Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Contents

Section 1. Materials and Materials Characterization

Materials

Luria Broth and Agar Bacteriological were purchased from Hopebio Company (Shandong, China). The reagents for the preparation of final materials, including the n-Hexane (99%, China), Ferrocene (98%, China), n-Butyllithium solution (China), *N,N,N,N*-Tetramethylethylenediamine (99%, China), Dichloromethane (99%, Chain), Petroleum ether (China), Ethylacetate (99%, China), cis-Cinnamaldehyde (99%, China), D-Mannitol (98%, China), and N,N-Dimethylformamide (99%, China) were purchased from the commercial suppliers, which was used directly without further purification.

Materials Characterization

The morphologies of powder samples were evaluated by the scan electron microscopy (SEM, Caisi Sigma 300) and the transmission electron microscopy (TEM, JEOL JEM2100PLUS) via dipping the prepared samples on a Cu-net. X-ray diffraction (XRD) parameters were obtained using a Rigaku Ultima IV diffractometer at a rate of 5^{o} min^{-1} from 5^{o} to 90^{o}. The Brunauer-Emmett-Teller (BET) method was utilized to calculate the specific surface area. The adsorption and desorption measurements for N_2 were performed on ASAP2460 (Micromeritics) at low temperature of 77 K. Fourier Transform Infrared Spectroscopy (FTIR) was performed on KBr pellets in the range from 4000 to 400 cm^{-1} using Thermo Nicolet iS5. Measurement of absorbance of samples by UV spectrophotometer (UV-8000), Shanghai Yuananalysis Instruments Co. The chemical composition of the as-synthesized monomer was analyzed by the ^{1}H NMR spectrometer (avance-400 MHZ NMR of Bruker company, Switzerland), mainly using deuterium DMSO as solvent

Section 2. Experimental Section

1. Synthesis of 1,1'-Ferrocenedicarboxaldehyde

The reaction was carried out in a Schlenk-type apparatus under argon. Ferrocene (5 g, 26 mmol) in 75 mL of dry hexane was treated with 24 mL of 2.5 M n-butyllithium (60mmol) in hexane, and subsequent addition of 8.5 mL of N,N,N,N-

Tetramethylethylenediamine (TMEDA)(56 mmol). The reaction mixture was stirred for 20 h, then 6.5 ml of DMF was added dropwise at -78oC. After 2 h stirring, the mixture was hydrolysed at -78 ^{o}C. The organic phase was extracted with CH2Cl2, and dried over Na2SO4, and the solvent then removed. 1,1-ferrocenecarboxaldehyde was purified with silica gel column when the flow phase was petroleum and ethyl acetate(5:1). A bright red crystal was obtained (4.15 g) with the yield of 63.5%. 1H NMR (400 MHz, Chloroform-d) δ 9.94 (s, 2H), 4.87 (s, 4H), 4.66 (s, 4H)

2. Synthesis of FMC-POP

Ferrocenedicarboxaldehyde (2 mmol), trans-cinnamaldehyde (1 mmol), mannitol (2.5 mmol) and N,N-methylformamide (DMF) were placed in a reaction vessel with a tetrafluoroethylene inner layer. The reaction was carried out at 180 °C for 24 h. After cooling to room temperature, the reaction kettle was opened and the solid was filtered under reduced pressure. Then, the solid was washed several times with distilled water until the supernatant was clear and colorless, and dried under vacuum at 80°C for 24 hours.

3. **Calculation of photothermal conversion efficiency:** The FMC-POP aqueous solution was irradiated by 808 nm laser for 10 min (1.2 W cm^{-2}). Then the heated aqueous dispersion was cooled down naturally and the temperatures during the cooling process were also carefully monitored every by a thermometer with a thermocouple probe. The photothermal conversion efficiency (η) was calculated by the following equation:

$$\eta = [hS\,(T_{max}-T_{surr})-Q_{Dis}]/I\,(1-10^{-A\lambda}) \quad (S1)$$

Where, ‘h’ is the heat transfer coefficients. ‘S’ is the surface area of the container; ‘T_{max}’ is the equilibrium temperature after 10 min irradiation; ‘Q_{Dis}’ refers to the heat dissipation by the test cell; ‘I’ represents 808 nm laser power (1.2 W cm^{-2}) and ‘A_{808}’ is the absorbance of the FCM-POP aqueous solution at 808 nm. The value of hS is determined according to the following equation:

$$hS = m_d C_d/\tau_S \quad (S2)$$

Where ‘m_d’ is the mass (1 g) and ‘C_d’ is the heat capacity (4.2 J/g) of the aqueous solvent; ‘τ_S’ is the sample system time constant and ‘θ’ is defined as the ratio of ΔT and ΔT_{Max}.

$$t = -\tau_S\,(In\ \theta) \quad (S3)$$

4. **Bacteria culture:** As a proof-of-concept, gram negative Escherichia coli (E.coli) and gram-positive Staphylococcus aureus (S. aureus) were employed in the experiment. Bacteria cells were prepared by inoculating a single bacterial colony from a LuriaBertani (LB) plate and then suspended in 5 mL sterile Luria-Bertani medium at 37 °C and shook at 110 rpm for 12 h. The number of bacteria was estimated by measuring the medium absorbance at 600 nm using a UV-Vis spectrophotometer.

5. **In vitro detection of ROS:** 3,3',5,5'-Tetramethylbenzidine (TMB) can be catalyzed by peroxidase to produce a soluble blue product with a specific absorption peak at 652 nm. This property allows us to detect the in vitro catalytic release of ROS from FcMC aggregates. Firstly, FMC-POP (200 μg/mL, 1 mL), TMB (1.5 mM, 1 mL), PBS (pH=4), and H_2O_2 (500 μL) were divided into four groups according to the control variables method, namely FMC+TMB+PBS, FMC+TMB+PBS+H_2O_2, FMC+PBS+H_2O_2, and TMB+PBS+H_2O_2. The mixtures were incubated for 10 min against light, and then the FcMC was filtered off with a 0.22 μm filter membrane, and the absorption of the mixtures in the range of 750-450 nm was detected by UV spectrophotometry. Different concentrations of FMC-POP (50, 100, 150, 200, 250, 300 μg/mL) were mixed with TMB (1.5 mM, 1 mL) + PBS (pH=4) + H_2O_2 (500 μL) and conditioned with PBS so that the final volume of the mixture was 4 mL. After incubation for 10 minutes protected from light, the mixture was filtered through a 0.22 μm filter membrane and measured under UV spectrophotometer. The absorption was measured under UV spectrophotometer in the range of 750-450 nm. Similar to the above operation, the concentration of FMC-POP was fixed at 150 μg/mL and the pH value of PBS was set at 1.5, 2.5, 3.5, 4.5, 5.5 and 6.5, and the absorption at 750-50 nm was measured after incubation and filtration with TMB and H_2O_2. The same mixture of FMC-POP (150 μg mL), TMB (1.5 mM, 1 mL), H_2O_2 (500 μL), and PBS (pH=4) was prepared. The same mixture 1 and mixture 2 were prepared, and mixture 1 was illuminated under an 808 nm laser (1.2 W cm^{-2}) for 10 min. Mix 1 was irradiated with 808 nm laser (1.2 W cm^{-2}) for 10 min, while Mix 2 was incubated for 10 min under low light, and then Mix 1 and 2 were filtered through a membrane to measure the UV absorption. The UV absorption was measured, and the data were collected and plotted.

6. **NIR Laser-Mediated PTT/enzyme Synergetic Antibacterial Effects In Vitro**: Before

investigating the NIR laser-mediated synergetic photothermal/enzyme antibacterial effects, the OD_{600} (optical density at 600 nm) method was used to evaluate the antibacterial capacities of FMC-POP. In vitro bacterial experiments were divided into seven groups by adding 400 μL of 10^4 CFU mL^{-1} bacterial solution (E. coli or S. aureus) to 2 mL EP tubes: PBS group (pH=5.5), FMC-POP group (200 μg/mL), H_2O_2 group (100 μL), laser group (808 nm, 1.2 W cm^{-2}, ten minutes), FMC+ H_2O_2 group FMC-POP +laser group, and FMC+H_2O_2+laser group. After the treatment was completed according to the grouping requirements, a constant temperature shaking window (110 rpm, 37°C) was placed to incubate for 12 hours, and then 100 μL of the blowing solution was transferred to the solid medium and coated well, and incubated at 37 °C for 24 hours to observe the morphology of the clones. Calculate the colonies and compare the bacterial activity with each group

7. **Live/Dead Bacterial Cell Staining:** SYTO-9 and PI were used to distinguish between live and dead microbial cells. SYTO-9 labels bacterial cells in green owing to penetrating all bacterial membranes (intact and damaged), while PI can only penetrate injured cell membranes labeling the cells red while diminishing the green resulting from SYTO-9 staining. In detail, 400 μL of *E. coli* or *S. aureus* (10^8 CFU mL^{-1}) culture suspensions treated with PBS, FMC-POP, H_2O_2, laser, FMC+ H_2O_2, FMC-POP +laser, and FMC+H_2O_2+laser group, were treated with 20 μL of SYTO-9 (1.0×10^{-3} m) and 20 μL of PI (1.5×10^{-3} m) in the dark for 15 min at 37 °C. After staining, the treated samples were centrifuged with PBS to remove excess SYTO-9 and PI. Bacteria were then re-suspended in 50 μL of PBS and placed on the surfaces of slides. The slides were used to capture the images of the stained *E. coli* or *S. aureus* using Inverted fluorescence microscope using a 60× magnification.

8. Transmission electron microscopy of bacteria: Take the bacterial solution of Staphylococcus aureus or Escherichia coli treated as described above. Bacterial cells were fixed with 2.5% glutaraldehyde solution (4°C, 24 h), washed three times with PBS, embedded in agar, and blocked. The bacteria were then dehydrated by sequential treatment of ethanol solutions (30%, 50%, 70%, 90%, 95%, and 100%) for 10 min each at room temperature, followed by dealcoholization with acetone for 3 h at room temperature, embedding by gradient penetration of the embedding medium, negative staining, and sectioning on a nickel mesh for TEM observation. Their morphology was obtained.

Section 3. ^{1}H-NMR Spectra

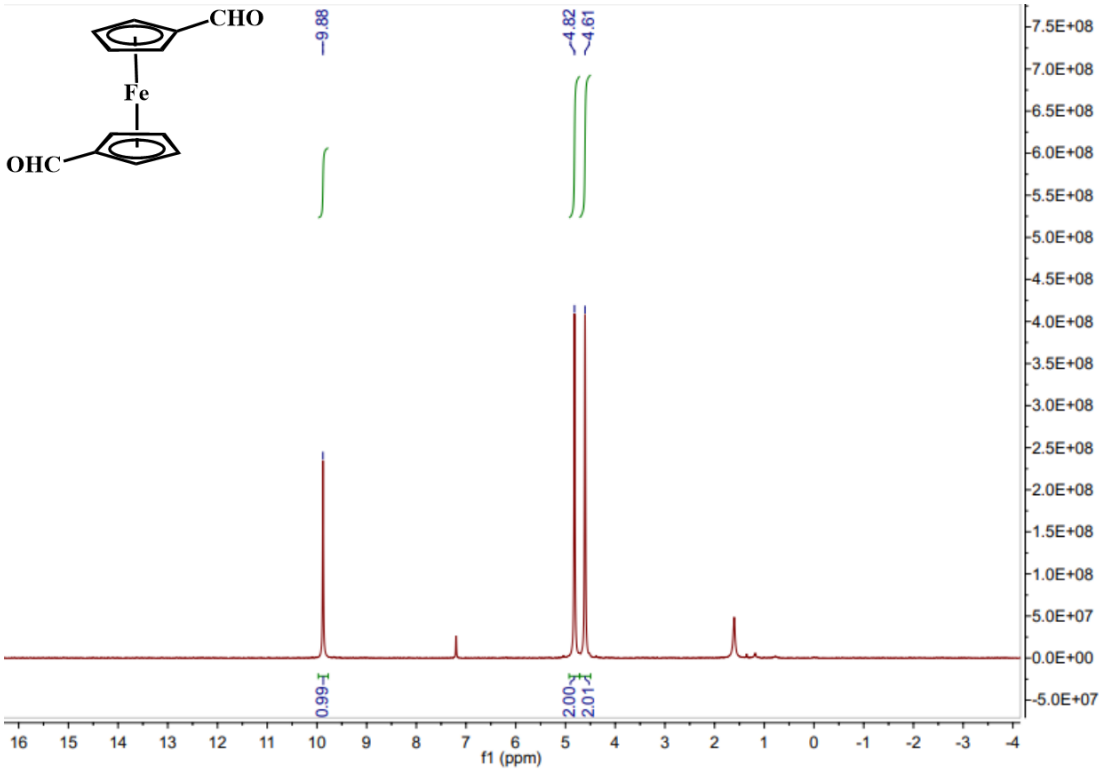

Figure S1. The ^{1}H NMR spectrum of the 1,1'-Ferrocenedicarboxaldehyde.

Section 4. Comparison of FMC-POP dispersion in different solvents

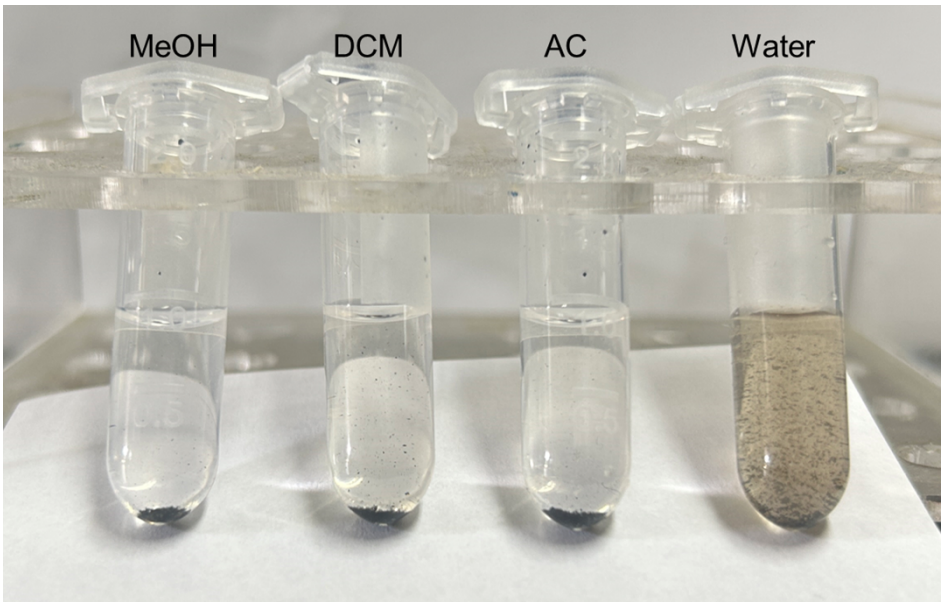

Figure S2. Comparison of FMC-POP dispersion in different solvents

Section 5. TGA of FMC-POP

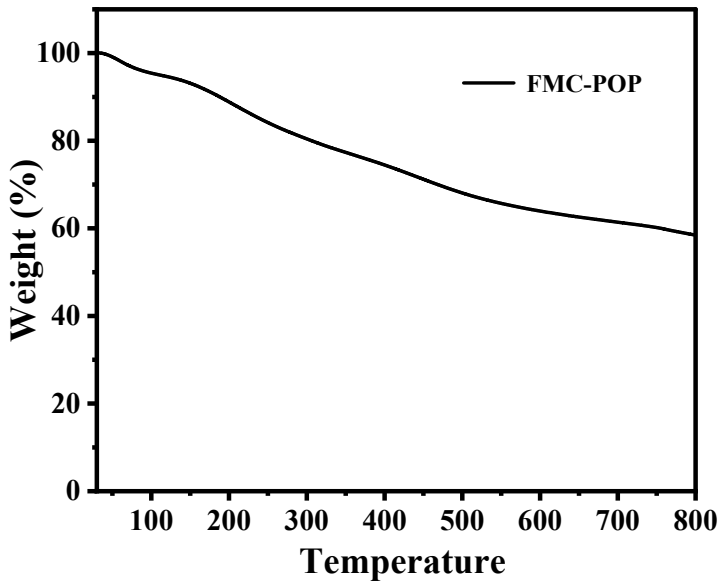

Figure S3. TGA of FMC-POP

Section 6. EDS and from TEM mapping

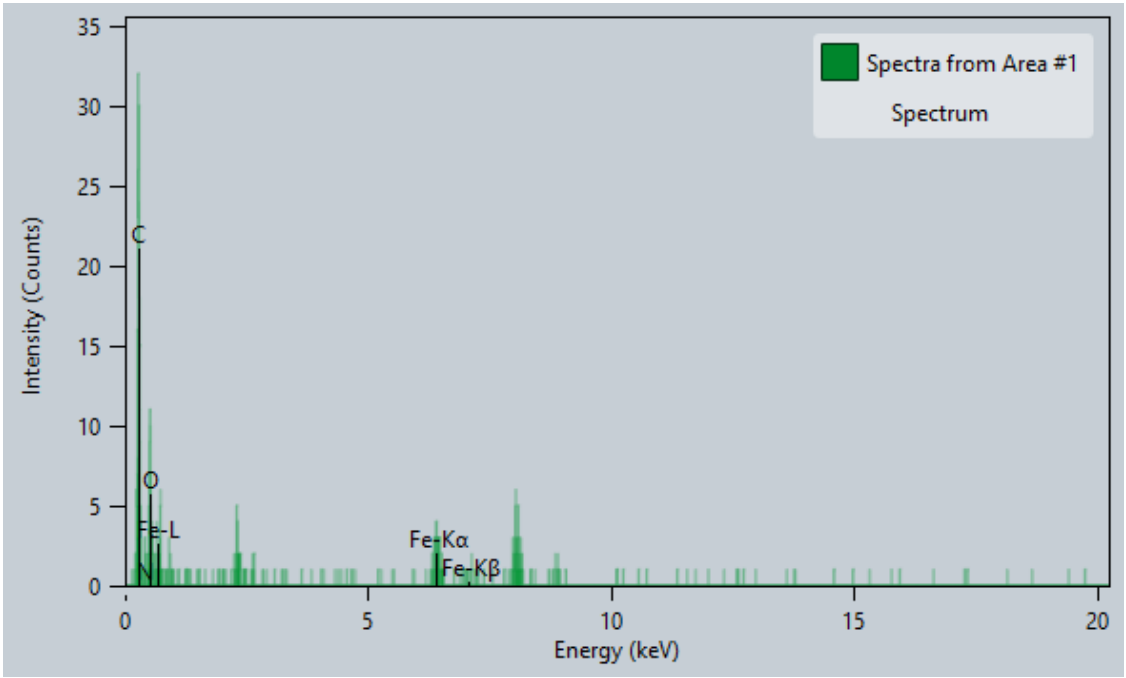

Figure S4. EDS and from TEM mapping

Section 7. UV-vis-NIR spectra

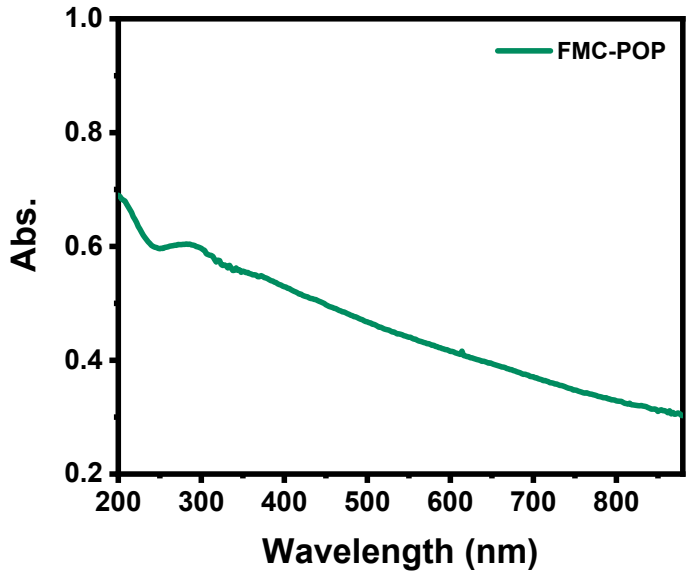

Figure S5. UV-vis-NIR spectra of FMC-POP. Linear time data vs. -lnθ obtained from the cooling stage

Section 8. Linear time data vs. -lnθ obtained from the cooling stage

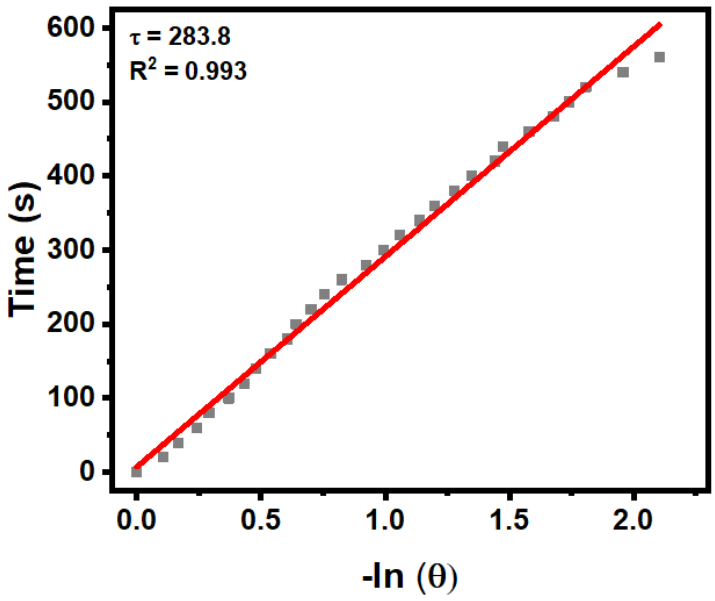

Figure S6. Linear time data vs. -lnθ obtained from the cooling stage

Section 9. UV-vis-NIR spectra of FMC-POP for photostability testing

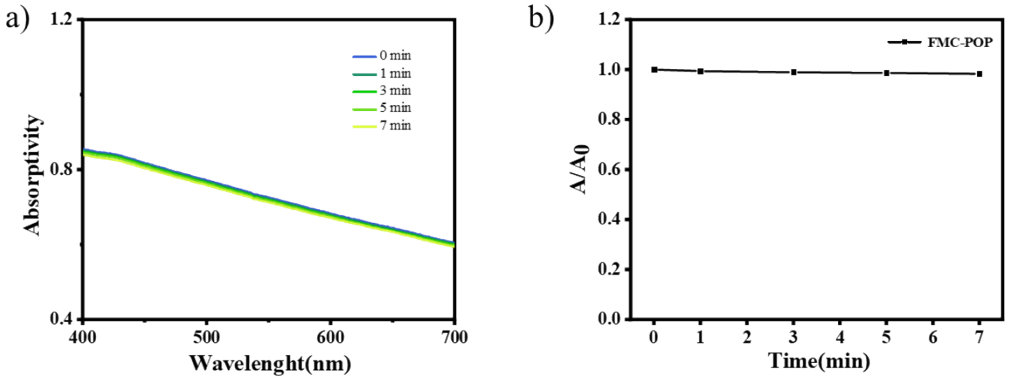

Figure S7. a) UV absorbance after irradiation with a 638 nm laser (1.0 W/cm^2) at different time intervals (0 min, 1 min, 3 min, 5 min, and 7 min) and b) Trend graphs of the changes

Section 10. EPR spectra

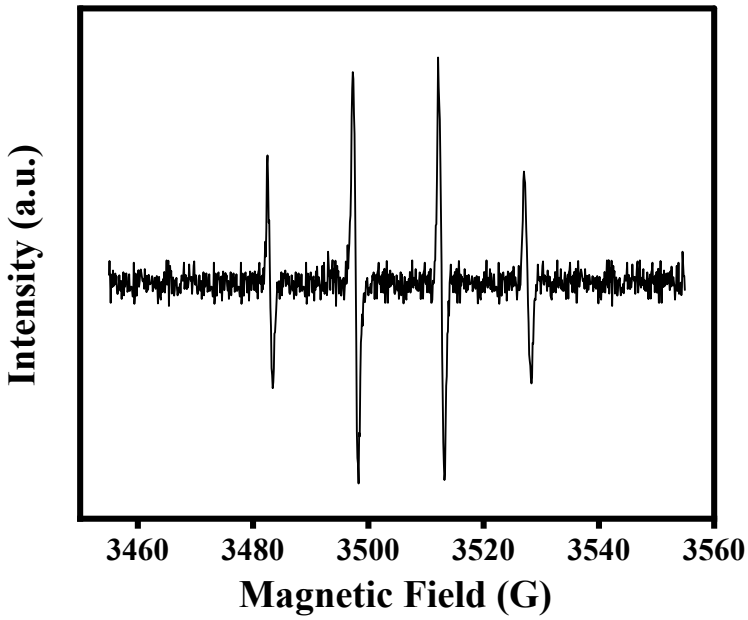

Figure S8. ESR spectra for detection of hydroxyl radical in the presence of DMPO

Section 11. Biocompatibility test of FMC-POP

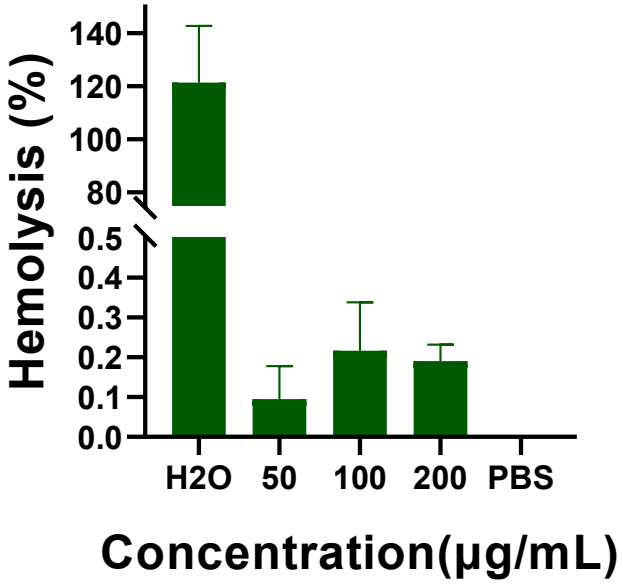

Figure S9. Hemolysis test of FMC-POP

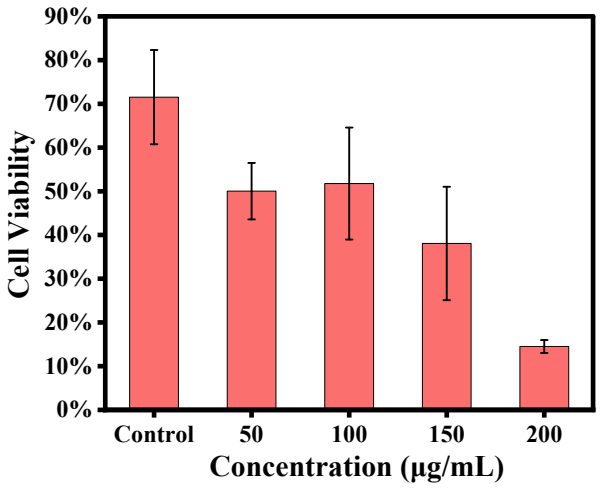

Figure S10. Cytotoxicity of FMC-POP toward L929 cells.