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Synthesis and bioimaging application of red-emissive carbon dots

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Experimental Section

Materials

Citric acid (CA), formamide, N,N-dimethylformamide (DMF), ethanol, and dimethyl sulfoxide (DMSO) were purchased from Sinopharm Group Chemical Reagent Co. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Aladdin Industrial Corporation (Shanghai, China). Fetal bovine serum (FBS), phosphate buffered saline (PBS), Dulbecco's modified Eagle's medium (DMEM) (high glucose), penicillin/streptomycin and trypsin were purchased from Hyclone (Utah, USA). All these chemicals are of analytical grade and used without further purification. In addition, deionized water was used during all experiments.

Apparatus and characterization

Morphological information was obtained by TEM (JEM-2100). The structure and composition of the samples were analyzed by X-ray photoelectron spectroscopy (XPS, ESCALAB250 spectrophotometer) and Fourier transform infrared spectroscopy (FT-IR) using a Nexus 870 FTIR (Thermo Nicolet, Waltham, MA, USA) spectrometer. Samples destined for FTIR studies were prepared by pressing in KBr powder. UV-Vis absorption and PL spectra were recorded using a UV-1800PC spectro-photometer and a fluorescence spectrophotometer (F-4500, Hitachi), respectively. Fluorescence images were recorded by using a CLSM (Leica TCS SP8X). A classical MTT assay was used to measure cytotoxicity by absorbance zymography (CMAX PLUS, SpectraMax® Absorbance Reader).

Synthesis of RCDs

In a typical experiment, citric acid (1.0 g) was dissolved in formamide solutions (10 mL) and N,N-dimethylformamide (10 mL) to form a clear solution. The resultant transparent solution was put into a Teflon lined stainless steel autoclave at 160 °C for 8 h. The autoclave was naturally cooled to room temperature. During this process the solution changed to a dark-brown solution, indicating the formation of carbon dots. These carbon dots were then purified by removing the larger nanoparticles by centrifugation at 10000 rpm for 10 min five times, the resultant carbon dots were named as RCDs. The yield of RCDs from the bulk material prepared is 19%.

In vitro cytotoxicity assay

The cytotoxicity of the RCDs on the HepG2 cells was determined by an MTT assay. Briefly, the HepG2 cells were inoculated at 1×10^4 cells per well in 96-well cell culture plates with 10% (v/v) fetal bovine serum and 1% (v/v) penicillin-streptomycin in DMEM and then incubated in a humidified atmosphere at 37 °C containing 5% CO_2 for 24 h. The RCDs were then placed under UV light and sterilized for 24 h. The RCDs were then dispersed in PBS and added to each well at increasing concentrations (0, 50, 100, 150, 200, 250, and 300 μg mL^{-1}) to incubate with the cells for 24 h. Then 10 μL of MTT was added to each well and left for 4 h in the dark. Finally, the supernatant was discarded, and 150 μL of DMSO was added to each well. The DMSO was incubated for 15 min on a shaker and the optical density (OD) was recorded at 562 nm with a reference wavelength of 630 nm using an enzyme marker (CMAX PLUS, SpectraMax® Absorbance Reader). The experiments were repeated three times.

Fluorescence imaging stained with the RCDs

Fluorescence imaging of the RCDs-treated HepG2 cells was recorded under 561 nm light excitation with a CLSM (CLSM, Leica TCS SP8X). In general, the HepG2 cells were incubated at 1×10^5 cells per well and incubated for a total of 12 h at 37 °C in 5% CO_2. Then, the medium was removed, the cells were washed three times with PBS (pH 7.2 to 7.4), and 2 mL of DMEM containing 300 μg mL^{-1} of the RCDs was added to the cell culture and then incubated at 37 °C, in a 5% CO_2 environment for 4 h. Finally, the HepG2 cells were washed three times with PBS and observed under a CLSM.

Imaging of RCDs-exposed zebrafish embryos and larvae

At 36 h_{pf} and 96 h_{pf}, zebrafish embryos and larvae, respectively, were treated with 300 μg mL^{-1} RCDs at 28 °C for 4 h. No apparent phenotypic changes were found in zebrafish embryos and larvae at this concentration. Zebrafish were then anesthetized using 0.04% MS-222 to be assessed via CLSM.

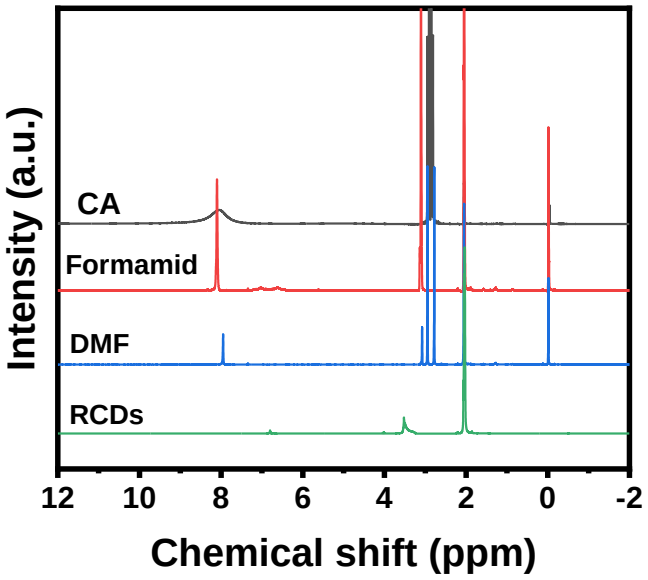

Figure S1. ^{1}H-NMR spectra of CA, formamide, DMF and RCDs.

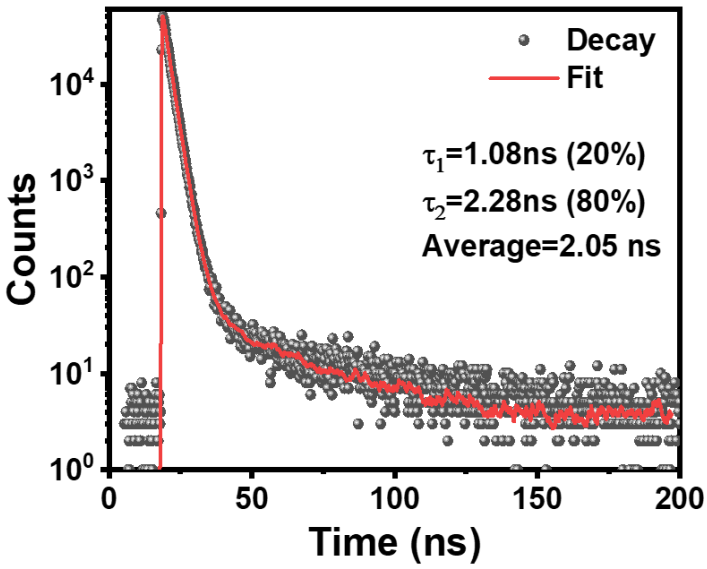

Figure S2. Time-resolved PL spectra and the corresponding fitting curves of RCDs.

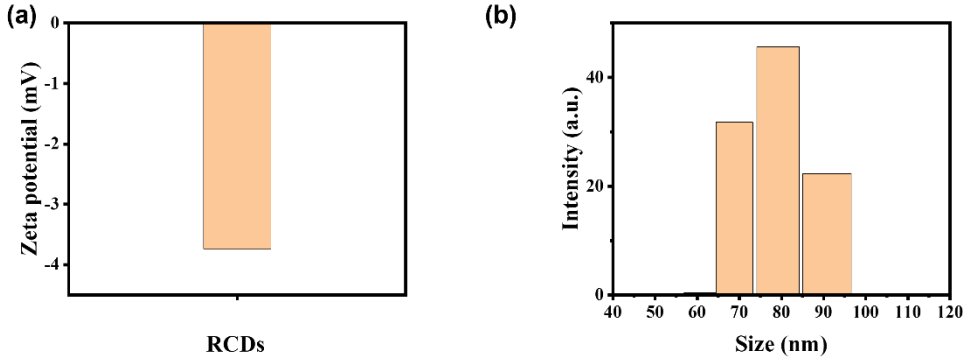

Figure S3. (a) Zeta potential and (b) DLS hydrodynamic diameter of RCDs.

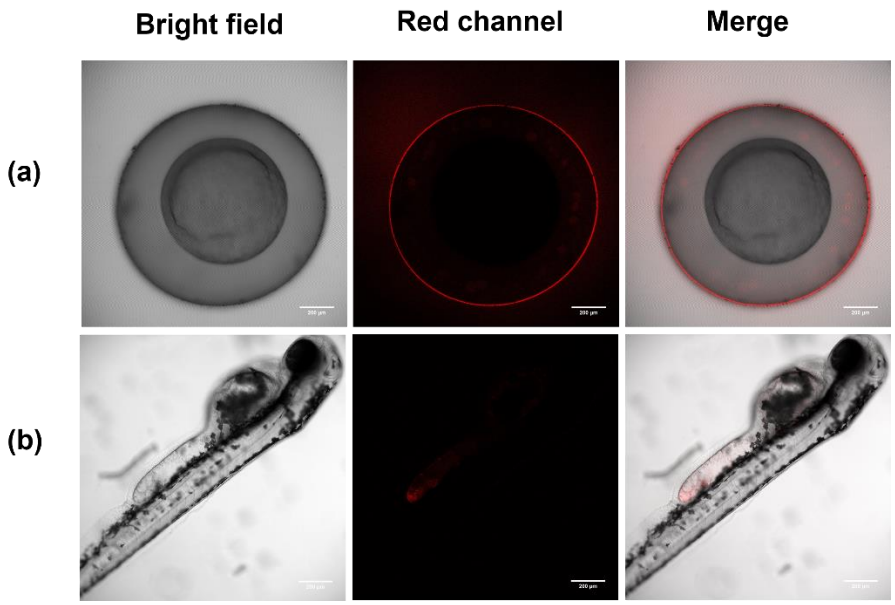

Figure S4. CLSM images of (a) embryos and (b) larval zebrafish were treated for 4 h with 300 μg mL^{-1} RCDs. The fluorescence signal was observed in embryos at 96 h_{pf}. Scale bar = 200 μm.