Supporting Information

for

A Responsive Mesoporous Silica Nanoparticle Platform for Magnetic Resonance Imaging-Guided High-Intensity Focused Ultrasound-Stimulated Cargo Delivery with Controllable Location, Time, and Dose

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## I. Supporting tables

<table>
<thead>
<tr>
<th></th>
<th>MSNs</th>
<th>MSNs-APTS</th>
<th>MSNs-PEG</th>
<th>Gd(DTPA)$^2$-loaded MSNs-PEG</th>
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<tbody>
<tr>
<td><strong>BET surface area</strong></td>
<td>1045</td>
<td>595</td>
<td>108</td>
<td>99</td>
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<tr>
<td><strong>(m$^2$/g)</strong></td>
<td></td>
<td></td>
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<tr>
<td><strong>Pore volume</strong></td>
<td>1.04</td>
<td>0.52</td>
<td>0.19</td>
<td>0.13</td>
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<tr>
<td><strong>(cc/g)</strong></td>
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<td></td>
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<tr>
<td><strong>Average pore diameter (nm)</strong></td>
<td>2.9</td>
<td>2.4</td>
<td>N/A</td>
<td>N/A</td>
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</table>

**Table S1.** Brunauer-Emmett-Teller (BET) surface area, total pore volume, and average pore diameter of MSNs, MSNs-APTS, MSNs-PEG, and Gd(DTPA)$^2$-loaded MSNs-PEG analyzed from the N$_2$ adsorption/desorption isotherms.
<table>
<thead>
<tr>
<th>HIFU parameters (power level, stimulation time)</th>
<th>Release efficiency</th>
<th>Temperature increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>(9 W, 3 min)</td>
<td>15.61 %</td>
<td>1 °C</td>
</tr>
<tr>
<td>(74 W, 3 min)</td>
<td>19.29 %</td>
<td>4 °C</td>
</tr>
<tr>
<td>(74 W, 5 min)</td>
<td>25.05 %</td>
<td>7 °C</td>
</tr>
<tr>
<td>(74 W, 10 min)</td>
<td>31.01 %</td>
<td>10 °C</td>
</tr>
<tr>
<td>(290 W, 3 min)</td>
<td>37.22 %</td>
<td>10 °C</td>
</tr>
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</table>

Table S2. Summary of the Gd(DTPA)$_2$ release efficiency and temperature increase using various HIFU parameters (power levels and stimulation times).
II. Supporting figures

Figure S1. Size distribution of 91.6 ± 15.1 nm mesoporous silica nanoparticles (MSNs).
Figure S2. Pore diameter distribution of MSNs, MSNs-APTS, MSNs-PEG, and Gd(DTPA)$_2$-loaded MSNs-PEG measured at 77 K.
Figure S3. The colloidal stability of MSNs-PEG (left tube) and MSNs-APTS (right tube) in PBS after being kept undisturbed for (a) 30 min, (b) 1 day, and (c) 3 days at room temperature.
Figure S4. TEM image of MSNs-PEG after 30 min of probe sonication.
Figure S5. (a)(b) Set-up of the MRgHIFU system. The agarose phantom was placed on top of the water-cooled HIFU transducer, which was secured on the patient bed in a 3T whole-body MRI scanner. (c) Top view of the HIFU transducer without cooling water and cap.
Figure S6. The percentage of $T_1$-weighted image intensity changes of the MRgHIFU-stimulated water-dispersed Gd(DTPA)$_2$-loaded MSNs-PEG (sample 1), unstimulated water-dispersed Gd(DTPA)$_2$-loaded MSNs-PEG (control 2), unstimulated Gd(DTPA)$_2$-loaded MSNs-PEG mixed in methylcellulose (2.5 wt %) and concentrated milk (v/v = 1/1) (controls 3 to 5), the mixture of methylcellulose (2.5 wt %) and concentration milk (v/v = 1/1) (control 6), and agarose phantom background (control 7). The amount of Gd(DTPA)$_2$ release of HIFU-stimulated sample 1 was quantified by ICP-OES.
Figure S7. Ultrasound-stimulated Gd(DTPA)$^{2-}$ release using a probe sonicator. (a) $T_1$ relaxivity ($r_1$) values of free Gd(DTPA)$^{2-}$ (blue), and Gd(DTPA)$^{2-}$-loaded MSNs-PEG without (black) and with (red) 30 min of ultrasound stimulation by the probe sonicator. (b) $T_1$-weighted images of each sample in (a) at different Gd(III) concentrations.
Figure S8. (a) $r_1$ and (b) $r_2$ values of Gd(DTPA)$^{2-}$ with or without HIFU stimulation (3 cycles of 1 min, 74 W).
Figure S9. (a) $r_1$ and (b) $r_2$ values of Gd(DTPA)$^{2-}$ with or without 30 min of probe sonication.
**Figure S10.** The colloidal stability of HIFU-stimulated MSNs-PEG (left tube) and unstimulated MSNs-PEG (right tube) in deionized H₂O after being kept undisturbed for 30 min. The HIFU-stimulated tube has aggregation at the bottom due to loss of colloidal stability after disruption of PEG caps.
Figure S11. Ultrasound-stimulated Gd(DTPA)$^2$-release using the probe sonicator and the resulting T$_1$ changes. (a) T$_1$ of Gd(DTPA)$^2$-loaded MSNs-PEG after 2, 5, 8, 10, and 30 min of probe sonication. Inset shows the corresponding T$_1$-weighted images. (b) Positive correlation between T$_1$ and the release efficiencies of Gd(DTPA)$^2$. 
Figure S12. The percentage of T1-weighted image intensity changes of the methylcellulose gel/milk-mixed Gd(DTPA)22-loaded MSNs-PEG (a) after 1, 3, 5, or 10 min of HIFU stimulation (74 W); (b) after 3 min of HIFU stimulation at electrical power levels of 9 W, 74 W, or 290 W. (c) Time-dependent T1-weighted image intensity of Gd(DTPA)22-loaded MSNs-PEG with multiple HIFU stimulations (3 cycles of 1 min, 74 W) (shown in black). T1-weighted image intensity of agarose background is shown in red. The yellow boxes show the ON time (3 min) of each HIFU stimulation. The OFF times between each ON time were 67 min and 57 min.

Discussion of Figure S12:

To demonstrate the potential of controlling the dose and timing of cargo release, three types of release studies were carried out as followed: (i) a single exposure to HIFU stimulation with different time durations, (ii) a single exposure to HIFU at different electrical power levels, and (iii) multiple HIFU stimulations of the same time duration. Gd(DTPA)22-loaded MSNs-PEG were dispersed in 2 mL of 2.5 wt% methylcellulose/milk (3 mg/mL) and filled in the sample wells in an agarose phantom.

In study (i), four Gd(DTPA)22-loaded MSNs-PEG samples were stimulated with HIFU at a power level of 74 W for 1, 3, 5, or 10 min, respectively. T1-weighted images of the samples were acquired before and after the HIFU stimulations. 1 min of HIFU stimulation resulted in a 7 % decrease in T1-weighted image intensity (Figure S12a). 9 %, 15 %, and 20 % decreases in T1-weighted image intensity were achieved after 3, 5, and 10 min of HIFU stimulations. Greater decrease in T1-weighted image intensity with longer HIFU stimulation time indicated more released Gd(DTPA)22.

In study (ii), three Gd(DTPA)22-loaded MSNs-PEG samples were stimulated with HIFU for a fixed duration of 3 min but at different power levels of 9 W, 74 W, and 290 W. The T1-weighted image intensity decreased by 7 %, 9 %, and 16 % at 9 W, 74 W, and 290 W, respectively (Figure S12b). Higher HIFU power level led to greater change in T1-weighted image intensity, since more Gd(DTPA)22 was released by the stronger cavitation generated at higher acoustic intensity. Studies (i) and (ii) support that the released amount of Gd(DTPA)22 can be controlled by adjusting HIFU parameters such as duration and power level.

In study (iii), after the first cycle of HIFU stimulation, the T1-weighted image intensity sharply decreased within 30 min and reached equilibrium until the next HIFU stimulation started, suggesting most Gd(DTPA)22 was released within 30 min (Figure S12c). Similarly, after the second and the third cycles of HIFU stimulation, the T1-weighted image intensity decreased within 20 min and reached equilibrium. Study (iii) supports that the cargo released from MSNs-PEG was temporally controllable to achieve the desired amount of released cargo within a certain time window. This result implies that the therapeutics delivered by this MRgHIFU-stimulated strategy could be released stepwise and finally achieve the required dosage in a desired therapeutic window by simply applying multiple HIFU stimulations.
**Figure S13.** TEM image of MSNs-PEG after 3 min of HIFU stimulation.
Figure S14. *Ex vivo* MRgHIFU-stimulated Gd(DTPA)\(^2\) release and the controllable MRI contrast changes in three-dimensional space. A piece of 3 × 6 cm\(^2\) chicken breast tissue was injected with methylcellulose gel containing Gd(DTPA)\(^2\)-loaded MSNs-PEG and stimulated with HIFU for 3 cycles of 3 min (2.5 MHz, 8 W). The T\(_1\)-weighted image of the chicken breast before the HIFU stimulation was shown in (a) axial, (b) sagittal, and (c) coronal orientations. The color-coded Δ T\(_1\)-weighted images, acquired by subtracting the T\(_1\)-weighted images before from the one after 3 cycles of HIFU stimulation, were shown in three-dimensional space.
Figure S15. Ex vivo MRgHIFU control experiment. (a) A piece of chicken breast tissue (3 cm × 5 cm) was injected with methylcellulose gel only. (b) The percentage of T1-weighted image intensity changes of the tissue background and the HIFU-stimulated gel after each cycle of HIFU stimulation (3 min, 2.5 MHz, 8 W). Total number of cycles= 2. (c) Color-coded Δ T1-weighted images of the chicken tissue after each cycle of HIFU stimulation. The position of HIFU-stimulated gel and tissue background was indicated.
Figure S16. Preparation of the agarose phantom. (a) Before the removal of the glass test tubes, and (b) after the removal of the glass test tubes. Five sample wells were molded in the agarose phantom.
III. Supplementary notes

Note S1: The methods for $T_1$ and $T_2$ fitting
The standard inversion-recovery MR signal model was used to calculate $T_1$ maps. Images acquired with different inversion times (TIs) were fit pixel-wise to the equation $M = M_0 (1 - 2e^{-\tau/T_1})$, where $M_0$ is the magnetization at thermal equilibrium, $M$ is the signal intensity of images acquired at TI = $\tau$. Similarly, the standard Car-Purcell-Meiboom-Gill (CPMG) signal model was used to calculate $T_2$ maps, where images acquired with different echo times (TEs) were fit pixel-wise to the equation $M = M_0 e^{-\tau/T_2}$, with $M_0$ being the magnetization at thermal equilibrium, $M$ being the signal intensity of images acquired at TE = $\tau$. 
IV. Movie descriptions

Movie S1:
Movie S1 shows the entire 3-D $\Delta T_1$-weighted image of the agarose phantom containing the sample wells after HIFU stimulation (3 cycles of 1 min, 74 W). The sample well with intense signal represents the HIFU-stimulated sample 1 as shown in Figure 3b.
**Movie S2a:**
Movie S2a shows the temperature map of water-suspended Gd(DTPA)$^2$-loaded MSNs-PEG (indicated as sample in agarose phantom 2) during 1 min of HIFU stimulation. The increase in temperature was monitored by dynamic MRI temperature mapping acquired using a 2D gradient-echo protocol (TE= 20 ms, TR= 30 ms, spatial resolution of $1 \times 1 \times 3$ mm$^3$, and temporal resolution of 2.8 s). The detailed temperature increase profile is shown in Figure 5a.
Movie S2b:
Movie S2b shows the MR temperature map of Gd(DTPA)\(^2\)-loaded MSNs-PEG mixed in the gel/milk mixture (indicated as sample) during 1 min of HIFU stimulation. The increase in temperature was monitored by dynamic MRI temperature mapping acquired using a 2D gradient-echo protocol (TE= 10 ms, TR= 20 ms, spatial resolution of \(1 \times 1 \times 3 \text{ mm}^3\), and temporal resolution of 1.8 s). The detailed temperature increase profile is shown in Figure 5a.