Erythrocyte Membrane-Enveloped Molybdenum Disulfide Nanodots for Biofilm Elimination on Implants Via Toxin Neutralization and Immune Modulation

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Research Article

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Abstract

Backgrounds:

Implant-related infections (IRIs) caused by bacterial biofilms remain a prevalent but tricky clinical issue, which are characterized by drug resistance, toxin impairment and immunity suppression. Recently, antimicrobial therapies based on reactive oxygen species (ROS) or hyperthermia have been developed to effectively destroy biofilms. However, all of them have failed to simultaneously focus on the immunosuppressive microenvironment of biofilms and tissue damage caused by bacterial toxins.

Results

Herein, we proposed a one-arrow-three-hawks strategy to orchestrate hyperthermia/ROS antibiofilm therapy, toxin neutralization and immunomodulatory therapies through engineering a bioinspired erythrocyte membrane-enveloped molybdenum disulfide nanodots (EM@MoS$_2$) nanoplatform. In biofilm microenvironment, pore-forming toxins actively attack the erythrocyte membranes on the nanodots and be detained, thus keeping toxins away from their targets and mitigating tissue damage. Under near-infrared laser irradiation, MoS$_2$ nanodots, with superb photothermal and peroxidase-like properties, exert a powerful synergistic antibiofilm effect. More intriguingly, we initially identified that they possess the ability to reverse the immunosuppressive microenvironment through skewing the macrophages from an anti-inflammatory phenotype to a proinflammatory phenotype, which would promote the elimination of biofilm debris and prevention of infection relapse. Systematic in vitro and in vivo evaluations have demonstrated that EM@MoS$_2$ achieves remarkable antibiofilm effect.

Conclusion

The current study integrated the functions of hyperthermia/ROS therapy, virulence clearance and immune regulation, which could provide an effective paradigm for IRIs therapy.

Background

Implant-related infections (IRIs), caused by pathogenic bacteria remain an intractable health problem. They are characterized by microbial biofilm formation on indwelling devices (e.g., artificial joints, catheters, cardiac valves, dental implants) and surrounding tissues[1–3]. Biofilms are sessile bacterial communities enveloped in self-generated extracellular polymer substances (EPSs), which commonly comprise polysaccharides, extracellular DNA (eDNA), proteins, and lipids[4, 5]. As EPSs form a physical and chemical barrier, they protect the microorganisms from antibiotics, host immune responses, or high shear stress, thus promoting resistance against available therapies[6, 7]. Thus, it means a lot to break down the EPSs structure for biofilm eradication. Among all the biofilm-positive strains, methicillin-resistant Staphylococcus aureus (MRSA) is well known for being resistant to nearly all the clinically used
antibiotics[8–10]. Moreover, biofilms formed by MRSA create an immunosuppressive microenvironment by switching the infiltrating macrophages from a proinflammatory phenotype (M1) to an anti-inflammatory (M2) phenotype, which inhibited the migration, and phagocytosis of macrophages and ultimately weakened their bactericidal capacity[11]. With this in mind, ideal antibiofilm strategies need to emphasize the immunomodulatory function of antibiofilm agents. Thus, selecting an alternative antibiofilm agent that can effectively disrupt EPSs structure and reverse the immunosuppressive states induced by biofilms, simultaneously, is an attractive countermeasure.

Recently, transition metal disulfides (TMDs) and their derivates have gained considerable attention for the development of antibacterial agents due to their unique merits, such as large specific surface-area, extraordinary photothermal conversion efficiency, and impressive catalytic activity[12–15]. Among the emerging diverse nanomaterials, molybdenum disulfide (MoS$_2$) possesses potent antibacterial properties both for planktonic bacteria and their biofilm counterparts[16, 17]. As previously reported, MoS$_2$ nanosheets[18], MoS$_2$ coatings[19], and MoS$_2$ nanoflowers[20] have been developed to simultaneously serve as photothermal conversion agents and reactive oxygen species (ROS) generators to eliminate bacteria without causing cytotoxicity. Also, sub-10-nm ultra-small nanodots of MoS$_2$ (MoS$_2$ NDs) have emerged and theoretically have the potential for antibiofilm applications. These nanodots have more active sites/edge atoms and higher in vivo clearance efficiency than their large-sized counterparts described above[21, 22]. Furthermore, we speculate that MoS$_2$ NDs can penetrate the biofilm structures because of decreased diffusional obstruction, thereby effectively increasing the interaction between the nanodots and biofilms. To the best of our knowledge, few attempts of utilizing MoS$_2$ NDs to conquer biofilm infection have been reported. Moreover, the performance of MoS$_2$ and other types of TMDs in immune modulation, particularly in macrophage polarization, remains unexplored.

More recently, the MRSA biofilm matrix has been proven to possess a huge quantity of toxins[5, 23]. Among them, pore-forming toxins (PFTs) are one of the most concerning and malignant one[7, 24]. This kind of toxins inherently anchor onto erythrocyte membranes to punch holes, which ultimately alters cellular permeability and induces cell dysfunction[25]. Some studies have demonstrated that neutralizing pore-forming toxins is extremely beneficial to alleviate the toxicity of $S$. $aureus$ infections[26, 27]. Nevertheless, conventional detoxification strategies rely heavily on antibodies which aim at a kind of structure-specific toxins, thereby lacking versatilities of use[28]. In recent years, cell membrane coating technology has emerged as a versatile and facile strategy, which could be employed to envelop nano-sized drugs to endow them with different biological functions[29]. Some reports have proved that nanoparticles coated with erythrocyte membranes could act as fake erythrocytes to absorb PFTs[26, 30]. Thus, we hypothesized that MoS$_2$ NDs could be enveloped in erythrocyte membranes to effectively neutralize bacterial toxins and eradicate biofilms simultaneously. To our knowledge, no relevant reports have been reported.

Herein, a “one-arrow-three-hawks” strategy was proposed to bridge direct antibiofilm therapy (hyperthermia/ROS), anti-virulence therapy and immunomodulatory therapy utilizing a MoS$_2$ NDs-based
biomimetic platform (Scheme 1). In this system, the erythrocyte membrane should absorb a large quantity of PFTs so as to ameliorate the symptoms of infection. Meanwhile, the MoS$_2$ NDs are expected to simultaneously cause sufficient hyperthermia and generate toxic ROS [mainly hydroxyl radicals (•OH)] under the activation of 808 nm laser irradiation and exposure to low concentrations of H$_2$O$_2$, respectively. This will collectively decompose the stiff EPSs matrix and kill most of the freed bacteria. Interestingly, MoS$_2$ NDs have an innate property to reverse macrophage phenotype from M2 to M1, consequently eliminating residual bacteria. Taken together, this study will further confirm the powerful antibiofilm effect of MoS$_2$ NDs, and also sheds a light on its immunomodulatory function against macrophages both in vitro and in vivo, proving their versatility as a possible immunomodulatory agent. Profoundly, EM@MoS$_2$ promises to effectively solve the intractability of biofilms in etiology (antibiofilm) and symptomology (antivirulence).

**Methods**

**Preparation of MoS$_2$ NDs.**

MoS$_2$ NDs were synthesized according to the previous report[31]. Briefly, (NH$_4$)$_2$MoS$_4$ (20 mg) and PVP (85 mg) were mixed in methanol (80 mL) and hydrazine hydrate (0.75 mL) was added dropwise during ultrasonication. The mixed solution was then transferred to a Teflon-lined stainless-steel autoclave. Subsequently, the resultant solution was heated to 140°C for 3 h. The mixture was filtered and methanol was removed with a rotary evaporator and then re-dispersed in water. Thereafter, the dispersion was dialyzed against deionized water for 48 h to obtain the final product.

**Fabrication of EM@MoS$_2$.**

EM@MoS$_2$ was synthesized by an “extrusion method”[32]. In detail, MoS$_2$ NDs (aqueous solution, aq) were mixed with obtained erythrocyte membrane fragments (EMFs, aq) at the mass ratio of MoS$_2$/membrane protein = 1:1, and the mixture was sonicated for 2 min (42 kHz, 100W). The resulting solution was sequentially extruded through 400 nm, 200 nm, and 100 nm polycarbonate membranes for 13 times respectively using a mini extruder, following by centrifugation at 18000g for 10 min to remove the free MoS$_2$ NDs and membrane vesicles. Finally, the purified EM@MoS$_2$ was collected and stored at 4°C for further use.

**Toxin-Neutralization Capacity of EM@MoS$_2$ in Vitro.**

α-hemolysin derived from staphylococcus aureus was used in this experiment. Five experiment groups were set as following: PBS, toxin (4 µg), toxin (4 µg) + MoS$_2$ NDs (200 mg/L), toxin (4 µg) + EM@MoS$_2$ (200 mg/L), toxin (4 µg) + anti-α-toxin antibody (20 mg/L). Initially, MoS$_2$ NDs, EM@MoS$_2$ and anti-Staphylococcal α-toxin antibody were incubated with α-hemolysin for 30 min at room temperature, respectively. Then 50 µL purified erythrocytes were added into each group of mixture, and the final volume was adjusted to 500 µL by PBS. After incubation for 10 min and subsequent centrifugation, the
extent of hemolysis of each group were estimated by measuring the absorbance of supernatants at 540nm using an Epoch-2 microplate reader (BioTek Instruments, Winooski, VT, USA). To quantitatively explore the efficiency of EM@MoS₂ to adsorb toxin, a series of graded amount of α-hemolysin (0.5, 1, 2, 5, 10 µg) were added into 500 µL EM@MoS₂ (200 mg/L), respectively. After incubation at room temperature for 30 min, 50 µL of purified erythrocytes were added into each group and incubated for 10 min. Subsequently, the absorbance of all samples at 540 nm was measured as described above. In addition, a complete hemolysis group was set as control by lysing RBCs with Triton-X100. Additionally, the biocompatibility of toxin-adsorbed EM@MoS₂ was further explored. In brief, L-929 cells were seeded in a 24-well plate at a density of 3×10⁴ cells/well and incubated at 37°C for 24 h. Then 5 µg of α-toxin or equivalent α-toxin-adsorbed EM@MoS₂ were added into the cells. After 12 h, the cells were fixed with 4% paraformaldehyde for 20 min and subsequently rinsed thrice with PBS. Then, F-actin and cell nuclei were stained separately with Rhodamine-labeled phalloidin for 20 min and DAPI for 5 min in the dark, respectively. Ultimately, each sample was observed and photographed by fluorescence microscope (DMi8, Leica Microsystems, German).

**In Vitro Antibiofilm Effect and Mechanisms.**

The MRSA biofilms formed on PEEK discs were used to evaluate the antibiofilm effects of nanomaterials in five experimental groups: control, NIR (808 nm, 1.0 W/cm², 10 min), MoS₂ NDs (200 mg/L, 500 µL), EM@MoS₂ (200 mg/L, 500 µL), and EM@MoS₂ (200 mg/L, 500 µL) + NIR laser (808 nm, 1.0 W/cm², 10 min). After treatment, all the samples were rinsed thrice for the following experiments. For scanning electron microscopy (SEM), all the samples were fixed overnight in 2.5% glutaraldehyde at 4°C. Thereafter, they were subjected to dehydration with a series of graded concentrations of ethanol (30%, 50%, 70%, 80%, 95%, 100%). Then the samples were freeze-dried and gold sprayed and observed under a Field Emission Scanning Electron Microscope (JSM-7800F, JEOL, Japan).

For CLSM observation, LIVE/DEAD BacLight Bacterial Viability Kit was used to stain the biofilms on the discs according to the manufacturer's instructions. Following this, excess dyes were removed and biofilms were observed and 3D-reconstructed using CLSM and its imaging software (Leica, TCS SP8, German). Further, the images were analyzed using COMSTAT plugin of ImageJ software to evaluate the biomass.

For ROS assay, the biofilms were incubated with DCFH-DA diluent (10 µmol/L) at 37°C for 20 min. Then excess dyes were removed and the samples were rinsed thrice with PBS for the subsequent observation using CLSM.

For bacterial resistance test, the standard plate counting method mentioned in supporting information was performed. In brief, a MRSA colony (termed as generation 1) was selected to prepare bacterial suspension and form bacterial biofilms on the PEEK substrates. After treated with EM@MoS₂ + NIR, the plate counting method is conducted to assess the bacterial survival rate in generation 1. Then, the obtained bacterial community on the plate of treated group was designated as generation 2 and underwent the next cycle of operation. This procedure was performed for 8 consecutive rounds.
**Immunoregulatory Potential and Mechanisms.**

The immunoregulatory potential of MoS\(_2\) NDs at different concentrations (0, 25, 50, 100, and 200 mg/L) and EM@MoS\(_2\) at a given concentration (200 mg/L) were assessed using RAW264.7 cells. After treatment, all the cell samples were rinsed thrice for the following experiments.

For immunofluorescence staining, obtained RAW264.7 cells were fixed with 4% paraformaldehyde (15 min), permeated with 0.5% triton X-100 (20 min), and then blocked with 5% BSA (30 min) at room temperature. Thereafter, cells were washed thrice with PBS and incubated with rabbit anti-mouse CD197 antibody (1: 200, Abcam) or rabbit anti-mouse Arg-1 antibody (1:200, Abcam) overnight at 4°C. The next day, after removing primary antibody dilutions, Alexa Fluor 594 labeled goat anti-rabbit antibody (1:500, Abcam) was added to conjugate CD197. Similarly, Alexa Fluor 488 labeled goat anti-rabbit antibody (1:500, Abcam) is designed for Arg-1. After incubation for 2 h, the cells were washed and stained with DAPI followed by observation and image acquisition using CLSM.

For flow cytometry analysis, the treated cells of various groups were carefully scraped off and transferred into respective EP tubes. Following centrifugation and blocking (5% BSA), cell samples were washed and further incubated with APC-labeled anti-CD86 antibody (0.25 µg/100 µL) or PE-labeled anti-CD206 antibody (0.5 µg/100 µL) at room temperature for 1 h. At last, CD86 and CD206 expression levels in each group were examined using a flow cytometer (Beckman Coulter, Brea, CA, USA).

For ELISA test, supernatants of each group were collected after incubation with nanomaterials for 24 h. Three classical pro-inflammatory cytokines (TNF-\(\alpha\), IL-1\(\beta\), MCP-1) and one anti-inflammatory cytokine (IL-10) were detected using ELISA kits according to the manufacturer’s instructions.

For RT-PCR analysis, the expressions of genes coding TNF-\(\alpha\), IL-1\(\beta\), MCP-1, and IL-10 were measured. In brief, all the cell samples were lysed for subsequent total RNA extraction in accordance with RNA Purification Kit (EZBionscience) instruction. Then equal amounts of RNA in each group were converted to cDNA by Color Reverse Transcription Kit (EZBionscience). Finally, obtained cDNA, \(2^{\Delta \Delta Ct}\) method was applied to calculate gene expression levels. Primer sequence information is listed in table S1.

For RNA-seq analysis, total RNA of control and MoS\(_2\) NDs groups (200 mg/L) were extracted using TRIzol reagent. Then the whole gene profiles were examined at Oebiotech Co. Ltd (Shanghai, China). Data processing, including correlation analysis, differential expressed genes (DEGs) analysis, Gene Ontology (GO) analysis and Kyoto Encyclopedia of Gene and Genomes (KEGG) analysis was performed on Oebiotech cloud platform (https://cloud.oebiotech.cn/task/).

**In Vivo Antibiofilm Effect and Immunoregulatory Role of EM@MoS\(_2\).**
Male BALB/c mice were provided by the Animal Experimental Center of Shanghai Jiao Tong University Affiliated Sixth People's Hospital. All animal experiments were conducted under approval of the Institutional Animal Care and Use Committee (IACUC) of Shanghai Jiao Tong University Affiliated Sixth People's Hospital (Animal Welfare Ethics acceptance number No: DWLL2021-0845).

30 mice were randomly divided into five groups (control, NIR, MoS$_2$ NDs, EM@MoS$_2$, and EM@MoS$_2$ + NIR). Prior to experiments, biofilms on the surfaces of PEEK discs were prepared (as mentioned in supporting information). To build a subcutaneous implant-related infection model, the mice were initially anesthetized by intraperitoneal injection of pentobarbital sodium (50 mg/kg), then their backs were shaved and disinfected. Subsequently, an incision (1 cm) was made across the operation area to implant prepared PEEK discs, following which the incision was sutured carefully. At last, 100 µL of MoS$_2$ NDs, EM@MoS$_2$ or PBS (for control and NIR groups) was administered subcutaneously onto the surface of discs using a syringe, respectively, according to grouping. Specifically, for NIR and EM@MoS$_2$ + NIR groups, the operation areas were irradiated using 808 nm NIR laser for 12 min on 2nd and 4th day after nanomaterials were injected.

For gross examination, we examined the lesions by taking gross photos of live mice on the 1st, 3rd, 7th, 10th, and 13th day. Also, MRI examination was performed using a small animal MRI scanner (CG NOVILA 7.0T, Shanghai Chenguang Medical Technologies Co., LTD) to observe the subcutaneous abscess on 13th day. Furthermore, the abscess volumes of each group were calculated through 3D reconstruction using ImageJ and its plugin, Measure Stack. After all the mice were sacrificed with anesthetic overdose, their impaired skins were cut for imaging and subsequent assessments.

For standard plate counting tests, the implants on their backs and the surrounding soft tissues were collected under aseptic condition. Whereafter, bacteria adhering to implants were obtained through vibration and sonication, then 100 µL lysate was used to plate on blood agar plates after serial dilution. Following 24 h incubation at 37°C, bacterial colonies were photographed and counted. Similarly, equal amounts of peri-implant soft tissues (2 g) of each group were homogenized and then used for plating and subsequent counting.

For SEM analysis, the harvested implanted discs were processed and observed as described above (section of In vitro antibiofilm effect and mechanisms).

For histological assessments, the surrounding soft tissues were fixed with 4% paraformaldehyde, dehydrated with gradient ethanol, embedded in paraffin, and finally cut into 5-µm thick tissue sections for subsequent staining and observation. H&E staining was employed to assess the inflammatory conditions including edema, connective tissue proliferation and inflammatory cell infiltration. Furthermore, the thickness of the connective tissue was measured by photoshop software. Meanwhile, tissue sections were stained with Giemsa to determine residual bacterial loads. In order to evaluate the effect of MoS$_2$ or EM@MoS$_2$ on the differentiation of macrophages in vivo, we stained the section with rat anti-mouse CD86 antibody (1:200, Abcam) and rabbit anti-mouse Arg-1 antibody (1:200, Abcam) for 12 h, followed
by Alexa Fluor 594 conjugated goat anti-rat antibody (1:200, Abcam) and Alexa Fluor 488 conjugated goat anti-rabbit antibody (1:200, Abcam) for 2h. Finally, the sections were observed and imaged by CLSM. To evaluate the in vivo biocompatibility of nanomaterials, the sections of the vital organs (the heart, liver, spleen, lung, and kidney) of each group were stained with H&E and observed using CLSM.

Statistical analysis

In vitro experiments were repeated for at least three times and in vivo experiments were repeated for at least six times. All data are presented as mean ± standard deviation. GraphPad Prism v9.0.0 (La Jolla, CA, USA) was used for data statistics. Statistical significance was calculated using the one-way ANOVA and student’s t test one-way ANOVA and student’s t test and was denoted as * (p<0.05), ** (p<0.01), *** (p<0.001), **** (p<0.0001).

Results And Discussion

Preparation and Characterization of EM@MoS$_2$.

The approach to prepare functional EM@MoS$_2$ with simultaneous toxin neutralization capability, hyperthermia/ROS antibiolm effect, and immunomodulatory property is illustrated in Scheme 1. In this work, uniform MoS$_2$ NDs were initially synthesized by one-step solvothermal process of (NH$_4$)$_2$MoS$_4$ under a reduced environment (hydrazine hydrate) in the presence of PVP.$^{[31]}$ The chemical composition of MoS$_2$ NDs was studied by X-ray photoelectron spectroscopy (XPS). The survey XPS spectrum (Fig. S1) confirmed the S and Mo elemental composition of the nanodots. In the Mo 3d region of XPS spectrum, three characteristic binding peaks at 228.8, 231.9, and 232.7 eV were observed (Fig. 1a), which can be assigned to 3d$_{5/2}$ and 3d$_{3/2}$ of Mo$^{4+}$. Moreover, the peak at 232.7 eV was indexed to the overlap of Mo$^{4+}$ with Mo$^{6+}$. On the other hand, the binding energies of S 2p (Fig. 1b) are corresponded to the S$^{2-}$ species. This result preliminarily demonstrated the construction of MoS$_2$ NDs. Additionally, transmission electron microscopy (TEM) revealed that the MoS$_2$ NDs featured high dispersity with a uniform diameter of 6.7 nm (Fig. 1c). The corresponding energy-dispersive spectrometry (EDS) line-scan elemental distribution profile shows the presence of Mo, S, and N elements (Fig. S2), confirming the successful modification of PVP molecular. Subsequently, MoS$_2$ NDs were camouflaged with erythrocyte membrane to obtain EM@MoS$_2$ nanocomposite through extrusion method. Compared to the spotty patterns of MoS$_2$ NDs, a distinct cell membrane structure (10 nm thick) containing some nanodots were observed in the EM@MoS$_2$ (Fig. 1d). This proved that the erythrocyte membrane provided prominent coverage to the nanodots. While the hydrodynamic diameter of EM@MoS$_2$ was determined to be 154.2 nm, that of a bare MoS$_2$ NDs was only about 8.7 nm (Fig. 1e). Simultaneously, the zeta potential of EM@MoS$_2$ was -25.6 mV (Fig. S3), which was similar to the potential of erythrocyte membrane vesicles (-23.2 mV) but lower than that of bare MoS$_2$ NDs (-14.5 mV). Subsequently, SDS-PAGE was utilized to confirm the existence of the membrane proteins in EM@MoS$_2$ (Fig. 1f). As expected, the EM@MoS$_2$ and erythrocyte membranes exhibited similar protein bands. These results reflected the successful formation of EM@MoS$_2$. 
In Vitro Photothermal Effect and Catalytic Performance of EM@MoS$_2$.

Owing to the presence of MoS$_2$ NDs, the obtained EM@MoS$_2$ exhibited intense NIR absorption (Fig. 1g). Thus, its photothermal conversion performance was examined. Both MoS$_2$ NDs and EM@MoS$_2$ displayed a substantial elevation in temperature compared with deionized water (DI water) after NIR irradiation (Fig. S4). With the increase of EM@MoS$_2$ concentration, the temperature of dispersion increased to 31°C for 25 mg/L, 37.9°C for 50 mg/L, 45 °C for 100 mg/L and 54.9°C for 200 mg/L as triggered by 808 nm laser (Fig. 1h, 1/Wcm$^2$, 10 min). In addition, the laser power density-dependent temperature increase of EM@MoS$_2$ was also observed (Fig. 1i). Furthermore, the photothermal stability of EM@MoS$_2$ was investigated and no remarkable temperature changes were observed during four heating/cooling cycles (Fig. S5). This excellent photothermal effect and desirable photostability suggested that EM@MoS$_2$ were suitable for photothermal antibacterial therapy.

It has been proved that MoS$_2$ can efficiently catalyze the decomposition of H$_2$O$_2$ to produce •OH, which is recognized as the most toxic ROS.[33, 34] Therefore, the peroxidase-like activity of EM@MoS$_2$ was investigated in the presence of H$_2$O$_2$ using TMB as a substrate. As shown in Fig. 1j, the absorbance of TMB at 635 nm effectively increased with the increase of H$_2$O$_2$ concentrations, demonstrating the generation of abundant •OH. Furthermore, the catalytic activity was found to be dependent on EM@MoS$_2$ concentrations (Fig. 1k) and pH values (Fig. S6). To verify the •OH generated by the MoS$_2$-based catalytic reactions, electron spin resonance (ESR) was performed under different conditions. Noteworthily, the presence of H$_2$O$_2$ and EM@MoS$_2$ under a mildly acidic environment could promote the yields of •OH, as demonstrated by the presence of the characteristic 1:2:2:1 signal in ESR spectrum (Fig. 1l), which was consistent with the TMB chromogenic reaction. Thus, our EM@MoS$_2$ nanocomposite exhibited integrated the multifaceted performance of a good photothermal conversion agent for photothermal therapy and a special peroxidase-like enzyme for •OH generation which is promising for biofilm disruption.

The Biocompatibility and Toxin-Neutralization Capacity of EM@MoS$_2$

The CCK-8 assay on RAW 264.7 cells was applied to assess the cytotoxicity of MoS$_2$ NDs and EM@MoS$_2$. As shown in Fig. S5, when the concentrations of two nanomaterials were increased to 200 mg/L, relative cell viabilities still maintained at a high level (approximately up to 90%), and there were no significant differences between MoS$_2$ NDs group and EM@MoS$_2$ group. However, the cell survival rate decreased to 56.77%, after treatment with 400 mg/L of MoS$_2$ NDs, which was notably lower than that of cells treated with 400 mg/L EM@MoS$_2$ (78.10%). These data indicated that both MoS$_2$ NDs and EM@MoS$_2$ were not cytotoxic to cells even at a concentration of 200 mg/L, consistent with previous studies[35]. Of note, EM@MoS$_2$ exhibited higher cytocompatibility than MoS$_2$ NDs at a high concentration (400 mg/L), which might be attributed to the toxicity isolation effect of erythrocyte membranes.
The capacity of EM@MoS$_2$ to neutralize toxins and further avoid hemolysis was tested using α-hemolysin derived from staphylococcus aureus. As presented in Fig. 2a and 2c, after pretreated with toxin + EM@MoS$_2$ or toxin + anti-α-toxin, supernatants in samples were clear and corresponding absorbance at 540 nm was relatively low, demonstrating that EM@MoS$_2$ had an α-hemolysin neutralizing ability comparable to that of anti-α-toxin antibody. On the contrary, severe hemolysis was observed in toxin and toxin + MoS$_2$ NDs group, implying MoS$_2$ NDs alone is ineffective to prevent α-haemolysin-induced erythrocyte lysis. Subsequently, the efficiency of EM@MoS$_2$ to absorb α-hemolysin was determined. As presented in Fig. 2b and 2d, after pretreated with EM@MoS$_2$ (200 mg/L, 500 µL), toxin with an amount up to 5 µg caused no apparent hemolysis, indicating 100 µg of EM@MoS$_2$ was able to neutralize at least 5 µg of α-haemolysin. However, when toxin amount increased to 10 µg, partial hemolysis was observed, indicating that the toxins cannot be completely captured and some unbound toxins attacked the erythrocyte membrane. Furthermore, to investigate the effect of EM@MoS$_2$-detained toxins on cell adhesion and proliferation, cell growth was assessed by cytoskeleton staining (Fig. 2e). In control group, cells with spindle-like morphology spread flat and packed tightly. In contrast, in α-toxin group, the number of adhering cells decreased to a large extent, and the cytoplasm became crumpled and even cracked (white circles), suggesting severe impairment caused by the toxin. As expected, there were still a large number of cells attaching and spreading well on the plates, with only minor injuries, after treatment with toxin-bound EM@MoS$_2$. Overall, EM@MoS$_2$ was able to efficiently absorb pore-forming toxins and keep them away from normal cells, thus contributing to reducing the virulence of biofilms.

In Vitro Antibiofilm Activity of MoS$_2$ NDs and EM@MoS$_2$.  

With the bacterial metabolic pathway spontaneously altering towards anaerobic glycolysis, biofilm microenvironment is featured by a low pH (4.5-6.5) and high H$_2$O$_2$ content (100-300 µmol/L), which would promote the generation of ROS. Based on good photothermal capacities and peroxidase-like properties, the in vitro anti-biofilm abilities of MoS$_2$ NDs and EM@MoS$_2$ were evaluated. Standard plate counting tests in vitro (Fig S8a and S8c) showed that MoS$_2$ NDs and EM@MoS$_2$ reduced MRSA viability by 0.28 lg CFU/mL and 0.23 lg CFU/mL, respectively, which is accordingly equivalent to a moderate antibiofilm efficiency of 46.72% and 41.27%, respectively. Hyperthermia therapy, wherein the treating temperature was maintained slightly above 50 °C for 5 min, had a higher antibiofilm efficiency of 70.61% comparing with MoS$_2$ NDs and EM@MoS$_2$ groups. Particularly, the bacterial viability in EM@MoS$_2$ + NIR group decreased by 2.47 lg CFU/mL (antibacterial rates of 99.66%).

To visualize the role of nanomaterials in disrupting the stacking biofilms, SEM was applied to further observe the changes in bacterial morphology and density after treatment. As shown in Fig. 3a, compared with control group, bacteria cells were distributed, to a moderate extent, more sparsely and some bacterial membranes became rough even ruptured (red arrows) following treated with MoS$_2$ NDs (or EM@MoS$_2$), indicating that a certain degree of impairment had occurred. This trend was more noticeable in the NIR group. When subjected to EM@MoS$_2$ and NIR simultaneously, the amount of adherent bacteria decreased considerably and a substantial fraction of bacteria had suffered irreversible damage, including...
deformation, perforation, and fragmentation. Additionally, although sub-10 nm MoS$_2$ NDs were hardly visible in MoS$_2$ NDs group, EM@MoS$_2$ or its aggregates (denoted as red points) could be found both on the bacterial surface and within the biofilms in EM@MoS$_2$ and EM@MoS$_2$ + NIR groups. Thus, these nanomaterials were able to penetrate into the tightly packed biofilm structure to cause hyperthermia and generate ROS in situ. In summary, these findings revealed that MoS$_2$ NDs or EM@MoS$_2$ have the potential to physically disintegrate the biofilm, particularly when combined with hyperthermia therapy.

Subsequently, the residual biofilm biomass of all samples was qualitatively and quantitatively assessed using confocal laser scanning microscopy (CLSM). As shown in Fig. 3b and 3d, the control group was completely covered with a dense green biofilm structure, with nearly no red signals. While in MoS$_2$ NDs or EM@MoS$_2$ groups, a slight redshift occurred and quite a few irregular voids of varying size appeared, suggesting that a small proportion of the biofilm fragments were depolymerized from the substrates. Consequently, there were 61.05% and 69.14% biomass left respectively. In the NIR group, the red fluorescence signals were more obvious and the biofilm biomass amounted to 42.54%. Remarkably, for the EM@MoS$_2$ + NIR group, only 15.53% of the biofilm debris was attached to the implant surface, implying that the biofilm was totally dissociated from the implant. Subsequently, crystal violet staining assays (Fig S8b and S8d) revealed comparable experimental data (100%, 61.75%, 71.10%, and 35.66% for control, NIR, MoS$_2$ NDs, EM@MoS$_2$, and EM@MoS$_2$ + NIR group, respectively). These results suggested that MoS$_2$ NDs itself possesses a certain degree of bactericidal ability in the biofilm environment, which, mechanically speaking, was mediated by toxic ROS. Obviously, this ability was barely affected by the cell membrane coating of EM@MoS$_2$. And it could be speculated that the antibiofilm effect of ROS could be enhanced by hyperthermia.

Therefore, the synergistic effect between hyperthermia and ROS was investigated by detecting the ROS concentrations in each group of biofilms using 2',7'-Dichlorodihydrofluorescein diacetate (DCFH-DA), which would be converted into green fluorescent 2',7'-dichlorofluorescein (DCF) in the presence of ROS[36]. As presented in Fig. 3c and 3e, there was hardly any green fluorescence signal in control group and it was slightly enhanced in NIR group, reflecting low levels of ROS. Whereas, this signal was moderately strengthened following pretreated with MoS$_2$ NDs or EM@MoS$_2$, implying intermediate levels of ROS and it was suitable for MoS$_2$ NDs (or EM@MoS$_2$) to induce ROS generation in acidic and hydrogen peroxide-containing microenvironment of biofilms. Notably, the EM@MoS$_2$ + NIR group emitted a robustly high green fluorescence, indicating that ROS production was strongly accelerated by hyperthermia. On the other hand, the standard plate counting tests and biofilm biomass analysis concurred that the antibiofilm effect of the EM@MoS$_2$ + NIR treatment was more superior than that of the NIR treatment. This suggested that ROS-induced oxidative damage to cell membranes rendered bacteria more sensitive to heat. Overall, we have confirmed that MoS$_2$ NDs or EM@MoS$_2$ acted as a synergistic therapeutic platform for hyperthermia- and ROS-based therapy.

It is well established that drug resistance of biofilms has been a persistent and non-negligible issue[37, 38]. Thus, it seemed indispensable to investigate whether drug resistance developed in response to
EM@MoS$_2$-mediated synergistic therapy (Fig. 3f). Our results indicated that MRSA viabilities had been steadily maintained at approximately 0.5% for 8 consecutive generations of screening. This confirmed our hypothesis that the synergistic therapy dose prevented the emergence of drug resistance and remains highly effective.

Consequently, these results indicated that both MoS$_2$ NDs and fabricated EM@MoS$_2$ were effective synergistic antibiofilm agents with superior photothermal and peroxidase-like performance. This is partly because MoS$_2$ NDs and EM@MoS$_2$ met the requirements to permeate biofilms, corroborating with previous reports that stated that ideal diameter for nanoparticles to penetrate the biofilms must not exceed 200 nm.[39, 40] Moreover, an ultra-small particle size increases the specific surface-area and active sites, which improves the catalytic activity of nanoparticles.[31] Thus, the characteristics above account for the effectiveness and efficiency of MoS$_2$ NDs and EM@MoS$_2$.

**In Vitro Immunomodulatory Properties of MoS$_2$ NDs and EM@MoS$_2$.**

Despite a relatively high efficiency of MoS$_2$ NDs-based synergistic therapy in destroying biofilms, inevitably, a small number of released bacteria can survive and reconstruct biofilms using cracked EPSs in an immunosuppressive microenvironment. Therefore, more attention needs to be paid to reverse this unfavorable immune state to avoid an infection recurrence. Macrophages have a powerful phagocytic ability and possess dynamic plasticity, conferring them as the most promising target to enhance immune therapy[41]. Macrophages can differentiate into the M1 phenotype, facilitating bacterial elimination, or into the M2 phenotype, that is correlated with immune suppression and infection recurrence[42]. To this end, we treated the macrophages with MoS$_2$ NDs and first-hand discovered its superior properties to reverse macrophage phenotype from M2 to M1.

Firstly, the results of immunofluorescence staining (Fig. 4a and 4c) revealed that red fluorescence corresponding to the expression of CCR7 (an M1 marker[43]) was gradually enhanced with increasing MoS$_2$ NDs concentrations and ultimately reached a maximum when the concentration of MoS$_2$ NDs or EM@MoS$_2$ increased to 200 mg/L. In contrast, the expression of Arg-1 (an M2 marker[44]) was marginally inhibited by MoS$_2$ NDs or EM@MoS$_2$ in a concentration-dependent manner. It’s worth noting that there seemed no obvious differences in CCR7 and Arg-1 expression levels between MoS$_2$ NDs (200 mg/L) and EM@MoS$_2$ (200 mg/L) groups according to semi-quantitative analysis. Similarly, flow cytometry (Fig. 4b) was subsequently conducted to verify the immunomodulatory effect of MoS$_2$ NDs and EM@MoS$_2$ using another pair of markers, CD86 (M1 marker[45]) and CD206 (M2 marker[46]). The proportion of CD86-positive cells increased remarkably and reached a maximum of 56.99% when treated with 200 mg/L MoS$_2$ NDs, while that of the control group is 4.89%. On the other hand, the proportion of CD206-positive cells remained low, and displayed a slight downward trend (11.49-6.89%) with increasing concentrations of MoS$_2$ NDs. Meanwhile, enzyme-linked immunosorbent assay (ELISA) (Fig. 4d) showed that the secretion levels of pro-inflammatory factors, TNF-α, IL-1β, MCP-1, were elevated in a concentrate-
dependent manner, whereas that of anti-inflammatory factor, IL-10, was decreased in the same way. An identical tendency was observed at gene level using RT-PCR tests (Fig. 4e).

Therefore, we explored the intrinsic mechanisms underlying MoS$_2$ NDs-induced macrophage polarization using transcriptome sequencing. Initially, the Pearson correlation analysis was applied to evaluate the reproducibility of the samples within the groups. As shown in Fig S9a, the Pearson coefficients within groups were very close to 1, significantly greater than that between groups, indicating a high gene expression stability. The volcano plot was chosen to present the gene expression profile of macrophages (Fig. 5a). Comparing with control group, there were 1274 genes up-regulated and 830 genes down-regulated in MoS$_2$ NDs group, which were together defined as differential expressed genes (DEGs). Next, some DEGs correlated with macrophage polarization were selected and shown in the heatmap (Fig. 5b).

In MoS$_2$ NDs group, the expression levels of M1 phenotype-related genes, including that coding for surface markers (CD86, CCR7, CD80), cytokines (IL-23, IL-1a, IL-1b, IL-6, TNF-α), and upstream regulators (NF-κB) were considerably up-regulated. Conversely, the expression levels of M2-related genes, like that coding for CD206, CD301, CD115, IL-10, PPARG, and KLF4 were substantially inhibited.[44,47,48] These findings supported and complemented the RT-PCR results. Furthermore, to classify all DEGs into various biological functions, Gene Ontology (GO) analysis was performed and top 30 enriched terms are shown in Fig S9b. Obviously, multiple DEGs were implicated in macrophage polarization-related terms (i.e., inflammatory response, immune response, cell migration, and response to lipopolysaccharide).

Subsequently, the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis was used to identify the signaling pathways underlying macrophage polarization. As depicted in bubble plots (Fig. 5c and 5d), signaling pathways mediating M1 macrophage activation, including TNF, NOD-like receptor, NF-κB, and MAPK signaling pathways, had comparably high q values. This implied an explicit pro-inflammatory effect of MoS$_2$ NDs on macrophages.[49] On the other hand, signaling pathways related to M2 polarization like Jak-STAT and PPAR were significantly down-regulated[43,49]. Thus, MoS$_2$ NDs were well substantiated to be an intrinsic immunomodulator, which can promote the differentiation of macrophages to M1 phenotype to establish a pro-inflammatory microenvironment. This finding broadens the biological applications of MoS$_2$ NDs and sheds light on the mechanisms underlying immuno-antibacterial therapy mediated by MoS$_2$ NDs.

**Immuno-Antibiofilm Therapy Mediated by EM@MoS$_2$.**

The ability of macrophages to penetrate EPS and engulf bacteria largely determines the host defense response against biofilm infection[11]. Inspired by MoS$_2$ NDs’ newfound property to activate macrophages, we initially explored the ability of activated macrophages (AM) to invade biofilms using CellTracker Red CMTPX dyes to trace macrophages and CFDA-SE to trace bacteria. As shown in Fig. 5e and 5f, after two hours of coculture, the number of macrophages infiltrating either an intact or a cracked biofilm increased when stimulated by EM@MoS$_2$. This indicated that AM possessed stronger chemotactic ability than unactivated counterparts (UM). Simultaneously, we also found that both AM and UM could easily enter the broken biofilm. These results illustrated that in the process of macrophage
attacking biofilms, the characteristics of the biofilm itself, that is, the degree of fragmentation, play a decisive role, while the states of macrophages play a supporting role. Subsequently, we compared the capacities of AM and UM to phagocytose and kill biofilm fragments. As presented in Fig. 5e, macrophages possessed an intrinsic trait to phagocytose biofilm fragments (green dots) and, obviously, this ability was enhanced by EM@MoS$_2$. After phagocytosis, bacteria survival rates were evaluated by plate counting test (Fig. S10 and Fig. 5g), which suggested that AM have a stronger bacteria-killing efficiency than UM. Thus, it could be concluded that EM@MoS$_2$ NDs could act as an immunological adjuvant to endow macrophages with potent bactericidal ability. In summary, it seems to be a more compelling antibiofilm strategy to initially destroy the biofilm structure and subsequently drive the macrophages to eliminate biofilm debris in comparison with monotherapy.

**Combinatory Antibiofilm Effect of EM@MoS$_2$ in Vivo.**

In order to assess the ability of EM@MoS$_2$ to synergistically eliminate biofilms on implants in vivo, we constructed a mouse subcutaneous implant-related infection model. Firstly, we took general pictures of all mice on 1st, 3rd, 7th, 10th, and 13th day to dynamically monitor skin lesions (Fig S11). Evidently, varying degrees of skin impairments, including skin edema, subcutaneous purulent exudates, and skin ulceration and necrosis, had occurred in all groups except for EM@MoS$_2$ + NIR group over the course of thirteen-day observation. Thereafter, magnetic resonance imaging (MRI) was employed to accurately evaluate the severity of the subcutaneous infections. As presented in Fig. 6a and 6g, the high signal oval regions around the PEEK discs on T2-weighted image represented inflammatory exudates, pus, or liquefactive tissues. The average abscess volume in MoS$_2$ NDs group (173.2 mm$^3$) was smaller than that in the control and NIR groups (393.3 mm$^3$ and 307.1 mm$^3$, respectively). Strikingly, the extent of infection was substantially reduced in mice treated with EM@MoS$_2$ and was almost negligent in those belonging to EM@MoS$_2$ + NIR group. After the mice were sacrificed, the infection status of peri-implant soft tissues was further assessed. As shown in Fig. S12, the PEEK discs of control and NIR groups were completely covered with pus and necrotic tissues. And there was hardly any visible bleeding in control group, implying an impaired blood supply. However, white pus was remarkably decreased in MoS$_2$ NDs group. In comparison, no purulent exudates were visible and the implants were encapsulated by a layer of vascularized fibrous tissues in EM@MoS$_2$ and EM@MoS$_2$ + NIR groups. Lastly, H&E staining was used to evaluate the degree of edema and inflammatory reaction (Fig. 6b and 6h). The thicknesses of skin connective tissues were decreased obviously after various treatment, especially in EM@MoS$_2$ and EM@MoS$_2$ + NIR groups. Similarly, a downward trend could be observed as for inflammatory cell infiltration. These results implied that MoS$_2$ NDs could appreciably relieve the infection symptoms, which was probably achieved through a combination of direct antibiofilm effect and immunomodulatory mechanisms. Moreover, EM@MoS$_2$ provided better protection on peri-implant soft tissues, indicating that the pore-forming toxins play a critical role in spreading of infection, and erythrocyte membrane coating of EM@MoS$_2$ exhibits a superb protective effect against bacterial toxins.
We further estimated the antibiofilm ability of MoS\(_2\) NDs and EM@MoS\(_2\) in vivo. Plate counting tests (Fig. 6c, d for implant, and i, j for peri-implant soft tissues) revealed that the bacterial burden after treatment with MoS\(_2\) NDs decreased by 0.82 lg CFU/ml (implant) and 0.60 lg CFU/ml (peri-implant tissue). This indicated a more remarkable antibiofilm effect in vivo than that in vitro, possibly attributable to the immunomodulatory role of MoS\(_2\) NDs in polarizing resident macrophages. Noteworthily, we also observed that the number of live bacteria in EM@MoS\(_2\) group was significantly reduced whether in implant (\(p<0.01\)) or in peri-implant tissues (\(p<0.05\)) compared with that in MoS\(_2\) NDs group. This was distinctly different from our in vitro analysis results wherein MoS\(_2\) NDs and EM@MoS\(_2\) showed similar antibiofilm effect. This difference may be attributed to the adsorption of a large amount of toxins by EM@MoS\(_2\) that could, otherwise, impair macrophage functions. Additionally, the EM@MoS\(_2\) + NIR group had only sporadic bacterial colonies on blood agar plates, suggesting a relatively powerful antibiofilm efficacy. Afterwards, residual biofilms were observed through SEM (Fig. 6e) and Giemsa staining (Fig S13). In control and NIR groups, a large number of dense bacterial communities were stacked on the implant surfaces or embedded in surrounding soft tissues (marked as red arrows). And the bacterial loads in other groups were correspondingly reduced in the same magnitude as plate counting tests. In general, MoS\(_2\) NDs or EM@MoS\(_2\) possess excellent in vivo antibiofilm abilities, especially combined with hyperthermia therapy.

Subsequently, immunofluorescence staining was performed to further evaluate the in vivo immunoregulatory properties of MoS\(_2\) NDs or EM@MoS\(_2\). As shown in Fig. 6f, Arg-1-positive M2 macrophages accounted for a higher proportion than CD86-positive M1 macrophages in control and NIR groups, which illustrated that the biofilm itself would skew macrophages from M1 to M2 phenotype. However, in other three groups treated with MoS\(_2\) NDs or EM@MoS\(_2\), the proportion was just the opposite, that is, M1 macrophages occupied the dominant position. Thus, the above results indicated that even in a complex in vivo biofilm microenvironment, the immunoregulatory mechanisms of MoS\(_2\) NDs still worked, which would contribute to reverse immunosuppressive microenvironment and prevent the recurrence of infection. Lastly, according to histologic examination of the vital organs (the heart, liver, spleen, lung, and kidney; Fig S14) of the mice, there was no visual damage among all the groups, implying that these nanomaterials were nontoxic and safe for in vivo application.

**Conclusion**

In conclusion, an erythrocyte membrane cloaked MoS\(_2\) nanodots-based biomimetic nanoplatform (EM@MoS\(_2\)) was prepared for the integrated treatment of implant-related infection. Through the membrane cloaking strategy, the merits of an erythrocyte membrane were coated to the nanoplatfrom, which can effectively absorb the bacterial toxins to alleviates the toxic effects of infection. MoS\(_2\) nanodots, the core components of nanoplatfrom, are characterized with several alluring merits: (i) ultra-small particle size that facilitates biofilm penetration and bacteria contact, which is one of the determinants for biofilm disruption; (ii) superior photothermal conversion efficiency and favorable
peroxidase-like activity, which achieves synergistic antibiofilm effect; (iii) unique immunomodulatory property to induce macrophage polarization from M2 to M1, thus leading an enhanced bactericidal ability. From the in vitro and in vivo evaluations, significant synergistic antibiofilm treatments and persistent immunomodulatory effects of EM@MoS$_2$ have been verified, simultaneously. The goal of our present study is to shed more light on the direction of MoS$_2$-based 2D nanomaterials for triggering macrophage-related immunity. Also, the combinatory strategies orchestrating hyperthermia/ROS antibiofilm strategy, anti-virulence therapy and immunomodulatory therapy will pave a new way for anti-IRI treatments.

**Declarations**

**Supporting Information**

Supporting Information accompanies this paper at: https://doi.org/xx.xxxx/xxxx xxxxxxxxx.

**Additional file 1.**

Materials; Cell lines and bacteria; Additional experimental details (including extraction of erythrocyte membrane fragments, characterization of EM@MoS$_2$, photothermal effect of MoS$_2$ NDs and EM@MoS$_2$, peroxidase activity of MoS$_2$ NDs and EM@MoS$_2$, biocompatibility of MoS$_2$ NDs and EM@MoS$_2$, standard plate counting tests and crystal violet staining for in vitro antibiofilm effect, cell culture and intervention for the assessment of immunoregulatory potential, immuno-antibiofilm therapy mediated by EM@MoS$_2$, and animal experiment ethics); Primers used in the RT-PCR experiment (**Table S1**); The XPS survey spectrum of MoS$_2$ NDs (**Fig S1**); Line-scan TEM-EDS elemental distribution curves of PVP-modified MoS$_2$ NDs (**Fig S2**); Zeta potentials of EMFs, MoS$_2$ NDs, and EM@MoS$_2$ dispersions in DI water (**Fig S3**); Temperature changes of EMFs, MoS$_2$ NDs, and EM@MoS$_2$ dispersions under irradiation of 808 nm laser at the power density of 1.0 W cm$^{-2}$ for 10 min (**Fig S4**); The temperature-time curve for EM@MoS$_2$ dispersion during four laser on/off cycles (**Fig S5**); UV-vis absorbance spectra of ox-TMB in the presence of H$_2$O$_2$ and EM@MoS$_2$ at different PH values (**Fig S6**); Cell viability of RAW 264.7 cells measured by CCK8 assay (**Fig S7**); In vitro antibiofilm effect of MoS$_2$ NDs and EM@MoS$_2$ (**Fig S8**); RNA-seq analysis of RAW 264.7 gene expression (**Fig S9**); Representative photographs of bacterial colonies from UM + CB and AM + CB groups (**Fig S10**); Typical photos of mouse subcutaneous implant-related infection models after different treatments over the course of thirteen-day observation (**Fig S11**); General observation of implants together with peri-implant soft tissues incised from infection area (**Fig S12**); Giemsa staining image showing residual biofilms in peri-implant soft tissues (**Fig S13**); Images of H&E-stained sections of major organs (heart, liver, spleen, lung and kidney) after different treatments (**Fig S14**).

**Authors’ contributions**

Tingwang Shi: Conceptualization, Investigation, Data curation, Methodology, Formal analysis, Validation, Writing – original draft. Zesong Ruan: Investigation, Data curation, Methodology, Resources, Software.
Weijuan Zou: Software, Investigation, Validation. Xiaojun Cai: Methodology, Formal analysis, Supervision, Resources. Jianrong Wu: Resources, Supervision, Writing – review & editing, Project administration. Yuanyi Zheng: Conceptualization, Funding acquisition, Writing – review & editing, Supervision, Project administration. Yunfeng Chen: Conceptualization, Funding acquisition, Supervision, Resources. All authors discussed the results and approved the final version.

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Availability of data and materials

The datasets and materials used in the study are available from the corresponding author.

Ethics approval and consent to participate

All the animal procedures were performed under the protocol approved by the Institutional Animal Care and Use Committee of Shanghai Jiao Tong University Affiliated Sixth People's Hospital (Animal Welfare Ethics acceptance number No: DWLL2021-0845). All the animal experimental operations were in compliance with the National Guidelines for Animal Protection.

Consent for publication

All authors have approved the manuscript be submitted.

Competing interests

The authors declare no competing interests.

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Supplementary

Schema 1 is available in supplementary section

Figures
Figure 1

(a) Mo 3d and (b) S 3p XPS spectra of MoS2 NDs. TEM image of (c) MoS2 NDs and (d) EM@MoS2. (e) Particle-size distribution of MoS2 NDs and EM@MoS2. (f) Protein analysis of EMFs, MoS2 NDs, and EM@MoS2 by using SDS-PAGE. (g) UV-vis absorption spectra of EMFs, MoS2 NDs, and EM@MoS2. (h) Concentration and (i) laser power densities-dependent photothermal curves of EM@MoS2 dispersion. UV-vis absorbance spectra of ox-TMB in the presence of various concentration of (j) H2O2 and (k) EM@MoS2. (l) ESR spectra of EM@MoS2 at different pH values with 5,5-dimethyl-1-pyrroline N-oxide (DMPO) as the spin trap.
Figure 2

In vitro toxin neutralization performance of EM@MoS2. (a) Hemolytic photographs of erythrocytes after different treatments. (b) Qualitative analysis of antihemolytic capacity of EM@MoS2 by toxin titrating experiment (c) Quantitative analysis of (a). (d) Quantitative analysis of (b). (e) Fluorescence staining of L-929 cells after treated with PBS (control), toxin, or toxin + EM@MoS2. The white circles indicate cell injury. Note: * (p<0.05), ** (p<0.01), *** (p<0.001), **** (p<0.0001).
Figure 3

In vitro antibiofilm activity of MoS2 NDs and EM@MoS2. (a) SEM images of biofilms after different treatments. Red arrows indicate damages, and pink dots indicate EM@MoS2. (b) Confocal 3D images of biofilms. (c) Representative fluorescent images of biofilms after stained with DCFH-DA. (d) Quantitative analysis of remaining biofilm biomass. (e) Quantitative analysis of ROS concentration in biofilms (f)
Detection of resistance of MRSA biofilms to EM@MoS2 + NIR during 8 consecutive generations. Note: * (p<0.05), ** (p<0.01), *** (p<0.001), **** (p<0.0001).

**Figure 4**

In vitro immunomodulatory properties of MoS2 NDs and EM@MoS2. (a) Represent immunofluorescent images of RAW 264.7 cells after stimulated by MoS2 NDs or EM@MoS2. CCR7 (red), Arg-1 (green), and DAPI (blue). (b) Flow cytometric analysis of CD86 (M1) and CD206 (M2) expression of RAW264.7 cells.
after treated for 24 h. (c) Semi-quantitative analysis of immunofluorescent images. (d) ELISA results of proinflammatory cytokines (TNF-α, IL-1β, MCP-1) and anti-inflammatory cytokines (IL-10). (e) RT-PCR results of M1-related genes (TNF-α, IL-1β, MCP-1) and M2-related genes (IL-10). Note: * (p<0.05), ** (p<0.01), *** (p<0.001), **** (p<0.0001).

Figure 5

(a) Volcano plot showing DEGs between control group and EM@MoS2 group. (b) Heatmap showing macrophage polarization-involved DEGs and their expression difference. (c) Top 15 up-regulated
pathways and (d) Top 15 down-regulated pathways implicated in macrophage polarization using KEGG enrichment analysis. (e) Confocal 3D images of biofilms after cocultured with macrophages for 2 h. (f) Confocal images showing phagocytosis of macrophages on cracked biofilms. (g) Quantitative analysis of infiltrating macrophages in biofilms. (h) Quantitative analysis of bacteria survival rates using standard plate counting tests. Note: * (p<0.05), ** (p<0.01), *** (p<0.001), **** (p<0.0001).

Figure 6
Combinatory antibiofilm effect of EM@MoS2 in vivo. (a) Representative MRI images of subcutaneous abscesses of different groups on 13th day. (b) H&E staining images (Scale bar: 100 μm) of peri-implant soft tissues. (c, d) Representative photographs of bacterial colonies derived from implants and peri-implant soft tissues. (e) SEM images of biofilms in vivo. (f) Immunofluorescence staining image showing iNOS (M1, red) expression level and Arg-1 (M2, green) expression level. (g) Quantitative analysis of abscess volume in MRI examination. (h) Quantitative analysis of the thickness of connective tissues in H&E staining. (i, j) Quantitative analysis of bacterial colonies from different groups using standard plate counting test. Note: * (p<0.05), ** (p<0.01), *** (p<0.001), **** (p<0.0001).

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