Electronic Supplementary Information

Construction of polydopamine-coated gold nanostars for CT imaging and enhanced photothermal therapy of tumors: an innovative theranostic strategy†

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Part of experimental details:

Characterization techniques. UV-vis spectra were acquired using a Lambda 25 UV-vis spectrophotometer (PerkinElmer, Waltham, MA). The morphology and structure of the Au-PEI@pD NSs were characterized by transmission electron microscopy (TEM) utilizing a JEM-2010F transmission electron microscope (JEOL, Tokyo, Japan). Fourier transform infrared (FTIR) spectra were recorded *via* a Nicolet Nexus670 FTIR spectrophotometer (Thermo, Waltham, MA). Thermal gravimetric analysis (TGA) was carried out using a TG 209 F1 thermal gravimetric analyzer (NETZSCH Instruments Co., Ltd., Selb/Bavaria, Germany). Zeta potential and dynamic light scattering (DLS) measurements were performed by a Malvern Zetasizer Nano-ZS system (Worcestershire, UK) equipped with a standard 633 nm laser. The Au concentration of the Au-PEI@pD NSs was measured by a Leeman Prodigy inductively coupled plasma-optical emission spectroscopy (ICP-OES) system (Hudson, NH). CT imaging was performed using a GE LightSpeed VCT imaging system (GE Medical Systems, Milwaukee, WI) with 100 kV, 80 mA, and a slice thickness of 0.625 mm. The solutions of Au-PEI@pD NSs with different Au concentrations ranging from 0.01 M to 0.08 M were prepared in water before measurements. The Au-PEI@pD NSs were irradiated with an 808 nm laser (Shanghai Xilong Optoelectronics Technology, Shanghai, China) with a power density of 1.3W/cm^2. The temperature of the solution was tracked by a DT-8891E thermocouple linked to a digital thermometer (Shenzhen Everbest Machinery Industry, Shenzhen, China).

Cell viability assay. HeLa cells were regularly cultured in DMEM supplemented with 10% FBS and 1% penicillin (100 units/mL)/streptomycin (100 μg/mL) in a 37 ^{o}C incubator with 5% CO_2.

To determine the *in vitro* cytotoxicity of the Au-PEI@pD NSs and AuNSs-PEI, a standard MTT assay was implemented. Briefly, HeLa cells were seeded into a 96-well culture plate at a

density of 1×10^4 cells per well. After overnight culture to bring the cells to confluence, the medium was substituted with 200 μL of fresh medium containing 20 μL of concentrated PBS suspension of the Au-PEI@pD NSs and AuNSs-PEI at different final Au concentrations **(**0, 0.07, 0.14, 0.28, and 0.41 mg/mL, respectively). The cells were regularly incubated for 24 h. Afterwards, the medium of each well was replaced with 180 μL of fresh medium and 20 μL of MTT solution (5 mg/mL, in PBS), and cells were cultured for additional 4 h. MTT assay was performed according to the manufacturer's instructions and the absorbance of each well was measured by a Multiskan MK3 ELISA reader (Thermo Scientific, Waltham, MA) at 490 nm. Mean and standard deviation (SD) of six parallel wells were reported for each sample.

CT imaging of a xenografted tumor model *in vivo*. The entire animal experiments were completed under the guidelines of the institutional committee for animal care and also the instruction of the National Ministry of Health. Male 4-6 weeks old BALB/c nude mice (15-20 g) were acquired from Shanghai Slac Laboratory Animal Center (Shanghai, China). To develop a xenografted tumor model, 2×10^6 HeLa cells were subcutaneously injected in the back of each nude mouse. When the tumor nodules reached a volume of 0.5-1.0 cm^3, the tumor-bearing mice were intratumorally injected with the Au-PEI@pD NSs ([Au] = 100 mM, in 100 μL PBS). CT scans were carried out before and at 10 min postinjection of the particles using a GE LightSpeed VCT imaging system using the same instrumental parameters as described above.

PTT of cancer cells *in vitro*. HeLa cells were plated in 96-well plates at a density of 1×10^4 cells/well and incubated overnight to allow the cells to be attached. Then the cells were carefully washed three times with PBS and incubated with 200 μL of fresh medium containing Au-PEI@pD NSs at different Au concentrations (0, 0.07, 0.14, 0.28, and 0.41 mg/mL, respectively) for each well. After 4 h of incubation, cells were irradiated by an 808 nm laser with a power density of 1.3 W/cm^2 for 300 s. Subsequently, the cell viability was evaluated *via* MTT assay as described above. In parallel, the morphology of cells treated with

PBS or the Au-PEI@pD NSs at diverse Au concentrations (0, 0.35, 0.7, 1.4, and 2.1 mM, respectively) for 4 h, followed by laser irradiation was visualized by Leica DM IL LED inverted phase contrast microscope with a 200 × magnification for each sample.

Histological examinations. Hematoxylin and eosin (H&E) and TdT-mediated dUTP Nick-End Labeling (TUNEL) staining of tumor sections were performed according to protocols described in the literature.[1] After different treatments, the HeLa tumor-transplanted mice were killed and the tumors were extracted, fixed in 4% paraformaldehyde, embedded in paraffin, and cut into 3 μm sections using a conventional microtome before H&E and TUNEL staining using a standard procedure, respectively. The stained tumor sections were visualized by Leica DM IL LED inverted phase contrast microscope with a 200 × magnification for each sample. The percentage of TUNEL-positive cells in every sample was quantified by selecting five random fields in the microscopic images.

References

(1) Zhu, J.; Zheng, L.; Wen, S.; Tang, Y.; Shen, M.; Zhang, G.; Shi, X. Targeted cancer theranostics using alpha-tocopheryl succinate-conjugated multifunctional dendrimer-entrapped gold nanoparticles. *Biomaterials* **2014,** *35*, 7635-7646.

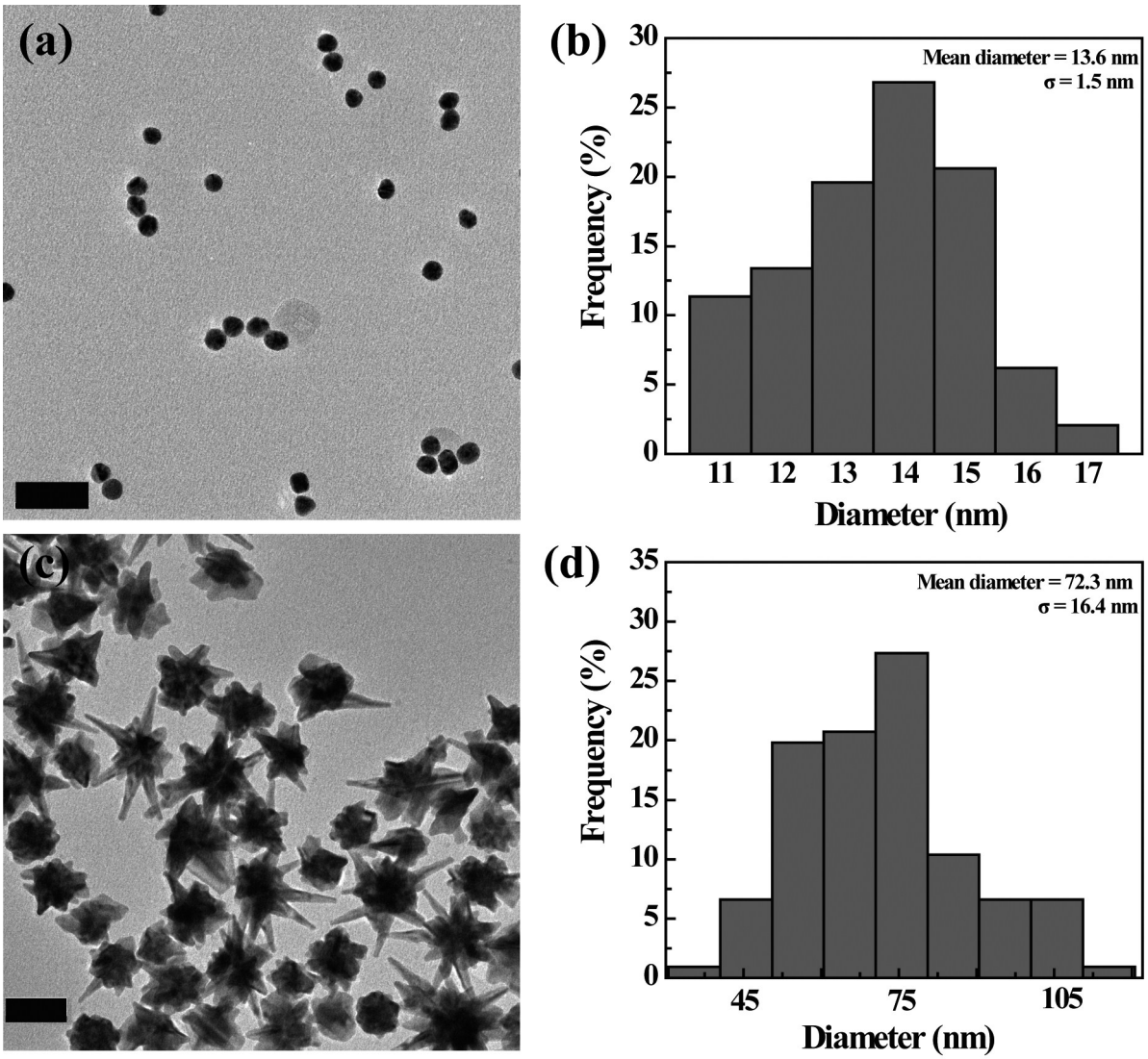

Figure S1. TEM images of Au seeds (a) and AuNSs-PEI (c). (b) and (d) show the size distribution histograms of the Au seeds and AuNSs-PEI, respectively. The scale bars in (a) and (c) represent 50 nm.

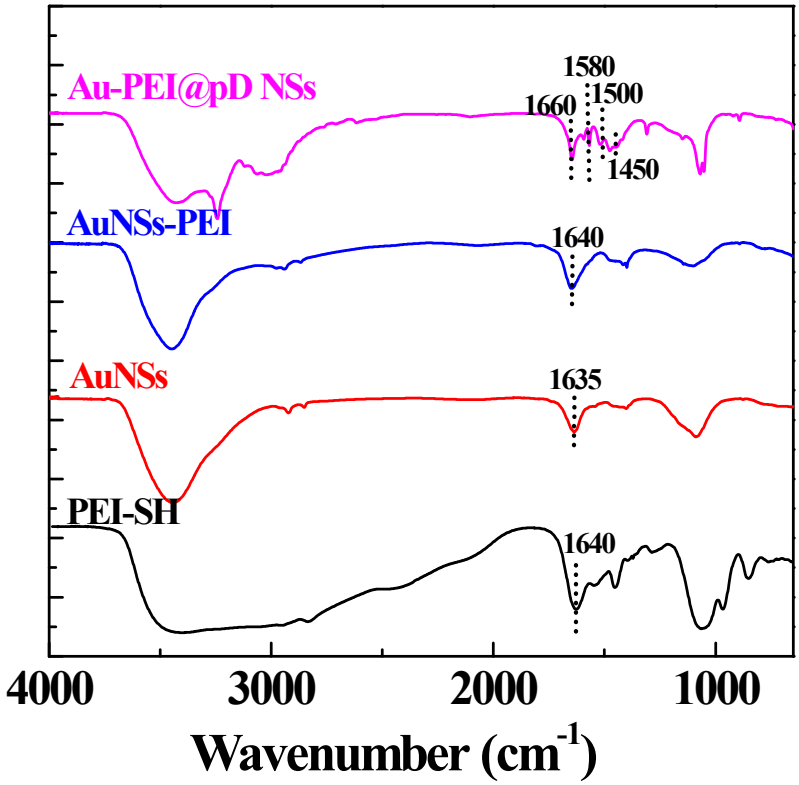

Figure S2. FTIR spectra of PEI-SH, AuNSs, AuNSs-PEI, and Au-PEI@pD NSs, respectively.

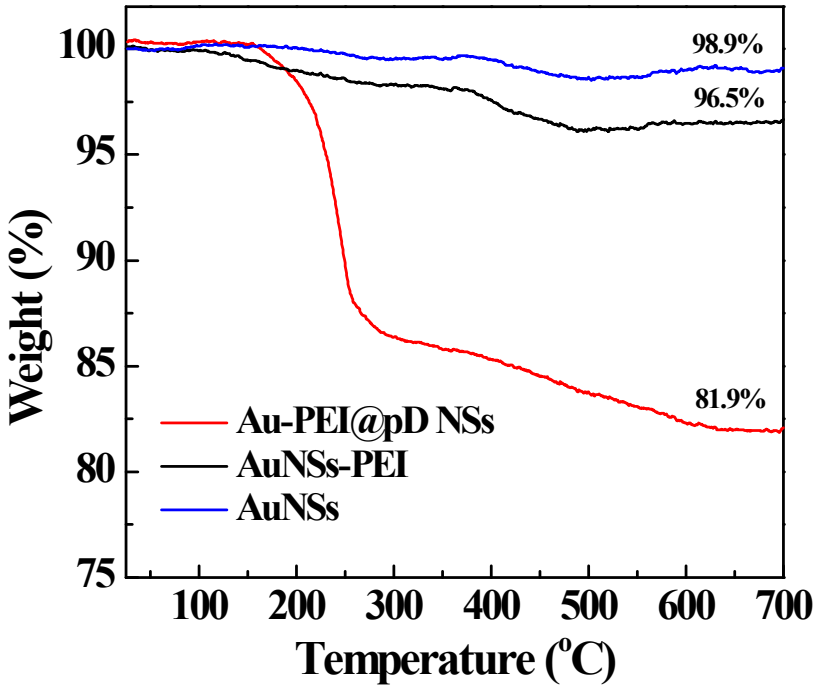

Figure S3. TGA curves of non-modified AuNSs, AuNSs-PEI, and Au-PEI@pD NSs, respectively.

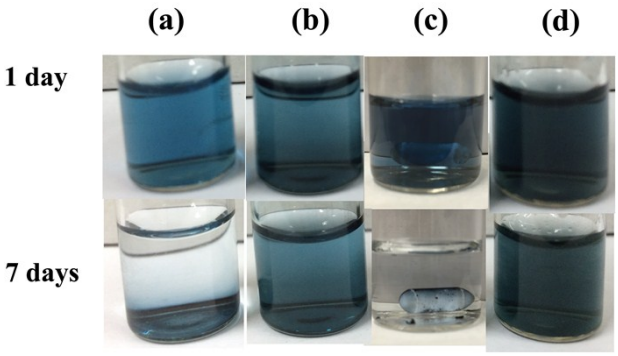

Figure S4. Photographs of AuNSs (a), AuNSs-PEI (b), AuNSs@pD (c) and Au-PEI@pD NSs (d) dispersed in water at the Au concentration of 0.35 mM for different time periods.

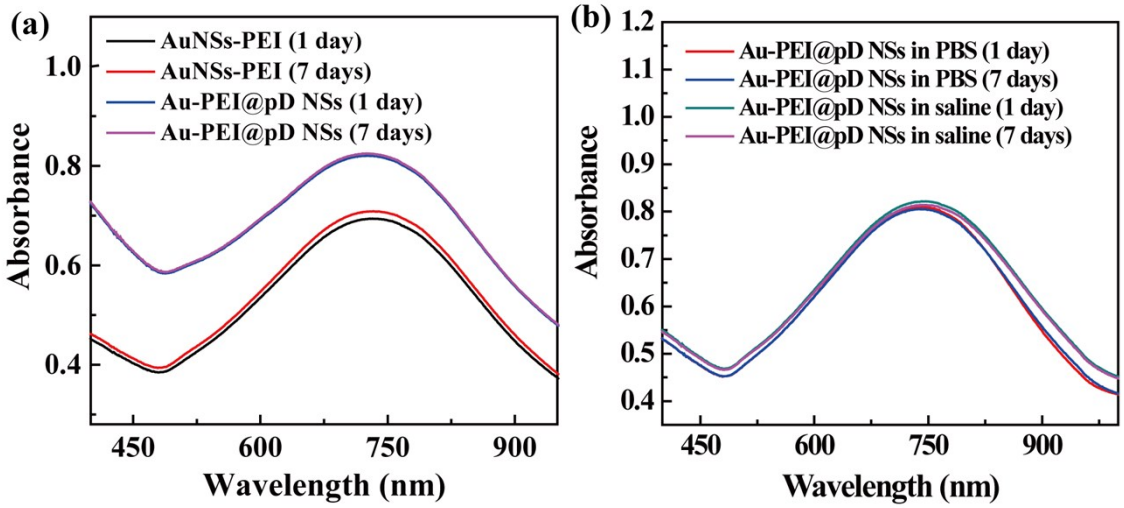

Figure S5. (a) UV-vis spectra of AuNSs-PEI and Au-PEI@pD NSs dispersed in water at the same Au concentration (0.35 mM) for different time periods. (b) UV-vis spectra of Au-PEI@pD NSs dispersed in PBS and saline at different time intervals.

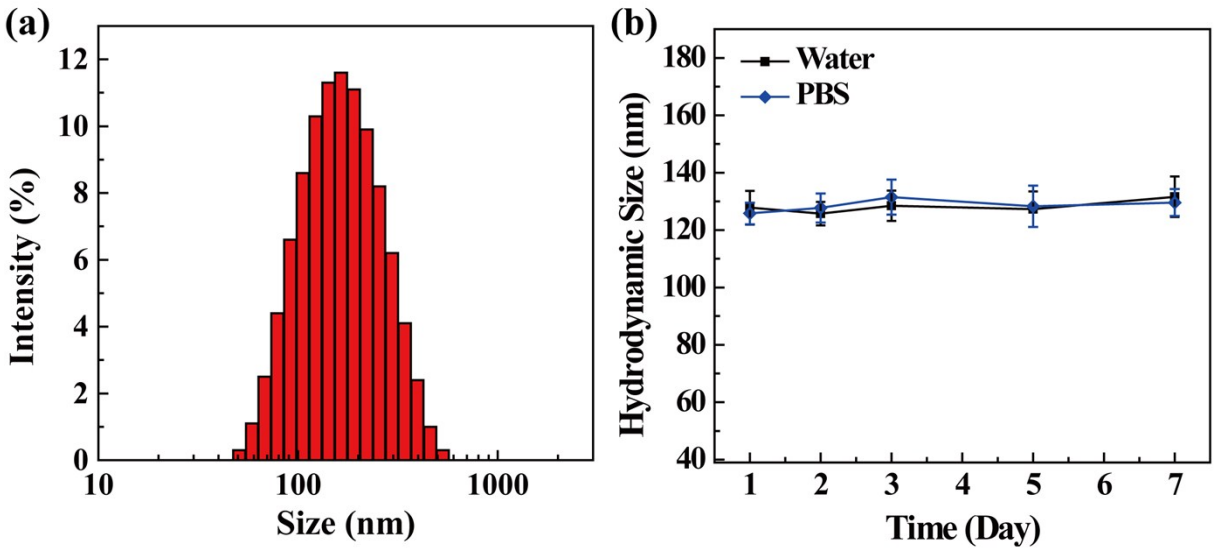

Figure S6. Hydrodynamic size of the Au-PEI@pD NSs dispersed in water (a) and Au-PEI@pD NSs dispersed in water and PBS at different time points (b).

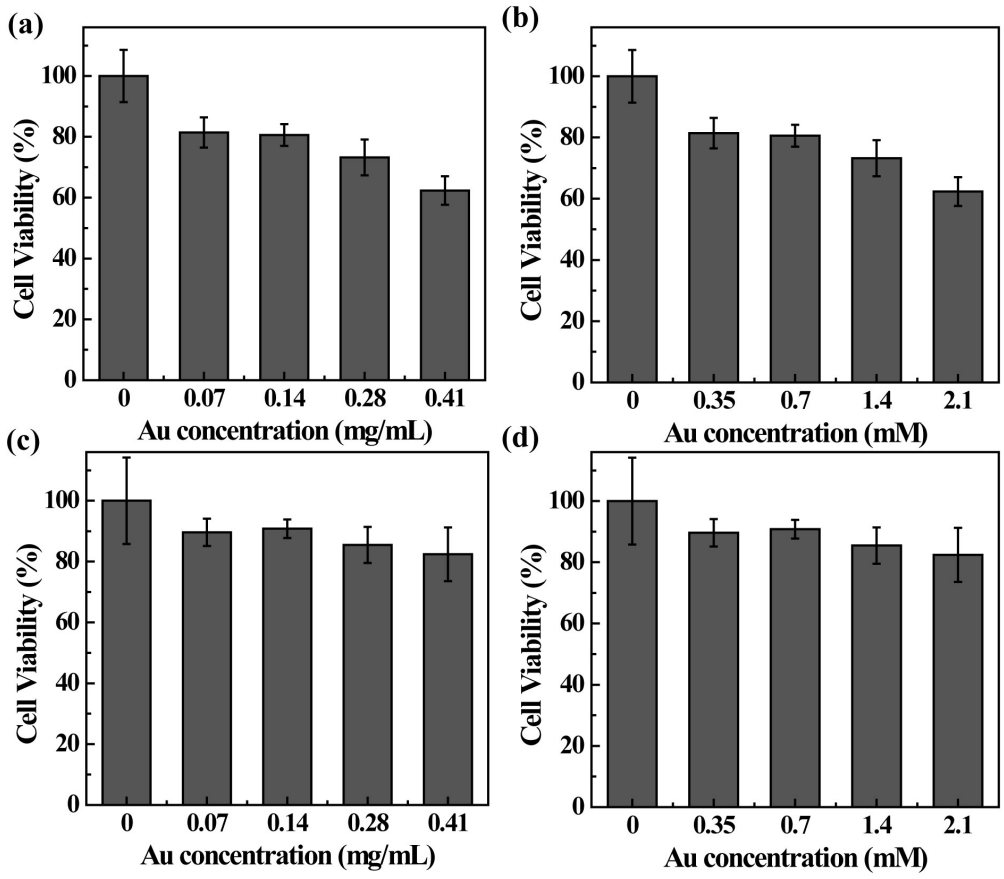

Figure S7. (a,b) MTT assay of HeLa cell viability after treatment with AuNSs-PEI in the Au concentration range of 0-0.41 mg/mL (a) or 0-2.1 mM (b) for 24 h, respectively. (c,d) MTT assay of HeLa cell viability after treatment with the Au-PEI@pD NSs in the Au concentration range of 0-0.41 mg/mL (c) or 0-2.1 mM (d) for 24 h, respectively.

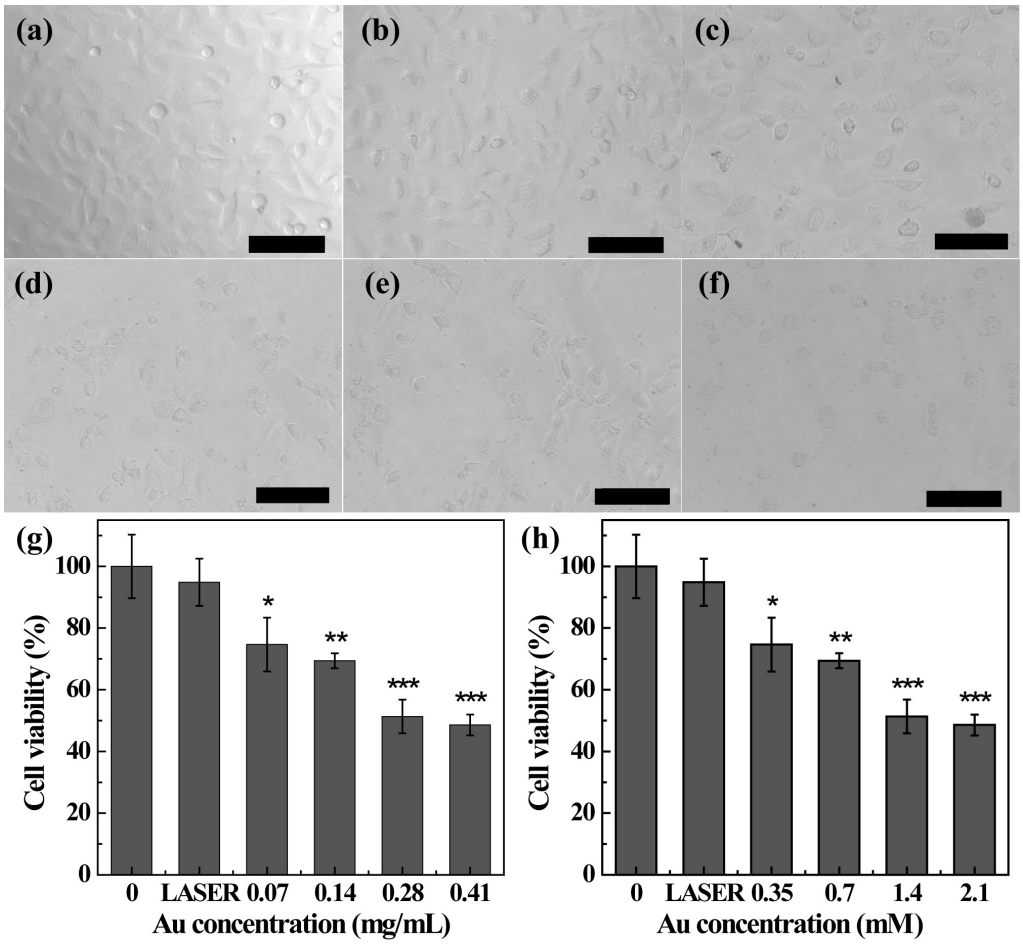

Figure S8. Phase contrast microscopic images of HeLa cells treated with PBS (a), treated with PBS and laser irradiation for 5 min (b), treated with the Au-PEI@pD NSs for 4 h at different Au concentrations (c-f: 0.07, 0.14, 0.28, and 0.41 mg/mL, respectively) and laser irradiation for 5 min. The scale bar in each panel represents 50 μm. (g,h) MTT viability assay of HeLa cells treated with the Au-PEI@pD NSs in the Au concentration range of 0-0.41 mg/mL (g) or 0-2.1 mM (h) for 24 h under different treatments. Cells treated with PBS without laser irradiation were used as control.

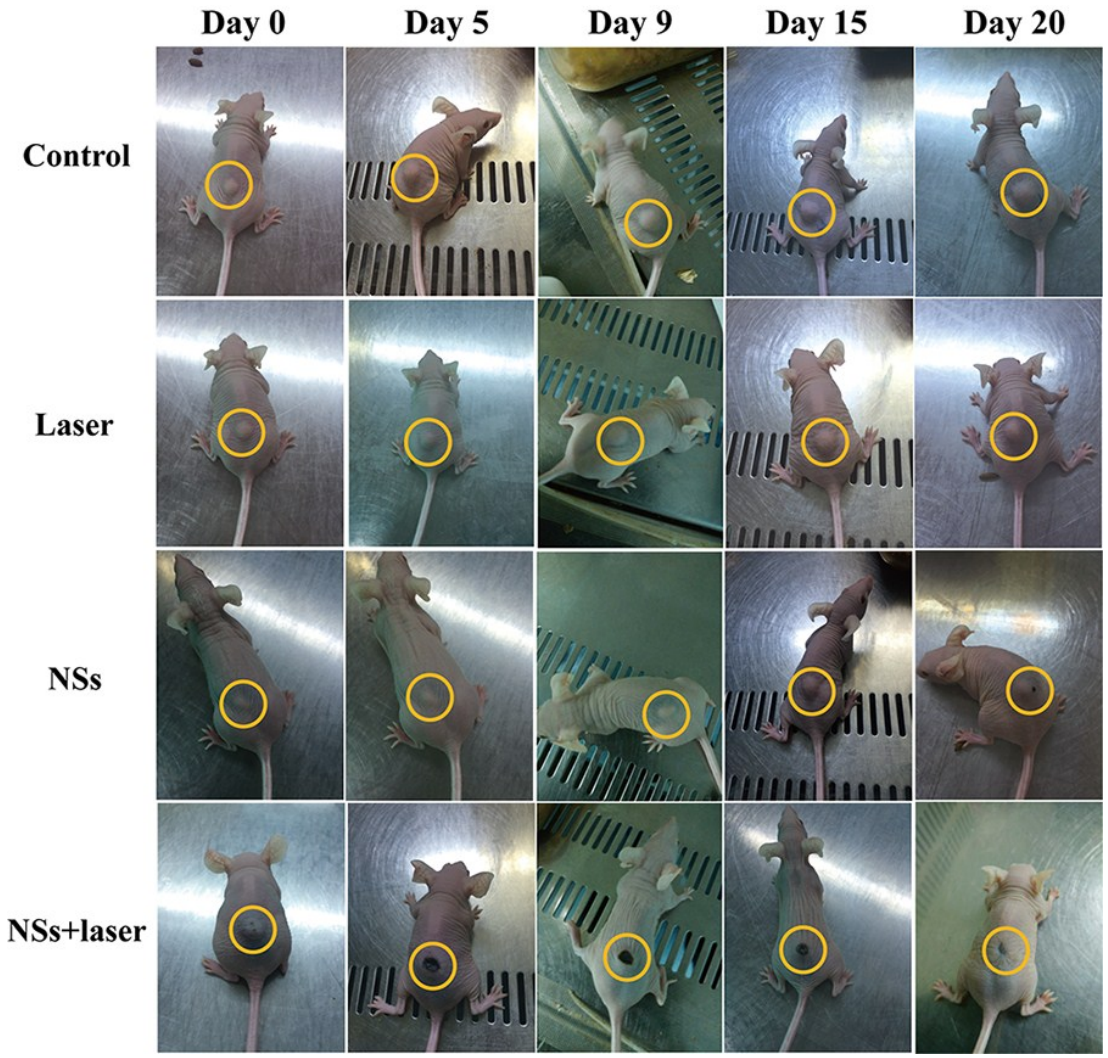

Figure S9. Photographs of the tumor mice after different treatments at different time periods.

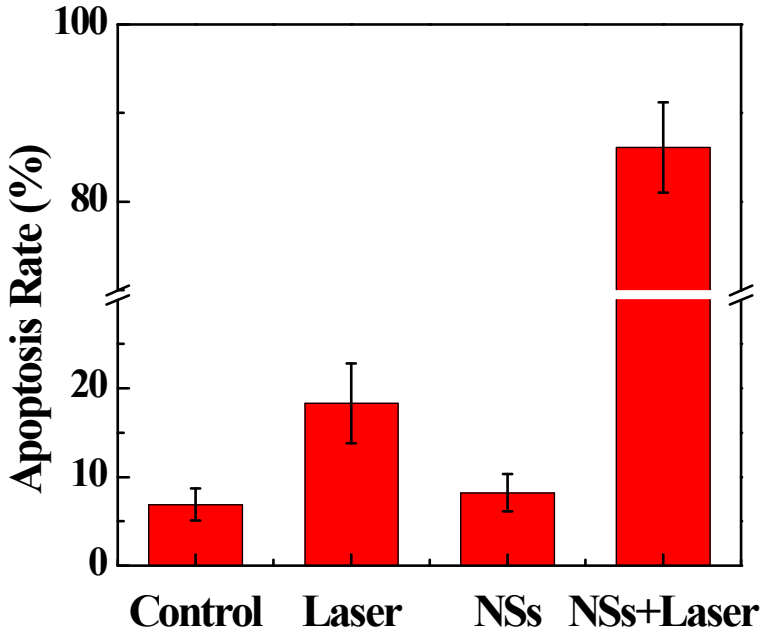

Figure S10. Apoptosis rates of tumor cells after different treatments by quantification of the TUNEL-positive tumor cells in tumor sections.