Supplementary Information

Antimicrobial Cotton Fabrics Modified with a Cationic Perylene Diimide Derivative for Durable and Superior Sterilization

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This supplementary information includes:

1. Experimental Section

Materials and measurements. All the chemical reagents used in organic synthesis were commercially obtained from Aladdin Industrial Corporation (Shanghai, China), Bidepharm. (Shanghai, China), or Sinopharm Chemical Reagent Co. Ltd. (Beijing, China) and were used without any further purification except special instruction. The cotton fabrics (CF) was obtained from YMSP Studios. The 2', 7'-dichlorodihydrofluorescein diacetate (DCFH-DA), 3-(4, 5-dimethylthiazol-2-yl)-2, 5 diphennyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO). Dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS), Penicillin-Streptomycin (P/S), 0.25% (1×) Trypsin and 10× phosphate buffer solution (PBS) were purchased from Sangon Biotech Co. Ltd (Shanghai, China). Ultrapure water was collected from the Milli-Q reference system.

^{1}H NMR and ^{13}C NMR spectra were measured on Bruker Avance 400 or 600 MHz spectrometers. HR-MS were measured on a Bruker Maxis II mass spectrometer. The UV-vis absorption spectra were taken on a Shimadzu UV-2600 spectrophotometer. The fluorescence spectra were recorded on a Hitachi F-7000 spectrophotometer. Fourier transform infrared spectroscopy (FTIR) was recorded on a Bruker Tensor II spectrometer in the range of 4000–400 cm^{-1}. Elemental analysis was performed on X-ray photoelectron spectroscopy (XPS) (Axis UltraDLD, Japan). The OD value was determined by the Spectra Max M5 Microplate Reader (Molecular Devices). Scanning electron microscopy (SEM) was carried out by SU 8220 scanning electron microscope

(Hitachi, Japan). The white light sources were purchased from Mejiro Genossen company. Cell numbers were quantitated using an Accuri C6 flow cytometer (Becton 110 Dickinson, Franklin Lakes, NJ). The absorbance for MTT analysis was performed on a microplate reader (SpectraMax M5).

Methods:

Synthesis of compound 4ClPDI. Under N_2 protection, 1, 6, 7, 12-Tetrachloroperylene tetracarboxylic acid dianhydride ($C_{24}H_4Cl_4O_6$, ≥ 97.0%; 265 mg, 0.5 mmol) was dissolved in toluene (15 mL), then N, N-dimethylethylamine ($C_4H_{11}N$, ≥ 97.0%; 176 mg, 2 mmol) was added to the above solution. The mixed solution was heated at 120 ℃ for 12 h. After the reaction solution was cooled to room temperature, the residual toluene in the reaction solution was removed under reduced pressure. Then the mixed solution was extracted with dichloromethane and washed three times with saturated NaCl solution. Then the organic phase was dried with anhydrous Na_2SO_4 and concentrated under vacuum. The crude product was separated and purified by silica gel column chromatography (the eluent was petroleum dichloromethane/methanol (V/V = 10/1)), and then dried in vacuo to obtain compound 4ClPDI (139 mg, 41%, orange-red solid). The ^{1}H NMR spectrum was shown in Figure S1. ^{1}H NMR (400 MHz, Chloroform-d) δ (ppm): 8.68 (s, 4H), 4.38 (t, J = 6.6 Hz, 4H), 2.71 (t, J = 6.8 Hz, 4H), 2.37 (s, 12H).

Synthesis of compound BPDI. Under N_2 protection, compound 4ClPDI (35.1 mg, 0.05 mmol) and 4-(bromomethyl) phenylboronic acid ($C_7H_8BBrO_2$, ≥ 98.0%; 215 mg, 1 mmol) were added to a 50 mL two-neck flask, and then ultra-dry DMF (15 mL) were

added to the flask. Then the mixture was degassed with N_2 for 30 min. The mixture was heated to 60 ℃ and stirred for 24 h. After the reaction stopped, concentrate DMF, and then precipitated with a large amount of dichloromethane, and this step is repeated at least three times. The precipitate was dried in vacuo to obtain compound BPDI (41 mg, 73%, red solid). The ^{1}H NMR spectrum was shown in Figure S2. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 8.64 (s, 4H), 8.24 (s, 4H), 7.91 (d, J = 7.8 Hz, 4H), 7.60 (d, J = 7.8 Hz, 4H), 4.72 (s, 4H), 4.61 (m, 5H), 3.60 (tt, J = 14.3, 6.9 Hz, 4H), 3.18 (s, 12H). ^{13}C NMR (125 MHz, DMSO-d6) δ = 34.41, 49.52, 62.98, 67.18, 86.13, 125.50, 129.44, 129.62, 129.97, 132.28, 134.09, 134.61, 144.51. HR-MS (ESI): calculated for $[C_{46}H_{40}B_2Cl_4N_4O_8]^{2+}$ 470.1330, found 470.0977. (Figure S3)

Evaluation of the ReactiveOxygen Species (ROS) Generation of OCF-PDA-PDI.

The ROS generation ability was studied using 2,7-dichlorofluorescein diacetate (DCFH-DA, $C_{24}H_{14}Cl_2O_7$, ≥ 98.0%) as an indicator. Under alkaline conditions, DCFH-DA was turned into 2,7-dichlorofluorescein (DCFH). In the presence of ROS, DCFH was followed by transforming into highly fluorescent 2,7-dichlorofluorescein (DCF, excitation 488 nm, emission at 525 nm). 0.97 mg DCFH-DA dissolved in DMSO (1 mM, 2000 μL) was mixed with NaOH (10 mM in dd water, 8 mL) followed by being reacted in dark place for 30 min to hydrolyze into DCFH. The activated DCFH-DA (DCFH, 40 μM) was placed in a quartz cuvette with OCF-PDA-PDI samples (1×1 cm^2 squares), which was exposed under white light (5 mW cm^{-2}) for various time interval. The fluorescence intensity of the solution at 525 nm was measured every minute with

excitation at 488 nm. In addition, as a control, the fluorescence signals of DCFH-DA (40 μM) stock solution (2 mL) without any sample were recorded.

Water Absorption Test.

The water absorption properties of the untreated cotton fabric (CF) and the modified fabric (OCF-PDA-PDI) were assessed via a capillary rise method in accordance with standard FZ/T 01071-2008. All tests were performed under controlled conditions (20 ± 2 °C; 65 ± 3% relative humidity). Triplicate samples of each fabric, measuring 250 mm × 25 mm, were prepared. Each strip was vertically suspended with its lower end immersed 15 ± 2 mm beneath the surface of ultrapure water. The maximum height of liquid rise was recorded at 1, 5, 10, 20, and 30 minutes after immersion.

Cytotoxicity by MTT assay.

The dark cytotoxicity of BPDI against HUVEC cells was evaluated by MTT assay. Briefly, cells were seeded in 96-well plates with a density of 8000 cells per well. After incubating for 24 h, the incubation medium was replaced with 100 μL fresh medium containing different concentrations of various BPDI solution, then cells were incubated for another 24 h. 10 μL MTT ($C_{18}H_{16}BrN_5S$, ≥ 98.0%; 5 mg mL^{-1}) was added into each well followed by incubated for 4 h, then the medium containing MTT was removed, and 100 μL DMSO was added into each well to dissolve the produced formazan. After shaking the plate for 10 min, the absorbance at 490 nm was measured via a microplate reader (SpectraMax M5). Human umbilical vein endothelial cells HUVECs were cultured in RPMI-1640 medium (Gibico) containing 10% FBS and 1% penicillin-streptomycin at 37 °C with 5% CO_2 in an incubator.

Antibacterial Experiments

For all tests, 1×1 cm^2 of the cotton fabric samples were sterilized by ultraviolet radiation.

To study the time-killing behaviors of positively charged QAS groups for BPDI, the antimicrobial assays of OCF-PDA-PDI were also carried out. The bacterial concentration was 1×10^7 CFU/mL. The cotton fiber fabric samples of each group were immersed in the *E.coli* solution, and treated under dark conditions for 3 h, 6 h, 12 h. After that, the bacterial solution was diluted to the same multiple with 0.9% NaCl, and 100 μL diluted bacterial solution were evenly spread on the solid medium and incubated in a thermostat for 20 h. The bacterial solution without any cotton fiber fabric was used as a control group.

The laundering test was used to evaluate the washing durability of OCF-PDA-PDI according to FZ/T 73023-2006. The 1993 AATCC Standard Reference Detergent (without optical brighteners) was used in the washing process. OCF-PDA-PDI were washed for 10, 20, and 50 laundering cycles, and the antimicrobial activities of washed OCF-PDA-PDI were tested according to the antimicrobial activity test described above.

SEM observation of the bacteria

The morphology of bacteria treated with the OCF-PDA-PDI was observed by SEM. The concentration of the bacterial suspensions (1×10^8 CFU/mL) were dropped onto the CF and OCF-PDA-PDI followed by exposure to dark or light for 30 min. Then, samples of each group were fixed in the 2.5% glutaraldehyde ($C_5H_8O_2$) solution at 4 °C for 12 h. The fabric samples were washed with 10 mM PBS buffer (pH 7.4) three times and

dehydrated by graded ethanol solutions (20-100%) and then the ethanol was replaced with tert-butanol: ethanol = 1:1, pure tert-butanol in turn. The samples were allowed to air-dry and placed on the conductive adhesive for further observation.

Comfortableness Test of Cotton Fabric.

Water Vapor Transmission of OCF-PDA-PDI: The moisture permeability of OCF-PDA-PDI was determined by measuring the WVTR according to the ASTM E96/E96M-2014. The test disk is a glass vial with an inner diameter of 6 mm and a height of 50 mm. Add a quantity of distilled water to the glass bottle so that the water level is about 19 mm below the mouth of the bottle. The fabric sample (CF or OCF-PDA-PDI) was attached onto the mouth, and the assembly was placed into a chamber with controlled temperature and humidity (maintained at 23 ± 2 °C, relative humidity 30 ± 2%). All experiments were performed in quadruplicate. The WVTR value was calculated based on the mass change during the test according to the following formula:

$$WVTR\ (g/m^2/h) = \frac{M}{tA}$$

where WVTR is expressed in g/m^2/h, M is the weight change of the assembly during the test, A is the test area (same as mouth area), and t is the test time.

2. Supporting Figures

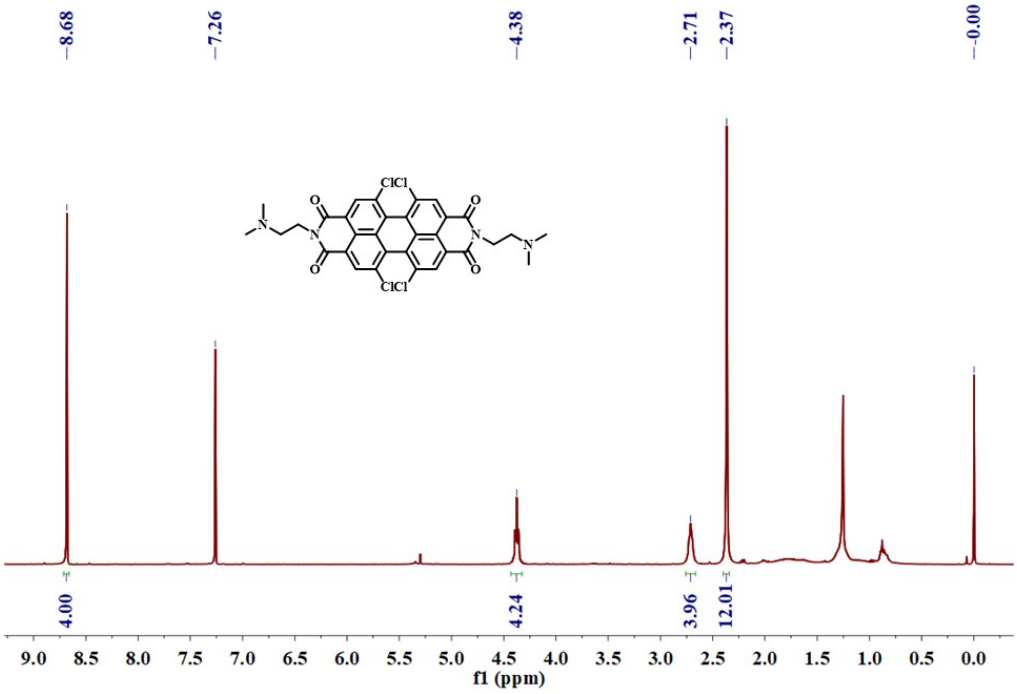

Figure S1. ^{1}H NMR spectrum of compound 4ClPDI in $CDCl_3$.

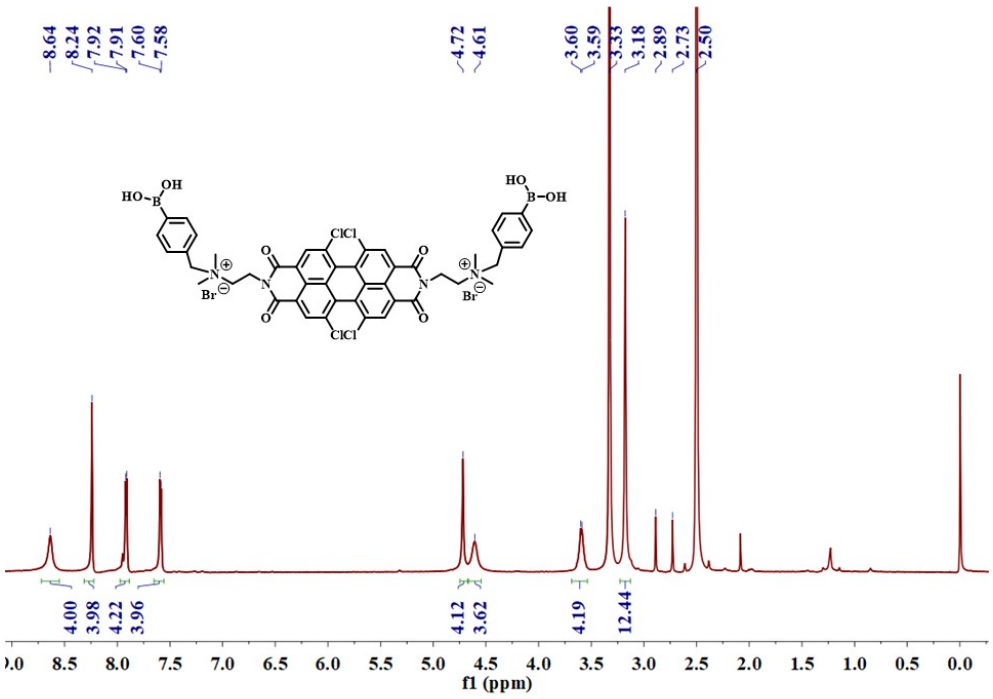

Figure S2. ^{1}H NMR spectrum of compound BPDI in DMSO.

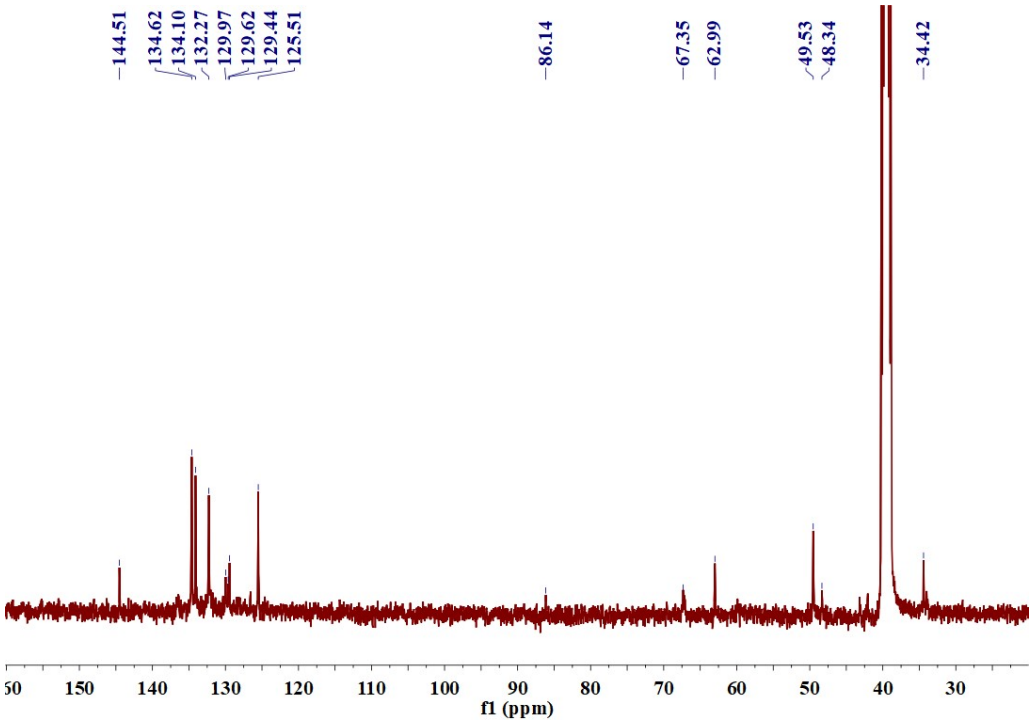

Figure S3. ^{13}C NMR spectrum of BPDI in $CDCl_3$.

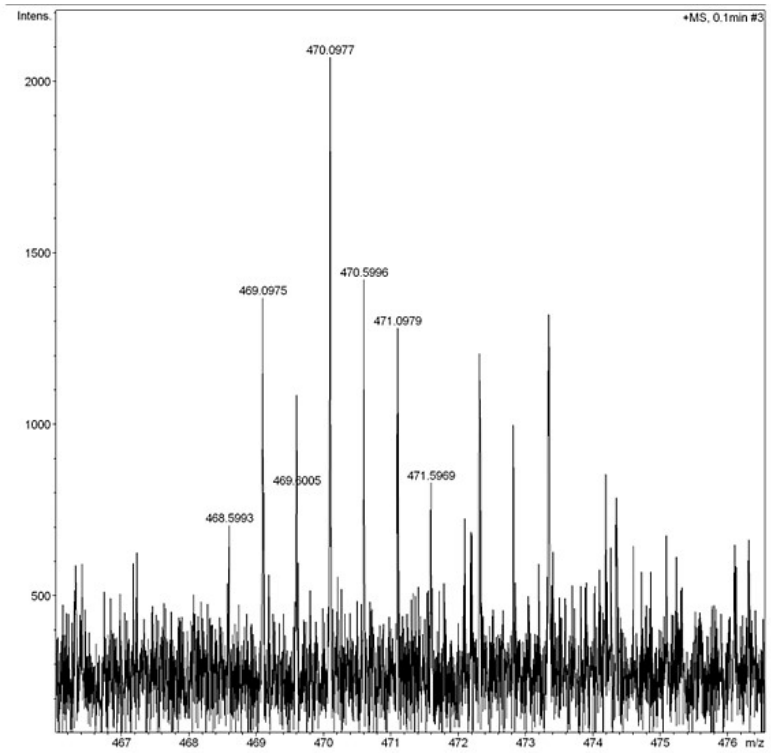

Figure S4. HR-MS spectrum of BPDI.

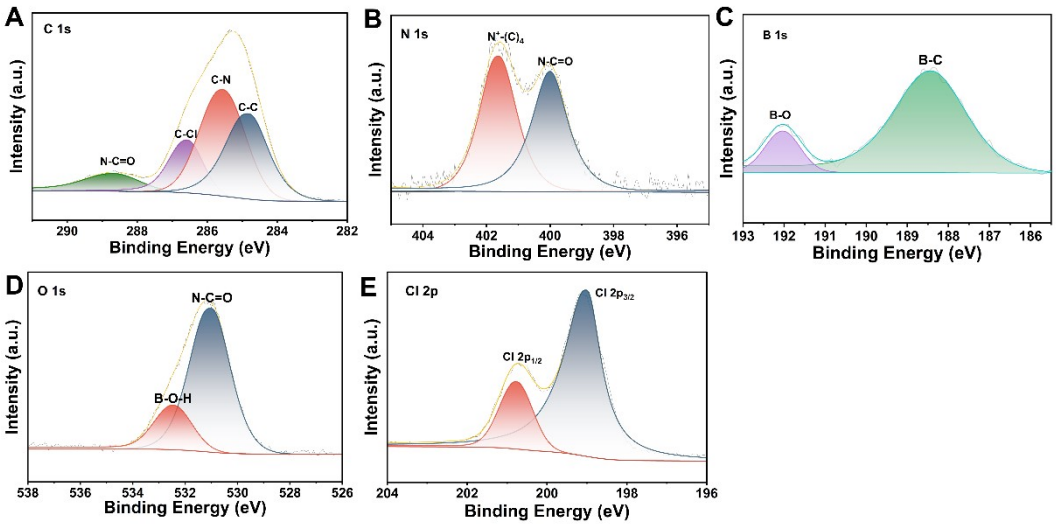

Figure S5. XPS analysis for BPDI. High-resolution C 1s (A), N 1s (B), B 1s (C), O 1s (D) and Cl 2p (E) spectra of BPDI.

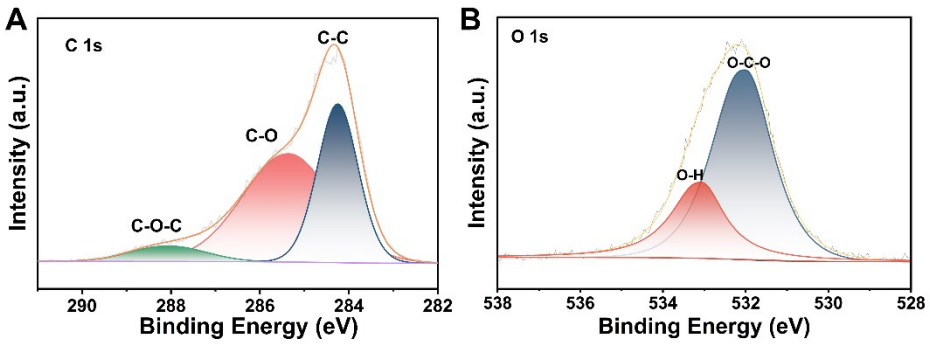

Figure S6. XPS analysis for CF. High-resolution C 1s (A) and O 1s (B) spectra of CF.

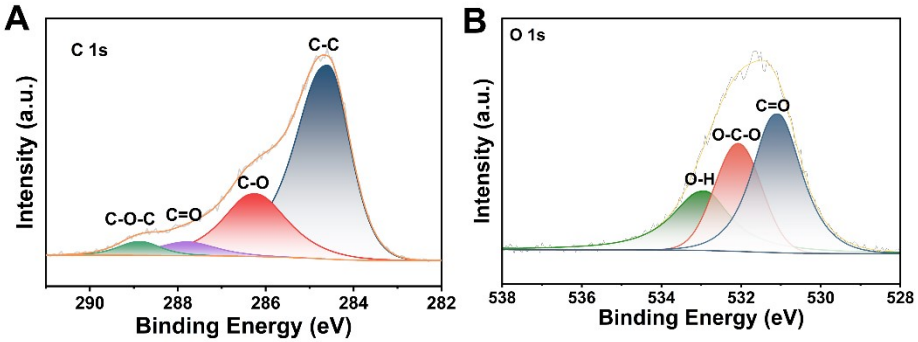

Figure S7. XPS analysis for OCF. High-resolution C 1s (A) and O 1s (B) spectra of OCF.

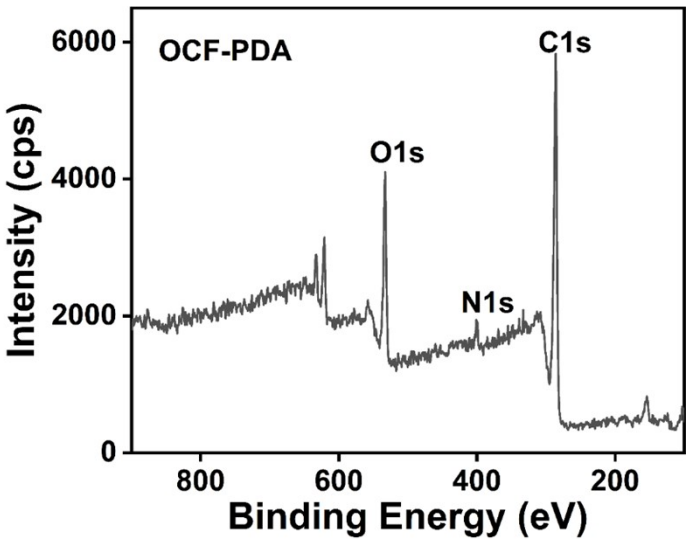

Figure S8. X-ray photoelectron spectra of OCF-PDA.

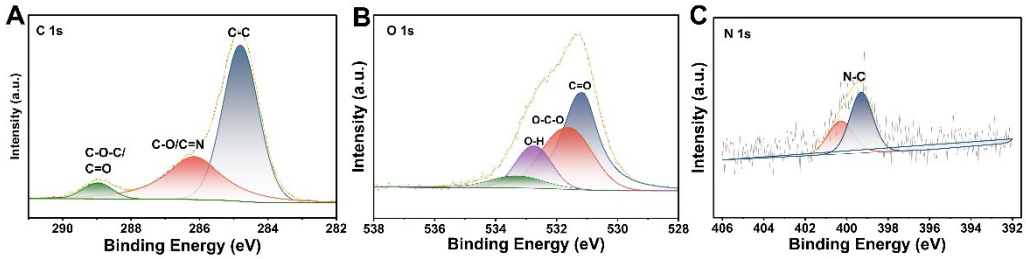

Figure S9. XPS analysis for OCF-PDA. High-resolution C 1s (A), O 1s (B) and N 1s (C) spectra of OCF-PDA.

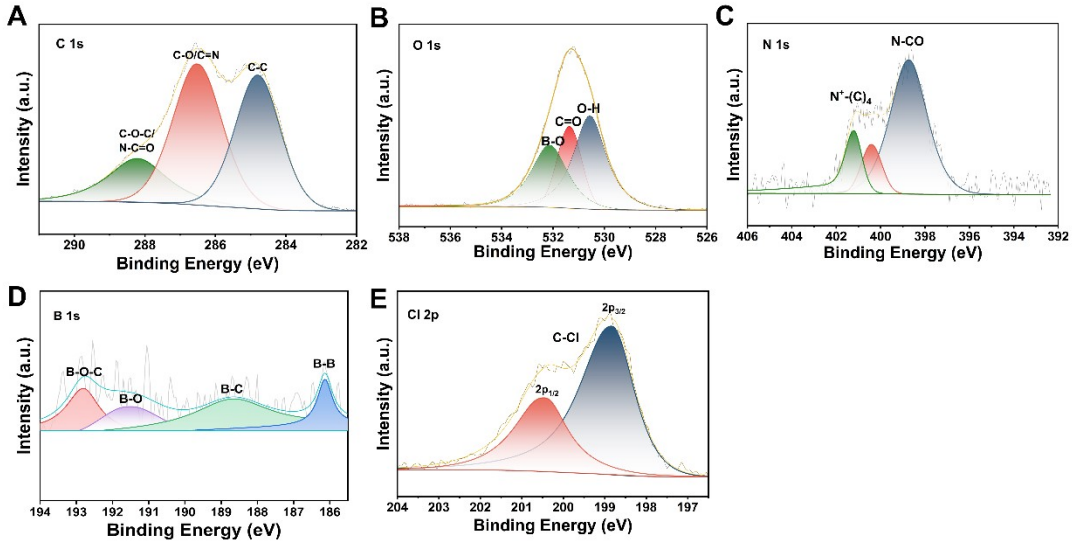

Figure S10. XPS analysis for OCF-PDA-PDI. High-resolution C 1s (A), O 1s (B), N 1s (C), B 1s (D) and Cl 2p (E) spectra of OCF-PDA-PDI.

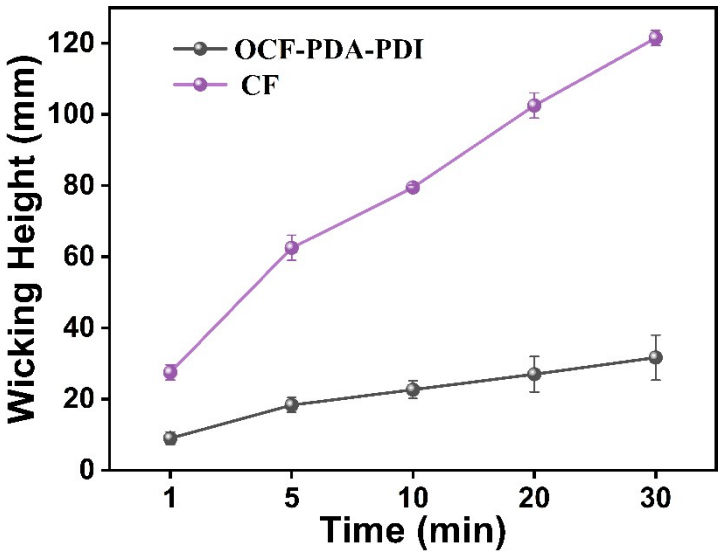

Figure S11. Change in core suction height over time for OCF-PDA-PDI and CF.

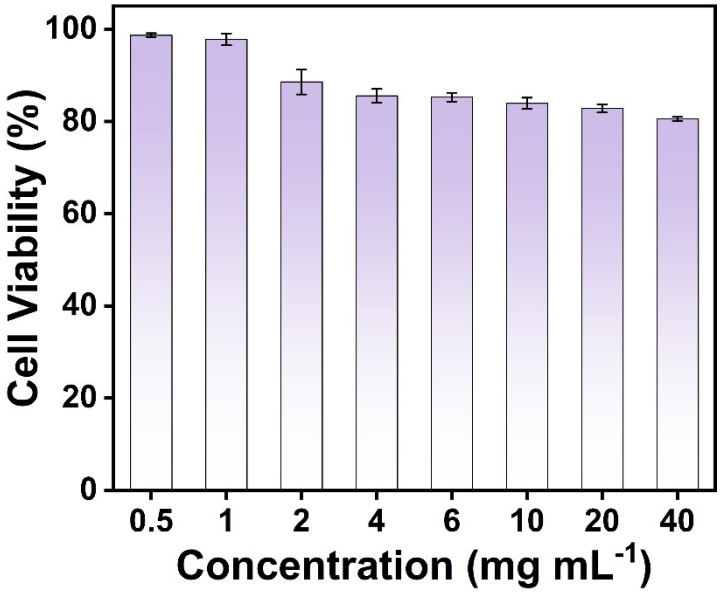

Figure S12. Cytotoxicity of HUVEC cells after 24 h of incubation with different concentrations of BPDI samples.

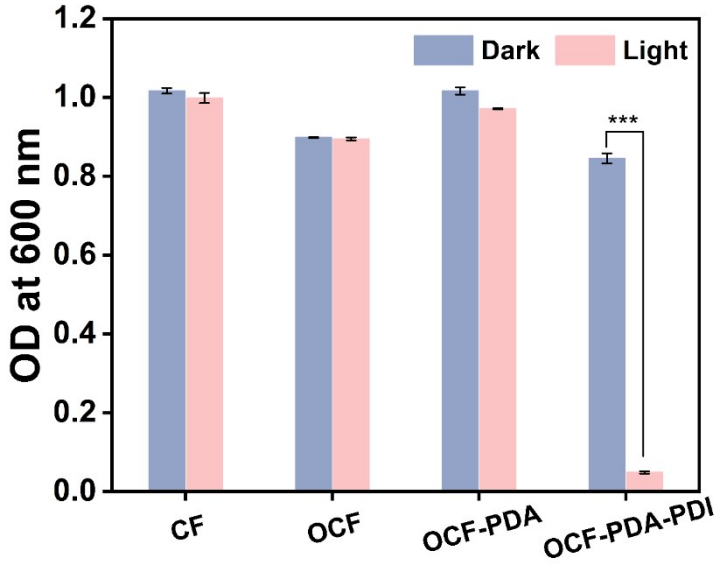

Figure S13. $OD_{600\ nm}$ value of liquid medium after incubation with *E.coli* on cotton fiber fabric for 12 h.

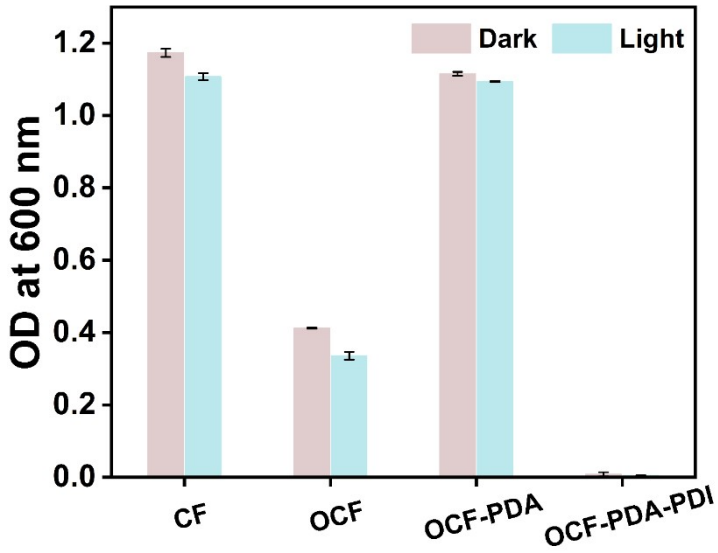

Figure S14. $OD_{600\ nm}$ value of liquid medium after incubation with *S. aurues* on cotton fiber fabric for 12 h.