

## Supplementary Materials

# Construction of AIE Molecule-Embedded Long-Persistent Luminescence Coating for Self-Activated Photodynamic Antibacterial Performance

Weizhe Li<sup>1</sup>, Li Xiang<sup>1</sup>, Sathishkumar Gnanasekar<sup>1,\*</sup>, Xiaodong He<sup>1</sup>, Yunjie Xiang<sup>1</sup>, Kun Xu<sup>1</sup>, Xi Rao<sup>1</sup>, En-Tang Kang<sup>2,\*</sup> and Liqun Xu<sup>1,\*</sup>

<sup>1</sup> BRICS Joint Laboratory on Biomedical Materials, School of Materials and Energy, Southwest University, Chongqing 400715, China

<sup>2</sup> Department of Chemical and Biomolecular Engineering, National University of Singapore, Kent Ridge, Singapore 117576, Singapore

\* Correspondence: sathegene@gmail.com (S.G.); cheket@nus.edu.sg (E.-T.K.); xulq@swu.edu.cn (L.X.)

**How To Cite:** Li, W.; Xiang, L.; Gnanasekar, S.; et al. Construction of AIE Molecule-Embedded Long-Persistent Luminescence Coating for Self-Activated Photodynamic Antibacterial Performance. *Advanced Antibacterial Materials* **2025**, 1(1), 37–48. <https://doi.org/10.53941/aam.2026.100004>

**Characterization:** FT-IR spectroscopic measurements were recorded on a PerkinElmer Spectrum Two™ FT-IR Spectrometer (PerkinElmer, Inc., Waltham, MA, USA). UV-visible absorption spectra were acquired using a Shimadzu UV-2550 spectrophotometer (Shimadzu Corporation, Kyoto, Japan), operating at a resolution of 1 nm. Hydrodynamic size and Zeta potential were determined utilizing a Nano ZS90 Zetasizer (Malvern Panalytical Ltd., Malvern, UK). The static water contact angle values were measured with a contact angle measuring device (SDC-350, SINDIN, Dongguan, China) using 3 µL of DI water. Surface compositions of the substrates before and after functionalization were analyzed by X-ray photoelectron spectroscopy (XPS) using a Thermo Scientific ESCALAB 250Xi photoelectron spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). The generation of ROS was assessed using electron spin resonance (ESR) spectroscopy on a Bruker EMXplus Nano instrument (Bruker BioSpin GmbH, Rheinstetten, Germany). Bacterial morphology and cross-sectional views of the coatings were observed using field-emission scanning electron microscopy (FE-SEM, JSM-7800F, JEOL Ltd., Tokyo, Japan). Live/dead bacteria were visualized via confocal laser scanning microscopy (CLSM) with a Carl Zeiss LSM 800 system (Carl Zeiss AG, Jena, Germany).

**Measurement of ROS generation:** 9,10-Anthracenediyl-bis(methylene)dimalonic acid (ABDA) and methylene blue (MB) were used as indicators of ROS. ABDA was dissolved in dimethyl sulfoxide and diluted with DI water to achieve a concentration of 1 mM, whereas MB was dissolved in DI water with a concentration of 1 µg/mL. PDMS-S@PA-CT was immersed in 3 mL of ABDA (or MB) solution. The absorption of the ABDA (or MB) solution was then monitored under UV light at 365 nm.

**Identification of ROS types:** 5,5-Dimethyl-1-pyrroline N-oxide (DMPO) was dissolved in DI water with a concentration of 2 mg/mL. All substrates PDMS-S, PDMS-S@PA-CMCS, and PDMS-S@PA-CT were immersed in 500 µL of the DMPO aqueous solution, and then charged with 365 nm UV light to detect the characteristic signal of ·OH generation. Similarly, 2,2,6,6-tetramethyl-4-piperidinol (TEMP) dissolved in DI water at a concentration of 20 mM was treated with PDMS-S, PDMS-S@PA-CMCS, and PDMS-S@PA-CT substrates under 365 nm UV light, resulting in the determination of <sup>1</sup>O<sub>2</sub> characteristic signal.

**In Vitro Assessment of the PDT Bactericidal Properties of PA-CB Coating:** *E. coli* (CMCC 44102) and *S. aureus* (CMCC 26003) were diluted to 1 × 10<sup>7</sup> cells/mL in phosphate-buffered saline (PBS), and the PDMS-S and PDMS-S@PA-CT substrates were co-cultured with 1 mL of bacterial suspension in 24-well plates for 4 h. Then, the wells containing the bacterial suspension and PDMS-S@PA-CT were exposed to 365 nm UV light, and the bacterial suspension was discarded. The substrates were washed with PBS thrice and sonicated in 3 mL of PBS for 6 min. The number of adhered bacteria on the PDMS-S and PDMS-S@PA-CT substrates was calculated using the spread plate method. The antibacterial rate was calculated using the following formula: antibacterial rate (%) = (CFU<sub>control</sub> – CFU<sub>experimental group</sub>)/CFU<sub>control</sub> × 100%.



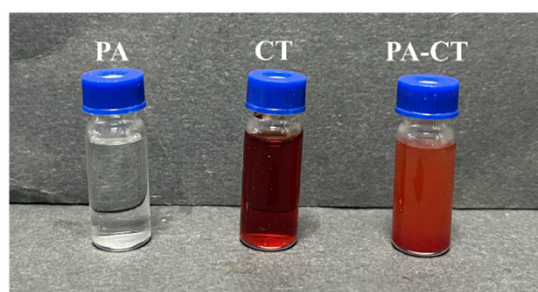
**ONPG hydrolysis assays:** In the ONPG hydrolysis assay, *S. aureus* and *E. coli* ( $10^7$  cells/mL) were cultured with the PDMS-S and PDMS-S@PA-CT substrates in a 24-well plate for 5 h. The white light (L+) group was charged with 365 nm UV light. Five hundred  $\mu$ L of ONPG agent (ONPG assay Kit) was added to each well, and the plate was incubated at 37 °C for another 12 h. The OD value of ONPG agent was measured at 420 nm.

**Bicinchoninic Acid (BCA) protein Assay:** For the BCA protein assay, bacterial suspensions (1 mL,  $1 \times 10^7$  cells/mL) were co-incubated with the PDMS-S and PDMS-S@PA-CT substrates at 37 °C for 4 h with/without 365 nm UV light. After that, 1 mL of BCA working solution was added to each well, and the plate was placed at 60 °C for 30 min. The absorbance of the purple solution was measured at 570 nm using a spectrophotometric microplate reader.

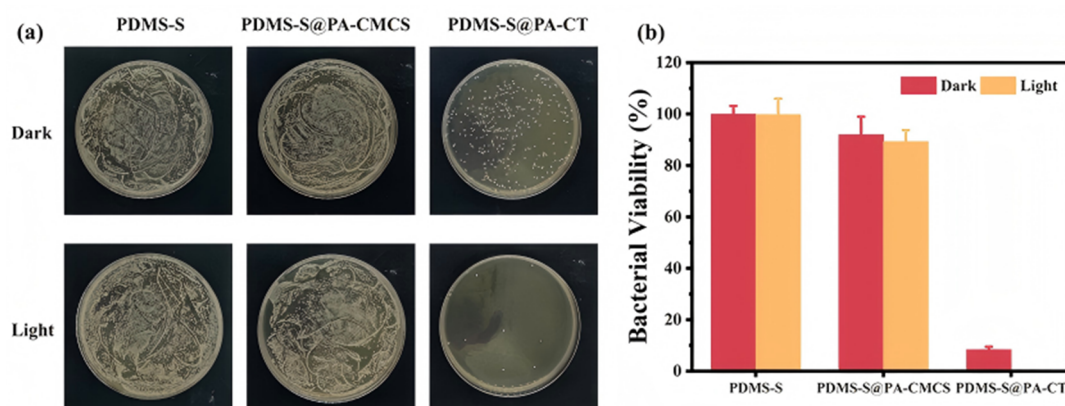
**Cell Counting Kit-8 (CCK-8) assay:** CCK-8 analysis was used to assess the cytotoxicity of the PA-CB coating on L929 cells. L929 cells were co-cultured with pristine PDMS-S and PDMS-S@PA-CT substrates for 48 h. Ten  $\mu$ L of CCK-8 solution was added to each well for 2 h. The OD values of three parallel samples at 450 nm were measured, and the average value was calculated.

**In Vivo Antibacterial Performance:** An in vivo antibacterial assay was conducted using a subcutaneous infection model in Sprague-Dawley (SD) rats. All animal procedures were approved by the Animal Use and Care Committee of Southwest University (UCUCA) (Ethical Approval Number: IACUC-20220607-03). Rats were anesthetized by intraperitoneal injection of 10% chloral hydrate. Once fully anesthetized, the dorsal hair was removed using depilatory cream. The sample substrates (PDMS-S, PDMS-S@PA-CMCS, and PDMS-S@PA-CT) were irradiated under 365 nm UV light for 15 min prior to implantation. A small incision was made on the dorsal skin of each rat, and the sterilized samples were implanted subcutaneously. Subsequently, 20  $\mu$ L of bacterial suspension ( $1 \times 10^7$  CFU/mL) was inoculated directly onto the surface of each implanted sample. The incisions were then sutured using sterile surgical sutures. After 7 days of normal feeding, the rats were euthanized, and the implanted samples were retrieved. The remaining bacteria on the sample surfaces were evaluated by inoculating them on TSB agar plates, following the same procedure as the in vitro antibacterial tests. Additionally, the skin tissues surrounding the implantation sites were collected, fixed, and stained with hematoxylin and eosin (H&E) for histopathological examination.

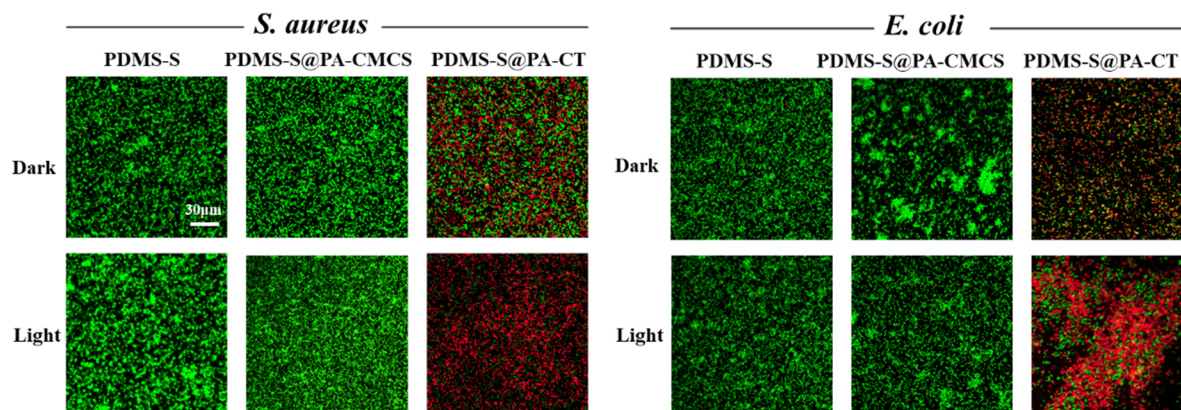
**Statistical analysis:** Statistical data are presented as mean values  $\pm$  SD. Statistical analysis was performed using a one-sample t-test with Origin Pro 9.1 software. All statistical data were collected from experiments with three replicates ( $n = 3$ ). The alpha value is 0.05, the  $p$  value \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , and n.s. indicates no significance.



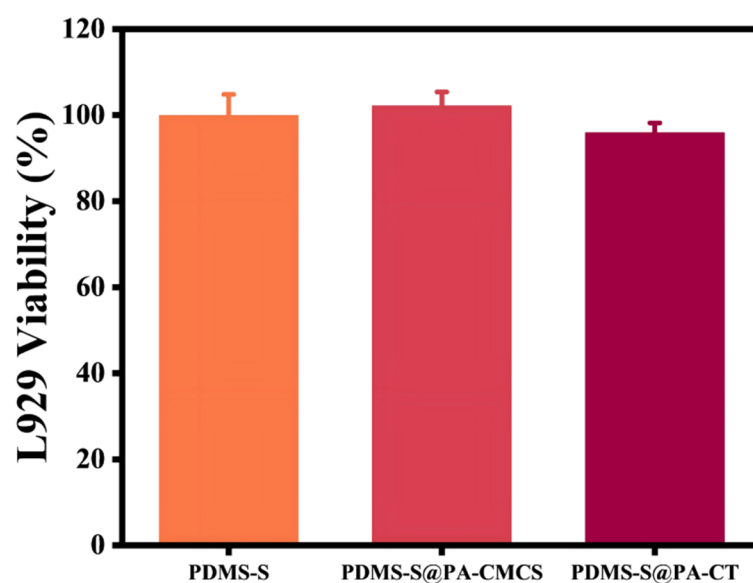
**Figure S1.** Digital photographs of aqueous PA, CT, and their mixture (PA-CT).



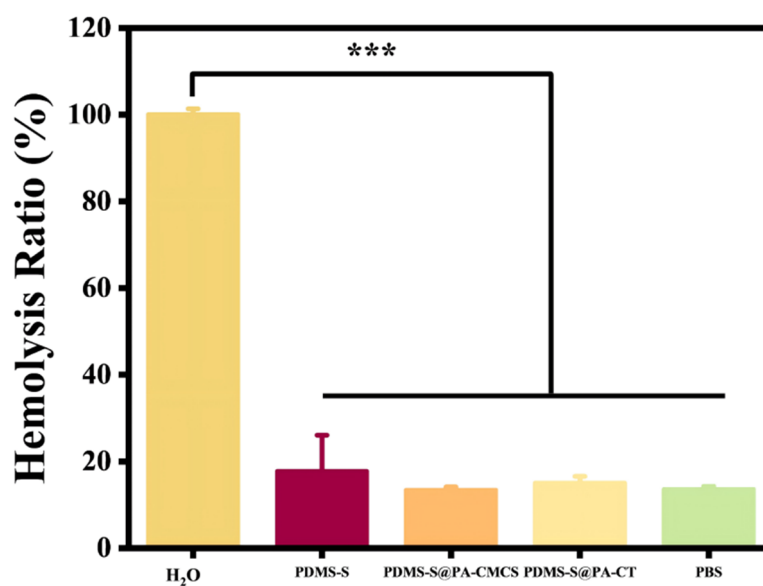
**Figure S2.** Antibacterial activity of PDMS-S@PA-CT against MRSA. (a) Photographs of TSB-agar plates inoculated with MRSA detached from pristine and modified PDMS-S surfaces; (b) Quantification of viable MRSA cells on different substrates.



**Figure S3.** *S. aureus* and *E. coli* biofilms inhibition on pristine and modified PDMS-S substrates observed by CLSM.



**Figure S4.** Cell viability of L929 fibroblasts cultured on pristine PDMS-S, PDMS-S@PA-CMCS, and PDMS-S@PA-CT substrates for 48 h.



**Figure S5.** Hemolysis assay of pristine PDMS-S, PDMS-S@PA-CMCS, and PDMS-S@PA-CT substrates after incubation with RBCs for 2 h. \*\*\*  $p < 0.001$ .