Supporting information

Fluorescent polymeric nanoparticles with ultra-low CMC for cell imaging

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Experimental Section

Materials

Phenothiazine, 1-bromooctadecane, N,N-dimethylformamide, 1,2-dichloroethane, phosphoryl chloride, 4-aminobenzyl cyanide, tetrabutylammonium hydroxide (0.8M in methanol), and glycidyl methacrylate (GM) purchased from Alfa Aesar were used as received. Poly(ethylene glycol) monomethyl ether methacylate (PEGMA, M_n = 950 Da) was purchased from ALDRICH company. All other agents and solvents were purchased from commercial sources and used directly without further purification. Ultra-pure water was used in the experiments.

Instrumentation and measurements

Gel permeation chromatography (GPC) analyses of polymers were performed using DMF as the eluent. The GPC system was a Shimadzu LC-20AD pump system comprising of an auto injector, a MZ-Gel SDplus 10.0 mm guard column (50×8.0 mm, 10^2 Å) followed by a MZ-Gel SDplus 5.0 μm bead-size columns (50-10^6 Å, linear) and a Shimadzu RID-10A refractive index detector. The system was calibrated with narrow molecular weight distribution polystyrene standards ranging from 200 to 10^6 g mol^{-1}. ^{1}H NMR spectra were measured on a Mercury-Plus 300 MHz spectrometer [d_6-DMSO as solvent and tetramethylsilane (TMS) as the internal standard]. The FT-IR spectra were obtained in a transmission mode on a Shimadzu Spectrum 8400 spectrometer (Japan). Typically, 8 scans at a resolution of 1 cm^{-1} were accumulated to obtain one spectrum. The X-ray photoelectron spectra (XPS) were performed on a VGESCALAB 220-IXL spectrometer using an Al Kα X-ray source (1486.6 eV). The energy scale was internally calibrated by reference to the binding energy (E_b) of the C1s peak of a carbon contaminant at 284.6 eV. Transmission electron microscopy (TEM) images were recorded on a HT7700 microscope (Hitachi, Japan) operated at 100 kV, the TEM specimens were made by placing a drop of the nanoparticles suspension on a carbon-coated copper grid. The size distribution of **PEG-GM-PhNH$_2$** FPNs in phosphate buffer solution (PBS) were determined using a zeta Plus apparatus (ZetaPlus, Brookhaven Instruments, Holtsville, NY). UV-Visible absorption spectra were recorded on UV/Vis/NIR 2600 spectrometer (Shimadzu, Japan) using quartz cuvettes of 1 cm path length. Fluorescence spectra were measured on an F-4600 spectrometer with a slit width of 3 nm for both excitation and emission. To measure the CMC value, six different concentrations of **PEG-GM-**

$PhNH_2$ FPNs (0.032, 0.01, 0.0032, 0.001, 0.00032, 0.0001 mg mL^{-1}) were prepared. The fluorescent excitation wavelength and emission wavelength were set as 488 and 595 nm, respectively.

Preparation of PEG-GM-$PhNH_2$ FPNs

To prepare **PEG-GM-$PhNH_2$** copolymer, a PEG monomer of PEGMA and an epoxy monomer of GM were copolymerized in ethyl acetate with the existence of AIBN through radical polymerization to afford **PEG-GM**, and then subsequent ring-opening crosslinking of **PEG-GM** with **$PhNH_2$** was conducted to obtain the resulting cross-linked copolymer, **PEG-GM-$PhNH_2$**. The preparation of **PEG-GM-$PhNH_2$** was shown in scheme 1. PEGMA (190 mg, 0.20 mmol), GM (28 mg, 0.20 mmol) and AIBN (5.0 mg) were dispersed in 6 mL of ethyl acetate and introduced into schlenk tube and purged by nitrogen flow for 30 min. Then the above mixture was put into an oil bath, which maintained at 80 °C for 12 h. Followed by adding **$PhNH_2$** (37 mg, 0.05 mmol) which was previously dissolved in 4 mL of ethyl acetate. The above mixture was stirred at room temperature for another 0.5 h. Then the reaction of crosslinking was stopped, and dialyzed against tap water for 24 h and ethanol for 6 h using 7000 Da M_w cutoff dialysis membranes. Finally, the solution in dialysis bag was carried out by freeze-drying to obtain **PEG-GM-$PhNH_2$**.

Cytotoxicity of PEG-GM-$PhNH_2$ FPNs

The cytotoxicity of **PEG-GM-$PhNH_2$** FPNs was examined by observing the cell morphology of A549 cells, which were incubated with different concentrations of **PEG-GM-$PhNH_2$** FPNs. The morphology of cells was observed by using an optical microscopy (Leica, Germany), the overall magnification was ×100. Briefly, cells were seeded in 6-well microplates at a density of 1×10^5 cells mL^{-1} in 2 mL of respective media containing 10% fetal bovine serum (FBS). After cell attachment, plates were washed with PBS and cells were treated with complete cell culture medium, or different concentrations of **PEG-GM-$PhNH_2$** FPNs prepared in 10% FBS containing media for 24 h. Then all samples were washed with PBS three times to remove the uninternalized nanoparticles.

The cell viability of **PEG-GM-$PhNH_2$** FPNs on A549 cells was evaluated by cell counting kit-8 (CCK-8) assay. Briefly, cells were seeded in 96-well microplates at a density of 5×10^4 cells mL^{-}

^{1} in 160 μL of respective media containing 10% FBS. After 24 h of cell attachment, the cells were incubated with 10, 20, 40, 80, and 120 μg mL^{-1} of **PEG-GM-PhNH$_2$** FPNs for 8 and 24 h, respectively. Then the nanoparticles were removed and cells were washed with PBS for three times. 10 μL of CCK-8 dye and 100 μL of DMEM cell culture medium were added to each well and incubated for 2 h at 37 °C. Plates were then analyzed with a microplate reader (VictorIII, Perkin-Elmer). Measurements of formazan dye absorbance were carried out at 450 nm, with the reference wavelength at 620 nm, as the values were proportional to the number of live cells. The percent reduction of CCK-8 dye was compared to control cells (no exposure to **PEG-GM-PhNH$_2$** FPNs), which represented 100% CCK-8 reduction. Three replicate wells were used per microplate, and the experiment was repeated for three times.

Confocal microscopic imaging of cells incubated with PEG-GM-PhNH$_2$ FPNs

Confocal microscopic imaging of A549 cells using **PEG-GM-PhNH$_2$** FPNs was conducted with a confocal laser scanning microscope (CLSM) Zeiss 710 3-channel (Zeiss, Germany), as the excitation wavelength was set as 488 nm. A549 cells were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% heat-inactivated FBS, 2 mM glutamine, 100 U mL^{-1} penicillin, and 100 μg mL^{-1} of streptomycin. Cell culture was maintained at 37 °C in a humidified condition of 95% air and 5% CO_2 in culture medium. Culture medium was changed every three days to maintain the exponential growth of the cells. On the day prior to treatment, cells were seeded in a glass bottom dish with a density of 1×10^5 cells per dish. On the day of treatment, the cells were incubated with **PEG-GM-PhNH$_2$** FPNs with a final concentration of 10 μg mL^{-1} for 3 h at 37 °C. Afterward, the cells were washed for three times with PBS to remove the **PEG-GM-PhNH$_2$** FPNs, and then fixed with 4% paraformaldehyde for 10 min at room temperature.

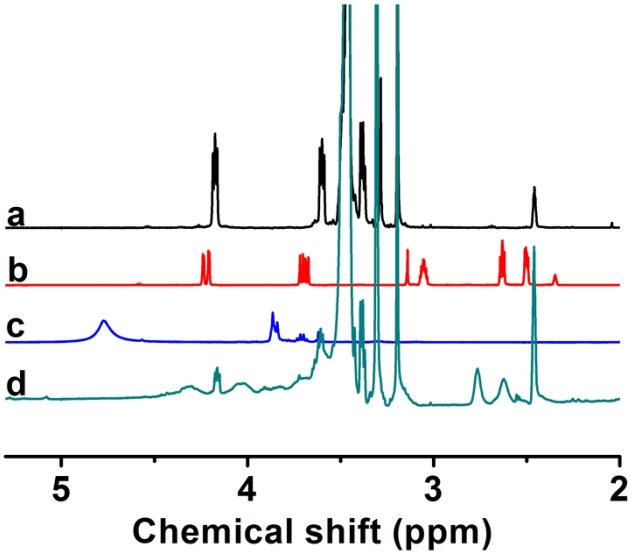

Fig. S1 Amplified ^{1}H NMR spectra of PEGMA (a), GM (b), **$PhNH_2$** (c), and **PEG-GM-$PhNH_2$** (d).

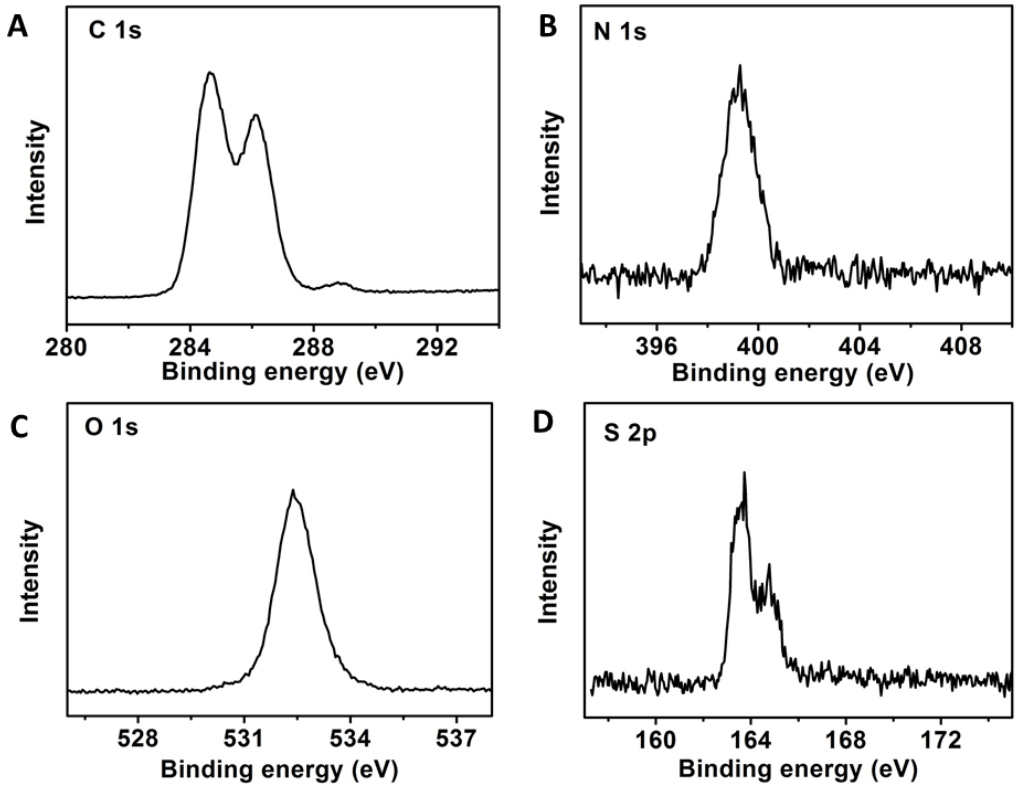

Fig. S2 XPS spectra of **PEG-GM-$PhNH_2$** FPNs: (A) C 1s spectrum; (B) N 1s spectrum; (C) O 1s spectrum; (D) S 2p spectrum.

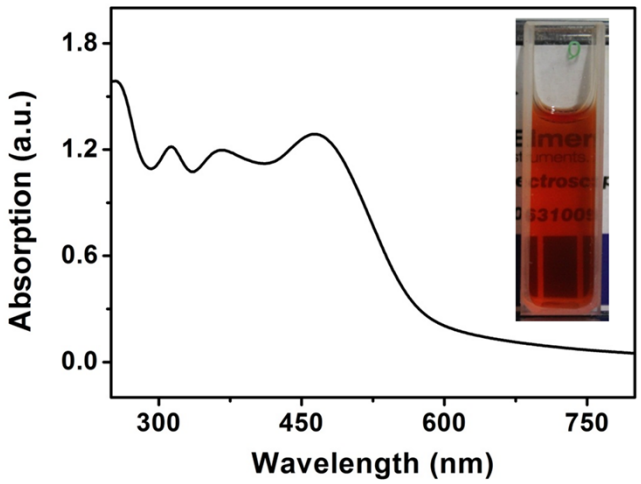

Fig. S3 UV-Vis spectrum of **PEG-GM-PhNH$_2$** FPNs, inset is the visible image of **PEG-GM-PhNH$_2$** FPNs in water.

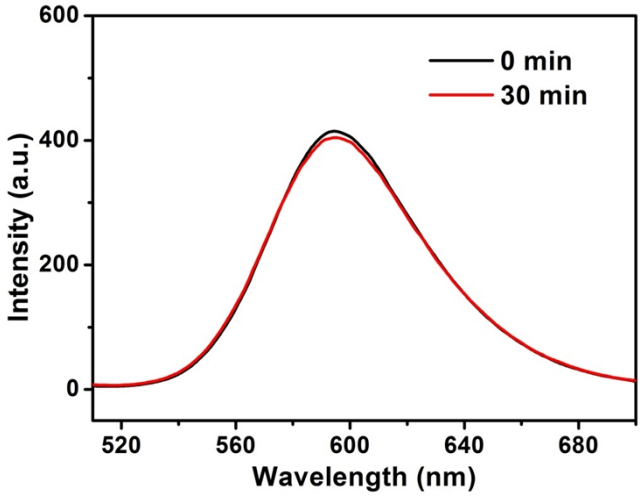

Fig. S4 PL spectra of **PEG-GM-PhNH$_2$** FPNs before and after they were irradiated with UV lamp at 365 nm for 30 min.

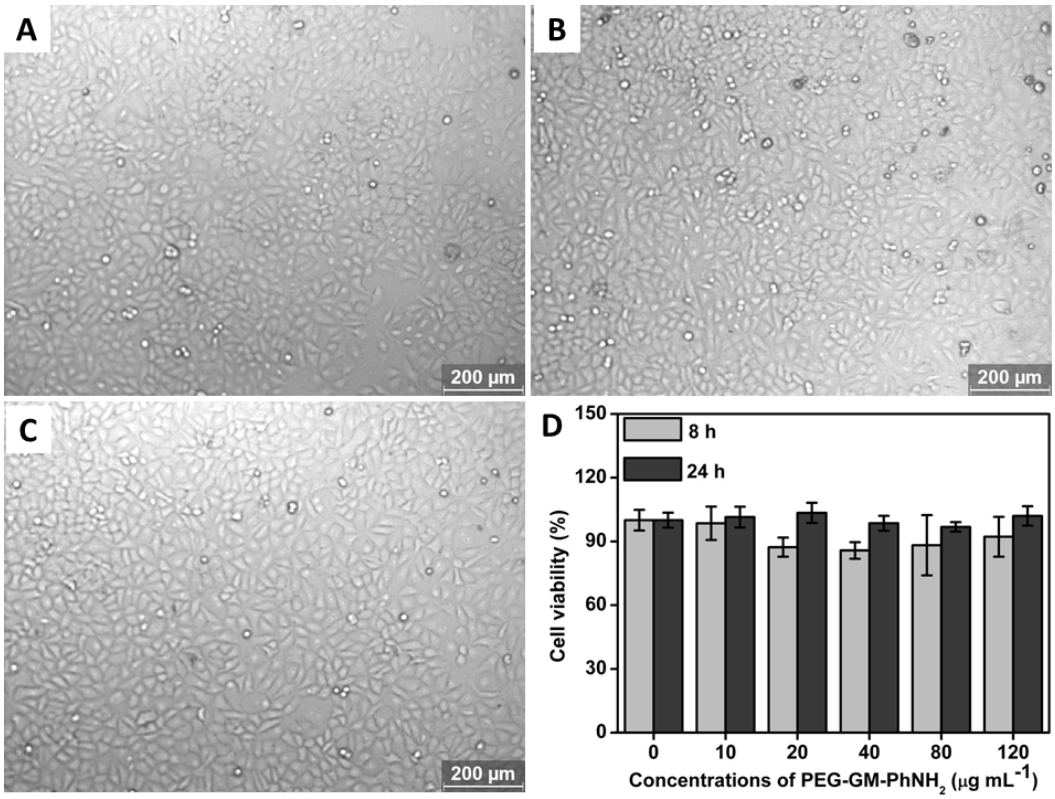

Fig. S5 Biocompatibility evaluations of **PEG-GM-PhNH$_2$** FPNs. (A-C) optical microscopy images of A549 cells incubated with different concentrations of **PEG-GM-PhNH$_2$** FPNs for 24 h: (A) control cells, (B) 10 μg mL^{-1}, (C) 80 μg mL^{-1}; (D) cell viability of **PEG-GM-PhNH$_2$** FPNs for 8 h and 24 h.