Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

NIR-responsive MoS_2-Cu_2WS_4 nanosheets for catalytic/photothermal therapy of methicillin-resistant *Staphylococcus aureus* infections

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Experimental Sections

Materials

Cuprous oxide (Cu_2O, 99.9%), ammonium tungsten sulfide ($(NH_4)_2WS_4$, 99.9%), 3-mercaptopropionic acid (MPA, 99%), molybdenum disulfide (MoS_2) powder (< 2 μm, 99%), and terephthalic acid (TA, 98%) were achieved from Sigma-Aldrich (Shanghai, China). Glutathione (reduced, GSH), ammonium hydroxide solution ($NH_3•H_2O$, 28% in H2O), and 3,3',5,5'-tetramethylbenzidine (TMB, 98%) were obtained from Aladdin (Shanghai, China). Luria-Bertani (LB) broth and LB broth with agar were purchased from Beyotime (Shanghai, China). Lactate dehyrogenase (LDH) cytotoxicity colorimetric assay kit was purchased from KeyGen (Nanjing, China).

Characterization

The morphology of samples was studied by using transmission electron microscopy (TEM, Hitachi-HT7700, Japan) and scanning electron microscopy (SEM, Hitachi-S4800, Japan). The crystal structure characterization and elemental mapping were performed on a high-resolution TEM (HRTEM, Talos-F200X, USA) at 200 kV, equipped with an energy dispersive spectrometer (EDS). The X-ray diffraction spectra were recorded on a X-ray powder diffractometer (XRD, Bruker, Germany) with Cu Kα line at 1.5418 Å. The sample thickness was investigated by atomic force microscopy (AFM, Nanoscope IIIa, Bruker, Germany). X-ray photoelectron spectroscopy (XPS) was carried out on PHI5000 VersaProbe spectrometer (Ulvac-Phi, Japan). The optical property was revealed by Ultraviolet-visible-near infrared (UV-vis-NIR) absorption spectrophotometer (UV-3600, Shimadzu, Japan) and microplate spectrophotometer (PowerWave XS2, BioTek, USA). The concentration of samples was determined by using inductively coupled plasma optical emission spectrometer (ICP-OES, Optima 5300DV, Perkin Elmer, USA).

Preparation of MS NSs

The single-layer MS NSs were prepared according to the previous work.[1] After MoS_2 powder (1 g) was added into the reaction tube, n-butyl lithium solution (5 mL, 2.4 M) was added under the protection of Ar. Then, the mixture was sonicated in ultrasonic

cleaner cooled with ice for 3 hours. After standing for 2 h, extra n-butyl lithium was taken out. Then, Ar-saturated ultrapure water (20 mL) was added into the reaction tube. After sonicated for 1 h, the mixture was centrifuged at 12000 rpm for 40 min. The precipitate was dispersed in 20 mL ethanol and centrifuged at 5000 rpm for 40 min. Finally, the as-prepared MS NSs was redispersed in ultrapure water and stored at 4°C. The height of CWS NSs was calculated according to the following equation:

$$\text{height (CWS)} = \frac{\text{height (MS - CWS)} - \text{height (MS)}}{2}$$

Hemolysis Assay of MS-CWS NSs

Animal experiments were carried out based on the Guidelines for the Care and Use of Laboratory Animals of Nanjing Medical University and approved by the Animal Ethics Committee of Nanjing Medical University. To obtain red blood cells (RBC), the blood of mouse was washed with saline for 3 times. RBC saline dispersion (100 μL) was added into MS-CWS NSs aqueous dispersion (1 mL) with different concentrations and incubated for 3 h. Saline and H_2O dispersions of RBC were used as negative and positive control, respectively. These mixtures were centrifuged at 14000 rpm for 10 min. The absorbance of the supernatants was monitored by microplate spectrophotometer at 540 nm.

$$\text{Hemolysis ratio (\%)} = \frac{A_S - A_N}{A_P - A_N} \times 100\%$$

where A_S is the absorbance of the supernatant containing MS-CWS NSs and RBC, A_N is the absorbance of the supernatant containing saline and RBC, and A_P is the absorbance of the supernatant containing ultrapure water and RBC.

Cytotoxicity Assay

Human normal liver (L-O2) cells were seeded into 96-well plates for 24 h at 37°C. Then, MS-CWS NSs dispersions of different concentrations (2, 5, 10, 20, 40, 80 and 160 μg/mL) were added and further incubated for 48 h. Cell viability was determined by LDH assay referring to previous work.[2]

***In Vivo* Toxicity Study**

Saline (200 μL), MS NSs saline dispersion (200 μL, 15 mg/kg), and MS-CWS NSs

saline dispersion (200 μL, 15 mg/kg) were intravenously injected into female Balb/c mice (3 mice for each group, Qinglong Mountain Company), respectively. The mice were sacrificed at 14th day, and their major organs were collected for histological analysis.

SEM Characterization

Glutaraldehyde solution (2.5%, 1 mL) was added to fix bacterial cells for 10 h at 4°C. After that, MRSA were serially dehydrated by ethanol solutions (20, 40, 80, and 100%). MRSA suspensions (50 μL) were dropped on clean silicon wafer, dried at room temperature, and used for SEM imaging.

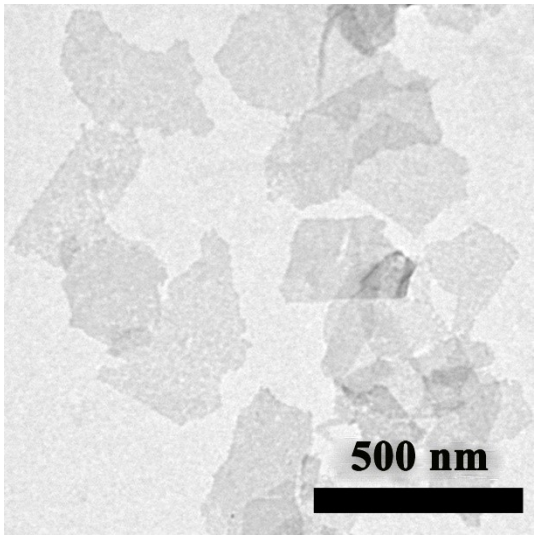

Figure S1 TEM image of MS NSs.

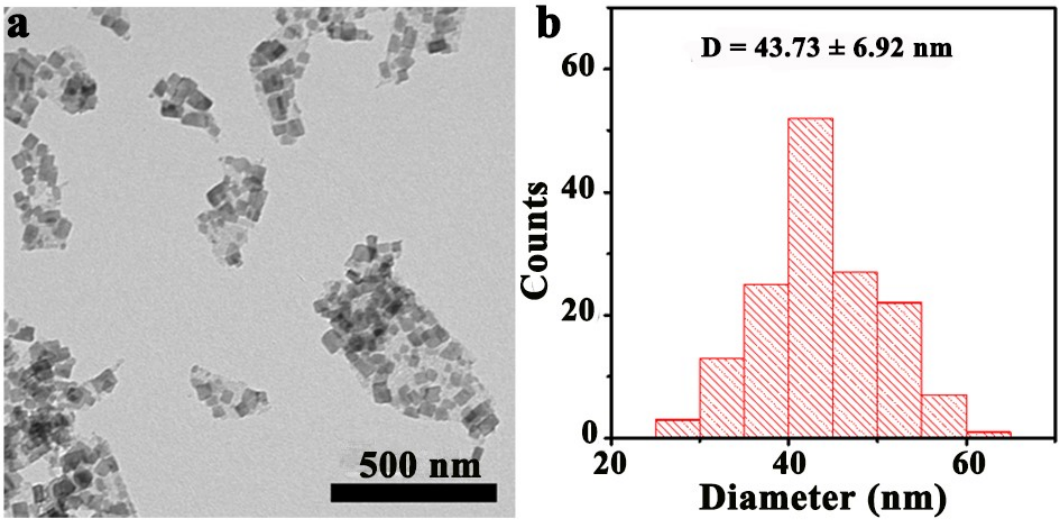

Figure S2 (a) TEM image of MS-CWS NSs and (b) size distribution histograms of CWS NSs.

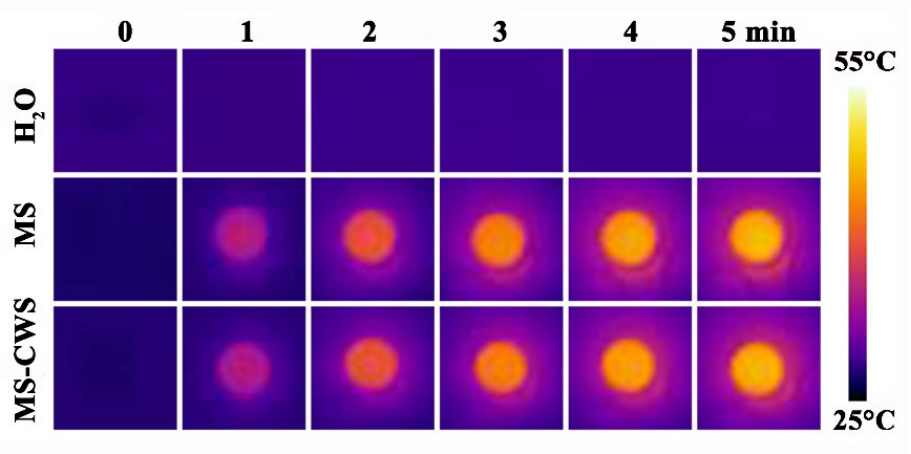

Figure S3 IR thermal images of H_2O, MS NSs, and MS-CWS NSs under the irradiation of 785 nm laser (0.8 W/cm^2).

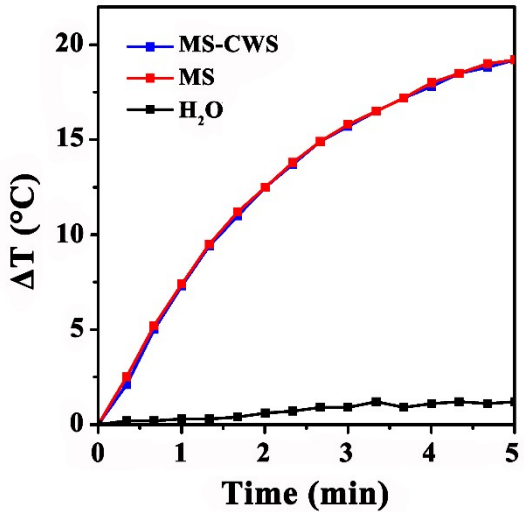

Figure S4 Photothermal-heating curves of MS NSs (20 μg/mL), MS-CWS NSs (20 μg/mL), and ultrapure water (H_2O) under the irradiation of 785 nm laser (0.8 W/cm^2).

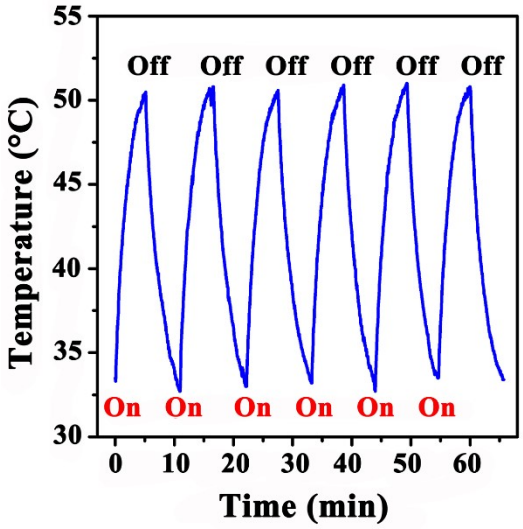

Figure S5 Heating/cooling cycle curves of MS-CWS NSs (20 μg/mL) under 785 nm laser irradiation (0.8 W/cm^2).

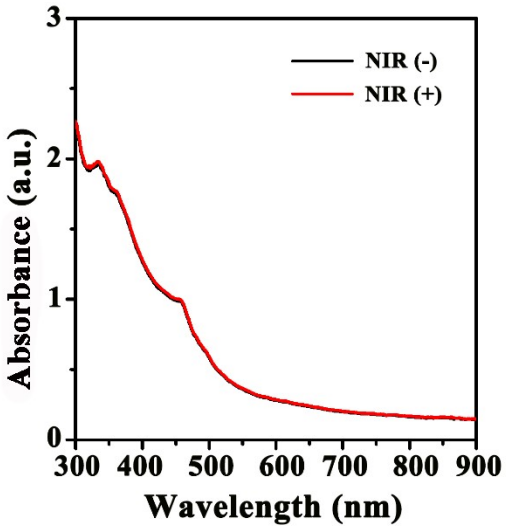

Figure S6 UV-vis-NIR absorption spectra of MS-CWS NSs before and after 785 nm laser irradiation (0.8 W/cm^2, 10 min).

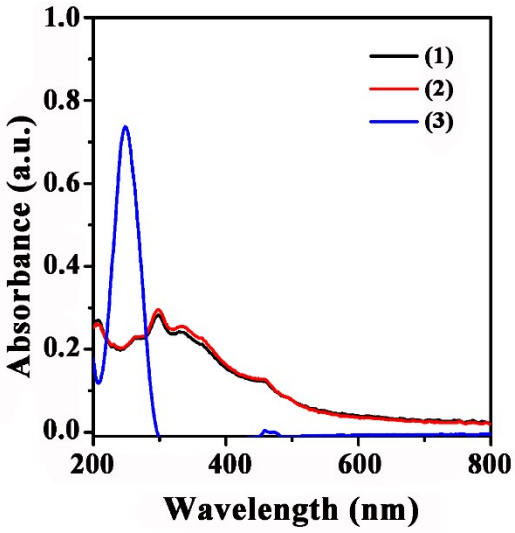

Figure S7 UV-vis-NIR absorption spectra of AA, MS-CWS NSs, and MS-CWS NSs + AA.

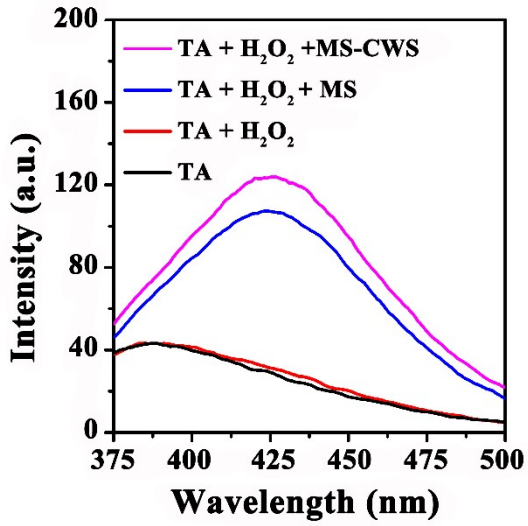

Figure S8 Dection of •OH according to PL spectra of TA in different reaction systems.

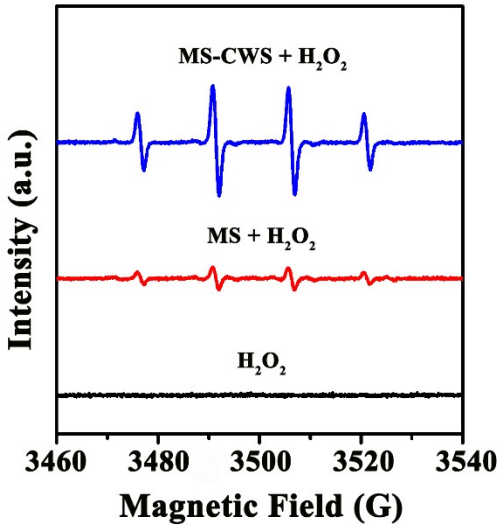

Figure S9 ESR spectra of •OH trapped by 5,5-dimethyl-1-pyrroline n-oxide (DMPO) in different reaction systems.

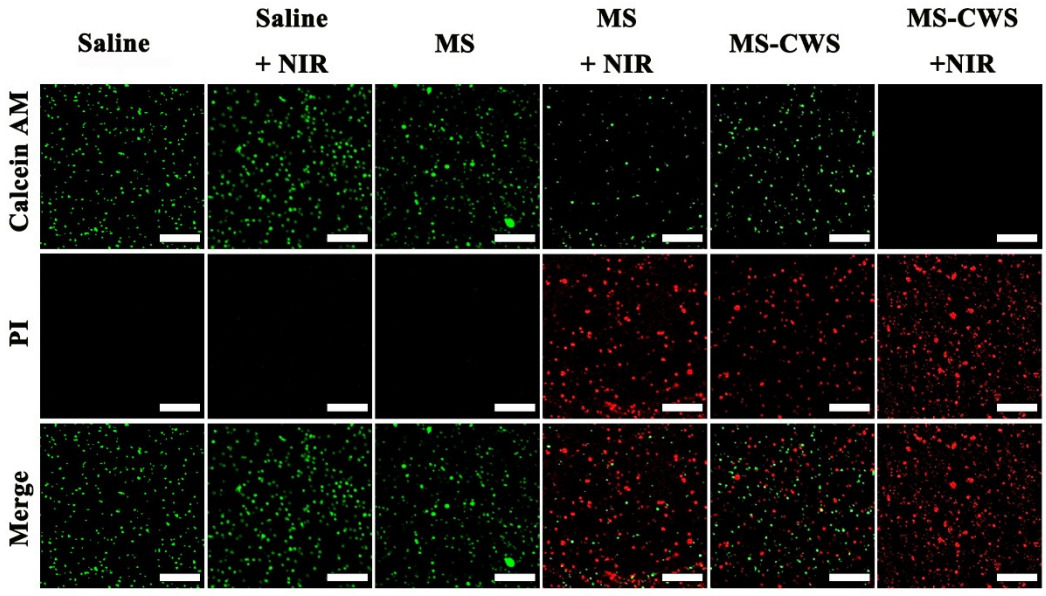

Figure S10 Fluorescence images of live/dead MRSA treated with saline, MS NSs, and MS-CWS NSs with or without laser irradiation. Scale bar, 50 μm.

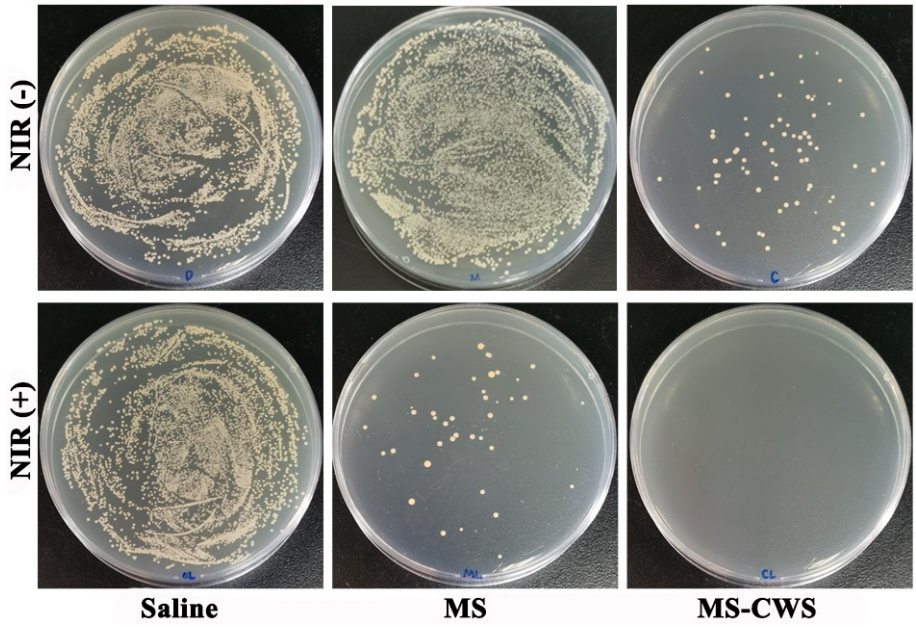

Figure S11 Photographs of bacterial colonies from MRSA-infected tissues in mice wounds after different treatments.

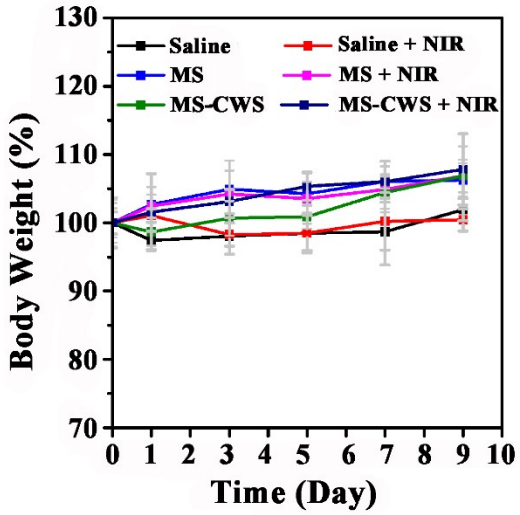

Figure S12 Time-dependent mice weight curves after different treatments.

Table S1 Comparison of kinetic parameters between MS-CWS NSs and nanozymes/enzymes.

Nanozymes / Enzymes	**Substrates**	**K_M (mM)**	**V_{max} (10^{-7} M s^{-1})**	**References**
MS-CWS	TMB	1.54	4.77	This work
MS	TMB	1.96	4.48	This work
HRP	TMB	0.43	1.00	[3]
Fe_3O_4 NPs	TMB	0.10	0.34	[3]
UiO-66-NH-CO-MoS_2	TMB	2.35	0.02	[4]
N-SCSs	TMB	0.15	2.21	[5]
N-PCNSs	TMB	2.25	0.45	[6]
MS-CWS	H_2O_2	14.36	3.11	This work
MS	H_2O_2	45.28	2.13	This work
HRP	H_2O_2	3.70	0.87	[3]
Fe_3O_4 NPs	H_2O_2	154.00	0.98	[3]
UiO-66-NH-CO-MoS_2	H_2O_2	0.23	1.57	[4]
N-SCSs	H_2O_2	81.53	2.33	[5]
N-PCNSs	H_2O_2	471.00	0.55	[6]

References

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