The positively charged BODIPY@carbon dots nanocomposites for enhanced photomicrobicidal efficacy and wound healing

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Materials

L-Aspartic acid and D-glucose were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. Agar, peptone, and yeast powder were purchased from Seans Biochemical Technology Co., Ltd. Sodium chloride, sodium hydroxide, citric acid, anhydrous ethanol, polyene polyamine were purchased from Sinopharm Chemical Reagent Co., Ltd. Live/dead double staining kit and SYTO 9/PI kit were purchased from Jiangsu Keygen Biotechnology Co., Ltd. MTT was purchased from Shanghai Yuanye Biotechnology Co., Ltd. Cell culture medium (DMEM) was purchased from Gibco.

Characterization

UV-Vis absorption spectra were performed on a UV-2450PC spectrophotometer (Shimadzu, Japan). The fluorescence spectra were measured by LS-55 fluorescence spectrometer (Perkin-Elmer, USA). Transmission electron microscopy (TEM) images were acquired by JEM-1011 electron microscope (JEOL Co., Japan). The hydrodynamic size and the potentials were measured by Zeta-sizer Nano ZS (Malvern Instruments Ltd., UK). The rheometer (Anton Paar, Physical MCR 302) was carried out to evaluate dynamic rheology behavior of adhesive hydrogels. The bacterial confocal images were obtained using Zeiss confocal laser microscope (ZEISS LSM 700).

Preparation of n-CDs

L-Aspartic acid (2.0 mmol, 266.0 mg) and D-glucose (8.0 mmol, 1.44 g) were dissolved

in water (10 mL), and then 1 M sodium hydroxide solution was gradually added to adjust to pH = 8.0. The beaker containing reaction solution was placed in an oven, heated to 125°C, held for 30 min, then heated to 200 °C, held for 20 min, and then cooled down. The crude product was dissolved in 20 mL of water, dialyzed against water for 24 h, and water was changed every 4 h. The solution was freeze-dried to obtain brown powder.

Preparation of p-CDs

The mixture of citric acid (2.10 g, 10.0 mmol) and polyene polyamine (PEPA, 10 mL) was heated to 170 °C and held at 170 °C for 1 h. After cooled to room temperature naturally, acetone was added and the precipitate was collected by centrifugation. The white solid was dissolved in water and dialyzed (Mw: 3.0 kDa) against water for 2 d. During this period, deionized water was changed every 4 h, and finally the CDs solution were lyophilized to a yellow solid.

Preparation of n-BDP

The BDP in tetrahydrofuran (THF) solution was slowly dropped into the n-CDs aqueous solution. After the complete addition of BODIPY to the negatively charged n-CDs solution, the mixed solution turned brown, and after stirring overnight, the resulting suspension was dialyzed against water for 24 h. The solution in the dialysis bag was freeze-dried to obtain a solid, and the BODIPY content was determined to be 55.7%, indicating the formation of n-BDP. The BDP content is 256 ng in the preparation of 1 mL of 460 ng/mL n-BDP solution.

Preparation of p-BDP

The BDP in tetrahydrofuran (THF) solution was slowly dropped into the p-CDs aqueous solution. After complete addition of BODIPY to the positively charged p-CDs solution, the mixture turned light pink, and after stirring overnight, the resulting suspension was dialyzed against water for 24 h. The solution in the dialysis bag was freeze-dried to obtain a solid, and the measured BODIPY content was 53.3%. Indicates the formation of p-BDP. Stirring an equal amount of BODIPY alone precipitates immediately, indicating that both positive and negative CDs can assemble with BODIPY to form stable nanostructures. The BODIPY content is 256 ng in the preparation of 1 mL of 480 ng/mL p-BDP solution.

In vitro antibacterial performance test

In the colony counting method, 1×10^7 CFU/mL *S. aureus* was co-treated with n-BDP (256 ng/mL) or p-BDP (128 ng/mL) with/without green LED light (18 mW cm^{-2}, 5 min) irradiation, and then 100 μL samples were taken for plating, then placed in a 37 °C incubator for 24 h and taken out for observation.

MTT assay

After NIH 3T3 cells and L929 cells grew to logarithmic growth phase in petri dishes, they were trypsinized and counted. The cells were seeded in 96-well plates and incubated at 37 ^oC for 24 hours. Various concentrations of n-BDP and p-BDP were added to the cell-seeded wells for a further 24 h of incubation. Then, the MTT solution was added to the well and incubated for another 4 h. DMSO was added to dissolve the formazan that produced by the reduction of MTT. Finally, the absorbance of formazan

at 490 nm was measured.

Live staining of NIH 3T3 cells

NIH 3T3 cells were cultured and seeded separately, and n-BDP or p-BDP was added to the ultra-clean bench for 30 min followed by light treatment (18 mW cm^{-2}, 5 min), and then incubated for 24 hours, and then calcein AM staining solution was added 30 min. All experiments were performed in three replicate wells.

Hemolysis test

Fresh blood from mice was centrifuged (3500 rpm, 5 min) to separate red blood cells (RBCs) from serum, and then washed with PBS solution until the supernatant became clear. PBS (Negative control), Triton-X 100 (positive control), n-CDs (256 ng/mL), n-BDP (256 ng/mL), p-CDs (128 ng/mL), p-BDP (128 ng/mL) were added to red blood cells, mixed gently, incubated at 37 ^{o}C for 4 h and then centrifuged. Subsequently, the OD value at 540 nm was measured by a microplate reader. Finally, the hemolysis rate was calculated according to the following formula.

$$\text{Hemolysis rate (\%)} = [(OD_x - OD_o) / (OD_y - OD_o)] \times 100$$

Where OD_x is the OD value of the sample, OD_o is the OD value of the negative control, OD_y is the OD value of the positive control.

In vivo Antibacterial experiments

All of the animal experiments were performed according to the legal requirements of the Animal Welfare and Ethics Committee of Changchun Institute of Applied

Chemistry, Chinese Academy of Sciences and all of the recommendations of the Guide for the Care and Use of Laboratory Animals were followed. Female Kunming mice were randomly divided into five groups of three mice each. A circular wound with a diameter of 8 mm was cut on the back skin of anesthetized mice, and a suspension of *S. aureus* was inoculated on the wound to establish an infection model. PBS, n-BDP (256 ng/mL) or p-BDP (128 ng/mL) were added to the wound. The wounds of the mice in the light group were irradiated with green LED light (18 mW cm^{-2}) for 5 min. The wound site was photographed on day 1, 3, 5, 7, and 14, respectively.

Plate counting method was used to evaluate and quantify wound colonies. The wound tissue was immersed in sterile PBS, ultrasonicated, and the bacterial suspension was spread on agar plates, incubated at 37 ^{o}C for 24 h. to observe the growth of plate colonies.

The wound surface was embedded, sliced, and stained with hematoxylin-eosin (H&E), and the wound healing of nanomaterials was analyzed and evaluated according to the staining of the skin tissue.

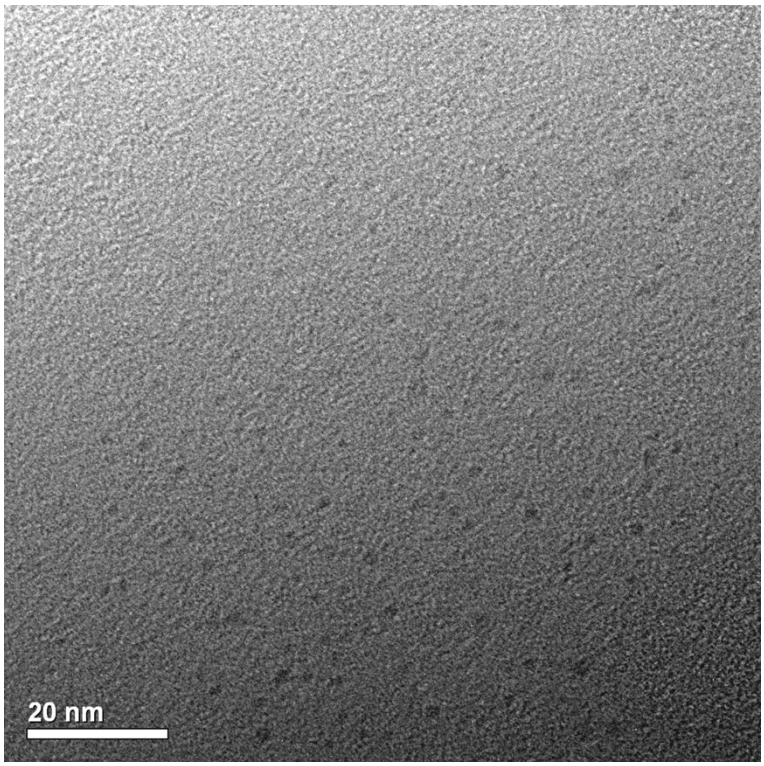

Fig. S1. TEM images of n-CDs.

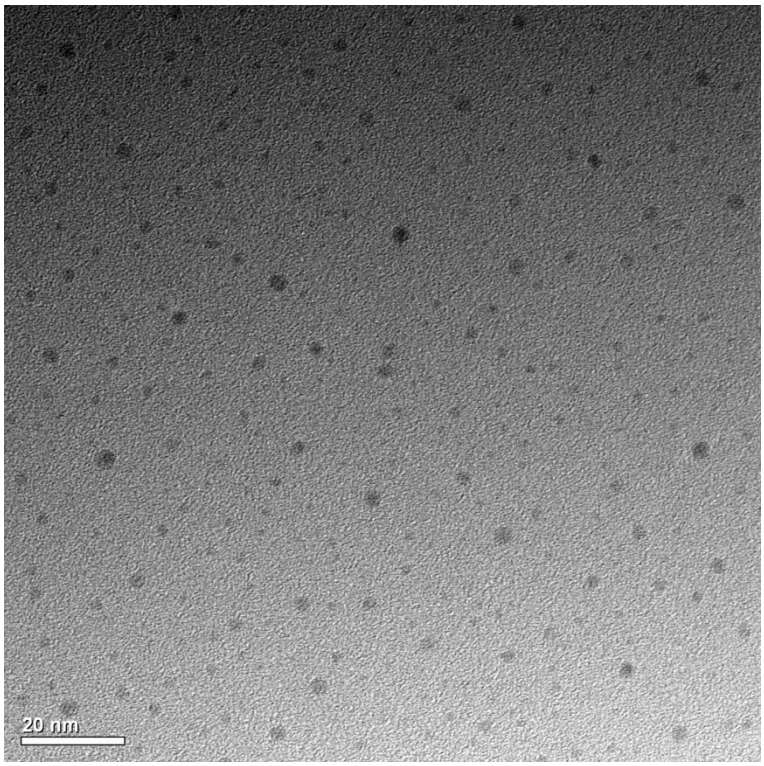

Fig. S2. TEM images of p-CDs.

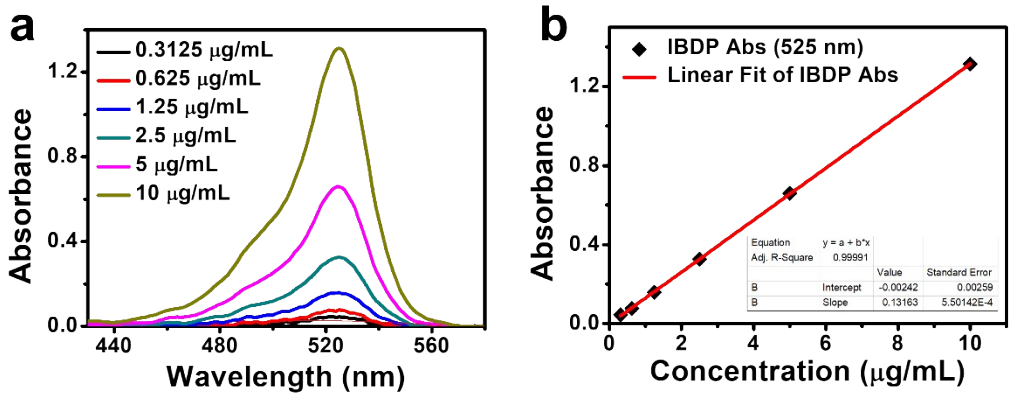

Fig. S3. (a) UV-Vis absorption spectra of BODIPY in DMF-water mixed solvent(v:v, 9:1). (b) The standard curve of the absorbance of BODIPY vs concentration.

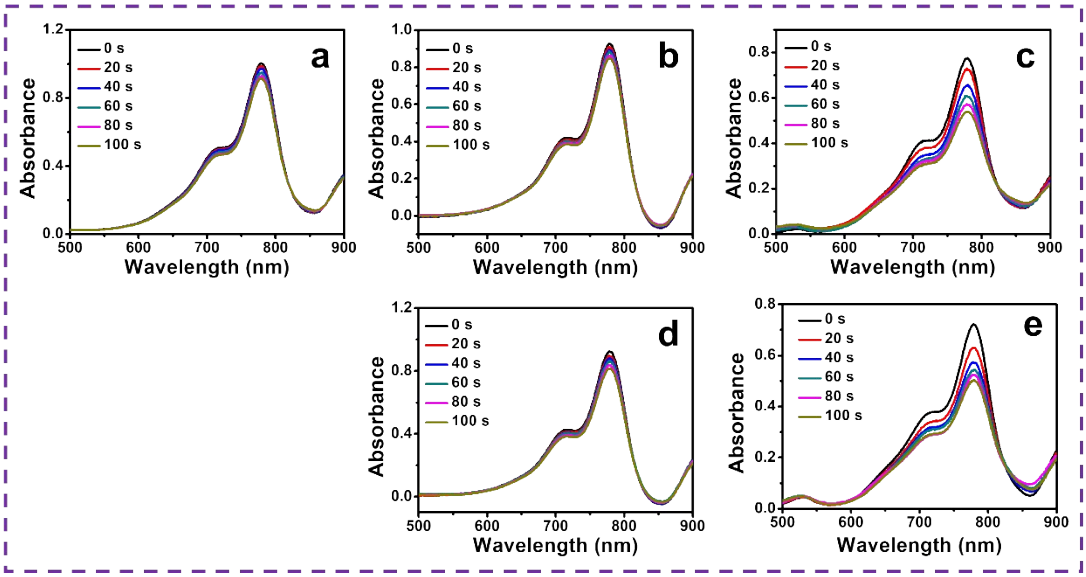

Fig. S4. UV-Vis absorption spectra of (a) ICG, (b) ICG + n-CDs, (c) ICG + n-BDP, (d) ICG + p-CDs and (e) ICG + p-BDP under green LED light (18mW·cm^{-2}).

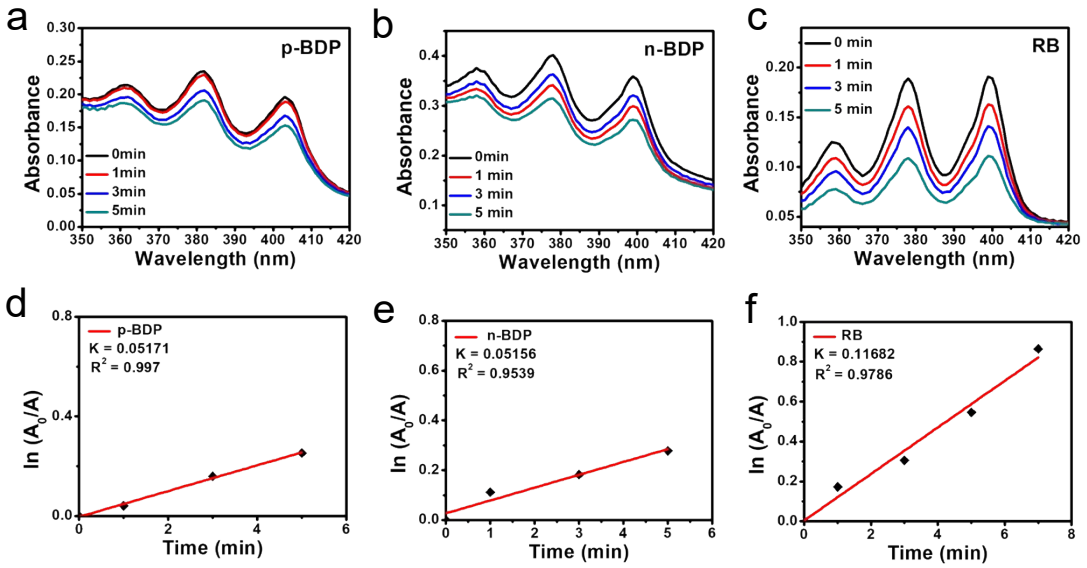

Fig. S5. Absorbance spectra of ABDA in the presence of (a) p-BDP, (b) n-BDP or (c) RB under green LED light irradiation over different periods of time. Decomposition rates of ABDA in the presence of (d) p-BDP, (e) n-BDP or (f) RB under light irradiation, where A_0 and A are the absorbance of ABDA at 400 nm.

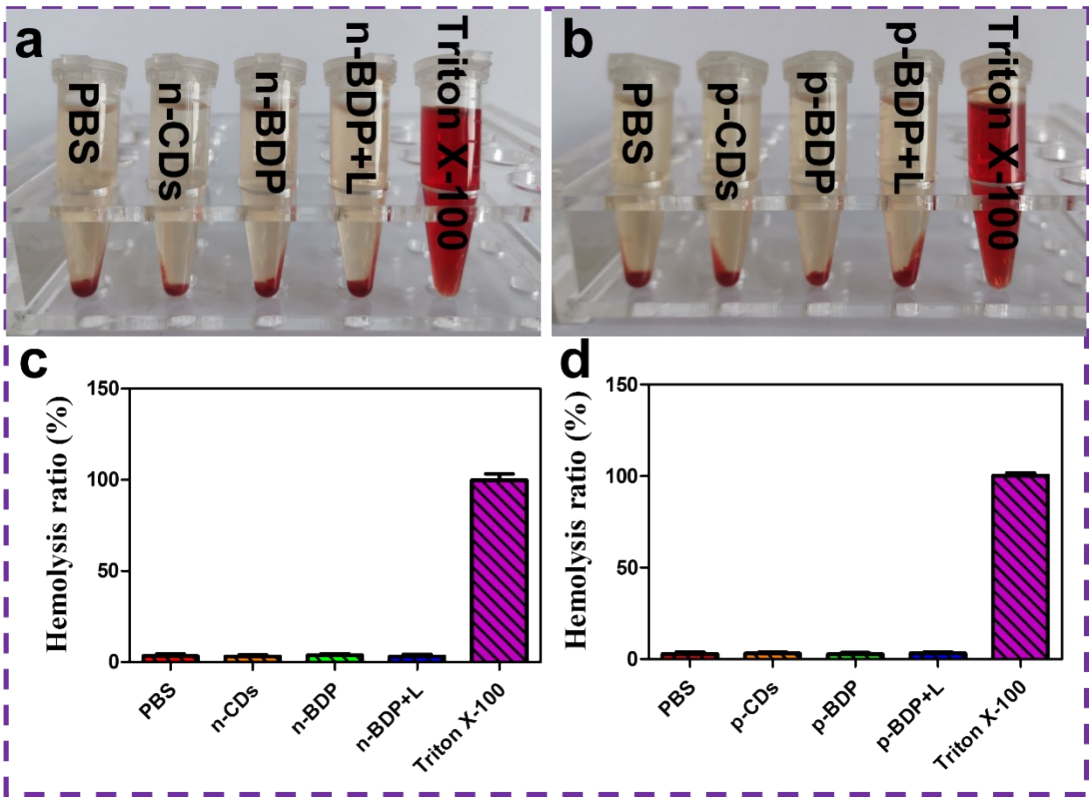

Fig. S6. (a) The hemolysis test of PBS, n-CDs, n-BDP, n-BDP+L and Triton X-100. (b) The hemolysis test of PBS, p-CDs, p-BDP, p-BDP+L and Triton X-100. (c) Quantitative analysis of (a). (d) Quantitative analysis of (c) .

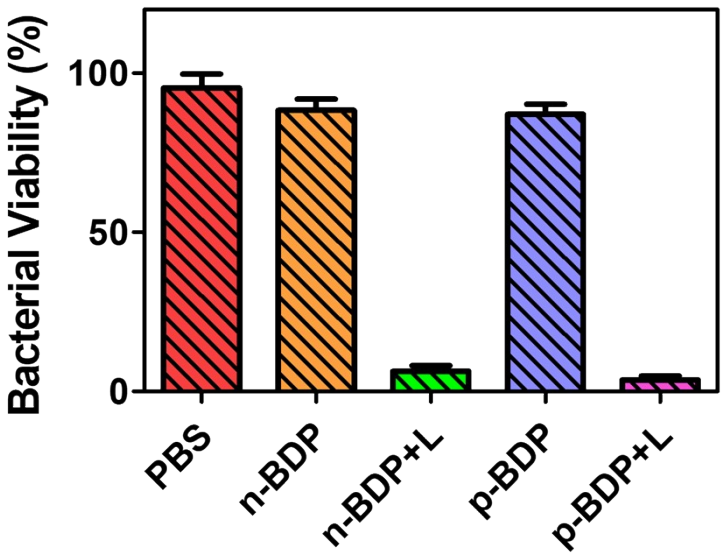

Fig. S7. Statistical analysis of the colonies of wound surface after treatments.