

Supporting Information

Janus Mesoporous Organosilica/Platinum Nanomotors for Active Treatment of Suppurative Otitis Media

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Experimental Section

Materials and Reagents.

Ammonia aqueous solution (25 wt%), n-hexane were purchased from Macklin. Tetraethoxysilane (TEOS), absolute ethanol, 1,4-bis (triethoxysilyl)-propane tetrasulfide (BTES), (3-aminopropyl) triethoxysilane (APTES), cetyltrimethylammonium bromide (CTAB) were purchased from Aladdin (Shanghai, China). Levofloxacin (LVF), glutathione (GSH), N,N-Dimethylformamide (DMF) and hydrochloric acid (HCl) was purchased from Sinopharm (Shanghai, China), phosphate buffered saline (PBS), Fetal bovine serum (FBS), penicillin, and streptomycin were purchased from Invitrogen. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), fluorescein isothiocyanate isomer I (FITC), SYTO 9/PI LIVE/DEAD BacLight Bacterial Viability Kit were purchased from Thermo Fisher Scientific. Deionized (DI) water obtained from a Milli-Q water system was used for all the experiments. All materials and reagents used were of analytical grade and without any further purification.

Characterization.

A scanning electron microscope (SEM, Hitachi SU8200) and a transmission electron microscope (TEM, Hitachi HT7800) were used to image the morphology of the nanoparticles and nanomotors. TEM and energy dispersive spectrometer (EDS) mapping images were acquired via a high-resolution transmission electron microscope (Talos F200x). The surface areas of the samples were determined by Bruner-Emmett-Taylor method (BET, ASAP 2020M). UV absorbance and spectrum were recorded via a UV spectrophotometer (Lambda 750, PerkinElmer). Fourier transform infrared (FT-IR) spectra were recorded via a Frontier FT-IR spectrometer (Nicolet IS50, Thermo Scientific). An electron beam evaporation system (Hitachi MC1000) was used to deposit the Pt layer. The absorbance for MTT analysis and the bacterial concentration was recorded using a microplate reader (Synergy NEO, BioTek, Vermont). A thermostatic shaker (THZ-300C), a super clean bench (SW-CJ-2FD), and a biochemical incubator (SPX-150BSH-type II) were used to culture the bacteria. Confocal images

were obtained by Leica SP8 confocal laser scanning microscope (CLSM). The flow cytometry analysis measured on BD Biosciences FACS AriaIII instrument.

Fabrication of MOS nanoparticles. Briefly, 0.32 g of CTAB was dissolved in a mixed solution containing 60 mL of ethanol, 150 mL of water, and 2 mL of concentrated aqueous ammonia solution (25 wt%). The mixture was stirred at a speed of 170 rpm for 1 h at 25 °C, and 50 mL of n-hexane was slowly added along the wall of the bottle. The water-ethanol/n-hexane biphasic solution was left in the water bath for 10 min without stirring. Then a mixture of TEOS (0.50 mL) and BTES (0.50 mL) was added dropwise into the n-hexane. The products were then collected by centrifugation at 13000 rpm for 10 min and washed 3 times with ethanol. To remove the CTAB template, the as-prepared materials were extracted 3 times in 200 mL ethanol containing 200 μ L concentrated HCl at 60 °C for 3 h. Finally, the products were washed three times with ethanol and dried under vacuum overnight.

Preparation of MOS/Pt nanomotors. The as-prepared MOSs were dispersed in a water/ethanol solution (V/V = 1:1) and dropped onto a piranha solution treated glass slides to form a monolayer structure. After evaporation of the ethanol in air, a MOSs monolayer formed on the glass slides which was then transferred to the sputter coater. A 5 nm Pt layer was then deposited on the surface of the MOSs by physical vapor deposition, and finally the MOS/Pt were released from the substrate by weak scrape.

Loading of LVF into MOS/Pt nanomotors. 20 mg LVF was dissolved in 5 ml acetonitrile/methanol (ACN/MeOH) (10/90). Then 40 mg of MOS/Pt were added to the drug solution and kept stirring for 12 h. The solutions were centrifuged at 20000 g for 10 min and save the supernatant. After that, the MOS/Pt nanomotors were washed and centrifuged again. The precipitation was the prepared LVF-MOS/Pt nanomotors, the supernatant was saved.

To determine the loading amount of LVF, the absorbance intensity of the LVF-MOS/Pt nanomotors suspension and the two saved supernatants were measured using a UV-vis spectrophotometer at the wavelength of 289 nm. The percent of LVF loaded was calculated using the following equation:

% loading = absorbance intensity of LVF-MOS/Pt nanomotors / (absorbance intensity

of LVF-MOS/Pt nanomotors + absorbance intensity of all supernatants).

Propulsion study of MOS/Pt nanomotors. The as-fabricated MOS nanomotors were dispersed into hydrogen peroxide solutions with different concentrations and the motion behaviors were recorded using an OLYMPUS IX73 inverted microscope equipped with an OLYMPUS DP74 camera and acquired at a frame rate of 20 frames/s using the OLYMPUS software. Particle tracking and velocity analysis were performed using the ImageJ with the plugin MTrackJ.

LVF Releasing Triggered by GSH

To investigate their release behavior, LVF-MOS/Pt (1 mg/mL) were dispersed in PBS (pH 7.4) solutions with or without 1 mM GSH and gently shaken at 120 rpm at 37 °C. At a predetermined time, the solution was centrifuged, and the supernatant was removed and replaced by the same amount of fresh PBS solution. The release amount of LVF was quantified by UV-vis measurement.

GSH-responsive degradation of MOS/Pt nanomotors. 1 mg of MOS/Pt nanomotors were mixed with 1 mM GSH in 10 mL of PBS buffer was stirred at 37 °C. At a predetermined time, aliquots were taken and washed with PBS before performing the TEM analyses.

***In Vitro* Cytotoxicity Evaluation of MOS/Pt nanomotors.** The standard MTT assay was used to evaluate cell viabilities. The L929 mouse fibroblasts cells were seeded in 96-well cell culture plates at a density of 1×10^5 cells per well and incubated overnight and then cultured with different conditions.

After 20 h, different concentrations of MOS/Pt and LVF-MOS/Pt nanomotors dispersed in DMEM medium were added to the cell culture. Five duplicate wells were set up for each concentration. After 8 h, the medium was replaced with 10 vol% MTT in culture medium and incubated for a further 4 h. The cell culture medium was then removed and replaced with 150 μ L DMSO. After shaking for 10 min, the absorbance value was measured using an automated microplate reader (BioTek) at a wavelength of 490 nm.

Antibacterial assays of LVF-MOS/Pt nanomotors. *S. aureus* were resuspended in LB medium and diluted to an optical density of 0.35 at 600 nm. Then 500 μ L of bacterial

solution was mixed with another 500 μL of PBS (pH 7.4), H_2O_2 , LVF, MOS/Pt, LVF-MOS/Pt and LVF-MOS/Pt- H_2O_2 and then placed in a biochemical incubator for 8 h (37 $^\circ\text{C}$, 0 rpm). As a result, the final concentrations of the *S. aureus* solutions contained 3% of H_2O_2 , the MOS/Pt concentration was 200 $\mu\text{g}/\text{mL}$, LVF-MOS/Pt concentration was 200 $\mu\text{g}/\text{mL}$, LVF concentration was 10 $\mu\text{g}/\text{mL}$. For membrane integrity studies, PI and SYTO9 DNA dye were added to the 1 mL bacterial suspension with a concentration of 10 $\mu\text{g}/\text{mL}$. After staining for 15 min, the bacteria were washed with 0.85% NaCl and resuspended in 0.85% NaCl. The suspension was then spotted onto a microscope slide and examined by CLSM. Then, the antibacterial difference was quantitatively measured using flow cytometry.

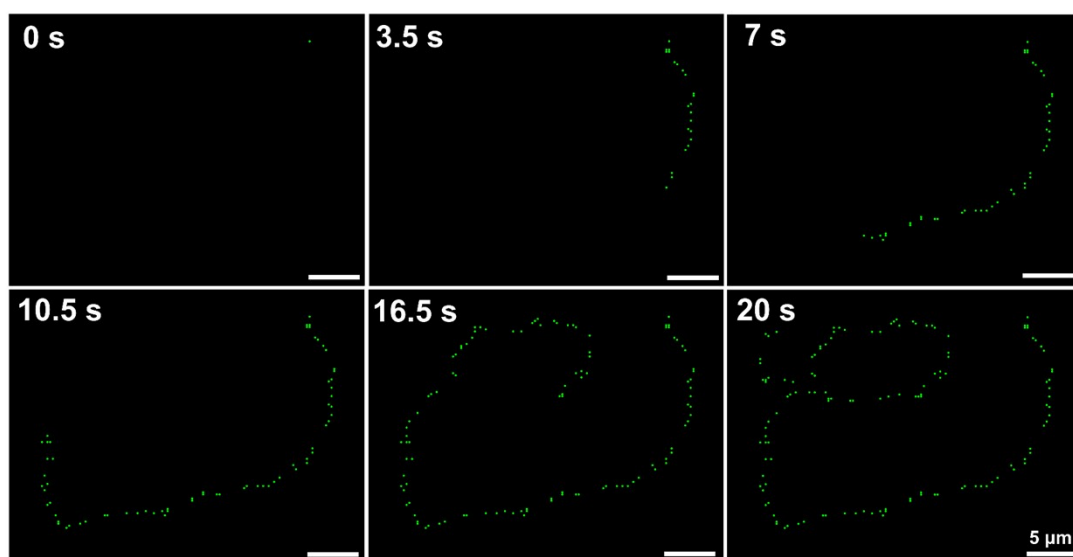


Figure S1. Time-lapse images of MOS/Pt nanomotors self-propulsion in 3% H₂O₂ solution.

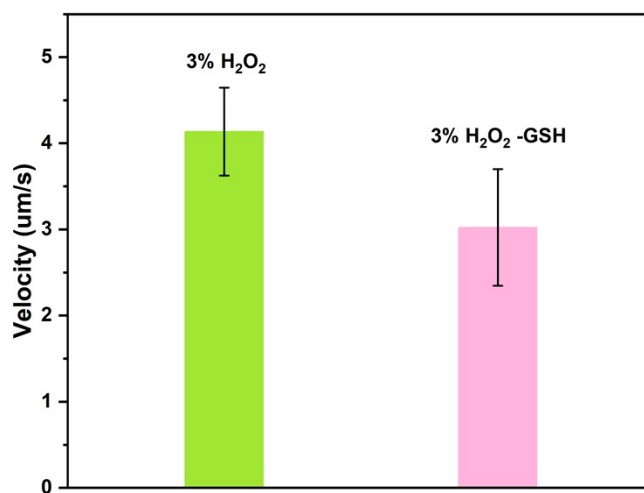


Figure S2. Velocity of MOS/Pt nanomotors in 3% H₂O₂ solution before and after being treated with GSH (1mM) for 2 h.

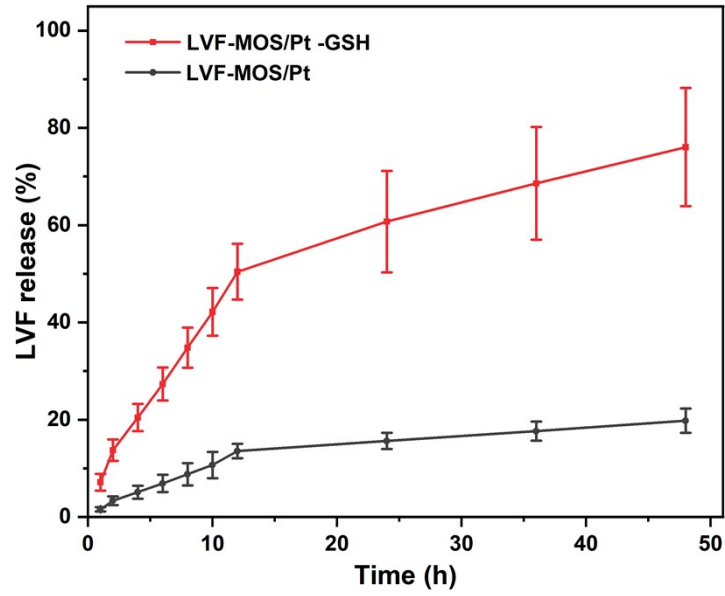


Figure S3. The LVF release plots of MOS/Pt nanomotors in the presence or absence of GSH (1 mM).

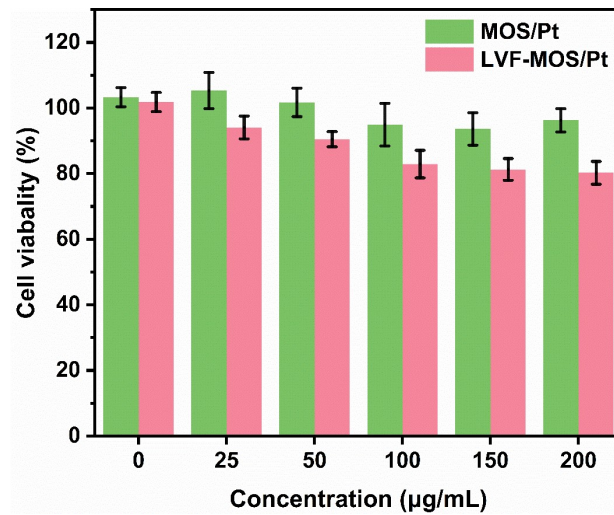


Figure S4. Cell viability of L929 mouse fibroblast cells incubated with the MOS/Pt and LVF-MOS/Pt at different concentrations.