Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.
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Supporting Information

Porphyrin-anthracene Covalent Organic Framework for Sustainable Photosterilization

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Supplementary Methods

Chemicals and materials. 5,10,15,20-tetra-(4-aminophenyl) porphyrin (TAPP) and 9,10-bis(4-formylphenyl) anthracene (DPA) were purchased from Jilin Chinese Academy of Sciences-Yanshen Technology Co., Ltd. (Jilin, China). Mesitylene, *n*-butanol (*n*-BuOH), acetonitrile (ACN), Ethanol (EtOH), acetic acid, *N,N*-dimethylformamide (DMF) and tetrahydrofuran (THF), were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Dioxane, *o*-dichlorobenzene (*o*-DCB) and 9,10-Anthracenediyl-bis (methylene) dimalonic acid (ABDA) were purchased from Macklin Biochemical Co., Ltd. (Shanghai, China). 1,3-Diphenylisobenzofuran (DPBF) and 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) was purchased from Aladdin (Shanghai, China). Ultrapure water was purchased from Hangzhou Wahaha Group Co., Ltd (Hangzhou, China). Luria-Bertani (LB) Broth Powder (FMB Grade), LB Agar Powder (FMB Grade) and phosphate buffer saline solution premixed powder were purchased from Shanghai Sangon Biotech (Shanghai, China). Escherichia coli (*E. coli*, ATCC 25922) and Staphylococcus aureus (*S. aureus*, ATCC 25923) were thawed from the frozen bacteria in our laboratory. Other reagents were at least of analytical grade and used without further purification.

Instrumentation and Characterization. Powder X-ray diffraction (PXRD) patterns were recorded on a D2 PHASER X-ray diffractometer (Bruker, German). Fourier transform infrared (FT-IR) spectra were carried out on a Nicolet IS50 spectrometer (Thermo, America) by using KBr pellet. The UV-vis absorption spectra were recorded on a UV-3600 PLUS (Shimadzu, Japan). Bacteria imaging experiments were obtained with a

FV3000 confocal laser scanning microscopy (CLSM) (Olympus, Japan). Scanning electron microscopy images (SEM) were carried out on a SU8100 SEM (Hitachi, Japan).

Synthesis of Por-DPA. A 35 mL Schlenk tube was charged with TAPP (67.5 mg, 0.10 mmol), DPA (77.3 mg, 0.20 mmol), and a mesitylene/*n*-butanol solution (9/1, V/ V, 2 mL), and the homogeneous suspension was obtained by sonicated for 10 min. Afterwards, aqueous acetic acid (6 mol L^{-1}, 0.2 mL) was added and sonicated for 5 min. Then, the tube was degassed through three freeze-pump-thaw cycles and sealed with a screw cap, and the resultant mixture was kept at 120 °C for 3 d. The yielded product was collected by centrifugation, washed several times with THF and DMF. Then, the collected powder was dried under vacuum at 45 °C to obtain Por-DPA COF.

Photothermal Properties of Por-DPA. To evaluate the photothermal property of Por-DPA, 1 mL of Por-DPA in different concentrations (0 μg mL^{-1}, 100 μg mL^{-1}, 200 μg mL^{-1} and 400 μg mL^{-1}) was exposed under the white light (100 mW cm^{-2}) for 30 min. The suspension of TAPP (200 μg mL^{-1}) under the same condition was chosen as the control group. The temperature changes and thermal images of the solution in real time were all recorded by a FLIR-50 thermal imaging camera. Meanwhile, the same method was used to determine the temperature changes and thermograms of 200 μg mL^{-1} of Por-DPA under different power densities of irradiation (50 mW cm^{-2}, 100 mW cm^{-2} and 150 mW cm^{-2}).

Photodynamic Properties of Por-DPA. To validate the photodynamic property of Por-DPA, ABDA as a ROS indicator was chosen to monitor the generation of $^1O_2$.[1] Briefly, 0.1 mL of 5 × 10^{-3} mol L^{-1} ABDA in DMF was blended with 9.9 mL Por-DPA in ultrapure water (the final concentration of Por-DPA was 0 μg mL^{-1}, 100 μg mL^{-1}, 200 μg mL^{-1} and 400 μg mL^{-1}, respectively). Then, the above solutions were exposed to white light (100 mW·cm^{-2}) irradiation, and 1 mL of the irradiated solution was taken out and centrifuged at different times (0 min, 3 min, 6 min, 9 min, 12 min, 15 min, 18 min, 21 min, 24 min and 30 min, respectively). After that, the absorbance spectra of each solution were recorded on a UV-vis-NIR spectrophotometer. TAPP (100 μg mL^{-1}) under 100 mW cm^{-2} irradiation was used as a control group. Meanwhile, the photodynamic properties of Por-DPA under different power of irradiation (50 mW cm^{-2}, 100 mW cm^{-2} and 150 mW cm^{-2}, respectively) were also measured by the same method. ABDA under 150 mW cm^{-2} irradiation was used as a blank group. It is worth noting that all operations in these experiments should be performed in the dark.

The sustainable $^1O_2$ generation of Por-DPA without irradiation was further investigated with DPBF as a probe.[2, 3] Briefly, 600 μL of 1 × 10^{-4} mol L^{-1} DPBF in ACN was blended with 400 μL Por-DPA in H_2O (the final concentration of Por-DPA was 200 μg mL^{-1}). Then, the above solutions were exposed to 808 nm laser (0.6 W·cm^{-2}) irradiation for 3 minutes, followed by stored in the absence of lightness. After being stored in the dark for different periods of time (0, 3, 6, 9, 12 and 24 min, respectively), 1 mL of the solution was taken out and centrifuged. Then, the absorbance spectra of each solution were recorded on a UV-vis-NIR spectrophotometer. All procedures after pre-excitation are carried out in the dark to avoid light interference.

Bacterial Cultures and Sterilization Experiment. The bactericidal properties of Por-DPA were evaluated using two typical bacteria, Gram-positive *S. aureus* and Gramnegative *E. coli*, as model bacteria. In brief, the frozen primitive bacteria were resuscitated and cultured in 5 mL Luria Bertani broth medium under 12 h shaking (200 rpm) at 37°C, and further experiments were carried out after culture to exponential growth stage.

100 µL of *E. coli* or *S. aureus* in PBS (colony concentration of 10^7 CFU mL^{-1} in final mixed solution) were mixed respectively with 900 µL of Por-DPA solution in centrifuge tubes to a final concentrations of 200 and 400 µg mL^{-1} and incubated at 37°C for 30 min. Then, the above two model bacteria were divided into the following groups: (1) bacteria in PBS without irradiation (blank group), (2) bacteria in PBS under irradiation for 30 min, (3) the bacteria treated with Por-DPA in PBS, (4) the bacteria treated with pre-irradiated Por-DPA in PBS, (5) the incubated bacteria with Por-DPA in PBS under irradiation for 10 min, 20 min, 30 min, respectively. Irradiation was performed with white light (100 mW cm^{-2}). 100 µL of above bacterial suspension was extracted from each group and gradually diluted 10^4 times. Then, 100 µL of each diluted bacteria solution was spread evenly onto Luria Bertani broth agar plates and cultured at 37°C for 24 h. Finally, the colonies number was counted.

Detection of intracellular $^1O_2$ in Bacteria. To further identify the photodynamic sterilization mechanism of Por-DPA, DCFH-DA was used as the indicator.[4] Briefly, 1 mL of *E. coli* solutions (10^7 CFU mL^{-1}) was cultured with Por-DPA (200 µg mL^{-1}) and DCFH-DA (10 µmol L^{-1}) for 30 min. Afterward, irradiation with or without white light (100 mW cm^{-2}, 10 min, 20 min, 30 min, respectively) was performed. Then, the bacterial suspension was centrifuged (5000 rpm, 10 min) and resuspended in 20 µL PBS. Finally, 10 μL of resuspended bacterial solution was dropped onto the glass slide and then corresponding CLSM images was acquired under excitation at 488 nm.

Morphological Observation of Bacteria. After incubation with PBS or Por-DPA dispersion (final concentrations of 200 μg mL^{-1}) at 37°C in the dark for 30 min, the *E. coli* suspension (10^7 CFU mL^{-1}) was irradiated by the white LED light (100 mW cm^{-2}) for 0 min, 10 min, 20 min, 30 min or not, respectively. Then, the above bacterial solutions were centrifuged (4000 rpm, 10 min) and fixed in 2.5% glutaraldehyde for 16 h. The resulting bacteria were further washed twice with PBS, and dehydrated through treating with 30%, 50%, 70%, 80%, 95% and 100% (v/v) gradient ethanol for 10 min. Finally, the resulting samples were freeze-dried and the morphologies of *E. coli* were observed on SEM.

Murine Infection Model. The animal experiment has passed the examination and approval of the Ethics Committee of Jiangnan University (Wuxi, China) (JN.No20220430b0180606[147]). The sustainable photosterilization of the Por-DPA COF on the wound healing process was evaluated using a mouse full-thickness cutaneous wound model infected with *E. coli*. First, female BALB/c mice (5-6 weeks, purchased from the Changzhou Cavens Experimental Animal Co. Ltd.) were anesthetized, and then their backs were created a round skin wound (d ≈ 7 mm). Subsequently, the back wound of the mice was added with 20uL of *E. coli* solution dropwise to establish an infection mode. According to different treatments, they were divided into five groups randomly, and each group had five parallel samples for contrast. The five groups were given the following treatments: no treatment, Por-DPA

(Por-DPA-treated without irradiation, irradiation (only irradiated with 30-min white light, 100 mW cm^{-2}), Por-DPA + pre-irradiation (treated with Por-DPA pre-irradiated for 30-min with white light, 100 mW cm^{-2}), Por-DPA + irradiation (coated with Por-DPA followed by 30-min irradiation treatment, 100 mW cm^{-2}). The dosage and concentration of Por-DPA in the above experimental groups were 50 µL and 400 µg mL^{-1}, respectively. Then, the wound allowed to heal naturally for 12 days, and the changes of body weight and wound area during the healing process were recorded.

The mice were sacrificed on the 12th day for evaluate the performance in biomedical science of the Por-DPA, and the wound skin tissues were collected for Masson and hematoxylin and eosin (H&E) staining and immunohistochemistry analysis (including tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6)).

Supplementary References.

1. X. Zhao, M.-Y. Yin, T.-Y. Gu, X.-M. Gao, L.-J. Chen, Z.-Y. Wang and X.-P. Yan, *ACS Appl. Nano Mater.*, 2023, **6**, 12402-12412.
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3. D. Yan, E. Lin, F. Jin, S. Qiao, Y. Yang, Z. Wang, F. Xiong, Y. Chen, P. Cheng and Z. Zhang, *J. Mater. Chem. A*, 2021, **9**, 27434-27441.
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Supplementary Figures

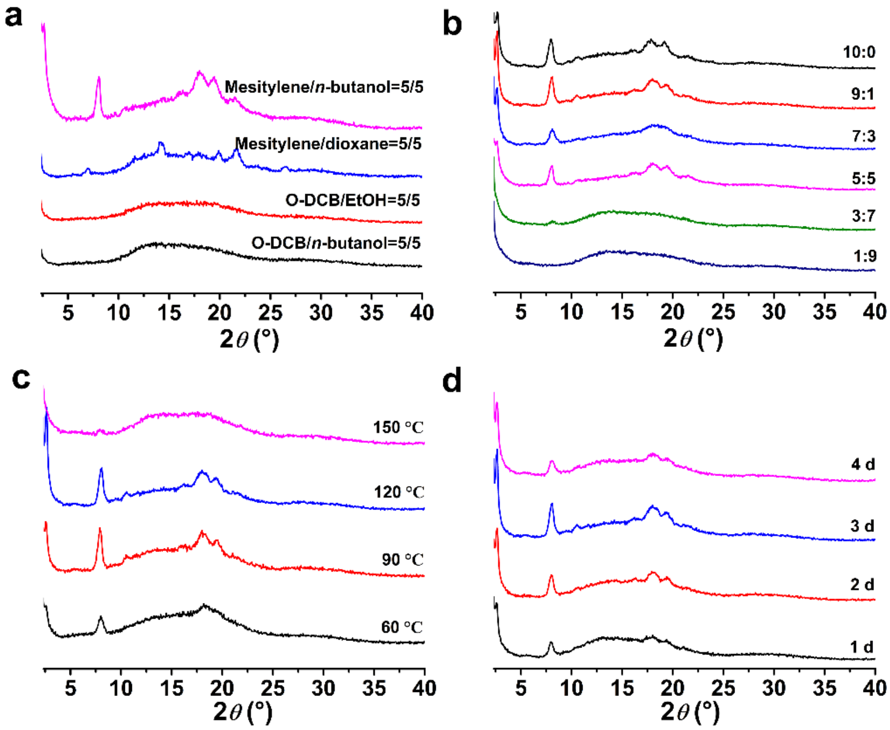

Fig. S1. (a) Effect of reaction solvent on the PXRD pattern of Por-DPA COF at 120 °C for 3 days. (b) Effect of the ratio of mesitylene to *n*-butanol (v/v) on the PXRD pattern of Por-DPA COF under the reaction at 120 °C for 3 days. (c) Effect of reaction temperature on the PXRD pattern of Por-DPA COF under 3 days of reaction with mesitylene and *n*-butanol (1/1, V/V) as solvent. (d) Effect of reaction time on the PXRD pattern of Por-DPA COF at 120 °C with mesitylene and *n*-butanol (1/1, V/V) as solvent.

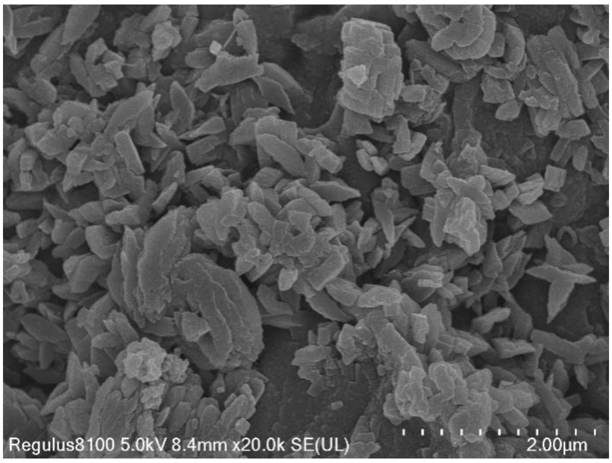

Fig. S2. SEM image of Por- DPA COF.

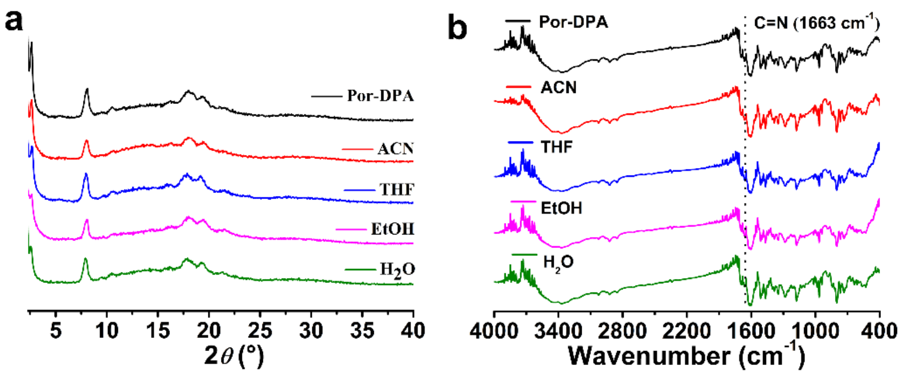

Fig. S3. PXRD patterns (a) and IR patterns (b) of Por-FPA COF after treatment with various solvents at 25 °C.

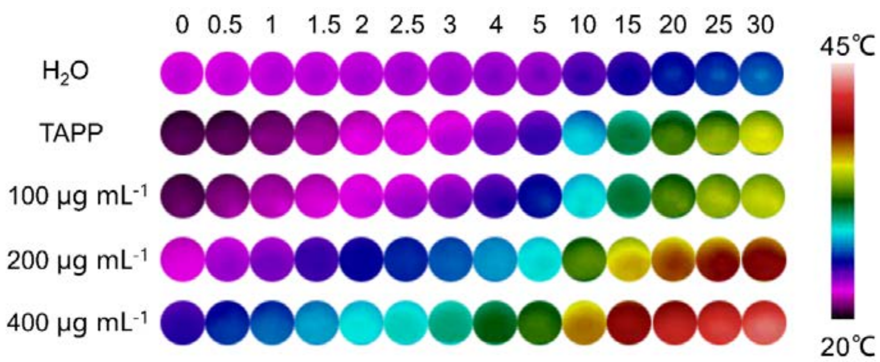

Fig. S4. The IR thermal images of different solutions including H_2O, TAPP (200 μg mL^{-1}) and different concentrations of Por-DPA under irradiation (100 mW cm^{-2}) (The unit of time is min).

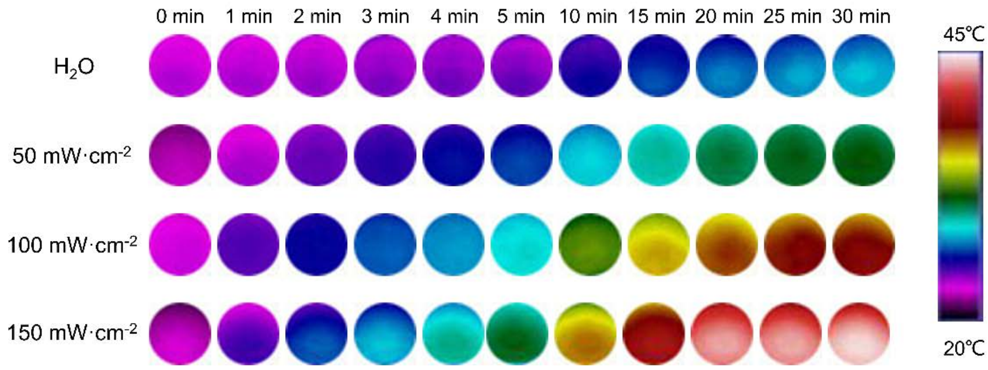

Fig. S5. The IR thermal images of different solutions including H_2O under irradiation (150 mW cm^{-2}) and Por- DPA (200 μg mL^{-1}) under different irradiations.

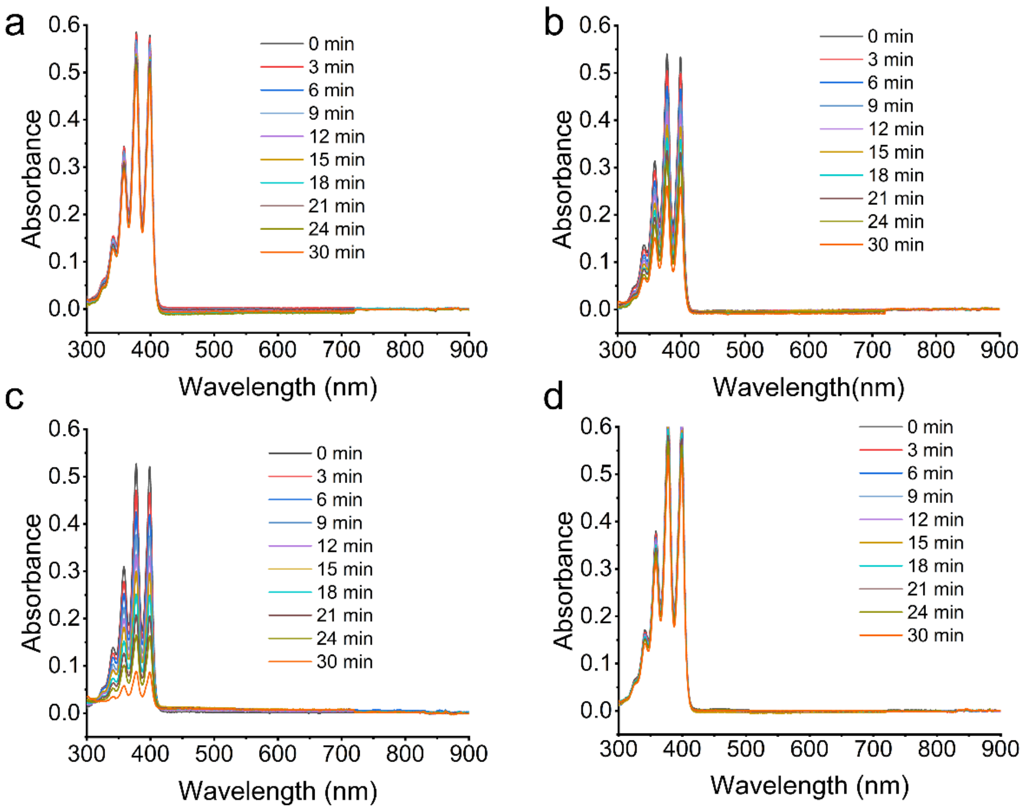

Fig. S6. Time-dependent absorption spectra changes of ABDA at 377 nm with different concentrations of Por-DPA (a, 0 µg mL^{-1}; b, 100 µg mL^{-1}; c, 200 µg mL^{-1}; d, TAPP 100 µg mL^{-1}) under irradiation (100 mW cm^{-2}).

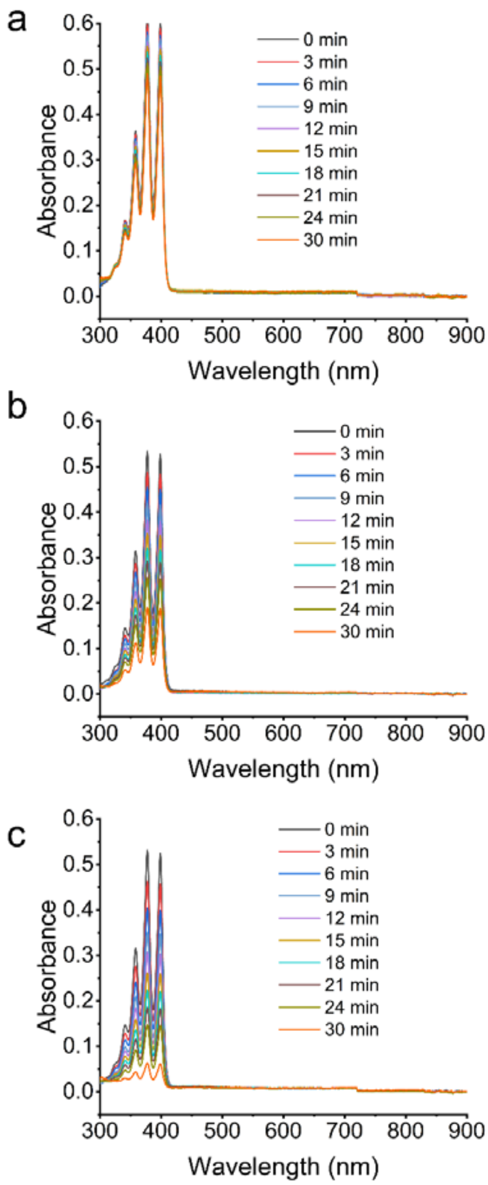

Fig. S7. (a) Time-dependent absorption spectra changes of ABDA at 377 nm under irradiation (150 mW cm^{-2}). (b and c) Time-dependent absorption spectra changes of ABDA at 377 nm with Por-DPA (200 μg mL^{-1}) under different irradiations (50 mW cm^{-2} and 150 mW cm^{-2}, respectively).

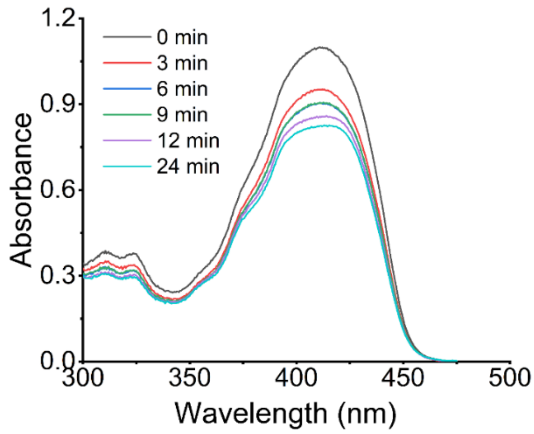

Fig. S8. Time-dependent absorption spectra change of DPBF (6×10^{-5} mol L^{-1}) at 410 nm with per-irradiated Por-DPA (200 μg mL^{-1}).

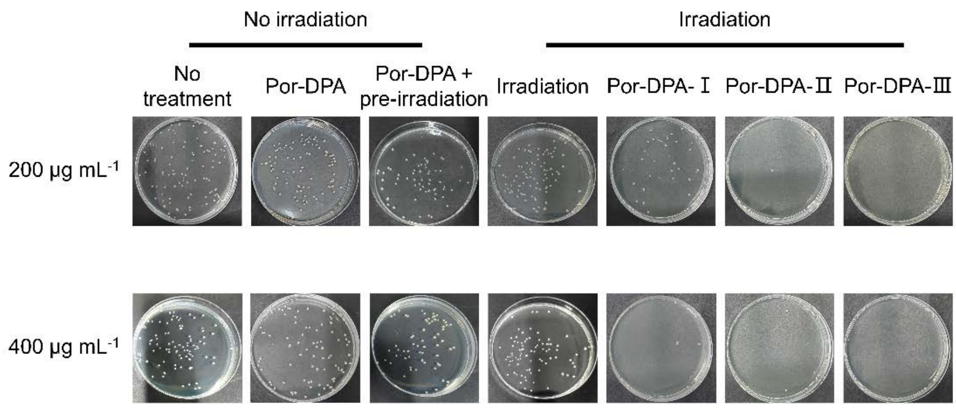

Fig. S9. Flat colony photographs of *E. coli* colonies after different treatments. Por-DPA-I, Por-DPA-II and Por-DPA-III represented irradiation for 10, 20 and 30 min, respectively.

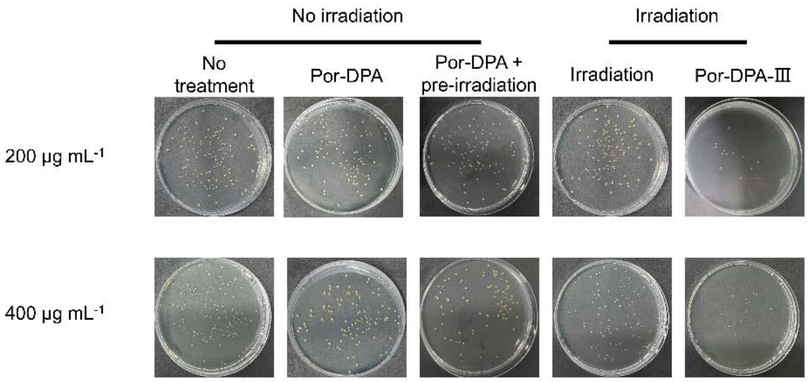

Fig. S10. Flat colony photographs of *S. aureus* colonies after different treatments. Por-DPA-III represented irradiation for 30 min.

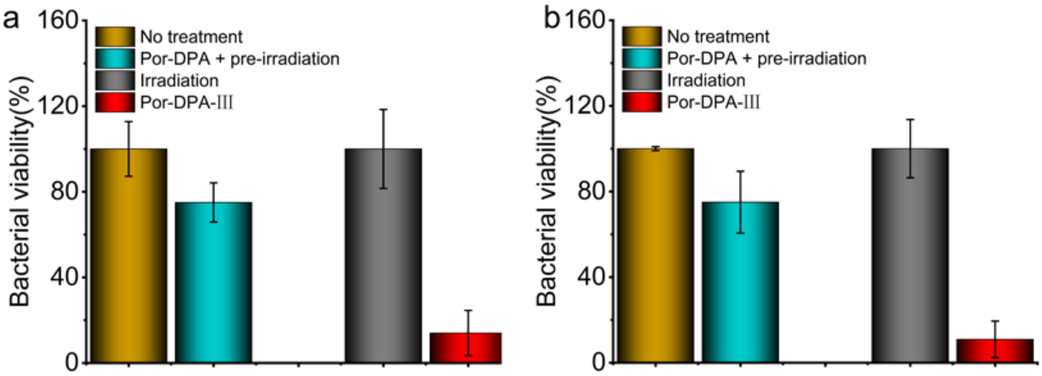

Fig. S11. In vitro bacterial viability of *S. aureus.* incubated with Por-DPA at (a) 200 μg mL^{-1} and (b) 400 μg mL^{-1}, respectively, under different treatments.

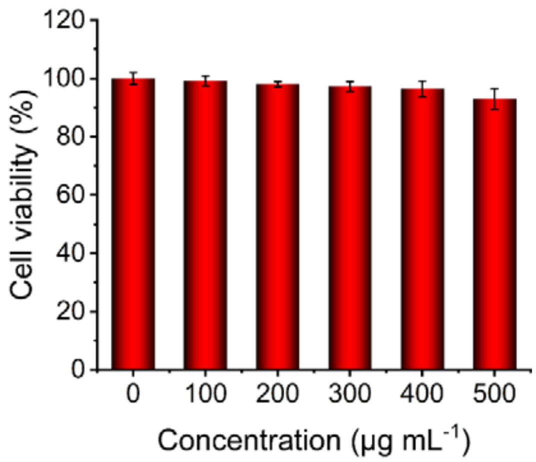

Fig. S12. Cell viability of 3T3 cells incubated with various concentrations of Por-DPA. Data were presented as the mean ± SD (n = 3).

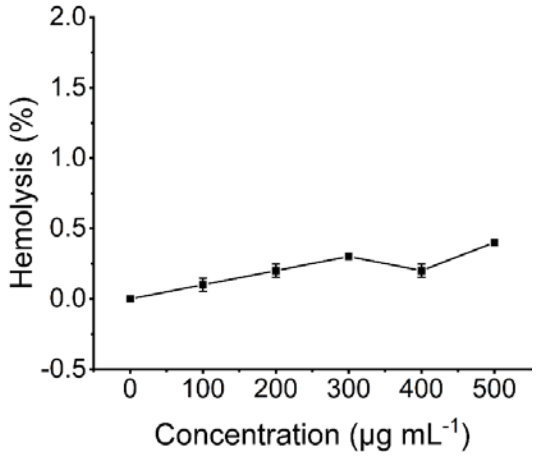

Fig. S13. Hemolysis rate of red blood cells incubated with different concentrations of Por-DPA. Data were presented as the mean ± SD (n = 3).

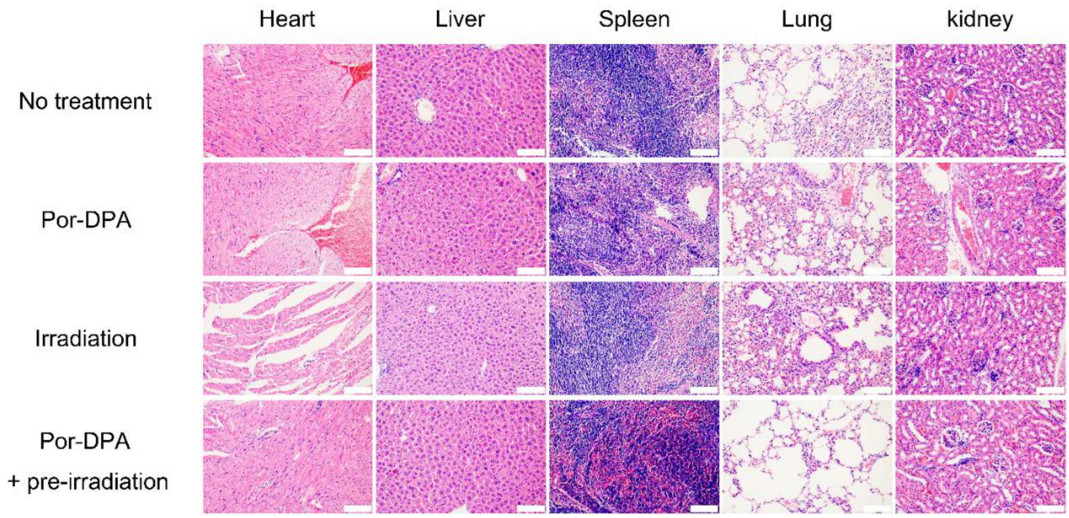

Figure S14. H&E staining of main organs of mice after various treatments for 12 days. (Scale bar, 200 μm).

Supplementary Table

Table S1 Fractional main atomic coordinates for the unit cell of TAPP-DPA COF after Pawley refinement

TAPP-DPA COF: Space group symmetry P4/m			
a = b = 33.7047 Å, c = 10.5931 Å, α = β = γ = 90°			
Atom	x	y	z
C1	1.22707	-0.50147	0.34284
C2	1.20778	-0.4655	0.36838
C3	1.16756	-0.46501	0.40065
C4	1.14562	-0.50054	0.41424
C5	1.16486	-0.53601	0.37659
C6	1.20495	-0.53643	0.34313
C7	1.10205	-0.50055	0.45115
C8	1.08288	-0.53473	0.47605
C9	1.09909	-0.56859	0.54405
C10	1.07075	-0.5954	0.54622
C11	1.03709	-0.57995	0.47321
N12	1.04304	-0.54343	0.43917
N13	1.26832	-0.50336	0.30934
C14	1.02253	-0.79502	0.34171
C15	1.01771	-0.8368	0.30292
C16	0.98896	-0.84817	0.21392
C17	0.98485	-0.888	0.1796
C18	1.00937	-0.91718	0.23309
C19	1.03714	-0.90585	0.3251
C20	1.04187	-0.86596	0.35756
C21	1.00418	-0.95969	0.1975
C22	0.96447	-0.97606	0.19555
C23	1.03614	-0.98356	0.1453
C24	0.89794	-0.0228	0.24436
C25	0.92481	-0.03362	0.15003
C26	1.09113	-0.00601	0.33189
C27	1.05546	-0.02638	0.31562
H28	1.22326	-0.43752	0.35946
H29	1.15417	-0.43623	0.40958
H30	1.14906	-0.56375	0.36879
H31	1.21886	-0.56421	0.31711
H32	1.12728	-0.57066	0.59252

H33	1.07217	-0.62311	0.59763
H34	1.04714	-0.78739	0.40279
H35	0.96967	-0.82635	0.17074
H36	0.96234	-0.89606	0.11173
H37	1.05468	-0.92784	0.37398
H38	1.06377	-0.85801	0.42803
H39	0.86983	-0.03806	0.25316
H40	0.9175	-0.05779	0.08707
H41	1.10988	-0.01199	0.41224
H42	1.04716	-0.04854	0.38492