Electronic Supplementary Information

Synthesis of PEGylated low generation dendrimer-entrapped gold nanoparticles for CT imaging applications

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Experimental Section

Materials: Ethylenediamine core G2.NH_2 PAMAM dendrimers were purchased from Dendritech (Midland, MI). PEG monomethyl ether with one end of carboxyl group (*m*PEG-COOH) was from Shanghai Yanyi Biotechnology Corporation (Shanghai, China). EDC was obtained from J&K Chemical Reagent Co., Ltd (China). Chloroauric acid and all other solvents and chemicals were of reagent grade and purchased from Sinopharm Chemical Reagent Co., Ltd (China). KB cells (a human epithelial carcinoma cell line) were from Institute of Biochemistry and Cell Biology, the Chinese Academy of Sciences (Shanghai, China). RPMI-1640 medium and fetal bovine serum (FBS) were purchased from Hangzhou Jinuo Biomedical Technology (Hangzhou, China). MTT was supplied from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd (China). Regenerated cellulose dialysis membranes (molecular weight cut-off, MWCO = 3500) were acquired from Shanghai Yuanye Biotechnology Corporation (Shanghai, China). All chemicals and reagents were used as received. The water used in all experiments was purified *via* a Milli-Q Plus 185 water purification system (Millipore, Bedford, MA) to have a resistivity higher than 18 mΩ cm.

Synthesis of G2-mPEG Conjugates: Firstly, *m*PEG-COOH (0.115 mmol) was dissolved in water (15 mL), in which an EDC aqueous solution (1.036 mL, 0.344 mmol) was dropwise added under magnetic stirring for 3 h to active the carboxyl group of the *m*PEG. After that, an aqueous solution of G2.NH_2 dendrimer (0.598 mL, 0.006 mmol) was added into the above mixture solution under vigorous magnetic stirring. The stirring process was continued for 3 days to complete the reaction. Then the reaction mixture was extensively dialyzed against water (6 times, 2 L) for 2 days to remove the excess of reactants and by-products, followed by lyophilization to obtain the G2-*m*PEG product.

Synthesis of PEGylated Au DENPs: The PEGylated Au DENPs were prepared using sodium borohydride reduction chemistry with the molar ratio of gold salt to G2-*m*PEG at 8:1. Briefly, G2-*m*PEG (0.003 mmol) was dissolved in water (30 mL), in which an aqueous solution of $HAuCl_4$ (0.334 mL, 0.024 mmol) was dropwise added under magnetic stirring. After 20 min, an aqueous solution of $NaBH_4$ (0.277 mL, 0.073 mmol) was added, and the reaction mixture turned wine red within a few seconds. The stirring process was continued for 10 h to complete the reaction. The reaction mixture was then extensively dialyzed against water (6 times, 2 L) for 2 days to remove the excess of reactants and by-products, followed by lyophilization to obtain the final product of PEGylated Au DENPs, which were denoted as $\{(Au^0)_8$-G2-*m*PEG$\}$.

Characterization Techniques: ^1H NMR spectra of PEGylated Au DENPs were recorded using a Bruker Avance 400 NMR spectrometer. D_2O was used as a solvent to dissolve the samples before measurements. UV-vis spectrometry was performed using a Lambda 25 UV-vis spectrophotometer (Perkin-Elmer, United States). All samples were dissolved in water before the measurements. Transmission electron microscopy (TEM) was carried out using a JEOL 2010F analytical electron microscope at an operating voltage of 200 kV. An aqueous sample solution (5 μL, 1 mg/mL) was dropped onto a carbon-coated copper grid and air dried before measurements. The size and size distribution of each sample were analyzed using ImageJ software (http://rsb.info.nih.gov/ij/download.html). For each sample, at least 300 NPs were randomly selected from different TEM images to measure the size. Zeta-potential and dynamic light scattering (DLS) measurements were performed using a Malvern Zetasizer Nano ZS model ZEN3600 (Worcestershire, UK) equipped with a standard 633 nm laser.

The stability of the PEGylated Au DENPs was evaluated by UV-vis spectroscopy under

different pH and temperature conditions. The aqueous solution of Au DENPs (0.5 mg/mL) was adjusted using HCl or NaOH (0.1 M) to have a pH range of 5-8 and maintained for 20 min before measurements. The Au DENPs dispersed in water were also kept at different temperatures (4, 25, 37, and 50 ^{o}C, respectively) for 30 min before measurements. The stability of the Au DENPs was further evaluated by exposing them to water, PBS, and cell culture medium for a period of one month at room temperature.

Cell Culture: KB cells were regularly cultured and passaged using RPMI 1640 medium supplemented with 10% FBS and 1% penicillin-streptomycin at 37 ^{o}C and 5% CO_2 in a humidified incubator.

Cytotoxicity Assay: An MTT colorimetric assay was used to quantify the viability of cells treated with PEGylated Au DENPs at different concentrations. Briefly, 1×10^4 KB cells per well were seeded into a 96-well plate. After overnight incubation to bring the cells to confluence, the medium was replaced with fresh medium containing PEGylated Au DENPs with concentrations ranging from 0 to 3000 nM. After 24 h incubation at 37 ^{o}C and 5% CO_2, MTT in PBS solution was added and the cells were incubated for additional 4 h under normal culture conditions. The assays were carried out according to the manufacturer's instructions and the absorbance of each well was recorded at 570 nm using a Thermo Scientific Multiskan MK3 ELISA reader (Thermo Scientific, USA). For each sample, mean and standard deviation for the quadruplicate wells were reported.

The cytotoxicity of {$(Au^0)_8$-G2-*m*PEG} DENPs was further confirmed by observation of the morphology of KB cells treated with the particles at different concentrations for 24 h *via* phase contrast microscopy (Leica DM IL LED inverted phase contrast microscope). The magnification was

set at 200× for all samples.

X-Ray Attenuation Measurements: {$(Au^0)_8$-G2-*m*PEG} DENPs or iohexol 300 (Omnipaque 300 mg I/mL, GE Healthcare, used as control) solutions with different concentrations of Au or iodine (0-60 mM) were placed in 1.5-mL Eppendorf tubes and put in a self-designed scanning holder. CT scanning was performed using a clinical LightSpeed VCT CT imaging system (GE Medical Systems, Milwaukee, WI, USA) with the tube voltage of 100 kV, the tube current of 200 mA, and the slice thickness of 0.625 mm. The X-ray attenuation intensity of Au DENPs was evaluated by loading the digital CT images in a standard display program and then analyzing a uniform round region of interest on the resultant CT image for each sample. Hounsfield units (HU) were used to quantify the contrast enhancement.

In Vivo CT Imaging of Mouse Heart: All experiments with live animals were performed in compliance with the relevant laws and institutional guidelines, and approved by the ethical committee of Shanghai First People's Hospital. Kunming mice (20 to 25 g) purchased from Shanghai Slac Laboratory Animal Center (Shanghai, China) were anesthetized by intraperitoneal injection of 1% pentobarbital sodium (4 mL/kg). {$(Au^0)_8$-G2-*m*PEG} DENPs dispersed in PBS (0.06 mL, [Au] = 0.12 M) were then intravenously injected into a mouse through the tail vein. Omnipaque was used as control and the injection dose was kept similar (iodine concentration similar to that of Au for Au DENPs) according to the body weight. The mice were scanned using a CT imaging system (GE Lightspeed VCT) with the following operating parameters: tube voltage, 80 kV; tube current, 100 mA; and slice thickness, 0.625 mm.

In order to investigate the excretion of the PEGylated Au DENPs, the mouse was euthanized

after one week post-injection of the particles. Then, the heart, liver, spleen, lung, and kidney were extracted and weighed. The mouse without injection of PEGylated Au DENPs was used as blank control. The organs were digested in aqua regia solution for 4 h. Au content was quantified by inductively coupled plasma-optical emission spectroscopy (ICP-OES, Leeman Prodigy, USA).

In Vivo CT Imaging of KB Xenograft Tumor Model: Male 4- to 6-week-old BALB/c nude mice (Shanghai Slac Laboratory Animal Center, Shanghai, China) were subcutaneously injected in their back with 5×10^6 cells/mouse. After approximately 3 weeks post injection, the tumor nodules reached a volume of 0.9 cm^3. Each nude mouse was anesthetized by intraperitoneal injection of 1% pentobarbital sodium (4 mL/kg). {$(Au^0)_8$-G2-*m*PEG} DENPs dispersed in PBS (0.06 mL, [Au] = 0.12 M) were then delivered to mice *via* intravenous and intraperitoneal injection, respectively. The tumor-bearing nude mice were scanned using the above CT imaging system with operating parameters similar to those used for CT imaging of mouse heart.

After 90 min post-injection of PEGylated Au DENPs *via* intravenous or intraperitoneal injection, the mice were euthanized. Then, the heart, liver, spleen, lung, kidney, and tumor were extracted and weighed. Following the same protocols above used to investigate the Au excretion in Kunming mice after one week injection, the Au distribution in different organs of the tumor-bearing mice were determined using ICP-OES.

Statistical Analysis: One-way ANOVA method was used to evaluate of the experimental data. A p value of 0.05 was considered as the significance level, and the data were indicated with (*) for $p < 0.05$, (**) for $p < 0.001$, and (***) for $p < 0.0001$, respectively.

Table S1. The hydrodynamic size, polydispersity index, and zeta-potential values of G2.NH_2, G2-*m*PEG, and {$(Au^0)_8$-G2-*m*PEG}.

	Hydrodynamic size (nm)	Polydispersity index	Zeta-potential (mV)
G2.NH_2	542.15 ± 39.67	0.60 ± 0.07	8.26 ± 1.44
G2-*m*PEG	820.25 ± 126.93	1.00 ± 0.00	4.65 ± 0.28
{$(Au^0)_8$-G2-*m*PEG}	276.10 ± 10.04	0.78 ± 0.10	3.61 ± 0.39

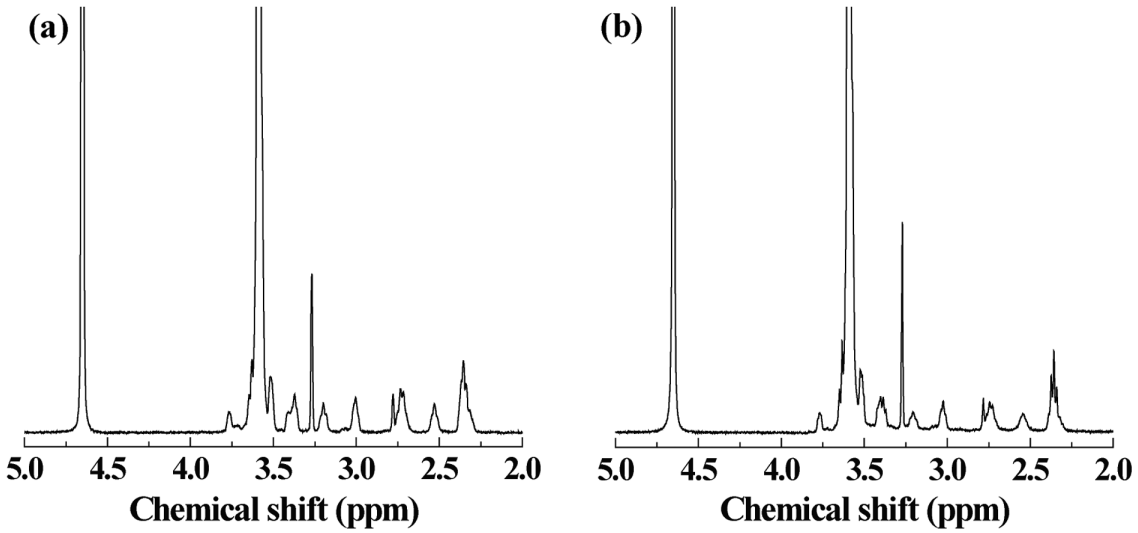

Figure S1. ^{1}H NMR spectra of the G2-*m*PEG dendrimers (a) and PEGylated Au DENPs (b).

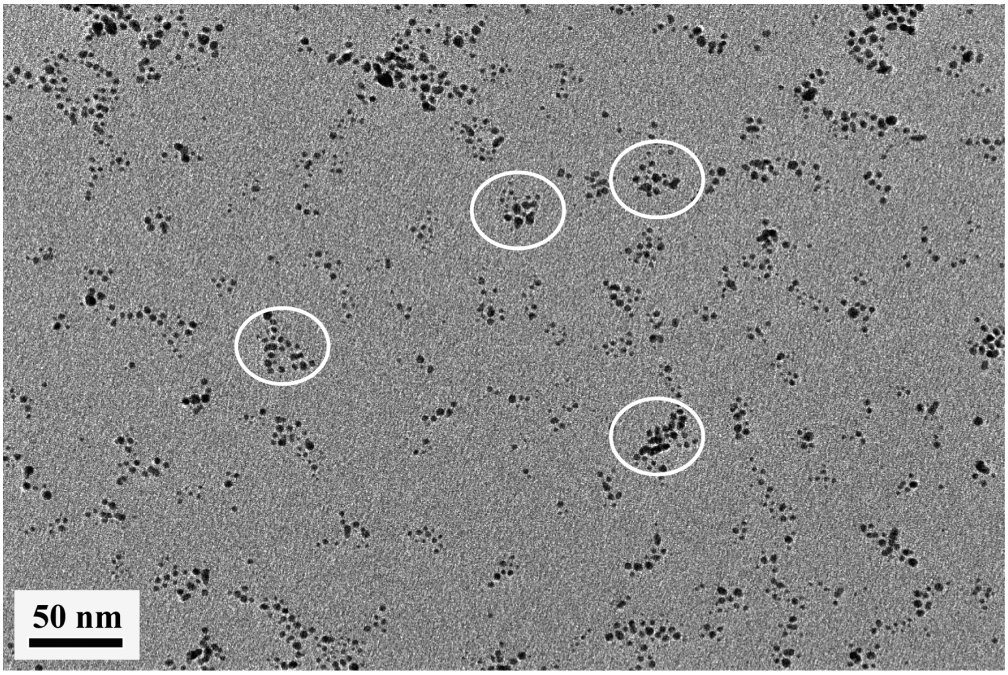

Figure S2. A large scale TEM image of the synthesized PEGylated Au DENPs.

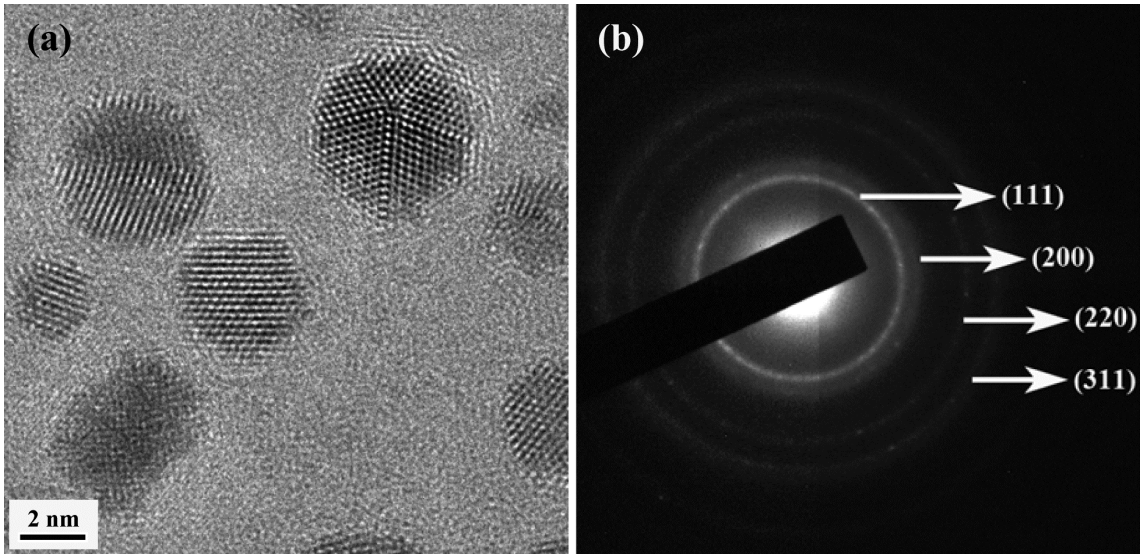

Figure S3. A typical high-resolution TEM image (a) and SAED pattern (b) of PEGylated Au DENPs.

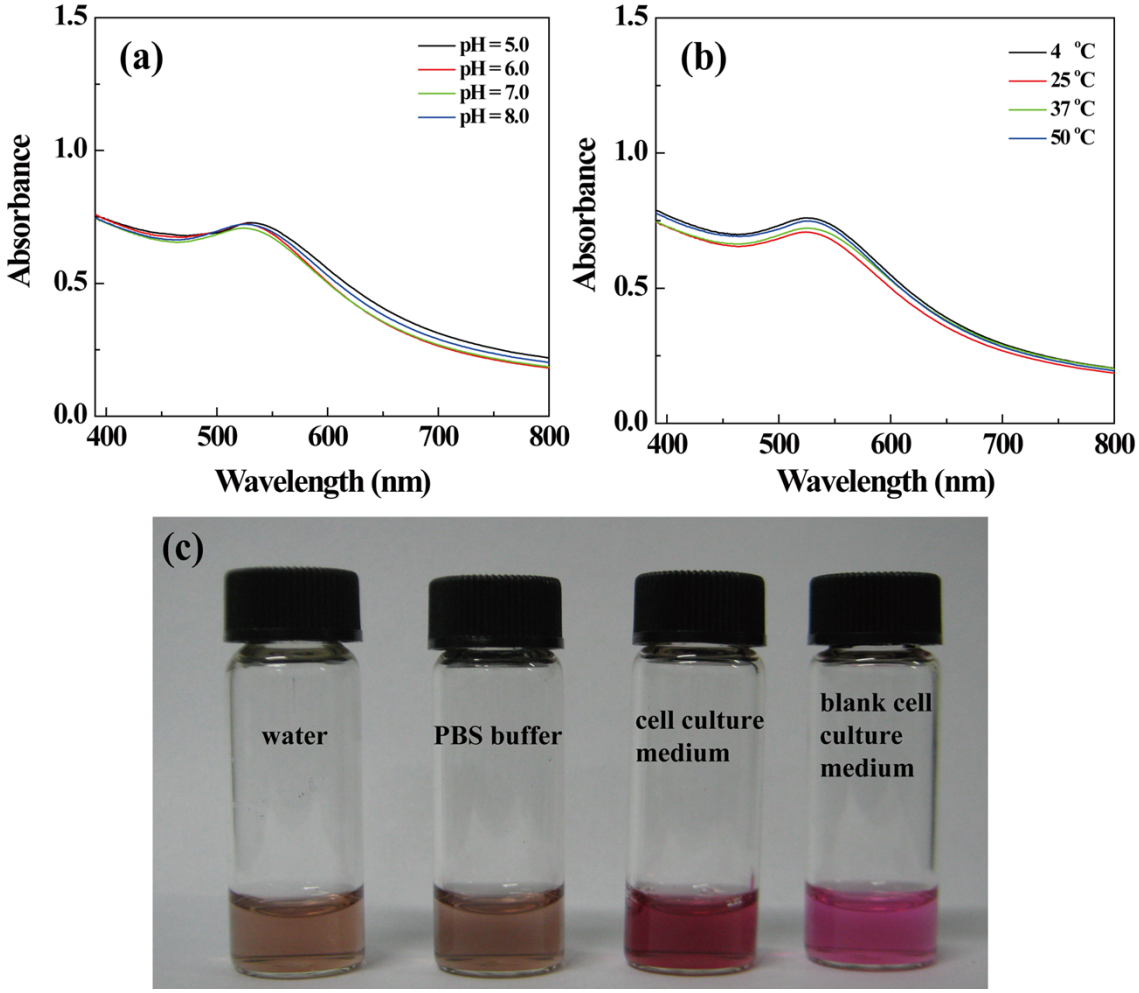

Figure S4. UV-Vis spectra of PEGylated Au DENPs under different pH (a) and temperature (b) conditions. The photographs of the as-prepared PEGylated Au DENPs dispersed in different media and the blank cell culture medium are shown in (c).

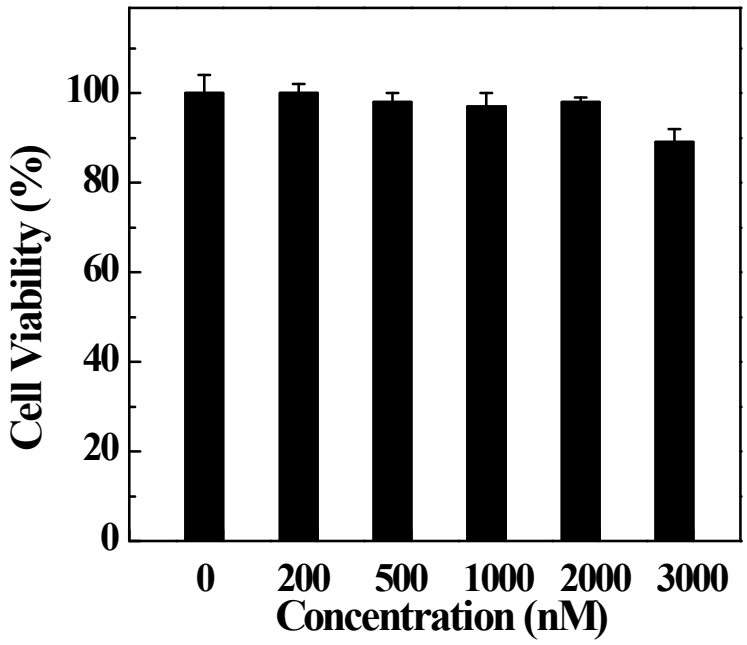

Figure S5. MTT viability assay of KB cells treated with PEGylated Au DENPs for 24 h. The cells treated with PBS were used as control. The data were expressed as mean ± S.D. (n = 4).

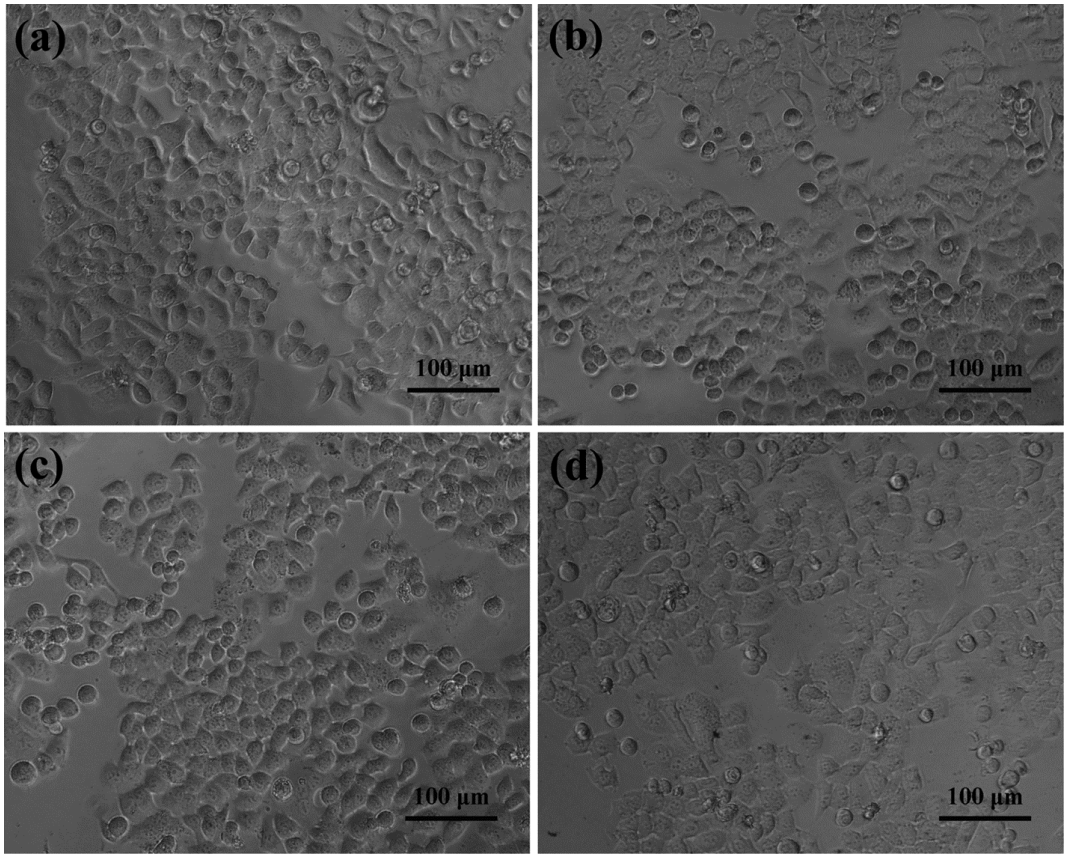

Figure S6. Phase-contrast photomicrographs of KB cells treated with (a) PBS as control and PEGylated Au DENPs at concentrations of (b) 1000 nM, (c) 2000 nM, and (d) 3000 nM, respectively.

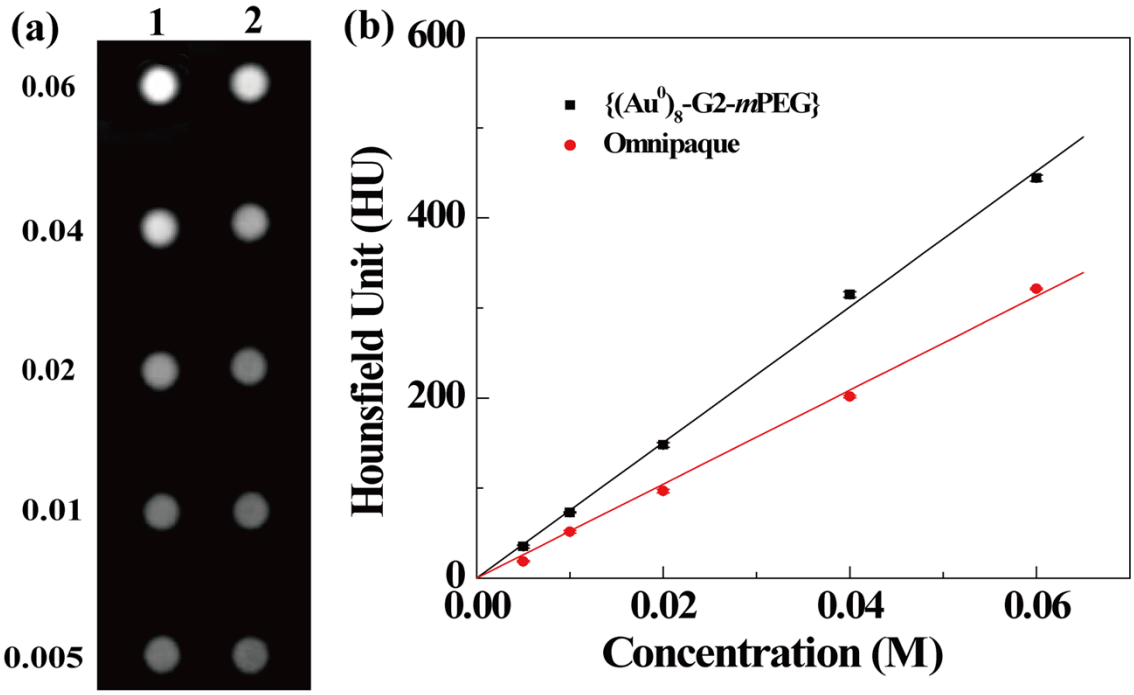

Figure S7. (a) CT images of (1) PEGylated Au DENPs and (2) Omnipaque with different concentrations. (b) X-ray attenuation intensity (HU) of PEGylated Au DENPs and Omnipaque as a function of the molar concentration of active element (Au or iodine).

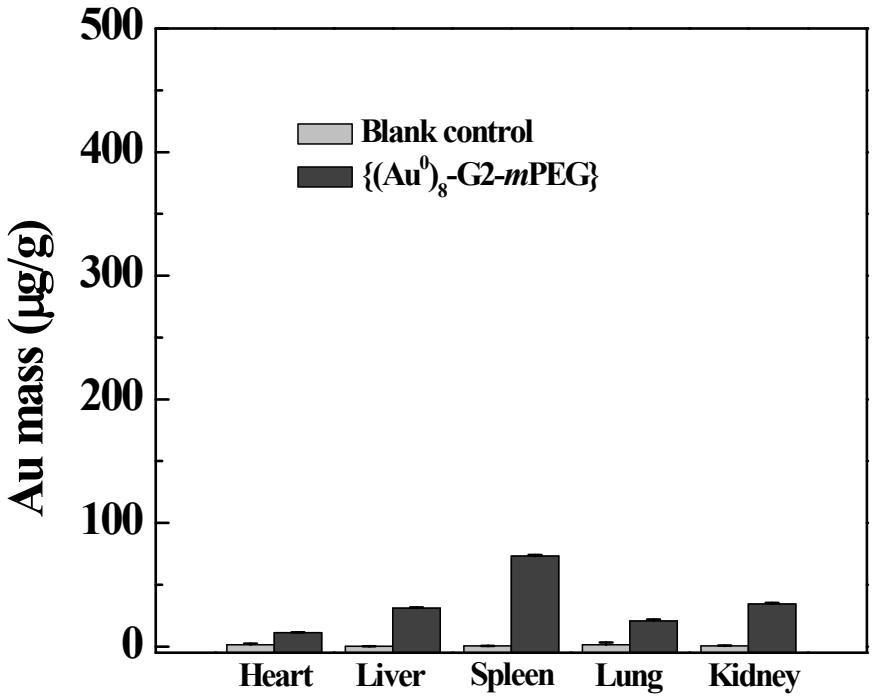

Figure S8. *In vivo* biodistribution of Au element in different organs at one week post intravenous injection of the PEGylated Au DENPs. The mouse without injection was used as blank control.

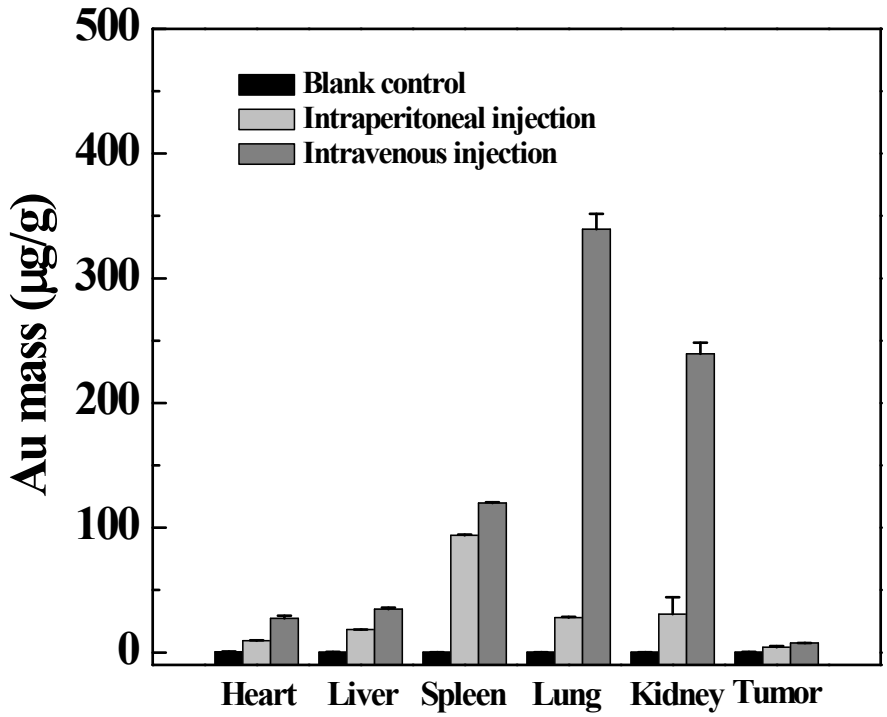

Figure S9. *In vivo* biodistribution of Au element in different organs of tumor-bearing mice at 90 min post intraperitoneal or intravenous injection of the PEGylated Au DENPs. The mouse without injection was used as blank control.