Supporting Information

Tunable phase-change materials @ Prussian blue microparticles for tri-modality biofilm removal based on gas-shearing microfluidics

Qingli Qu[a,c,d,1], Xiaoli Zhang[a,1], Jules Muhire[c,d], Anquan Yang[e], Min Xie[e], Ranhua Xiong[a], Weixia Cheng[b*], Dong Pei[c,d*] and Chaobo Huang[a*]

[a]Joint Laboratory of Advanced Biomedical Materials (NFU-UGent), Jiangsu Co-Innovation Center of Efficient Processing and Utilization of Forest Resources, College of Chemical Engineering, Nanjing Forestry University (NFU), Nanjing 210037, China

[b]Children's Hospital of Nanjing Medical University, Nanjing 210008, China

[c]CAS Key Laboratory of Chemistry of Northwestern Plant Resources and Key Laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou 730000, China

[d]University of Chinese Academy of Sciences, Beijing 100049, PR China

[e]Zhejiang OSM Group Co., Ltd, Huzhou 313000, China

*Corresponding authors. E-mail: Chaobo.Huang@njfu.edu.cn (C. Huang); dongpei@licp.cas.cn (D. Pei); weixiacheng@126.com (W. Cheng).

[1]These authors contributed equally to this work.

S1. Materials and methods

S1.1. Materials

Chitosan and olive oil were purchased from Energy Chemical (Shanghai, China). $FeCl_3$ and $K_4Fe(CN)_6$ were purchased from Macklin (Shanghai, China). Alginate was purchased from Aladdin (Shanghai, China). Lauric acid, stearic acid, Dulbecco's Modified Eagle's medium (DMEM), trypsin and phosphate-buffered saline (PBS) were acquired from Adamas-beta® (Shanghai, China). Rifaximin and Coomassie brilliant blue G250 were purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). Methylene blue and 1,3-Diphenylisobenzofuran were purchased from Leyan.com (Shanghai Haohong Scientific Co., Ltd). Fetal bovine serum was acquired from Sijiqing (Hangzhou, China). Mouse fibroblast (L929) cells were provided by BioCambridge (Nanjing, China). *Escherichia coli* (*E. coli*, ATCC 25922) and *Staphylococcus aureus* (*S. aureus*, ATCC 25923) were provided by Fuxiang Biotechnology Co., Ltd. (Shanghai, China).

S1.2. Fabrication of PCM@PB MPs

PCM@PB MPs were prepared by gas-shearing microfluidics. 1.5% alginate solution with 0.01 mol/L $K_4Fe(CN)_6$ was as outer phase. 3% lauric acid / stearic acid (8:2, w/w) with 6% w/v Rifaximin solution (chloroform / anhydrous ethanol (2:8, v/v) as solvent) dissolved in olive oil as the inner phase. The outer phase and inner phase were pumped into the tri-coaxial needle by a micro syringe pump and cut into droplets by the shearing force generated by gas flow. Unless otherwise specified, the gas flow rate required for the preparation of MPs in this study was 4 L min^{-1}; the liquid flow rate of the outer phase was 1 mL h^{-1}; the liquid flow rate of the inner phase was 0.5 mL h^{-1}. 2%$FeCl_3$, 2%$CaCl_2$ and 3%CS (8:1:1, v/v) mixing aqueous solution was used as the collection bath to harvest the PCM@PB MPs. Bright-field images of microparticles

were collected by microscope (OLYMPUS IX53).

S1.3. The photothermal effect of PCM@PB MPs

About 200 MPs were dispersed in 1 mL PBS (pH 7.4) and irradiated with 808 nm laser (0, 0.6, 0.8, 1.0, 1.2 W cm^{-2}), and removed the laser when the temperature no longer rose in this test. During this NIR laser irradiation, the temperature was recorded at specified time intervals; images were acquired with an 875-1i infrared camera (Testo, Germany). As a control sample, PBS (pH 7.4) was irradiated with the NIR laser.

S1.4. ROS generated by PCM@PB MPs

The generation of ROS was detected by UV-vis spectrophotometer in the presence of methylene blue as the sensor. In brief, about 200 MPs (all MPs were prepared according to S1.2. The liquid flow rate of the outer phase during MPs preparation was all 1 mL h^{-1}. The MPs prepared using a liquid flow rate of inner phase of 0.5 mL h^{-1} are denoted as 0.5 PCM@PB MPs; prepared using a liquid flow rate of inner phase of 0.3 mL h^{-1} are denoted as 0.3 PCM@PB MPs; prepared using a liquid flow rate of inner phase of 0.1 mL h^{-1} are denoted as 0.1 PCM@PB MPs) were mixed with H_2O_2 (3% v/v, 200 μL) and methylene blue (0.5% w/v, 3 mL) under stirring. The absorbance of methylene blue solution at 630 nm was measured using a UV-vis spectrophotometer at predetermined time intervals. ROS generation was then assessed by monitoring changes in the light absorbance of 1,3-diphenyl isobenzofuran (DPBF) in the same manner. A solution containing 200 MPs, 200 μL of H_2O_2, and 3 mL of DPBF (in absolute ethanol, 25 μg/mL) was prepared, and its absorbance at 410 nm was monitored over time.

S1.5. NIR-responsive drug release of PCM@PB MPs

Approximately 200 PCM@PB MPs (encapsulated with 6% Rifaximin) were prepared, and the MPs were washed with excess water. Subsequently, the particles were immersed in a 3 mL release medium (PBS, pH 7.4), followed by sustained irradiation

with the 808 nm NIR laser (0.6, 0.8 or 1.2 W cm^{-2}). Then, the absorbance at 441 nm of the release medium was recorded using a UV-vis spectrophotometer as a time function.

S1.6. Antibacterial experiments of PCM@PB MPs

E. coli and *S. aureus* were incubated in LB broth in a thermostatic oscillator (SHA-B; Lichen, Changzhou, China) overnight (37 °C, 30 rpm). There were 6 groups in this test. ①Negative control: 2 mL untreated bacterial solution; experimental group: ②PTT: 2 mL bacterial solution treated with 200 PCM@PB MPs (without Rifaximin) under 808 nm irradiation for 5 min; ③CDT: 2 mL bacterial solution treated with 200 PCM@PB MPs (without Rifaximin) and 100 μL 3% H_2O_2; ④CT: 2 mL bacterial solution treated with 200 alginate microparticles with 6% w/v Rifaximin; ⑤Rif: 2 mL bacterial solution containing 6% w/v Rifaximin; ⑥PTT/CDT/CT: 2 mL bacterial solution treated with 200 PCM@PB MPs (with 6% w/v Rifaximin) and 100 μL 3% H_2O_2 under 808 nm irradiation for 5 min.

In the proliferation assay, various groups were cultured with a medium, after which 2 mL of bacterial solution (treated differently) was extracted and its absorbance at 600 nm was measured using a BIO-TEK EL800 microplate reader at specified time points. For CFU testing, bacteria in the logarithmic growth phase, following different treatments, were plated on LB dishes. After 4 hours of incubation at 37 °C, colonies were photographed for documentation. To measure electrical conductivity, nucleic acid, and protein levels, tested strains were cultured in 100 mL liquid medium overnight at 37 °C with shaking, and centrifuged at 5000 rpm for 15 minutes after reaching the logarithmic phase. After collecting the bacteria and washing them three times with PBS, 10 mL of PBS was added. The sample group (200 PCM@PB MPs and 20 μL of 3% H_2O_2) and the control group were added to the bacterial solution, with the sample group

irradiated with NIR light (808 nm, 1.2 W/cm²). To assess conductivity, 5 mL of the solution was centrifuged at 10,000 rpm for 3 minutes after 3 hours, and conductivity was measured using a DDS-307 device (China). Nucleic acid content was determined by measuring the supernatant's absorbance at 260 nm, with the control group corrected using PBS. The effect of MPs on bacterial protein synthesis was tested using a Bradford assay, with the absorbance peak recorded at 630 nm using a BIO-TEK EL800 microplate reader.

S1.7. Cell viability of PCM@PB MPs

To make extracts from the MPs, MPs at a number of about 100, 200 and 300 were respectively immersed in 1 mL DMEM at 37 °C for 72 h. L929 cells were seeded in 96-well plates at a cell density of 6000 cells per well for 24 h. Then, cell culture was done using the MPs extracts instead of DMEM in the incubator (Thermo Forma 3111) for another 24 h. Subsequently, 20 μL 5 mg/mL MTT was added to each well, and the cells were incubated at 37°C for 4 h. Replace 150 μL DMSO with the medium for 20 min to dissolve the formazan crystals. The absorbance of each well was measured with a microplate reader at 492 nm.

S1.8. Hemolytic activity test of PCM@PB MPs

Blood was centrifuged at 1000 rpm for 10 minutes to isolate erythrocytes, which were then washed three times with PBS and diluted to create a 10% (v/v) erythrocyte stock. To this, 500 μL PBS with approximately 20 MPs was added to 500 μL of the erythrocyte stock, followed by incubation at 37 °C for 1 hour with shaking. Positive control was 0.1% (v/v) Triton X-100, and PBS served as a negative control. The mixtures were then centrifuged at 8000 rpm for 1 minute, and the absorbance of the supernatant was recorded at 540 nm using a microplate reader.

S2. Figure:

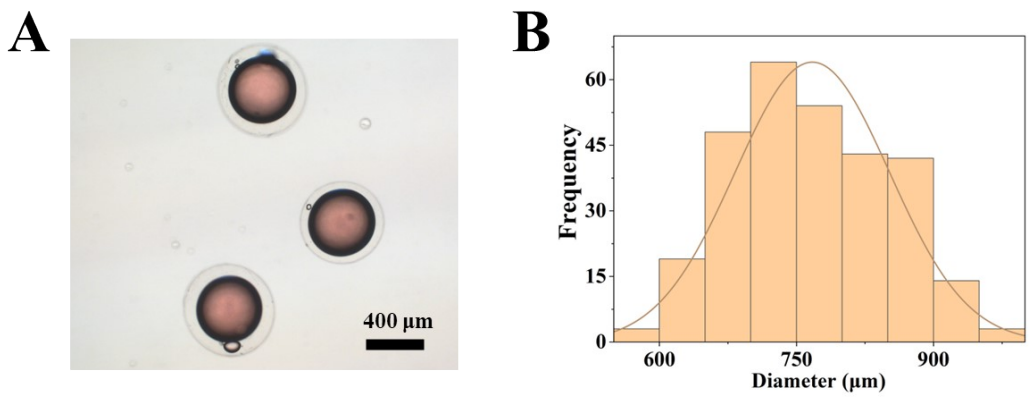

Figure S1 (A) Optical images for core-shell MPs, which are composed of alginate as the shells and oil dyed with Oil Red O as cores. (B) Size distribution for the MPs.

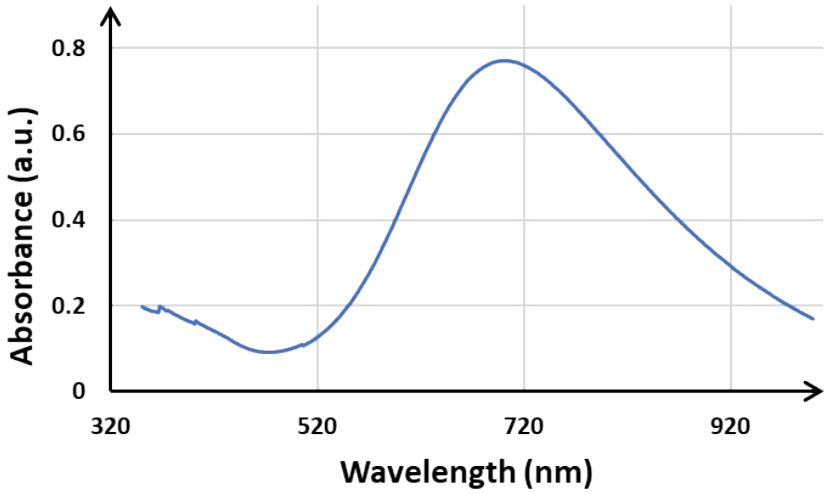

Figure S2 UV-vis absorption spectra of PCM@PB MPs at the range from 350 to 1000 nm.

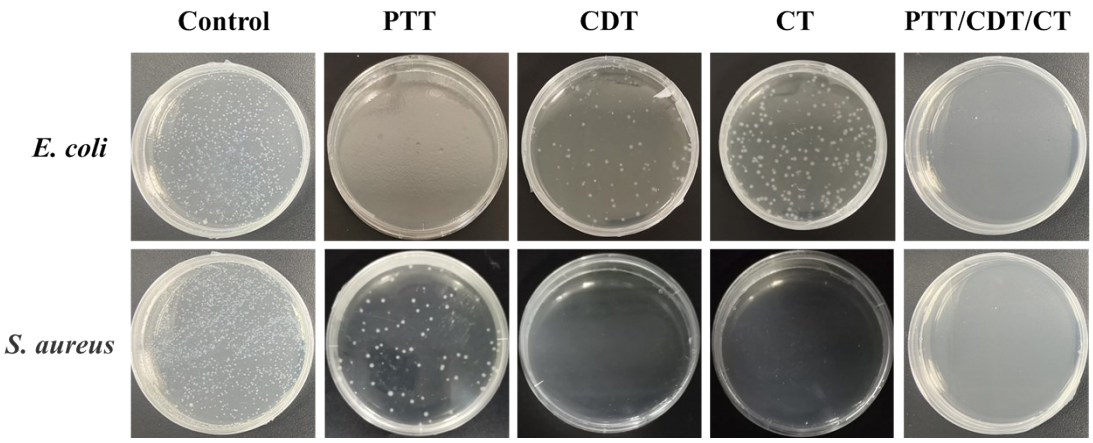

Figure S3 Optical images of antibacterial effect on *E. coli* and *S. aureus* after treatment of PTT (0.3 PCM@PB MPs with NIR only), CDT (0.3 PCM@PB MPs with H_2O_2 only), CT (0.3 PCM@PB MPs encapsulated rifaximin) or PTT/CDT/CT.

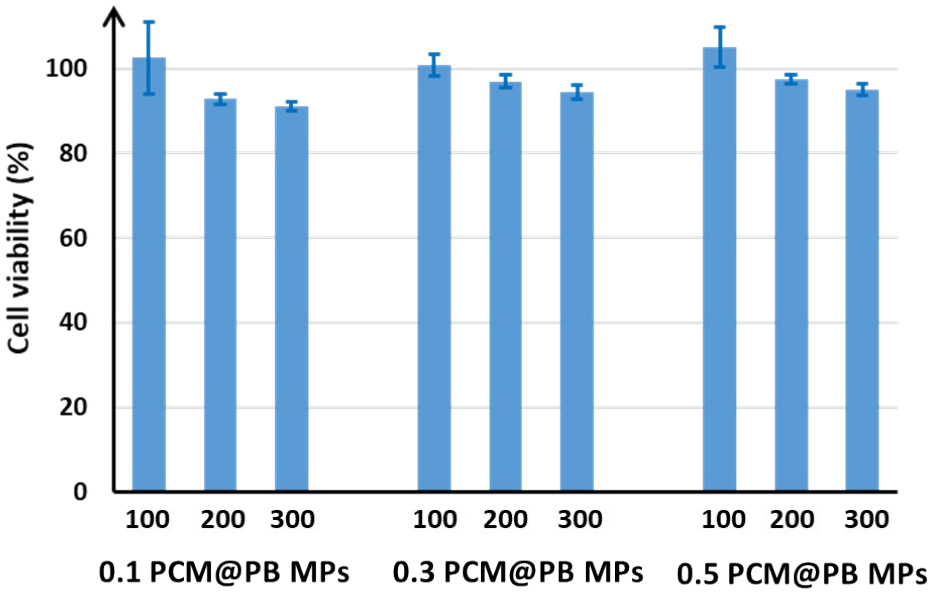

Figure S4 Cell viabilities of L929 cells treated with different concentrations and kinds of MPs. 100, 200, and 300 stand for the number of MPs per 1 mL DMEM.