Supporting Information

Reductive microenvironment responsive gadolinium-based polymers as potential safe MRI contrast agents

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Materials and Methods:

^{1}H NMR data was recorded via a 400 MHz Bruker Advanced Spectrometer under room temperature. The short peptide GFLG content was measured by amino acid analysis as previously reported.[1] Particle size and zeta potential were measured in distilled water by a Zetasizer Nano ZS (Malvern Instruments, UK). The ex vivo fluorescent images were obtained using a Maestro In-Vivo Imaging System (Cri, USA). The content of the thiol groups in the copolymers was tested as previously reported.[1] The cancer cell was 4T1 cell line (murine breast cancer cell). The animals are female BALB/c mice obtained from Chengdu DaShuo Biological Technology Co., Ltd. China.

MW and PDI

The MW and polydispersity (PDI) of the copolymer were tested via size-exclusion chromatography (SEC) on a Superose 6 HR10/30 column and on an ÄKTA/FPLC system (GE Healthcare). Sodium acetate buffer/methanol (7:3, pH 6.5) was used as mobile phase with a corresponding flow rate of 0.4 mL/min. The copolymers were purified by SEC via a Superose 6 HR10/30 column, while the mobile phase was sodium acetate buffer/methanol (7: 3, pH 6.2), and the flow rate was 2.5 mL/min, and the temperature was 4 ^{o}C. The products were fractionated/purified by size exclusion chromatography using Superose 6 HR10/30 (MW range for hydrophilic neutral polymers 15-300 kDa/14 mL separation volume) column on an ÄKTA FPLC system (GE Healthcare) column with sodium acetate buffer containing 30% methanol (pH = 6.5) as the mobile phase.

Biodegradability of the Gd-pDHPMA CAs

Biodegradation studies of the Gd-pDHPMA CAs were studied in McIlvaine's buffer (pH 5.4) with 2.8 μM cathepsin B. The concentration of the copolymer (pDHPMA-Cy5.5-DOTA-Gd and pDHPMA-Cy5.5-SS-DOTA-Gd) was 3 mg/mL. The mixture

was incubated for 0, 2, 4, 6, 10 h at 37 °C. Thereafter, tested samples were measured by SEC.

Table S1. The amino acids content of the Gd-pDHPMA CAs by weight percent.

Copolymer	Gly%	Phe%	Leu%
pDHPMA-Cy5.5-DOTA-Gd	0.10	0.11	0.08
pDHPMA-Cy5.5-SS-DOTA-Gd	0.13	0.15	0.11

Table S2. The results of the degraded products after incubation of the Gd-pDHPMA CAs in McIlvaine's buffer with cathepsin B (2.8 μM, pH = 5.4) at 37 °C.

Gd-pDHPMA CAs	0 h	2 h	4 h	8 h
pDHPMA-Cy5.5-DOTA-Gd	95 kDa, PDI 1.20	66 kDa, PDI 1.84	48 kDa, PDI 1.34	42 kDa, PDI 1.15
pDHPMA-Cy5.5-SS-DOTA-Gd	92 kDa, PDI 1.21	61 kDa, PDI 1.64	42 kDa, PDI 1.35	39 kDa, PDI 1.11

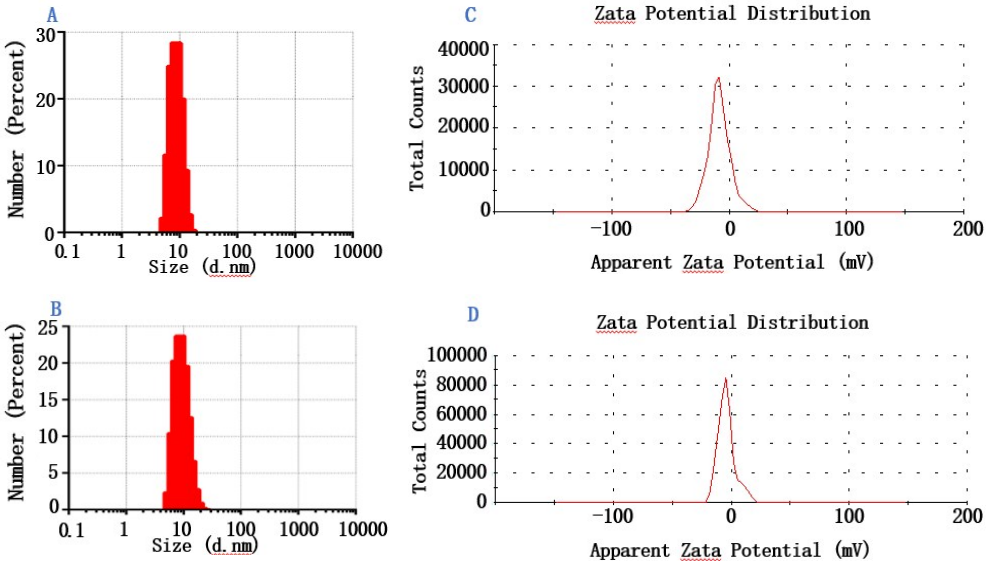

Figure S1. Particle size of (A) pDHPMA-Cy5.5-DOTA-Gd; (B) pDHPMA-Cy5.5-SS-DOTA-Gd and zata potential distribution of (C) pDHPMA-Cy5.5-DOTA-Gd; (D) pDHPMA-Cy5.5-SS-DOTA-Gd by DLS.

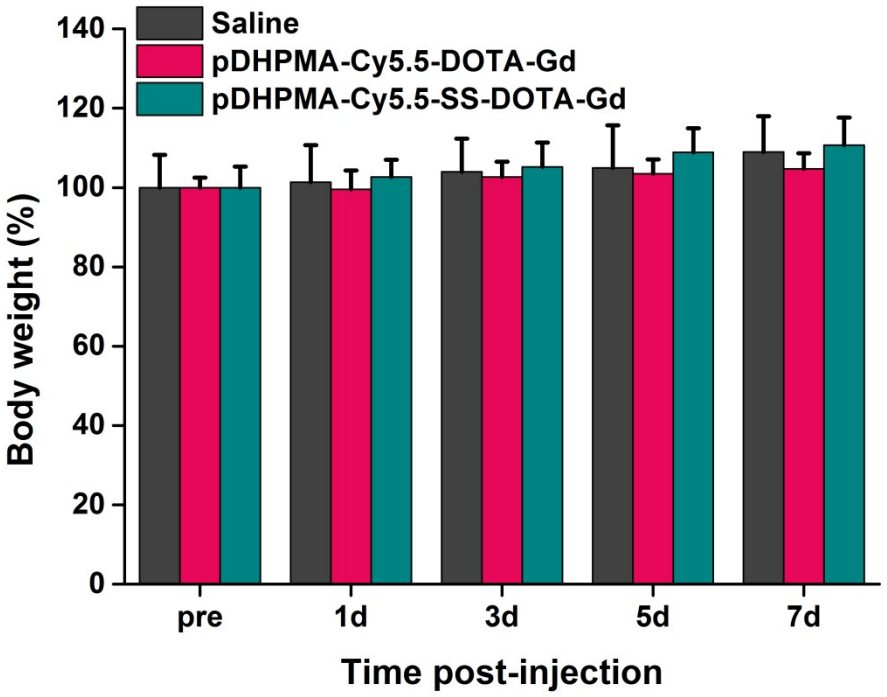

Figure S2. Body weight of mice post-injection. The weight pre-injection was standardized to 100%.

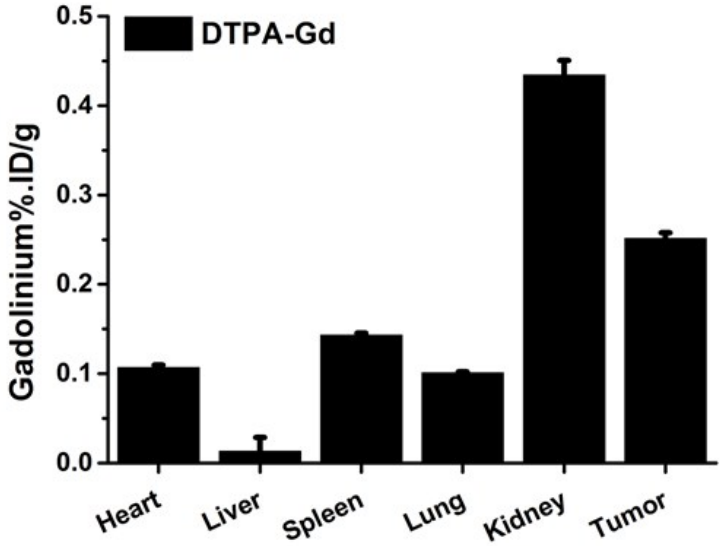

Figure S3. Tissue distribution at 24 h post injection.

In vitro release of gaodolinium

The release profiles of Gd(III) from copolymers (pDHPMA-Cy5.5-DOTA-Gd and pDHPMA-Cy5.5-SS-DOTA-Gd) were established by a dialysis method. The copolymers were dissolved in PBS (pH 7.4, 10 Mm DTT) or PBS (pH 5.4) and placed in different dialysis tube (MWCO 2000), the initial concentration of Gd(III) was 10.0 μg/mL. All the tubes with 3 mL solution were immersed in PBS (50 mL, pH 7.4, 10 Mm DTT) or PBS (50 mL, pH 5.4) at 37 °C. At pre-specified time points, the solution in tubes was collected and diluted with RO-water to 4 mL. The solutions were further

diluted and treated, and the concentration of Gd(III) was determined by inductively coupled plasma mass spectrometry (ICP-MS).

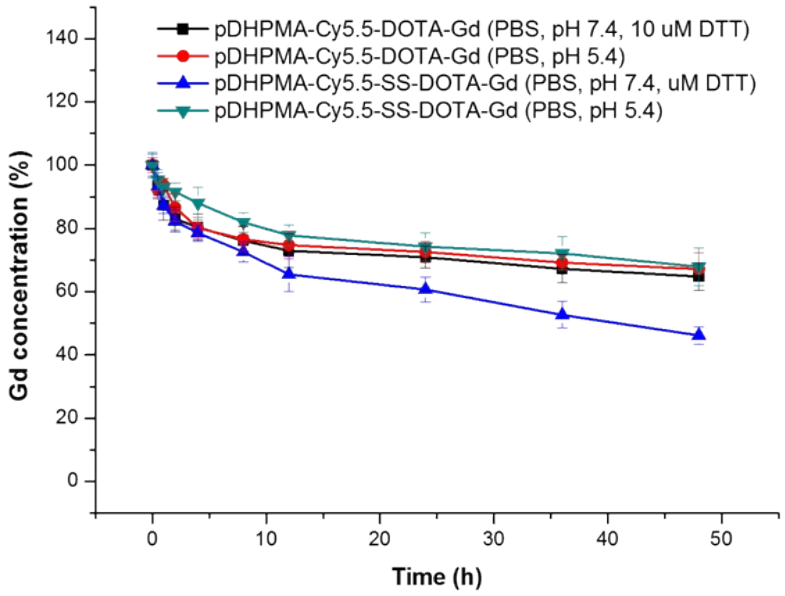

Figure S4. Release profiles of Gd(III) after incubation at 37 °C in PBS at (1) pH 7.4 with 10 μM DTT (2) pH 5.4 without DTT.

Cytotoxicity study in vitro

4T1 and HUVEC cells were chosen to evaluate cytotoxicity of the conjugates. The cells were seeded in a 96-well plate at a density of 3000 (4T1) or 5000 (HUVEC) cells per well and cultured in 5% CO_2 at 37 ℃ for 24 h. DTPA-Gd, the pDHPMA-Cy5.5-DOTA-Gd and pDHPMA-Cy5.5-SSDOTA-Gd at an equivalent Gd(III) concentration were added into these wells. After incubation for another 48 h, the cytotoxicity of DTPA-Gd and the Gd-pHPMA CAs were evaluated through the cell viability using the CCK-8 assay (Dojindo, Japan). The values and cell viability for untreated cells were standardized to be 100%.

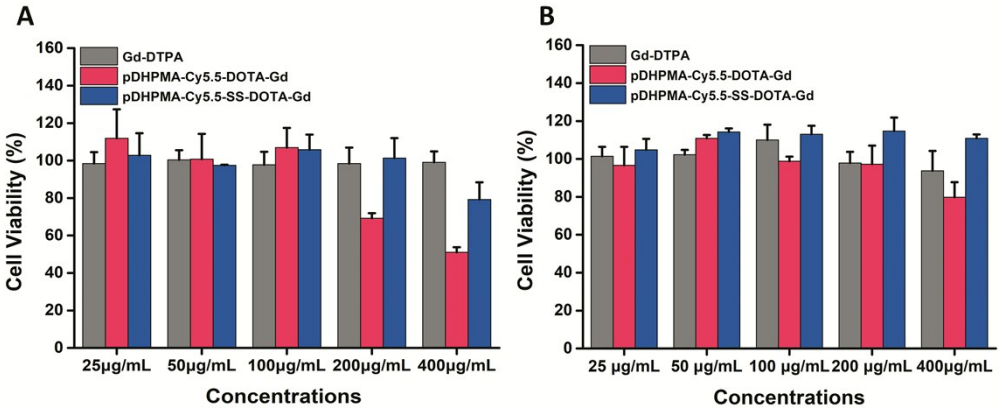

Figure S5. Cytotoxicity of DTPA-Gd, pDHPMA-Cy5.5-S-DOTA-Gd and pDHPMA-Cy5.5-SS-DOTA-Gd, (A) 4T1 cells, (B) HUVEC cells.

References

1. L. Sun, X. Li, X. Wei, Q. Luo, P. Guan, M. Wu, H. Zhu, K. Luo and Q. Gong, *Acs Applied Materials & Interfaces*, 2016, **8**, 10499-10512.