Supporting information

Nonclustered magnetite nanoparticle encapsulated biodegradable polymeric micelles with enhanced properties for in vivo tumor imaging

Synthesis of allyl-terminated block copolymer of PCL and PEG (allyl-PEG-PCL)

The copolymer was synthesized by sequential anionic ring-opening polymerization of EO and ε-CL in one pot using potassium alkoxide as an initiator. [16] THF solution (4 mL) of potassium naphthalide was allowed to mix with 0.5 mL allyl alcohol, and then the mixture was stirred for 30 min into a flame-dried reaction flask equipped with a magnetic stirring bar and two capillary gas inlets for EO and argon, respectively. Subsequently, 20 mL anhydrous THF and 1.5 g 18-crown-6 predissolved in 5 mL anhydrous THF in another flamed flask were then transferred into the first reaction flask under argon. After stirring for another 30 min, the mixture was cooled with a salted ice-water bath of -5°C. A precalculated amount of dry EO was slowly blown and condensed into the reaction mixture. Afterwards, the EO polymerization was conducted at 0°C for 24 h and then at room temperature for 3 days to ensure a thorough conversion of EO. In the second step, a predesigned amount of ε-CL was injected into the reaction flask under argon protection and then polymerized at room temperature for 48 h. The polymerization was finally quenched by adding a small amount of acetic acid. The crude copolymer collected by precipitation in hexane was redissolved in dichloromethane and added to ten-fold diethyl ether under vigorous stirring. A white powder was sequentially isolated by filtration and washed with hexane and diethyl ether. ^{1}H-NMR ($CDCl_3$): δ = 1.40 (m, 2H, -$COCH_2CH_2C\boldsymbol{H}_2CH_2CH_2O$-), 1.65 (m, 4H, -$COCH_2C\boldsymbol{H}_2CH_2C\boldsymbol{H}_2CH_2O$-), 2.31 (t, 2H, -$COC\boldsymbol{H}_2CH_2CH_2CH_2CH_2O$-), 3.65 (s, 4H,

-C***H***$_2$C***H***$_2$O-), 4.05 (t, 2H, -COCH$_2$CH$_2$CH$_2$CH$_2$C***H***$_2$O-), 4.2 (s, 2H, -CH$_2$C***H***$_2$OCO-), 5.15~5.30 (q, 1H, CH$_2$=C***H***-), 5.80 (m, 2H, C***H***$_2$=CH-).

Synthesis of NH_2-PEG-PCL

The reaction was conducted in an aqueous micelle solution, which was prepared by slowly adding a THF solution (2 mL) of allyl-PEG-PCL (0.5 g) into distilled water (20 mL) under stirring, and then allowing evaporation of THF and formation of micelles. The micelle solution was first bubbled with nitrogen for 1 h to remove oxygen, and then $K_2S_2O_8$ (0.8 molar equivalent of allyl-PEG-PCL) and 2-aminoethanethiol hydrochloride (10-fold molar equivalent of allyl-PEG-PCL) were added into the above solution. Subsequently, the micelle solution was sealed in a nitrogen atmosphere and stirred for 5 h at 52°C. Unreacted 2-aminoethanethiol hydrochloride and $K_2S_2O_8$ were removed by dialysis against water for 24 h at room temperature (MW cut-off: 8kDa). The obtained micelle solution was immediately freeze-dried (yield = 78%). ^{1}H-NMR ($CDCl_3$): δ = 1.40 (m, 2H, -COCH$_2$CH$_2$C***H***$_2$CH$_2$CH$_2$O-), 1.65 (m, 4H, -COCH$_2$C***H***$_2$CH$_2$C***H***$_2$CH$_2$O-), 2.31 (t, 2H, -COC***H***$_2$CH$_2$CH$_2$CH$_2$CH$_2$O-), 2.65~2.70 (m, 4H, -C***H***$_2$SC***H***$_2$-), 2.94 (t, 3H, H$_2$NC***H***$_2$CH$_2$S-), 3.65 (s, 4H,-C***H***$_2$C***H***$_2$O-) 4.05 (t, 2H, -COCH$_2$CH$_2$CH$_2$CH$_2$C***H***$_2$O-), 4.2 (s, 2H, -CH$_2$C***H***$_2$OCO-).

Synthesis of folate-conjugated copolymer (Fa-PEG-PCL)

Briefly, folic acid (1 g) dissolved in anhydrous DMSO (30 mL) was reacted overnight with NHS (0.9 g) in the presence of DCC (0.5 g) under argon at room temperature, and 1,3-dicyclohexylurea (DCU) was removed by filtration. Subsequently, the above activated folate solution (3 mL) was added to a DMSO solution (5 mL) containing NH_2-PEG-PCL (0.4 g) and triethylamine (0.05 mL). The reaction was performed at room temperature for 10 h under argon. The resulting solution was centrifuged and filtered. The filtrate thus obtained was dialyzed against water for 24 h (M_W cut-off: 1kDa). The aqueous solution inside the dialysis bag was then freeze-dried. The powdery sample was redissolved in THF (3 mL), and the filtrate was added dropwise to distilled water under stirring. After overnight evaporation

of THF, the resultant micelle solution was dialyzed against water for 5 days to completely remove unreacted folic acid and any residual THF. The micelle solution was finally freeze-dried to yield a solid powder (yield = 82%).

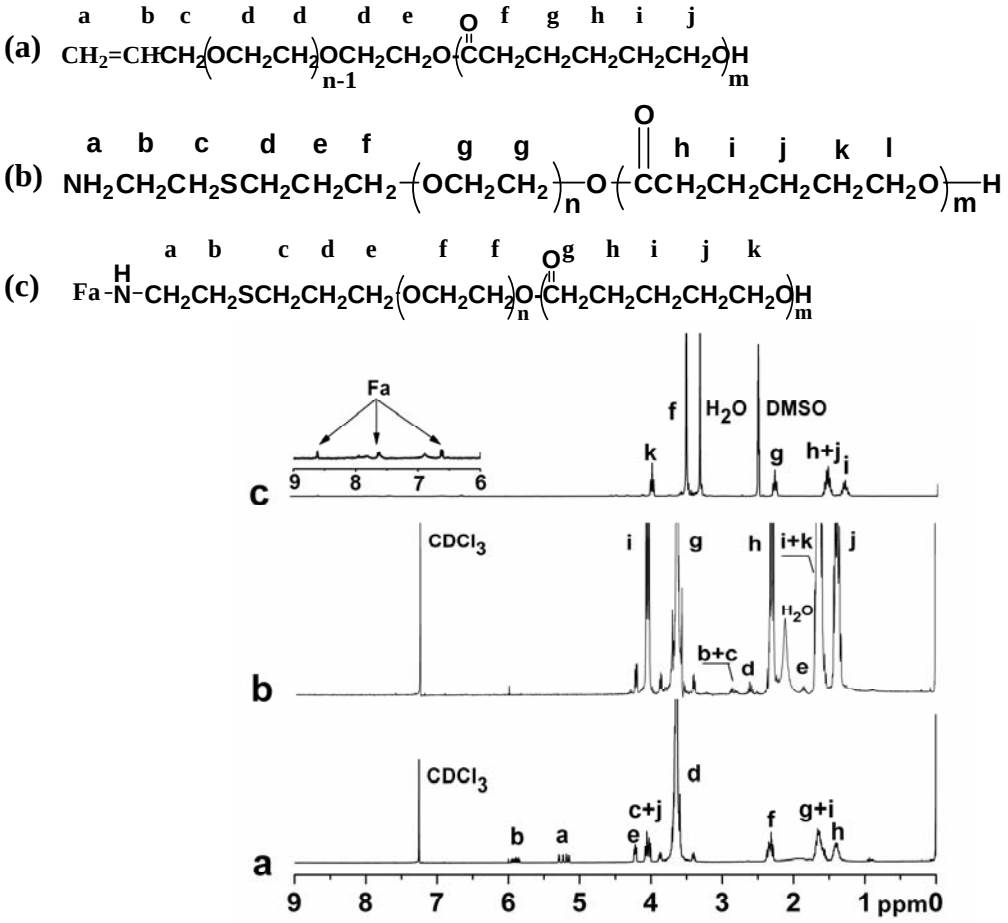


Figure S1. ^{1}H NMR spectra of ally-PEG4.3k-PCL1k in $CDCl_3$(a), NH_2-PEG4.3k-PCL1k in $CDCl_3$ (b) and Fa-PEG4.3k-PCL1k in DMSO-d_6(c)

To evaluate the conversion rate of NH_2-PEG-PCL into folate-PEG-PCL, copolymer was dissolved in DMSO and folate absorbance at 363 nm was measured by a Unico UV-2000 UV-Vis spectrophotometer to quantify the folate mass content in the sample. Absorbance of folate at 363 nm in DMSO with various concentrations was measured to generate a calibration curve. ^{1}H-NMR (DMSO-d_6): δ = 1.40 (m, 2H, -COCH$_2$CH$_2$C***H***$_2$CH$_2$CH$_2$O-), 1.65 (m, 4H, -COCH$_2$C***H***$_2$CH$_2$C***H***$_2$CH$_2$O-), 2.31 (t, 2H, -COC***H***$_2$CH$_2$CH$_2$CH$_2$CH$_2$O-), 2.65~2.70

(m, 4H, -C***H***$_2$SC***H***$_2$-), 3.2 (t, 3H, H_2NC***H***$_2$$CH_2$S-), 3.65 (s, 4H, -C***H***$_2$C***H***$_2$O-), 4.05 (t, 2H, -$COCH_2CH_2CH_2CH_2C$***H***$_2$O-), 4.2 (s, 2H, -CH_2C***H***$_2$OCO-), 4.45 (d, 2H, C_9-H_2 of folic acid), 6.61 (d, 2H, aromatic protons of folic acid), 7.60 (d, 2H, aromatic protons of folic acid), 8.62 (s, 1H, C_7-H of folic acid).

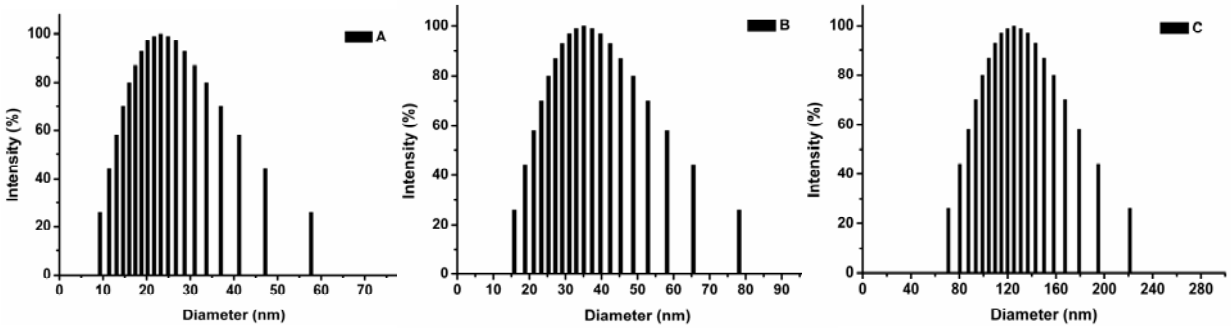


Figure S2. DLS histograms of PEG4.3k-PCL1k empty micelle (A), and SPION-encapsulated micelles PEG4.3k-PCL1k-SPION (B) and PEG4.3k-PCL7.2k-SPION (C). Measured in distilled water at a 90° scattering angle.

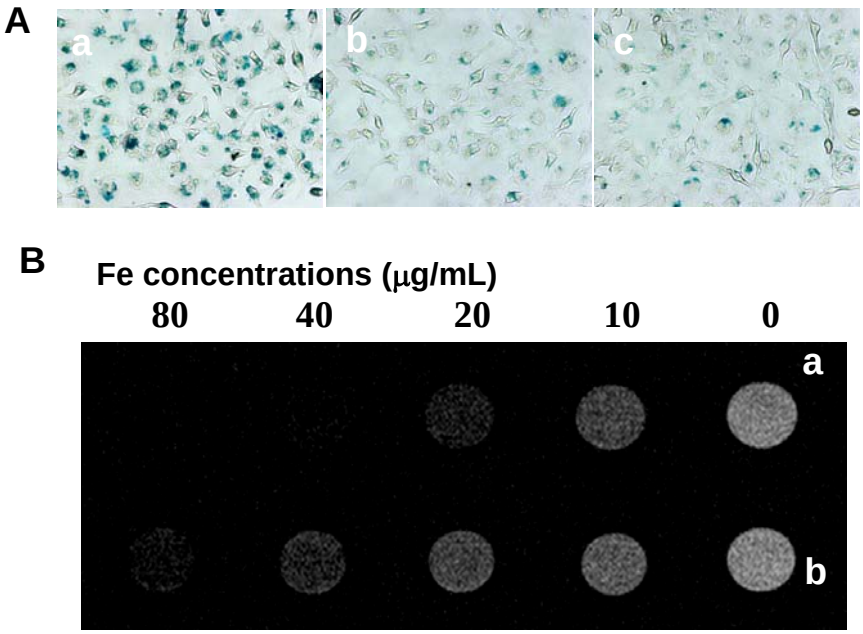
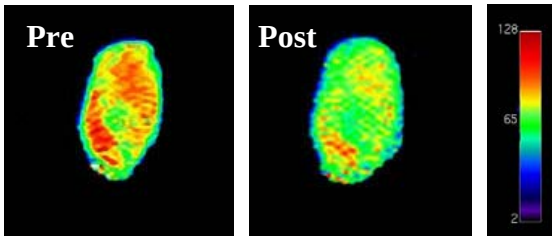
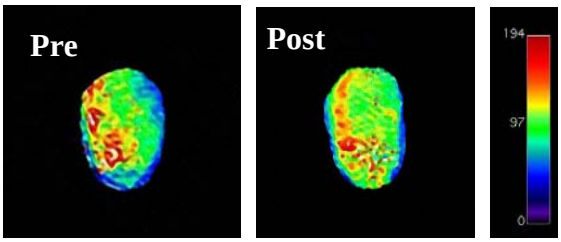


Figure S3. (A) Prussian blue staining images (×200) of Bel 7402 cells treated with Fa-PEG4.3k-PCL1k-SPIONs (a), non-targeting PEG4.3k-PCL1k-SPION (b), and Bel 7402 cells pre-treated with folic acid (1 mM) for 30 min and then with Fa-PEG4.3k-PCL1k-SPION (c). Cells incubated at 80 μg/mL Fe for 1 h in a folate-free RPMI 1640 medium. (B)

T_2-weighted MRI images (1.5 T, TR=5000 ms, TE= 65 ms) of Bel 7402 cells after 1 h incubation with Fa-PEG4.3k-PCL1k-SPION (a) and PEG4.3k-PCL1k-SPION (b) respectively, at various Fe concentrations in folate-free RPMI 1640 medium. Cells were mixed with 2% agarose solution in PBS before being scanned on 1.5 T MRI scanner (Philips Intera,Netherlands, B.V.).



Fa-PEG4.3k-PCL1k-SPION



PEG4.3k-PCL1k-SPION

Figure S4. T_2 mapping of tumors picked from two mice before and 3h after receiving administration of folate targeted and nontargeted micelles. Mice showed more obvious T_2 shortening in tumor upon injection of the targeting nanomicelle rather than the nontargeting one.