Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Lactic-co-glycolic acid-coated methylene blue nanoparticles with enhanced antibacterial activity for efficient wound healing

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Preparation of MPNPs

MB (Sigma-Aldrich, USA) solution (10mg/ml) with 3% NaCl was used as the internal aqueous phase. 1% and 0.5% PVA (Jinan Daigang biological engineering co. LTD) solution was configured as the external aqueous phase. Weigh 200 mg PLGA (Jinan Daigang biological engineering co. LTD) and dissolve it in 5.5 mL ethyl acetate. Put 0.5 mL the inner water phase into the oil phase, and the colostrum is formed by ultrasound under the ice bath. The colostrum was poured into 10 mL of 1% PVA external water phase, and the pre-mixed milk was prepared by ultrasonic ice bath. The premixed milk was poured into 10ml 0.5% PVA solution to produce the premixed milk, stirred at 300-400rpm at room temperature for 3-4h and obtained PLGA nanoparticles .Then, the solution was centrifuged at 4℃ at 10000rpm for 10min and distilled water was added for ultrasonic dispersion.

Bacteria Growth.

The Gram-negative bacteria E. coli (ATCC25922) and Gram-positive bacteria S. aureus were used to study the activities of MPNPs antibacterial. Methicillin-resistant Staphylococcus aureus (MRSA) used in the experiment as drug resistant strain was isolated from the swine and gifted from College of Veterinary, China Agricultural University. All apparatus and materials were autoclaved and handled under sterile conditions during the experiments. E. coli and MRSA were revived with Luria−Bertani (LB) broth and nutrient agar at 37 °C for 24 h. The density of bacterial cells in the liquid cultures was estimated by optical density (OD) measurements at 600 nm wavelength. The cell suspensions used for antibacterial activity contained 1×10^5 colony-forming

units (CFU) mL^{-1}.

In Vitro Antibacterial Evaluation

PBS, MB and MPNPs stock solution was diluted to concentrations (100 μg mL^{-1}) followed by bacterial staining process. Next, the bacterial suspensions were exposed to light irradiation for 10 min (1 W cm^{-2}), respectively. Meanwhile, the PBS groups, MB groups and MPNPs groups were incubated in the darkness at 37℃. The bacterial suspensions were diluted to 1×10^5 fold with 1×PBS. 100 μL diluted bacterial cells wasspread on the solid LB agarplate, followed by culturing at 37 ℃ for 24 h before colony forming units (CFU) counting and taking photos.The killing efficiency was determined by dividing the number of CFU with PBS, MB and MPNPs groups without light and PBS, MB and MPNPs groups with light irradiation. The five repetitions of each sample were performed and each experiment was carried out in duplicate.

Minimum Inhibitory Concentration Test.

A sterile 96-well plate was used in MIC test. The modified resazurin method was used. Briefly, a volume of 100 μL of test material in sterile water was pipetted into the 1−9 columns of the plate. Tips were discarded after use such that each well had 50 μL of the test material in serially descending concentrations. To the wells in columns 10−12 of the plate, 100 μL of nutrient broth or sterile saline was added. Serial dilutions were performed using a multichannel pipet. Finally, 10 μL of bacterial suspension (5×10^6 CFU mL^{-1}) was added to each well to achieve a concentration of 5×10^5 CFU mL^{-1}. Each plate had a set of controls: the 10 column with streptomycin as positive control, the 11 column with all solutions with the exception of the test compound as negative

control, and the 12 column with all solutions with the exception of the bacterial solution adding 10 μL of nutrient broth instead as blank control. The plates were incubated at 37 °C for 18 h. Then 0.0675% resazurin solution was prepared, and a 10 μL aliquot was added to each well and incubated at 37 °C for 4 h. The color change was then assessed visually. Any color changes from purple to pink or colorless were recorded as positive. The lowest concentration at which color change occurred was taken as the MIC value. The average of three values was calculated, and that was the MIC for the test material and bacterial strain.

Cell Culture and Treatment.

4T1 cells (Mouse mammary tumor cell line) were maintained in high-glucose Dulbecco's modified Eagle's medium (DMEM), which was supplemented with 10% fetal bovine serum, 100 units mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin at 37 °C in a humidified atmosphere of 5% CO2. The cytotoxicity of MPNPs was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) viability assay. After coincubating the cells with MPNPs for 24 h at a series dosage, MTT solution was added to each well. After 4 h of incubation at 37 °C, colorimetric measurements were performed at 495 nm on a scanning multiwell spectrometer. Data were expressed as mean ± standard error of mean of at least five independent experiments.

Detection of Single Oxygen.

MPNPs and MB were dispersed in water solution for single oxygen tests. The detailed procedure according to the method described previously. Trapping agent utilized in this test was DMPO. Trapping agent was pipetted into solution and mixed homogeneously

as preparation and then capillaries were introduced to load samples. Then the capillaries were placed into Electron Paramagnetic Resonance (JEOL, JES-FA200) to collect information of radicals.

SEM of Bacteria Samples.

MRSA were incubated with MPNPs at 37℃ for 6 h and washed with PBS for three times. The cells were suspended by PBS solution and drop on 9 mm cover glasses. The specimens were fixed in 3% glutaraldehyde for 3 h then submerged in acetone solutions at concentrations of 10%, 20%, 40%, 60% and 80% for 15 min and later in 100% acetone for 1 h. After 24h desiccation at 37°C, they were mounted on aluminum stubs with copper tape, afterwards coated with gold in a low-pressure atmosphere using an ion sputter coater. The surface topographies of the bacteria cells were visualized and photographed using the SEM.

Animal Anti-infection Model Assay.

Every five mice were housed in stainless steel cages containing sterile paddy husk as bedding in ventilated animal rooms. Mice were anaesthetized with isofluorane and were disinfected with ethanol (70%) and shaved on the middle of the back (approximately a one-inch by one-inch square region around the injection site) one day prior to infection. Skin on the back was punctured with sterile syringe needle and inoculated with 5×10^7 CFU mL^{-1} MRSA 100 μL sterile PBS. MRSA was transferred to fresh TSB and shaken at 37°C until an OD600 value of 1.0 was achieved. The cells were centrifuged, washed once with PBS, re-centrifuged, and then re-suspended in PBS. Clinical examination and washes bacterial culture and biochemical identification were performed to verify mice

skin infection model. Mice received no treatment were used as blank control. 100 μL MPNPs and MB solutions in PBS with 2 mg mL^{-1} final levels were inoculated the wound site at 8 h later after infection. All animals were treated single time and followed 7 days recovery. Each group of mice receiving a particular treatment regimen was housed separately in a ventilated cage with appropriate bedding, food, and water. Mice were checked twice daily during infection and treatment to ensure no adverse reactions were observed. Infected animals were divided into three groups including. On the 24 hours, 3 days and 7days, clinical examination, bacterial culture and biochemical identification were performed. Colonies were then enumerated and reported as recovered colony forming units (CFU) per mL of washing fluid. The skin tissue from each mouse was fixed in 10% buffered formalin phosphate at room temperature followed by paraffin embedding. Histological slide preparation and H&E staining were performed. H&E stained mouse histology sections collected above were visualized on an optical microscope (Olympus X71, Japan). All the identity and analysis of the pathology slides were blind to the pathologist.

Statistical Analysis:

Results were expressed as mean ± standard deviation (S.D). Multigroups comparisons of the means were carried out by one-way analysis of variance (ANOVA) test using SPSS 16.0 (SPSS Inc., Chicago, IL). The statistical significance for all tests was set at $p< 0.05$.

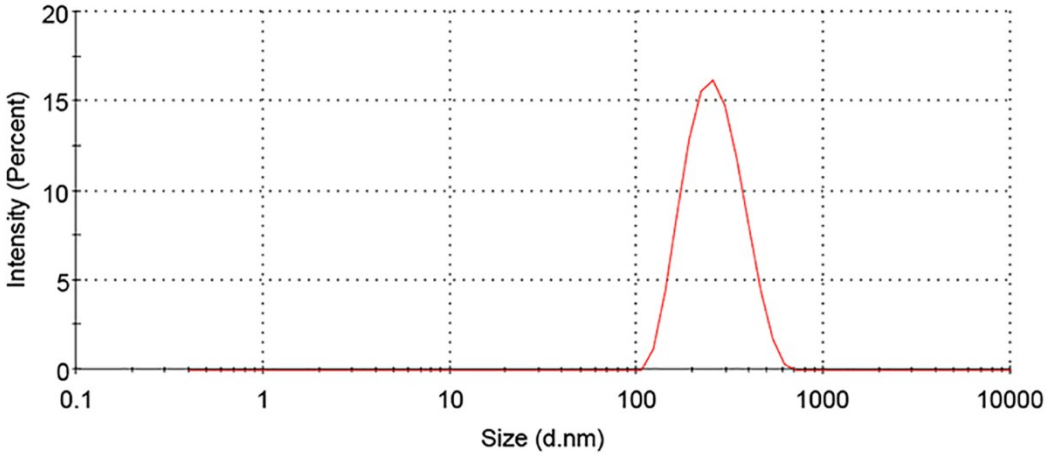

Fig.S1 The DLS analysis of **MP NPs** in water.

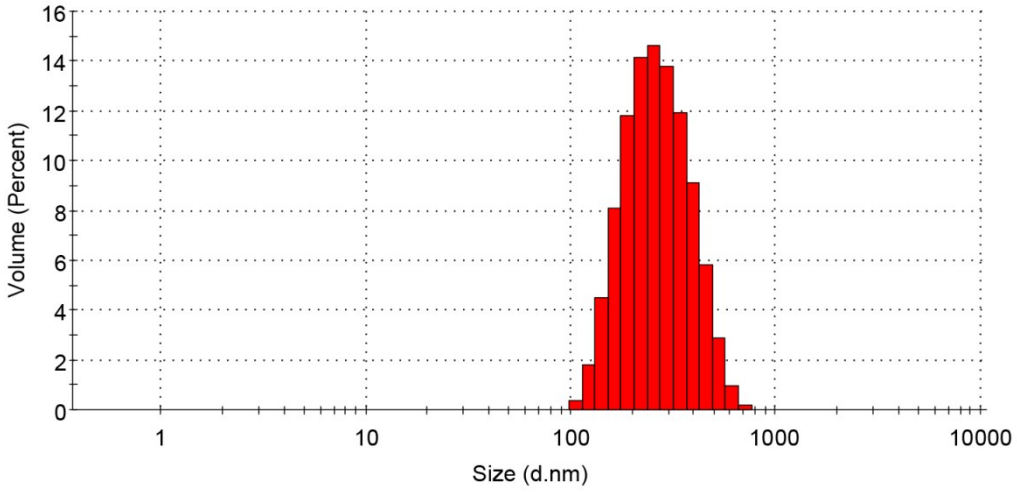

Fig.S2 Hydration diameter distribution of MPNPs in water.

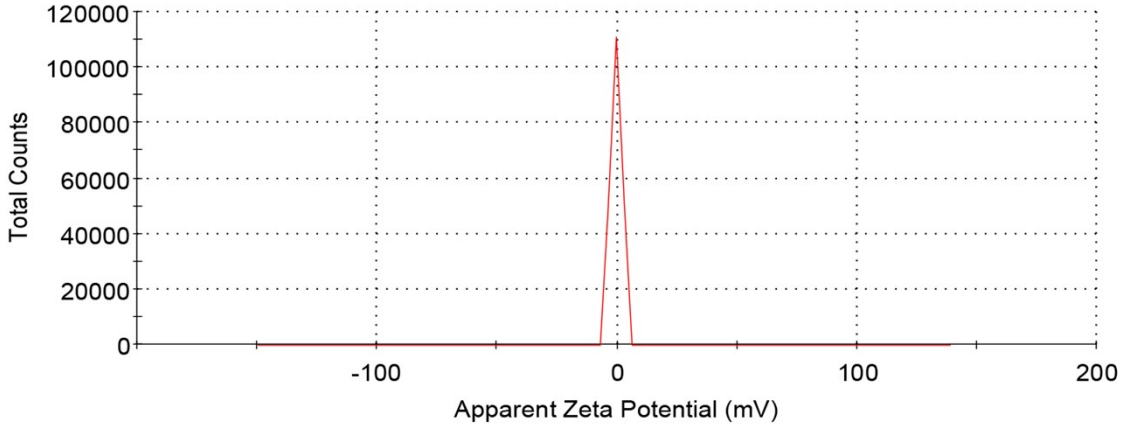

Fig.S3 Zeta of MP NPs in pure water.

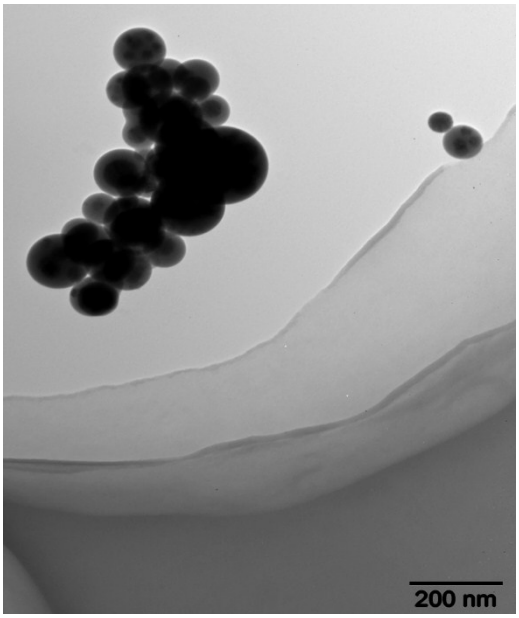

Fig.S4 Transmission electron microscopy (TEM) image of **MP NPs**.

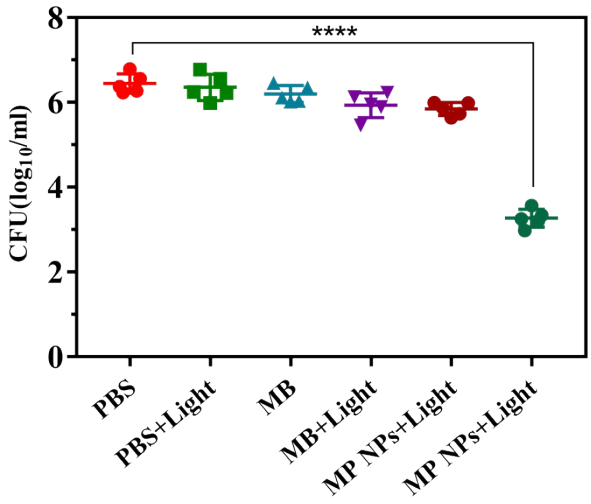

Fig.S5 The numbers of viable *S.aureus* in infected wounds after different treatments determined by the plate counting method.

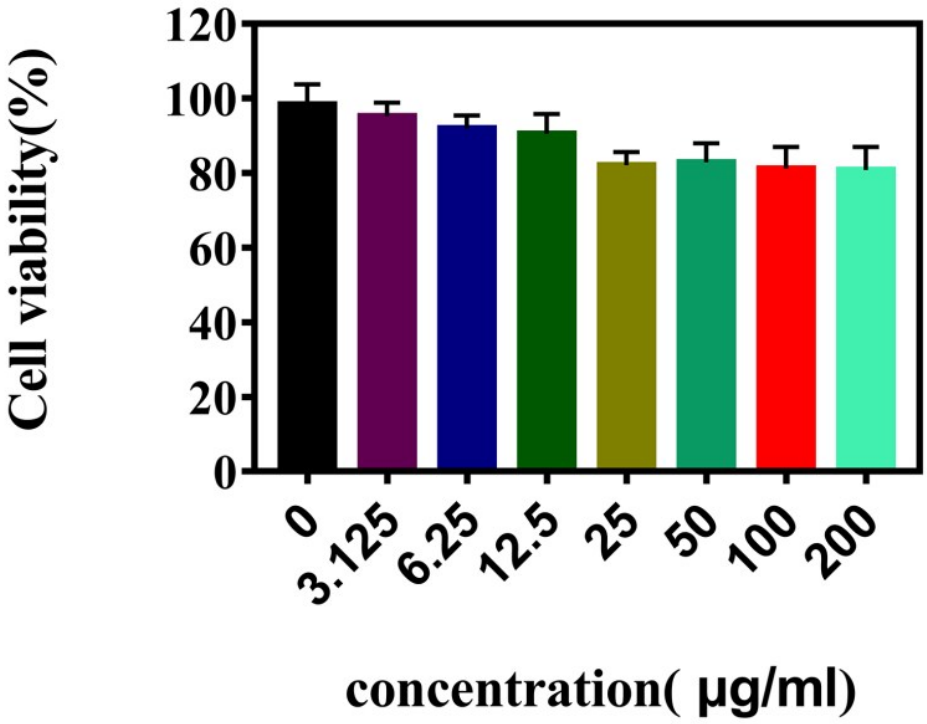

Fig.S6 Relative cell viability of 4T1 cells after 24 h incubation with **MPNPs** (3.125, 6.25, 12.5, 25, 50, 100, and 200 μg mL^{-1}).

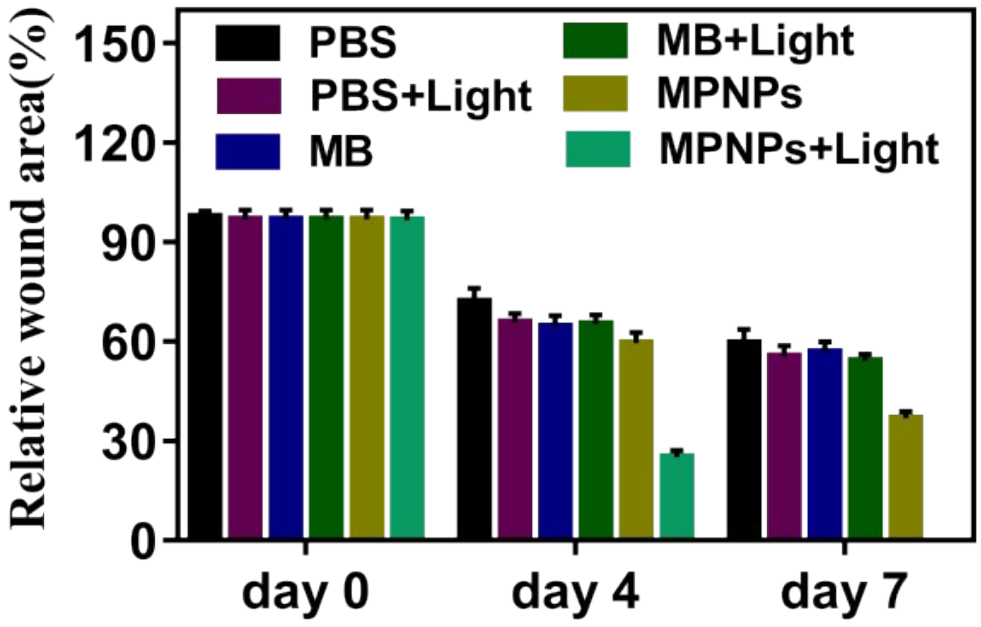

Fig.S7 The relative wound area in (PBS, PBS+Light, MB, MB+Light, MBNPs and MPNPs+Light group).

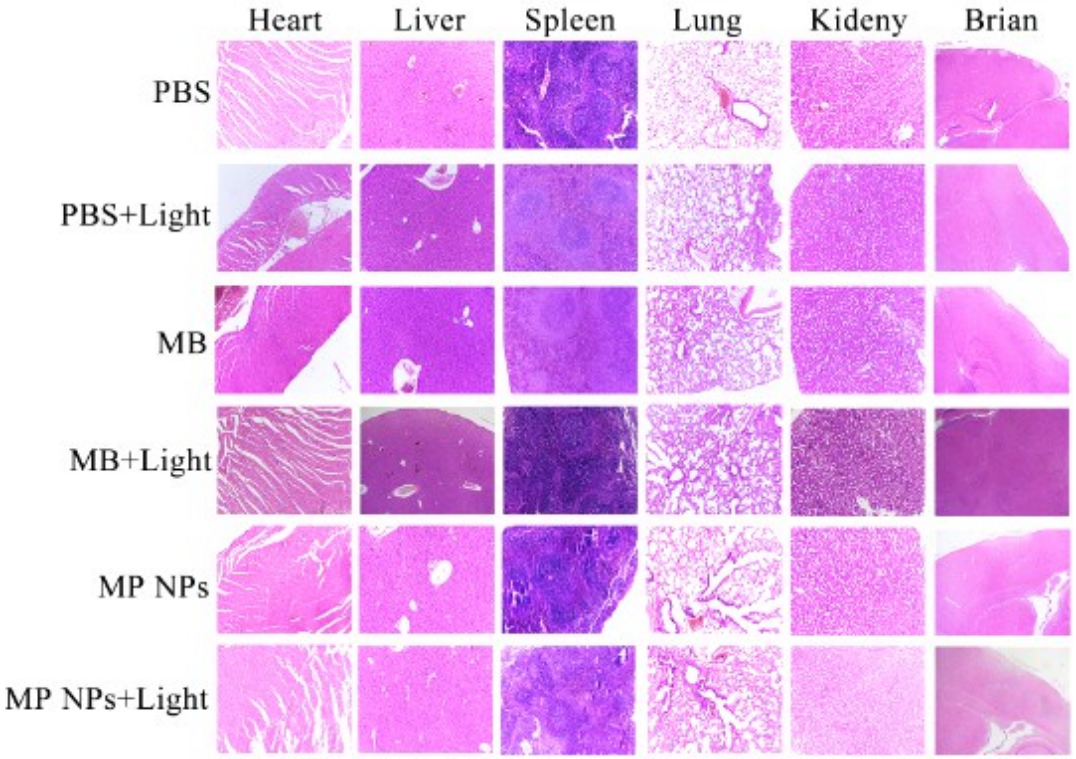

Fig.S8 Hematoxylin and eosin stained images of major organs of all groups.

Table S1 Restrain rate of different groups against *E. coli* and MRSA.

Group	*E. coli*	MRSA
MPNPs+light	99.99%	99.67%
MPNPs	35%	30%
MB	36%	27%
MB+light	80%	75%
PBS	0%	0%
PBS+Light	25%	40%