Supplementary information

Injectable Thermosensitive Magnetic Nanoemulsion Hydrogel for Multimodal-Imaging-Guided Accurate Thermoablative Cancer Therapy

Haoan Wu, Lina Song, Ling Chen, Yixin Huang, Yang Wu, Fengchao Zang, Yanli An, Hanbai Lyu, Ming Ma, Jun Chen, Ning Gu* and Yu Zhang*

a State Key Laboratory of Bioelectronics, Jiangsu Key Laboratory for Biomaterials and Devices, School of Biological Science and Medical Engineering & Collaborative Innovation Center of Suzhou Nano Science and Technology, Southeast University, Nanjing 210096, P. R. China. E-mail: zhangyu@seu.edu.cn

b CAS Key Laboratory for Biological Effects of Nanomaterials & Nanosafety, National Center for Nanoscience and Technology, Beijing 100190, P. R. China

c Jiangsu Province Tumor Hospital, Nanjing 210009, P. R. China

d Jiangsu Key Laboratory of Molecular and Functional Imaging, Medical School, Southeast University, Nanjing 210009, P. R. China
Experimental Section

Materials. Iron(III) acetylacetonate \([\text{Fe(acac}_3\text{) (98%)})\], OA \((\text{C}_{17}\text{H}_{33}\text{COOH, 85%)})\), and OAm \((\text{C}_{18}\text{H}_{35}\text{NH}_2, 90\%)\) were purchased from Aladdin Chemical Reagent Co. Ltd. Zinc 2,4-pentanedioniate monohydrate \([\text{Zn(acac)}_2\cdot\text{H}_2\text{O}]\) and benzyl ether (98%) were purchased from Alfa Aesar. Indocyanine green (ICG) was purchased from Sangon Biotech (Shanghai) Co. Ltd. Silicone oil (viscosity = 5 cp, PDMS), PEG-DA \((\text{M}_w \sim 700 \text{ g mol}^{-1})\) were purchased from Sigma Aldrich. The commercially available reagents, including Chloroform (98%), ethanol (95%), Sodium dodecyl sulfate (SDS, 98%), were all purchased from Sinopharm Chemical Reagent Co. Ltd. All the chemicals were used as obtained without any further purification.

Characterization. A dynamic light scattering (DLS) device from Malvern Instruments (mode) was used to measure the hydrodynamic size of MNH. Before the measurement, 30 μL MNH was diluted with 3 mL distilled water. A JEM-2100 electron microscope (JEOL) with a working voltage of 200 kV was used to observe the morphology of the MNH. Transmission electron microscopy (TEM) image negatively stained by 2% sodium phosphotungstate \((\text{PH} = 7.0)\) was taken from the 1:10 diluted MNH. Morphologies of the porous MNH films were studied by scanning electron microscopy (SEM, Ultra Plus, Carl Zeiss, Germany) after thermal gelation using water bath and alternating magnetic field heating. The M–H curves and magnetism of Oleic acid (OA)-coated Zn ferrite MNPs were obtained by a vibrating sample magnetometer (VSM, Lakeshore VSM 7407). Rheological properties were studied using a Physica MCR 302 (Anton Paar, Germany) equipped with coaxial cylinder geometry (PP25). Temperature-ramp experiments were carried out by heating the sample at a rate of 1 °C min\(^{-1}\) while monitoring the viscoelastic moduli under oscillatory shear at a frequency of \(f = 1 \text{ Hz}\) and strain amplitude of \(\gamma_0 = 1\%\). The temperature at which \(G' = G''\) represents the gelation temperature. Viscosity change at
specific temperature was carried out by rotating speed over a range of 1-20 s⁻¹ at a strain amplitude of $\gamma_0 = 1\%$.

For biological TEM (JEOL/JEM-2000E, Japan) analysis, MNH was cut into small pieces of ~1 mm³ and embedded in resin, which was then cured in an oven at a temperature of 60 °C (2 days) for TEM observation.

For the Prussian blue staining of frozen section, MNH was embedded in OCT (Optimal Cutting Temperature) Compound, and then was cut into small pieces of 50 μm on glass slides by Frozen Slicer (Leica CM 1850). In succession, MNH specimens were stained 5 min using Prussian Blue containing 1% K$_3$[Fe(CN)$_6$] and 3% HCL at room temperature, and then examined using optical microscopy.

**Preparation of PEGylated magnetic nanoparticles.** A DSPE-PEG2000 (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)], Shanghai A.V.T. Pharmaceutical L.T.D. China) was dissolved in 3 ml chloroform. OA-capped Fe$_3$O$_4$/Zn ferrite MNPs (dispersed in 1 ml chloroform) and DSPE-PEG2000 were mixed at 1:5 weight ratio (iron:DSPE-PEG2000) in a 25 ml round-bottom flask, and then, 3 ml deionized water was added gradually to the mixture. After chloroform was completely vaporized by slow evaporation (60 °C, 15 min), the MNPs became water soluble.

**Thermal response gelation performance in vitro** 1 mL of MNH packed in glass bottle was allowed to stand at 37 °C water bath for 5 minutes. Then the bottle was inverted to observe its flowability.

**Animal protocol.** All animal care and experimental procedures were approved and performed in accordance with the Animal Management Rules of the Ministry of Health of the People’s
Republic of China and the guidelines for the Care and Use of the Southeast University Laboratory Animal Center. Female BALB/c mice (4 weeks of age, 20-25 g in weight) were purchased from the Model Animal Research Center of Southeast University. To establish the experimental model of the breast tumor, 4T1 cells ($2 \times 10^6$, in 150 $\mu$L serum free RPMI-1640 medium) were subcutaneously injected into the right hind flank of each mouse. The tumors were used for therapy after implantation at 2 weeks when the tumor diameter reached ~5 mm.

**In vivo ultrasound imaging.** In vivo US imaging was performed using a small animal US imaging system (Visualsonics Vevo2100, Canada). The BALB/c mice bearing 4T1 xenograft were firstly anesthetized by chloral hydrate (0.08 - 0.1 mL, 10%). The tumors were scanned in three dimensions to confirm a clear background signal and then the pinpoint of syringe was put into the tumor center by the guidance of ultrasound imaging (30 MHz, 20 dB). Next, the liquid MNH (30 $\mu$L) was injected slowly into the tumor. The enhancement in the intensity of the US signal of MNH was in-situ and real-time monitored.

**In vivo MRI and NIRF imaging.** When the tumor size reached ~60 mm$^3$, tumor-bearing mice were intratumorally injected with 30 $\mu$L MNH. MRI was performed at 7.0 T Micro-MRI (PharmaScan, Brukers, Germany) with a 35 mm birdcage coil and small animal-cradle. 4% isoflurane/air gas mixture was delivered through a nose cone to anesthetize the mice. During the experimental period, the mice body temperature was maintained by a circulating warm water (37 °C). T2-weighted and T2*-weighted images were acquired by a flash sequence and the parameters were as follows: repetition time (TR) = 400 ms, echo time (TE) = 8.0 ms, Flip Angle = 30°, number of excitations (NEX) = 1, matrix size = 256 × 256, field of view (FOV) = 35 mm × 35 mm, slice thickness = 1 mm. Signal intensities were measured in defined regions of interest (ROIs) with Image J software.
For in vivo NIRF imaging, the tumor-bearing (4T1) mice was in-situ injected with MNH (30 μL) into the center of tumor tissues. After five minutes of the administration, the mice were imaged using the Maestro in vivo optical imaging system. Then, the mice were placed into the center of electromagnetic induction heating coil for 5 min magnetic thermal ablation. Two hours after magnetic irritation, the mice were imaged by a Maestro in vivo imaging system again. The optical imaging parameters were as follows: a magnification of ×2, a wavelength of 704 nm and a NIR emission filter.

**Ex vivo histological staining.** Tumor tissues from tumor-bearing mice in different groups were collected and fixed in 5% neutral buffered formalin at room temperature. Then the tissues were processed into paraffin and sectioned at a thickness of 3-5 mm. Ferric ions and cell nucleus were stained successively by Prussian blue and nuclear fast red (PB&NFR) respectively, haematoxylin and eosin (H&E) staining slides were also prepared and then observed by bright field microscopy.
**Supplementary Figures**

**Figure S1.** (a) Photographs of tumor-bearing mice. (b) After intratumoral injection of PEGylated Fe$_3$O$_4$. (c) Thermal image of intratumorally with PEGylated Fe$_3$O$_4$ under the influence of ACMF.
**Figure S2.** SEM image of Zn ferrite magnetic nanoparticles.

![SEM image of Zn ferrite magnetic nanoparticles](image)

**Figure S3.** (a) SEM, (b: Fe, c: Zn) corresponding element mapping and (d) SEM-EDS elemental analysis of Zn ferrite magnetic nanoparticles at the same observing position.

![Element mapping and SEM-EDS analysis](image)

**Figure S4.** Enlarged TEM image of MNH taken at 25 °C.

![Enlarged TEM image of MNH](image)
**Figure S5.** Negatively stained TEM image of the MNH taken at 37 °C.

**Figure S6.** SEM (a) and corresponding element mapping (b-d; b: Fe, c: Si and d: Merged image of b and c) of MNH at the same observing position.
Figure S7. MNH was tightly adhered to the tumor tissue when the temperature reached 60-65°C during the treatment.

Figure S8. Photograph of MNH when the temperature reaches eighty degrees.
Figure S9. SEM image of MNH after thermal gelation under ACMF (410 kHz, 1.8 kA m$^{-1}$).

Figure S10. Prussian blue staining images of frozen section from MNH, gelation occurred in the exposure of ACMF (a) and in water bath (b), respectively.
**Figure S11.** Digital photos of tumor-bearing mice which was placed into the center of a water-cooled magnetic induction copper coil.

**Figure S12.** Magnetic resonance image of PEGylated Fe$_3$O$_4$ before and after injected into the tumor.
**Figure S13.** Digital photos of 4T1 xenografted tumor mice three months after treatment.

**Figure S14.** The PB&NFR staining image of border parts between tumor tissue and normal tissue (magnification: 200 ×).
Figure S15. H&E stained tissue sections of major organs, including the heart, liver, spleen, lung, and kidney from three control groups mouse and MNH-implanted mouse post ACMF irradiation at day 28 (magnification: 100 ×).