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Supporting Information

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Double-Effector Nanoparticles: A Synergistic Approach to Apoptotic Hyperthermia**

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Supporting Information

I. Synthesis of double-effector nanoparticles (GdTx MNPs)

a. Surface modification of $Zn_{0.4}Fe_{2.6}O_4$ nanoparticles

15 nm $Zn_{0.4}Fe_{2.6}O_4$ nanoparticles[1] are coated with 16 nm SiO_2 shell using a modified base-catalyzed sol-gel process.[2] Polyoxyethylene (5) nonylphenylether (1.95 mL, 4.40 mmol, Igepal CO-520 containing 50 mol% hydrophilic group) and 2.5 mg of the nanoparticles are dispersed in cyclohexane and subject to vortex mixing. Treatment with a solution of 30% *aq.* ammonium hydroxide (0.26 mL) followed by tetraethyl orthosilicate (0.46 mL, 2.04 mmol, TEOS) provides silica-coated nanoparticles. With these in hand, 3-aminopropyltrimethoxysilane (12.5 µL, 0.07 mmol, APTMS) is added to introduce the amine functional group. After 72 h of aging at room temperature, amine-functionalized nanoparticles are precipitated via the addition of methanol. After collecting by centrifugation, the precipitates are treated with succinic anhydride (2.50 mg, 0.10 mmol, SA) in dimethyl sulfoxide (DMSO) and stirred for 24 h. After work-up, the carboxylate nanoparticles are redispersed in DMSO.

b. Synthesis of gadolinium texaphyrins - $Zn_{0.4}Fe_{2.6}O_4$ magnetic nanoparticles (GdTx MNPs)

1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (50 mM, EDC) and *N*-hydroxysulfosuccinimide (5 mM, Sulfo-NHS) are added to a solution of carboxylated $Zn_{0.4}Fe_{2.6}O_4$ nanoparticles in DMSO. Then, 3500 molecular weight poly(ethylene glycol) bis(amine) (50 mg, 0.03 mmol, PEG) is added to the reaction mixture. After 2 h at room temperature, the PEGylated $Zn_{0.4}Fe_{2.6}O_4$ nanoparticles (MNPs) are isolated by centrifugation and redispersed in DMSO. Gadolinium texaphyrins (34 mg, 0.03 mmol, GdTx) and disuccinimidyl suberate (10 mg, 0.03 mmol, DSS) in anhydrous *N, N*-dimethyl-formamide (DMF) are stirred for 4 h under argon atmosphere.[2] The above MNPs are then added to the mixture. After 4 h, the GdTx MNPs are precipitated, via the addition of acetone, and redispersed in DMSO. The GdTx MNPs as dispersion in DMSO are then transferred to aqueous solution. A transmission electron microscopy (TEM) image of GdTx MNPs in aqueous solution is shown in Figure 1b(ii).

II. T1, T2 MR imaging of MNPs, GdTx, and GdTx MNPs

MR imaging of the GdTx MNPs of this study is performed using a 1.5 T MRI instrument (Philips, Germany). T1 scans are obtained using a standard sequence (TR = 625 ms, TE = 10 ms, FOV = 75 mm, matrix = 256 × 256, slice thickness = 0.7 mm, acquisition number = 1). T2 scans are obtained by using fast spin-echo sequence (TR = 4000 ms, TE = 80 ms, FOV = 75 mm, matrix = 256 × 256, slice thickness = 0.7 mm, acquisition number = 1) (Figure 1c).

III. UV-VIS analysis of GdTx MNPs

The presence of the GdTx on the MNPs is confirmed by UV-VIS absorption spectroscopy, which reveals the presence of spectral features ascribable to both the GdTx and MNPs in the spectrum of GdTx MNPs.

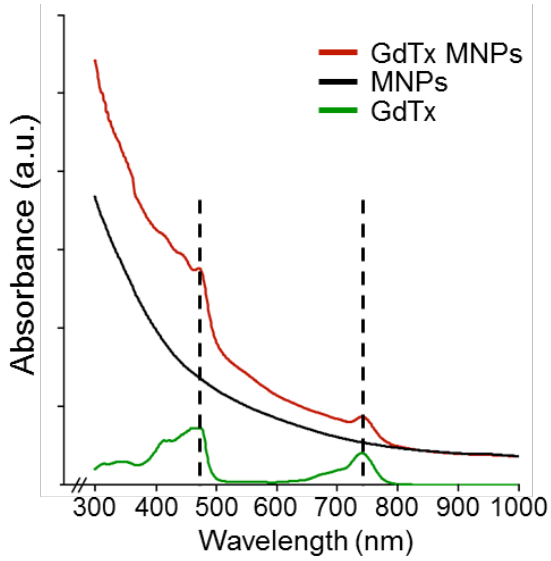

Figure S1. UV-VIS absorption spectra of GdTx (green), MNPs (black), and GdTx MNPs (red). The spectrum of GdTx MNPs corresponds to a linear combination of the individual spectra of GdTx and MNPs.

IV. Measurement of specific loss power

Magnetic heating of aqueous suspensions of GdTx MNPs with a concentration of 0.2 mg/mL is performed under an alternating magnetic field (500 kHz at 37.4 kA/m, AC magnetic field), provided by a high-radiofrequency heating machine (HF 10K, Taeyang System Co., Korea). The temperature change is monitored by fiber optic thermometer (M602, Lumasense technologies, Inc. USA). The specific loss power (SLP) values (Wg^{-1}) for the samples are calculated using equation (1), where d*T*/d*t* is the initial slope of the graph (temperature change vs. time) in Figure S2.

$$SLP = \frac{CV_s}{m}\frac{dT}{dt} \qquad (1)$$

C: volumetric specific heat capacity of the sample solution ($JL^{-1}K^{-1}$)
V_s: sample volume (L)
m: mass of magnetic material in the sample (g)
dT/dt: initial slope of the change in temperature versus time curve (Ks^{-1})

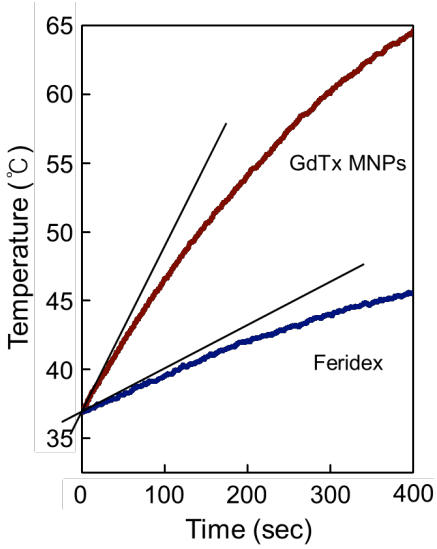

Figure S2. Temperature profile of GdTx MNPs and Feridex under an AC magnetic field application. Each black linear line indicates the initial slope of GdTx MNPs (red) or Feridex (blue). The fourfold increase in the initial slope for the GdTx MNPs experiments (0.12) compares favorably to the one produced by Feridex (0.03). The SNP values are 471 Wg^{-1} and 115 Wg^{-1} for GdTx MNPs and Feridex, respectively.

V. Tests of MNP cytotoxicity

Breast cancer cells, MDA-MB-231, are cultured in culture media composed of minimal essential medium (MEM, gibco) supplemented with 10% fetal bovine serum (FBS, gibco), 25 mM HEPES buffer, and penicillin/streptomycin at 37 °C in a 5% CO_2 and 95% air atmosphere. The cytotoxicity of the MNP is assessed using a cell counting kit-8 (CCK-8, Dojindo Molecular Technology). 1×10^5 MDA-MB-231 cells are seeded in a 24-well plate and incubated overnight. The cells are then incubated with 200 µL of a fresh media containing 12.5 – 300 µg/mL concentrations of MNPs for 24 h. After the solution is replaced with a fresh media (OPTI-MEM, gibco), cells are incubated with 10 µL of the CCK-8 solution for each well for 2 h. Then, the absorbance of each sample is measured at 450 nm.

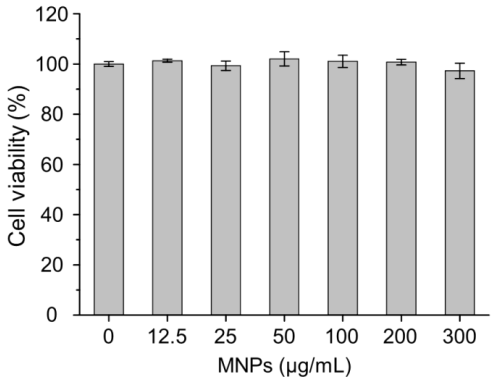

Figure S3. *In vitro* antiproliferative effect of MNPs on the MDA-MB-231 cancer cell line. The results indicate that the inherent viability of these cells is not perturbed by the MNPs even at high (300 µg/mL) nanoparticle concentrations.

VI. Cancer cell killing effect of GdTx

The experiment is carried out in the same way described in Supporting Information Section V but using GdTx instead of the MNPs.

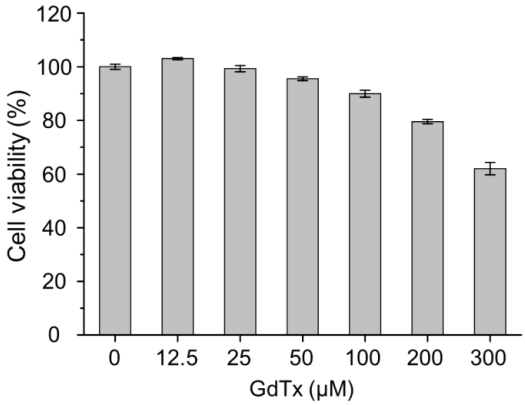

Figure S4. Anticancer effect of GdTx. The result shows the decrease in cell viability as the concentration of GdTx increases. At concentrations less than 25 μM, no cell death is observed.

VII. Detection of reactive oxygen species (ROS) in MDA-MB-231 breast cancer cells

ROS production is measured in live cells by monitoring the oxidation of 2′,7′-dichlorofluorescein diacetate (DCFA, Molecular Probes) to 2′,7′-dichlorofluorescein (DCF).[4] In these experiments, 5×10^4 MDA-MB-231 cells/well are incubated in MEM supplemented with 10% fetal bovine serum (FBS) containing 0.2 mg/mL GdTx-MNPs and 100 μM ascorbate for 24 h. After washing several times in phosphate buffered saline (PBS), the cells are incubated in 4 μM DCFA for 15 min at 37 °C in the dark. Cells are washed with PBS and imaged immediately using a FV1000 confocal microscope (Olympus).

VIII. Monitoring of apoptosis induced by mild hyperthermia in the MDA-MB-231 cell line

For monitoring cell death pathways, the Annexin V-FITC (fluorescein isothiocyanate) Apoptosis Detection Kit (Biovision) is used. Here, MDA-MB-231 cells are incubated with 0.2 mg/mL of GdTx MNPs for 5 h and then subjected to AC magnetic field for 30 min. At 6 h and 24 h post treatment, 5 μL of Annexin V-FITC and PI in binding buffer are added to cells. Treated cells are fixed with 4% paraformaldehyde at 4 ^{o}C for 30 min and washed twice with cold PBS solution. At this junction, a mounting solution containing DAPI (4',6-diamidino-2-phenylindole) is added. The cells are observed under a FV1000 confocal microscope (Olympus).

IX. Intracellular T1-, T2- MR images of GdTx MNPs.

MDA-MB-231 cells are incubated with GdTx MNPs for 24 h and their T1-, T2-weighted MR images are obtained. Untreated cells serve as control. Cells are incubated in OPTI-MEM containing 40 μg/mL of GdTx MNPs and 1.6 μg/mL of poly L-lysine as a transfection agent for 24 h. After washed several times in cold PBS, the cells are fixed with 4% formaldehyde solution for 30 min at 4 ^{o}C. Cells are washed with cold PBS, resuspended in PBS, and an aliquot of 1 x 10^5 cells is placed in a PCR tube. MR imaging of GdTx MNPs incubated cells is performed by using 1.5 T MRI. T1-, T2-MR scans are obtained by using the same method described in Section II of the Supporting Information (*vide supra*).

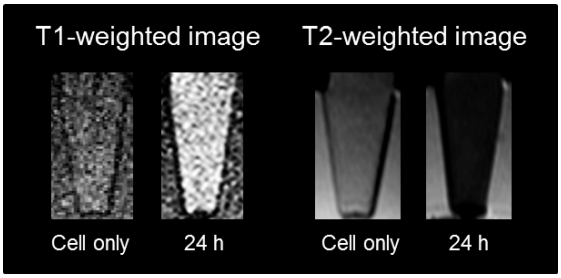

Figure S5. T1-, T2- weighted MR images of cells incubated with GdTx MNPs.

References

1. J.-t. Jang, H. Nah, J.-H. Lee, S. H. Moon, M. G. Kim, J. Cheon, *Angew. Chem.* **2009**, *121*, 1260–1264.

2. W.-H. Wei, M. Fountain, D. Magda, Z. Wang, P. Lecane, M. Mesfin, D. Milesb, J. L. Sessler, *Org. Biomol. Chem.* **2005**, *3*, 3290–3296.

3. D. K. Yi, S. S. Lee, G. C. Papaefthymiou, J. Y. Ying, *Chem. Mater.* **2006**, *18*, 614–619.

4. A. M. Evens, P. Lecane, D. Magda, S. Prachand, S. Singhal, J. Nelson, R. A. Miller, R. B. Gartenhaus, L. I. Gordon, *Blood* **2005**, *105*, 1265–1273.