Electronic Supplementary Information

Design of dual drug-loaded dendrimer/carbon dot nanohybrids for fluorescence imaging and enhanced chemotherapy of cancer cells

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Part of the experimental details

Materials

Ethylenediamine core amine-terminated generation 5 (G5) poly(amidoamine) (PAMAM) dendrimers were purchased from Dendritech (Midland, MI). Thiolated cyclic arginine-glycine-aspartic (RGD) peptide was purchased from GenicBio (Shanghai, China). Polyethylene glycol (PEG) monomethyl ether with one end of carboxyl group (*m*PEG-COOH) and PEG with maleimide group at one end and carboxyl group at the other end (MAL-PEG-COOH) were purchased from Shanghai Yanyi Biotechnology Corporation (Shanghai, China). D-α-Tocopherol polyethylene glycol 1000 succinate (TPGS 1000) was from Aldrich (St. Louis, MO). Sodium citrate and ammonia were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 1-Ethyl-3-(3-dimethylaminopropy) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), and N, N'-carbonyldiimidazole (CDI) were from J&K Chemical Ltd. (Shanghai, China). DOX·HCl was from Beijing Huangfeng Pharmaceutical Co., Ltd. (Beijing, China). Cell counting kit-8 (CCK-8) was acquired from 7sea Biotech. Co., Ltd. (Shanghai, China). A549 cells (a non-small cell lung carcinoma cell line) were acquired from Institute of Biochemistry and Cell Biology (the Chinese Academy of Sciences, Shanghai, China). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin, and streptomycin were purchased from HyClone Lab., Inc. (Logan, UT). All chemicals and materials were used as received. Water used in all experiments was purified using a MilliQ Plus185 water purification system (Millipore, Bedford, MA) with a resistivity higher than 18.2 MΩ·cm. Regenerated cellulose dialysis membranes with molecular weight cut-off (MWCO) of 1000, 5000 and 8000 were acquired from Fisher (Pittsburgh, PA).

Characterization techniques

UV-vis spectroscopy was carried out using a Lambda 25 UV-vis spectrophotometer (PerkinElmer, Waltham, MA). Fluorescence spectroscopy was performed using a QuantMaster-40 fluorescence

spectrophotometer (Protein Technologies, Inc., Tucson, AZ). Zeta potentials were measured using a Malvern Zetasizer Nano ZS model ZEN3600 (Worcestershire, UK) equipped with a standard 633 nm laser. Transmission electron microscopy (TEM) imaging was executed using a JEOL 2010F analytical electron microscope (JEOL, Tokyo, Japan) at an operating voltage of 200 kV. Samples were prepared by dropping an aqueous particle suspension onto a carbon-coated copper grid and air dried before measurements. Fourier transform infrared (FTIR) spectra were recorded on a Nicolet 6700 FTIR spectrophotometer (Thermo Electron Corporation, Madison, WI).

***In vitro* drug release kinetics**

An aqueous solution of (CDs/DOX)@G5-RGD-TPGS complexes (2 mg/mL, 1 mL) was placed in a dialysis bag having an MWCO of 8000, and then exposed to 9 mL of phosphate buffered saline (PBS, pH 7.4) or phosphate buffer (pH 5.5). The entire system was kept in a constant temperature vibrator at 37 °C. One milliliter was taken out from the outer phase at different time intervals and the samples were measured by UV-vis spectrometry. The volume of outer phase was maintained constant by adding 1 mL of the corresponding buffer solution.

Cytotoxicity assay

A549 cells were regularly cultured and passaged under normal cell culture conditions. To test the therapeutic activity of the complexes, A549 cells were seeded in 96 well plates at the density of 1×10^4 cells per well with 100 μL of DMEM containing 10% FBS, 100 U mL^{-1} penicillin, and 100 U mL^{-1} streptomycin for each well and incubated at 5% CO_2 and 37 °C. After 24 h, the medium was replaced with fresh medium containing (CDs/DOX)@G5-RGD-TPGS complexes, (CDs/DOX)@G5-RGD-*m*PEG complexes, CDs/DOX, G5-RGD-TPGS or free DOX·HCl with different DOX concentrations (1, 5, 15, 25 and 35 μg/mL, respectively). After 24 h, the cells were washed three times with PBS and treated with 100 μL of DMEM containing 10% CCK-8 for each well for additional 2 h. A Thermo Scientific Multiskan MK3 ELISA reader (Waltham, MA) was used to record the absorbance of each well at 450 nm.

Flow cytometry analysis

A549 cells were seeded in 12 well plates at a density of 2×10^5 cells per well and incubated at 5% CO_2

and 37 °C. After 24 h, the medium was replaced with fresh medium containing (CDs/DOX)@G5-RGD-TPGS complexes or (CDs/DOX)@G5-*m*PEG-TPGS complexes with different DOX concentrations (10, 15 and 20 μg/mL, respectively) and PBS was used as control. After 6 h, the cells were washed with PBS for three times, trypsinized, resuspended in PBS, and analyzed using a Becton Dickinson FACScan flow cytometer (BD Biosciences, Franklin Lake, NJ).

Fluorescence microscopy

The cells were plated on a 24 well plate at a density of 5×10^4 cells per well and incubated for 24 h. Then the medium was replaced with fresh medium containing CDs with different concentrations (400-800 μg/mL), and DOX·HCl or (CDs/DOX)@G5-RGD-TPGS complexes with the same DOX concentration (20 μg/mL). PBS was used as control. The cells were incubated for 6 h, washed with PBS for three times, fixed with glutaraldehyde (4%), and observed using an Axio Vert.A1 Carl Zeiss fluorescence microscope (Jena, Germany).

Statistical analysis

One way analysis of variance (ANOVA) statistical analysis was used to assess the significance of the experimental data by selecting a p value of 0.05 as a significance level. The data were indicated with *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$, respectively.

Table S1. The fluorescence quantum yield of CDs, and CDs/DOX and (CDs/DOX)@G5-RGD-TPGS complexes.

Sample	Quantum Yield (%)
CDs	36.8%
CDs/DOX	24.8%
(CDs/DOX)@G5-RGD-TPGS	16.4%

Table S2. The drug loading content (DLC) and the drug loading efficiency (DLE) of CDs/DOX and (CDs/DOX)@G5-RGD-TPGS complexes.

Sample	Drug loading content (DLC)	Drug loading efficiency (DLE)
CDs/DOX	11.4 %	22.3 %
(CDs/DOX)@G5-RGD-TPGS	7.8 %	22.3 %

Table S3. NMR diffusion coefficients (D) and radius (R) of CDs, G5-RGD-TPGS conjugates, and (CDs/DOX)@G5-RGD-TPGS complexes.

Sample	Diffusion coefficient (m^2/s^{-1})		δ（ppm）	Radius (nm)
CDs	$D_{CDs} = 5.06\times10^{-10}$	$D_{D_2O} = 1.96\times10^{-9}$	2.39-2.59 for citrate	3.94
G5-RGD-TPGS	$D_{dendrimer} = 3.53\times10^{-11}$	$D_{D_2O} = 1.95\times10^{-11}$	2.10-3.40 for dendrimer	5.65
(CDs/DOX)@G5-RGD-TPGS	$D_{dendrimer} = 3.41\times10^{-11}$	$D_{D_2O} = 1.94\times10^{-11}$	2.64-3.40 for dendrimer	5.85

Table S4. The half maximal inhibitory concentrations (IC_{50}) of the CDs/DOX, (CDs/DOX)@G5-RGD-*m*PEG, (CDs/DOX)@G5-RGD-TPGS complexes and free DOX·HCl.

Sample	IC_{50} (μg/mL)
CDs/DOX	30.40
(CDs/DOX)@G5-RGD-*m*PEG	27.18
(CDs/DOX)@G5-RGD-TPGS	10.0
free DOX·HCl	10.47

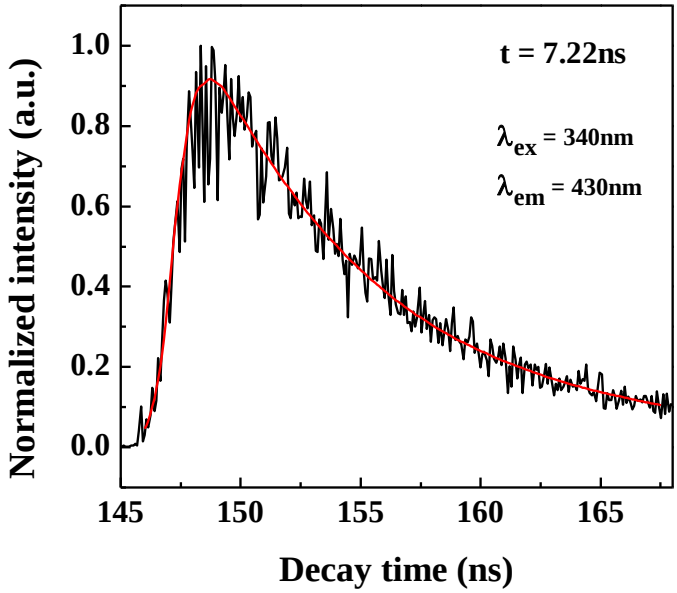

Figure S1. Fluorescence decay profile of CDs (λ_{ex} = 340 nm; λ_{em} = 430 nm).

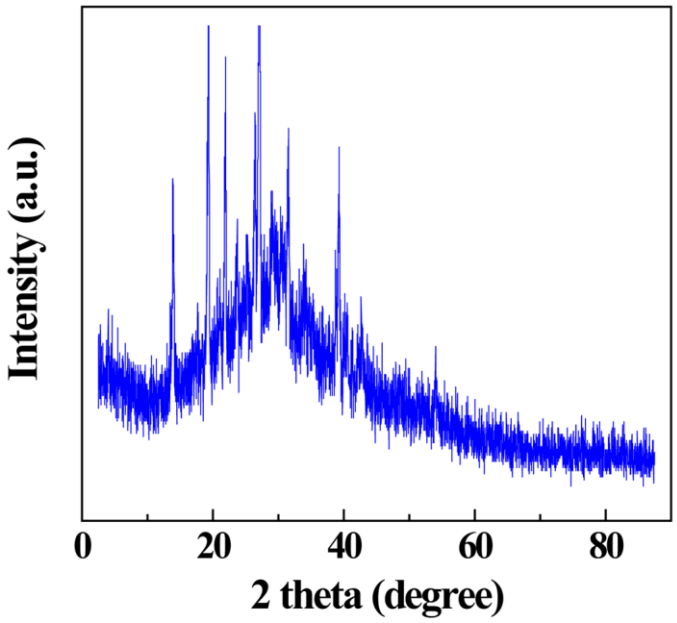

Figure S2. XRD pattern of the formed CDs.

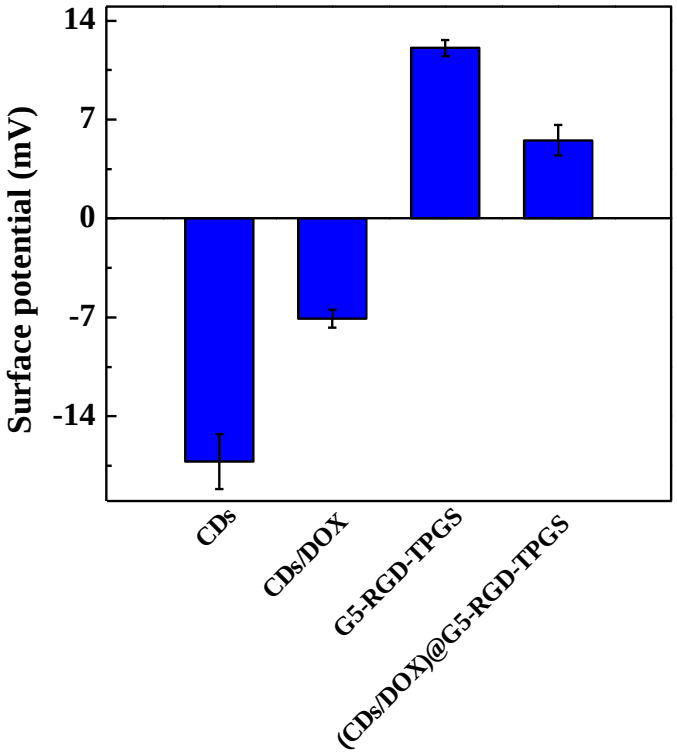

Figure S3. Surface potential of CDs, CDs/DOX complexes, G5-RGD-TPGS conjugates and (CDs/DOX)@G5-RGD-TPGS complexes, respectivity.

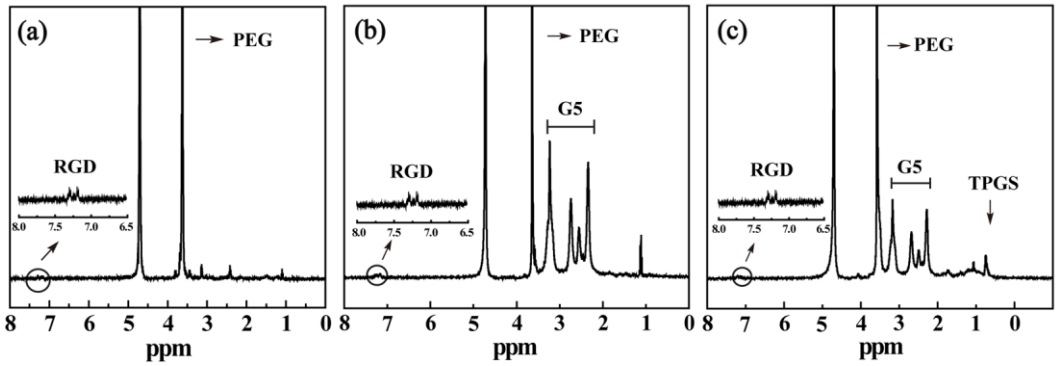

Figure S4. ^{1}H NMR spectra of RGD-PEG-COOH (a), G5-PEG-RGD (b), and G5-RGD-TPGS (c), respectively.

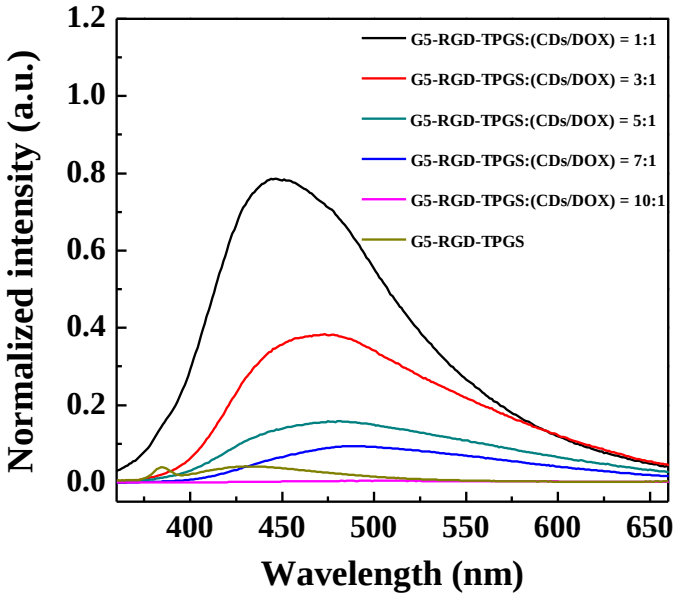

Figure S5. Fluorescence emission spectra of G5-RGD-TPGS conjugates and (CDs/DOX)@G5-RGD-TPGS complexes at different mass ratios formed *via* covalent bonding (λ_{ex} = 340 nm).

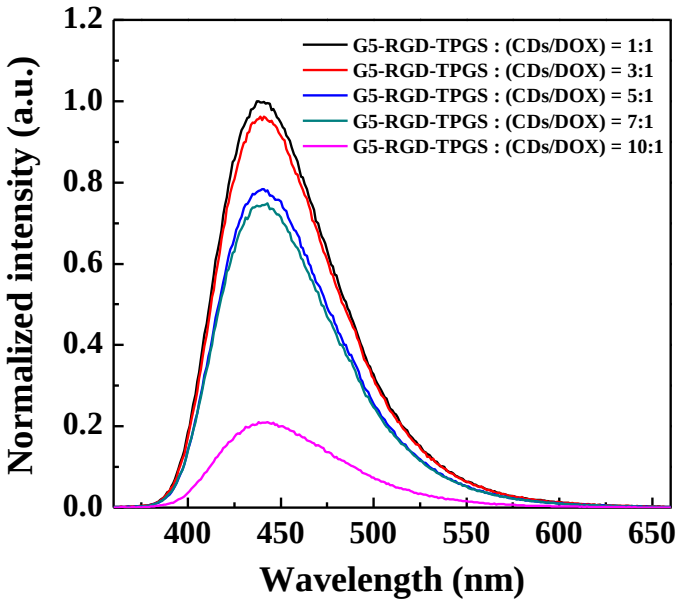

Figure S6. Fluorescence emission spectra of (CDs/DOX)@G5-RGD-TPGS complexes formed *via* electrostatic interaction at different mass ratios (λ_{ex} = 340 nm).

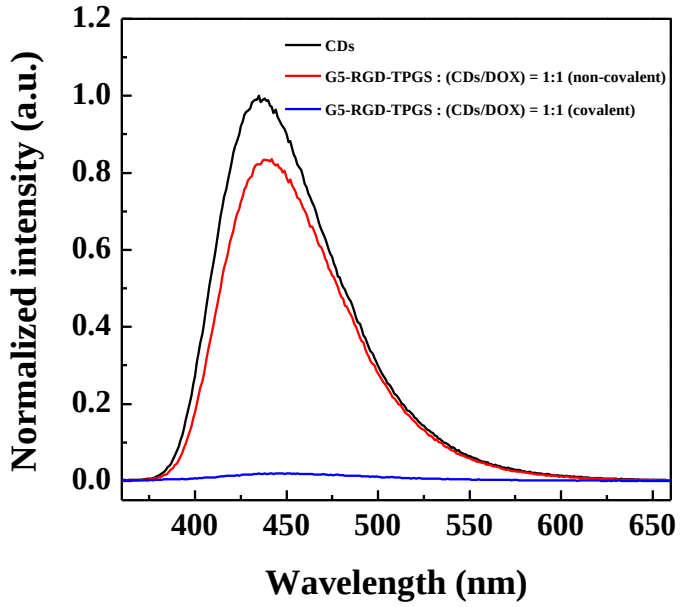

Figure S7. Fluorescence emission spectra of CDs and (CDs/DOX)@G5-RGD-TPGS complexes formed by covalent interaction and by non-covalent interaction at a mass ratio of 1:1, respectively.

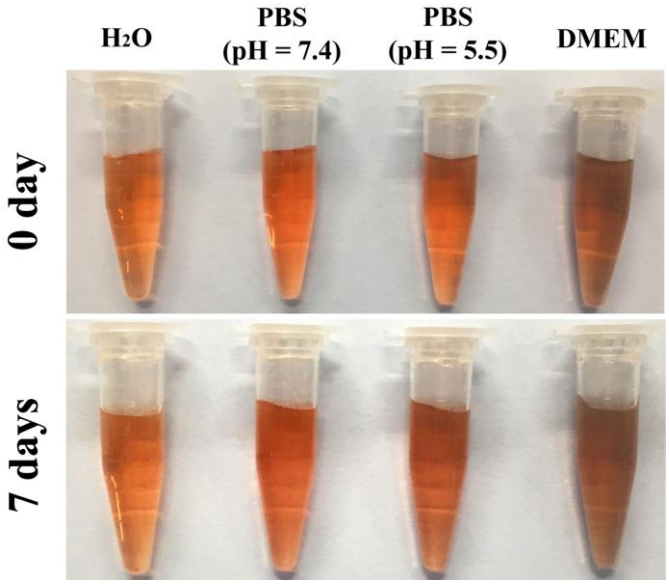

Figure S8. Photographs of (CDs/DOX)@G5-RGD-TPGS (1.0 mg/mL) dispersed in water, PBS (pH = 7.4), phosphate buffer (pH = 5.5) and DMEM at day 0 and day 7, respectively.

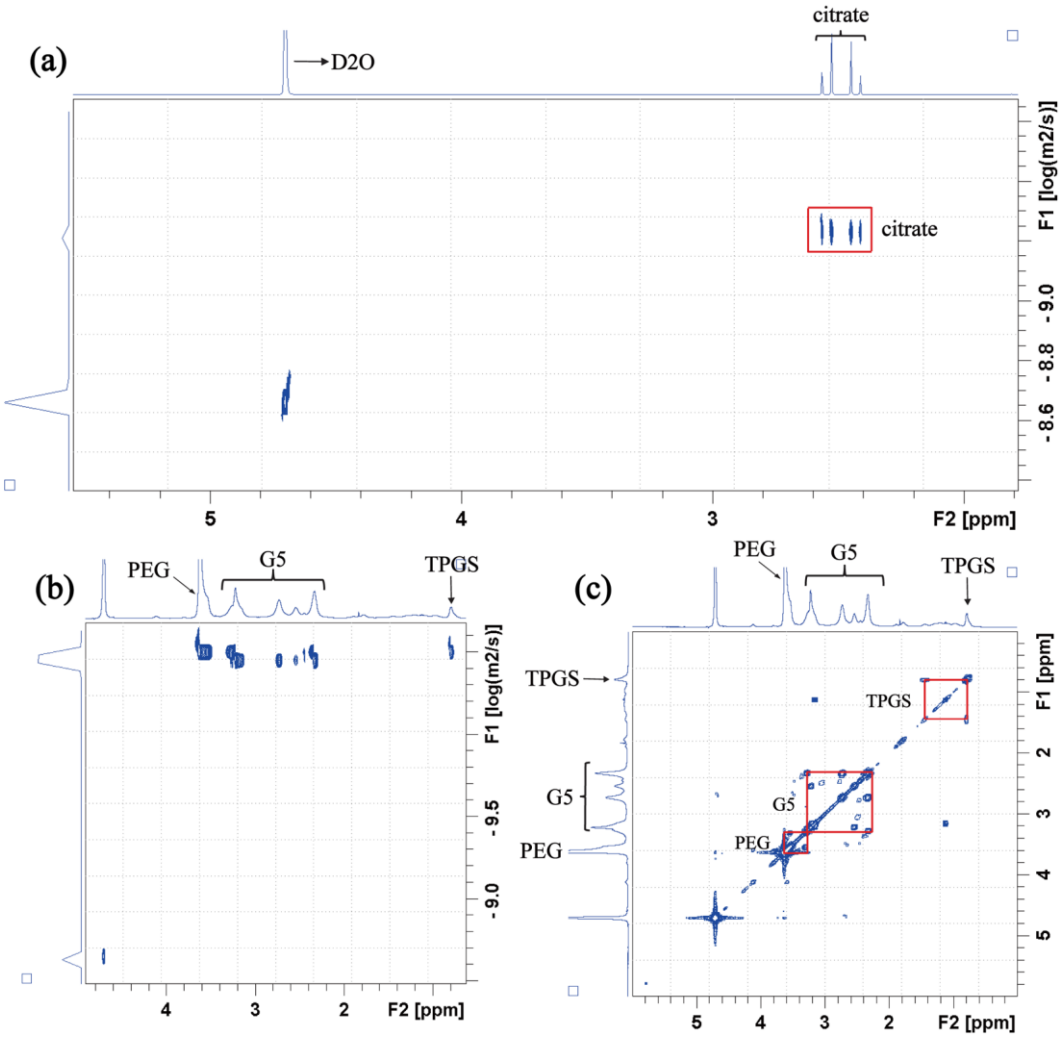

Figure S9. 2D DOSY spectra of CDs (a) and G5-RGD-TPGS conjugates (b). 2D NOSY spectrum of G5-RGD-TPGS conjugates (c).

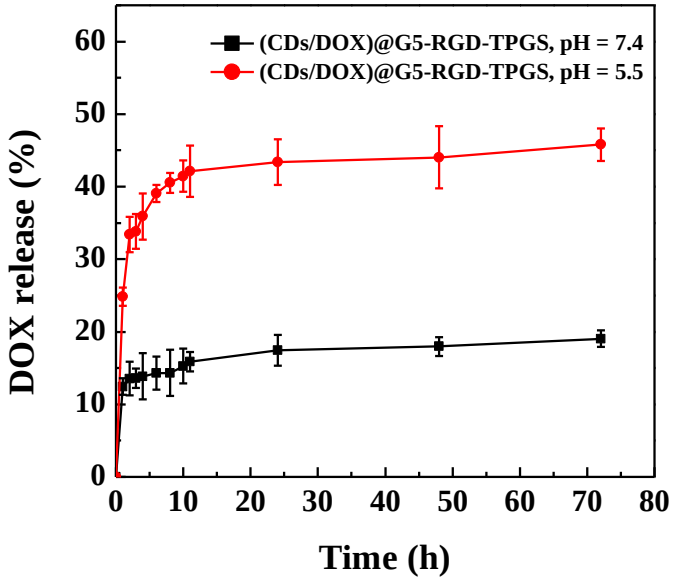

Figure S10. Release kinetics of DOX from the (CDs/DOX)@G5-RGD-TPGS complexes at pH 5.5 and 7.4, respectively.

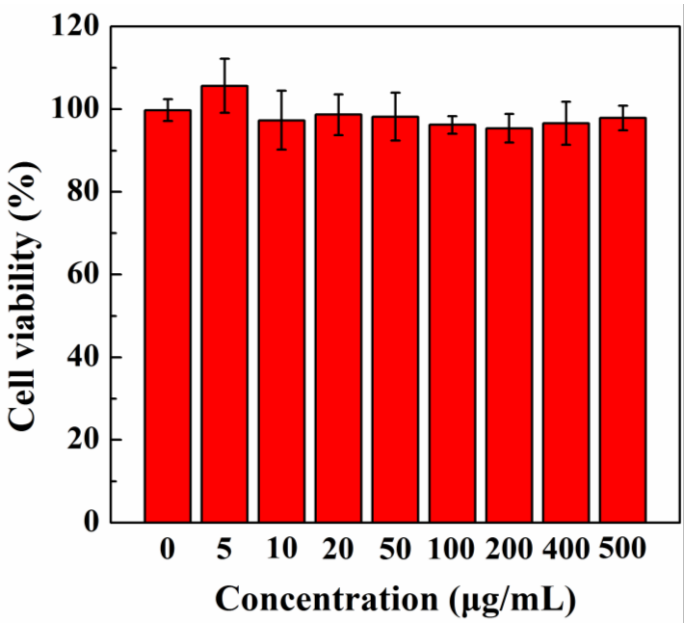

Figure S11. CCK8 assay of the viability of A549 cells after treated with CDs at varying concentrations for 24 h.