Supplementary Material

Visible light induced bactericidal properties of a novel thiophene-based linear conjugated polymer/TiO_2 heterojunction

1. Experimental Section.

1.1. Photocatalytic disinfection tests

The photocatalytic disinfection abilities of PTCD, TiO_2, PTCD/TiO_2 were evaluated via the inactivation of *S. aureus* under visible light irradiation. Before the microbiological experiments, all the glassware and the culture medium solution were sterilized by autoclaving at 121 °C for 20 min. And all the experiments were processed under sterile conditions. *S. aureus* cells(10^8 cfu mL^{-1}, 100 μL) were grown in TSB (6 mL) at 37 °C for 16 h with shaking, and collected by centrifugation (10000 rpm, 2 min). After washing with PBS (0.01 M, pH 7.4), the *S. aureus* cells were re-suspended in PBS (0.01 M, pH 7.4) to obtain a initial cell density of 10^7 cfu mL^{-1}. For each antibacterial experiment, 20 mL of *S. aureus* suspension and 4 mg catalyst powders were pipetted into a container. Then, the antibacterial experiment was initiated by irradiating the suspension with a commercial 300 W xenon lamp equipped with a UV cut off filter (λ > 420 nm). The light source was located 13 cm from the reaction solution (100 mW cm^{-2} illumination intensity). At given irradiation time interval (0, 1, 2, 3, 4 h), 100 μL of tested suspension samples were spread on freshly prepared TSA agar plates, and the colonies were counted to determine the survival bacterial numbers after incubation at 37 °C for 24 h. Experiments on different concentration of photocatalysts were also perform by this method. The bacterial inactivation efficiencies of PTCD, TiO_2, PTCD/TiO_2 were calculated using by the equation : Bacterial survival ratio (%) = (the number of bacterial colony with material for different time after the reaction)/(the numbers of bacterial colony before the reaction) ×100%

1.2. Rabbit Plasma Test

Staphylococcal coagulase is an important virulence factor for *S. aureus*. Coagulase converts host prothrombin to staphylothrombin, leading to activation of the protease activity of thrombin. It was predicted that coagulase could protect bacteria from phagocytic and immune defenses by causing localized clotting. The expression of coagulase of *S. aureus* was examined after the interaction between 30% PTCD/TiO_2 and the bacteria. A tube coagulation assay based on freeze-dried rabbit plasma was performed. 30% PTCD (4 mg) was added to bacteria culture (20 mL) and allowed to react for 0, 1, 2, 3, and 4 h. Then 300 μL of reaction solution collected at different times were added to 500 μL of rabbit plasma in small ampules of glass tubes. The mixture samples were incubated at 37 °C for 6 h, and rabbit plasma coagulation was examined.

1.3. Fluorescent-based cell live/dead test

Fluorescent-based cell live/dead tests were conducted for investigating the integrity of bacterial cell membranes. In brief, the bacterial liquids after 4 h irradiation treatment in the control and experimental groups were collected and centrifuged at 8000 rpm for 2 min with the supernatant being discarded. The obtained bacteria were dispersed in 50 μL sterile PBS solution and stained with 25 μL of SYTO-9 (6 mg/mL in sterilized deionized water) and 25 μL of PI (6 mg/mL in sterilized deionized water) solution for 15 min in the dark at room temperature. Lastly, 10 μL of stained bacterial liquid was taken out and dropped on the center of slide and then imaged using a laser scanning confocal microscope (Nikon Ti-s, Japan).

1.4. Cell Toxicity Assay

The cytotoxicity of 30% PTCD/TiO_2 with different concentrations (100 μg mL^{-1}, 200 μg mL^{-1}, and 400 μg mL^{-1}) was investigated by the CCK-8 test on NIH3T3 cells. The cells were cultured in Dulbecco's modified eagle medium (DMEM), then diluted to yield a cell count of approximately 5×10^4 cells/mL, 100 μL of suspension were put on a 96-well plate so that they adhered to the plate wall. The old medium was removed, replaced with fresh medium containing heterojunctions of different concentrations, and co-cultured with NIH3T3 cells for 24 h. Afterwards, 10 μL of the CCK-8 solution were added to the 96-well and cultured for another 4 h at 37 °C. Finally, the solution was subjected to the optical density measurement on a microplate reader (450 nm).

2. Supplementary figures

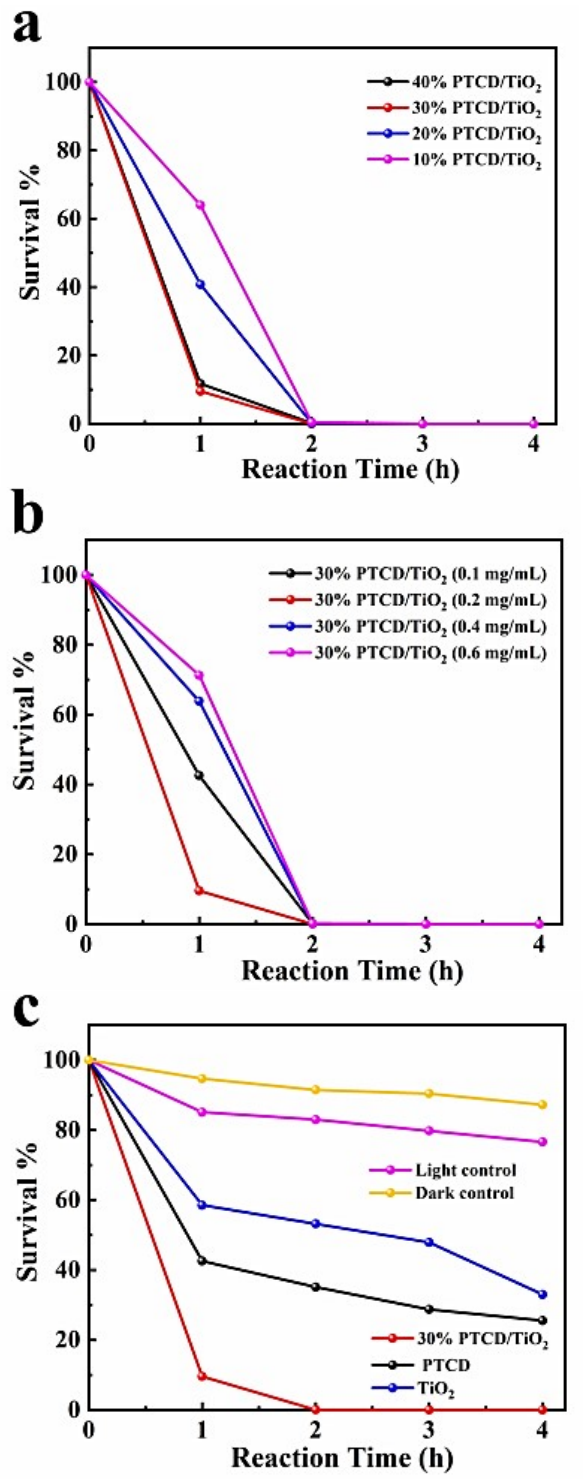

Fig. S1. Photocatalytic inactivation efficiency of *S. aureus* under visible-light irradiation with (a) different composite mass ratios of PTCD/TiO_2, (b) the different concentrations of 30% PTCD/TiO_2 and (c) the samples (0.2 mg mL^{-1}) against *S. aureus* under light and dark conditions.

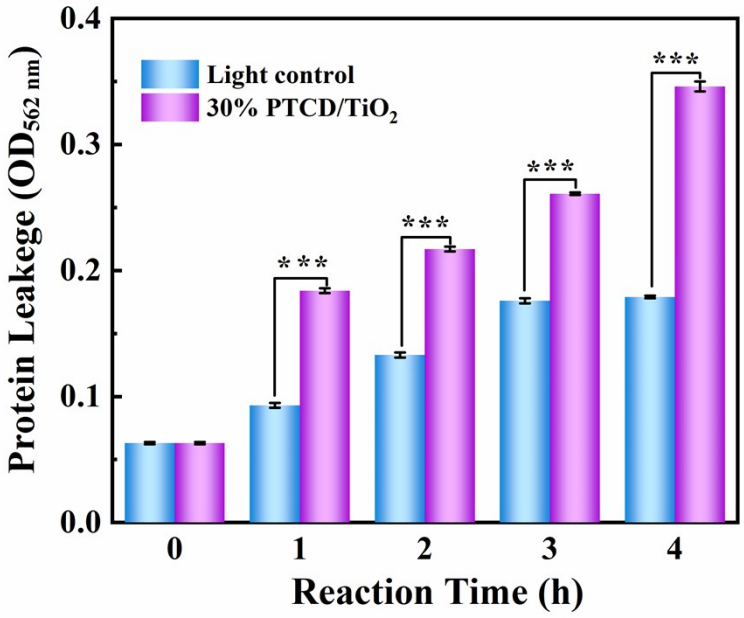

Fig. S2. The $OD_{562\ nm}$ absorbance value of BCA Protein leakage of the *S. aureus* suspensions treated with 30% PTCD/TiO_2 under visible light irradiation. Student's t test, ***P < 0.001.

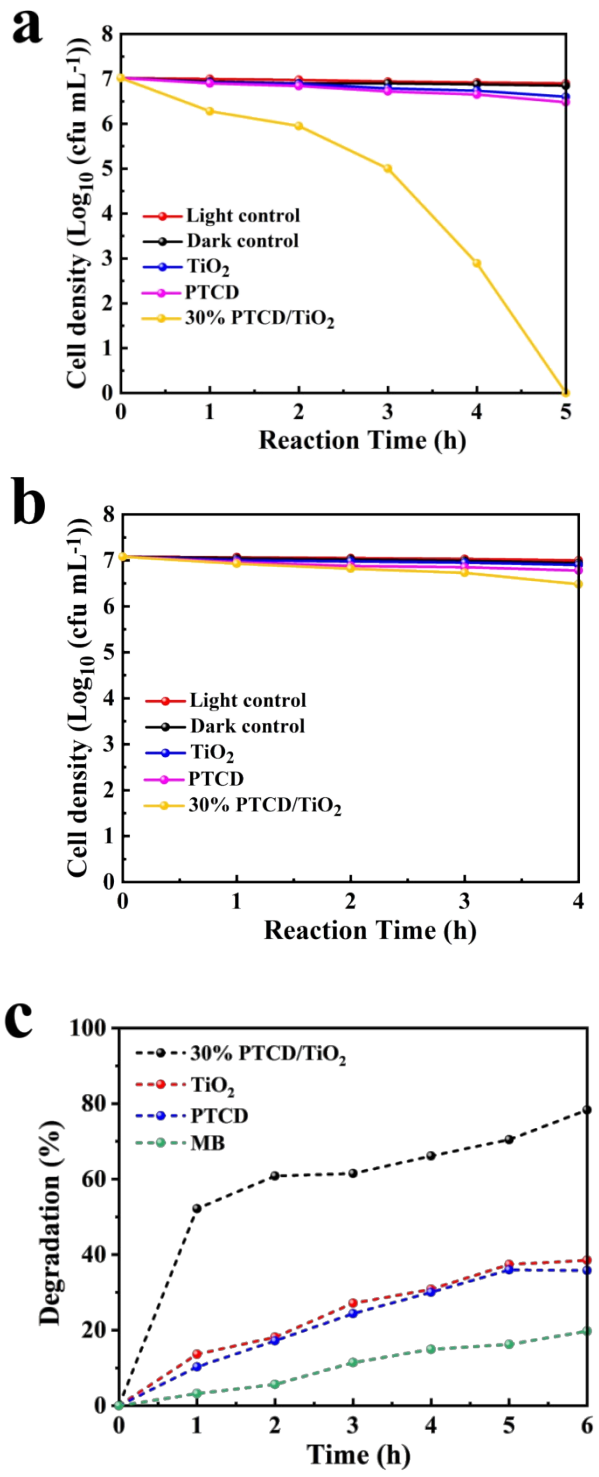

Fig. S3. (a) Photocatalytic inactivation cell density of PTCD, TiO_2, 30% PTCD/TiO_2 against *S. epidermidis* and (b) *E. coli*, (c) photodegradation efficiencies of MB solution(50.0 mL 10 mg/L) between degradation and irradiation time under visible-light irradiation in the presence of PTCD, TiO_2, 30% PTCD/TiO_2 composites.

3. Detection of H_2O_2

The KI-starch method was used to detect H_2O_2 formed in the reaction with minor changes [1]. The experimental results are shown in Fig. S4, which indicated that H_2O_2 was produced during the reaction.

Fig. S4. Detection of H_2O_2. (a) starch+KI; (b) starch+KI+HCl; (c) starch+KI+HCl+molybdate; and (d) starch+KI+HCl+molybdate+supernatant of the reaction mixture.

Reference

[1] M.K. Sahoo, G. Jaiswal, J. Rana, E. Balaraman, Organo-Photoredox Catalyzed Oxidative Dehydrogenation of N-Heterocycles, Chem. Eur. J., 23 (2017) 14167-14172.