< Supplementary Information >

Poly(amino acid)s micelle-mediated assembly of magnetite nanoparticles for ultra-sensitive long-term tumor imaging

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Synthesis of poly-α,β-(N-2-dimethylaminoethyl $_L$-aspartamide)-g-octadecyl (PDMAEA-g-C_{18}): As-synthesized PSI (5 mmole, 485 mg, DP = 125 by GPC) was dissolved in DMF (5 mL) and added with octadecylamine (0.5 mmole, 135 mg). Grafting reaction was maintained at 70 ^{o}C for 24 hours and followed by aminolysis with *N,N*-dimethylethylenediamine (6 mmole, 0.665 mL) at 40 ^{o}C for 12 hours. The polymer product was obtained by precipitation into diethyl ether.

Aggregation of 6 nm-sized magnetite nanoparticles enclosed with PDMAEA-g-C_{18} (P-SPION): Mixture of PDMAEA-*g*-C_{18} solution in water (50 mg in 5 mL) magnetite nanoparticle solution in chloroform (10 mg in 0.2 mL) was vortexed at 3000 rpm for 25 minutes and followed by sonication for 5 minutes to generate an emulsion. Chloroform was evaporated using a rotary evaporator and unloaded magnetite nanoparticles were removed by centrifugation at 7000 rpm for 10 minutes. P-SPION micelles were selectively collected by centrifugation at 15000 rpm for 1 hour.

T_2 relaxivity measurement: Solutions of P-SPION and Feridex® containing Fe at concentrations of 31.25, 62.5, 125, and 250 μM were prepared in DDI water. MR T_2 mapping experiments to obtain T_2 relaxation time and T_2 relaxivity coefficient were performed with a 4.7-T clinical MRI instrument (Bruker BioSpec 47/40) with the following TR (repetition time) and TE (echo time): TR = 10000 msec, TE = 5.707 - 1460.992 msec with an interval of 5.707 msec.

In vitro/in vivoMR imaging: MR imaging of both CT26 cells *in vitro* and a mouse implanted with CT26 tumor were performed with a 1.5-T MR scanner (GE Signa Exite Twin-speed, GE Health Care System, Milwaukee, USA) using an animal coil (4.3 cm Quadrature volume coil, Nova Medical System, Wilmington, DE). CT26 cells were treated with P-SPION at a concentration of 100 $\mu g \cdot mL^{-1}$ for 24 hours and then collected using gelatin. For T_2-weighted MR imaging of CT26 cells, parameters were set as follows: TR/TE, 3000/102 msec; flip angle, 90°; echo train length, 10; field of view, 5 cm; section thickness, 2 mm; intersection gap, 0.2 mm; matrix, 256 × 160. A CT26 tumor bearing mouse anesthetized by inhalation of 1.5 % isoflurane in 1:2 O_2/N_2 was intravenously injected with P-SPION through the tail vein with a dose of 15 mg $Fe \cdot kg^{-1}$. T_2-weighted *in vivo* MR images were obtained before injection and at 0.5, 2, 3, 22, and 72 hours after injection with the same parameters same as applied for the *in vitro* experiments. The signal intensity (SI) was measured in defined regions of interest (ROIs), which were in similar locations within the tumor center. The SI of ROIs in the back muscle adjacent to the tumor and in the liver was also measured with the size of each ROI being two-thirds the maximum diameter of the tumor. Relative signal enhancement was calculated by using SI measurements before (SI pre) and after (SI post) injection of P-SPION according to the following formula: relative signal enhancement (%) = 100 × [1-(SI post in tumor/SI post in muscle)/(SI pre in tumor/SI pre in muscle)]. *Ex vivo* Prussian blue staining was performed with CT26 tumor tissue collected from the mouse immediately after MR imaging of 72 hour postinjection.

Fig. S1. Synthetic route of PDMAEA-*g*-C_{18}.

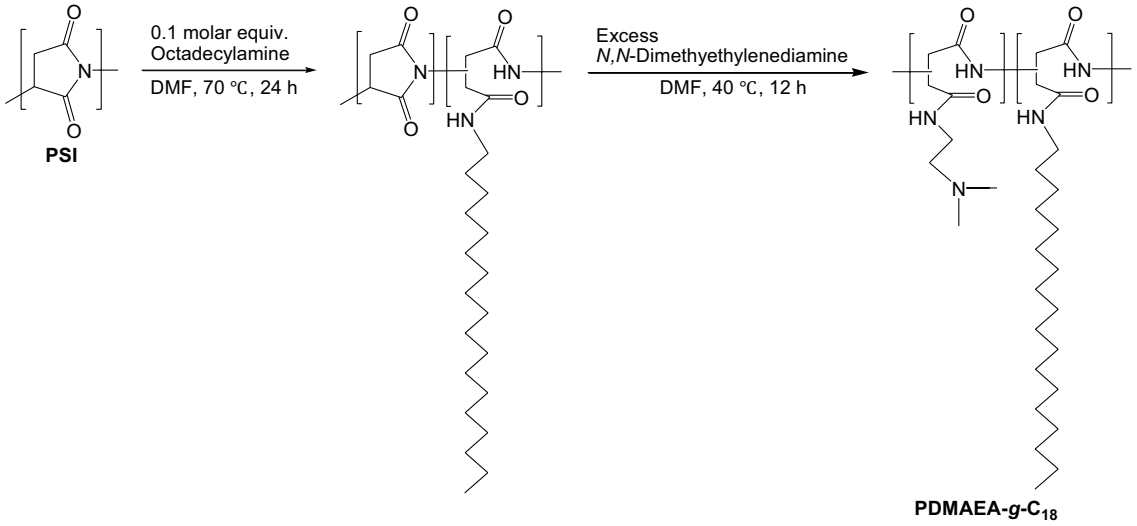

Fig. S2. Molecular formula of PDMAEA-*g*-C_{18} and the ^{1}H NMR spectrum in DMSO-d_6.

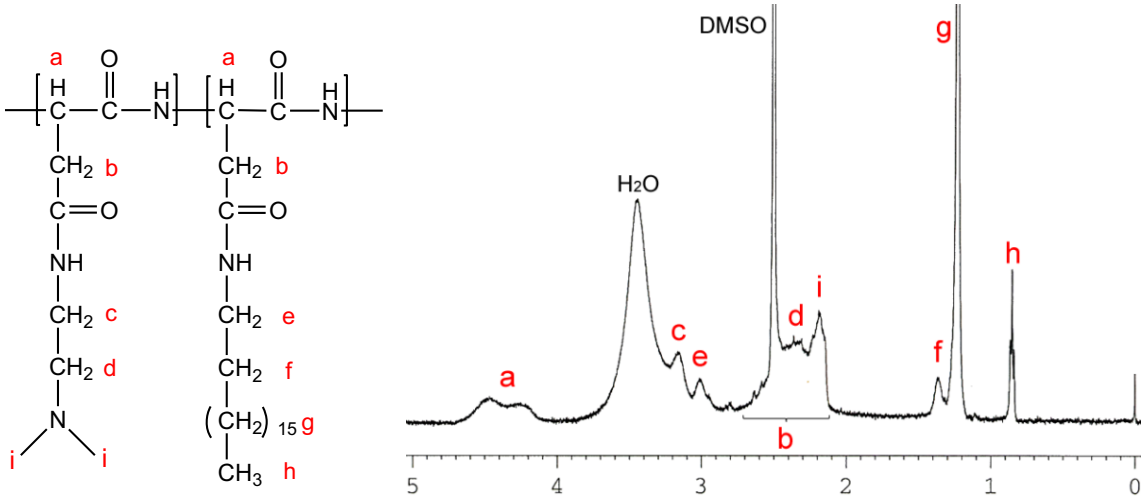

Fig. S3. Change of hydrodynamic mean diameter with time and pH variance.

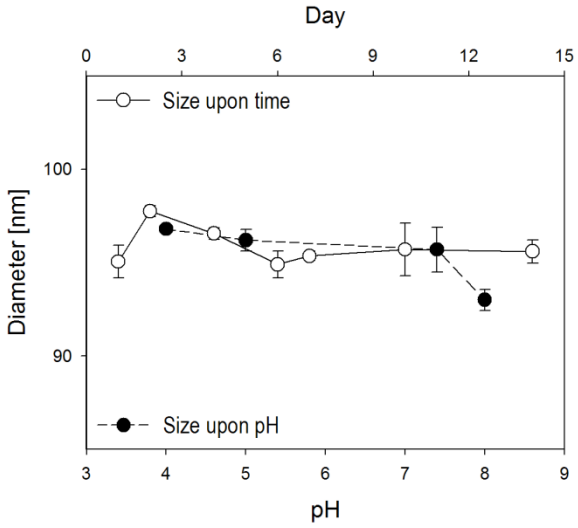

Fig. S4. (a) SEM image of P-SPION along with (b) EDS analysis. Scale bar in (a) represents 100 nm.

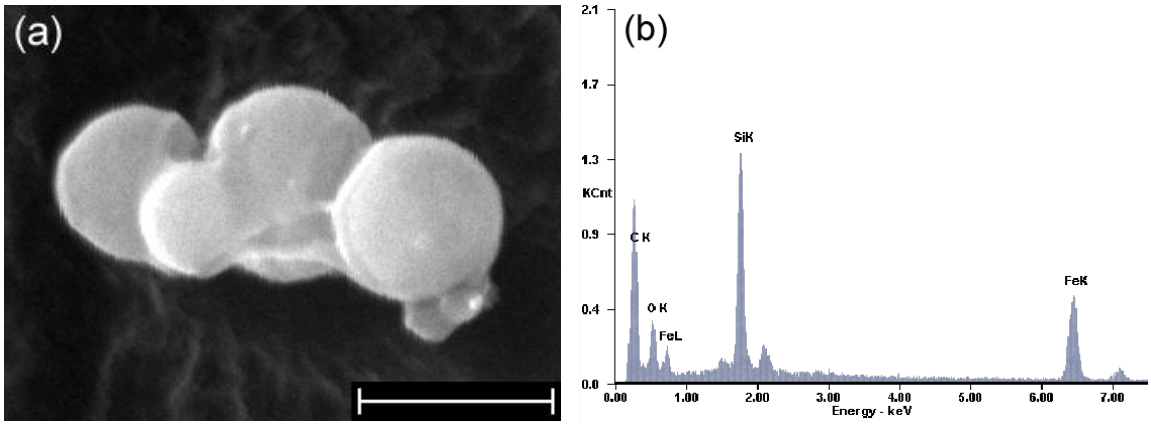

Fig. S5. Magnetic field-dependent magnetization curve of P-SPION and MNP6 obtained by using SQUID magnetometer at 300 K.

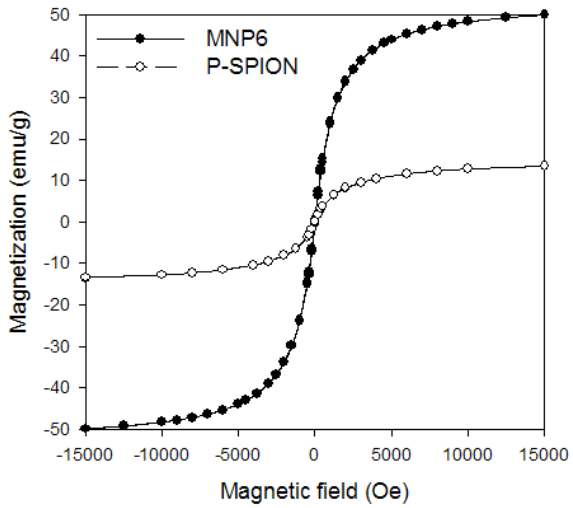

Fig. S6. Cell cytotoxicity of P-SPION determined by MTT assay. CT26 cells were incubated with P-SPION solutions of various concentrations for 24 hours. (*: $p < 0.05$)

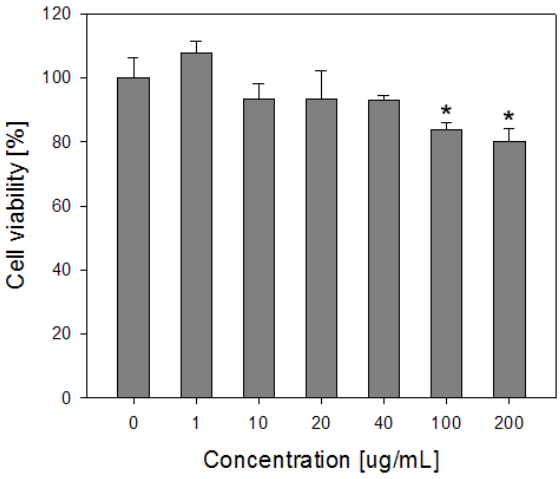

Fig. S7. Change of tumor-to-muscle and liver-to-muscle signal intensity ratios compared to preinjection.

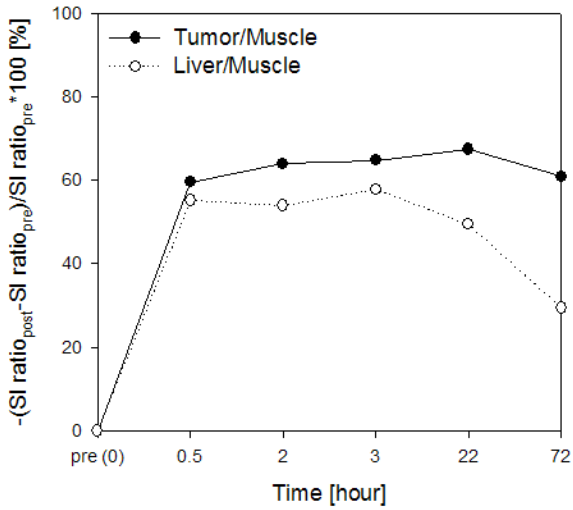

Fig. S8. *Ex vivo* Prussian blue staining image of tumor tissue extracted from the mouse at 72-hour postinjection.