Supplementary Information

Zinc-Manganese Sulfide Nanoplatform for Tumor-Microenvironment-Responsive Chemodynamic/Gas Therapy and Immunomodulation

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Experimental Sections

Materials.

Manganous chloride ($MnCl_2·4H_2O$), Oleic acid were purchased from Alfa Aesar. 1, 2-hexadecanodiol was purchased from Sigma-Aldrich. Anhydrous zinc chloride ($ZnCl_2$) was purchased from Alfa. Oleylamine and benzyl oxide were purchased from Thermo Fisher Scientific. Distearoylphosphatidylethanolamine-Polyethylene Glycol-Amino (DSPE-PEG-NH_2 M.W 2000) was purchased from J&K Chemical Ltd. Isopropanol, chloroform, hexane and ethanol were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). The PBS, RPMI 1640 Medium, fetal bovine serum (FBS), CCK-8 kit, Methylene blue, 2-(4-amidinophenyl)-6-indolecarbamidine dihydrochloride (DAPI), Mitochondrial membrane potential assay kit with mitochondrial probe 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide (JC-1), 2′,7′-dichlorofluorescein diacetate (DCFH-DA), Calcein acetoxymethylester (calcein-AM), and propidium iodide (PI) double staining kit were purchased from Dalian Meilun Biotechnology Co., Ltd. phosphorylated STING (p-STING) antibody was purchased from Abclonal and collagen I antibody was purchased from Sevier. Catalase activity assay kit was purchased from Nanjing Jiancheng，all enzyme-linked immunosorbent assay (ELISA) kits were purchased from Quanzhou Jiubang Biotechnology Co., Ltd. All chemical agents were of analytical grade and were used directly with no further purification.

Characterizations.

The morphology of the nanoparticles was characterized using transmission electron microscopy (TEM) on a JEM-2100 microscope at an accelerating voltage of 200 kV. Elemental composition was determined by energy-dispersive X-ray spectroscopy (EDS) on an FEI Tecnai G2 F30 field-emission TEM operated at 300 kV. Crystal structure was analyzed by X-ray powder diffraction (XRD) using a Rigaku Ultima IV X-ray diffractometer. Surface chemical states were

examined by X-ray photoelectron spectroscopy (XPS) on a Thermo Scientific K-Alpha X-ray photoelectron spectrometer. Magnetic properties were assessed by measuring field-dependent magnetization (M-H) curves with a Quantum Design PPMS-9. The hydrodynamic size and surface charge (zeta potential) of the nanoparticles in dispersion were measured by dynamic light scattering (DLS) using a Brookhaven NanoBrook 90 Plus PALS instrument. The elemental concentrations of zinc and manganese in the particles were quantified by inductively coupled plasma optical emission spectrometry (ICP-OES) with an Agilent 5110 instrument. UV-Vis absorption spectra were recorded using a Shimadzu UV-2600i UV-vis spectrophotometer. The T1 relaxation times were measured using a 0.5 T NMR analyzer (PQ001). The magnetic resonance imaging (MRI) contrast efficiency was further validated on a 7.0 T Bruker BioSpec 70/20 USR scanner. T1-weighted phantom images and in vivo mouse MRI data were acquired using the same 7.0 T Bruker BioSpec 70/20 USR animal MRI scanner. Fluorescence imaging was conducted with a Leica DMILLED FLUO system.

Synthesis of Zinc Oleate.

Sodium oleate (4.56 g) was dissolved in a mixture of 20 mL of water and 10 mL of anhydrous ethanol under ultrasonic agitation. The solution was then stirred and heated. In a separate step, zinc chloride (1.02 g) was dissolved in 5 mL of water and added dropwise to the initial solution. The resulting mixture was heated to 70 °C and stirred continuously for 4 hours to complete the reaction. Following the reaction, the product was washed with distilled water, dried in an oven, and stored at room temperature for subsequent use.

Synthesis of Manganese Oleate.

Manganese oleate was synthesized according to a previously reported method. Briefly, 0.80 g of $MnCl_2 \cdot 4H_2O$ and 2.53 mL of oleic acid were dissolved in 20 mL of methanol under sonication. The mixture was then heated to 70 °C, followed by the dropwise addition of a solution containing 0.33 g of NaOH in 20 mL of methanol. The reaction was allowed to proceed under continuous

stirring for 2 hour. After the reaction was complete, the resultant adherent product was washed with distilled water and subsequently dissolved in hexane. This hexane solution was transferred to a glass dish and left in a fume hood to allow for complete solvent evaporation, yielding a waxy manganese oleate product. The final product was stored at room temperature for further use.

Synthesis of Zinc-Manganese Sulfide nanoparticles (ZMSN).

Initially, a reaction solution was prepared by combining zinc oleate (0.31 g), manganese oleate (0.31 g), and sulfur (0.06 g) in 7.5 mL of 1-octadecene. To this mixture, 500 μL of oleylamine and 500 μL of oleic acid were added and thoroughly dissolved. To remove oxygen, the system was subjected to three vacuum-purge cycles with nitrogen at room temperature. The reaction solution was then heated to 260 °C and maintained at this temperature for 30 minutes. After cooling to room temperature, the nanocrystals were precipitated with isopropanol and collected by centrifugation. The product was isolated via centrifugation and purified by washing twice with anhydrous ethanol. Finally, the purified nanocrystals were redispersed in hexane for storage at room temperature.

Synthesis of Manganese Sulfide Nanoparticles (MSN).

A reaction solution was prepared by combining manganese oleate (0.31 g) and sulfur (0.06 g) in 7.5 mL of 1-octadecene. Oleylamine (250 μL) and oleic acid (250 μL) were added to the mixture and thoroughly dissolved. To prevent oxidation, the reaction vessel was subjected to three vacuum-nitrogen purge cycles at room temperature. The solution was subsequently heated to 260 °C and held at this temperature for 30 minutes. Upon cooling to 70 °C, an additional 1 mL of oleylamine was introduced, and stirring was continued until the solution reached room temperature. The nanocrystals were then precipitated with isopropanol, collected by centrifugation, and purified by washing twice with anhydrous ethanol. The final product was redispersed in hexane for storage at room temperature.

Synthesis of Zinc Sulfide Nanoparticles (ZSN).

A reaction solution was prepared by combining zinc oleate (0.31 g) and sulfur (0.06 g) in 7.5

mL of 1-octadecene. Oleylamine (250 μL) and oleic acid (250 μL) were added to the mixture and thoroughly dissolved. To prevent oxidation, the system was degassed via three vacuum-refill cycles with nitrogen at room temperature. The solution was then heated to 260 °C and maintained at this temperature for 30 minutes. After cooling to room temperature, the nanocrystals were precipitated with isopropanol and collected by centrifugation. The final nanocrystals were redispersed in hexane and stored at room temperature for subsequent use.

Synthesis of PEGylated ZSN, MSN, and ZMSN.

The nanoparticles were initially mixed with DSPE-PEG-NH_2 (MW: 2000) in chloroform. Specifically, ZMSN, ZSN, and MSN were combined with DSPE-PEG-NH_2 at weight ratios of 1:2, 1:4, and 1:2, respectively. The mixture was sonicated for 15 minutes to form a homogeneous dispersion. Subsequently, chloroform was slowly evaporated at 45 °C, and any residual solvent was removed under vacuum. Finally, the product was redispersed in water and stored at 4 °C for further use.

pH-Triggered Release of Zinc Ions (Zn^{2+}) from ZMSPN.

To investigate the pH-dependent release profile, 50 μL of ZMSPN was introduced into 9 mL of phosphate-buffered saline (PBS) at various pH values. After thorough mixing by vortexing, samples were collected at predetermined time points. The samples were then centrifuged at 16,000 rpm for 6 minutes to separate the nanoparticles, and the resulting supernatants were collected. Finally, the concentration of Zn^{2+} ions released into the supernatant was quantified using inductively coupled plasma optical emission spectrometry (ICP-OES).

pH-Triggered Release of H_2S from ZMSPN.

To quantitatively assess H_2S generation, 50 μL of ZMSPN (containing Zn+Mn at 18 μg/mL) was added to 2 mL of phosphate-buffered saline (PBS) at various pH values in sealed vials. The mixtures were incubated at room temperature, and the amount of H_2S gas produced was monitored

in real-time at designated intervals using a gas detector. In a parallel qualitative analysis, 20 μL of ZMSPN was introduced into 1 mL of PBS at different pH values in small vials. The vials were immediately sealed with lead acetate test strips, and the color changes of the strips were recorded over time to visually confirm H_2S production.

pH-Triggered Release of Manganese Ions (Mn^{2+}) from ZMSPN.

50 μL of ZMSPN (Mn: 8 μg/mL) was added to 9 mL of phosphate-buffered saline (PBS) at different pH values. The solutions were thoroughly mixed by vortexing, and aliquots were collected at designated time points. The aliquots were then centrifuged at 16,000 rpm for 6 minutes, and the supernatants were collected for analysis. To the supernatant, the following reagents were sequentially added: 50 μL of ammonia solution, 10 μL of formaldoxime, 10 μL of hydroxylamine hydrochloride, and 20 μL of EDTA disodium salt.Finally, measure the absorbance at 450 nm.

Assessment of chemodynamical activity.

To evaluate the Fenton-like catalytic activity, ZMSPN (with varying Mn^{2+} concentrations), 100 mM H_2O_2, and 100 μg/mL methylene blue (MB) were mixed in a 50 mM sodium bicarbonate ($NaHCO_3$) solution. The mixture was then incubated at 37 °C for 30 minutes. After incubation, the solution was centrifuged to obtain the supernatant. The absorbance of the supernatant was subsequently measured between 500 and 800 nm using a UV-Vis spectrophotometer.

0.5 T magnetic resonance measurements of r_1.

To evaluate the pH-dependent relaxivity, ZMSPN were incubated in PBS buffers of varying pH for 2 hours. The solutions were then embedded in agarose gel phantoms with a series of final Mn^{2+} concentrations (0, 0.05, 0.1, 0.2, and 0.4 mM). The T_1 relaxation times of these phantoms were measured using a 0.5 T NMR analyzer (PQ001).

In vitro MR imaging.

ZMSPN nanoparticles were dispersed in phosphate-buffered saline (PBS) at different pH values

and incubated for 2 hours. The resulting mixtures were then embedded in agarose gel phantoms with final Mn^{2+} concentrations of 0, 0.05, 0.10, 0.20, and 0.40 mM.T_1-weighted images and T_1 relaxation times were acquired using a 7.0 T magnetic resonance imaging (MRI) scanner. The T_1 quantification parameters were set as follows: echo time (TE) = 6.5 ms; repetition time (TR) = 550.0 ms; slice thickness = 0.9 mm. Quantitative analysis was performed by measuring the signal intensity within defined regions of interest (ROIs) in the acquired images.

Cytotoxicity Assay

The cytotoxicity of ZMSPN, ZSPN, and MSPN was evaluated in vitro using the Cell Counting Kit-8 (CCK-8) assay. 4T1 cells were seeded in 96-well plates at a density of 2×10^4 cells per well in 100 μL of culture medium and incubated for 24 hours. The medium was then replaced with 100 μL of fresh medium containing varying concentrations of ZMSPN, ZSPN, or MSPN. After an additional 24 hours of incubation, cell viability was assessed following the standard CCK-8 protocol.

Live/Dead Cell Staining Assay

To visually assess cytotoxicity, 4T1 cells were seeded in 96-well plates at a density of 1×10^4 cells per well in 100 μL of culture medium and incubated for 24 hours. After the initial incubation, the medium in each well was replaced with 100 μL of fresh medium containing ZSPN, MSPN, ZMSPN (50 μg/mL Zn+Mn content, all at equivalent Zn or Mn concentrations), or PBS as a control. Following 24 hours of further incubation, the cells were washed with PBS, stained with calcein acetoxymethyl ester (calcein-AM) and propidium iodide (PI), and observed under a fluorescence microscope.

Preparation of ZMSPN-DiD.

During the surface modification of ZMSPN with PEG, 0.5 μL of DiD Perchlorate dye was incorporated into the mixture. All subsequent steps were performed under light-protected conditions to prevent photobleaching.

Determination of Intracellular ZMSPN-DiD.

To evaluate cellular uptake of the nanoparticles, 4T1 cells were seeded in 96-well plates at 1×10^4 cells per well in 100 μL of culture medium and incubated for 12 hours. The medium was then replaced with fresh medium containing 25 μg/mL ZMSPN-DiD (based on Zn+Mn content) and incubated for predetermined time intervals (0, 2, 4, and 6 hours). Following incubation, the cells were washed with phosphate-buffered saline (PBS), harvested, and analyzed by flow cytometry to quantify intracellular ZMSPN-DiD levels.

Determination of Intracellular Zn^{2+}

To measure intracellular zinc levels, 4T1 cells were stained with the Zinquin fluorescent probe. Initially, cells were seeded in 96-well plates at a density of 1×10^4 cells per well in 100 μL of culture medium and incubated for 12 hours. The medium was then replaced with fresh medium containing ZSPN, MSPN, or ZMSPN (50 μg/mL Zn+Mn content, all at equivalent Zn concentrations) and incubated for predetermined durations. After 6 hours incubation, the cells were washed with phosphate-buffered saline (PBS). Intracellular zinc levels were subsequently visualized using fluorescence microscopy following Zinquin staining.

MMP-2 expression was detected by ELISA kit.

4T1 cells were seeded in 6-well plates at a density of 2×10^5 cells per well and incubated for 24 hours. The culture medium was then removed, and the cells were washed with PBS.The medium was then replaced with fresh serum-free medium containing ZMSPN, MSPN, MOPN, ZSPN (50 μg/mL Zn+Mn content, all at equivalent Zn or Mn concentrations), or PBS as a control, followed by an additional 24 hours of incubation. The cells were washed with PBS, collected, and then lysed for protein extraction. The level of MMP-2 was detected using an ELISA kit.

Detection of Collagen I

Cells were seeded in 96-well plates at a density of 1×10^4 cells per well and cultured for 24

hours. After removing the medium and washing with PBS, the cells were treated with fresh medium containing ZMSPN, MSPN, ZSPN (100 μg/mL Zn+Mn content, all at equivalent Zn or Mn concentrations), or PBS as a control. Following 24 hours of incubation, the cells were fixed with 4 % paraformaldehyde for 10 minutes. The fixed cells were then incubated overnight at 4 °C with an anti-collagen I primary antibody (1:400), followed by a 1 hour incubation at 37 °C with a Cy3-conjugated goat anti-rabbit IgG secondary antibody. Finally, nuclei were counterstained with DAPI for 10 minutes, and the samples were imaged using fluorescence microscopy.

Determination of Intracellular H_2S

To detect intracellular H_2S production, 4T1 cells were stained with the H_2S-sensitive fluorescent probe WSP-1. Initially, cells were seeded in 96-well plates at a density of 1×10^4 cells per well and cultured for 12 hours. The medium was then replaced with fresh medium containing ZSPN, MSPN, or ZMSPN (25 μg/mL Zn+Mn content, all at equivalent Zn or Mn concentrations) and incubated for predetermined durations. After 6 hours incubation, the cells were washed with phosphate-buffered saline (PBS), and H_2S generation was visualized using fluorescence microscopy following WSP-1 staining.

Catalase (CAT) activity assay

4T1 cells were seeded in 6-well plates at a density of 2×10^5 cells per well and incubated for 24 hours. The culture medium was then removed, and the cells were washed with PBS. Subsequently, the cells were treated with fresh serum-free medium containing different concentrations of ZMSPN, or PBS as a control. After an additional 24 hours of incubation, the cells were washed with PBS, harvested, and intracellular CAT activity was measured using a commercial CAT activity assay kit.

Determination of Intracellular H_2O_2

4T1 cells were seeded in 96-well plates at a density of 1×10^4 cells per well and cultured for 12 hours. The medium was then replaced with fresh medium containing ZSPN, MSPN, or ZMSPN (50

μg/mL Zn+Mn content, all at equivalent Zn or Mn concentrations) and incubated for predetermined durations. After 6 hours incubation, the cells were washed with phosphate-buffered saline (PBS), and H_2O_2 level was visualized using fluorescence microscopy following ROSGreenTMH_2O_2 probe staining.

Detection of ROS generation by Cell staining

4T1 cells were seeded in 96-well plates at a density of 1×10^4 cells per well and cultured for 24 hours. The medium was then replaced with fresh serum-free medium containing ZMSPN, MSPN, ZSPN (50 μg/mL Zn+Mn content, all at equivalent Zn or Mn concentrations), or PBS as a control, followed by an additional 8 hours of incubation. The cells were subsequently washed with PBS, stained with the fluorescent probe DCFH-DA, and analyzed using fluorescence microscopy to detect intracellular reactive oxygen species (ROS) levels.

Detection of ROS generation by flow cytometry

4T1 cells were seeded in 6-well plates at a density of 2×10^5 cells per well and incubated for 24 hours. The culture medium was then removed, and the cells were washed with PBS.The medium was then replaced with fresh serum-free medium containing ZMSPN, MSPN, MOPN, ZSPN (25 μg/mL Zn+Mn content, all at equivalent Zn or Mn concentrations), or PBS as a control, followed by an additional 8 hours of incubation. The cells were subsequently washed with PBS, stained with the fluorescent probe DCFH-DA, After incubation, the cells were washed with PBS, collected, and subjected to flow cytometry analysis.

Detection of Mitochondrial Membrane Potential

To assess the impact of ZMSPN on mitochondrial function in 4T1 cells, the cells were seeded in 96-well plates at a density of 1×10^4 cells per well and cultured for 24 hours. The medium was then replaced with fresh serum-free medium containing ZMSPN, MSPN, ZSPN (all at equivalent Zn or Mn concentrations), or PBS as a control, followed by another 24 hours of incubation. After

treatment, the cells were washed with PBS, stained with the 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethyl-imidacarbocyanine iodide (JC-1) and Mito-Detector Red as probes, and examined under a fluorescence microscope to evaluate mitochondrial membrane potential.

Detection of p-STING

Cells were seeded in 96-well plates at a density of 1×10^4 cells per well and cultured for 24 hours. The medium was then removed, and the cells were washed with PBS before being treated with fresh medium containing ZMSPN, MSPN, ZSPN (100 μg/mL Zn+Mn content, all at equivalent Zn or Mn concentrations), or PBS as a control. After 24 hours of further incubation, the cells were fixed with 4 % paraformaldehyde for 10 minutes. Following fixation, the cells were incubated overnight at 4 °C with an anti-phospho-STING (p-STING) primary antibody (1:400), followed by a 1 hour incubation at 37 °C with a Cy3-conjugated goat anti-rabbit IgG secondary antibody. Finally, the nuclei were counterstained with DAPI for 10 minutes, and the samples were imaged using fluorescence microscopy.

High mobility group box 1 (HMGB1) Release Detection.

4T1 cells were seeded in 6-well plates at a density of 2×10^5 cells per well and cultured for 24 hours. After removing the culture medium and washing with PBS, the cells were treated with fresh medium containing ZMSPN, MSPN, ZSPN (100 μg/mL Zn+Mn content, all at equivalent Zn or Mn concentrations), or PBS as a control, followed by an additional 24 hours of incubation. The cell culture supernatants were then collected by centrifugation, and the release of high mobility group box 1 (HMGB1) was quantified using a commercial enzyme-linked immunosorbent assay (ELISA) kit.

ATP Release Detection.

4T1 cells were seeded in 6-well plates at a density of 2×10^5 cells per well and incubated for 24 hours. After removing the medium and washing with phosphate-buffered saline (PBS), the cells

were treated with fresh medium containing ZMSPN, MSPN, ZSPN (100 μg/mL Zn+Mn content, all at equivalent Zn or Mn concentrations), or PBS as a control. Following an additional 24-hour incubation, the culture supernatants from each treatment group were collected by centrifugation, and extracellular ATP release was quantified using a commercial ATP assay kit.

Calreticulin (CRT) Exposure Release Detection.

4T1 cells were seeded in 96-well plates at a density of 1×10^4 cells per well and cultured for 24 hours. After removing the medium and washing with phosphate-buffered saline (PBS), the cells were treated with fresh medium containing ZMSPN, MSPN, ZSPN (100 μg/mL Zn+Mn content, all at equivalent Zn or Mn concentrations), or PBS as a control. Following an additional 24-hour incubation, the cells were fixed with 4 % paraformaldehyde for 10 minutes. The fixed cells were then incubated overnight at 4 °C with an anti-calreticulin (CRT) primary antibody (1:400), followed by incubation with a Cy3-conjugated goat anti-rabbit IgG secondary antibody at 37 °C for 1 hour. Finally, nuclei were counterstained with DAPI for 10 minutes, and the samples were imaged using fluorescence microscopy.

MR imaging (MRI) in Vivo

For in vivo magnetic resonance imaging (MRI), a subcutaneous tumor model was established using 6 week old female BALB/c mice. When tumor volumes reached 60-100 mm^3, the mice were intravenously administered ZMSPN at a dose of 5 mg per kg body weight. T_1-weighted MR images were acquired using a 7T MicroMRI scanner with the following parameters: repetition time/echo time (TR/TE) = 500/6.5 ms, slice thickness = 0.9 mm, number of slices = 14. Images were collected before injection and at 0.5, 1, and 2 hours post-injection. The signal-to-noise ratio (SNR) and tumor-to-normal contrast (T/N ratio) were calculated to quantify T_1 signal enhancement.

Accumulation of major organs.

Following intravenous administration of ZMSPN-DiD, fluorescence imaging was performed at

predetermined time points (0, 1, 2, 4, 8, 24, and 48 hours). At 48 hours post-injection, the mice were euthanized, and major organs (heart, liver, spleen, lungs, and kidneys) along with tumor tissues were harvested for ex vivo fluorescence imaging analysis.

Pharmacokinetics of ZMSPN-DiD.

Blood samples were collected at 0, 0.5, 1, 2, 4, 6, 8, and 24 hours post-intravenous injection of ZMSPN-DiD (5mg Zn+Mn per kg body weight). At each time point, 20 μL of blood was drawn from each mouse and mixed with 180 μL of anticoagulant solution (EDTA, 2 mg/mL), followed by storage under light-protected conditions. After centrifugation, the supernatant was collected, and the relative fluorescence intensity was quantified using 678 nm excitation and 701 nm emission wavelengths.

Histology Analysis.

Upon completion of the treatment regimen, all mice were euthanized, and tumor tissues were harvested for weighing and photographic documentation. Major organs (heart, liver, spleen, lungs, and kidneys) along with tumor tissues were fixed in 4% paraformaldehyde solution. Following fixation, the tissues were embedded in paraffin, sectioned, and subsequently subjected to hematoxylin and eosin (H&E) staining, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assays, immunohistochemical staining for MMP-2, and immunofluorescence staining for Collagen I, p-STING, as well as the infiltration of $CD4^+$ (green) and $CD8^+$ (red) T cells for histological analysis.

Label the nanoparticles with CY5.5 monosuccinimidyl ester

The surfaces of ZMSPN, MSPN, and ZSPN were all modified with DSPE-PEG-NH_2 (MW: 2000). In their respective aqueous solutions, each nanoformulation was reacted with CY5.5 monosuccinimidyl ester (Cy5.5) at a mass ratio of 50:1 (based on the metal ion content of the nanoparticles) for 2 hours at room temperature, followed by an additional 30 minutes of ultrasonic

dispersion.

Drug penetration in tumor sections

After two administrations of saline, ZSPN-Cy5.5, MSPN-Cy5.5, and ZMSPN-Cy5.5.(5mg Zn+Mn per kg body weight, all at equivalent Zn or Mn concentrations), all tumor-bearing mice were euthanized, and tumor tissues were collected and fixed in 4% paraformaldehyde solution. Following fixation, the tissues were embedded in paraffin, sectioned, and subsequently stained with DAPI for nuclear visualization. After staining, the sections were observed and photographed under a Leica inverted fluorescence microscope for histological analysis.

Activated immune system inhibits lung metastasis.

Female BALB/c mice (8 weeks old) were subcutaneously injected with 2.50×10^6 4T1 cells in the dorsal flank to establish tumor-bearing models. When the tumor volume reached approximately 50-100 mm^3, 4T1 cells (1.80×10^6)were intravenously injected into mice via the tail vein to establish the experimental metastasis model. The treatment was administered every three days according to the therapeutic schedule. On day 10 post-cell inoculation, the mice were euthanized, and lung tissues were collected. The surface metastatic nodules on the lungs were counted, and the internal metastases were quantified through hematoxylin and eosin (H&E) staining.

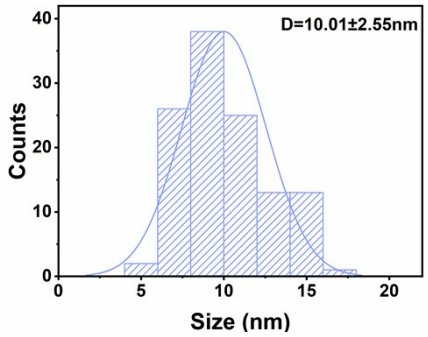

Figure S1. Particle size distribution histogram of ZMSN.

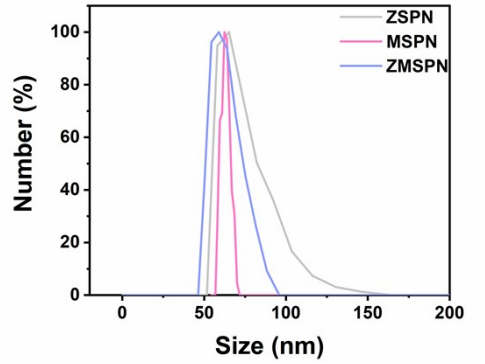

Figure S2. Hydrated particle size of ZSPN, MSPN, and ZMSPN.

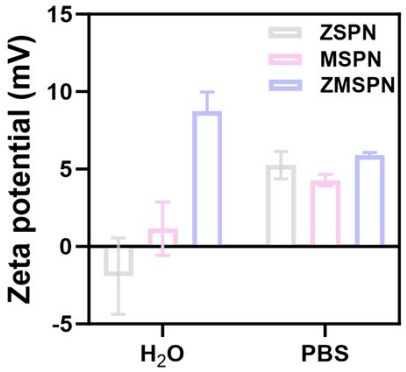

Figure S3. Zeta potential of ZSPN, MSPN, and ZMSPN (Data are presented as mean ± SD, n = 3/group).

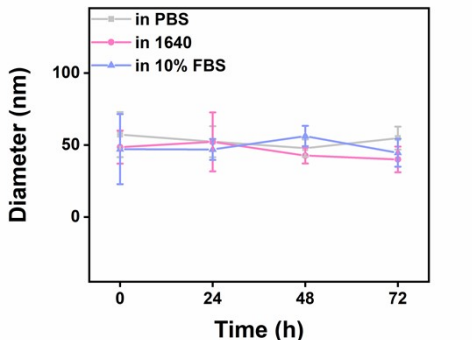

Figure S4. Stability analysis of ZMSPN after incubation in PBS, 1640 medium, and complete medium containing 10% fetal bovine serum (FBS) for 0, 24, 48, and 72 hours (Data are presented as mean ± SD, n = 3/group).

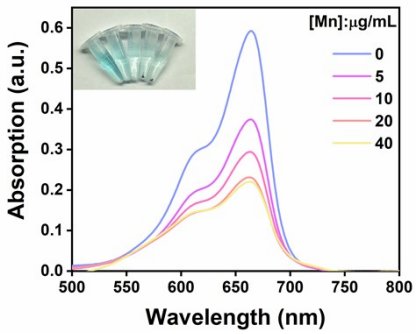

Figure S5. MB degradation by ·OH generated by distinct concentrations of ZMSPN.

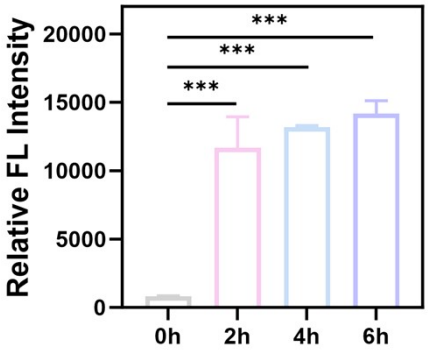

Figure S6. Uptake levels of ZMSPN-DiD by 4T1 cells at varying times (Data are presented as mean ± SD, ***p < 0.001 by one-way ANOVA, n = 3/group).

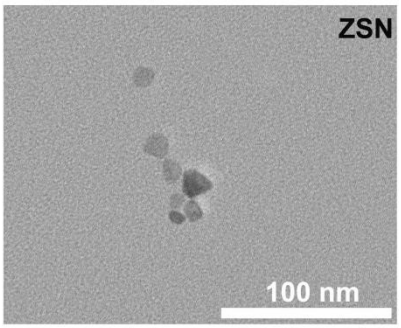

Figure S7.TEM image of ZSN.

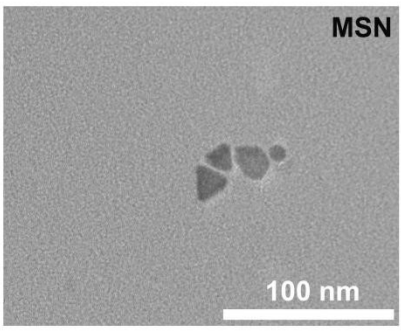

Figure S8. TEM image of MSN.

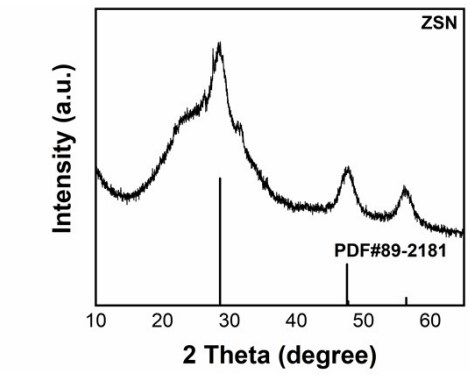

Figure S9. XRD pattern of ZSN.

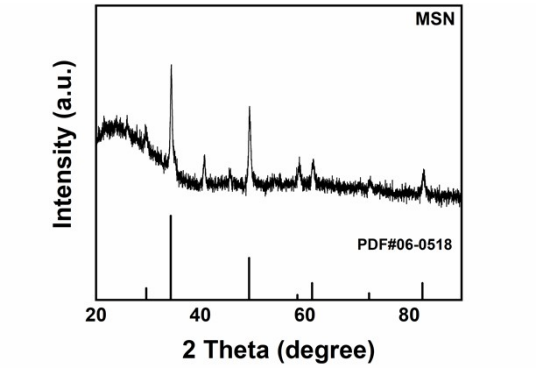

Figure S10. XRD pattern of MSN.

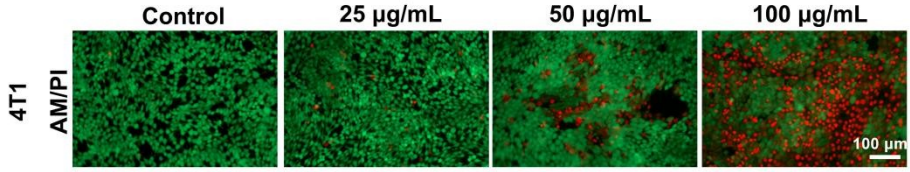

Figure S11. Live/dead staining images of 4T1 cells with distinct treatments. Red, dead cells; green, live cells. Scale bar is 100 μm.

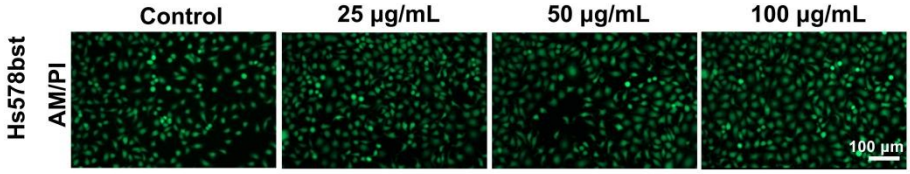

Figure S12. Live/dead staining images of Hs578bst cells with distinct treatments. Red, dead cells; green, live cells. Scale bar is 100 μm.

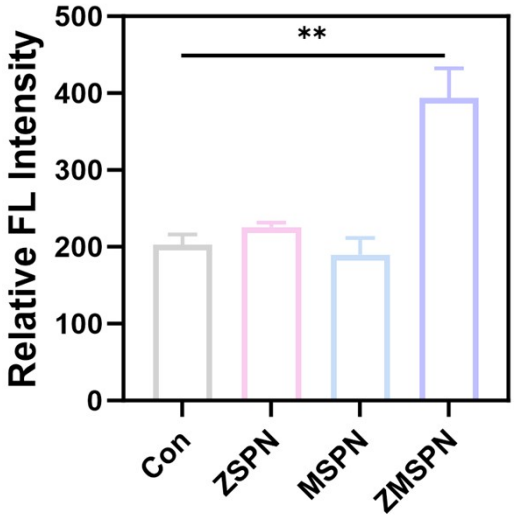

Figure S13. MMP-2 expression in 4T1 cells was detected using an ELISA kit after 24 hours of treatment with various agents.(Data are presented as mean ± SD, **p < 0.01 by one-way ANOVA, n = 3/group).

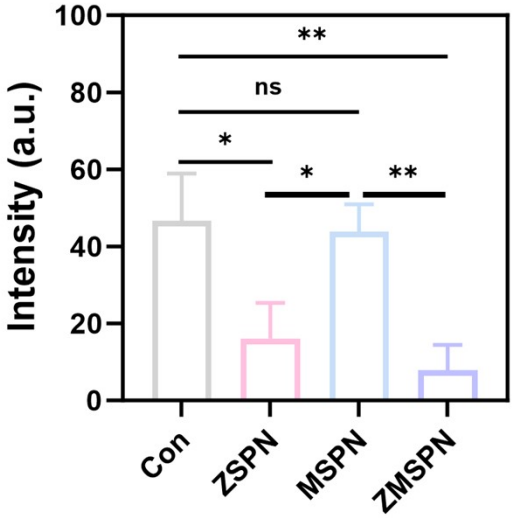

Figure S14. Quantitative analysis of Collagen I expression following treatment with distinct agents (Data are presented as mean ± SD,*p < 0.05, **p < 0.01 by one-way ANOVA, n = 3/group).

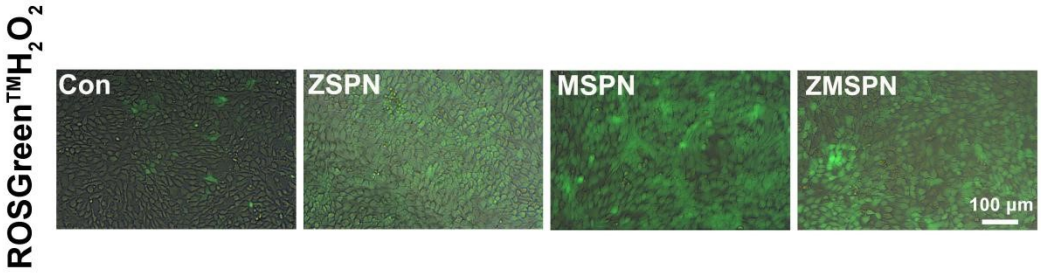

Figure S15. Fluorescence images of 4T1 cells with distinct treatments to detect intracellular H_2O_2 levels via ROSGreen™H_2O_2 probe for 6h. Scale bar is 100 μm.

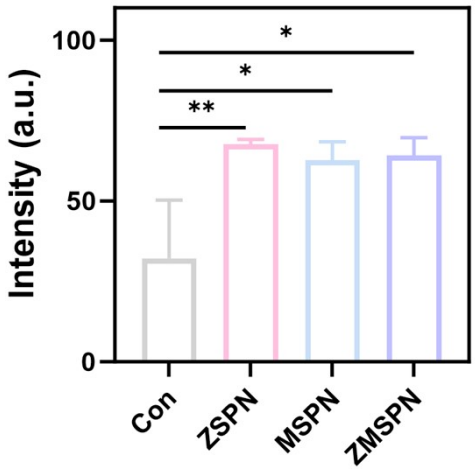

Figure S16. Quantitative analysis of ROSGreen™H_2O_2 fluorescence of distinct treatments (Data are presented as mean ± SD,*p < 0.05, **p < 0.01 by one-way ANOVA, n = 3/group).

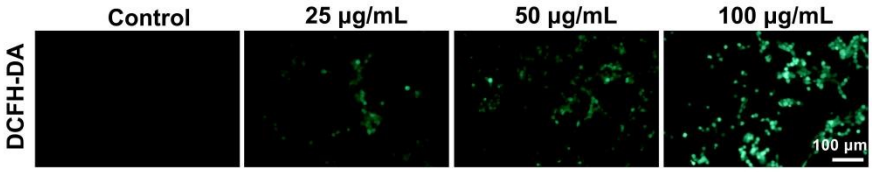

Figure S17. Fluorescence images of 4T1 cells with distinct treatments to detect intracellular ROS levels via DCFH-DA fluorescent probe. Scale bar is 100 μm.

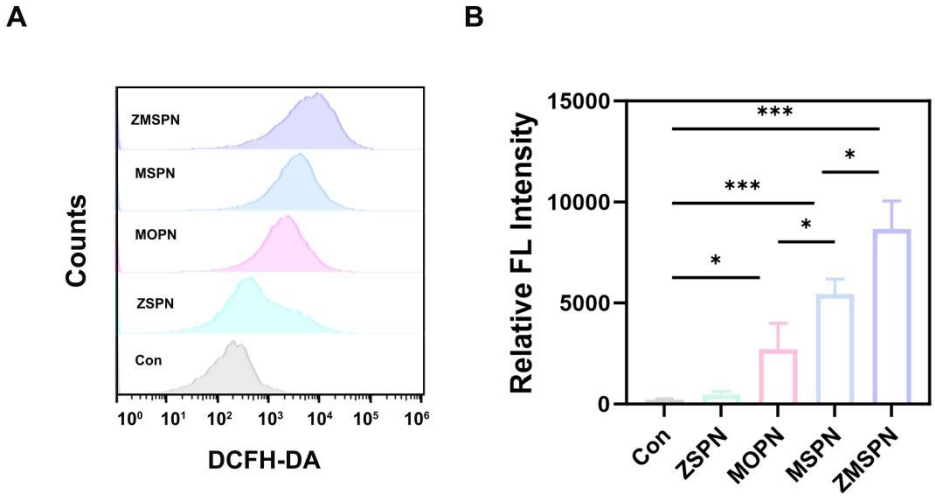

Figure S18. (A) Flow cytometry analysis of intracellular ROS treated with distinct treatments for 8h. Scale bar is 100 μm. (A) Quantitative analysis of DCFH-DA fluorescence by flow cytometry (Data are presented as mean ± SD,*p < 0.05, ***p < 0.001 by one-way ANOVA, n = 3/group).

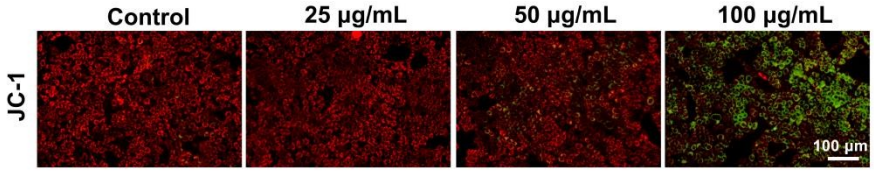

Figure S19. Fluorescence images to detect mitochondrial membrane potential measurement by the JC-1 probe. Scale bar is 100 μm.

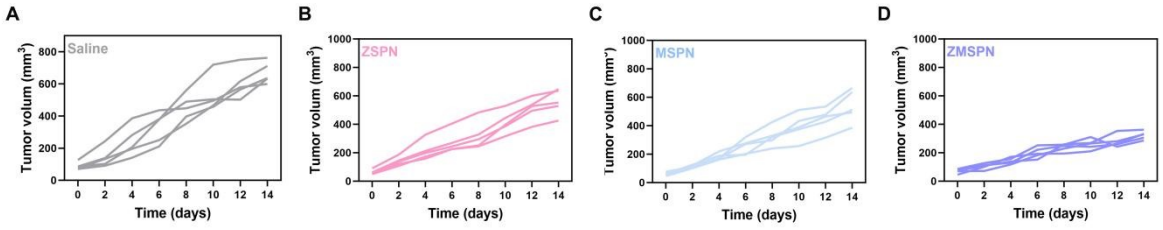

Figure S20. Individual tumor growth kinetics in mice following diverse therapeutic interventions.

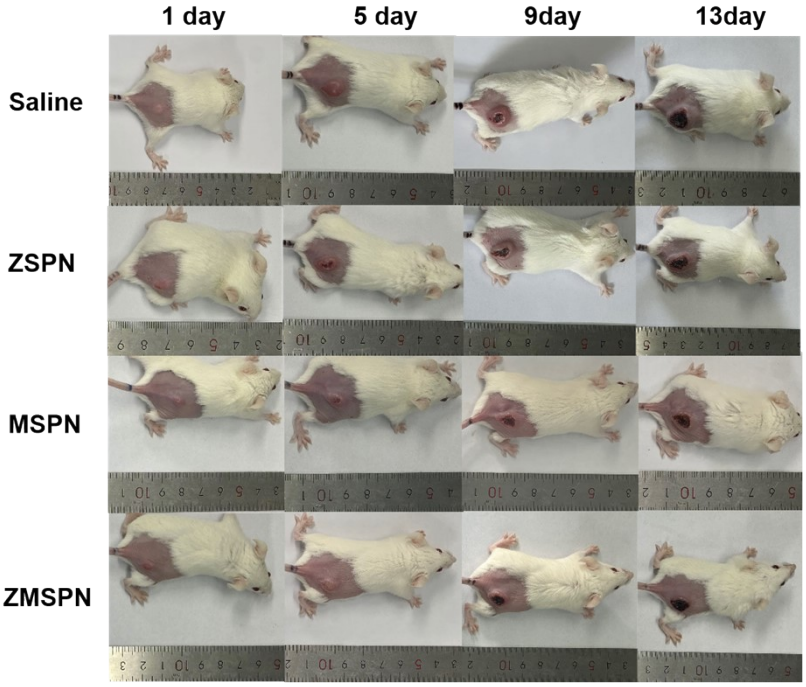

Figure S21. The representative digital photographs of diverse group.

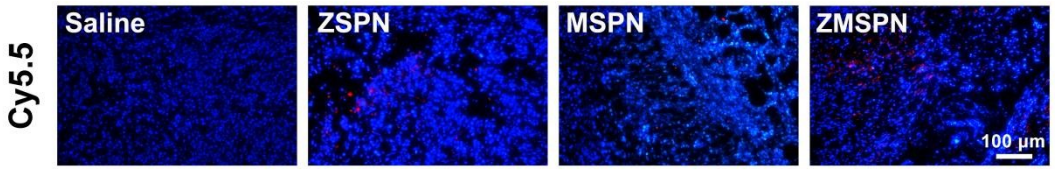

Figure S22. Tumor sections from different groups after two administrations. Scale bar is 100 μm.The particles were labeled with the fluorescent probe: Cy5.5 (red).

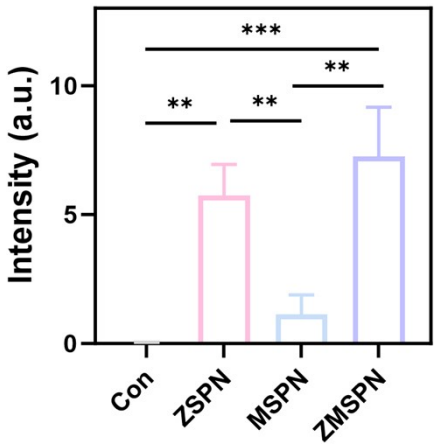

Figure S23. Quantitative analysis of Cy5.5 fluorescence of distinct treatments (Data are presented as mean ± SD,*p < 0.05, **p < 0.01 by one-way ANOVA, n = 3/group).The particles were labeled with the fluorescent probe: Cy5.5 (red).