Supporting information

Facile One-pot Synthesis of Multifunctional Protamine Sulfate-derived Carbon Dots for Antibacterial and Fluorescent Imaging Applications of Bacteria

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1. Experimental section

1. 1. Chemicals and materials

Yeast extract was purchased from OXOID Co., Ltd (Shanghai, China). MH broth was obtained from Gaokeyuan Haibo Biotechnology Co., Ltd (Qingdao, China). Peptone was purchased from Double Spin Micro Medium Product Factory (Beijing, China), and agar power was acquired from Beijing Aoboxing Biotechnology Co., Ltd. NaCl powder and glucose was obtained from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Quinoline sulfate was purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphen-yltetra-zolium bromide (MTT) was acquired from Sigma-Aldrich (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM) was purchased from Gibco (Carlsbad, CA, USA). Trypsin, Dulbecco phosphate-buffered saline (D-PBS) and phosphate buffered saline (PBS) were acquired from HyClone Company (Logan, Utah, USA). Fetal Bovine Serum (FBS) was purchased from Sijiqing Company (Hangzhou, China). Anticoagulant rabbit whole blood was obtained from Jushi Biotechnology Co., Ltd (Henan, China).

1. 2. QYs measurement

The QYs of PS-CDs were calculated by comparing the integrated PL intensities and

absorbance values of the samples (λ_{ex} = 360 nm), with quinine sulfate (dissolved in 0.1 mol/L H_2SO_4 aqueous solution (refractive index (η) of 1.33)) as the standard (QYs = 54%). All samples dissolved in water have absorbance less than 0.1 at 360 nm. The relative QYs can be calculated by the following equation:

$$Q_S=Q_R(I_S/I_R)(A_R/A_S)(\eta_S^2/\eta_R^2)$$

Where subscripts Q is QYs, I_S and I_R are the integrated emission intensity of the sample and the reference, A is the absorbance at the excitation wavelength and η is the solvent refraction index.

1. 3. Preparation of medium

Preparation of MH broth liquid medium: 2.1 g MH broth powder was added to 100 mL of DI water. Preparation of Luria-Bertani (LB) liquid medium: 0.5 g of yeast extract, 1.0 g of peptone and 1.0 g of NaCl were added to 100 mL of DI water. Preparation of yeast liquid medium: 0.5 g yeast extract, 1.0 g peptone and 1.0 g glucose were added to 100 mL of DI water to dissolve completely.

1. 4. MTT assay

The biocytotoxicities of PS-CDs was shown by standard MTT method. The LO2 cells were seeded at a density of 10^5 cells/well in 96-well plates and were preincubated for 24 h in a DMEM medium containing 10% FBS (37 °C, 5% CO_2). Then for another 24 h by dissolving PS-CDs in DMEM medium and adjusting to the required concentration (0, 0.5, 1, 10, 50, 100, 200 μg/mL). And 20 μL of 5 mg/mL MTT solution was added to each well for further incubating 4 h, followed by discarding the culture medium with MTT. Then 150 μL DMSO was transferred to every cell well, and the plates were shaken for 10 min. The optical density (OD) of the solution was performed at 490 nm. The cell viability was evaluated as the following equation:

$$\text{Cell viability (\%)} = OD/OD_0 \times 100\%$$

Where OD is the absorbance of the experimental group (OD was obtained in the presence of PS-CDs) and OD_0 is the absorbance of the control group (OD_0 was obtained in the absence of PS-CDs).

1. 5. Hemolysis test

Solutions of different concentrations of PS-CDs (12.5, 62.5, 125, 312.5, and 625 μg/mL) were prepared with D-PBS. Then, fresh rabbit whole blood was separated by centrifugation at 9000 rpm for 3 min. The supernatant was carefully removed, and the red blood cells (RBCs) were washed with D-PBS. Then, diluted RBCs suspension (0.2 mL) was systematically added to 0.8 mL of different concentrations of PS-CDs solutions. These the mixed samples were incubated at 37 ℃ for 3 h, and then centrifuged at 9000 rpm for 3 min after incubation. The supernatant was transferred to a 96-well plate and the absorbance was measured at 577 nm with a microplate reader. The mean value of three measurements was calculated. As a positive control, 0.2 mL of diluted RBC suspension was added to 0.8 mL of DI water, and as a negative control, 0.2 mL diluted RBC suspensions was added to 0.8 mL of D-PBS solution. Hemolysis rate is calculated by the following equation:

$$\text{Hemolysis rate (\%)} = [(ODt-ODn)/(ODp-ODn)]\times 100\%$$

Where ODt, ODn and ODp are the absorbance values of the test sample, a negative control group and a positive control group at 577 nm, respectively.

2. Characterization

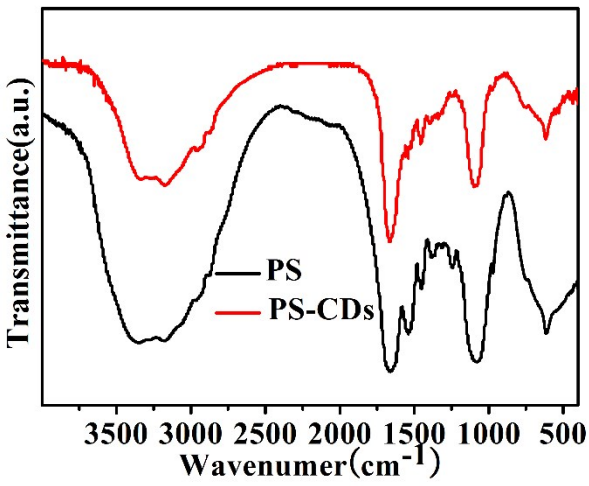

Fig. S1. FTIR spectra of PS and PS-CDs.

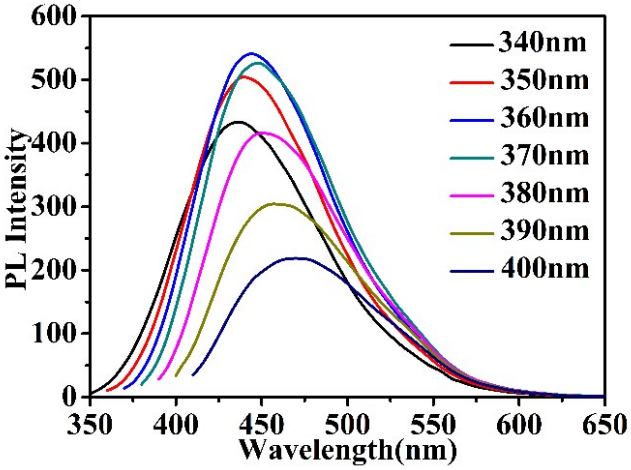

Fig. S2. Emission spectra of PS-CDs at different excitation wavelengths.