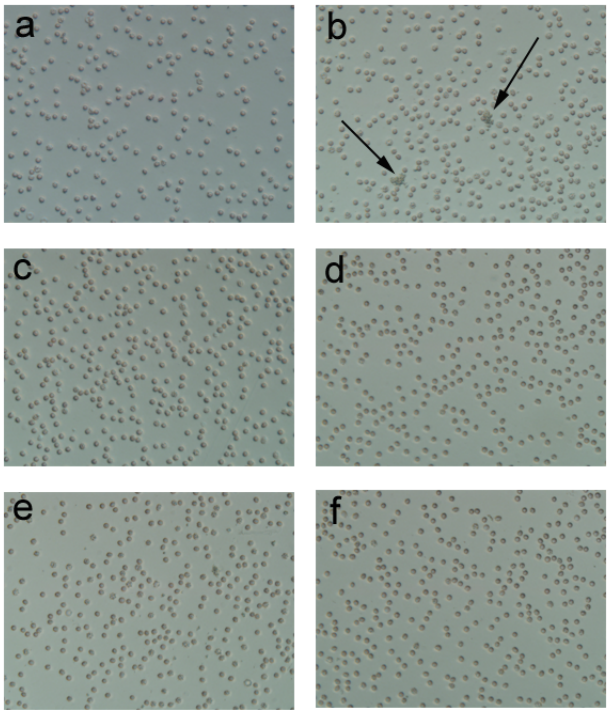

Figure S3. Microscopic images (400* magnification) of erythrocytes after being incubated at 37 °C for 2 h with (a) PBS control, (b) DNA/PPMS polyplex, (c) DNA/42%PEG5K-PPMS-10%PDL micelles, (d) DNA/42%PEG5K-PPMS-20%PDL micelles, (e) DNA/ 42%PEG2K-PPMS-11%PDL micelles, and (f) DNA/42%PEG2K-PPMS-20%PDL. The micelle and polyplex samples were formulated at N/P ratio of 150 and were used at 500 μg/mL concentration.

As shown in Figure S3-b, agglutination of erythrocytes was observable after incubation of the erythrocytes with DNA/PPMS polyplexes. In contrast, similar agglutination phenomena did not occur for the four DNA-loaded PEG-PPMS micelle samples (Figure S3-c, d, e and f). Clearly, erythrocyte agglutination was suppressed by the PEG conjugation in the PEG-PPMS copolymers. It has been reported that erythrocyte agglutination is often induced by cationic polyplexes due to strong electrostatic interactions between the positively charged polyplex particles and the negatively charged cellular membrance.[2] The reduced positive surface charge of the DNA-loaded 42%PEG-PPMS micelles vs. DNA/PPMS polyplex particles is anticipated to decrease the micelle-erythrocyte interactions, thus minimizing erythrocyte agglutination.

References

1. A. Besheer, J.r. Vogel, D. Glanz, J.r. Kressler, T. Grothand K. Mäder, *Molecular Pharmaceutics*, 2009, **6**, 407.
2. J.J. Gu, X. Wang, X.Y. Jiang, Y.Z. Chen, L.C. Chen, X.L. Fangand X.Y. Sha, *Biomaterials*, 2012, **33**, 644.