Supplementary Information for:

Versatile Molybdenum Disulfide Based Antibacterial Composites for in Vitro Enhanced Sterilization and in Vivo Focal Infection Therapy

Wentao Zhang,a Shuo Shi,b Yanru Wang,a Shaoxuan Yu,a Wenxin Zhu,a Xu Zhang,a Daohong Zhang,a Baowei Yang,a Xin Wang,a Jianlong Wang*a

aCollege of Food Science and Engineering, Northwest A&F University, Yangling, 712100, Shaanxi, P. R. China.
bFaculty of Pharmacy, School of Medicine, Xi’an Jiaotong University, Xi’an, 710061, Shaanxi, P. R. China.

* Corresponding authors: wanglong79@nwsuaf.edu.cn

1. Experimental Section

1.1 Exfoliation of MoS_2 nanosheets

Natural MoS_2 powder with a bulk particle size <2 μm (Sigma Aldrich) was exfoliated using a modified ionic liquid assisted grinding method.1 Typically, using an agate mortar with a pestle, 300 milligrams of bulk MoS_2 was ground with 0.4 mL of 1-butyl-3-methylimidazolium hexafluorophosphate (BMIPF_6) for 1 h. The grinding mixture was collected from the mortar and pestle, and then washed with acetone for three times to remove the ionic liquid. Finally, the sediment was dispersed in 50 mL mixture of ethylene glycol (EG) and diethylene glycol (DEG) with volume ratio of 1:9 and centrifuged at a speed of
1500 rpm for 20 min to remove the large/thick MoS$_2$. The obtained MoS$_2$ nanosheet dispersion was stored in 4 °C for the following investigations.

1.2 Preparation of CFM

MoS$_2$ nanosheets were transferred from the as-made suspension into a 50 mL beaker. Then, 94.2 mg FeCl$_3$·6H$_2$O was added. After ultrasonication and stirring for 20 min, 250 mg of sodium acrylate and 250 mg of sodium acetate (NaOAc) were added into the suspension under magnetic stirring or ultrasonication and allowed to dissolve completely. The resulting homogeneous solution was transferred to a Teflon-lined stainless steel autoclave and sealed before heating at 200 °C for 10 h. The as-prepared magnetic MoS$_2$ was washed several times with water. To modify with chitosan, the obtained magnetic MoS$_2$ was mixed with 0.05 wt % chitosan dissolved in 0.5% glacial acetic acid solution. After bath sonication for 10 min, the mixture was further stirred for another 24 h. CFM thus obtained was collected by magnetism and washed thoroughly several times with deionized water.

1.3 Characterization

Fourier transform infrared (FT-IR) spectroscopy was recorded on a Vetex70 (Bruker Corp., Germany). The UV-vis spectra were recorded with a UV-2550 spectrophotometer (Shimadzu, Japan) at room temperature. The weight loss curves were obtained with thermo gravimetric analyzer apparatus (STA449F3, Netzsch, Germany) from room temperature to 600 °C at a rate of 10 °C min$^{-1}$ in N$_2$ calcinations. Field emission scanning electron microscopy (SEM) image was taken by an S-4800 (Hitachi, Japan). The magnetic properties of samples were measured on a VSM-7307 vibrating sample magnetometer (Lake Shore Cryotronics, USA) at room temperature with a maximum magnetic field of 10 kOe.
1.4 Conjugating Bacteria with CFM Composite

The bacterial concentration was diluted to a desired level (OD$_{600}$ = 1.0 or 0.5), and 200 μL of a certain amount of CFM composites was then added into the bacterial solution (1.8 mL). The mixed solution was incubated in a rotary shaker at 250 rpm for different time to determine conjugation kinetics of CFM, so that nanomaterials can conjugate with the bacteria effectively. Subsequently, the resulting nanocomposites-bacteria conjugates were magnetically confined for 1 min with an external magnet. The supernatant was then carefully pipetted out and determined by optical density method and the conventional surface plate count method.

1.5 In Vitro Photothermal ablation of Pathogenic Bacteria

For NIR photothermal ablation of pathogenic bacteria, cells with a desired level (OD$_{600}$ = 0.5) were mixed with CFM. The interaction between CFM and bacteria was carried out by vortexing for 0.5 min. The CFM-bacteria conjugates were aggregated at the bottom with the help of an external magnet. The conjugate was subsequently exposed to NIR laser irradiation (808 nm, 2.0 W cm$^{-1}$) for 10 min. Finally, the conjugate was collected for further evaluation.

The metabolic activities of bacterial cells with different treatments were analyzed using the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Following treatment, bacterial cells were rinsed with saline and treated with 100 μL 5 mg mL$^{-1}$ MTT. After incubation for further 3 h, formazan crystals in each tube were solubilized in 1.0 mL of dimethyl sulfoxide (DMSO). The final solution was centrifuged at 13 000 rpm to remove any solid residues. The optical absorbance at 490 nm was then recorded by using a Microplate reader (Thermo Multiskan MK3).
After treatment and further incubation for 2.5 h at 37 °C, the bacteria were collected by centrifugation, stained with propidium iodide (PI, 1 μg mL\(^{-1}\)) for 15 min, counterstained with 4′-6-diamidino-2-phenylindole (DAPI, 5 μg mL\(^{-1}\)) for 5 min in the dark, and then imaged using a fluorescence microscope (Olympus IX71, Tokyo, Japan).

### 1.6 Biocompatibility Examination of CFM Composite

The biocompatibility of CFM composite was examined by cytotoxicity experiments, skin irritation test. The in vitro cytotoxicity experiments were firstly carried out using a human hepatoma (HepG2), mouse microglia (BV2) and buffalo rat liver 3A (BRL-3A) cells. Cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), benzylpenicillin (100 kU L\(^{-1}\)) and streptomycin (100 mg L\(^{-1}\)) at 37 °C and under an atmosphere of 5% CO\(_2\). To determine the cell viability under dark conditions (i.e. without laser treatment), cells (2 × 10\(^5\) cells per well) was seeded into 24 well cell-culture plates and incubated for 24 h at 37 °C. Then, cells were treated with different concentrations of CFM for 24 h. Following treatment, cells were rinsed with DPBS and treated with 50 μL 5 mg mL\(^{-1}\) MTT in serum-free media. After incubation for further 3 h, formazan crystals in each well were solubilized in 0.5 mL of dimethyl sulfoxide (DMSO). The final solution in each well was centrifuged at 13 000 rpm to remove any solid residues. The optical absorbance at 490 nm was then recorded by using a Microplate reader (Thermo Multiskan MK3).

For the acute skin irritation test, mouse, purchased from the Fourth Military Medical University, was kept under conventional care for one week before the skin irritation tests to make sure the acclimation of mouse. The following operations were similar with a pervious
Then, each mouse was accommodated separately and evaluations were made after 1 and 5 day, respectively.

1.7 In Vivo Photothermal Ablation Therapy of Focal Infection

Balb/c mice were used in the animal study. To evaluate the capacity of the CFM as a photothermal agent for focal infection treatment, a subcutaneous abscess was experimentally created in each test mouse. After shaving by a shaver and disinfection with 75% alcohol, a subcutaneous injection of \( S. aureus \) (~10\(^7\) CFU mL\(^{-1}\), 50 µL) was conducted on the shaved back of the test mice. After 24 h following the injection of bacteria, an infected abscess was formed subcutaneously. CFM solution (10 mg mL\(^{-1}\), 20 µL) was then directly injected into the local infection. Before irradiated by laser beam with the power density of 2 W cm\(^{-2}\) for 5 min in situ, the abscess site was exposed to an external magnet to concentrate CFM-bacteria conjugation. The thermographic images of test animal were then recorded by an IR thermal camera ((FLIR ThermaCAM E45, USA). Following the thermographic analysis, the mice were sacrificed, and their skins of infected tissues were harvested and analyzed the bacterial content. Briefly, excised skin specimens were homogenized using a TissueLyser II (QIAGEN, Hilden, Germany) for 1 min at 20 Hz and \( S. aureus \) survival was determined by plating serially diluted homogenized samples on LB plates after 24 h incubation. For histological analysis, the skin including the entire wound with adjacent normal skin were excised and immersed in 4% formaldehyde, dehydrated with a graded series of ethanol, embedded in paraffin and then stained by hematoxylin and eosin (H&E).

1.8 Ethics statement
This study was performed with the approval of the Experimental Animal Management Committee (EAMC) of Northwest A&F University. Animals were treated as the guidelines of EAMC.

2. Detail Discussion of Characterization of CFM Hybrids

As shown in Fig. 1A, the FT-IR spectra of chitosan, MoS$_2$ and CFM nanocomposite are compared. Obviously, CFM produced very similar absorption for native chitosan. The peaks at 1595 and 1440 cm$^{-1}$ are assigned to the –NH and –CH$_2$ bending, respectively. CH$_3$ and C–OH wagging are located at 1380 and 1340 cm$^{-1}$, respectively, while 1050–1260 cm$^{-1}$ resulted from skeletal vibrations of C–O–C stretching. These groups are assigned to chitosan. Peak located around 570 cm$^{-1}$ is designated as the Fe–O of Fe$_3$O$_4$ nanoparticles. Moreover, the band at about 468 cm$^{-1}$ presented in both cases of MoS$_2$ and CFM corresponds to Mo–S vibration. The results indicate the coexistence of chitosan, Fe$_3$O$_4$ and MoS$_2$, meaning the formation of the CFM hybrids. The UV-$\text{vis}$ spectra of MoS$_2$ and CFM were subsequently recorded (Fig. 1B). Typical characteristic absorption bands of MoS$_2$ located at 665, 605, 443 and 387 nm are observed, which are in good agreement with our previous work.$^1$ For the spectrum of CFM, significant changes in curve shape were observed and the optical absorbance in the NIR regions was enhanced relative to pristine MoS$_2$, likely owing to the formation of Fe$_3$O$_4$ nanoparticle on MoS$_2$ sheets. The decreased intensity of peaks at about 443 nm and 387 nm might attribute to plasmon coupling and refractive index effects between Fe$_3$O$_4$ and MoS$_2$. To better demonstrate the combination, Thermogravimetric analysis (TGA) measurements under a N$_2$ atmosphere at a heating rate of 10 °C min$^{-1}$ were employed. About 2.39% weight loss can be observed during the heating process from room temperature to 600
°C for FM (Fig. 1C). For the TGA curve of CS, two weight losses are observed (Fig. S1a). The weight loss below 200 °C is due to the moisture vaporization, while the weight loss over 200 °C is attributed to the degradation of CS molecules. Once processed with chitosan, compared with the TGA curve of magnetic MoS$_2$ without chitosan, the intercalation compound reveals two decomposition onsets and a significant weight loss over 200 °C that agrees well with that of pure CS, further indicating the successful formation of the CFM composite.

3. Supporting Figures

![TGA curve of chitosan](image.png)

**Fig. S1** Thermal gravimetric (TGA) analysis of chitosan. Two weight losses are observed. The weight loss before 200 °C is due to the moisture vaporization, while the weight loss over 200 °C is attributed to the degradation of chitosan molecules.
**Fig. S2** Scanning electron microscopy (SEM) images of MoS$_2$ nanosheets (a) and CFM at low magnification (b).
Fig. S3 The X-ray diffraction (XRD) of bulk MoS$_2$ and the as-prepared CFM. In the representative XRD pattern of CFM, three diffraction lines, assigned to the (220), (400), (511) reflections of the pure cubic spinel crystal structure of Fe$_3$O$_4$, were presented, indicating the coexistence of MoS$_2$ and Fe$_3$O$_4$. Diffraction peaks are indexed from the MoS$_2$ phases (reference code. 37-1492) and Fe$_3$O$_4$ (reference code. 19-0629).
Fig. S4 Size distribution of Fe$_3$O$_4$ nanoparticles on MoS$_2$ nanosheets, over 200 particles were counted, indicating Fe$_3$O$_4$ nanoparticles possess average particle size, about 18.19 nm.
Fig. S5 Photographs of *E. coli* bacterial colonies after removal by different concentrations of CFM. (A), (B), (C), (D) corresponds to 0, 50, 100, 200 μg mL⁻¹, respectively. The initial concentration of *E. coli* is OD₆₀₀=0.5.
Fig. S6 Capture kinetics of CFM (200 μg mL\(^{-1}\)) for *S. aureus* and *E. coli* (OD\(_{600}\)=0.5) in a regular shaking incubation at 250 rpm. The concentration of both bacteria in the supernatant solution quickly decreases with the increase of incubation time; even in 0.5 min of incubation in a shaker, bacterial OD\(_{600}\) drops by more than 90%, indicating fast and efficient capture of bacteria by CFM.
Fig. S7 Capture capacity of magnetic MoS$_2$ for *S. aureus* and *E. coli* at a bacterial concentration of $\text{OD}_{600}=0.5$. Even at the highest concentration used for the capture experiment of CFM, 500 $\mu$g mL$^{-1}$, magnetic MoS$_2$ can only remove about 50% bacteria.
Fig. S8 Photographs of bacterial colonies after different treatments. (A) Bacteria solution mixed with CFM without further treatment. (B) Bacteria solution mixed with CFM and treated with NIR irradiation in dispersion solution. (C) Photothermally treating CFM-bacteria aggregates enriched by an external magnet.

The well bacterial conjugation performance of CFM allows the formation of CFM–bacteria conjugation in aqueous medium, then forming CFM–bacteria aggregates with assistance of external magnet. To confirm whether magnetic enrichment can make contribution to improve the photothermal conversion efficiency of CFM to obtain enhanced photothermal antibacterial activity, different treatments of bacteria conjugated with CFM are conducted. As shown in Fig. S8B, after being mixed with bacterial solution, CFM showed strong photothermal antibacterial activity in solution even without further magnetic enrichment. Most importantly, compared to photothermal treatment of CFM–bacteria conjugation in dispersion solution, photothermal treatment on CFM–bacteria aggregates shows more efficient bacterial inactivation activity (Fig. S8C). The results demonstrate that magnetic enrichment can improve the photothermal conversion efficiency of CFM with enhanced photothermal antibacterial activity.
Fig. S9 Photographs of (A) *S. aureus* and (B) *E. coli* bacterial colonies after interaction with CFM in the absence or presence of NIR laser irradiation.
To clearly understand the photothermally antibacterial mechanism of CFM, SEM characterization of CFM–bacteria conjugates treated with NIR irradiation was conducted. As shown in Fig. 2 and Fig. S10, compared to CFM–bacteria conjugates without the treatment of NIR irradiation, the laser exposed bacteria exhibited significantly collapsed morphology including fragments, rupture. The photothermal property of CFM in the presence of NIR laser could be the key reason for the complete thermal destruction of bacteria. \(^5\)
Fig. S11 (A) Illustration of three areas including back, left and right pelvis of the rat for skin irritation studies. (B) Lacerate treatment to form uniform damage by sterile injection needles. Then treated with CFM nanocomposites (right pelvis), ethanol and H$_2$O$_2$ (left pelvis), saline (back), respectively. And photographs of three areas with different treatments after 1 day (C) and 5 days (D).
In vivo accumulation of the CFM in different time intervals after subcutaneous injection of CFM determined by ICP-MS measurements of Mo element in tissue lysates.

As a subcutaneous injection for bacterial administration, CFM was subcutaneously injected to evaluate in vivo accumulation of CFM. Following in vivo focal infection therapy, the content of Mo element in various organs, including heart, liver, spleen, lung, kidney, skin and scab, was measured by ICP-MS. The result was shown in Fig. S12. In the experimental groups before 6 days, the Mo content in scab was determined to be equal to injected dose and no obvious increase of Mo content was found in various organs, indicating continuous high concentration accumulation of CFM in the skin where subcutaneous injection occurs. This result is reasonable as CFM-bacteria aggregates are too large to be cleared from the injection site upon subcutaneous administration and magnetic enrichment. With the repair of wound, the scab and the implanted CFM almost thoroughly flaked off within 14 days, which agrees well with a previous report. Thus, as a subcutaneous injection for bacterial administration, CFM shows favourable treatment effect with weak in vivo toxicity.
Reference