Supporting information:

Hyaluronic Acid-Methotrexate Conjugates Coated Magnetic Polydopamine Nanoparticles for Multimodal Imaging-Guided Multistage Targeted Chemo-Photothermal Therapy

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Figure S1

The as-obtained MPDA nanoparticles were re-dispersed in water for further use. Concentrated hydrochloric acid was added to adjust the pH of MPDA nanoparticles, and then the zeta potential of MPDA nanoparticle solution under different pH conditions were measured by DLS. Figure S1 show that the isoelectric point of MPDA≈4.6. MPDA nanoparticles showed different charges in the solution of different pH.

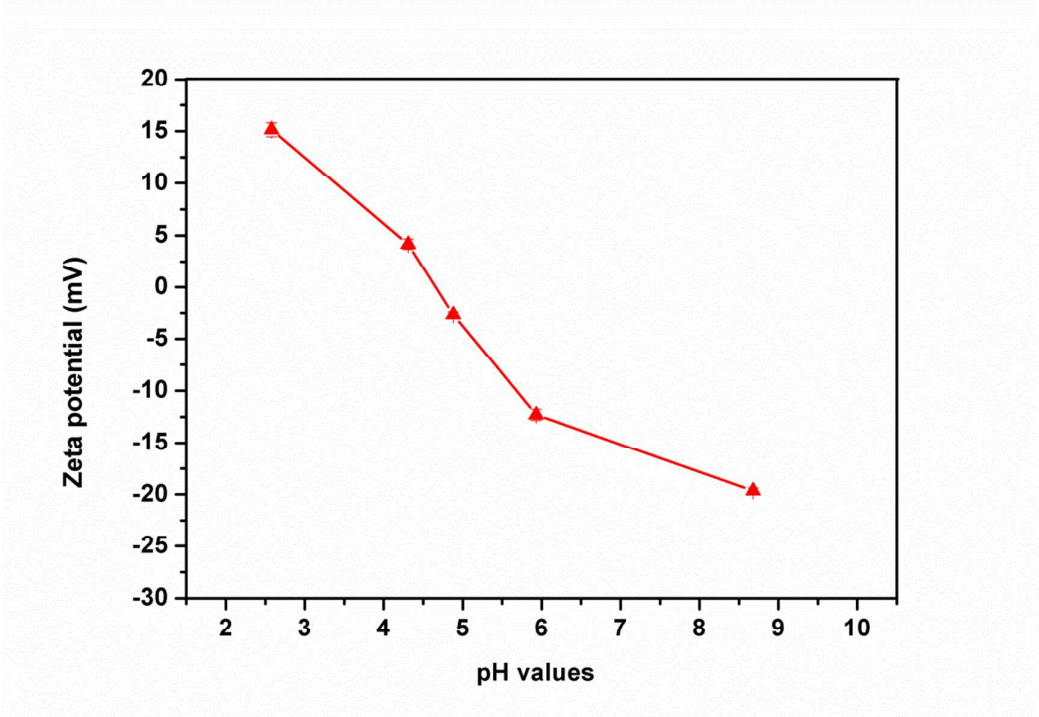

Figure S1. Changes of zeta potentials of MPDA nanoparticles in aqueous solutions at different pH values.

Table S1

The particle size and zeta potential of the Fe_3O_4 nanoparticles, MPDA, MPDA/DOX, and MPDA/DOX@HA were measured by dynamic light scattering （DLS, Nano-ZS, Malvern, Instruments, Ltd., U. K.). As shown in the Table S1, the Fe_3O_4 nanoparticles and MPDA had a hydrodynamic diameter of 81.5 nm and 167.10 nm respectively, due to the existence of a hydration layer in aqueous solutions. After loading DOX into MPDA, the hydrodynamic diameter of nanoparticles changed dramatically. Additionally the zeta potential increased from -25.3±0.67 mV to 24.9±3.12 mV, indicating that the driving force for DOX loading was mainly by electrostatic interaction. It was worthwhile to note that the particle size of MPDA/DOX@HA and MPDA/DOX@HA-MTX are 267.1 and 236.50 nm, which were large enough to avoid rapid renal clearance but small enough to avoid rapid clearance by the RES.

Table S1 The particle size and zeta potential of Fe_3O_4 nanoparticles, MPDA, MPDA/DOX, MPDA/DOX@HA

Formulations	Size (nm)	Zeta potential (mV)
Fe_3O_4 nanoparticles	81.5±0.53	-36.5±1.91
MPDA	167.10±2.01	-25.3 ±0.67
MPDA/DOX	248.0±4.60	24.9 ±3.12
MPDA/DOX@HA	267.1±4.39	-43.3 ±0.87

Figure S2

MPDA nanoparticles and MPDA@HA nanoparticles were analyzed using an infrared spectrometer, the obtained FT-IR spectra was show in Figure S2. MPDA@HA had a broad peak around 3400 cm^{-1} ascribed to C−NH, C=NH, and −OH stretching vibrations. The coatings also presented the peaks at 1050 cm^{-1} (C−O−C stretching vibrations), suggesting that the HA were successfully prepared on to MPDA and HA coatings may be due to the strong electrostatic interaction between PDA and HA. Because the amino group of PDA and the carboxyl group of HA have electrostatic interaction or hydrogen bonding or a combination of both effect.

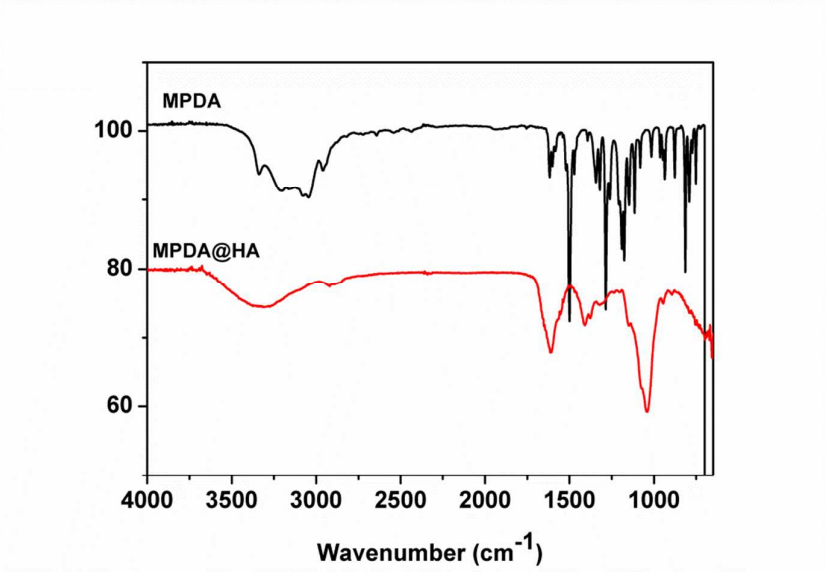

Figure S2 FT-IR spectra of MPDA and MPDA@HA

Figure S3

Hemolysis test was performed on MPDA and MPDA@HA-MTX which were dispersed in PBS Identical volume of 2% (v/v) rabbit red blood cells (RBCs) was added to the solution,and make the final concentrations of nanoparticles to 5, 12.5, 25, 50, 125, 250, and 500 mg/mL. The mixtures were then vortexed and incubated at 37°C for 1 h followed by the centrifugation at 8, 000 rpm for 5 min. The absorbance values of supernatant were measured at 560 nm by an Multiskan FC microplate reader. An equal volume of PBS was set as a negative control and deionized water as a positive control. The hemolysis percentage was calculated by the following formula: hemolysis percentage = (experimental sample absorbance - negative control sample absorbance)/ (positive control sample absorbance - negative control sample absorbance) × 100%. There was no significant hemolytic phenomenon detected on MPDA@HA-MTX at a series of

concentrations between 25 and 500 μg/mL, and MPDA@HA-MTX could be used *in vivo*. These results were explained by the fact that the surface coating of HA-MTX conjugates on MPDA could remarkably improve the hemocompatibility of MPDA nanoparticles.

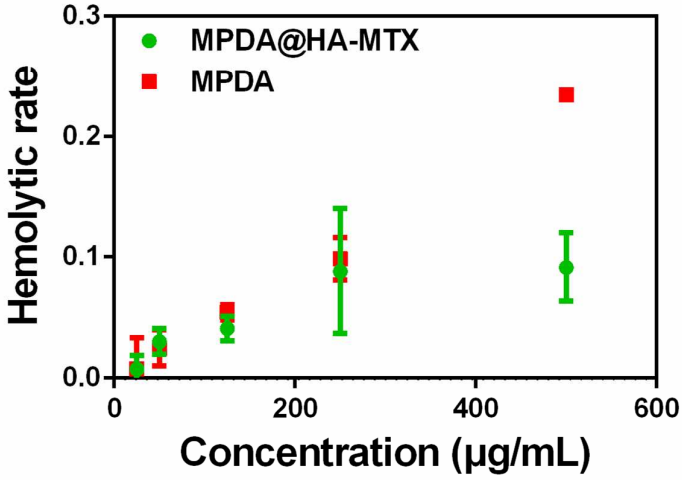

Figure S3 Hemolysis assay of the DOX-free MPDA@HA-MTX and MPDA at different concentrations, using PBS as a negative control and distilled water as a positive control.

Figure S4

The pharmacokinetics investigation was conducted to examine the behavior of MPDA/DOX@HA-MTX *in vivo*. MPDA/DOX@HA-MTX was intravenously injected into tumor-free healthy SD mice. At each time point, blood was collected from eyes and then centrifuged at 10, 000, and then 0.1 mL of supernatant was added with acid ethanol to extract DOX. Subsequently, the mixture was incubated in dark overnight. The concentration of remaining DOX was determined by fluorescence intensity after centrifugation. As shown in Figure S4, the DOX signals gradually reduced over time, and the half-life (t1/2) of MPDA/DOX@HA-MTX was determined to be 45.37 ± 3.03 h, indicating a long circulation time of nanosystems in bloodstream.

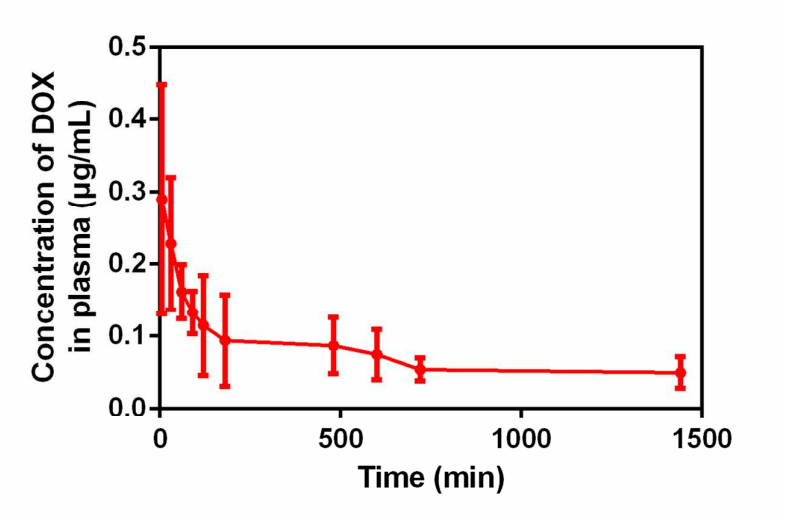

Figure S4 *In vivo* pharmacokinetics of DOX in SD rats after intravenous administration of

MPDA/DOX@HA-MTX (mean ± SD, n = 6).