Supporting information

NIR Light Degradable Antimony Nanoparticle Based Drug Delivery Nanosystem for Synergistic Chemo-Photothermal Therapy *In Vitro*

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Additional Experimental Section

Characterizations

High resolution transmission electron microscopy (HRTEM) and transmissions electron microscopy (TEM) images of AMNP and AMNP-DOX-PAA were collected using JEM-2100F high and normal resolution transmission electron microscope functioned at 120 kV. Fourier transform infrared spectra (FT-IR) for wave numbers in between 4000 and 400 cm^{-1} of AMNP, DOX, PAA and the final nanostructure (AMNP-DOX-PAA) were obtained using Thermo Nicolet 6700 spectrometer with KBr pellet. Shimadzu UV-2500PC ultraviolet-visible spectrometer was utilized to get UV-vis spectra. Potentiometric Analyzer Zetasizer 3000HS interfaced with computer was operated to acquire zeta potential. PTT effect was investigated by using 808 nm continuous wave lasers having 5 mm laser module with adjustable power. The concentration of antimony (Sb) was determined by using inductively coupled plasma atomic emission spectroscopy (ICP-AES, Perkin Elmer DV7300). A microplate reader was utilized to study feasibility of the HeLa cells through measurement of optical density (OD) of all the 96-wells used in the experiment at wavelength of 450 nm. The live and dead cells were identified using fluorescence microscope, DMI3000.

AMNP-DOX-PAA as Photothermal Agents (PTA)

PTT effect of our final samples (AMNP-DOX-PAA) was investigated by irradiating 1 mL of AMNP-DOX-PAA dispersed in DI water at given ranges of concentrations (50.0, 100, and 200 μg/mL) with 808 nm laser (continuous wave) at power density of 1.5 W/cm^2. Then, the temperature increment for each of the selected concentration of the aqueous solution was measured every 20 s using a digital thermometer.

DOX Release of AMNP-DOX-PAA

For DOX releasing measurement, 3.0 mL of AMNP-DOX-PAA dispersion (2.0 mg/mL) with and without 808 nm laser treatment for 10 min were added in a dialysis bag (8000 MW) and dialyzed in 4.0 mL PBS solution at pH 5.0 and 7.4 in the dark by stirring at 37 °C. At different

time points (2, 4, 6, 8, 10, and 20 h), the PBS solution with the released DOX from AMNP-DOX-PAA was collected and replaced with an equal amount of fresh PBS solution with the corresponding pH values. The amount of DOX released at each time point was calculated by using UV-Vis absorbance of the collected PBS solution at 480 nm. This DOX release experiment was performed three times.

Cytotoxicity Assessment *In Vitro*

First of all, biocompatibility of AMNP was confirmed *via* cytotoxicity assay experiment conducted using HeLa cells. Accordingly, feasibility of the cells was studied by treating cells with 50.0, 100, and 200 μg/mL concentrations of AMNP using previously reported methods with some modifications.[S1,S2] Briefly, HeLa cells were kept in high glucose Dulbecco's Modified Eagle's medium (DMEM, 4.50 g/L glucose) supplemented with 10 % FBS and 1 % penicillin/streptomycin in an atmosphere of 5% CO_2/95% air (at 37 °C). Then, the cells were seeded into 96-well plates at density of 5000 cells per well in 100 μL culture medium and grown overnight. After that, the medium was removed and 200 μL of fresh culture medium containing AMNP with concentrations of 50.0, 100, and 200 μg/mL were added to the HeLa cells for 24 h at 37 °C under 5% CO_2/95% air. After that the culture medium was removed and 100 μL of CCK-8 solution that containing 10 % CCK-8 was added to each well and kept for 1.5 h. The viability of the cells was then measured by a microplate reader (Thermo, VarioskanFlash, Boston, MA, USA) at the wavelength of 450 nm. The cell viability was calculated using the following equation which is the same as previous report.[S3]

$$Cell\ viability = \frac{OD_{test} - OD_{blank}}{OD_{control} - OD_{blank}} x100\% \quad (1)$$

where OD_{test} is the optical density (OD) of the cells exposed to AMNP-DOX-PAA, $OD_{control}$ is the optical density of the control, and OD_{blank} is the optical density of the well without cells. All of the tests were independently performed six times to confirm the cell compatibility. And another replicate was performed for each experiment to confirm reproducibility of the data. If reproducibility of the data is ok, the data of replicate experiment were not used.

Based on the result of biocompatibility test of AMNP, 0, 18.12, 37.50, and 75.00 μg/mL of AMNP was chosen to synthesize the final drug delivery system (AMNP-DOX-PAA) with the concentration of 0, 50.0, 100, and 200 μg/mL respectively for further investigation of cytotoxicity of HeLa cells with and without laser treatment. All cytotoxicity experiment of the HeLa cells were independently performed six times. Accordingly, the HeLa cells treated with AMNP at concentration of 0, 18.12, 37.50, and 75.00 μg/mL for 4 h were exposed to 808 nm laser (1.5 W/cm^2, 10 min) to investigate only PTT effect. At the same time the cells were incubated for 4 h with the final AMNP-DOX-PAA at concentration of 0, 50.0, 100, and 200 μg/mL that have the same concentration of AMNP mentioned above respectively. Then, the HeLa cells incubated with AMNP-DOX-PAA were divided into two groups, a group without laser treatment and a group exposed to 808 nm laser (1.5 W/cm^2, 10 min) in order to investigate the chemotherapy and the synergistic chemo-phototherapy efficacy of the sample, respectively. In addition for comparison, the control group (HeLa cells without the sample) was also irradiated with 808 nm laser for 10 min (power density: 1.5 W/cm^2) to observe the effect of the laser irradiation on the viability of the cells. Followed by incubation of the cells in each group for additional 24 h, the culture medium was avoided and 100 μL of CCK-8 solution containing 10 % of WST-8 was added to every well. After incubation of cells for extra 1.5 h, the OD of wells was determined by using a micro-plate reader at wavelength of 450 nm. Finally, the cell feasibility of each experimental and control group discussed above was obtained according to the cell viability equation.

Live and Dead Staining of HeLa Cells after Treated with the Samples.

A live and dead cell assess was done to image the cells exposed to the 75.0 μg/mL and 200 μg/mL of AMNP and AMNP-DOX-PAA, respectively by staining the live and dead cells with the mixture of calcein AM (calceinacetoxymethyl) and PI (propidium iodide) which can distinguish live cells represented with green emission at 515 nm (λ_{ex} = 495 nm) from dead cells represented with red emission at 617 nm (λ_{ex} = 535 nm). All groups of cells were treated according to different concentrations mentioned above (see section 2.8). After treatment of the cells for 24 h, the culture medium was avoided and the cells were washed with D-hanks and then incubated with the mixture of calcein AM and PI for another 30 min at room temperature in dark. After the surplus dyes were

avoided and the cells were washed again with D-hanks, 100 μL of D-hanks solution was introduced to each well. Then the cells were investigated under a fluorescence microscope.

Cellular Uptake

Confocal laser scanning microscopy was used to investigate the uptake of AMNP-DOX-PAA by HeLa cells. Briefly, HeLa cells were plated in glass bottom cell culture dish (1.5×10^5 cells per well) and cultured overnight. Then, culture medium was replaced with fresh culture medium containing 200 μg/mL AMNP-DOX-PAA. After incubated for 0.5, 2, 4 h, the cells were carefully washed with D-Hanks solution for three times, fixed with 4 % paraformaldehyde and then suspended in 200 μL culture medium for Confocal laser scanning microscopy image.

Computational Methodology to Investigate Degradability of Antimony

In order to study the reactivity of AMNP in the water medium, we used the density functional theory based code CASTEP for the geometry optimizations and the molecular dynamics calculations, as implemented in Materials Studio 2016. As the size of the nanoparticle is large enough to be considered, in the first approximations, as bulk, we modeled the AMNP surface as a periodic surface. In CASTEP we used norm-conserving pseudo-potentials with reciprocal space representation for all atoms. The valence shells of the atoms Sb and O contain 5 and 6 electrons in the orbitals ($5s^2\ 5p^3$) and ($2s^2\ 2p^4$) respectively. In the geometry optimizations, the Brillouin zone was sampled by using a mesh commensurate to the specific lattice dimensions, but with the actual spacing below 0.03 $Å^{-1}$. In the molecular dynamics we used Γ-point calculations. We employed the Perdew-Burke-Ernzerhof (PBE) form of the generalized gradient approximation (GGA) of the exchange-correlation functional. For the dispersion interactions we used the dispersion correction scheme proposed by Grimme, as implemented in CASTEP. In the molecular dynamics, we used the microcanonical ensemble (NVE) to simulate an isolated system with a fixed total energy and the canonical ensemble (NVT) at different temperatures, from 300 K to 2000 K. The time step was set to 2 fs and the total simulation time was set to 5 ps. The Sb (0 1 2) surface, which is the experimentally obtained surface of AMNP, was modelled starting with the 3x3x3 supercell of the trigonal Sb, space group R-3m. The model surface contains 162 atoms on three parallel slabs. The atomic positions of Sb on the last two slabs were kept fixed. Though this is a small model surface,

it is quite demanding for the first-principles calculations. On top of Sb surface an increasing number (from 1 to 70) of water molecules were added, in order to reach the water molecule coverage of up to 16 molecules/nm^2. In addition, a reduced model surface, cut through the 3x3x3 supercell by symmetry considerations and containing 4 layers of the Sb (0 1 2) surface, was used with 50 H_2O molecules to reach a coverage of 38 molecules/nm^2. This helped to clarify the coverage dependence of the reactivity of antimony surface with thermally activated water molecules.

REFERENCES

S1. Li, P.; Yan, Y.; Chen, B.; Zhang, P.; Wang, S.; Zhou, J.; Fan, H.; Wang, Y.; Huang, X. Lanthanide-doped Upconversion Nanoparticles Complexed with Nano-Oxide Graphene Used for Upconversion Fluorescence Imaging and Photothermal Therapy. *Biomater. Sci.* **2018,** *6*, 877-884.

S2. Ge, X.; Sun, L.; Ma, B.; Jin, D.; Dong, L.; Shi, L.; Li, N.; Chen, H.; Huang, W. Simultaneous Realization of Hg^{2+} Sensing, Magnetic Resonance Imaging and Upconversion Luminescence in vitro and in vivo Bioimaging Based on Hollow Mesoporous Silica Coated UCNPs and Ruthenium Complex. *Nanoscale* **2015,** *7*, 13877-13887.

S3. Chen, H.; Zheng, D.; Liu, J.; Kuang, Y.; Li, Q.; Zhang, M.; Ye, H.; Qin, H.; Xu, Y.; Li, C.; Jiang, B. pH-Sensitive Drug Delivery System Based on Modified Dextrin Coated Mesoporous Silica Nanoparticles. *Int. J. Biol. Macromolecules* **2016,** *85*, 596-603.

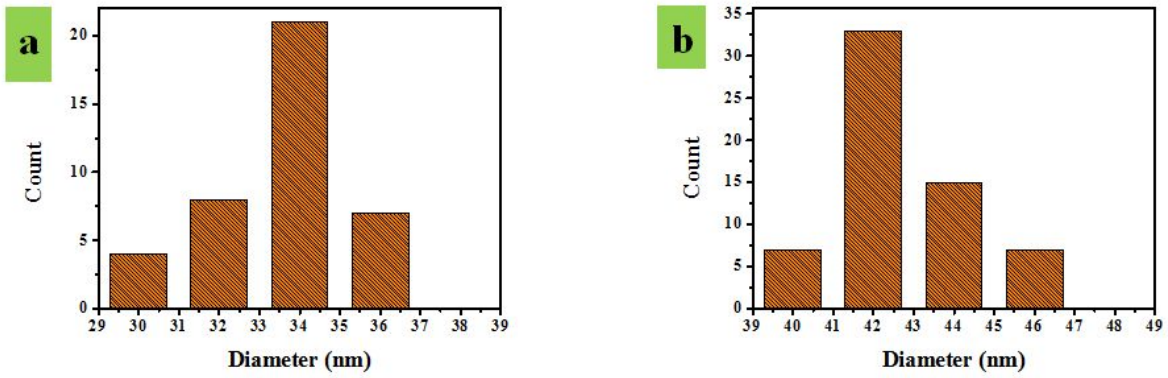

Figure S1. Size distributions of (a) AMNP and (b) AMNP-DOX-PAA obtained from TEM images.

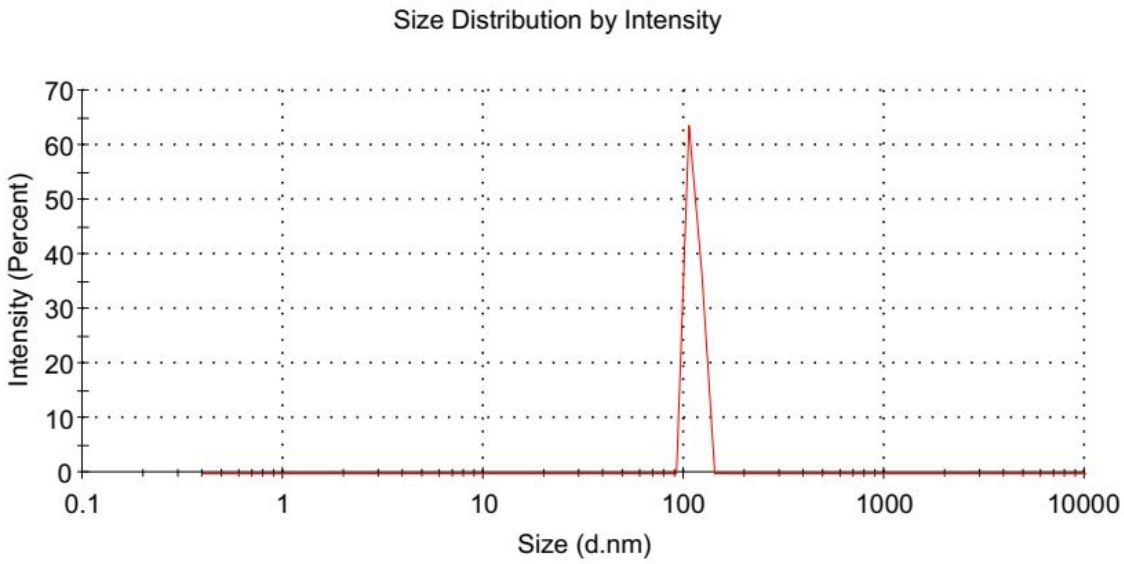

Figure S2. Dynamic light scattering (DLS) distribution of AMNP-DOX-PAA in H_2O (intensity−average diameters is ~111.8 nm).

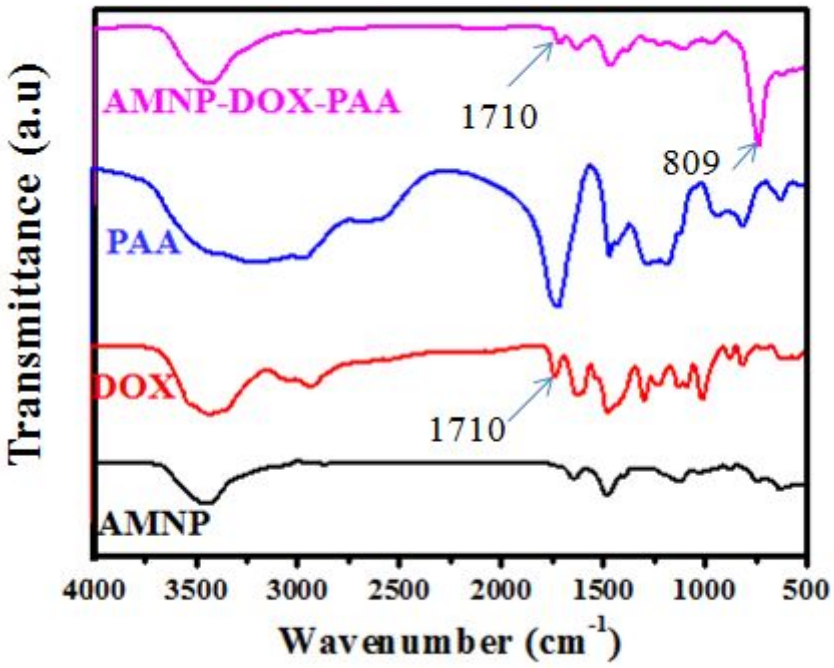

Figure S3. FTIR spectra of AMNP, DOX, PAA, and AMNP-DOX-PAA, respectively.

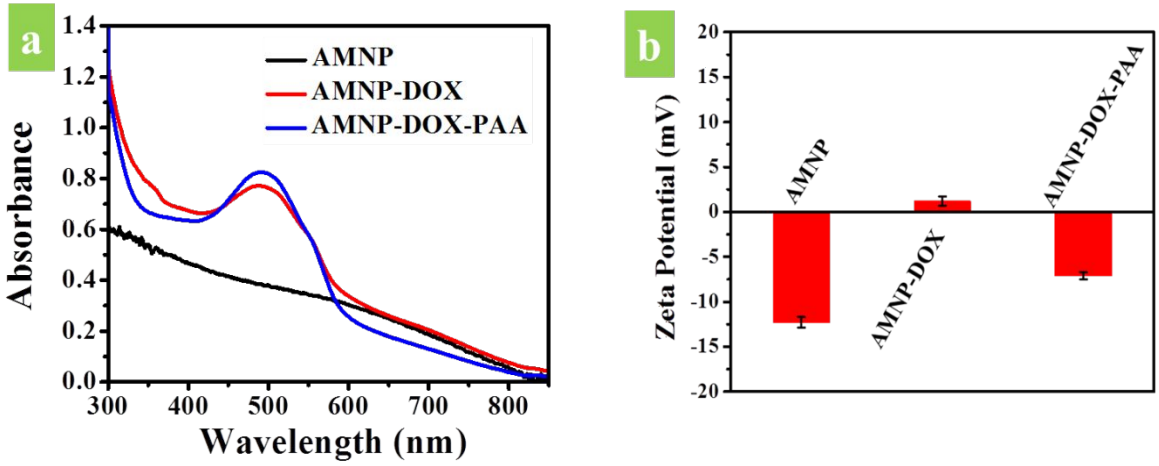

Figure S4. (a) UV-vis absorption spectra and (b) zeta potentials of AMNP, AMNP-DOX, and AMNP-DOX-PAA, respectively. Data are mean zeta potential (mV) (n = 3) ± standard deviation.

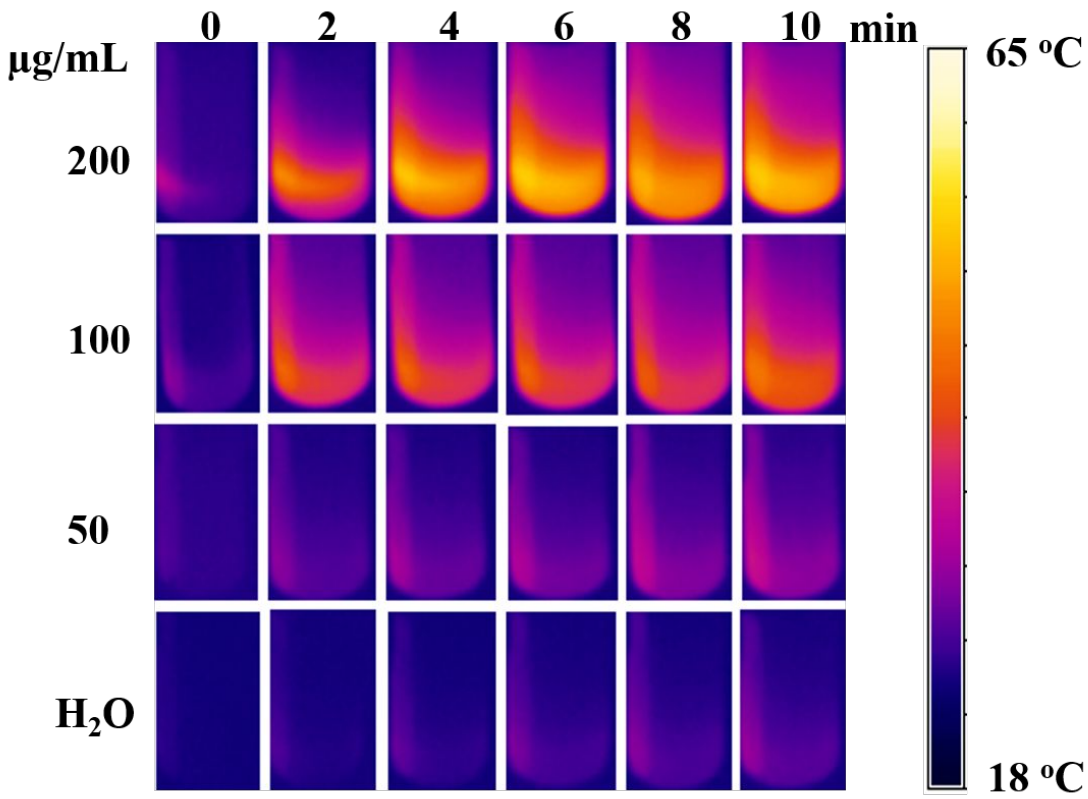

Figure S5. Infrared thermal images of different concentrations (50-200 μg/mL) of ANP-DOX-PAA irradiated with 808 nm laser (1.5 W/cm^2) at different time points (0-10 min). Water is used for comparison.

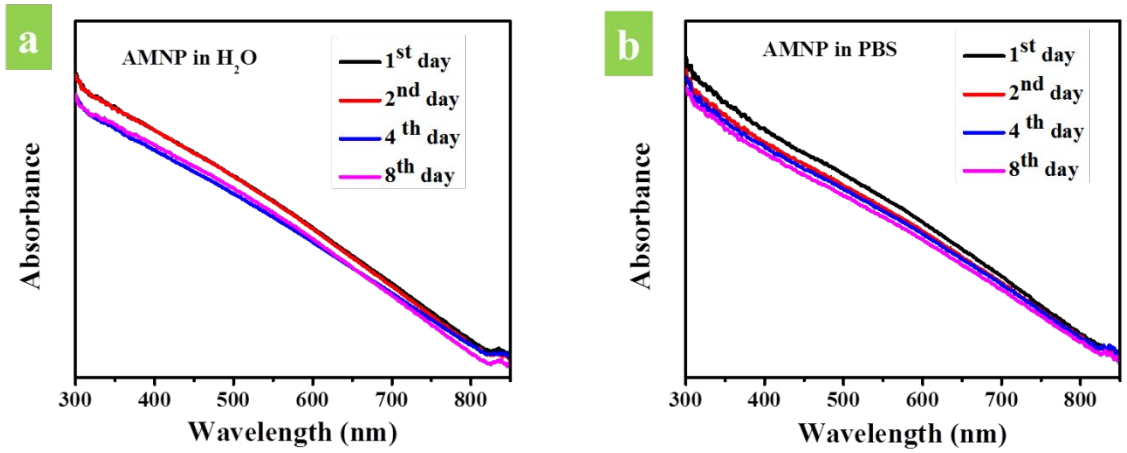

Figure S6. UV-Vis-NIR spectra of AMNP dispersed in (a) water (b) PBS at concentration of 200 µg/mL for 1 to 8 days.

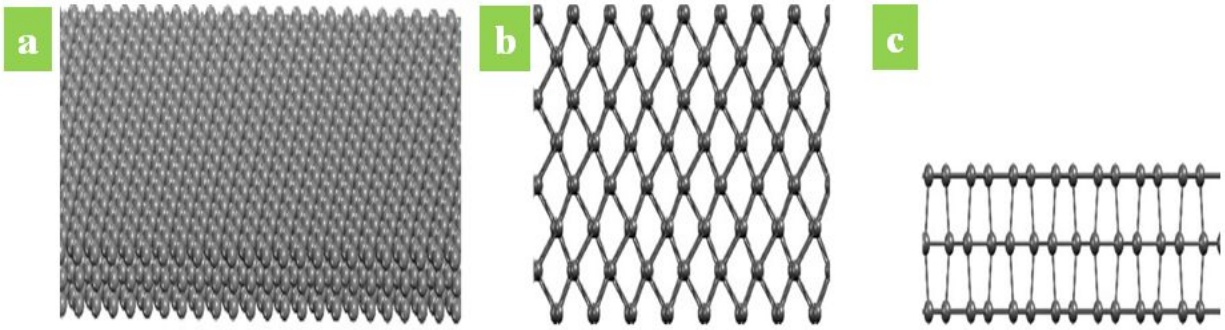

Figure S7. (a) A 3D-view, (b) a top view and (c) lateral view of the Sb (0 1 2) surface.

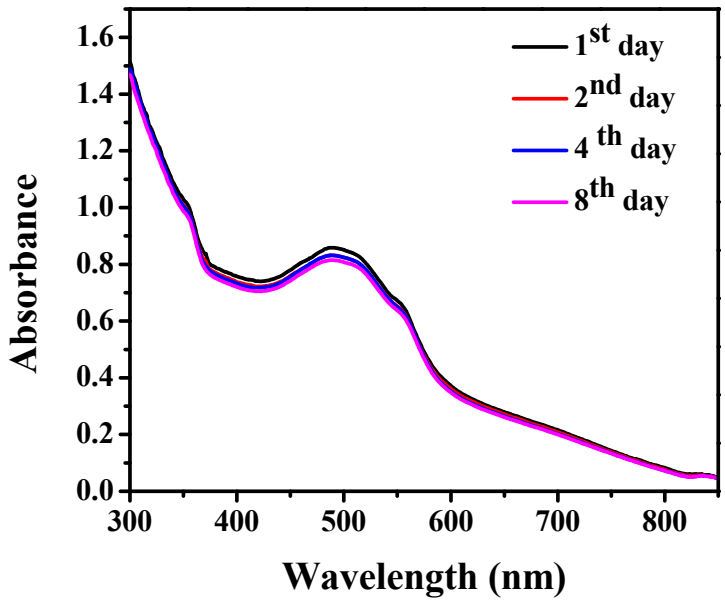

Figure S8 UV-Vis-NIR absorption spectra of AMNP-DOX-PAA dispersed in water at concentration of 200 μg/mL for 1 to 8 days.

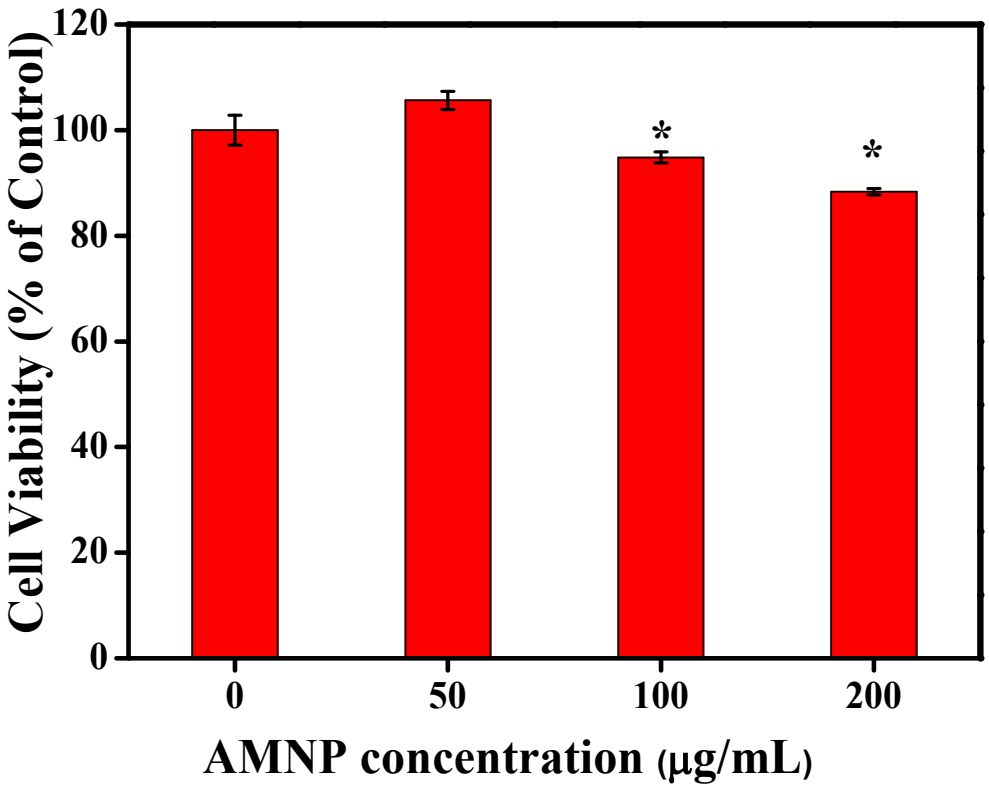

Figure S9. Cytotoxicity assays of HeLa cells incubated with AMNP nanoparticles with different concentrations for 24 h.