Supplementary Information

Glioma cell targeting doxorubicin delivery and redox-responsive release using angiopep-2 decorated carbonaceous nanodots

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Experimental section

Materials

Cysteine was obtained from Aladdin Industrial Inc (Shanghai, China). NH_2−PEG (Amino poly-(ethylene glycol), MW = 5000) and NH_2−PEG-COOH (Amino poly-(ethylene glycol) carboxyl) was purchased from Seebio Biotech, Inc (Shanghai, China). Glutamic acid was purchased from Sinopharm Chemical Reagent (Shanghai, China). C6 cells was purchased from the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). Angiopep-2 was synthesized by Sangon Biotech Co., Ltd (shanghai, China). 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide Hydrochloride (EDC) and N-hydroxy-succinimide (NHS) were obtained from Keddia Reagent (Chengdu, China). Plastic cell culture dishes and plates were obtained from Wuxi NEST Biotechnology Co. Ltd. (Wuxi, China). RPMI-1640 medium and FBS were purchased from Life Technologies (Grand Island, NY, USA). Other chemicals and reagents were of analytical grade.

Synthesis of CDs and DOX loaded CDs

The CDs were produced though our previous protocol. After heated to 280 °C, glutamic (1 g) acid was added into the beaker and kept on the electric stove for 1 min to make the glutamic acid carbonized. Deionized water (5 mL) was added after the beaker cooled to 60 °C. The solution was sonicated for 10 min and followed centrifugation (30 min) at 12000 rpm. Then, the supernatant was harvested as CDs.

For DOX-CDs synthesis, carboxyl units of CDs were activated by EDC and NHS in PBS for 0.5 h, and then cysteine was added in the solution. After stirred for 6 h, carboxyl units of the cysteine coated CDs were further activated by EDC and NHS for 0.5 h, and then the DOX (0.5 mg/mL) was added and stirred. 6 h later, the unconjugated DOX was removed by 12 h of dialysis (cutoff size = 10 kDa). For PEG-DOX-CDs synthesis, as described above, after the DOX was added and stirred for 6 h, the NH_2-PEG was added and stirred for another 6 h. Other steps were the same as DOX-CDs synthesis. For AN-PEG-DOX-CDs synthesis, firstly, the angiopep-2 was modified with NH_2-PEG-COOH for further synthesis. As described above, after the DOX was added and stirred for 6 h, the modified angiopep-2 was added and stirred. 6 h later, the NH_2-PEG was added and stirred for another 6 h. Other steps are the same as DOX-CDs synthesis. For DOX-PEG-CDs synthesis, firstly, the DOX was modified with NH_2-PEG-COOH for further synthesis. carboxyl units of CDs were activated by EDC and NHS in PBS for 0.5 h, and then the modified DOX was added in the solution. 6 h later, the unconjugated DOX was removed by 12 h of dialysis (cutoff size = 10 kDa). The hydrated diameter and zeta potential were carried out by a Malvern Zetasizer (Malvern, NanoZS, UK)

In vitro DOX release study

Dialysis method was used for in vitro DOX release study. PBS (pH 7.4) containing 0.1% (v/v) Tween 80 (including 10 mM GSH) was used as the release media, while the PBS (pH 7.4) containing 0.1% (v/v) Tween 80 (without GSH) was used as the control media. DOX-loaded CDs were placed into dialysis tubes (cutoff size =1000 Da) and gently sealed. The dialysis tubes were added into 50 mL release media and tightly oscillating at 37 °C for 48 h. 0.1 mL release media was sampled and replaced with fresh release media of equal volume at different time points. These samples were analyzed by HPLC to determine the DOX concentrations.

Cell culture

C6 glioma cells were cultured in RPMI-1640 medium contained 10% FBS, 100 U/mL penicillin and 100 mg/mL streptomycin in a 5% CO_2 humidified incubator (Thermo Scientific, USA) at 37 °C.

Cellular uptake study

For quantitative studies, C6 cells were seeded in 6-well plates at a density of 5×10^5 cells per well and cultured for 24 h. DOX-CDs, PEG-DOX-CDs and AN-PEG-DOX-CDs were added into the wells at a final DOX concentration of 5.2 μg/mL. After incubation for 1 h and 4 h respectively at 37 °C, the cells were washed three times with PBS, trypsinized, and resuspended in 0.5 mL PBS. The fluorescent intensity of cells was analyzed by a flow cytometer (Cytomics FC 500, Beckman Coulter, USA). The quantitative studies of DOX-CDs and DOX-PEG-CDs were similar with the method

described above expect the final DOX concentration of DOX-CDs and DOX-PEG-CDs was up to 15 μg/mL.

For qualitative measurement, C6 cells were seeded at a density of 1×10^5 cells per well on cover slips in 6-well plates and cultured for 24 h. DOX-CDs, PEG-DOX-CDs and AN-PEG-DOX-CDs were added into the plates as described in the quantitative studies. After incubation for 1 h and 4 h respectively at 37 °C, the cells were washed with PBS for three times, then fixed with 4% paraformaldehyde for 30 min at room temperature. DAPI was added for 5 min for nuclei staining. Finally, the cells were imaged by a confocal microscope (FV1000, Olympus, USA).

Cytotoxicity study

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to determine the cytotoxicity of DOX-CDs, PEG-DOX-CDs and AN-PEG-DOX-CDs. C6 cells were seeded in 96-well plates at a density of 5×10^3 cells per well. And the cells were cultured in RPMI-1640 at 37 °C for 24 h, then DOX-CDs, PEG-DOX-CDs and AN-PEG-DOX-CDs were diluted in the culture medium without FBS and added into the wells at a series of DOX concentrations ranging from 2.4 ng/mL to 7.35 μg/mL for 24 h incubation, 20 μL MTT solution (5 mg/mL) was added into each well and incubated for another 4 h. Then the medium was replaced by 200 μL DMSO (dimethyl sulfoxide), and the absorbance was measured by a microplate reader (Thermo Scientific Varioskan Flash, USA) at 490 nm.

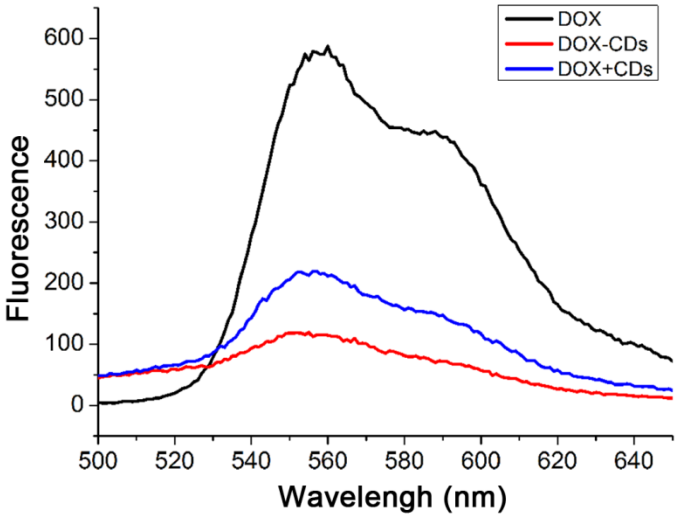

Figure S1. Fluorescence spectra of DOX-CDs and DOX+CDs (DOX dissociated from DOX-CDs)

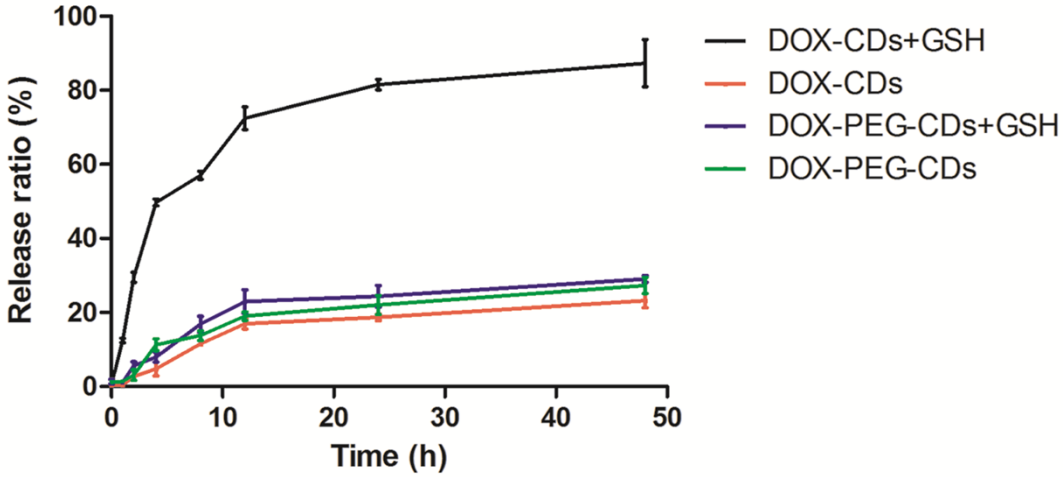

Figure S2. In vitro release profiles of DOX-CDs and DOX-PEG-CDs with and without GSH (10mM) (n=3, mean ± SD).

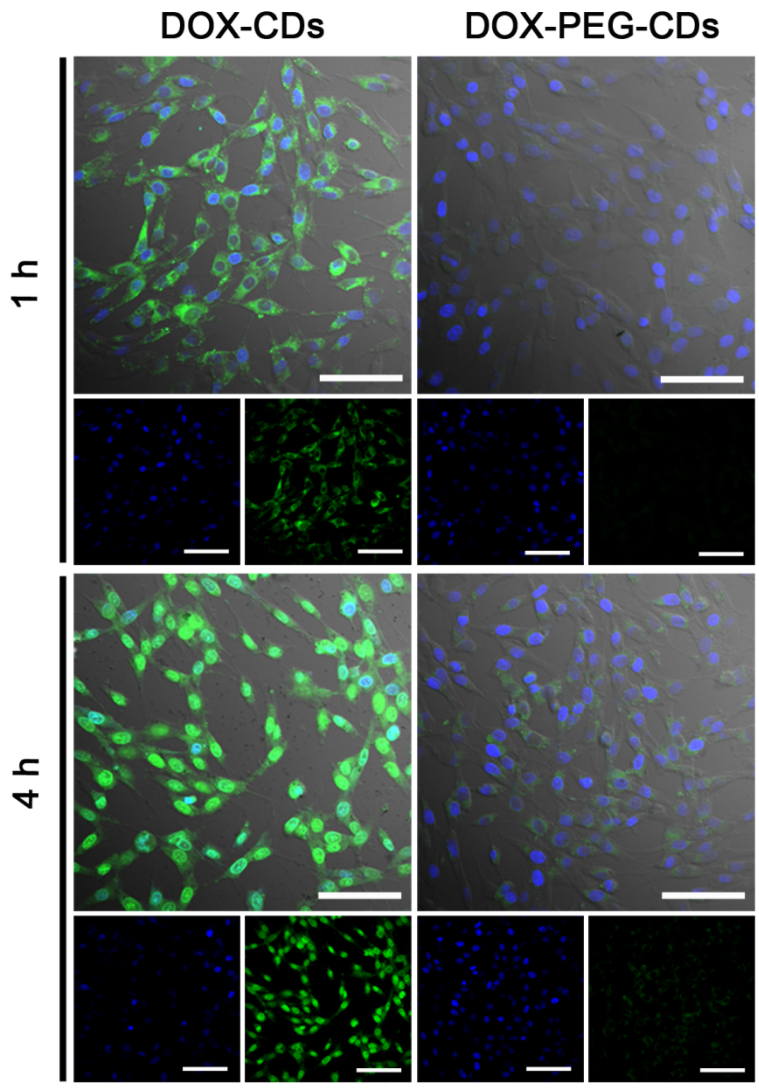

Figure S3. Confocal images of cellular uptake of DOX-CDs and DOX-PEG-CDs on C6 cells after incubation of 1 h and 4 h. Blue represents nuclei, green represents DOX and bar represents 50 μm