Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting information

A Nanoparticle Enabled Focused Ultrasound-Stimulated Magnetic Resonance Imaging Spotlight

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Experimental session

Chemicals

Tetraethylorthosilicate (TEOS; 99%, Aldrich), cetyltrimethylammonium bromide (CTAB; 98%, Aldrich), sodium hydroxide (99%, Fisher Scientific), absolute ethanol (EtOH; Aldrich). 3-aminopropyl triethoxysilane (APTES; 99%, Aldrich), poly(N-isopropylacrylamide) (PNIPAm, carboxylic acid terminated, Mn=7000, Aldrich), EDC•HCl (99%, Covachem), sulfo-NHS (99%, Covachem), diethylenetriaminepentaacetic acid gadolinium(III) dihydrogen salt hydrate (Gd-DTPA; 97%, Aldrich), nitric acid (TraceMetal grade, Fisher), methyl cellulose (4000 cP, Sigma), agarose BP160-100 (Molecular Biology Grade, Fisher) were used as received. Anhydrous toluene was obtained by distillation from CaH_2 under dry nitrogen.

Synthesis of Gd-DTPA and PNIPAm modified MSNs (Gd-P-MSNs)

Synthesis of mesoporous silica nanoparticles (MSNs)

0.25 g CTAB and 875 μL of sodium hydroxide solution (2 M) were dissolved in 120 mL of water under stirring. The solution was heated at 80 °C for 30 minutes, followed by the addition of 1.2 mL of TEOS and 0.79 mL ethyl acetate under vigorous stirring. Stirring was continued for 2 h at 80 °C, and then the solution was allowed to cool to room temperature. The nanoparticles were collected by centrifugation (15 min at 7830 rpm), washed 3x with ethanol (3x 30 mL) and dispersed in 20 mL ethanol for further use. Approximately 200 mg of MSNs was obtained in each batch.

Amine modification on MSN surface

Around 200 mg unfunctionalized MSNs was washed 3x with toluene (3x 30 mL), and redispersed in 30 mL of dry toluene stirring in a flame-dried 50 mL round bottom flask under nitrogen. Then 120 μL (3-aminopropyl)triethoxysilane was added drop by drop and resulting mixture was refluxed in 110 °C oil bath under nitrogen overnight. The amine-modified MSNs was collected by centrifugation (10 min at 7830 rpm) and washed 3x with ethanol (3x 30 mL). Product was redispersed in 20 mL ethanol for further use.

Extraction of APTES-functionalized MSNs (NH_2-MSNs)

APTES-functionalized MSNs dispersed in toluene were washed 2x with ethanol (2x 30 mL). To extract the organic template from the pores, the nanoparticles were dispersed in 80 mL of an acidic ethanolic solution (EtOH:HCl(conc.) = 90/10 (v/v)), refluxed for 1 h, collected by centrifugation (10 min at 7830 rpm), and repeated above procedure one more time. The product was washed 2x with ethanol (2x 30 mL) and stored in ethanol.

Gd-DTPA modification of NH_2-MSNs

NH_2-MSN dispersed in ethanol was washed 3x with DI water (3x 30 mL), then dispersed in HEPES buffer (pH=7.4) for future use. 1.2 mL of Gd-DTPA water solution (0.10 g/mL, pH=6.7) was mixed with 4.8 mL of MES buffer (100 mM, pH = 6.0), 17.2 mg of EDC•HCl and 19 mg of sulfo-NHS, and the mixture was stirred for 20 min. 0.3, 0.6, 2.1, 3 mL of mixture were added to 5 mL of HEPES buffer with 60 mg NH_2-MSN dispersed, and stirred in room temperature for 24 h. Gd-DTPA modified MSNs (Gd-MSNs) products were washed 3x with DI water (3x 30 mL) and labeled as sample 1 to sample 4 (S1, S2, S3, S4).

PNIPAm modification of Gd-MSNs

Wash S1-S4 with HEPES buffer (20 mL) and redisperse it in 20 mL HEPES buffer for further use. 30 mg of PNIPAm was dissolved in cold MES buffer and stirred for 15 min, then 8.25 mg of EDC•HCl and 9.3 mg of sulfo-NHS were added. The mixture was stirred for one hour in room temperature, then add to 5 mL HEPES buffer with 50 mg of dispersed S1-S4. The mixture was stirred for 24 h, and products were collected by centrifugation (15 min at 7830 rpm) at 20 °C followed by washing 8x with cold DI water (8x 20 mL). S1-S4 were redispersed in 20 mL DI water for further use.

Tissue mimicking gel and MRI-guided HIFU sample preparation

1 g methyl cellulose was slowly added to 15 mL boiled water and stirred for 3 min. Then 25 mL condensed milk was added followed by 10 mL cold water. The mixture was stored in refrigerator overnight to eliminate air bubble. 3 mg Gd-P-MSNs were dispersed in 0.5 mL water and then mixed with 1 mL gel/milk mixture, resulting a 2 mg/mL Gd-P-MSNs gel/milk mixture. The Magnevist (Mgv) control was made by similar method. Mgv was first diluted to 0.5 mL water, then was mixed with 1 mL gel/milk mixture.

Agarose phantom

17.5 g agarose was slowly added to hot water with stirring. Then the solution was heated up to boiling, and then poured

to sample holder model. After agarose was solidified under room temperature, it was stored in refrigerator for further use.

Characterization

Transmission electron microscopy (TEM)

TEM images were recorded on a Tecnai T12 Quick CryoEM at an accelerating voltage of 120 kV. A suspension (8 μL) of nanoparticles in ethanol was dropped on a 200 mesh carbon coated copper grid and the solvent was allowed to evaporate at room temperature.

Zeta-potential analysis and dynamic light scattering (DLS)

Zeta-potential analysis and DLS were carried out on a ZetaSizer Nano (Malvern Instruments Ltd., Worcestershire, U.K.) in DI water.

Thermogravimetric analysis (TGA)

TGA was performed using a Perkin-Elmer Pyris Diamond TG/DTA under air (200 mL/min). Approximately 5-10 mg of sample was loaded into aluminum pans. The sample was held at 100 °C for 30 minutes, and then the data were recorded during a temperature scan from 100 to 600 °C at a scan rate of 10 °C/min and an isothermal process of 600 °C for 80 min. The plotted values are normalized to the weight at 100 °C. An empty aluminum pan was used as a reference.

Quantification of Gd-DTPA by inductively coupled plasma atomic emission spectroscopy (ICP-OES)

ICP-OES measurements were made using ICPE-9000 Shimadzu. 0.1 mL of sodium hydroxide solution (2 M) was added to approximately 0.5-1 mg sample (Gd-MSNs or Gd-P-MSNs) dispersed in 0.05 mL of Milli-Q water, and the mixture was sonicated for 1 h. Then 0.05 mL of nitric acid was added, and the mixture was sonicated for 1h. The solution was then diluted to 10 mL with 2% nitric acid for measurement.

MRI-HIFU Experiment

All MRI-guided HIFU experiments were conducted using a research-dedicated HIFU system (Image Guided Therapy, Bordeaux, France) integrated with a whole-body 3 T scanner (Prisma, Siemens Healthineers, Erlangen, Germany). The HIFU system had an 8-element annular transducer array with a diameter of 25 mm, frequency of 2.5 MHz, a focal point of 0.7×0.7×3mm^3 in size, and a peak electrical power output of 200 W. The electrical power output during experiments ranged from 18 W to 24.5 W.

T_1-weighted images were acquired before and after HIFU stimulation with a 3D Cartesian gradient-echo sequence using the following parameters: field of view (FOV)=280×140×54 mm^3, matrix size=256×128×18, echo time (TE)=1.89 ms, repetition time (TR)=5 ms, flip angle=10°. T_1 relaxation times were measured before, during, and after HIFU stimulation using a Cartesian variable flip angle sequence with the following parameters: FOV=180×90×48 mm^3, matrix size=192×96×16, TE=2.29 ms, TR=6 ms, flip angles=1, 2, 5, 7 and 9°. To correct B_1+ field variations, a separate B_1 mapping protocol was ran before, during, and after HIFU with matching FOV and matrix size with the T_1 mapping protocol and TE=1.87 ms, TR=2 s and flip angle=10°. These images were reconstructed in-line with the scanner software. A standard DESPOT1 T_1 fitting algorithm[1] was then carried out in an offline MATLAB 2018a (MathWorks, Natwick, MA) script to produce 3D T_1 maps. They were saved as DICOM images and imported into Horos where regions of interest (ROIs) of 9 voxels in size were carefully drawn to exclude the thermal probe and/or air bubbles inside the heated region of the sample to compute the average T_1 value.

For simultaneous acquisition of the change in temperature and T_1-weighted image signal, a 3D multi-echo gradient-echo stack-of-radial sequence was used with FOV=109×109×30 mm^3, matrix size=96×96×10, six echoes, $TE_1/\Delta TE$=1.43/1.29 ms, TR=11.1 ms, flip angle=6° and number of radial spokes=3000. Reconstruction was performed offline in MATLAB. To increase the temporal resolution, a k-space weighted image contrast (KWIC) filter was employed with eight annuli in total and the number of spokes in each annulus following the Fibonacci numbers[2], e.g., 3 (innermost annulus), 5, 8, 13, 21, 34, 55 and 87 (outermost annulus). The filter then moved 5 radial spokes at a time for a temporal resolution of 0.33 s and a temporal footprint of 9.88 s. Gridding, density compensation, and coil combination then followed to produce magnitude and phase images[3]. Magnitude images of all echoes were combined with sum-of-squares and a fast Fourier transform was performed on a voxel-by-voxel basis along the time dimension for spectral analysis and producing the modulation enhancement map (MEM). Phase images of all echoes were also combined to an effective TE=10 ms and relative temperature change was extracted from the phase difference between a dynamic image acquired at a time point t and the first dynamic image at baseline temperature before HIFU ablation using $\Delta T(t) = \frac{\Phi_t - \Phi_0}{\alpha \cdot TE \cdot \gamma \cdot B_0}$, where ΔT is the change in temperature at t, Φ_t is the phase at t, Φ_0 is the phase of the first dynamic image, α is the proton resonant frequency shift (PRF) temperature coefficient of -0.01 ppm/°C, TE is the effective echo time (10 ms in this study), γ is the gyromagnetic ratio of protons (267.522×10^6 rad·s^{-1}·T^{-1}), and B_0 is the magnetic field strength. Similar to

the measurement of T_1 relaxation times, ROIs of 9 voxels in size were drawn to compute the average relative temperature change and magnitude change. The same ROIs were also transferred to MEMs to measure the intensity of the 0.1 Hz peak. For comparison, ROIs of 9 voxels and 100 voxels were drawn in unheated regions of the agar gel phantom and background noise, respectively, in the same images.

Figures

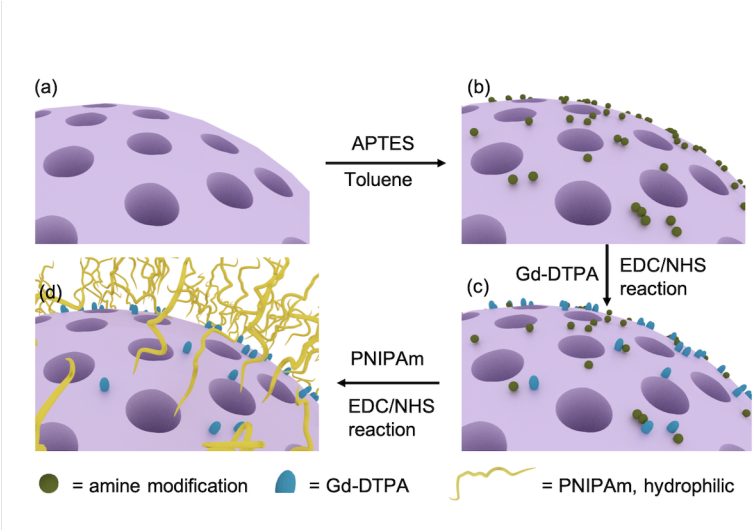

Fig. S1 Synthesis route of Gd-P-MSNs. (a) bare MSNs (b) amine modified MSNs (NH_2-MSNs) (c) Gd-DTPA modified MSNs (Gd-MSNs) (d) Gd-P-MSNs

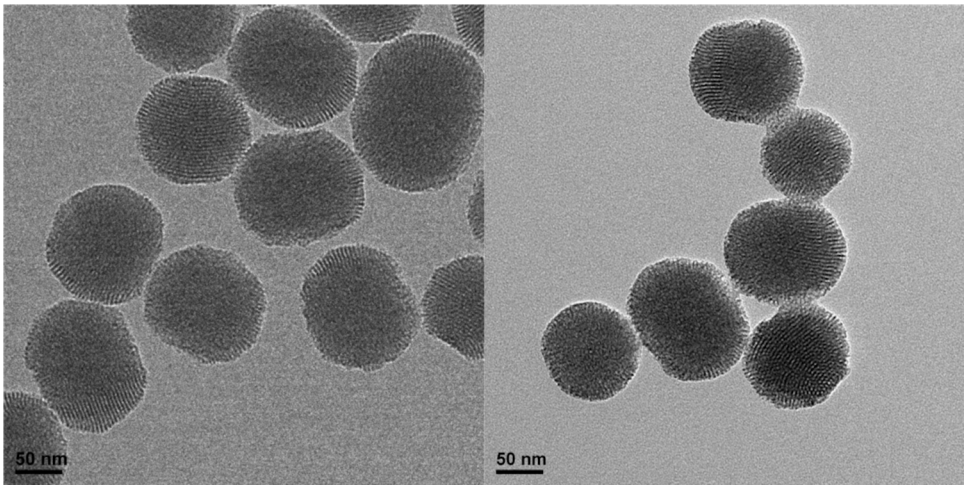

Fig. S2 TEM images of bare MSNs (left) and Gd-P-MSNs (right). MSNs stay intact after all modification steps.

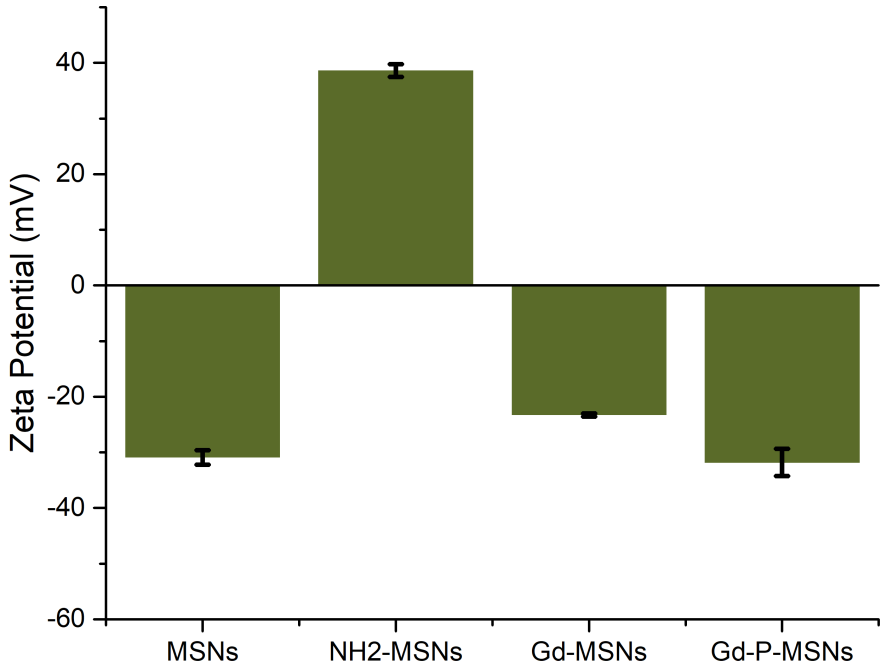

Fig. S3 Zeta-potential results after each modification step.

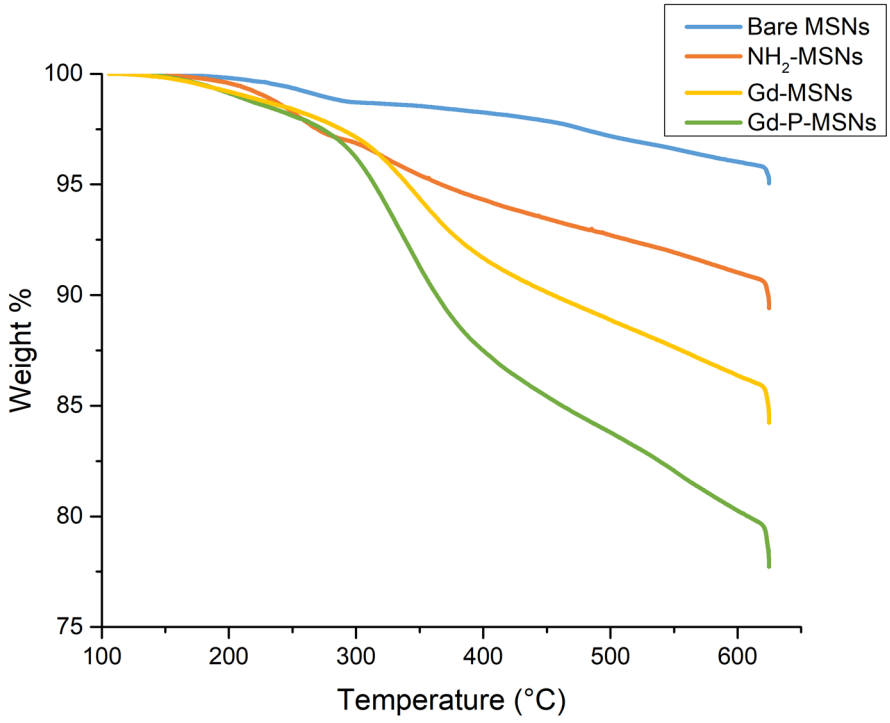

Fig. S4 TGA results after each modification step.

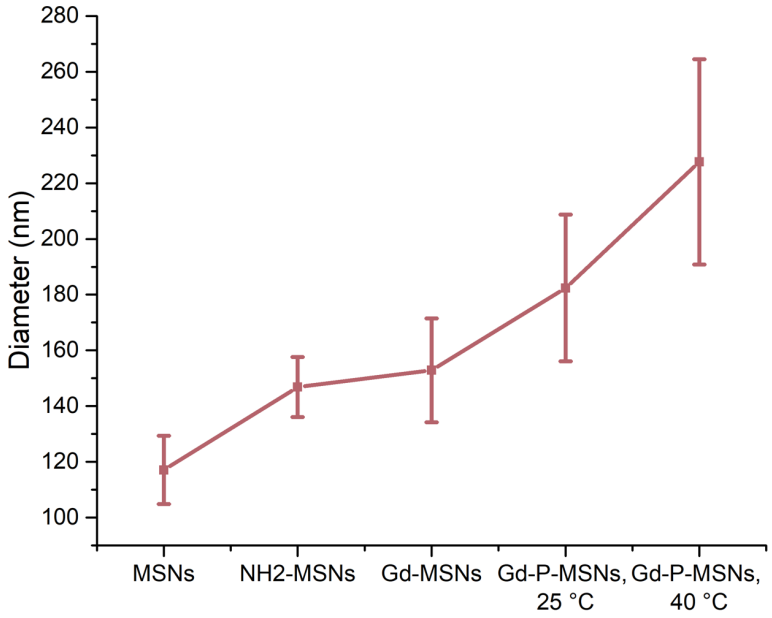

Fig. S5 DLS results after each modification step. Gd-P-MSNs showed larger diameter at 40 °C than 25 °C due to aggregation.

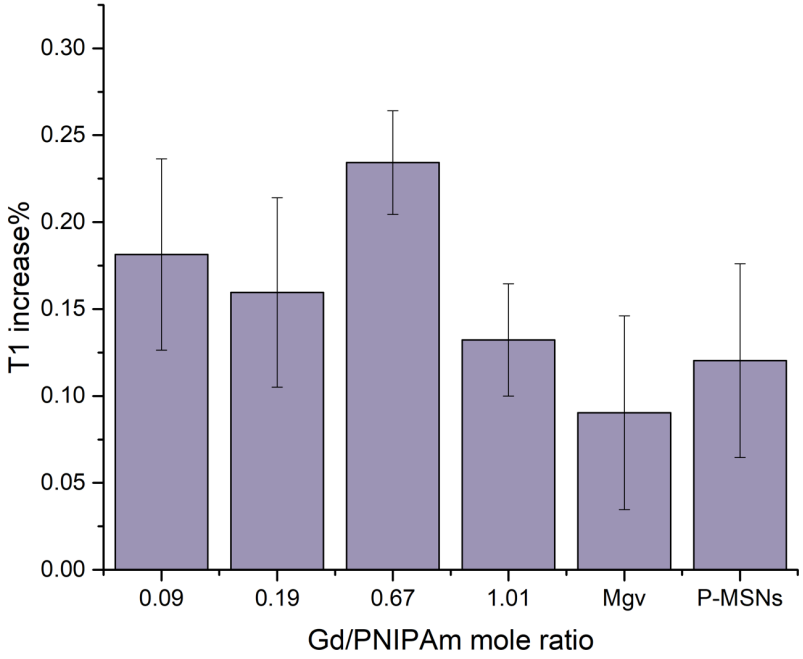

Fig. S6 ΔT_1% of Gd-P-MSNs with different Gd/PNIPAm mole ratio and Magnevist control (Mgv) and PNIPAm modified MSNs (P-MSNs) control.

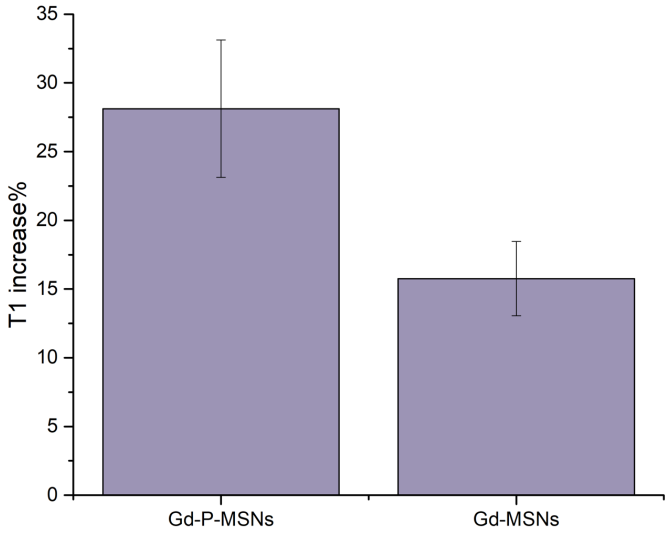

Fig. S7 ΔT_1% of Gd-P-MSNs and Gd-MSNs control

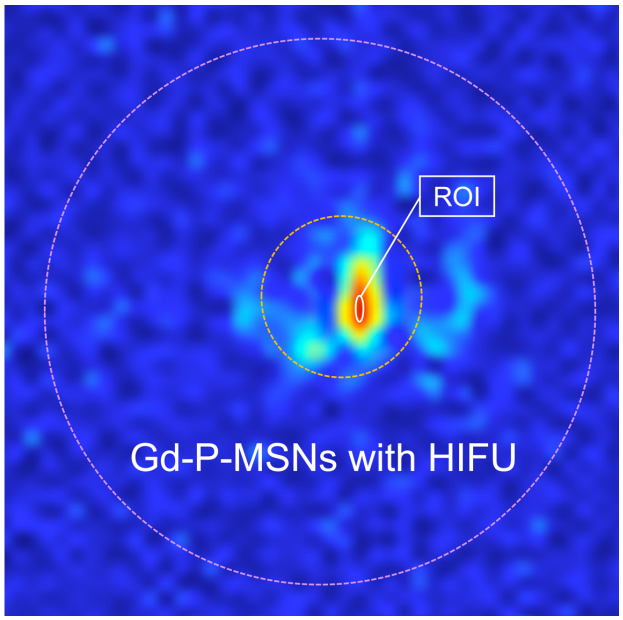

Fig. S8 ROI of the HIFU focal point.

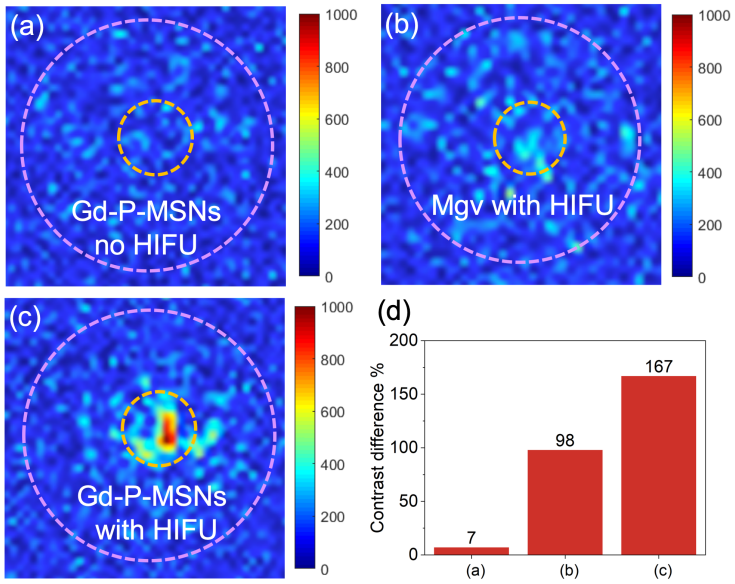

Fig. S9 Modulation enhancement maps (MEMs) constructed from the modulation in first 30 s and contrast difference% (CD%) of samples and controls. In (a) through (c), the edge of the agarose phantom is delineated with the purple circle (outer circle), and the sample/control region is delineated by a yellow circle (inner circle). (a)-(c) are MEMs of Gd-P-MSNs no HIFU, Mgv with HIFU and Gd-P-MSNs with HIFU. (d) CD% of (a)-(c). The CD% of Gd-P-MSNs with HIFU is 167, which is 23-fold higher than CD% of 7 in Gd-P-MSNs no HIFU, close to 2-fold higher than CD% of 98 in Mgv with HIFU and 15-fold higher than CD% of 11 in Mgv T1W before HIFU in Fig. 5 (a). From MEM of Gd-P-MSNs with HIFU in Fig. S9 (c), we observe that the CD% at the 1.5 mm^3 HIFU focal point is 512 (ROI showed in Fig. S8), which is 46-fold higher than CD% of Mgv T_1W before HIFU.

Table S1. Gd-P-MSNs sample1 (S1) to sample 4 (S4) with different Gd-DTPA/PNIPAm mole ratio.

	Gd-DTPA/MSNs wt %	PNIPAm/MSNs wt %	Gd/PNIPAm mole ratio
S1	0.09%	17.55%	0.09
S2	0.18%	16.58%	0.20
S3	0.58%	15.54%	0.67
S4	0.83%	14.54%	1.01

Wt%: weight percentage normalized to bare MSNs

Reference

1 S. C. L. Deoni, B. K. Rutt and T. M. Peters, 2003, **526**, 515–526.

2 H. K. Song and L. Dougherty, *Magn. Reson. Med.*, 2000, **832**, 825–832.

3 L. Zhang, T. Armstrong, X. Li, H. H. Wu, *Magn. Reson. Med.*, 2019, DOI: 10.1002/mrm.27883