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Biodegradable $Zn_xNi_{1-x}S$ hollow nanospheres for NIR-driven photothermal antibacterial therapy

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Materials

All chemicals were of analytical grade and used without further purification.

Hexadecyl trimethylammonium bromide (CTAB, $C_{19}H_{42}BrN$, 99%) and Poly (maleic anhydride-alt-1-octadecene) ($PMHC_{18}$, 99.0%) were obtained from Shanghai Macklin Biochemical Co., Ltd. L(+)-Ascorbic acid (AA, $C_6H_8O_6$, 99.7%), ethylene glycol ($C_2H_6O_2$, 99%), hexamethylenetetramine (HMTA, $C_6H_{12}N_4$, 99%) and zinc nitrate hexahydrate ($Zn(NO_3)_2{\cdot}6H_2O$, 99%) were gained from Sinopharm Chemical Reagent Co., Ltd. Nickel acetate tetrahydrate ($C_4H_6NiO_4{\cdot}4H_2O$, 99%), thiourea (H_2NCSNH_2, 99%) and mPEG-NH_2 (95.0%) were obtained from Aladdin Industrial Corporation. BacLight™ Kit L-7012 was purchased from Thermo Fisher Scientific Inc. Glutaraldehyde ($C_5H_8O_2$, 2.5 wt%) was purchased from Beijing Leagene Biotechnology Co., Ltd. Both LB broth medium and LB agar slants were bought from Hangzhou Microbial Reagent Co., Ltd. Gram-negative bacteria, DH5α (*E. coli*), and Gram-positive bacteria, ATCC43300 (*MRSA*) were grown in Luria-Bertani (LB) broth medium at 37°C. Human umbilical vein endothelial cells (HUVEC), normal human hepatocytes (L02) and mouse mammary epithelial cells (HC11) were obtained from the American Type Culture Collection (ATCC). Deionized water was supplied through a Milli-Q water system. 3-(4, 5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide (MTT) were obtained from Gibco Life Technologies.

Live/Dead Bacterial Staining Assay

The bacterial suspensions were labeled using a Live/Dead BacLight bacterial viability kit containing PI and SYTO 9. Typically, the bacterial samples treated with different conditions

were co-cultured with PI and SYTO 9 for 20 min in dark, and then the live and dead bacteria could be observed by the fluorescence microscopy.

Morphological Characterization of Bacteria

The bacteria samples treated with different conditions were washed with PBS for several times and then fixed with 2.5% glutaraldehyde for 12 h, followed by dehydration of ethanol of gradient concentrations (30%, 50%, 70%, 90%, 100%). Finally, the samples after freeze-dry could be observed with SEM.

Minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) Determination

E. coli (DH5α) and methicillin-resistant *S. aureus* (ATCC43300) were used for antibacterial assay. 100 μL of bacterial stored at -80°C was added to 100 mL sterile LB broth medium at 37°C within a shaking incubator under 150 rpm overnight. ZNSP with different concentrations (from 0 to 100 μg mL^{-1}) were respectively mixed with 200 μL of diluted bacteria (1×10^6 CFU mL^{-1}) in a 96-well plate. Each group contained three parallel experiments. After irradiated by NIR laser (808 nm, 2.0 W cm^{-2}) for 3 min, the mixtures were incubated at 37°C for 12 h in the shaking incubator. The minimum inhibition concentration (MIC) of the antibacterial samples could be observed in the 96-well plate where the mixtures were clear with no visual turbidity of bacteria. Then the MBC was evaluated by plate count method. 100 μL of the mixtures from MIC were symmetrical delivered onto the surface of LB agar plates and then incubated at 37°C for 24 h. The MBC could be obtained where the bacteria was killed completely.

Supporting Figures

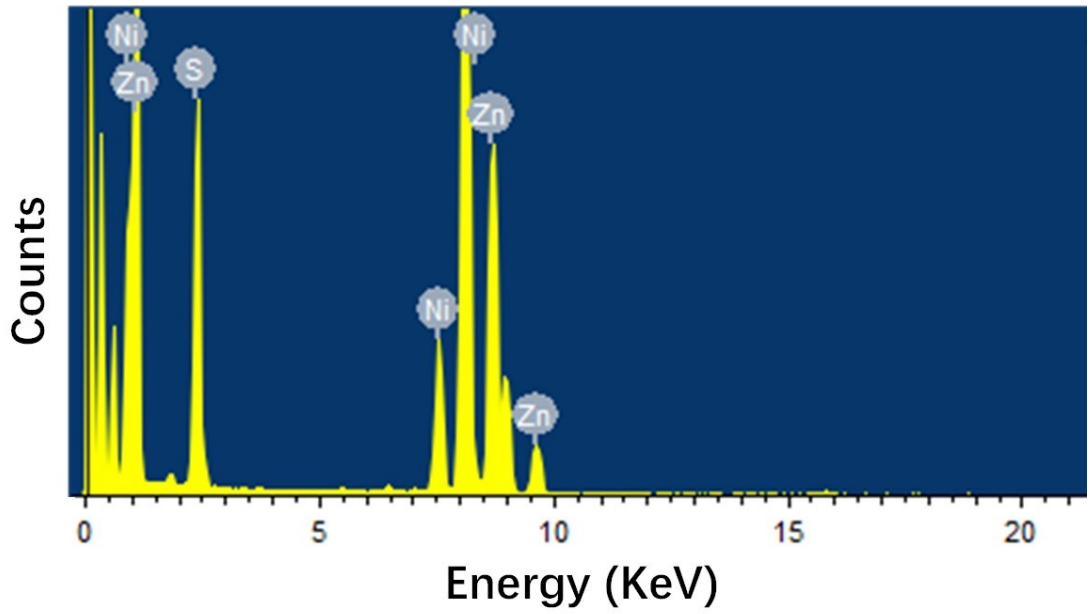

Fig. S1. Energy dispersive X-ray spectra of the as-obtained ZNSP nanostructures.

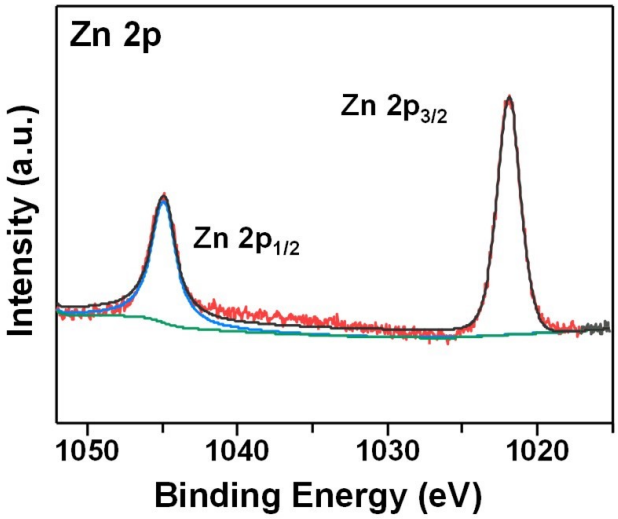

Fig. S2. Zn 2p of as-obtained ZNS.

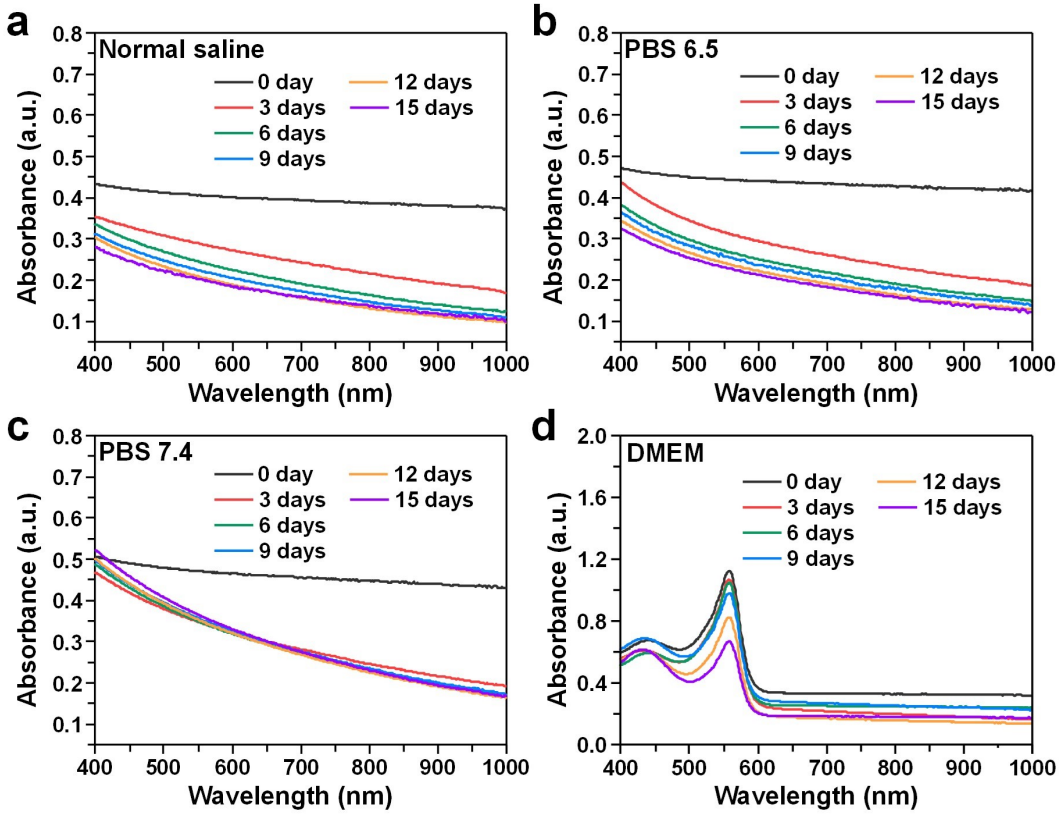

Fig. S3. UV-Vis-NIR absorption spectra of the ZNSP nanostructures in (a) normal saline, (b) PBS 6.5, (c) PBS 7.4, (d) DMEM over time related to Fig. 3b.

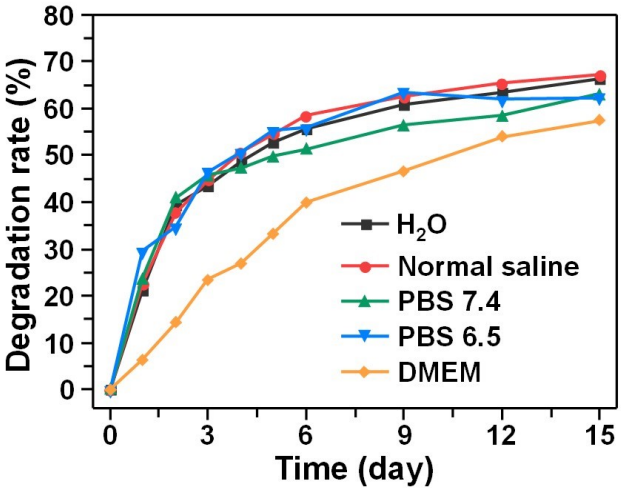

Fig. S4. Comparison of the degradation rate for ZNSP in various medium on the 15 days.

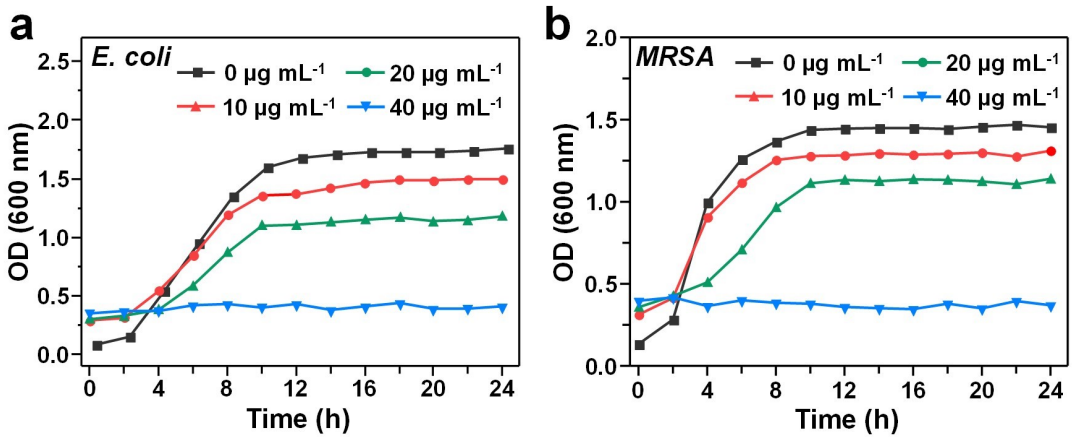

Fig. S5. Growth curves of (a) *E. coli* and (b) *MRSA* treated with ZNSP nanostructures at different concentration after NIR irradiation.

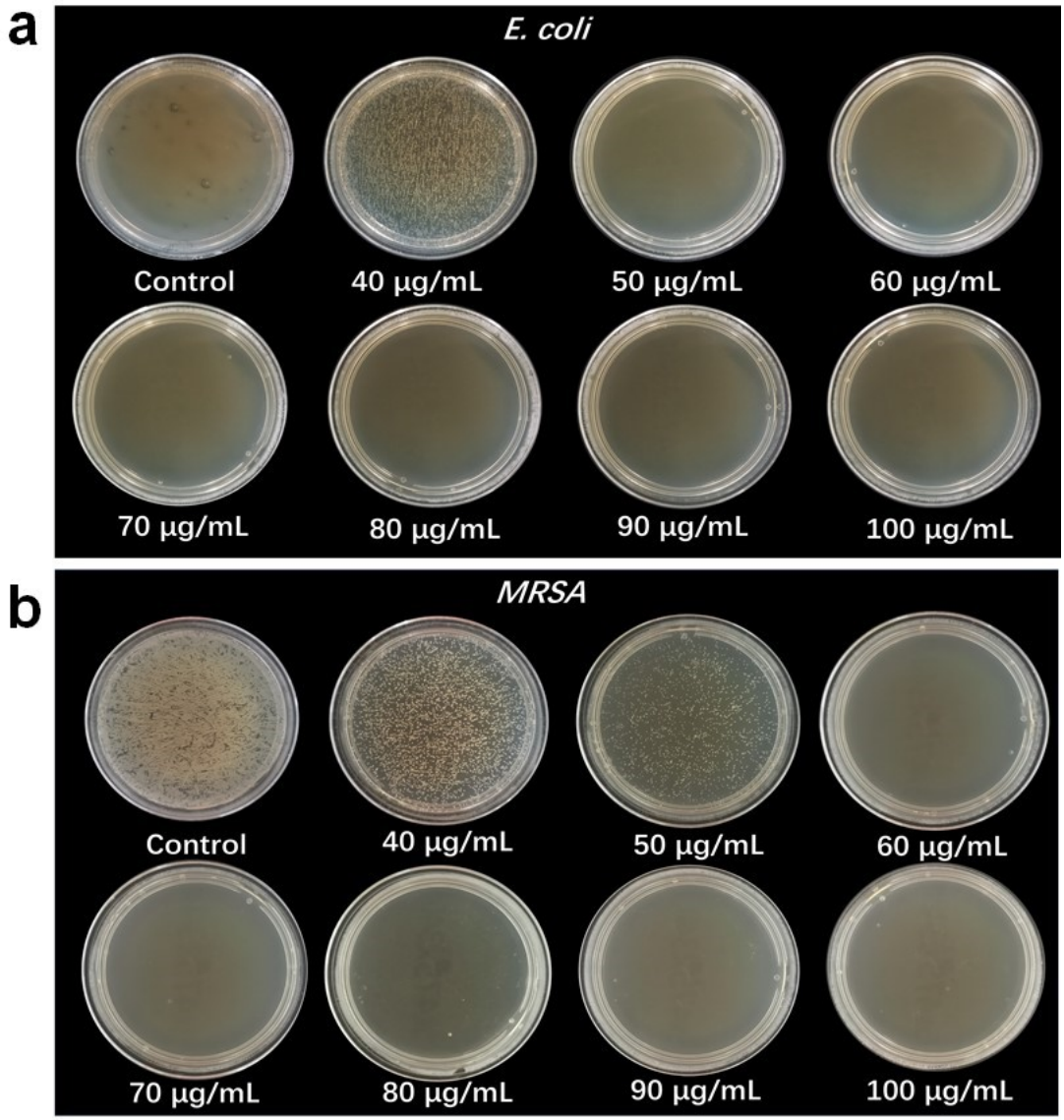

Fig. S6. Photographs of colonies of (a) *E. coli* and (b) *MRSA* treated with ZNSP nanostructures at different concentration after NIR irradiation.

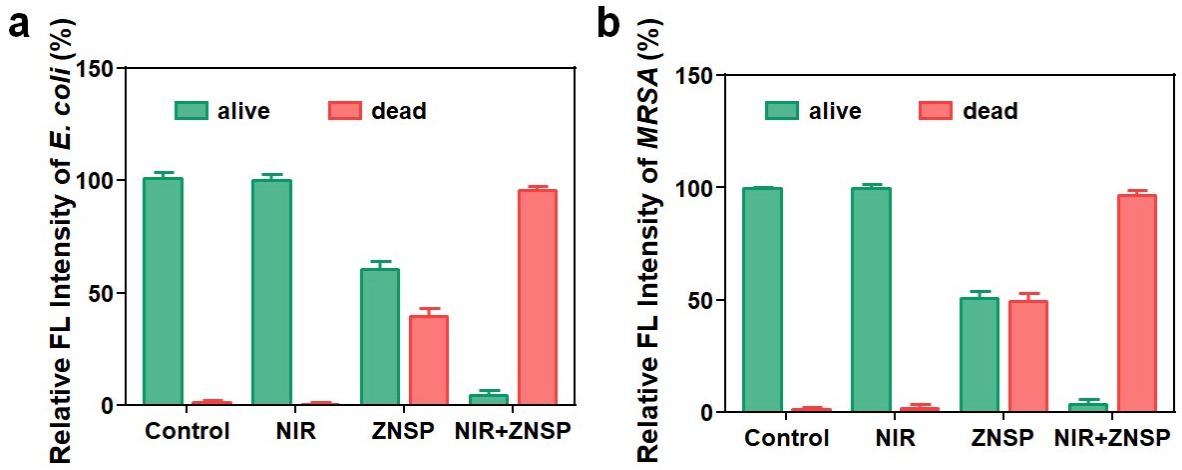

Fig. S7. The fluorescence intensity in Fig. 4e and 4f calculated by Image J.

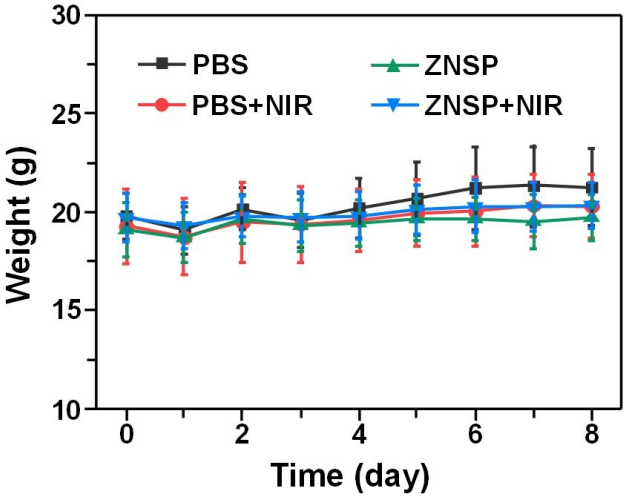

Fig. S8. The body weights change during various treatments. Related to Fig. 5.

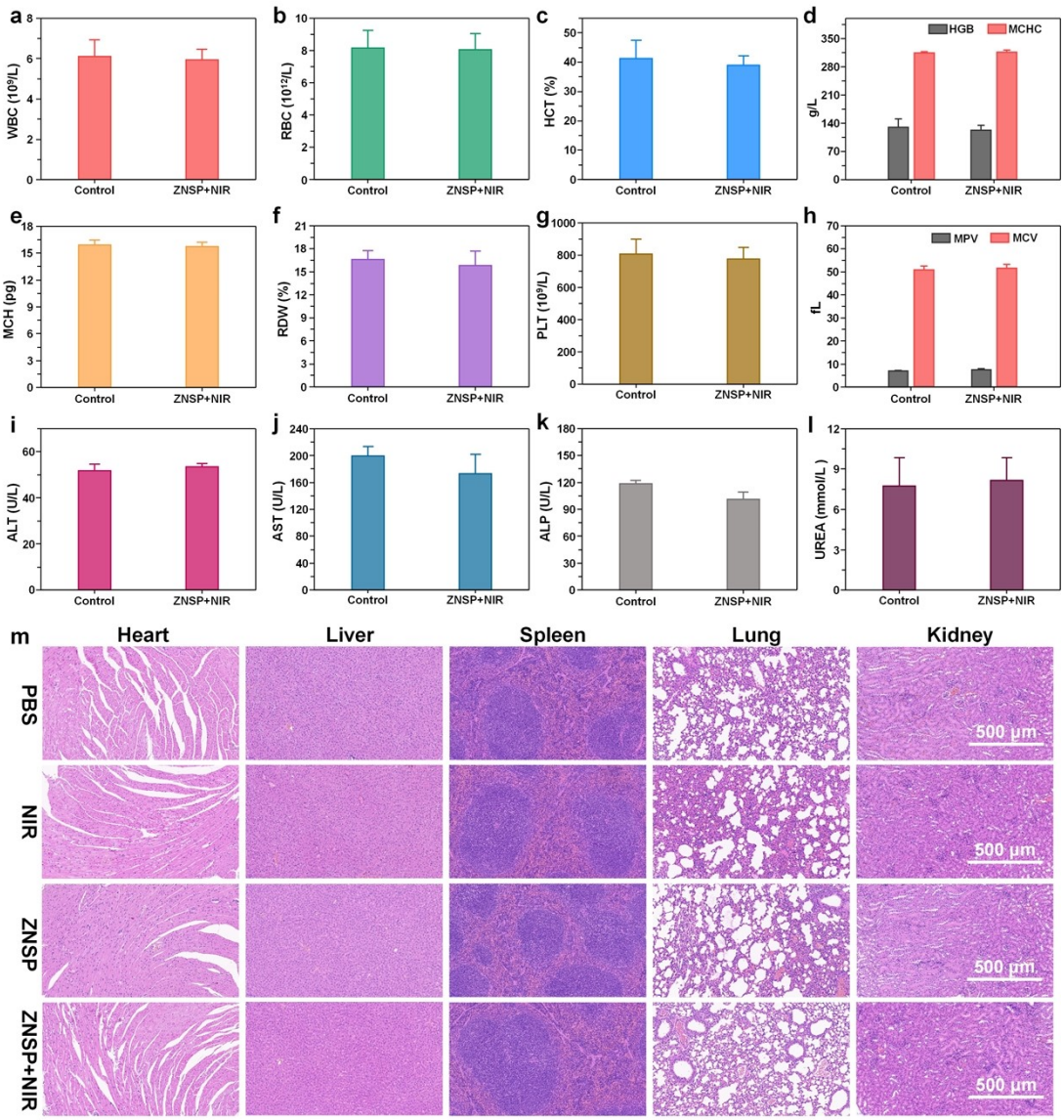

Fig. S9. The blood panel analysis (a) and the blood biochemistry test; (b) H&E staining images of major organs (including heart, liver, spleen, lung, and kidney) of the mice after treatment.

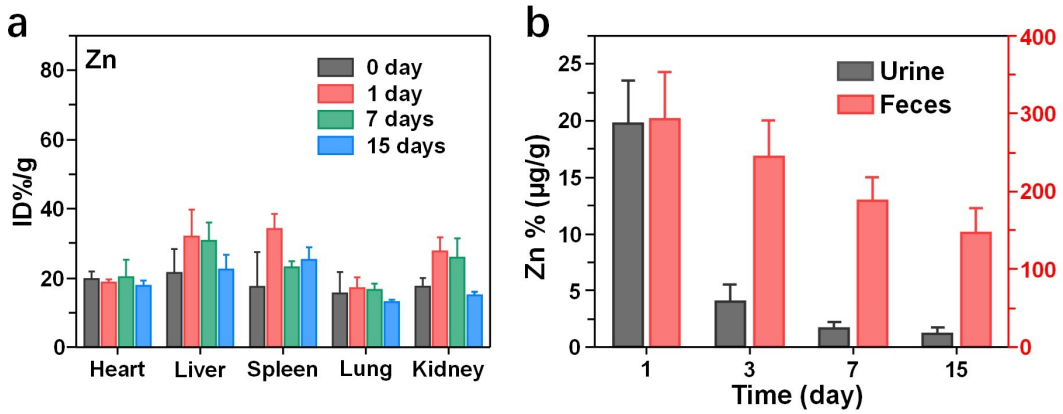

Fig. S10. (a) Biodistribution of Zn *in vivo* and (b) emission manner at different time.

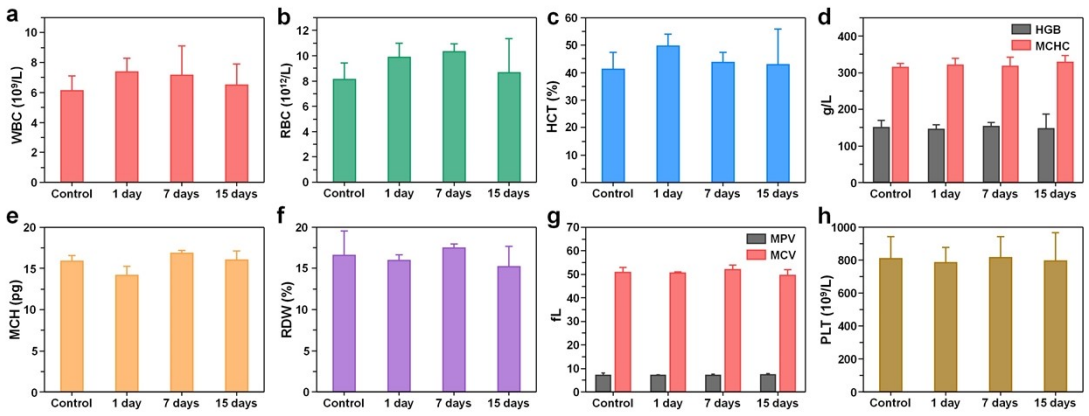

Fig. S11. The blood panel analysis after intravenous injection of ZNSP naostruetures at different days (dose: 5 mg kg^{-1}).

Table S1. The concentration of released metal ions measured by ICP-MS.

Parameters	Ni (ppb)	Zn (ppb)
pH=7.4, 37°C	4014.544	206.958
	3639.848	281.382
	5002.346	235.094
pH=6.5, 37°C	5213.441	366.344
	6023.790	471.826
	5789.237	562.674
pH=7.4, 45°C	5151.495	554.862
	5551.331	704.430
	6451.762	631.552
pH=6.5, 45°C	9336.835	1284.982
	8579.301	1023.876
	8906.643	1118.854

Table S2. MIC and MBC of ZNSP nanostructures against *E. coli* and *MRSA*

Bacteria	MIC (μg mL^{-1})	MBC (μg mL^{-1})
E. coli	40	50
MRSA	40	60