Artificially engineered "tumor bio-magnet" for collecting blood-circulating nanoparticles and magnetic hyperthermia

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Experimental section

1 Materials

Polylactic-co-glycolic acid (PLGA, Mw: 40000 Da, 50:50,) was purchased from Jinan Daigang Bioengineering Co., Ltd (Jinan, China). N-methyl pyrrolidone (NMP) was obtained from Sigma-Aldrich (AL,USA). Neodymium iron boron ($Nd_2Fe_{14}B$) particles were silver gray powder (diameter: 30 μm), obtained from Guangzhou Xinnuode Rotatable Parts (Guangzhou, China). Ferroferric oxide (Fe_3O_4) nanoparticles were black powder, and the diameter of particles were 20-50 nm, obtaining from Chengdu AikeDa Chemical Reagent Co., Ltd (Chengdu, China). The formation and preparation process of $Nd_2Fe_{14}B$/ Fe_3O_4-PLGA were shown in Video-S1. Nude mice and Kunming mice were purchased from and fed at Animal Experiment Center of Chongqing Medical University. All animal studies were performed following protocols approved by Animal Ethics Committee of Chongqing Medical University.

Ethical statement:

All animal procedures were performed in accordance with the Guidelines of the Ministry of Science and Technology of Health Guide for Care and Use of Laboratory Animals, China, and approved by the institutional ethical committee (IEC) of Second Affiliated Hospital of Chongqing Medical University.

2 Preparation of liquid $Nd_2Fe_{14}B/Fe_3O_4$-PLGA bio-magnet

Typically, polylactic-co-glycolic acid (1.1 g) was put into a penicillin bottle, followed by adding NMP (2 mL) as our previous research. The PLGA was thoroughly dissolved into NMP at 37℃, 120 r/min overnight to obtain a homogeneous PLGA liquid bio-injection. Certain amount of $Nd_2Fe_{14}B$ power and Fe_3O_4 particles before being charged were dispersed into PLGA liquid gel *via* a mechanical vibration process at the mass ratio of $Nd_2Fe_{14}B$ and Fe_3O_4 was 2.5, 4.5 and 6.5.

3 Characterization of solid $Nd_2Fe_{14}B/Fe_3O_4$-PLGA

The morphology of liquid bio-injection was recorded by digital photos. The solid form of bio-magnet samples were randomly chosen for the structural and compositional analysis by scanning

electron microscope (SEM) (Olympus Tokyo, Japan) and ESD manpping. The saturation magnetization was determined on Gauss meter (TJSH-035).

4 Evaluation of syringeability of $Nd_2Fe_{14}B/Fe_3O_4$-PLGA

The as-obtained $Nd_2Fe_{14}B/Fe_3O_4$-PLGA liquid bio-injection was freely loaded into a standard 1 mL syringe (21 gauge pinpoint), then injected into phosphate buffered saline (PBS) at the volume of 50 μL, 75 μL and 100 μL, respectively. The progress of the liquid bio-magnet transforming into solid form after contacting with water were evaluated and the post-formed $Nd_2Fe_{14}B/Fe_3O_4$-PLGA solid bio-injections were monitored by video (Video-S2) and digital photos.

5 Ultrasound imaging to *in-situ* observe the phase transformation of $Nd_2Fe_{14}B/Fe_3O_4$-PLGA injection

Agarose (9 g) was poured into a plastic square box, followed by further adding degassed water (300 mL). The mixture was stirred and heated in a microwave oven for 200 seconds repeatedly. The space was created within agarose by dispersing certain numbers of 100 μL pipette tips into solution, which were then taken out immediately after the chilling down and solidification of agarose solution. The agarose gel model was stored at 4 ^oC for further usage.The prepared liquid $Nd_2Fe_{14}B/Fe_3O_4$-PLGA bio-injection was directly injected into the agarose gel model filling with PBS. As the meanwhile, the phase-transformation procedure was dynamically guided by ultrasound (US) imaging (Esoate, L5 − 12 MHz) and the ultrasound images were recorded every 10 seconds.

6 Magnetism charging of solid $Nd_2Fe_{14}B/Fe_3O_4$-PLGA

The specific magnetism of bio-magnet was endowed by charging with a magnetizing apparatus, which was kindly provided by Chongqing University of Science and Technology. The magnetizer (MT-4060) included direct current high voltage capacitor and a discharge coil resistance. The input power was AC 220 V, 30A. The center field strength is 8 Tesla, the peak charging voltage is DC 2000 V and the peak electric fields is 10,000 A. The current pulse produces a strong magnetic field in the coil, and the $Nd_2Fe_{14}B$ component of $Nd_2Fe_{14}B/Fe_3O_4$-PLGA solid implant was magnetized in the coil to change its property to a permanent magnet.

7 Evaluations of magnetic properties of the post-formed bio-magnet

Magnetic flux density and magnetic hysteresis loop of bio-magnet were characterized by Gauss Tesla meter and physical property measurement system (PPMS). Magnetic physical quantities such as the maximum magnetic field intensity, coercivity and magnetic flux were obtained from magnetic hysteresis loop. As the meantime, *in vitro* fluorescence imaging, using winding scalp-acupuncture to simulate a blood vessel. Fluorescent superparamagnetic microspheres (200 μL) were injected into the blood vessel model, and made sure the fluorescent superparamagnetic microspheres were flowing. Bio-magnets were prepared before then put it into the middle of blood vessel model. Digital photos and fluorescence imaging photos were recorded the progress of collecting fluorescent superparamagnetic microspheres.

The study was performed after the nude mice were allowed to acclimate for one week. SMMC-7721 cancer cells (the Chongqing Key Laboratory of Ultrasound Molecular Imaging, Chongqing, China) were collected by centrifugation and dispersed into RPMI-1640 cell culture medium containing FBS(10% v/v), penicillin (100 U/mL) and streptomycin(100 μg/mL), which were then subcutaneously injected into the back of nude mice (0.1 - 0.2 mL, 1×10^6 cells/mouse). After further feeding for 3-4 weeks, the animal experiment was carried out when the tumor diameter reached around 1 cm.

The nude mice bearing the SMMC-7721 xenograft (n =20) were divided into four groups, including saline as group A, pure PLGA as group B, $Nd_2Fe_{14}B$ /Fe_3O_4-PLGA without magnetizing as group C, $Nd_2Fe_{14}B$/Fe_3O_4-PLGA bio-injection followed by magnetism charging as group D. All of the nude mice were injected with 75 μL liquid bio-magnet, $Nd_2Fe_{14}B$/Fe_3O_4-PLGA, PLGA gel and saline in the center of the tumor, respectively. After that, the magnetism of tumor bio-magnets which were group A endowed by charging with a magnetizing apparatus. Then, the fluorescent superparamagnetic nanoparticles (excitation wavelength: 660 nm, emission wavelength: 690 nm.) were injected *via* intravenous administration. The fluorescent images were recorded after the intravenous administration for different durations (24 h, 48 h and 72 h), and the intensities were recorded for quantitative analysis. The mice were sacrificed after *in vivo* fluoresce imaging, the bio-magnet was removed from the the tumor before cutting the tumor tissues into slices for Prussian-blue staining at different time points (before injecting, after injecting 24 h, 48 h, and 72 h), in order to observe the targeting efficiency of MNPs.

8 *In vivo* and *in vitro* magnetic hyperthermia evaluation

For the *in vitro* assay, a variety of solid bio-magnets were randomly chosen, containing different mass fraction of $Nd_2Fe_{14}B$ powder (25%, 45% and 65%), different volumes of bio-injection (50 μL, 75 μL, 100 μL), as well as magnetizing and without magnetizing ones. They were dropped into saline solution (1 mL) in Eppendorf tubes (2 mL). The Eppendorf tubes were put in the center of the electromagnetic induction-heating coil of a homemade magnetic hyperthermia analyzer (frequency: 626 KHz, output current: 28.6A and coil diameter: 3 cm). After the exposure to external alternating current ((a. c.) magnetic field), the infrared thermal images of the Eppendorf tube and bio-magnets were taken (time interval: 10 s with a total of 180 s). The Eppendorf tube containing only saline solution and solid PLGA (75 μL) were tested under the same condition for the blank controls. The temperatures were analyzed using the thermal images by Smart View 3.3 software.

The fresh *ex vivo* bovine livers were taken for the assessment. The liquid bio-magnet (65%wt-$Nd_2Fe_{14}B$, 75 μL) was injected into the middle of isolated bovine liver tissue. After the liquid-to-solid transformation of $Nd_2Fe_{14}B/Fe_3O_4$-PLGA followed by magnetism charging, the tissues containing solid $Nd_2Fe_{14}B/Fe_3O_4$-PLGA bio-magnet were put into the center of the electromagnetic induction-heating coil and was exposed to a. c. magnetic field for 1 min, 2 min, 3 min, 4 min and 5 min, respectively. The temperature variations in tissues were recorded every 10 s and analyzed by using the thermal images assisted by Smart View 3.3 software. The bovine livers without bio-magnet were treated under the same condition for the blank controls. Coagulative necrosis area after magnetic-hyperthermia ablation was measured and calculated. The volumes of coagulative necrosis liver tissues were calculated by the following formula: $V\ (mm3) = \pi/6 \times length \times width \times depth$[1].

The nude mice bearing the SMMC-7721 (n=5) xenograft were injected with certain amount of bio-injection (75 μL) in the center of the tumor under the guidance of ultrasound imaging after anesthesia. The mice were transferred to the center of heating coil for magnetic-induced hyperthermia ablation after anesthesia. The a. c. magnetic field was directly acted at the tumor site for 3 minutes. The tumor volume was monitored before and after magnetic hyperthermia for 5 d, 10 d and 15 d, and the tumor image was taken at the selected time intervals.

9 Biocompatibility assay

The as-prepared liquid $Nd_2Fe_{14}B/Fe_3O_4$-PLGA bio-injection were subcutaneous injected into Kunming mice, which were then fed under the guidelines approved by the Chongqing Medical University. The biodegradation process was monitored every day until two months feeding. After the biodegradation was completed, the mice were sacrificed, and the main organs (heart, liver, spleen, lung and kidney) were harvested for pathological analysis including hematoxylin and eosin, as well as Prussian blue staining.

Supplementary figures

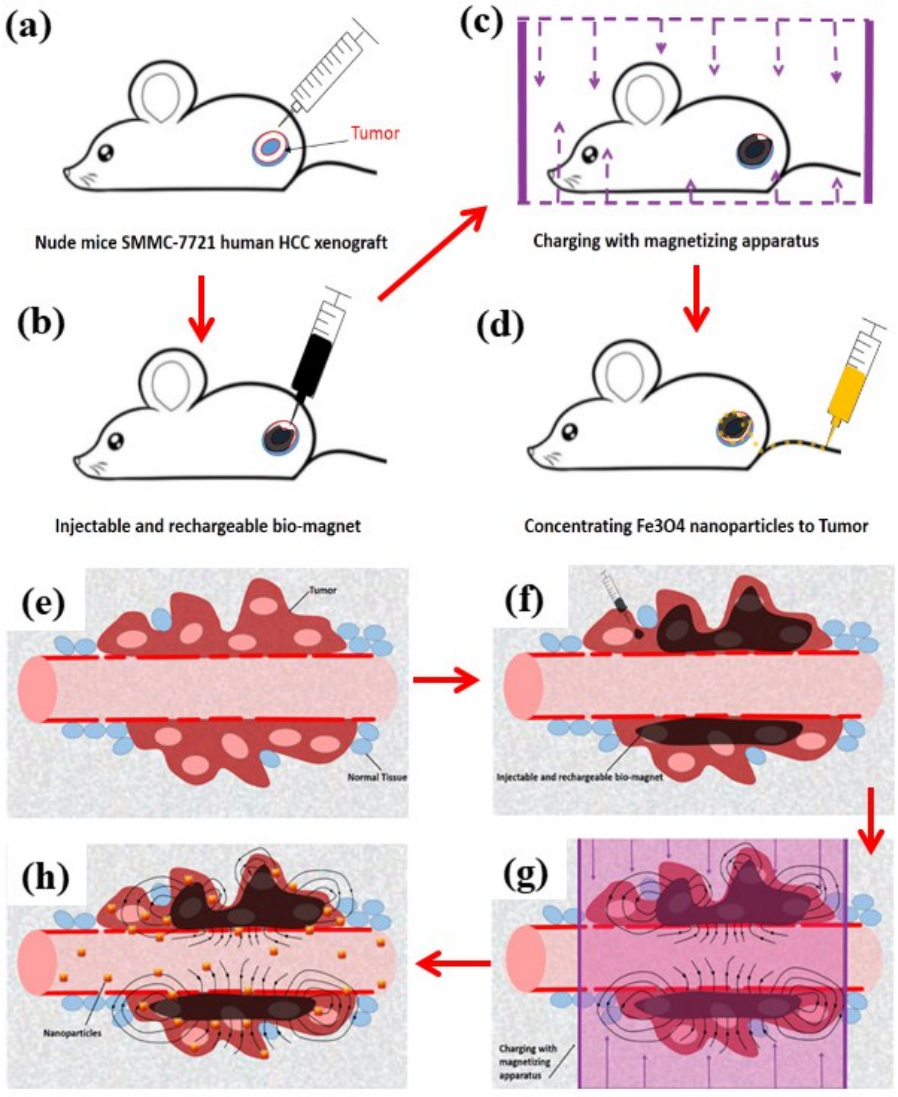

Figure S1. (a) Establishing nude mice SMMC-7721 human liver cancer xenograft, (b) followed by

the injection of $Nd_2Fe_{14}B/Fe_3O_4$-PLGA/NMP bio-injection, (c) further magnetic charging of solid $Nd_2Fe_{14}B$-Fe_3O_4-PLGA to form bio-magnet with magnetizing apparatus and (d) collecting blood-circulation MNPs. *In vivo* tumor microenvironment model showing (e, f) the implantation of $Nd_2Fe_{14}B/Fe_3O_4$-PLGA bio-injection, (g) endowed localized magnetic field within tumor tissue and (h) collecting MNPs by magnetic guidance assisted by the implanted bio-magnet.

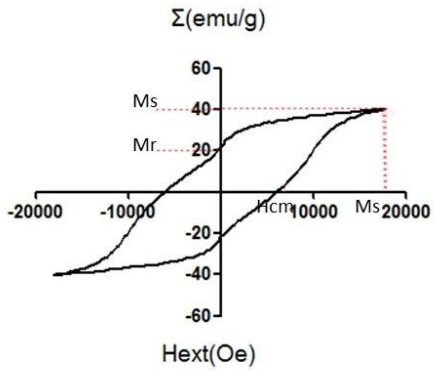

Figure S2. The magnetic hysteresis loop of the initial $Nd_2Fe_{14}B$ particles.

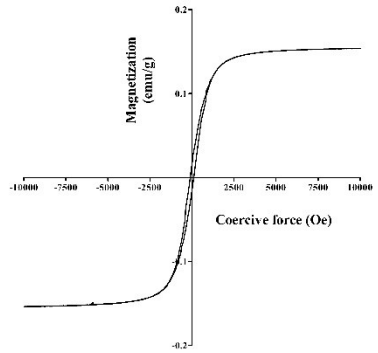

Figure S3. The magnetic hysteresis loop of the initial Fe_3O_4 nanoparticles.

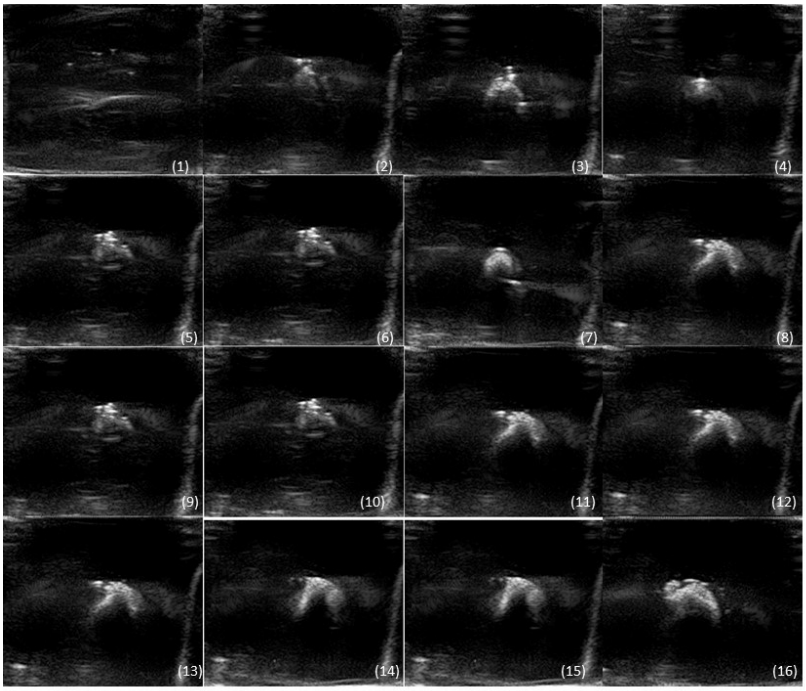

Figure S4. *In vitro* ultrasound-imaging characterizing the phase-transformation procedure of $Nd_2Fe_{14}B/Fe_3O_4$-PLGA from liquid (1: 0 s) to its solid state (2: 0.8 s, 3: 1.6 s, 4: 2.4 s, 5: 3.2 s, 6: 4.0 s, 7: 4.8 s, 7: 4.8 s, 8: 5.6 s, 9: 6.4 s, 10: 7.2 s, 11: 8.0 s, 12: 8.8 s, 13: 9.6 s, 14: 10.4 s, 15: 11.2 s and 16: 12 s).

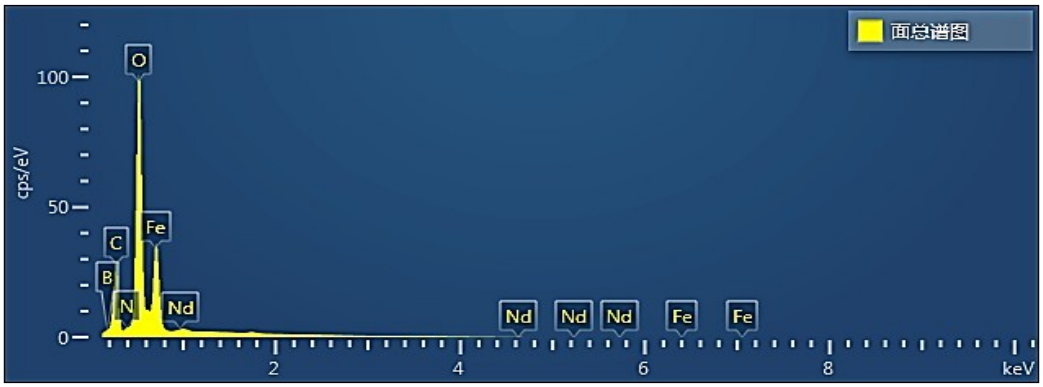

Figure S5. Energy dispersive spectrometer (EDS) result of post-formed $Nd_2Fe_{14}B/Fe_3O_4$-PLGA solid implant.

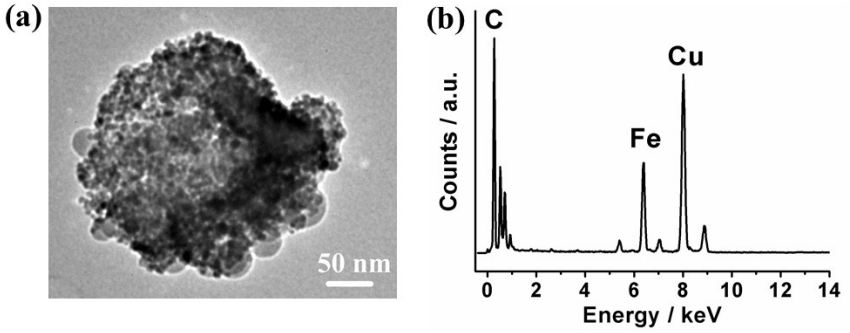

Figure S6. (a) TEM image and (b) corresponding EDS result of the adopted MNPs.

Video S1. Preparation process

Video S2. Liquid solid transformation of bio-magent.

Video S3. Magnetism test of 60 μL of bio-magnet.

Video S4. Magnetism test of 80 μL of bio-magnet.

Video S5. Magnetism test of 100 μL of bio-magnet.

Reference:

1. H. Tang, G. Yuan, P. Li, F. Hui, Z. Wang, Y. Zheng, H. Ran and C. Yu, *ACS applied materials & interfaces*, 2018, **10**, acsami.8b01967.