Molecular dipole regulated pyrene-based polymers for highly efficient natural-light-driven photocatalytic disinfection

Xiaohuan Li[a], Xin Wang[a], Haiyong Guo*[b], Enwei Zhu*[c], Chunbo Liu*[a,c]

a Jilin Joint Technology Innovation Laboratory of Developing and Utilizing Materials of Reducing Pollution and Carbon Emissions, College of Engineering, Jilin Normal University, Siping, Jilin 136000, P. R. China.

b Department of Biological Science, School of Life Science, Jilin Normal University, Siping, Jilin 136000, P. R. China.

c Key Laboratory of Preparation and Application of Environmental Friendly Materials, Ministry of Education, Jilin Normal University, Changchun, Jilin 130103, P. R. China.

Chunbo Liu

E-mail: chunboliu@jlnu.edu.cn

College of Engineering

Jilin Normal University

Haifeng Road. #1301

Jilin, Siping

136000, China

Tel/Fax: +86-434-3290623

Materials

2, 7-dibromopyrene, (P-2Br) was purchased from Henan Wittig Chemical Technology Co., Ltd. (Henan, China). 2, 7-dibromopyrene-4,5,9, 10-tetrone (PTO-2Br) was purchased from Henan Weitixi Chemical Technology Co., Ltd. (Henan, China) [(E)-1, 2-vinyldiethyl-5, 2-thidiyl] bis (trimethylstannane)(TVT-2Sn) was purchased from Nanjing Zhiyan Technology Co., Ltd., PVA was acquired from Shanghai McLean Biochemical Technology Co., Ltd. Tetris (triphenylphosphine) palladium($Pd(PPh_3)_4$) was purchased from Anhui Zesheng Technology Co., Ltd. Nitrogen gas (N_2) was purchased from Siping Hongyuan Gas Co., Ltd. (Siping, China). Phosphate buffered saline (PBS) (pH = 7.2) was supplied by Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). Tryptic soy agar (TSA) and tryptic soy broth (TSB) were supplied by Qingdao Hope Bio-Technology Co., Ltd. (Qingdao, China). Isopropanol was purchased from Tianjin Fuyu Co., Ltd. (Tianjin, China). Propidium iodide (PI) and SYTO-9 were supplied by Invitrogen (USA). Ethylenediamine tetraacetic acid disodium salt (EDTA-2Na) was supplied by Changchun Tianjia Biological Techonogy Co., Ltd. (Changchun, China).

Methanol, tetrahydrofuran (THF), acetic acid, toluene, hexane, $K_2Cr_2O_7$, NaN_3 and L-Ascorbic acid were supplied by Sinopharm Chemical Reagent Beijing Co., Ltd. (Beijing, China). NIH-3T3 cells were purchased from Huake Cell Biotechnology (Beijing, China). 4-hydroxy-2,2,6,6-tetramethyl-1-piperidine (TEPM) were obtained from Sigma Aldrich. 5,5-dimethyl-1-pyrroline N-oxide (DMPO) and 3,4-Dihydro-2-methyl-1,1-dimethylethyl ester-2H-pyrrole-2-carboxylic acid-1-oxide (BMPO) were obtained from Dojindo (Japan). Cell Counting Kit-8 (CCK-8), fetal bovine serum (FBS) and Dulbecco′s modified eagle medium (DEME) were supplied by HyClone (USA) and Peak Serum (USA), respectively. The water used in all experiments was deionized. MRSA, Staphylococcus epidermidis and E. coli were provided by Microbiology Lab, School of Life Sciences, Jilin Normal University. The freeze-dried rabbit plasma was purchased from Qingdao high-tech Industrial Park Haibo Biotechnology Co., LTD. NIH-3T3 was purchased from Shanghai Qida Biotechnology Co., LTD.

Characterization of materials

The photocatalytic antibacterial experiment was carried out by using Perfectlight photochemical reaction system (PCX-50C Multi-channel, 420-700 nm). The Fourier transform infrared (FT-IR) spectra of samples were recorded using an FT-IR spectrometer (IRAffinity-1, Shimadzu, Japan), high-resolution TEM (HR-TEM, FEI Tecnai G2 F20, Japan), and scanning electron microscopy (SEM, JSM-7800F, Japan). A UV-vis-NIR spectrophotometer (UV-2550, Shimadzu, Japan) was used to record the UV-visible diffuse reflectance spectra (DRS) of samples. Photoluminescence (PL) (time-resolved) spectra were recorded using a continuous 365 W Xe lamp/Xe flash tube-equipped spectrofluorometer (Horiba Jobin Yvon Fluorolog-3). The electron spin resonance (ESR) was conducted using a spectrometer (Bruker EMX-Plus, Germany). Electrochemical cyclic voltammetry (CV), photocurrent, electrochemical impedance spectroscopy (EIS) and Mott–Schottky were carried out on an electrochemical workstation (CHI760D Chenhua, Shanghai).

Photocatalytic experiments' details

Using Gram-positive Methicillin-resistant *Staphylococcus aureus* (MRSA) as inactivated bacteria, the photocatalytic disinfection effect of P-TVT and PTO-TVT with different mass ratios was evaluated. All medium solutions (TSA, TSB, and PBS) and glassware were autoclaved at 121 ℃ for 30 min prior to the experiment. MRSA cells were cultured in TSB at 37 ℃ for 12 h and centrifuged to obtain 10^7 colony-forming units per mL (cfu mL^{-1}) in PBS. The photocatalytic disinfection experiment was carried out under LED white light. The temperature is maintained at 30 ℃ and the visible light intensity is fixed at 80 mW cm^{-2}. The circulating water temperature control system is used to control the water temperature and remove the photothermal effect in the reaction process. The concentration of MRSA in antibacterial test was about 10^7 cfu mL^{-1}. At different light intervals, 0.5 mL of the sample to

be tested was extracted, diluted continuously with PBS, and spread on the TSA board. Finally, the relative survival rate and cell density of MRSA incubated at 37 ℃ for 12 h were calculated by counting the number of cells. Dark control (MRSA with PTO-TVT) and light control (MRSA without photocatalyst) were performed. All tests were repeated three times.

Other experiments' details

Rabbit plasma test

The coagulase of MRSA protects the bacteria from phagocytosis and provides immunity and prevention by causing local clotting. After the interaction of PTO-TVT with MRSA bacteria, the survival of the bacteria was studied by detecting the expression of coagulase in MRSA. Therefore, coagulation tests based on freeze-dried rabbit plasma were performed. PTO-TVT (2 mg) was added to the reaction solution containing bacteria (20 mL) for 2.5 h, sampling every 1 h. Finally, the glass bottles containing PTO-TVT and bacterial reaction liquid samples taken at different time periods were incubated at 37 ℃ for 6 h to check the coagulation of rabbit plasma.

Fluorescence microscopy observation of bacteria

Furthermore, fluorescent-based cell live/dead tests were carried out to explore the integrity of bacterial cell membranes. In brief, the bacterial liquids before and after 2 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^{-1} in sterilized deionized water) and 25 μL of PI (6 mg mL^{-1} 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 Tis, Japan).

Protein Leakage

10^7 cfu mL^{-1} MRSA were cultured with PTO-TVT (0.1 mg mL^{-1}) and irradiated with LED white light for 2.5 h. The bacterial solution without PTO-TVT was used as the control group. Centrifuge at 12000 rpm for 2 min at 4 °C. The supernatant was transferred to a 96-well plate and the protein leakage concentration was detected using an enhanced BCA protein detection kit (BL521A 500T, Biosharp, China).

Electrical conductivity test

The PTO-TVT (2 mg) was added to the MRSA bacterial culture medium (20 mL) and reacted with light for 0, 0.5, 1, 1.5, 2, and 2.5 h. Then, the 3 mL reaction solution collected at different times was centrifuged for 2 min at a rotating speed of 8000 rpm, and the supernatant was taken and the conductivity was measured by a conductivity meter.

Active species capture experiment

In order to explore the active species produced in photocatalytic process, isopropanol (10 mmol L^{-1}), $K_2Cr_2O_7$ (2 mmol L^{-1}), L-ascorbic acid (2 mmol L^{-1}), NaN_3 (2 mmol L^{-1}) and EDTA-2Na (0.5 mmol L^{-1}), were employed as the traps of hydroxyl radicals (·OH), electrons (e^-), superoxide ($\cdot O_2^-$), $^1O_2$ and holes (h^+), respectively. Electron spin resonance (ESR) analyses were performed on the Bruker EMX-Plus spectrometer with the concentration of spin traps of DMPO, BMPO and TEMP being 0.22 mmol L^{-1} in deionized water.

Cell Toxicity Assay

The CCK-8 assay was applied to study the cytotoxicity of the PTO-TVT. First, the NIH-3T3 cells were cultured with dulbecco's modified eagle medium (DEME, HyClone) (6 mL) containing 1% penicillin-streptmycin solution (HyClone) and 10% fetal bovine serum (FBS, PEAK) in a humidified atmosphere containing 5% CO_2 at 37 °C. The cell culture medium was refreshed every 3 days. Then, then NIH-3T3 cells (5×10^4 cells mL^{-1}) were cultured in DMEM (100 μL) for 24 h on a 96-well plate so that they adhered to the plate wall, 5 replicates per well. Afterwards, DMEM was discarded and fresh DMED medium containing PTO-TVT with different concentrations of 10 μg mL^{-1}, 30 μg mL-1, 50 μg mL^{-1}, 100 μg mL^{-1}, 200 μg mL^{-1}, 500 μg mL^{-1}, 1000 μg $mL^{-1}$and 800 μg mL-1 was added in each well respectively under LED white light for 2.5 h. After another 12 and 24 h co-cultivation, each well was incubated with 10 μL CCK-8 solution at 37 °C for 2 h, the absorbance at 450 nm was measured using Multimode Plate Reader.

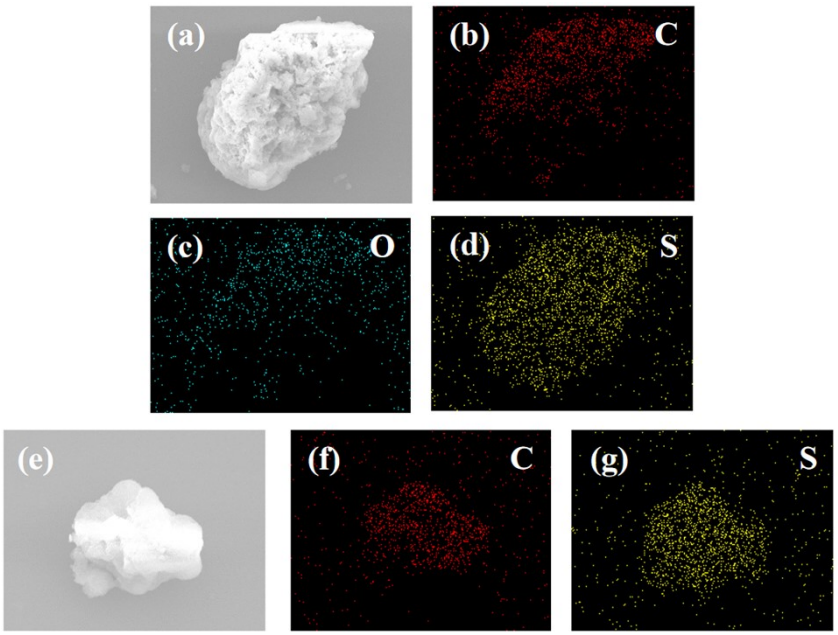

Fig. S1. (a) SEM image and (b-d) element mappings of PTO-TVT, (e) SEM image and (f, g) element mappings of P-TVT.

	element	Weight percentage	Atomic percentage
PTO-TVT	C	65.90	75.09
	S	24.17	20.68
	O	9.93	4.24
P-TVT	C	87.93	95.11
	S	12.07	4.89

Table S1 Elements and their proportion in PTO-TVT and P-TVT

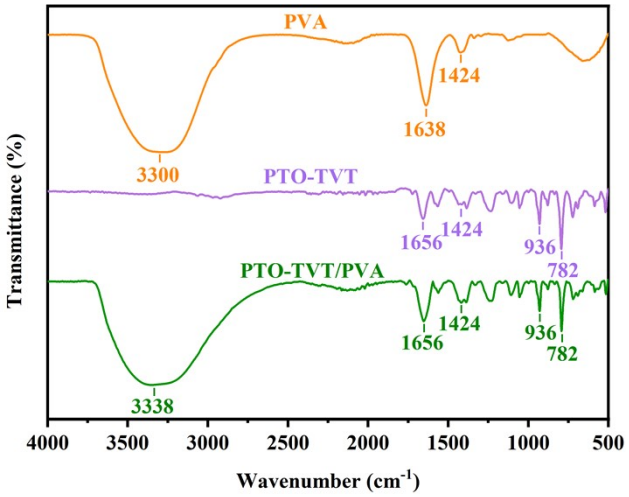

Fig. S2. FT-IR spectra of PTO-TVT/PVA

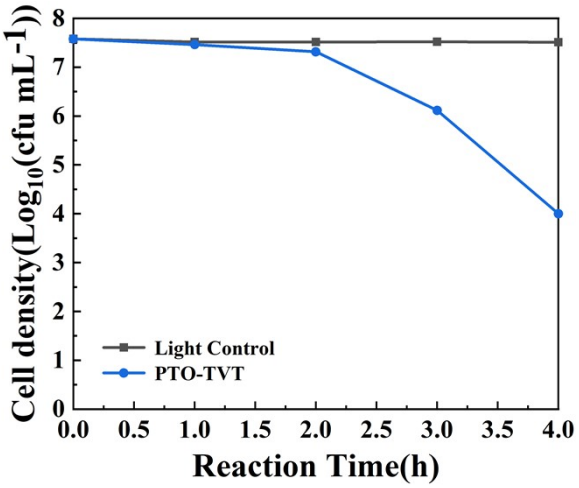

Fig. S3. Photocatalytic antibacterial effect of PTO-TVT (0.1 mg mL^{-1}) on Staphylococcus epidermidis (~10^7 cfu mL^{-1}) under LED white light.

Fig. S4. Photocatalytic antibacterial effect of PTO-TVT (0.1 mg mL^{-1}) on E.coli (~10^7 cfu mL^{-1}) under LED white light

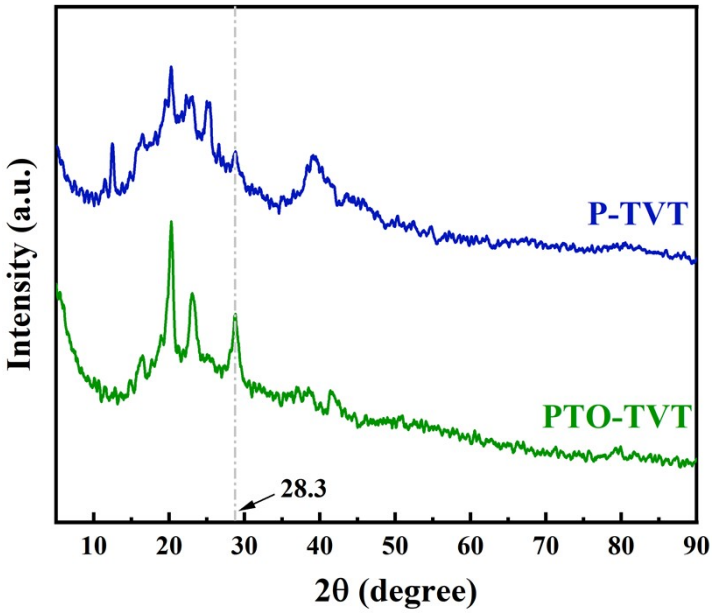

Fig. S5. The XRD of P-TVT and PTO-TVT

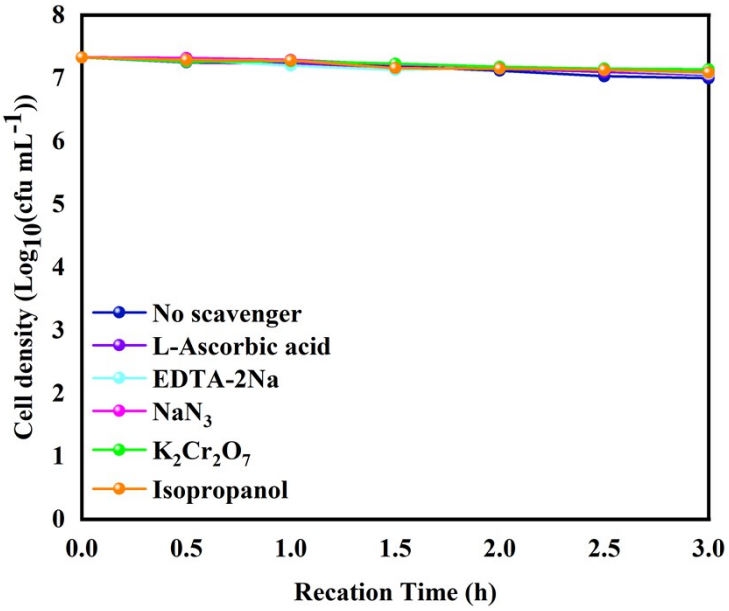

Fig. S6. The effects of different trapping agents on MRSA inactivation under visible light irradiation without photocatalyst