Supporting information

Facile synthesis of highly luminenscent carbon nanodots@silica nanorattle for *in vivo* bioimaging

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1. Experimental

1.1 Materials

All the reagents used in this work are analytical purity and used without further purification. Citric acid and quadrol are purchased from Beijing Chemical Company. Distilled water was used throughout the experiments. Calcein-AM, Propidium Iodide (PI) were purchased from the sigma. WST-1 kit was obtained from beyotime.

1.2 Preparation of CDs@SN

A typical synthesis of CDs@SN was described as follows. Firstly, rattle-type silica (SN) was prepared by our previous report. Afterwards, 30 mg SN was dispersed in 10 mL water. Then, 1.05 g citric acid and 670 μL quadrol were dissolved in above suspension. The above mixture was poured into a conical flask that was subsequently treated by sonication for 30 minutes to introduce citric acid and quadrol into the cavity of the SN under vacuum condition. The mixture was transferred to a stainless steel autoclave and was heated at 200 ℃ for 8 h. Finally, the reactor was automatically cooled to room temperature. The resulting solution was centrifuged at 12000 rpm for 10 min to collect the precipitate. Finally, the precipitates were alternatively washed with water for several times to remove residual CDs to obtain the light yellow CDs@NS with bright fluorescence.

1.3 Characterization

Transmission electron microscopies (TEM) images operated by JEOL JEM-2100 (Hitachi, Tokyo, Japan) instrument at an accelerating operating at 200 kV. Prior to TEM analysis, the sample was dropped on a Cu grid coated with an ultrathin amorphous carbon film. UV-Vis

absorption was carried out using a Varian Cary 500 ultraviolet-visible-near infrared (UV-Vis-NIR) spectrophotometer. Fluorescence spectroscopy was performed on a fluorescence spectrophotometer (Model Cary eclipse) equipped with a 120 W Xenon lamp as the excitation source. Fourier transform infrared spectra (FT-IR) investigation was recorded on an Excalibur 3100 infrared spectrophotometer using KBr pellets as the sample matrix in the frequency range 500-4000 cm^{-1}. Powder X-ray diffraction (XRD) was obtained on a German Bruker D8 Focus XRD with a graphite monochromatized Cu Ka radiation source (λ = 1.54056 Å).

1.4 Cell imaging

The HepG2 cells were maintained in Dulbecco's modied Eagle's medium supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin and cultured in a incubator with 5% CO_2 at 37 ℃. For performing the cell imaging, HepG2 cells were dispersed on confocal dishes (35mm, purchased from MatTek Corporation) for overnight. The same amount of CDs and CDs@SN were added to the HepG2 cells and co-incubated for 4 h. Then the cells were washed thrice with PBS and observed with a confocal laser scanning microscope (Nikon Multiphoton Microscpe, AIR MP).

1.5 Bioimaging of CDs@SN *in vivo*

All animal experiments were conducted following the local ethics committee. Female ICR mice of 4-6 weeks, purchased from Vital River Laboratory Animal Technology Co. Ltd were used for the studies. Five mice were raised in a stainless steel cages with sterile paddy husk. They were acclimated in the new environment for a week with free access to water and food before experiments. The mice were injected subcutaneously in the right axillary region with 0.1 mL of cell suspension containing 2×10^6 hepatoma 22 (H22) cells. After tumor size had reached about 200 mm^3, the mice were intravenously administered with (A) physiological saline, (B) CDs and (C) CDs@SN to examine the different distribution. The concentration of SN in group C was 80 mg kg^{-1}. CDs and CDs@SN have the same fluorescence intensity. The mice were killed and the tissues of the liver, spleen, kidneys and the tumors were removed at 1 h, 4 h and 12 h. Five micrometer frozen section were prepared and the images were captured with Nikon fluorescence microscope (Nikon Eclipse Ti-S, CCD: Ri1).

1.6 Stability of the CDs@SN in the biological simulation medium

Firstly, CDs and CDs@SN were adjusted to the same fluorescence intensity in PBS.

Afterwards, the same concentration of CDs and CDs@SN were dispersed in the simulated biological fluids, which were prepared according to the previous reference.[33] Fluorescence spectroscopy was performed at various time intervals to detect the change of fluorescence intensity.

1.7 Cytotoxicity *in vitro*

HepG2 cells were seeded in 96-well plates at an initial cell density of 8000 cells for overnight, then were exposed to CDs@SN at various concentration for 24 h and 48 h. The viability of HepG2 cells were determined by WST-1 assay following the manufacturer suggested procedures. The viability of the cells was also confirmed by the calcein-AM and PI staining. HepG2 cells were seeded in confocal dishes at a 10000 cells per plate for overnight. Then HepG2 cells were incubated with CDs@SN at the concentration of 100 μg mL^{-1} for 24 h. Calcein-AM and PI were added. Fifteen minutes later excess CDs@SN were removed by washing with water for three times. The images were captured with Nikon fluorescence microscope (Nikon Eclipse Ti-S, CCD: Ri1).

1.8 Hemolysis assay

Rabbit whole blood was obtained from the heart and anticoagulated using the heparin sodium. Then the whole blood was washed with PBS and was centrifuged at 4000 rpm min^{-1} for three times. RBC incubation with water and PBS were used as the positive and negative controls. 500 μL CDs@SN at the concentration of 800 and 600 μg mL^{-1} was respectively mixed with 500 μL whole blood (2%) and kept in static condition at room temperature for 3 h. Finally, all samples were centrifuged at 12000 rpm min^{-1} for 10 min at 4 ℃. The absorbance of the supernatant was tested at 570 nm. The hemolysis rate (R) of RBCs was calculated by the following equation:

$$R = (I_s - I_{nc}) / (I_{pc} - I_{nc}) \times 100$$

where I_s is the sample absorbance, I_{nc} is the negative control absorbance, and I_{pc} refers to the positive control absorbance.

1.9 Statistics

Results were expressed as a mean ± standard deviation (S.D). Multigroup comparisons of the means were carried out by one-way analysis of variance (ANOVA) test using SPSS 14.0 (SPSS Inc., Chicago, IL). The statistical significance for all tests was set at $p < 0.05$.

2. Results

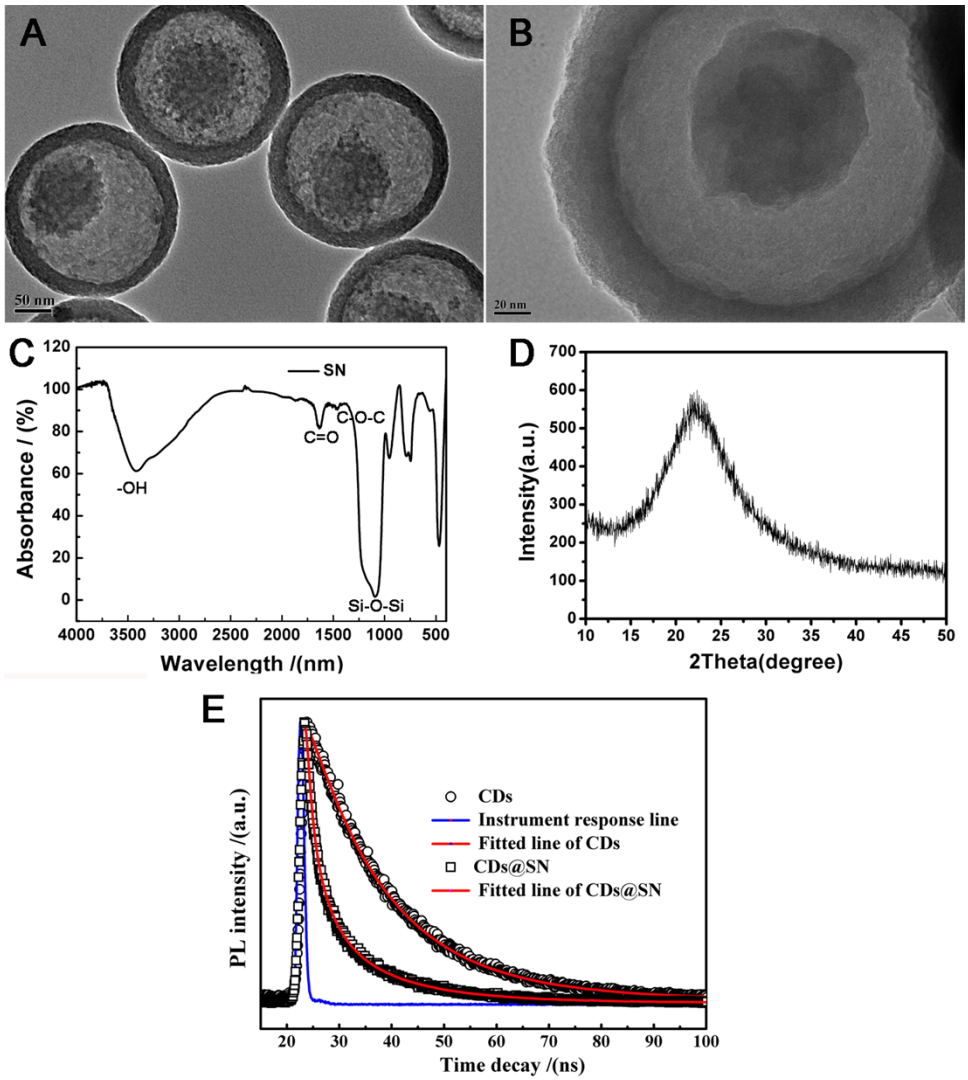

Fig.S1 (A-B) TEM image of SN alone. (B) the high magnified image of SN. (C) FTIR spectrum and (D) XRD pattern of the as-synthesized SN. (E) PL life time of CDs@SN and CDs dispersed aqueous solution.

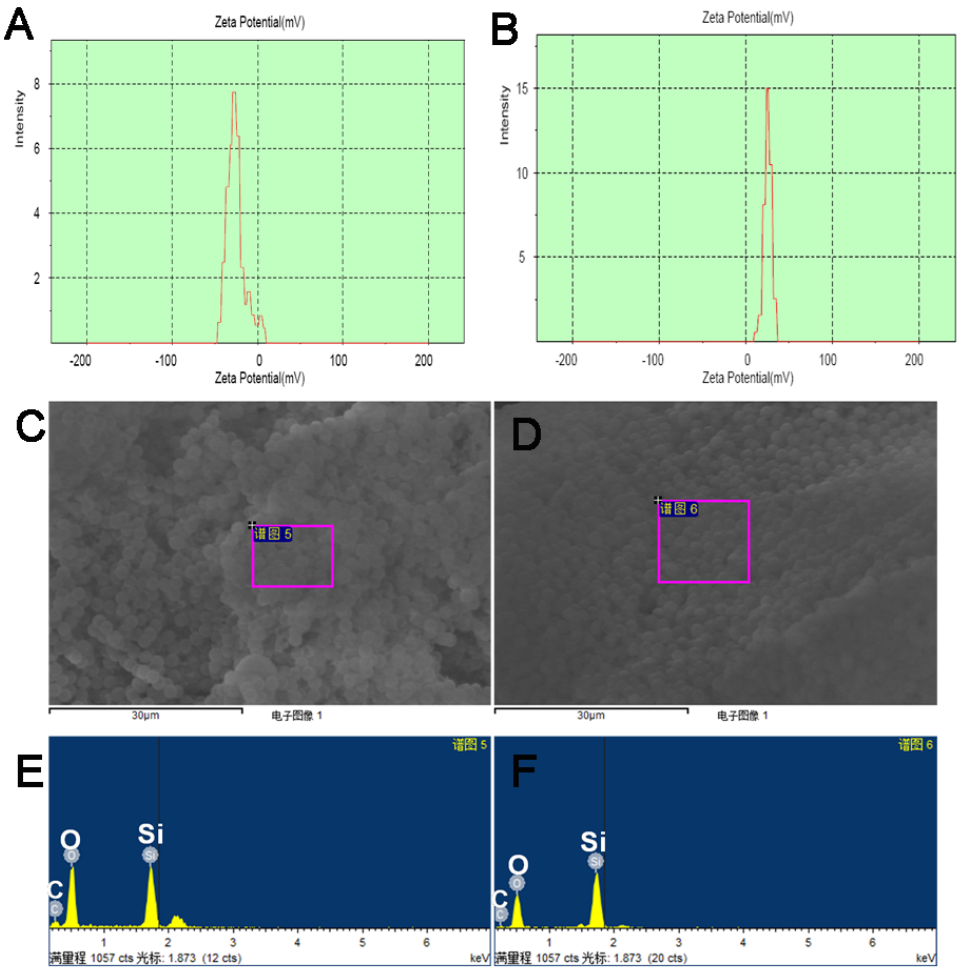

Fig.S2 Zeta potential of carbon dots (A) and SN alone (B). SEM images and the corresponding EDS patterns of CDs@SN (C, E) and SN (D, F).

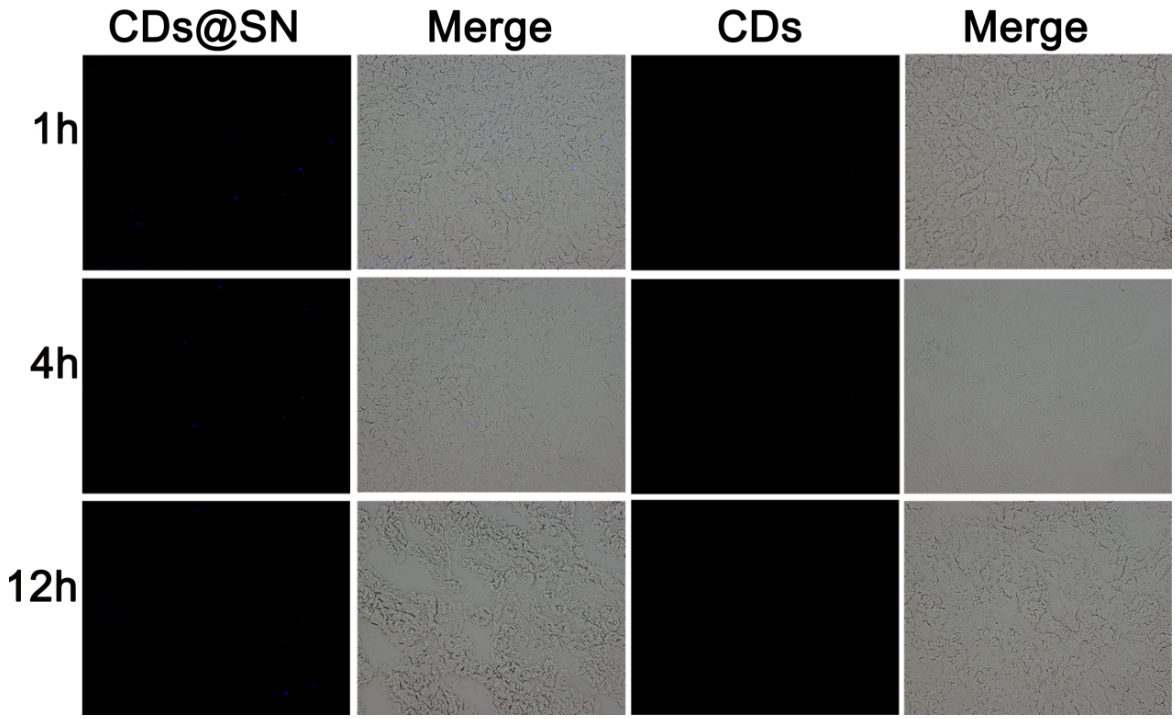

Fig. S3 The frozen section of kidney from the mice administrated with CDs@SN and CDs after intravenous injection at different time intervals.

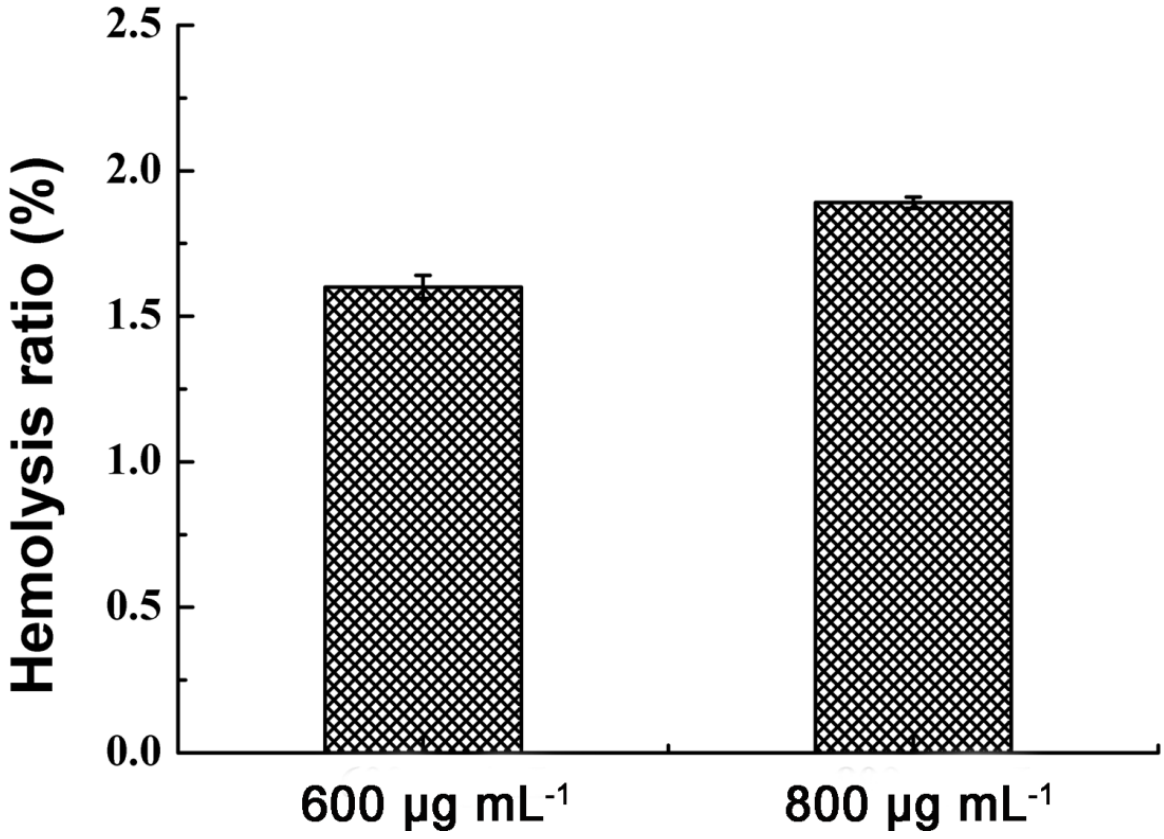

Fig. S4 The effect of CDs@SN on the hemolysis of red blood cell at 800 and 600 μg mL^{-1}.