Hollow spherical $Mn_{0.5}Zn_{0.5}Fe_2O_4$ nanoparticles with magnetic vortex configuration for enhancing magnetic hyperthermia

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2.1. Chemical. Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), Zinc chloride ($ZnCl_2$), tetrahydrate manganese chloride ($MnCl_2 \cdot 4H_2O$), Ethylene glycol (EG), Sodium acetate (CH_3COONa), Hexadecyl trimethyl ammonium bromide (CTAB), Acetone (C_3H_6O), N-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), NH_2-PEG-NH_2, and Urea (CH_4N_2O) were obtained from Shanghai Aladdin Industrial Corporation, Shanghai China. The human breast cancer cells (MCF-7, BT549), and mouse cells (4T1) were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin at 37 °C in an atmosphere with 100% humidity, and 5% CO_2. FBS and DMEM were obtained from Shanghai Beyotime Biotechnology Co., Ltd., Shanghai, China., Live/Dead cell double staining kit, lysobrite green, and methyl thiazolyl tetrazolium (MTT), and doxorubicin (DOX) were obtained from Shanghai Beyotime Biotechnology Co., Ltd., Shanghai China. All chemicals and reagents were of analytical grade and used without any further purification.

2.6. In Vitro Cell Culture and Incubated Conditions. The MCF-7 cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% (V/V) fetal bovine serum (FBS), 1% penicillin/streptomycin at 37°C in an atmosphere with 100% humidity, and 5% CO_2. The MCF-7 cells were subcultured regularly using trypsin/EDTA.

2.8. Cellular Uptake. For carrying out cell uptake experiments, doxorubicin (DOX) labeled

$Mn_{0.5}Zn_{0.5}Fe_2O_4$-PEG (MZF-HS-PEG / DOX) was prepared such that the DOX was loaded in $Mn_{0.5}Zn_{0.5}Fe_2O_4$-PEG. MCF-7 cells were seeded onto a laser confocal petri dish (Φ=20 mm) with a density of 2×10^5 cells/well and cultured overnight. The MZF-HS-PEG / DOX (DOX = 15 μM) was incubated with the cells at 37°C for 2 h. After washing 3 times, the cells were stained with Hoechst 33342 (10 μg/mL) and LysoBrite Green (1 μM) in DMEM at 37°C for 20 min. After washing with PBS 3 times, the cells were observed using a Zeiss LSM800 confocal microscope.

2.7. In Vitro Viability Assays of MZF-HS-PEG. The MCF-7 cells were used to evaluate the biocompatibility of MZF-HS-PEG. First, the MCF-7 cells were dispensed onto a 96-well plate at a density of 1×10^4 cells/well. After a 24 h culture period for cell attachment, the media were taken out from the wells, followed by washing three times with PBS, and then incubated with various concentrations of MZF-HS-PEG (0, 20, 50, 100, and 200 μg/mL). After further incubation for 24 h and 48 h as different control groups, the cell viability was determined by a standard MTT assay.

2.9. Evaluation of the Magneto-thermal toxicity. The MCF-7 cells were seeded onto a laser confocal petri dish at a density of 2×10^5 cells/well and cultured for 24 h. The cells were first incubated with various concentrations of MZF-HS-PEG (0, 20, 50, 100, 150, and 200 μg/mL) for 4 h. After washing with PBS several times and being replaced with fresh DMEM, the cells were kept under both an AMF with an amplitude of 6.7 kA/m for 10 min. After MHT treatment, the cells were incubated with fresh DMEM containing 10% FBS at 37°C for 30 min. The cells were stained with propidium iodide (PI) and Calcein-AM Live/Dead cell double staining kit and then observed by a fluorescent inverted microscope. The cell survival efficiency of the dual-mode ablation of cancer cells with MZF was also further investigated using an MTT assay.

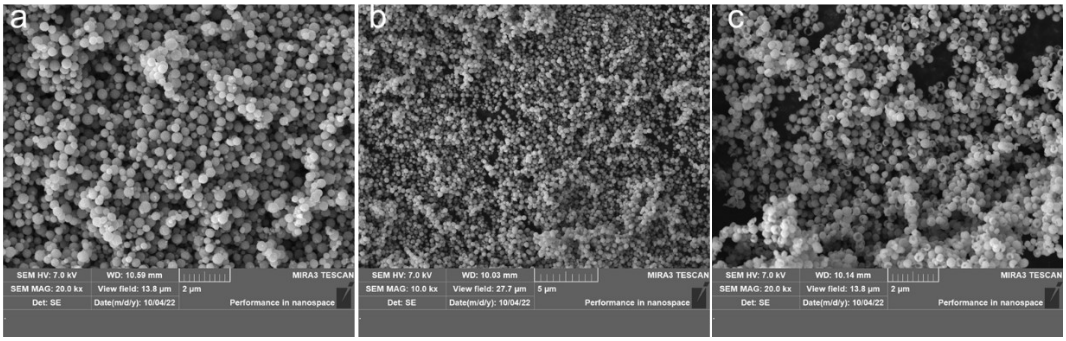

Figure S1. The SEM images of (a,b) n_{urea} = 32 mmol (1:8), (c) n_{urea} = 40 mmol (1:10)

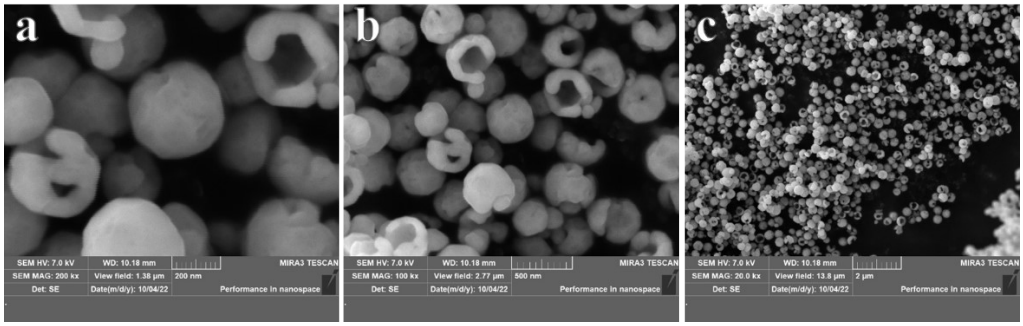

Fig. S2 The SEM images of spherical $Mn_{0.5}Zn_{0.5}Fe_2O_4$ (1:10)

The reaction mechanism of the sphere morphology is Figure S4. First of all, EG reacts with urea to produce Ethylene Carbonate (EC, $CO(OCH_2)_2$) and NH_3. At the same time, a dehydration reaction occurs between EG and EG, then H_2O is released. NH_3 and H_2O provide OH^- in an alkaline environment. Besides, NH_3 provides internal hollow structure support during formation and growth of $Mn_{0.5}Zn_{0.5}Fe_2O_4$ grains. An increase of urea content can raise PH value of the solution, promoting to form the $Mn_{0.5}Zn_{0.5}Fe_2O_4$ spheres in smaller size.

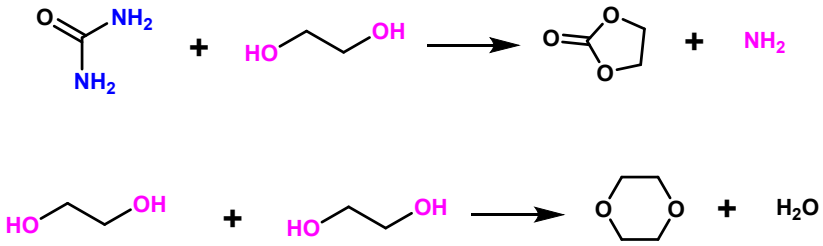

Fig. S3. The Reaction Mechanism of $Mn_{0.5}Zn_{0.5}Fe_2O_4$ Hollow Sphere Morphology.

In all reactions, EG (Ethylene Glycol, $(CH_2OH)_2$) acts as solvent and reducing agent, and CTAB is a surfactant. Urea (H_2NCONH_2) provides an alkaline environment and releases NH_3 to produce hollow structure. When $Mn_{0.5}Zn_{0.5}Fe_2O_4$ spheres are formed, the reduction of Fe (III) to Fe (II) is first occurred, accompanying by the decomposition of the intermediate of Fe (II) at high temperature [38]. Under the action of an appropriate amount of reducer EG, there are enough precursors — metal salts supplied during the growth stage of the crystals. At high temperature, the particles are prone to grow thermodynamically, which means that particles grow uniformly in all directions, thus generating spheres.

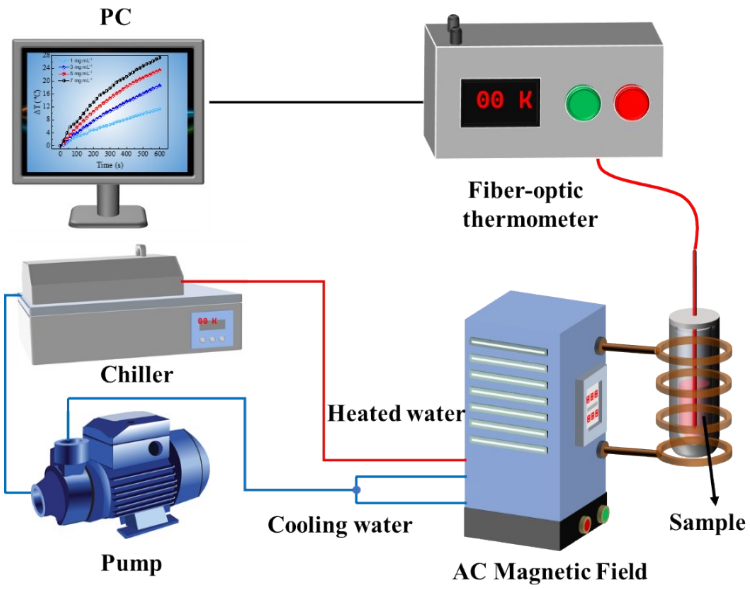

Fig. S4. Schematic diagram of magnetothermal experiment system

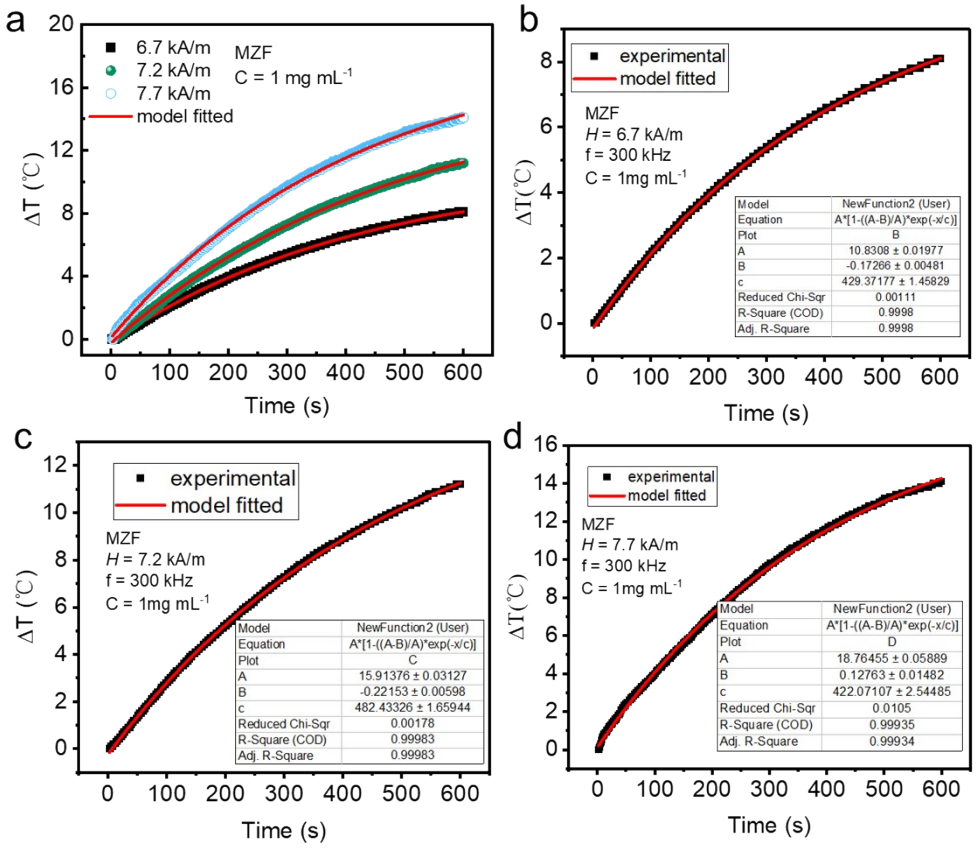

Fig. S5. Temperature-time curves of MZF at a fixed combination of the frequency (*f*) and various amplitudes (*H*) of the AC magnetic field, and their fitted data based on Box-Lucas Model

Table S1 The parameters (τ , T_∞ and T_0) that were extracted from the fitting of the T versus t data

at a fixed combination of the frequency (f) and various amplitudes (H) of the AC magnetic field.

Sample	τ (s)	T_∞ (°C)	T_0 (°C)	f (kHz)	H (kA/m)	C (mg mL^{-1})
MZF	429.3 ± 1.4	10.8 ± 0.019	-0.17 ± 0.004	300	6.7	1
MZF	482.4 ± 1.6	15.9 ± 0.03	-0.22 ± 0.005	300	7.2	1
MZF	422.0 ± 2.5	18.7 ± 0.05	0.12 ± 0.014	300	7.7	1
MZF-HS	313.6 ± 1.2	14.3 ± 0.02	-0.14 ± 0.011	300	6.7	1
MZF-HS	303.6 ± 1.2	17.9 ± 0.02	-0.08 ± 0.011	300	7.2	1
MZF-HS	307.1 ± 1.2	20.8 ± 0.02	-0.62 ± 0.017	300	7.7	1
MZF	1033.2 ± 34	30.1 ± 0.78	-0.8 ± 0.03	300	6.7	3
MZF	506.2 ± 5.5	25.1 ± 0.15	0.1 ± 0.027	300	6.7	5
MZF	791.6 ± 13	45.0 ± 0.54	-0.39 ± 0.03	300	6.7	7
MZF-HS	839.7 ± 8.0	36.3 ± 0.24	-0.18 ± 0.015	300	7.7	3
MZF-HS	548.6 ± 1.4	35.4 ± 0.05	-0.28 ± 0.008	300	7.2	5
MZF-HS	460.4 ± 6.0	37 ± 0.26	0.56 ± 0.05	300	6.7	7

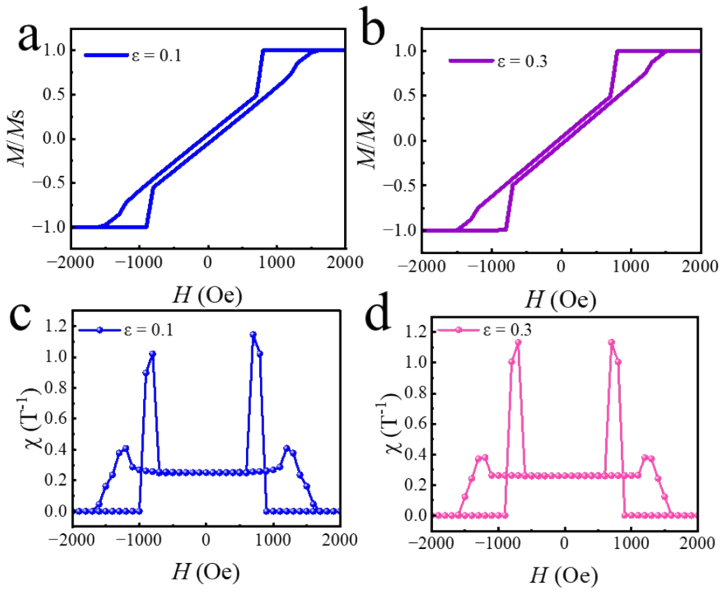

Fig. S6 *The simulated normalized hysteresis loops of MZF-HS with ε = 0.1 (a), and ε = 0.3 (b). (c) The differential magnetic susceptibility χ (χ = dM/dH) of MZF-HS with ε = 0.1 (c), and ε = 0.3 (d).*

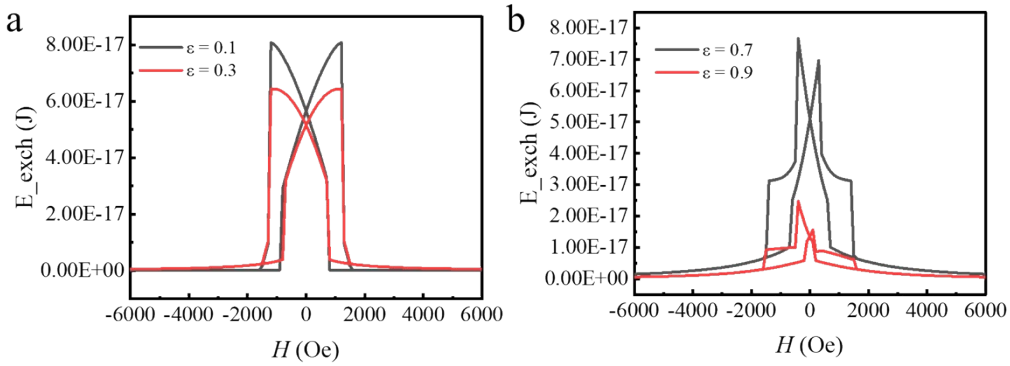

Fig. S7 *The plot of exchange energy for ε = 0.1 , ε = 0.3* (a) *and ε = 0.7 , ε = 0.9* (b).

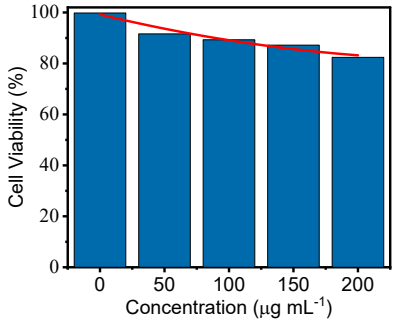

Fig. S8. Cell viability of MCF-7 cells incubated with different concentrations of MZF-HS for 24 h