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Supporting Information

Thermosensitive Curcumin/Silver/Montmorillonite-F127 Hydrogel with Synergistic Photodynamic/Photothermal/Silver Ions Antibacterial Activity

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1. Thermo-sensitivity and injectability studies of CAM-F hydrogels

The thermos-sensitivity and injectability of F127 hydrogel, CAM-F-1, CAM-F-2 and CAM-F-3 hydrogels were recorded by cell phone camera. The hydrogels were placed at 4 , 25, 37 and 55℃, respectively. Their morphology was photographed to verify their temperature sensitivity. The CAM-F-3 hydrogel was then placed into the syringes and squeezed them out of the syringe into the water at 37 ℃.

2. Photothermal performance of hydrogel

To evaluate the photothermal performance of CAM-F hydrogels with different Cur/Ag/Mt-3 content (1, 2, 3 wt%), 2 mL CAM-F hydrogels were placed in the 24-well plate and irradiated with different power densities (0.3, 0.6, 0.9 and 1.2 W/cm^2) 808 lasers for 10 min. The temperature measurement probe was submerged below the hydrogel surface, and the temperature at this point was recorded as the initial temperature. During irradiation, the temperature was measured 30 second with digital thermal probe. The F127 hydrogel was used for control. The photothermal stability of the samples were determine by NIR on–off. During irradiation, the temperature was recorded 30 s with a thermometer.

3. Ag^+ and Curcumin release

The Ag^+ and Cur release experiment was conducted to investigate the stability and antibacterial mechanism of sample. CAM-F-3 hydrogel was placed in the PBS solution (pH 7.4). Then, it was shaken in a constant temperature oscillator with slow stirring at 37 ℃ for 16 h. At each predetermined time point, 5 mL of a solution that contained the released Ag^+ and Cur was taken out. The amount of Ag^+ released into the solution was measured using ICP-OES, to evaluate the stability of the materials. The concentration of the released Cur was determined with UV−vis

spectroscopy at 425 nm.

4. Characterization of Cur/Ag/Mt and CAM-F Hydrogel

X-ray diffraction (XRD) analysis was carried out on a PANalytical X'Pert PRO X-ray diffraction analyzer (PANalytical, Netherlands) using a Cu target Kα (λ=0.1542 nm) operating at 40 kV and 40 mA. The X-ray photoelectron spectroscopy (XPS) data of the sample powder were recorded on a Kratos AXIS Ultra DLD. The standard internal reference used to calibrate the binding energies was the C1s spectrum, which has a 284.8 eV value. Fourier Transform Infrared Spectroscopy (FTIR) was taken with a Nicolet 6700 FTIR spectrometer (Thermo-Nicolet, USA) in transmission mode. For morphology observation, the samples were put on a conductive adhesive, coated with gold, and then scanned using a Hitachi S-4700(II) scanning electron microscope (SEM). Using a Mettler Toledo Star thermos gravimetric analysis (TGA) instrument, the curves of the sample's mass loss which are temperature-dependent were studied. Under N_2 gas flow conditions, the samples were examined from room temperature to 800 ℃ at a heating rate of 10 ℃/min. High-Angle Annular Dark Field Scanning Transmission Electron Microscopy (HAADF-STEM) measurements and EDX analysis were collected on the Talos 200 kV analytical FEG S/TEM from FEI. Inductively coupled plasma optical emission spectroscopy (ICP−OES) (Optima 8000) was used to determine the Ag^+ contents in the solution.

5. Rheological evaluation of CAM-F hydrogel

All the rheological measurements of the nanocomposite hydrogel were performed on Malvern Kinexus Rheometer (Malvern Kinexus Lab+ Rheometer) with stainless steel plate geometry (diameter 25 mm having cone angle 21 and truncation gap 0.105 mm) in rotational and oscillatory modes. The samples were thermally balanced for 1 min prior to the measurement, and the storage modulus (G′) and loss modulus (G″) were determined at an oscillation frequency of 6.283 rad/s and constant strain of 1%. The temperature was varied from 4 to 55°C, and the samples were covered with sealed caps to reduce evaporation during the measurement.

For rotational tests, viscosity was recorded as a function of temperature (temperature ramp) by keeping the shear rate constant at $\gamma=50s^{-1}$ and oscillatory tests in a small oscillatory shear strain

amplitude sweep at a constant angular frequency w= 10 rad· s^{-1} and frequency sweep are performed in the linear viscoelastic region (LVR), keeping the strain amplitude (g = 0.5%) constant.

6. Antibacterial activity evaluation of CAM-F hydrogel

The antibacterial properties of different samples against *E. coli* and *S. aureus* were assessed by the plate coating method. To determine the antibacterial activity of F127 hydrogel, CAM-F-3 hydrogels against *E. coli* and *S. aureus,* 400 μL of precursor solutions of PF127 hydrogel and CAM-F hydrogels were added to the wells of a 48-well plate, and the sterile deionized water was used as control. The hydrogels were first made to form a hydrogel at 37 ℃. Next, 20 μL of bacterial suspension (1×10^8 CFU mL^{-1}) was added to each well. 405nm laser irradiation for 20 min (400 mW/cm^2), NIR for 8 min (1.2 W cm^2), and no irradiation were used, respectively, while the samples were incubated with shaking at 200 rpm at 37 ℃ for 4 h. Then 400 μL of the liquid medium was added to each well of the hydrogel group to resuspend the bacteria. The bacteria obtained after co-incubation of each group were diluted and spread evenly on the solid plate medium. Then they were incubated in a biochemical incubator at 37 ℃ for 12~16 h. The inhibition ratio was determined by calculating the number of colony-forming units (CFUs).

7. Disrupting biofilm research

S. aureus (10^8 CFU mL^{-1}) was added to a 48-well plate. Soon afterward, the well plates were incubated in a 37 ℃ biochemical incubator for 72 h. At the end of incubation, the plate was washed several times with PBS to remove the remaining suspended bacteria. Then 150 μL of F127 hydrogel, CAM-F-1,CAM-F-2 and CAM-F-3 hydrogels were added to the wells and incubated in a 37 ℃ biochemical incubator for 24 h. After incubation, the wells were washed several times with PBS, fixed with 10% formaldehyde, and washed again with PBS. The wells were then fixed with 1% crystalline violet at room temperature for 15 min, aspirated, and washed with PBS. The wells were then fixed with 1% crystalline violet at room temperature for 15 min, aspirated, and washed with PBS. After air-drying, 33% acetic acid was added to dissolve the crystalline violet bound to the bacterial biofilm.

8. SEM image of bacteria

The bacterial solution was treated with or without 405+808 nm laser irradiation. The bacteria were collected (8000 rpm, 1 min), cleaned twice with phosphate buffer saline (PBS), and fixed for 12 h with glutaraldehyde solution (2.5 wt%) at 40 times of the bacteria volume. The fixed bacteria were cleaned with PBS twice, and then dehydrated n ethanol solution with different concentrations (30 %, 50 %, 70 %, 80 % and 90 %) respectively, and finally dehydrated with 100 % ethanol twice. The SEM sample was prepared by taking appropriate amount of thallus on the cover glass, freeze drying and gold spraying treatment.

9. Bacterial imaging processed by PI

The bactericidal effects were evaluated by the live/dead staining assay. The bacteria treated with or without laser irradiation were added with 200 μL of mixed solution containing PI, and incubated in dark environment at 37 ℃ for 15 min. The concentrations of PI were 10 μg/mL. After the probe staining was completed, the bacteria were cleaned twice with PBS (8000 rpm, 1 min), and then 100 μL of PBS suspended bacteria were added. 10 μL of mixed bacterial droplets were placed on the glass slide and observed under an inverted fluorescence microscope (Olymplus BX53).

10. Cytotoxicity test

The *in vitro* cytotoxicity of the CAM-F hydrogel was evaluated by the Cell Counting Kit-8 (CCK-8) using L929 in the logarithmic growth phase. Cell suspension containing 10 % fetal bovine serum (FBS) and DEME nutrient solution was added to each well of 96- well plates and incubated for 24 h in an incubator at 37 ℃ with 5 % CO_2. Afterward, the cells were treated with the F127 hydrogel and CAM-F hydrogels with different concentration for 48 h at 37 ℃ with 5 % CO_2. CCK-8 (5 %, 200 μL) was added to each well and the cell solution was incubated for 3 h. At the end of incubation, the absorbance at a wavelength of 450 nm was measured using a microplate reader. The cells without the samples and bare CAM-F hydrogel were the positive control.

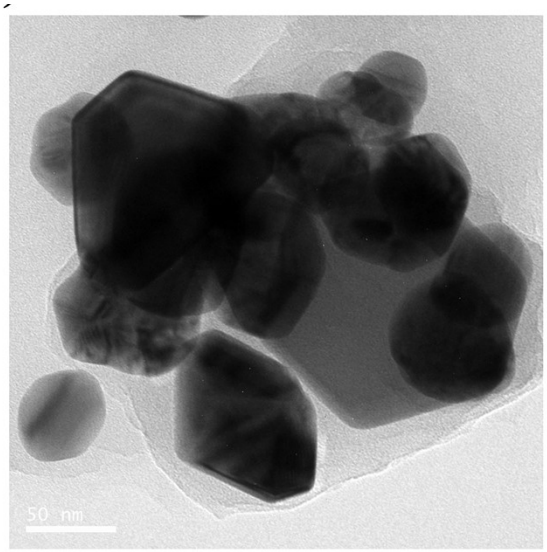

Figure S1. TEM image of Cur/Ag

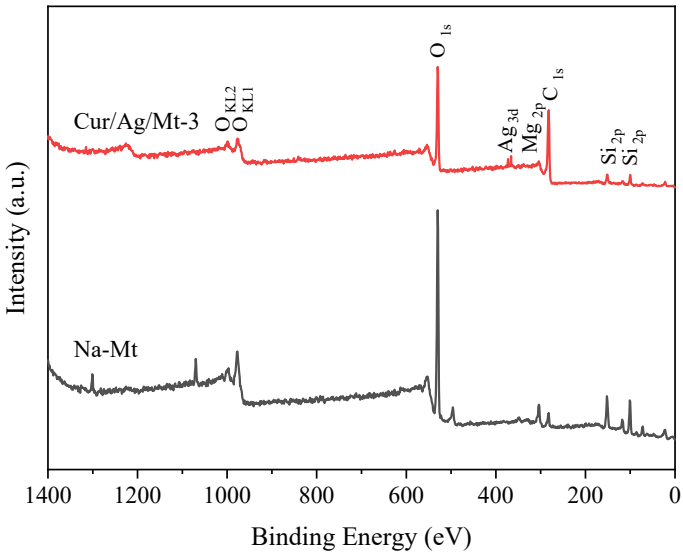

Figure S2. XPS spectra of Na-Mt and Cur/Ag/Mt-3.

Cur/Ag-Mt-3: Curcumin/silver/montmorillonite synthesized with a 2:1 molar ratio of curcumin and silver nitrate

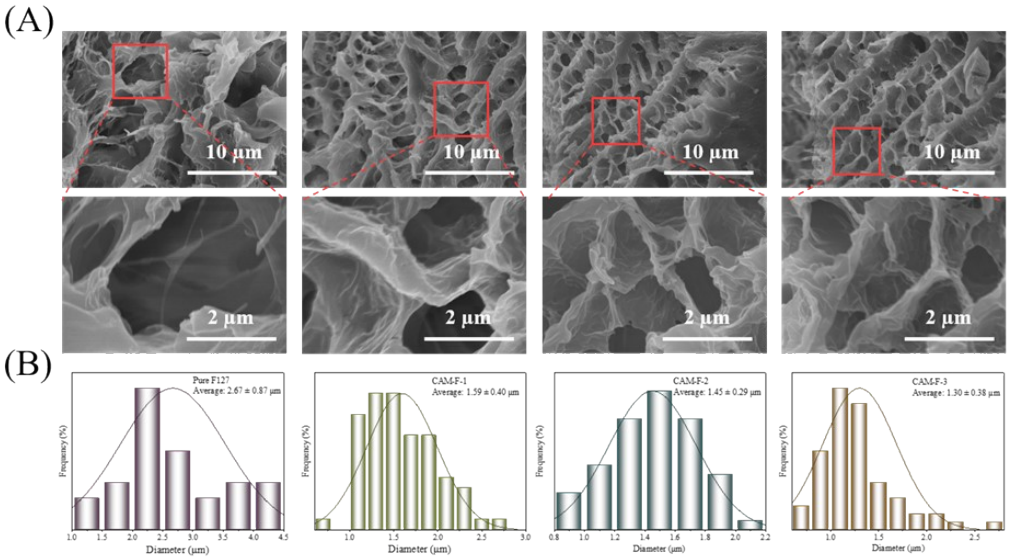

Figure S3. SEM image (A) and pore diameter distributions (B) of F127 hydrogel CAM-F-1, CAM-F-2 and CAM-F-3 hydrogels. CAM-F-1, 2, 3 hydrogel：F127 hydrogels containing curcumin/silver/montmorillonite (1, 2, 3 wt%)

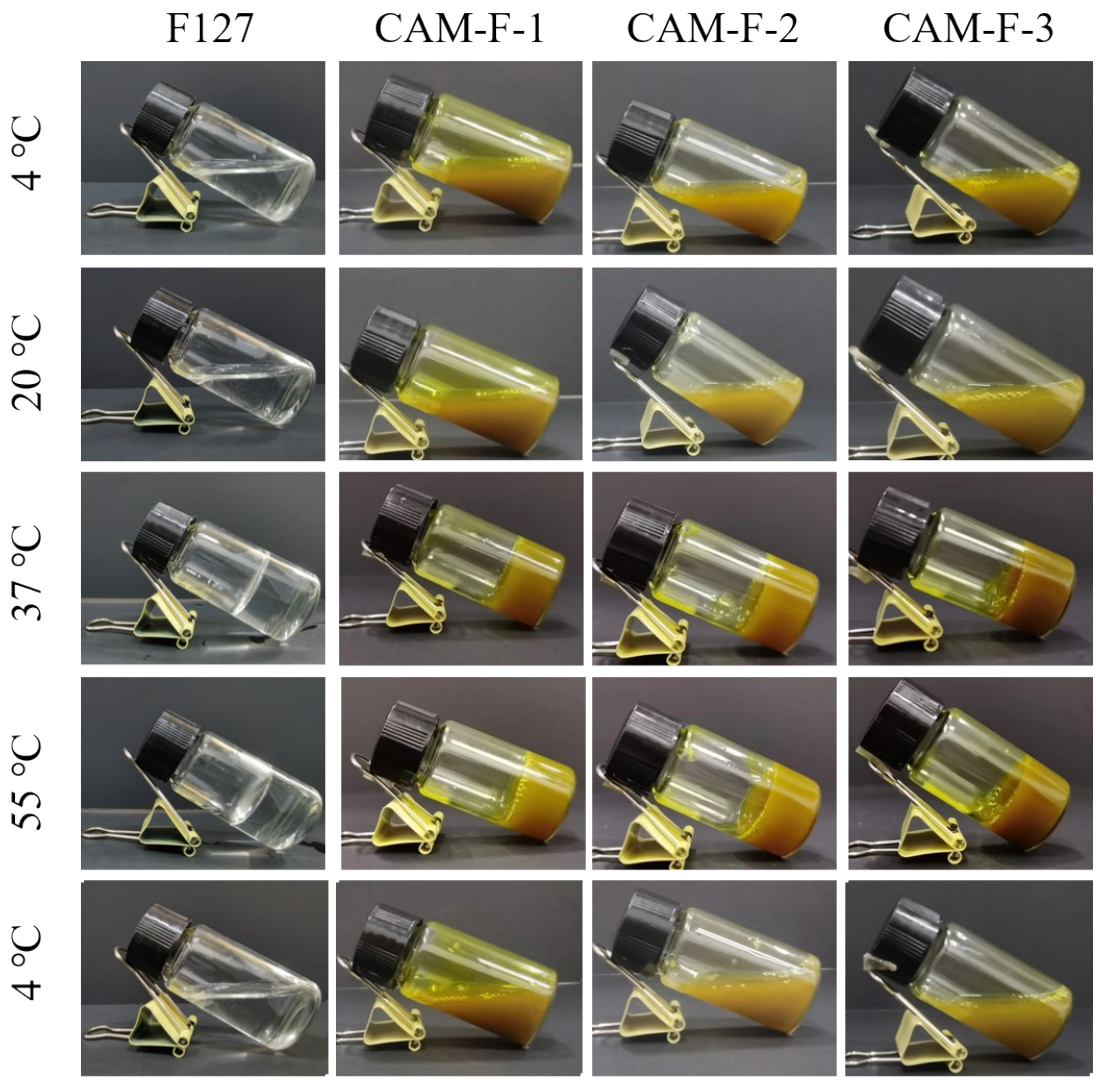

Figure S4. Photograph of the F127 hydrogel and CAM-F-1, CAM-F-2 and CAM-F-3 hydrogel at different temperature. CAM-F-1, 2, 3 hydrogel：F127 hydrogels containing curcumin/silver/montmorillonite (1, 2, 3 wt%)

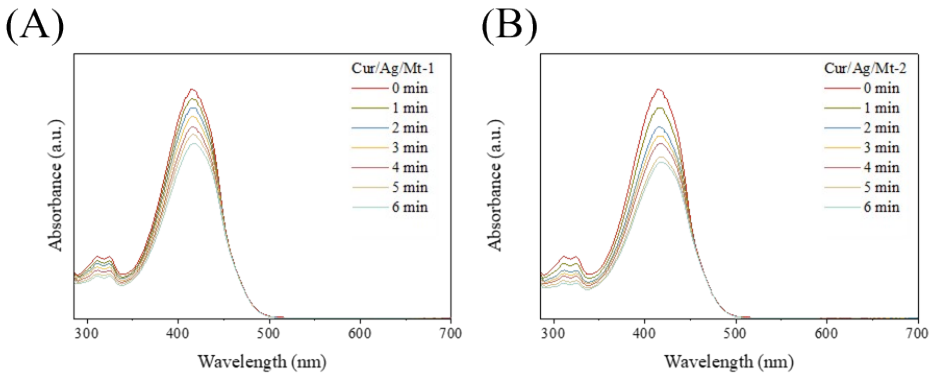

Figure S5. UV-vis spectra of DPBF irradiated by 405 nm laser (400 $mW \cdot cm^{-2}$) in the presence of (A) Cur/Ag/Mt-1 and (B) Cur/Ag/Mt-2. Cur/Ag/Mt-1, 2, 3 correspond to different molar ratios of silver nitrate and curcumin 2:1; 1:1; 1:2 for synthesized curcumin/silver/montmorillonite, respectively

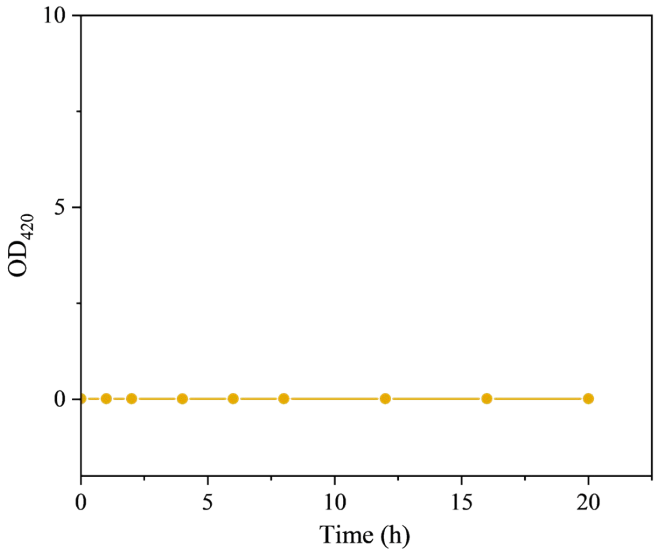

Figure S6. The Cur-release behavior of CAM-F-3 hydrogel at different times. CAM-F-3 hydrogel：F127 hydrogels containing 3 wt% curcumin/silver/montmorillonite

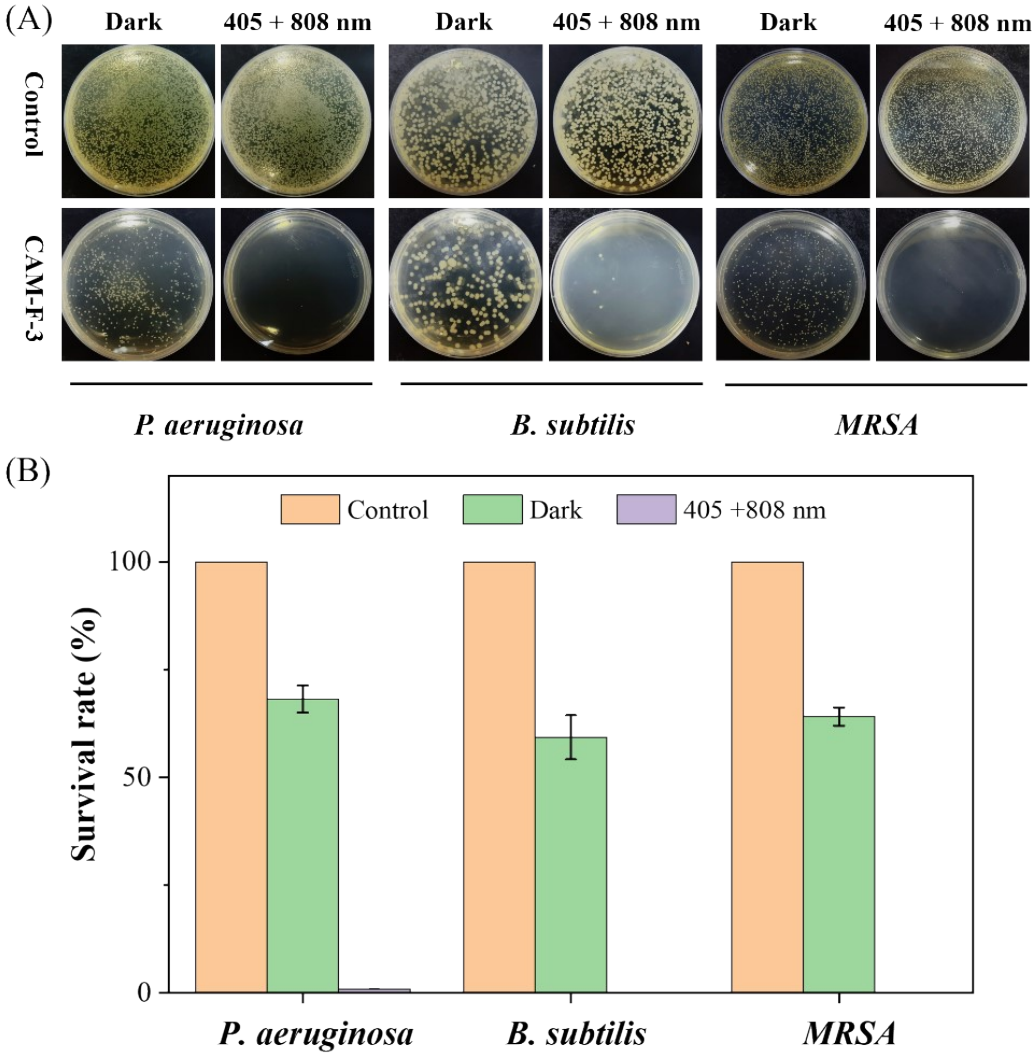

Figure S7. The antibacterial activity of CAM-F-3 hydrogels against *P. aeruginosa*, *B. subtilis* and *MRSA*. (A) Representative agar plate photographs and (B) The corresponding survival rates of *P. aeruginosa*, *B. subtilis* and *MRSA* after dark or under 405 + 808 nm laser irradiation, respectively. The power of 405nm and 808nm laser were 0.4 and 0.9 W/cm^2; *B. subtilis: Bacillus subtilis*; CAM-F-3 hydrogel: F127 hydrogels containing 3 wt% curcumin/silver/montmorillonite; *MRSA: methicillin-resistant Staphylococcus aureus*; *P. aeruginosa: Pseudomonas aeruginosa*

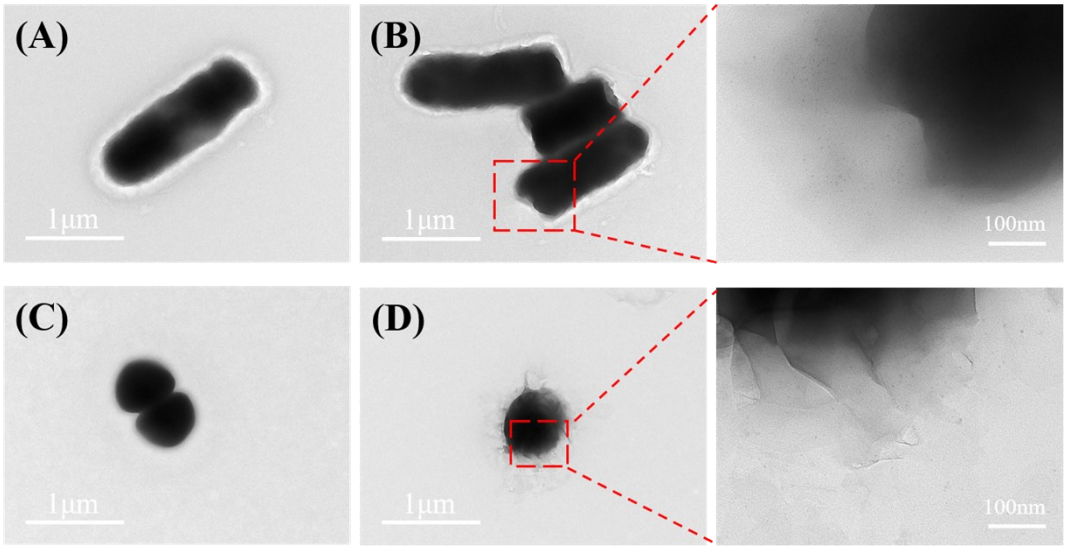

Figure S8. TEM image of (A) *E. coli* and (B) *E.coli* treated by CAM-F-3 hydrogel under 405+808 nm laser irradiation; C) *S. aureus* and (D) *S. aureus* treated by CAM-F-3 hydrogel under 405+808 nm laser irradiation. CAM-F-3 hydrogel：F127 hydrogels containing 3 wt% curcumin/silver/montmorillonite