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Supplementary materials

Novel quaternized carbon dot-papain complex for double-target anti-biofilm activity and visualization-ratio fluorescence dual-mode detection of H_2O_2

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Materials

Staphylococcus aureus (*S. aureus*, CCTCC AB 91093), methicillin-resistant *Staphylococcus aureus* (MRSA, CCTCC AB 2015107), *Bacillus subtilis* (*B. subtilis* CCTCC AB 90008), *Pseudomonas aeruginosa* (*P. aeruginosa*, CCTCC AB 2013184), *Saccharomyces cerevisiae* (*S. cerevisiae*, CCTCC AY 92003) and *Escherichia coli* (*E. coli*, CCTCC AB 93154) were purchased from the China Center for Type Culture Collection (CCTCC) in Wuhan.

Characterization

Fluorescence spectra were read on an LS55 spectrofluorometer (PerkinElmer Company). UV-visible absorption spectra were recorded using a Lambda-35 UV-visible spectrophotometer (PerkinElmer Company). Fourier-transform infrared (FT-IR) spectra were recorded using a Nicolet 6700 spectrometer (Thermo Fisher Scientific), and the samples tested were powder. Transmission electron microscopy (TEM) images were obtained using a JEM2100F transmission electron microscope (Japan Electron Optics Laboratory Company). X-ray photoelectron spectra (XPS) were assessed using a VG Multilobe 2000 X-ray photoelectron spectrophotometer (Thermo Electron Corporation). Images of *S. aureus* were captured using a scanning electron microscope (SEM, Zeiss SIGMA). The zeta potentials of qCD-P were measured with a Zetasizer (Nano ZS, Malvern Instruments, Worcestershire, UK). A fluorescence optical microscope (Nikon ECLIPSE Ti) was used for capturing the images of the laser-scanning confocal fluorescence of bacterial cells.

Quantum yield (QY) measurement

The quantum yield (QY) of prepared carbon dots was calculated by using established methods [1]. Quinine sulphate in 0.1 M H_2SO_4 was chosen as a standard reference sample which has fixed and known fluorescence quantum yield value (QY = 54% at 360 nm). The QY of prepared carbon dots was calculated according the following equation:

$$QY_x = QY_{std} (I_x A_{std} n_x^2) / (I_{std} A_x n_{std}^2) \quad (1)$$

where I is the measured integrated emission intensity, n is the refractive index of the solvent,

and *A* is the optical density (OD). The subscript "std" refers to the standard sample with known QY and "x" for the unknown samples.

MIC test

The MIC of qCD-P was determined by microdilution using a 96-well cell culture plate. Bacterial cultures grown overnight were adjusted to a concentration of 2×10^7 CFU mL^{-1}. Each well contained 50 µL of bacterial cell suspension and various concentrations of 50 µL qCD-P to obtain a final volume of 100 µL $well^{-1}$. MIC analysis was performed in quadruplicates. The treated bacterial samples were then incubated at 37 °C for 12 h. At the end of the incubation period, 100 µL of the suspension was co-incubated with 5 µL of MTT and further incubated for 20 min. The precipitates formed were dissolved in DMSO for visual observation. The experiment was repeated three times.

SEM images for bacteria and biofilm

S. aureus cells (≈ 10^9 CFU mL^{-1}, 950 µL) were incubated with qCDs (0.5 mg mL^{-1}) for 4 h. Bacterial that were not treated with qCDs were used as blank controls. The cells were subject to high-speed centrifugation (8000 rpm, 10 min, 4 °C), and the substrate was removed using DI water. The cells were fixed with 2.5% glutaraldehyde for 12 h at 4 °C. The bacterial cells were dehydrated using ethanol, vacuum dried, and sputter coated with gold. The samples were observed under a Zeiss SIGMA FESEM apparatus.

The procedure for obtaining SEM images for mature biofilm was the same as above, and the experiment was carried out with mature biofilm on coverglass.

Analysis of *S. aureus* DNA by gel electrophoresis

To study the degradation of DNA, genomic DNA of *S. aureus* cells was extracted using the Bacterial Genomic DNA Kit. Three groups of bacterial DNA genomes were required to be extracted: *S. aureus* (OD ≈ 0.3) after qCDs (OD ≈ 1) treatment, *S. aureus* with PBS treatment at initial concentration, and *S. aureus* with PBS treatment at diluted concentration (OD ≈ 0.3). In these later experiments, the nucleic acid lysis products were identified by agarose gel

electrophoresis and EB staining. In these later experiments, nucleic acid cleavage products were identified with agarose gel electrophoresis and EB staining.

Treatment of *S. aureus* protein for circular dichroism analysis

Total protein of *S. aureus* was extracted using a bacterial protein extraction kit. Two equal parts of *S. aureus* protein were diluted with 0.01 M PBS or 1000 μg mL^{-1} qCDs solution to the same concentration, respectively. The two parts were then shaking at 25 °C for 1-2 h. Protein was stored at 4 °C before circular dichroism spectrum analysis.

Results and discussion

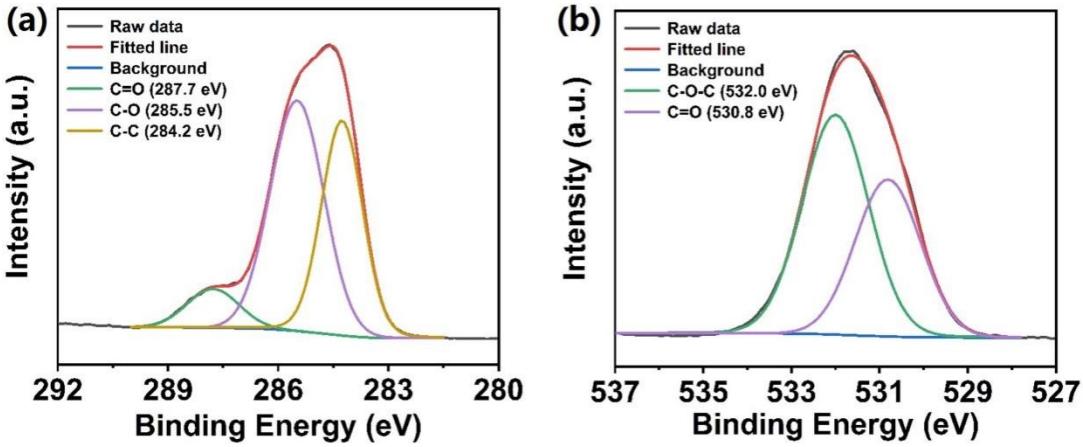

Fig. S1 XPS spectra (a) C 1s and (b) O 1s of qCD-P.

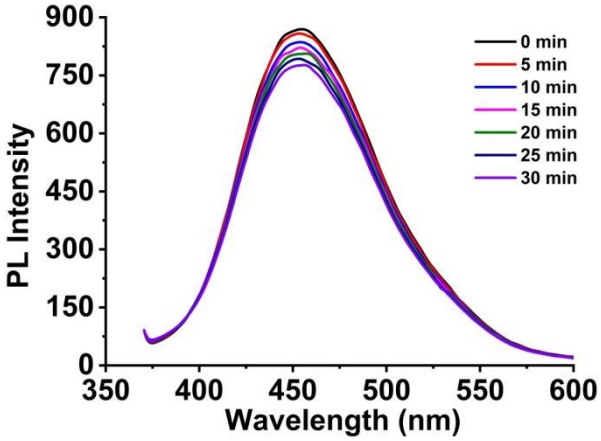

Fig. S2 Fluorescence spectra of qCD-P under ultraviolet irradiation within 30 minutes.

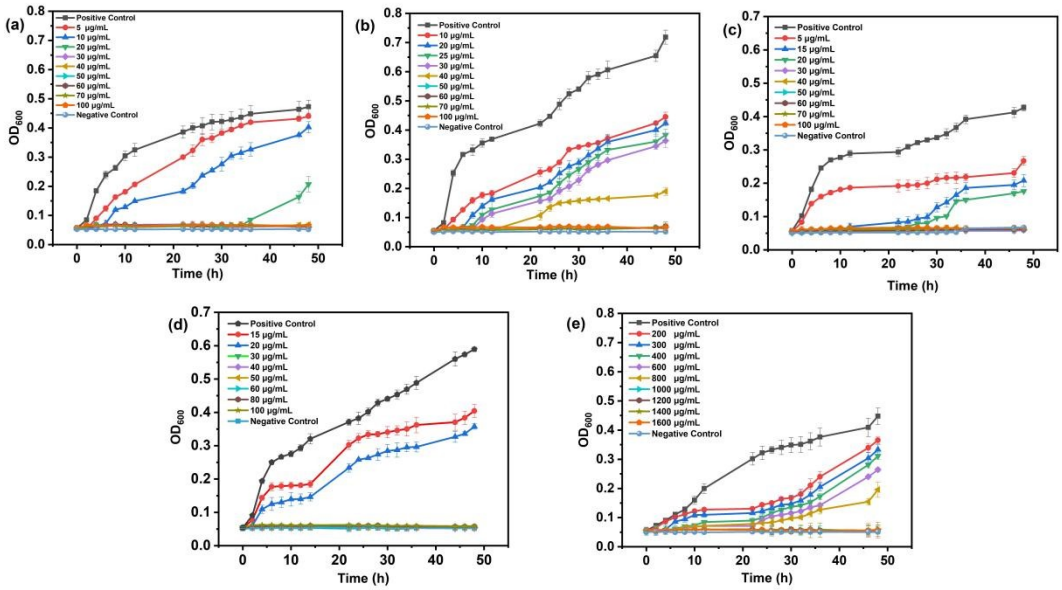

Fig. S3 The OD_{600} of (a) MRSA, (b) *P. aeruginosa*, (c) *B. subtilis,* (d) *E. coli* and (e) *S. cerevisiae* solution treated with different concentrations of qCD-P at different times within 48 h.

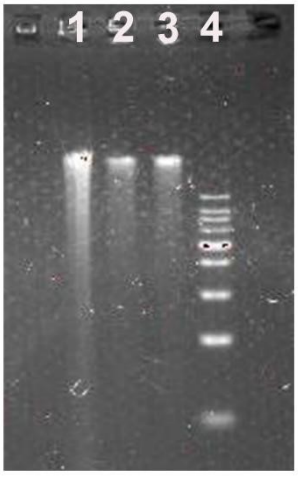

Fig. S4 Agarose gel electrophoresis analysis of the DNA of *S. aureus* with or without treatment of qCDs. Lanes 1-4: (1) genomic DNA of *S. aureus* incubated with qCDs (0.1 mg mL^{-1}); (2) genomic DNA of pristine *S. aureus* (diluted); (3) genomic DNA of pristine *S. aureus* (undiluted); (4) DNA marker.

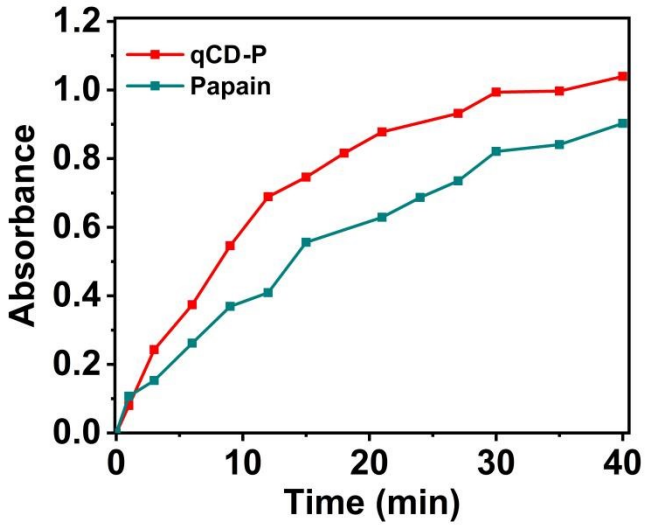

Fig. S5 Time curves of casein hydrolysis by qCD-P and papain.

Reference

[1] Lakowicz J R (Ed.) 2013 *Principles of fluorescence spectroscopy* Springer science & business media.