Supporting Information

Multifunctional Carbon Dots-Based Nanoplatform for Bioimaging and Quaternary Ammonium Salt/Photothermal Synergistic Antibacterial

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Experimental Details

Materials. Citric acid (99%) and urea (99%) were purchased from Shanghai Macklin Biochemical Co., Ltd. N-hydroxysuccinimide (NHS, 98%), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, 98%), dimethyl sulfoxide (DMSO, 99.5%), cocoamidopropyl betaine (CAB-35, 35%), and 2-(N-morpholino) ethanesulfonic acid (MES) were purchased from Aladdin Chemical Reagent Co., Ltd. All chemicals were employed immediately without any depuration. Deionized water was used in all experiments with a resistivity of 18.2 MΩ·cm.

Bacterial strains *Staphylococcus aureus* (*S. aureus*, ATCC 25923) and *Escherichia coli* (*E. coli*, ATCC 25922) were purchased from Nanjing Lezhen Biotechnology Co., Ltd. Human breast cancer cells (MCF-7) and mouse fibroblast cells (L929) were obtained from the Cell Bank, Cultural Collection Center, Chinese Academy of Sciences (Shanghai, China). Fresh whole blood was obtained from the Jiangsu Blood Center. Dulbecco's modified eagle medium (DMEM), phosphate buffered saline (PBS, pH

7.4), and fetal bovine serum (FBS) were purchased from Jiangsu KGI Biotechnology Co., Ltd. Propidium iodide (PI), Calcein-AM/PI, Hoechst 33342, and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) were obtained from Aladdin Chemical Reagent Co., Ltd. (Shanghai, China). The mice (Female, Balb/c, 6 weeks) were purchased from Qinglong Mountain Animal Breeding Farm, Jiangning District, Nanjing. Animal studies were conducted in accordance with the Guide for the Care and Use of Laboratory. All studies were conducted according to the institutional guidelines of Nanjing Normal University (Permit Number: SYXK 2015-0028).

Characterization. The morphologies of RCDs-C_{35} and bacterial cells were observed by transmission electron microscopy (TEM; H-7650, Hitachi, Japan) and scanning electron microscopy (SEM, S-4800II, Hitachi, Japan), respectively. The microstructures, functional groups, and chemical compositions of RCDs-C_{35} were characterized using X-ray diffraction (XRD, D8 Advance, Bruker, Germany), Nexus 670 (Thermo Nicolet, US) FTIR thermal spectrophotometer, and X-ray photoelectron spectroscopy (XPS, ESCALAB 250Xi, Thermo Fisher Scientific, USA), respectively. The hydrodynamic diameters of RCDs-C_{35} were measured using dynamic light scattering spectrometer (DLS, Malvern Nano-ZS90, UK). UV-vis absorption and photoluminescence (PL) spectra were measured on Cary 50 (Agilent, Australia) and F-7000 (Hitachi, Japan) spectrophotometer, respectively. Fluorescent graphs of cells were imaged via confocal laser scanning microscopy (CLSM) using a TI-E-A1R (Nikon, Japan) instrument.

Cell culture. MCF-7 and L929 cells were cultured in DMEM containing 80 U/mL penicillin, 80 μg/mL streptomycin and 10% FBS. The cells were incubated in a humidified atmosphere with 5% CO_2 at 37 ℃.

Biocompatibility evaluation and cellular imaging. The biocompatibility of RCDs-C_{35} was explored by MTT, live/dead cells staining, and hemolysis assay. *In vitro* dark cytotoxicity and phototoxicity of RCDs-C_{35} were determined via MTT and live/dead cells staining assays using L929 cells. In brief, *in vitro* dark cytotoxicity was evaluated as follows: 200 μL of the L929 cells were seeded in 48-well plates with a density of 2×10^5 cells per/mL and cultured for 24 h in incubator (37 ℃, 5% CO_2). Then, the

culture medium was discarded and incubated with different doses of RCDs-C_{35} (0, 100, 200, 400, 800, and 1600 μg/mL) for 24 h. After co-cultivation, the L929 cells were washed twice with PBS, and 200 μL of fresh culture medium containing 20 μL MTT (5 mg/mL) was added to each well and incubated for another 4 h. Finally, 100 μL DMSO was added to dissolve the formed formazan crystals. Finally, the absorbance of the mixtures was measured at 570 nm using a BioTek Synergy 2 Multi-Mode Microplate Reader. To evaluate the phototoxicity of the sample, different concentrations of RCDs-C_{35} solution were co-cultured with L929 cells for 2 h and then irradiated with 808 nm laser (2.0 W/cm^2, 10 min), afterwards, incubated for another 22 h. The following process was the same as the evaluation of dark cytotoxicity. The cell viability was obtained as the following formula:

Cell viability=A_{ex}/A_{con} ×100%

where A_{ex} and A_{con} represent the absorbance of the experimental group and control group, respectively.

The live/dead cells staining assay was also performed to determine the dark cytotoxicity and phototoxicity of the RCDs-C_{35} via Calcein-AM/PI co-staining. In short, L929 cells were seeded into 35 mm Peri culture dishes and cultured for 24 h, then divided into four groups, including Control, Control+L, RCDs-C_{35}, and RCDs-C_{35}+L group. To explore the cytotoxicity of RCDs-C_{35}, the sample was incubated with cells for 2 h, and PBS was regarded as a control. For the evaluation of phototoxicity, the L929 cells were incubated with RCDs-C_{35} and PBS for 30 min, respectively, then irradiated with 808 nm laser for 10 min and co-cultured for another 1.5 h. Subsequently, a mixture solution containing 2 μM calcein-AM and 8 μM PI was added to the above culture dishes, then incubated for 30 min in dark conditions. Finally, the L929 cells were immediately observed and imaged via CLSM after being washed twice with PBS.

The evaluation of blood compatibility of the RCDs-C_{35} was performed through hemolysis assay. Briefly, 2 mL anticoagulated blood was centrifuged at 3000 rpm for 10 min, then the supernatant was discarded, and the red blood cells (RBCs) were collected. The RBCs were mixed with normal saline (0.9%) at a volume ratio of 1:24

after being washed with normal saline for three times. The various concentrations of RCDs-C_{35} solutions (100, 200, 400, 800, and 1600 μg/mL) were added to diluted blood solution at a volume ratio of 1:1 and incubated for 3 h at 37 ℃. Next, the mixture was centrifuged at 1500 rpm and the supernatant was transferred to 96-well plates, then the absorbance of the supernatant was measured at 545 nm using a BioTek Synergy 2 Multi-Mode Microplate Reader. Additionally, the RBCs were also collected, and the cell morphologies were observed through microscopy. The RBCs that mixed with normal saline solution and deionized water were considered as negative control and positive control, respectively. The hemolysis rate was calculated as the following formula:

$$\text{Hemolysis rate}=[(D_s - D_{nc})/(D_{pc} - D_{nc})] \times 100\%$$

where D_s, D_{nc}, and D_{pc} represent the absorbance of the sample, negative control, and positive control, respectively.

The application of cellular imaging of the RCDs-C_{35} was explored using MCF-7 cells. In brief, MCF-7 cells were incubated in 35 mm Peri culture dishes for 24 h. Then, the culture medium was discarded and washed twice with PBS. After that, 1600 μg/mL of RCDs-C_{35} was added and incubated for 0.5 h in a humidified incubator (37 ℃, 5% CO_2). The group without RCDs-C_{35} was regarded as the control group. Then, the cells were washed three times with PBS and immediately imaged via CLSM.

Bacterial culture. The colonies of *E. coli* and *S. aureus* were transferred to liquid Luria-Bertani (LB) culture medium and incubated for 24 h at 37 ℃ under 120 rpm rotation, respectively. In antibacterial assays, the resuscitated bacteria were diluted with LB culture medium until the optical density at 600 nm ($O.D._{600}$) was reached 0.1.

Antibacterial activities. *E. coli* and *S. aureus* were chosen as the bacterial model for antibacterial activity assay. The antibacterial performance of RCDs-C_{35} was evaluated according to the number of colony-forming units (CFUs) using the plate-counting method. Series of concentrations of RCDs-C_{35} (0, 100, 200, 400, 800, and 1600 μg/mL) were incubated with *E. coli* and *S. aureus*, respectively. After cultured for 2 h, these mixtures were irradiated by 808 nm NIR laser for 10 min and then incubated at 37 ℃ for 22 h with gentle shaking. The bacterial solutions without irradiation were considered

as control groups. Additionally, to prove the RCDs-C_{35} possess synergistic antibacterial properties, the antibacterial performance of CAB-35 and RCDs was also evaluated, and the experimental operation process was the same as the RCDs-C_{35}. After that, the bacterial solution was diluted 10^5 times with Luria-Bertani (LB) culture medium. 30 μL of the diluted bacterial solution was spread on LB plates and incubated for 24 h at 37 ℃. After that, the bacterial colonies on the plates were counted. The antibacterial ratio was calculated in terms of the following formula:

Antibacterial ratio=$(CFU_{cg}-CFU_{eg})/CFU_{cg}\times100\%$

where CFU_{cg} and CFU_{eg} represent the numbers of colonies which formed in the control and experimental group.

Additionally, the morphologies of bacteria after receiving various treatments were investigated by SEM imaging. The *E. coli* and *S. aureus* suspensions were incubated with CAB-35, RCDs, and RCDs-C_{35} (1600 μg/mL) in the presence or absence of 808 nm laser irradiation (2.0 W/cm^2, 10 min), respectively, and the untreated bacteria with samples were regarded as negative control groups. After that, the bacteria were fixed with 2.5% glutaraldehyde for 4 h at 4 ℃. After removing the excess glutaraldehyde solution, these bacteria were dehydrated by different doses of ethanol aqueous solutions (30, 50, 70, 90, and 100 vol%) for 15 min successively. Finally, these bacteria were resuspended in deionized water and dripped onto silicon wafer and dried at room temperature before SEM characterization.

To further confirm the synergistic antibacterial performance of RCDs-C_{35}, the live/dead bacterial cells staining assay was also conducted. The bacterial suspensions were treated with CAB-35, RCDs, and RCDs-C_{35} (1600 μg/mL) in the presence or absence of 808 nm laser irradiation (2.0 W/cm^2, 10 min), respectively, and the untreated bacteria with samples were regarded as negative control groups. Then, the *E. coli* and *S. aureus* suspensions were collected via centrifugation and washing with PBS. 100 μL of Hoechst 33342 (100 μg/mL) was incubated with *E. coli* and *S. aureus* suspensions in the dark for 10 min, then centrifuged and washed with PBS. After that, 100 μL of Propidium iodide (PI, 100 μg/mL) was added and cultured for another 10 min, then centrifuged and resuspended in PBS. All mixtures were placed on the surface of slides,

covered with a cover glass, and imaged in CLSM.

***In vivo* animal antibacterial and anti-infective therapy.** To further evaluate the *in vivo* antibacterial and anti-infective performance of RCDs-C_{35}, the *S. aureus*-infected wound model was constructed using Balb/c mice, and all animal experiments were monitored and authorized by the school of Nanjing Normal University. Firstly, the rats were anesthetized by injecting 10% chloral hydrate solution, the hair on the back of the rats was shaved to create a round skin wound with a diameter of 0.8 cm followed by treating with *S. aureus* ($O.D._{600}$=0.1, 100 μL), and then infected for 24 h. After that, the mice were separated into four groups: Control, Control+L, RCDs-C_{35}, and RCDs-C_{35}+L. 100 μL of preparations from various treatment groups were directly dropped on the infected site, and the irradiation groups were irradiated with 808 nm NIR laser (2.0 W/cm^2) for 5 min, then fastened with sterile nonwoven. The dressing was changed every 2 days for 14 days. The wounds were photographed at 0, 1, 3, 7, 10, and 14 days and the wound healing rate was calculated through the following formula:

Wound healing rate (%)=1-S_t / S_0 ×100%

where S_t represent the wound area of the mouse at various times and S_0 represent the wound area of the mouse at 0 days.

The antibacterial and anti-infective activities of RCDs-C_{35} were evaluated via plate-counting method and hematoxylin and eosin (H&E) staining of wound site. After 14 days of treatment, all the mice were sacrificed, and their skin tissues were excised. Using a sterile cotton swab to wipe the skin tissues, and then placed into sterile PBS (2.0 mL), stirring and forming homogeneous solution. Then, 100 μL of the 10^3-fold diluted bacterial solution was spread on agar plates and cultured for 24 h at 37 ℃. Additionally, these skin tissues were fixed with 4% paraformaldehyde solution and stained with H&E for histology analysis.

***In vivo* biosafety evaluation.** The *in vivo* biosafety of RCDs-C_{35} was evaluated based on the bodyweight of mice and the H&E staining of major organs, including heart, liver, spleen, lungs, and kidneys. The bodyweight of these mice was recorded every 2 days. The major organs were excised and collected after these mice were sacrificed, then fixed with 4% paraformaldehyde solution and stained with H&E. Afterwards, these

histological images were observed under optical microscopy.

Figure

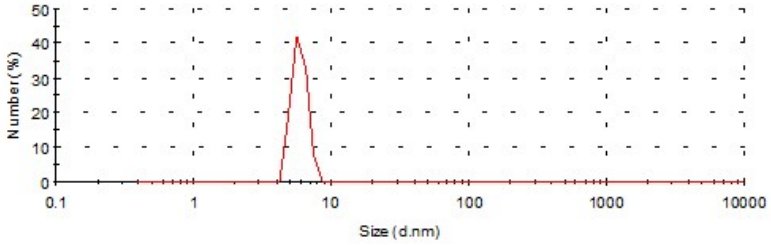

Fig. S1. The hydrodynamic diameter of RCDs-C_{35}.

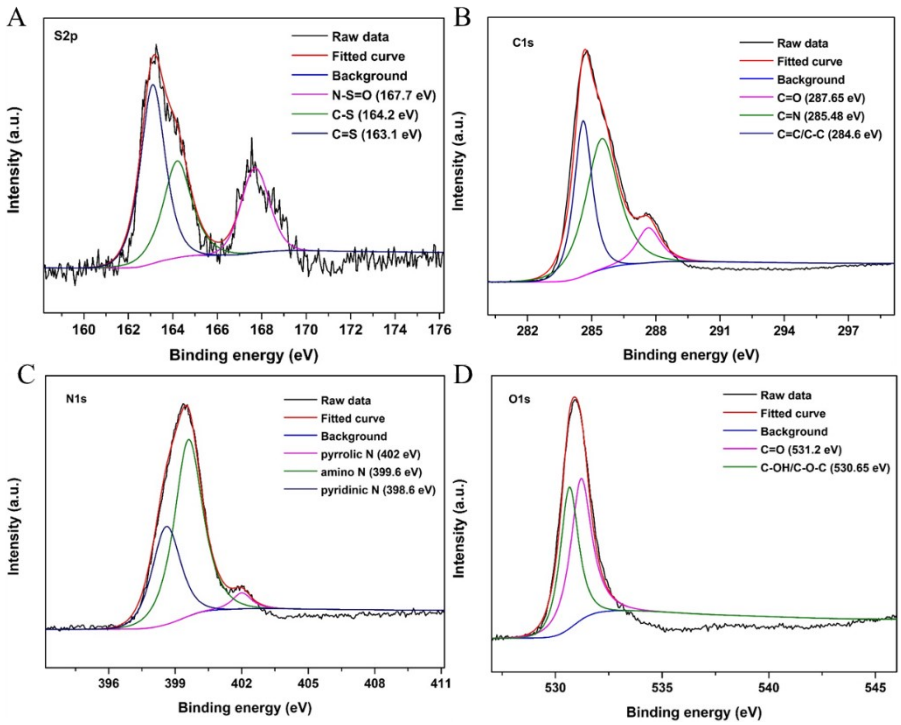

Fig. S2. The high-resolution XPS spectrum of (A) S2p, (B) C1s, (C) N1s, and (D) O1s.

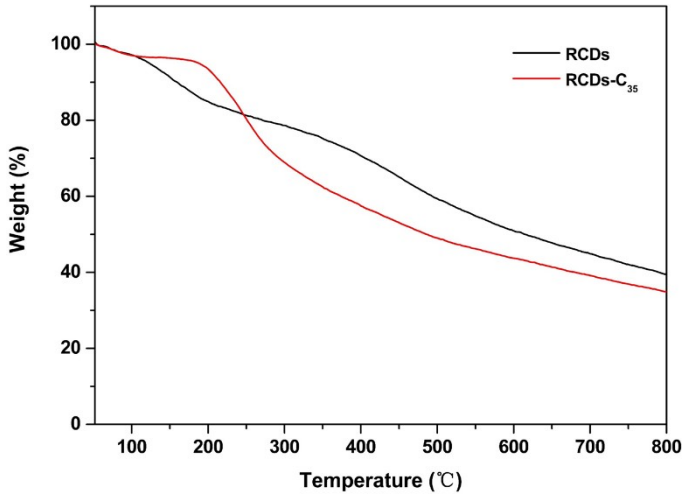

Fig. S3. TGA curves of RCDs and RCDs-C_{35}.

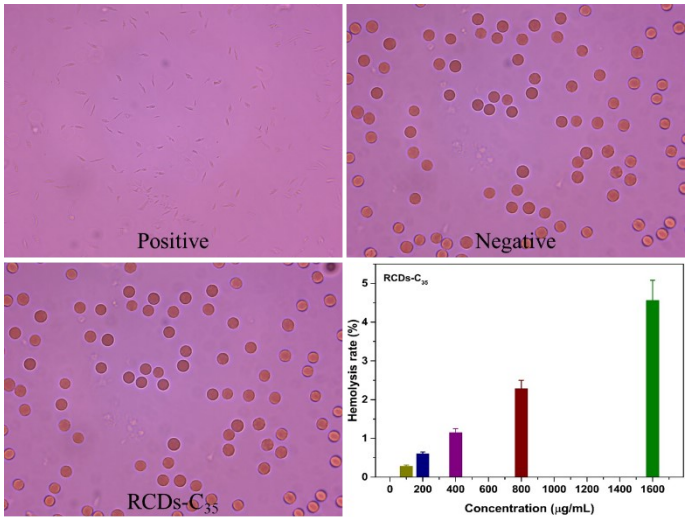

Fig. S4. Optical images of RBCs from the positive control, negative control, and 1600 μg/mL of RCDs-C_{35}, and the hemolysis rate of RCDs-C_{35} with different solution concentrations. The error bars showed standard deviation (n=3).

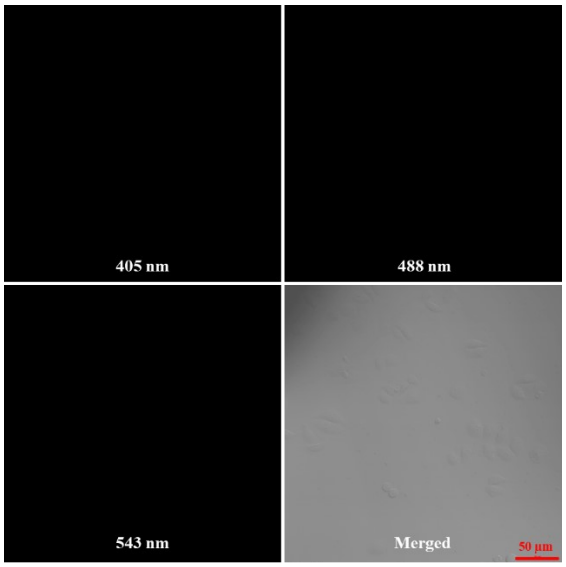

Fig. S5. Confocal fluorescence images of MCF-7 cells without co-cultured with RCDs-C_{35}.