Supporting information

Advanced Micelles-based Biodegradable HPMA Polymer-Gadolinium Contrast Agent MR Imaging of Murine Vasculature and Tumor

Haonan Li [a, ‡], Qiang Luo [a, ‡], Hongyan Zhu [a], Zhiqian Li [a], Xiaoming Wang [a, b], Neil Roberts [d], Hu Zhang [c], Qiyong Gong [a], Zhongwei Gu [a], Kui Luo [a, *]

[a] Laboratory of Stem Cell Biology, and Huaxi MR Research Center (HMRRC), Department of Radiology, Functional and molecular imaging Key Laboratory of Sichuan Province, West China Hospital, Sichuan University, Chengdu 610041, China

[b] Department of Radiology, Chongqing General Hospital, UCAS, 104 Pipashan Zheng Street, Chongqing 400014, PR China

[c] Amgen Bioprocessing Centre, Keck Graduate Institute, Claremont, CA 91711, USA

[d] School of Clinical Sciences, The Queen's Medical Research Institute (QMRI), University of Edinburgh, 47 Little France Crescent, EH16 4TJ, UK

*Corresponding author.

E–mail: luokui@scu.edu.cn

‡Li H. and Luo Q. contributed equally to this work.

Materials and Methods:

Monomers HPMA, MA-GFLG-OMe and MA-DOTA, and peptide functionalized CTA CTA-GFLG-CTA were prepared as previously reported [1-3]. VA044 and $GdCl_3 \cdot 6H_2O$ were purchased from Sigma-Adrich. Size and zeta potential were detected by Zetasizer (Malvern, Worcestershire, UK).

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

^{1}H NMR spectroscopy: ^{1}H NMR spectroscopy data were obtained via a 400 MHz Bruker Advanced Spectrometer, and the chemical shifts were reported in ppm on the δ scale.

Percentage of amino acids and loaded-Gd: For both copolymers, the amino acids content was assayed through the amino acid analysis method [5]. Inductively coupled

plasma mass spectrometry (ICP-MS, Elan DRC-e) was used for measuring Gd(III) content of the final product and qualitatively assessing Gd(III) uptake by different tissues in vivo.

***In vitro* MRI scanning parameters:** The MRI scanner (SIEMENS 3.0 T) was from West China Hospital. T_1-weighted images were acquired with a spin echo (SE) sequence under the following parameters: TE = 8.7 ms, TR = 25, 30, 50, 70, 90, 110, 130, 150, 170, 190, 210, 230, 250, 300, 400, 500, 600, 700 and 800 ms, Fov = 200 mm, Slice thickness = 2.0 mm and Matrix dimensions = 256×256.

***In vivo* tumor MRI scanning parameters:** Before scanning, mice were anesthetized using pentobarbital sodium, and they were equipped with a customized mice coil for transmission and reception of the signals using a multi-section single-echo T_1 weighted TSE sequence. Parameters were set: TR = 450 ms, TE = 11 ms, Slices = 11, Voxel size = 0.2 × 0.2 × 1.5 mm and Fov = 51 mm. The intensity of tumor and bladder could be quantitatively measured by using the noose tool in the scanning system. To obtain more precise results, the same size circles and nearly identical location in tumors and bladders were applied in every measurement.

Hemolysis Test: Fresh blood was extracted from healthy human (in anti-sodium citrate tubes), then centrifuged at 1000 g for 5 min and washed 3 times with PBS. Red blood cells were suspended in PBS to obtain 16 wt% of cells (volume ratio, the OD of the ratio of around 1). Then 50 μL of the red cell suspension was added into the EP tube with 1 mL of the polymeric agent solution (1 mg/mL, 3 mg/mL and 5 mg/mL). After incubation for a certain period of time, the solution was vortexed and

centrifuged at 1000 g for 5 min. 100 μL of the supernatant was pipetted into a 96-well plate. The OD values of the samples were determined at 540 nm (absorption of hemoglobin) using a microplate reader.

Effect on RBCs' morphologies and aggregation: RBCs were separated from fresh blood from healthy human by centrifuging at room temperature (1,000 ×g, 5 min) and washing 3 times with PBS solution. Then 20 μL of RBCs were added into 100 μL of the solution of the polymeric agent, and the final solution had a concentration of 2.5 mg/mL after incubating for 15 min. The RBCs (20 μL) were added into the PBS solution (100 μL) as the control group. After washing with PBS twice, all samples were fixed with 4% paraformaldehyde for at least 1 h. Erythrocyte suspensions (10 μL) were transferred onto clean glass slides and dehydrated with ethanol at gradient concentrations as previously reported [1,4]. Samples were dried in a static platform at room temperature. Finally, all sample photos were obtained under the SEM after coating with gold.

Table S1. The characterization of different copolymers and the final products pGFLG-block-pHPMA-DOTA-Gd.

Comp.	MW[a]	PDI[b]	Gd[c]	Gly[c]	Phe[c]	Leu[c]
pGFLG-block-pHPMA-DOTA	81.82	1.22	-	17.8	19.3	15.6
pGFLG-block-pHPMA-DOTA-Gd	82.11	1.24	6.9	16.1	18.3	14.5

[a]Molecular weight (MW) with a unit of kDa, and [b] polydispersity index (PDI); [c,d]The contents of Gd and amino acids content with weight percent (%).

As gadolinium can be cheated with the DOTA-moiety via a known molar ratio, thus, the DOTA content were calculated from the content of gadolinium. The content and the mole ratio of MA-DOTA in the copolymer were also calculated. Additionally, the amino acids content and the mole ratio of MA-GFLG-OMe in the copolymer can be

calculated. From the contents of gadolinium and amino acids, the content and the molar ratio of HPMA in the copolymer can be derived. Based on the content of HPMA, gadolinium and amino acids, the mole ratio of three monomers may be calculated. The nMA-GFLG-OMe: nHPMA: nMA-DOTA is about 105: 1240: 140, and m: n: o in the copolymer is about 21: 248: 28.

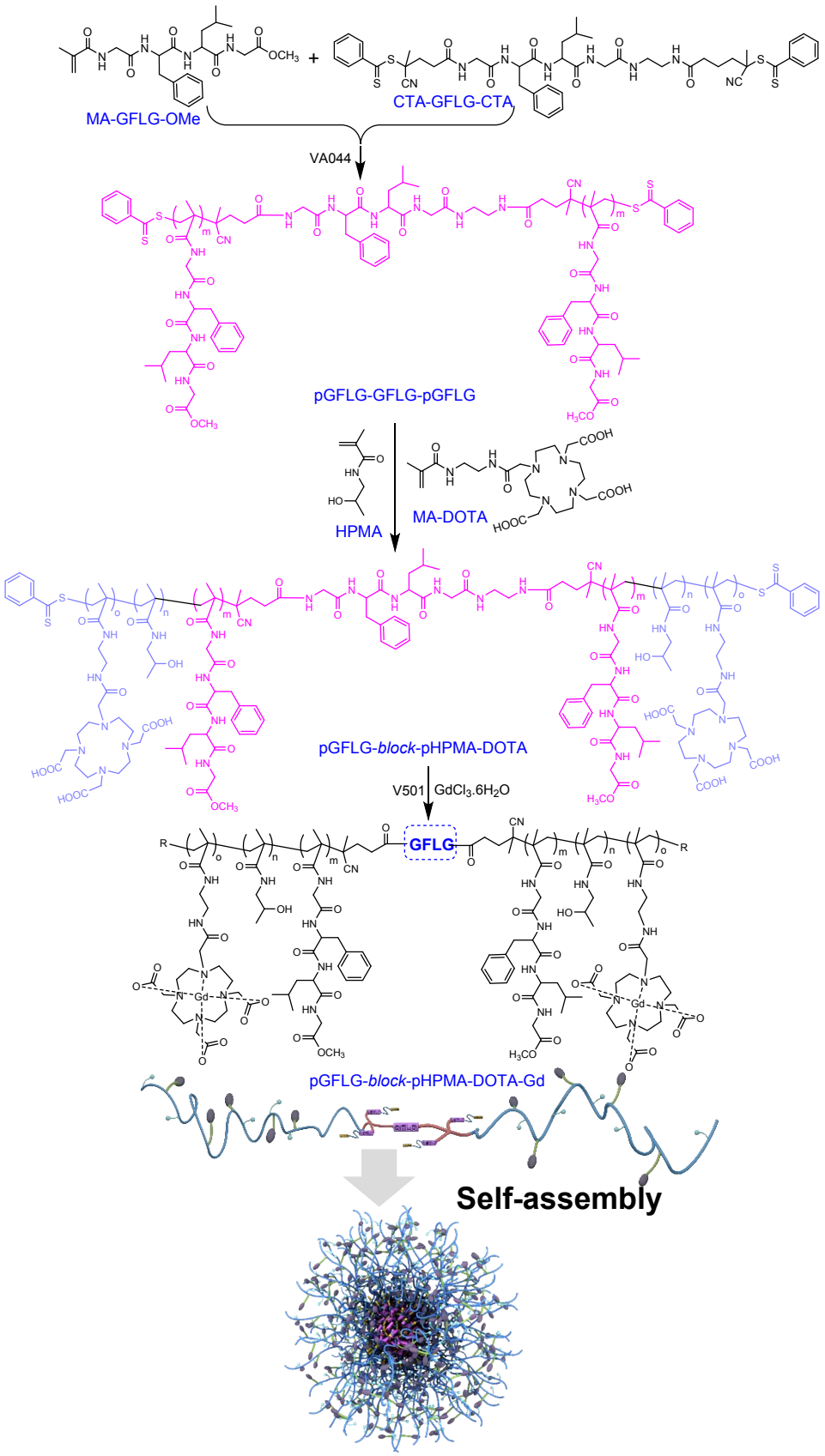

Fig. S1. The preparation route for the gadolinium-containing polymer and illustration of the micelle-based polymeric agent.

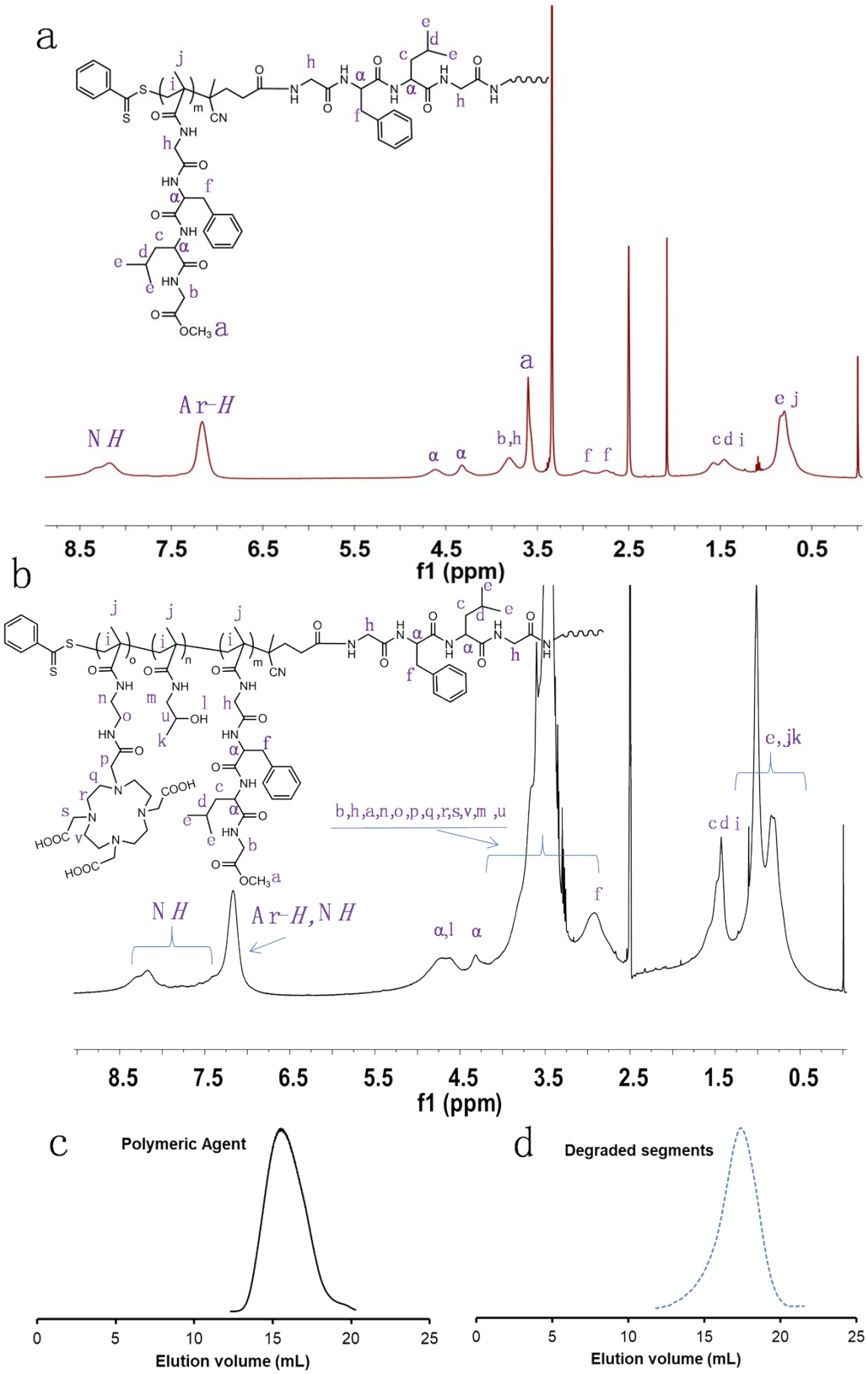

Fig. S2. ^{1}H NMR spectra of the polymer pHPMA-DOTA (a) and pGFLG-block-pHPMA-DOTA (b). SEC profiles of the final gadolinium-based product (polymeric agent) (c) and its degraded products (d).

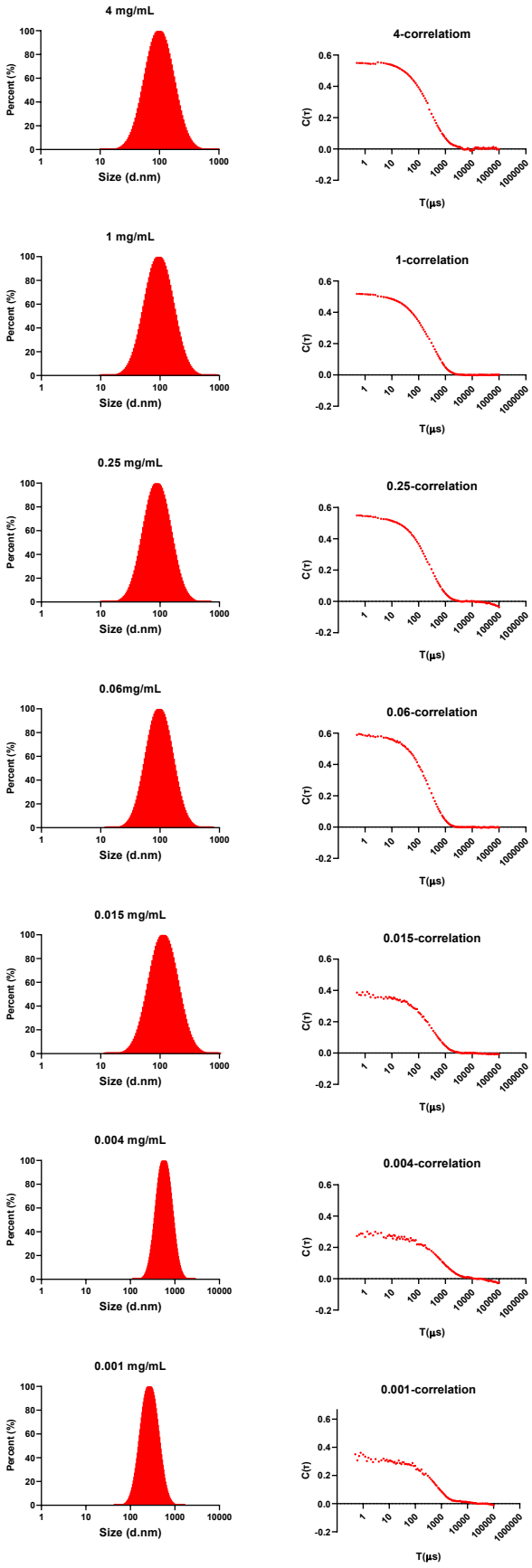

Fig. S3. The DLS results of the polymeric agent at different concentrations.

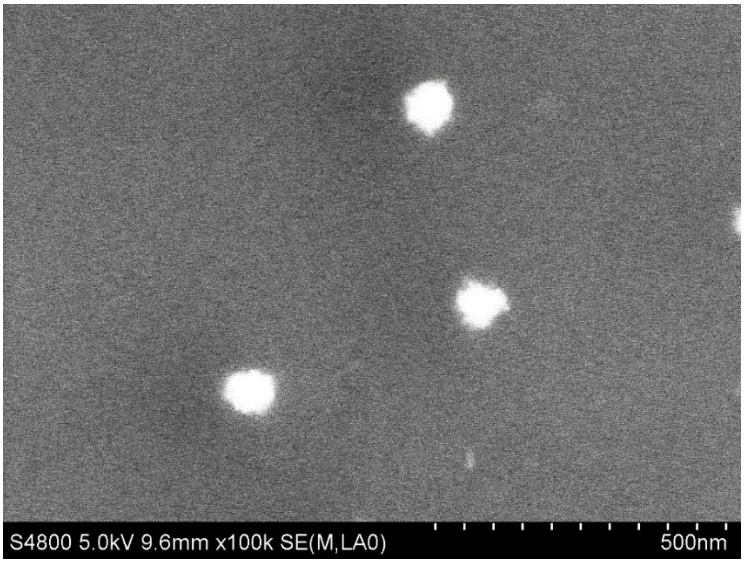

Fig. S4. The SEM result of the polymeric agent.

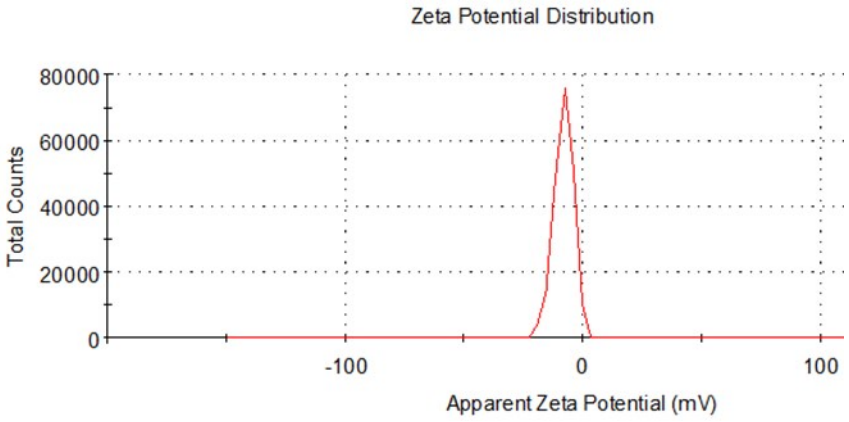

Fig. S5 The zeta potential of the polymeric agent.

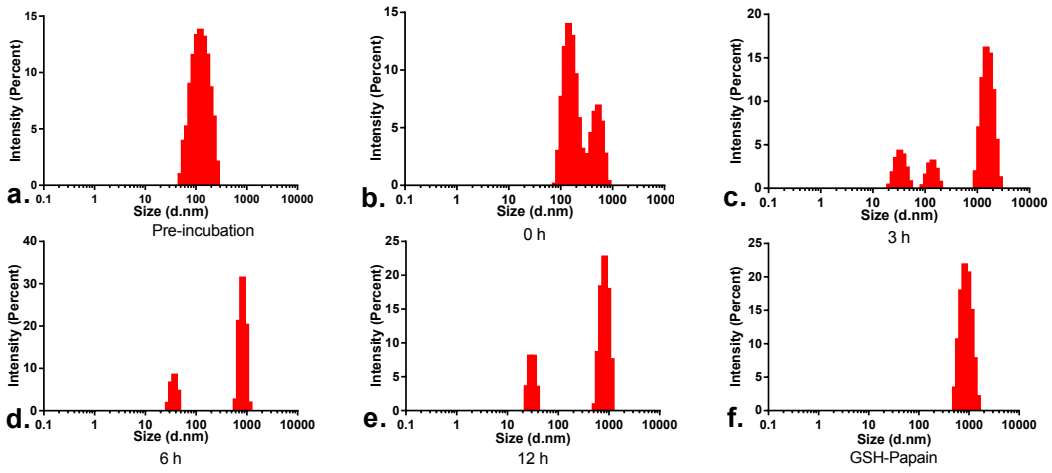

Fig. S6. (a) Size of the polymeric agent. (b-e) size of the agent after incubation with GSH-Papain at various time-points. (f) size of GSH-Papain (about 1000 nm).

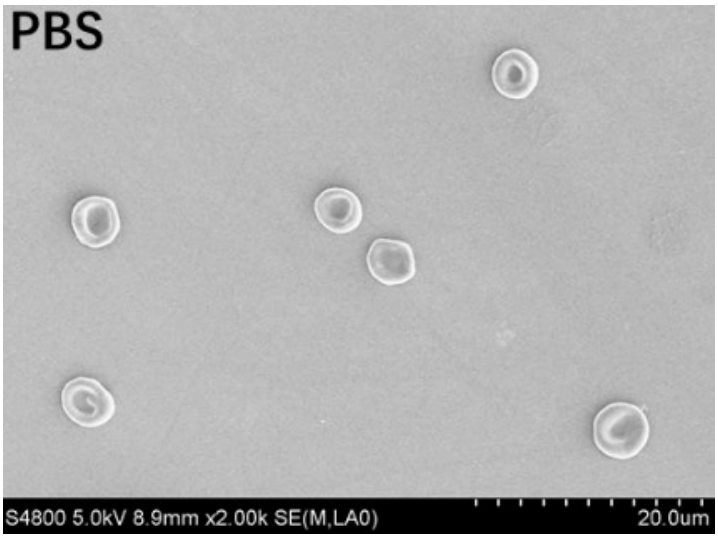

Fig. S7. RBC morphology after incubation with PBS.

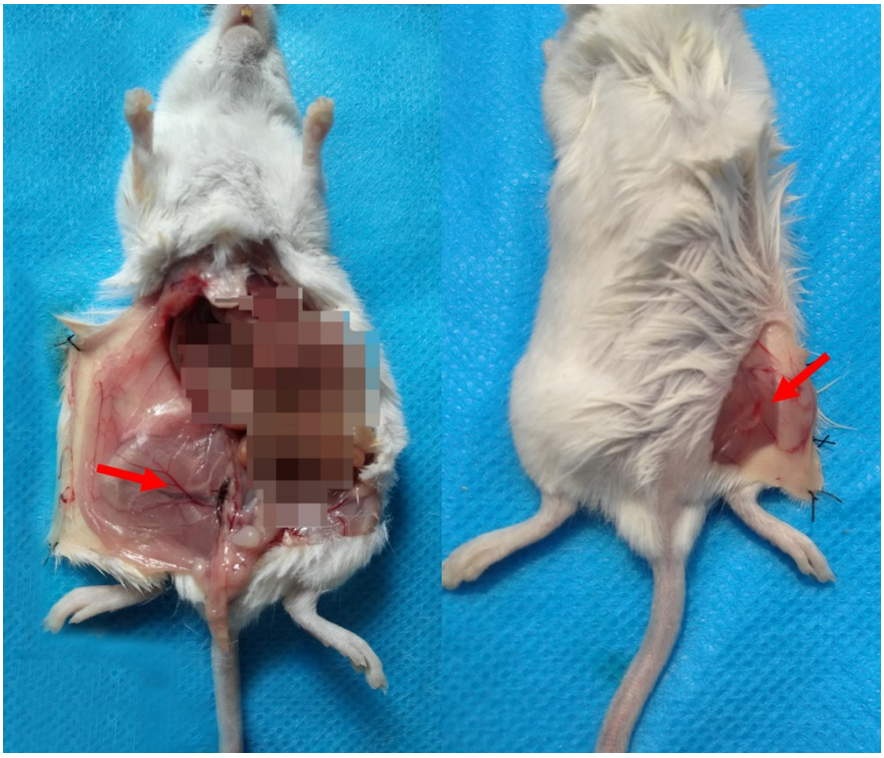

Fig. S8. Vessels detected by MRI. Mice were anesthetized and sacrificed, and the vessel detected by MRI was found and captured (red arrow).

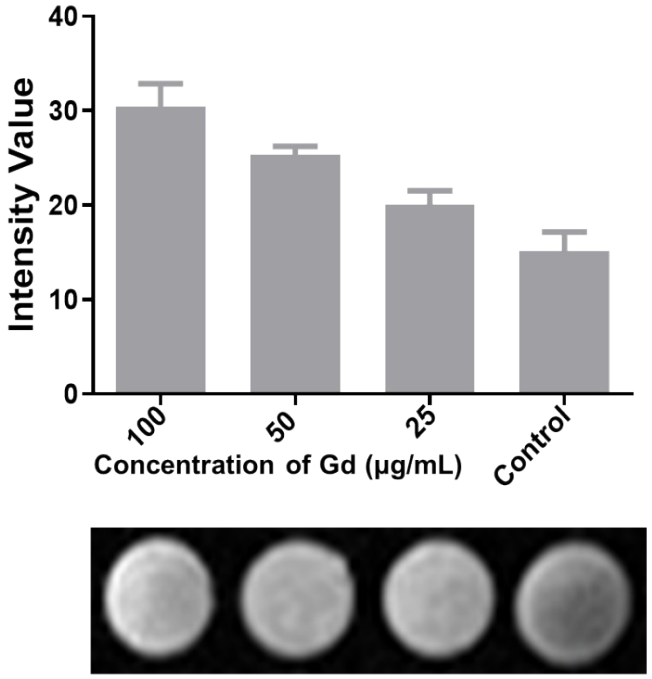

Fig. S9. MR images of tumor cells after incubating with the polymeric agent.

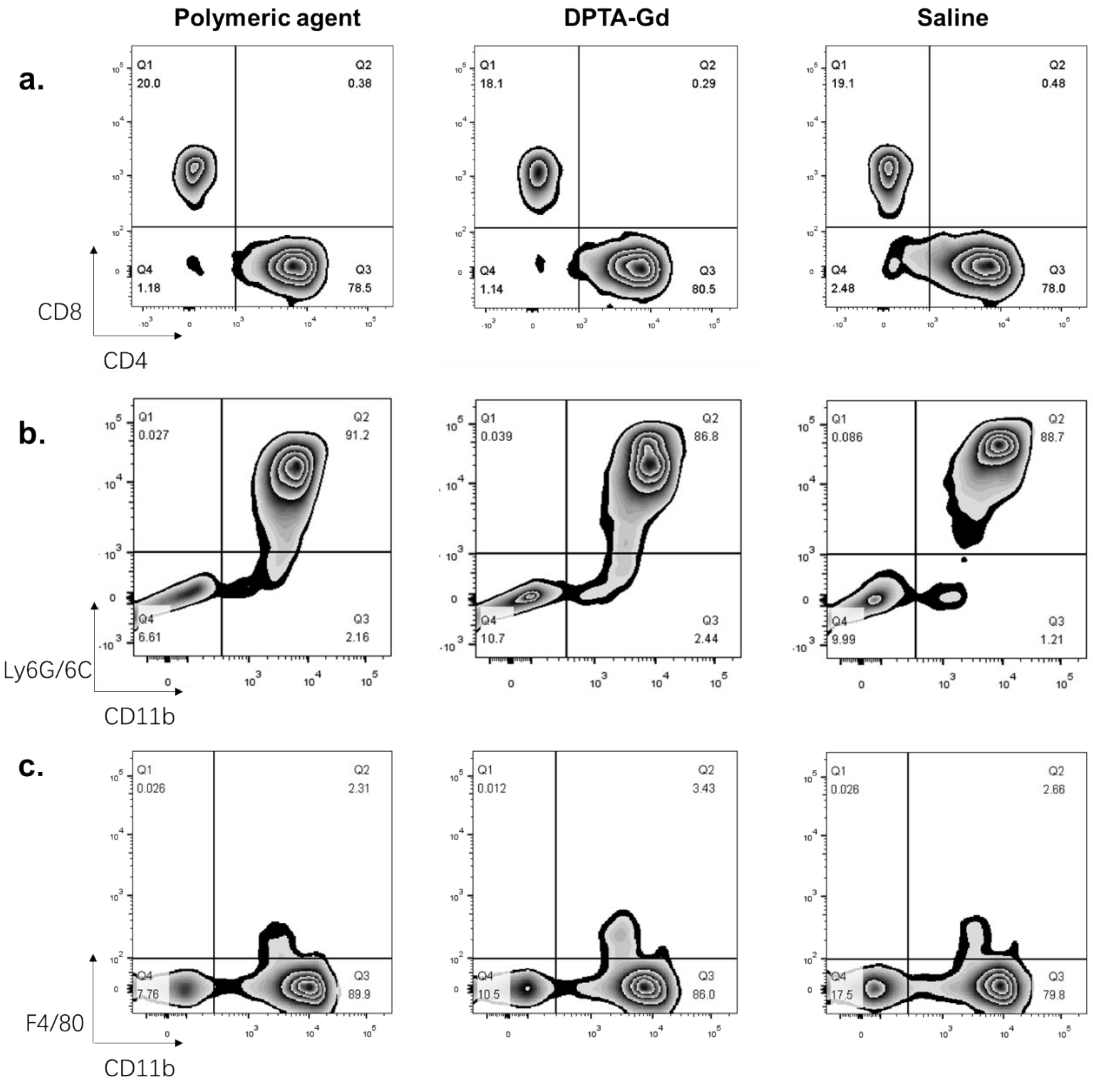

Fig. S10. In vitro flow cytometric analysis of blood after injection of the contrast agent. (a) lymphocytes ($CD4^+CD8^-$ or $CD4^-CD8^+$ in $CD45^+CD3^+$), (b) monocytic-granulocytic cells ($CD11b^+Ly6G/6C^+$ in $CD45^+$), (c) macrophages ($CD11b^+F4/80^+$ in $CD45^+$).

References

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