Real-Time Monitoring of Arsenic Trioxide Release and Delivery by Activatable T₁ Imaging

Zhenghuan Zhao, Xiaomin Wang, Zongjun Zhang, Hui Zhang, Hanyu Liu, Xianglong Zhu, Hui Li,
Xiaoqin Chi, Zhenyu Yin, and Jinhao Gao*

Supporting Information

Experimental Sections

Evaluation the pH-induced cargo release. The pH-responsive As and Mn release behavior was studied by ICP-MS. The MnAsO_x@SiO₂ was dispersed in 3000 μL of PBS buffer (pH ~ 7.4) and citrate buffer (pH 5.4) in the bath with 37 °C for 24 h. At the selected time, 200 μL of solution was centrifuged (14000 rpm, 20 min) to collect the As and Mn and analyzed their release amount.

Activatable T₁ imaging *in vitro*. Prior to test the contrast ability of Mn ions and MnAsO_x@SiO₂ in activatable T₁ imaging, we incubated the MnCl₂ and MnAsO_x@SiO₂ in the buffer of pH 7.4 and 5.4 for 6 h, respectively. The phantom study of MRI samples was prepared in 1% agarose with the Mn concentrations range of 400, 200, 100, 50, and 25 μM. The T₁ relaxation times were measured by a 0.5 T NMI20-Analyst NMR system and used to calculate the relaxation rates (r_1) of the samples.

Monitoring the cargo release by activatable T₁ MR imaging. We incubated MnAsO_x@SiO₂ in citrate puffer (pH 5.4) for 0, 4, and 8 h. After testing the released Mn and As ions by ICP analysis, we mixed them with 1% agarose to form MRI phantom with the Mn concentration range of 400, 200, 100, 50, and

25 μ M. The T_1 relaxation times for all the samples were measured by a 0.5 T NMI20-Analyst NMR system and used to calculate the r_1 values. The T_1 -weighted MRI images for the samples were acquired using MSE sequence as following parameters: TR/TE=100/12 ms, 256 matrices, thickness =1mm, NS=2.

Cell culture. All cells (HeLa, HepG2, and SMMC-7721) were purchased from Cell Bank of Chinese Academy of Sciences (Shanghai, China). HeLa and HepG2 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM medium) supplemented with 10% fetal bovine serum (FBS, Hyclone) and antibiotics (100 mg/mL streptomycin and 100 U/mL penicillin). The SMMC-7721 cell was cultured in Roswell Park Memorial Institute 1640 (RPMI-1640) supplemented with 10% FBS and antibiotics. All cells maintained in a humidified atmosphere of 5% CO_2 at 37 $^\circ\text{C}$.

Cytotoxicity evaluation. Cells were seeded into a 96-well plate with a density of 5×10^3 cells/well in culture medium, and incubated in the atmosphere of 5% CO_2 at 37 $^\circ\text{C}$ for 12 h. The cells were then incubated with ATO and $\text{MnAsO}_x\text{@SiO}_2$ at a serial of As concentrations for 48h. Each experiment in the same concentration was performed in five times. Subsequently, after removing the culture medium, we replaced the growth medium with DMEM or RPMI-1640 containing 0.5 mg/mL of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) and incubated for another 4 h at 37 $^\circ\text{C}$. After discarding the culture medium, 100 μL of DMSO was added to dissolve the precipitates and the resulting solution was measured for absorbance at 492 nm using a MultiSkan FC microplate reader (Thermo scientific).

Monitoring the ATO drug release inside living cells. We incubated 1×10^7 SMMC-7721 cells with $\text{MnAsO}_x\text{@SiO}_2$ at 37 $^\circ\text{C}$ for 2 h, 4h, 6h, and 8h, respectively. After harvesting the cells, we washed them with PBS buffer three times to remove the free $\text{MnAsO}_x\text{@SiO}_2$ nanomaterials. Then we concentrated the cells at the button of EP tube by centrifugation and performed T_1 -weighted MRI on a

0.5 T NMI20-Analyst NMR system. The samples were scanned using a multi-echo T_1 -weighted fast spin echo imaging sequence (TR/TE=100/12 ms, 256 matrices, thickness =1mm, NS=16).

Circulation in mice. We analyzed the concentrations of Mn and As ions in mice blood as follows. After injection of $\text{MnAsO}_x@\text{SiO}_2$ and $\text{MnAsO}_x@\text{SiO}_2\text{-GSH}$, we gathered the mice blood with the amount of 20 μL at 10 min, 30 min, 1 h, 1.5 h, 2 h, 3 h, and 4.5 h. The isolated bloods were completely lysed at room temperature for one night in 500 μL nitrolysis solution (mixture of concentrated nitric acid and hydrogen peroxide). Subsequently, we analyzed the solution by ICP-MS.

Drug delivery *in vivo*. We tested As concentrations in liver and tumor as follows. The isolated liver and tumor with known weight were completely lysed in 2 mL nitrolysis solution. Further, the samples heated to 120 $^{\circ}\text{C}$ in the oil bath until complete evaporation of mixture. After adding 2% nitric acid to re-dissolve the residue, we analyzed the solution by ICP-MS.

***In vivo* subcutaneous tumor MR imaging.** We established the subcutaneous tumor model by injection of murine hepatocellular carcinoma cells, H22 (5×10^6) cells, into the subcutaneous tissue of mice forelegs. When the H22 carcinoma reached approximately 3-4 cm in diameter, we intravenously injected $\text{MnAsO}_x@\text{SiO}_2$ and $\text{MnAsO}_x@\text{SiO}_2\text{-GSH}$ into the mice (1 mg Mn/kg body weight each). The coronal and transverse plane MR images were acquired using the following parameters: TR/TE = 400/10 ms, 256×256 matrices, thickness = 1mm, FOV = 50×50 . The MR images were sequentially obtained at 0, 20 min, 30 min, 1 h, and 2 h post-injection ($n = 3/\text{group}$) To quantify the efficacy of contrast enhancement, we calculated the $\text{SNR}_{\text{post}}/\text{SNR}_{\text{pre}}$ values of liver and tumor.

***In vivo* liver tumor MR imaging.** We established the orthotopic liver tumor model by injection of H22 cells (1×10^6) to the liver of BALB/c mice. When the tumor reached 1-2 mm in diameter, mice were intravenously injected with $\text{MnAsO}_x@\text{SiO}_2\text{-GSH}$ at a dose of 1 mg Mn/kg body weight. The coronal and transverse plane MR images were acquired using a sequence (TR/TE =400/ 10 ms, 256×256 matrices, Averages =1, FOV = 40×40) on a 7 T MRI scanner. The MR images were sequentially

obtained at 0, 0.5, 1, and 4 h post-injection ($n = 3/\text{group}$). To quantify the efficacy of contrast enhancement, we calculated the $\text{SNR}_{\text{post}}/\text{SNR}_{\text{pre}}$ values of liver and tumor.

Statistical analysis. Statistical analysis was performed using the Student's t -test for unpaired data, p value of less than 0.05 was accepted as a statistically significant difference compared to control.

Figure S1. Characterization of MnAsO_x@SiO₂ MDDS in aqueous solution. (a) DLS profiles of hollow silica nanoparticles (HSNs) and MnAsO_x@SiO₂. (b) The stability of MnAsO_x@SiO₂ in PBS for 1 week. The error bars represent the standard deviation of three independent experiments.

Figure S2. Analysis of MnAsO_x@SiO₂ by energy dispersive X-ray spectroscopy (EDS). The results of EDS to (a) blank region and (b) accumulative MnAsO_x@SiO₂ nanoparticles in the copper mesh, respectively. Inserts are the TEM images corresponding to the blank region and the region of MnAsO_x@SiO₂ in the copper.

Figure S3. MR relaxivity of the Mn ions and MnAsO_x@SiO₂. Analysis of relaxation rate R_1 ($1/T_1$) vs Mn concentration for (a) Mn ions and (c) MnAsO_x@SiO₂ in the buffer of pH 7.4 and 5.4 for 6 h. Analysis of relaxation rate R_2 ($1/T_2$) vs Mn concentration for (b) Mn ions and (d) MnAsO_x@SiO₂ in the buffer of pH 7.4 and 5.4 for 6 h. It is noted that the r_2 values in here are extremely low, which is not capable for T₂ contrast imaging.

Figure S4. *In vitro* cytotoxicity of HSNs and Mn ions. (a) The MTT assay of HeLa cells incubated with multi-concentrations of HSNs for 48 h ($n = 5/\text{group}$). (b) The MTT assay of HeLa cells incubated with various concentrations of MnCl_2 for 48 h ($n = 5/\text{group}$).

Figure S5. Surface and interfacial properties of MnAsO_x@SiO₂ and MnAsO_x@SiO₂-GSH. The DLS analysis of (a) MnAsO_x@SiO₂ and (b) MnAsO_x@SiO₂-GSH incubated with or without FBS. These results indicated the GSH modification could significantly reduce the interaction between MnAsO_x@SiO₂-GSH and serum proteins. (c) The zeta-potential analysis of hollow SiO₂ and hollow SiO₂-GSH in the buffer of pH 5.4, 7.5, and 9.4.

Figure S6. Circulation of MnAsO_x@SiO₂ and MnAsO_x@SiO₂-GSH in blood. Circulation curves of MnAsO_x@SiO₂ and MnAsO_x@SiO₂-GSH in mice by the measurements of (a) As ions and (b) Mn ions. The concentrations of As and Mn were tested by ICP-MS ($n = 3/\text{group}$).

Figure S7. Quantification of the efficacy of contrast enhancement in the liver of mice.

Quantification of relative $\text{SNR}_{\text{post}}/\text{SNR}_{\text{pre}}$ values in liver collected at different time after administration of (a) $\text{MnAsO}_x@\text{SiO}_2$ and (b) $\text{MnAsO}_x@\text{SiO}_2\text{-GSH}$. These results indicated that the GSH modification could obviously reduce uptake of the nanoparticles by liver.

Figure S8. Identifying the location of orthotopic liver tumor. (a) The optical image shows the orthotopic liver tumor in the mice. The white arrow indicates the H22 tumor. (b) *In vivo* MR images of mice bearing H22 tumors after intravenous injection of $\text{MnAsO}_x@\text{SiO}_2\text{-GSH}$. The white arrow indicates the H22 tumor.

Figure S9. *In vivo* therapeutic study. The optical photos of SMMC-7721 tumor-bearing mice were taken at different times after treatment by PBS, ATO, MnAsO_x@SiO₂, and MnAsO_x@SiO₂-GSH.

Figure S10. *In vivo* study on the tumor model mice. (a) Tumor growth curves after intravenous injection of HSNs, PBS, and $MnCl_2$. The arrows indicate the time of treatment. (b) The body weight change curves of the mice during the treatment of HSN, PBS, and $MnCl_2$.

Figure S11. *In vitro* cytotoxicity of MnAsO_x@SiO₂ and MnAsO_x@SiO₂-GSH to SMMC-7721. The MTT assay of SMMC-7721 cells incubated with multi-concentration of MnAsO_x@SiO₂ and MnAsO_x@SiO₂-GSH for 48 h ($n = 5/\text{group}$).

Figure S12. Biocompatibility of MnAsO_x@SiO₂ and MnAsO_x@SiO₂-GSH. The organ histology images of heart, liver, spleen, lung, and kidney of the mice after administration of PBS, ATO, MnAsO_x@SiO₂, and MnAsO_x@SiO₂-GSH with the dose of 2.0 mg As per kg for 21 days. Scale bar, 200 μm.

Figure S13. Analysis of biochemistry index. Quantitative analysis of biochemistry indices (a) alkaline phosphatase, (b) albumin, (c) total protein, (d) total cholesterol, (e) hemoglobin, (f) lymphocyte, (g) total bilirubin, and (h) blood urea nitrogen of the mice treated by PBS, ATO, $\text{MnAsO}_x@SiO_2$, and $\text{MnAsO}_x@SiO_2\text{-GSH}$. The results indicated that the rational designed multifunctional agents have acceptable biocompatibility.

Table S1. Comparison of IC₅₀ values (48 h) of ATO and MnAsO_x@SiO₂ MDDS to HeLa, HepG2, SMMC-7721, and H22 cells (*n* = 5/group).

Cell lines	IC ₅₀ (μM As)	
	ATO	MnAsO _x @SiO ₂
HeLa	2.5 ± 0.3	0.9 ± 0.1
HepG2	75.0 ± 6.8	20.0 ± 2.3
SMMC-7721	22.0 ± 1.5	2.5 ± 0.2
H22	7.7 ± 0.5	2.9 ± 0.3

Table S2. MR signal-to-noise ratio (SNR) changes of liver before and after intravenous injection of $\text{MnAsO}_x@/\text{SiO}_2$ and $\text{MnAsO}_x@/\text{SiO}_2\text{-GSH}$ ($n= 3/\text{group}$). We calculated the signal-to-noise ratio (SNR) by the equation: $\text{SNR}_{\text{liver}} = \text{SI}_{\text{liver}} / \text{SD}_{\text{noise}}$, where SI represent signal intensity and SD represent standard deviation. Then we calculated the SNR changes of ROI by the equation: $\Delta\text{SNR} = |\text{SNR}_{\text{post}} - \text{SNR}_{\text{pre}}| / \text{SNR}_{\text{pre}}$.

	$\text{MnAsO}_x@/\text{SiO}_2$	$\text{MnAsO}_x@/\text{SiO}_2\text{-GSH}$
$\text{SNR}_{\text{pre}} (\%)$	100	100
$\text{SNR}_{20 \text{ min}} (\%)$	314.6 ± 2.9	277.1 ± 1.0
$\Delta\text{SNR}_{20 \text{ min}} (\%)$	214.6 ± 2.9	177.1 ± 1.0
$\text{SNR}_{30 \text{ min}} (\%)$	273.8 ± 3.4	304.8 ± 6.5
$\Delta\text{SNR}_{30 \text{ min}} (\%)$	173.8 ± 3.4	204.8 ± 6.5
$\text{SNR}_{1 \text{ h}} (\%)$	211.0 ± 0.7	314.5 ± 9.0
$\Delta\text{SNR}_{1 \text{ h}} (\%)$	111.0 ± 0.7	214.5 ± 9.0
$\text{SNR}_{2 \text{ h}} (\%)$	213.8 ± 1.2	238.3 ± 7.8
$\Delta\text{SNR}_{2 \text{ h}} (\%)$	113.8 ± 1.2	138.3 ± 7.8
$\text{SNR}_{4 \text{ h}} (\%)$	188.9 ± 3.5	222.5 ± 1.6
$\Delta\text{SNR}_{4 \text{ h}} (\%)$	88.9 ± 3.5	122.5 ± 1.65
$\text{SNR}_{6 \text{ h}} (\%)$	185.4 ± 6.0	191.9 ± 2.3
$\Delta\text{SNR}_{6 \text{ h}} (\%)$	85.4 ± 6.0	91.9 ± 2.3

Table S3. SNR changes of tumor after intravenous injection of $\text{MnAsO}_x@\text{SiO}_2$ and $\text{MnAsO}_x@\text{SiO}_2\text{-GSH}$ ($n = 3/\text{group}$). We calculated the SNR changes of ROI by the equation: $\Delta\text{SNR} = |\text{SNR}_{\text{post}} - \text{SNR}_{\text{pre}}| / \text{SNR}_{\text{pre}}$.

	$\text{MnAsO}_x@\text{SiO}_2$	$\text{MnAsO}_x@\text{SiO}_2\text{-GSH}$
$\text{SNR}_{\text{pre}} (\%)$	100	100
$\text{SNR}_{20 \text{ min}} (\%)$	124.4 ± 2.9	253.8 ± 17.8
$\Delta\text{SNR}_{20 \text{ min}} (\%)$	24.4 ± 2.9	153.8 ± 17.8
$\text{SNR}_{30 \text{ min}} (\%)$	116.4 ± 1.6	330.6 ± 8.1
$\Delta\text{SNR}_{30 \text{ min}} (\%)$	16.4 ± 1.6	230.6 ± 8.1
$\text{SNR}_{1 \text{ h}} (\%)$	120.3 ± 6.1	379.4 ± 23.2
$\Delta\text{SNR}_{1 \text{ h}} (\%)$	20.3 ± 6.1	279.4 ± 23.2
$\text{SNR}_{2 \text{ h}} (\%)$	119.7 ± 0.7	313.5 ± 8.4
$\Delta\text{SNR}_{2 \text{ h}} (\%)$	19.7 ± 0.7	213.5 ± 8.4

Table S4: SNR changes of tumor after intravenous injection of MnAsO_x@SiO₂-GSH (*n* = 3/group). We

calculated the SNR changes of ROI by the equation: $\Delta\text{SNR} = |\text{SNR}_{\text{post}} - \text{SNR}_{\text{pre}}| / \text{SNR}_{\text{pre}}$.

	MnAsO _x @SiO ₂ -GSH
SNR _{pre} (%)	100
SNR _{0.5 h} (%)	183.0 ± 11.6
ΔSNR _{0.5 h} (%)	83.0 ± 11.6
SNR _{1 h} (%)	208.8 ± 12.7
ΔSNR _{1 h} (%)	108.8 ± 12.7
SNR _{4 h} (%)	125.0 ± 8.4
ΔSNR _{4 h} (%)	25.0 ± 8.4