Supporting Information

"Fabrication of a Silica Sphere with Fluorescent and MR Contrasting GdPO4 Nanoparticles from Layered Gadolinium Hydroxide"

Young-su Yoon,[†] Byung-Il Lee,[†] Kyung Sig Lee,[†] Hyejung Heo,[‡] Jung Hee Lee,[‡,*] Song-Ho Byeon,[†,*] and In Su Lee,[†,*]

[†]*Department of Applied Chemistry, College of Applied Science, Kyung Hee University, Gyeonggi-do 449-701, Korea, and* [‡]*Department of Radiology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul 135-710, Korea.*

e-mail: hijunghee@skku.edu (J. H. L.), shbyun@khu.ac.kr (S. H. B.), insulee97@khu.ac.kr (I. S. L.)

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Synthesis of silica spheres having $GdPO_4$:Eu nanoparticles at the surface.

General consideration. Any reagent including $GdCl_3 \cdot 6H_2O$ (Aldrich), $EuCl_3 \cdot 6H_2O$ (Aldrich), tetraethylorthosilicate (TEOS, Acros), NH_4OH solution (0.28~0.3 M, Samchun), sodium 3-(trihydroxylsily)propylmethylphosphonate (TSPMP,Aldrich), 2-[methoxy(polyethylenoxy)propyl]trimethoxysilane (MPEOPS, Gelest) was used as purchased without any purification. Analyses of transmission electron microscopy (TEM) and energy dispersive X-ray spectroscopy (EDX) were conducted with JEOL JEM-2010. Scanning electron microscopy (SEM) was carried out with LEO SUPRA 55 (Carl Zeiss, Germany). X-ray diffraction patterns were obtained by using a rotating anode installed X-Ray Diffractometer (18kW) (Mac Science, Japan). UV absorption and fluorescence were observed by using V670 UV-Visible-NIR spectrophotometer (JASCO) and FP-6600 spectrofluorometer (JASCO), respectively.

Synthesis of LGdH:Eu and SiO_2-P. A colloid of delaminated layers of 10% Eu^{3+} doped LGdH (LGdH:Eu), $[Gd_{1.8}Eu_{0.2}(OH)_5(H_2O)_x]Cl$, and silica spheres with a surface modified anionically with methylphosphonate groups (**SiO_2-P**) were prepared by a modification of a previously reported procedure.[S1,S2] For the synthesis of LGdH:Eu, a KOH solution (0.1 M, 100 ml) was slowly added to the aqueous solution (100 ml) containing $GdCl_3 \cdot 6H_2O$ (1.67g, 6.34 mmol) and $EuCl_3 \cdot 6H_2O$ (0.182g, 0.634mmol) and reacted for 15 min at 60 ºC. After cooling the reaction suspension, the resulting LGdH:Eu was isolated by the centrifugation and purified by three times washes with water. For the preparation of **SiO_2-P**, TEOS (6.32 ml, 28.5mmol) was added to a water/EtOH mixture solution (63.5 ml) of NH_4OH and stirred for 30 min at room temperature. Then an aqueous solution of sodium 3-(trihydroxylsily)propylmethylphosphonate (TSPMP, 2ml, 10.5 mmol) was injected to the reaction solution and stirred for additional 2 hr. The resulting silica spheres, **SiO_2-P**, with methylphosphonate groups at the surface were collected by centrifugation. The collected **SiO_2-P** spheres were redispersed in EtOH and recovered by the centrifugation. The dispersion

of **SiO_2-P** into EtOH suspension and the centrifugation was repeated three times for the purification.

Synthesis of SiO_2-L and SiO_2-A. In order to deposit the LGdH:Eu layers on the **SiO_2-P** surface, the **SiO_2-P** spheres (100 mg) and the LGdH:Eu (20 mg) were mixed in a formamide solution (20 ml) and sonicated for 20 min. The resulting spheres, **SiO_2-L**, were collected by the centrifugation with a low speed spin (3000 rpm). The collected spheres of **SiO_2-L** were redispersed in water and recovered by the centrifugation. The isolated solid of **SiO_2-L** were completed dried *in vacuo* at room temperature. The powder of **SiO_2-L** was heated up with 5 ºC/min heating rate in a furnace and annealed in air condition for 4 hr at 400 ºC, 500 ºC, 600 ºC, 700 ºC, and 800 ºC generating the nanospheres of **SiO_2-A**.

Synthesis of SiO_2-PEG. The spheres of **SiO_2-A-700** (30mg) and 2-[methoxy(polyethylenoxy)propyl]trimethoxysilane (MPEOPS) (1 ml) were mixed in an ethanol suspension. Then small amount of NH_4OH solution was added to the suspension to initiate the reaction and stirred for 12 hr. The resulting PEG-modified silica sphere, **SiO_2-PEG**, hybrid were recovered by the centrifugation and washed with water for several times.

Measuring MRI relaxation properties

T_1 and T_2 relaxation times of the aqueous suspension of the **SiO_2-PEG** that were prepared in test tubes with varying concentrations were measured on a 3.0 T clinical MRI scanner (Philips, Achieva ver. 1.2, Philips Medical Systems, Best, The Netherlands) equipped with 80 mT/m gradient amplitude and 200 ms/m slew rate. A Look-Locker sequence (TR/TE = 10/1 ms and flip angle = 5) was used to measure T_1 by acquiring 17 gradient echo images at different inversion delay times using minimum inversion time of 87 ms with a phase interval of 264 ms with in-plane image resolution= 625×625 mm^2 and slice thickness = 500 mm. The images were fitted into a 3-parameter function to calculate T_1 values using Matlab program. T_2 measurements were performed by using 10 different echo times in a multislice turbo spin

echo sequence (TR/TE = 5000/20, 40, 60, 80, 100, 120, 140, 160, 180, 200 ms, in-plane resolution = 200 × 200 mm^2, slice thickness = 500 mm). The images were fitted into Levenberg-Margardt method to calculate T_2 values using Matlap program. We calculated the specific relaxivities (r_1 and r_2) of the nanoparticles from the plot of T_1^{-1} and T_2^{-1} vs concentration of contrast agent. The signal intensities of each ROIs (regions of interest) in the T_1 map (60 – 80 pixels) and the T_2 map (200 – 300 pixels) were measured for each concentration, which were then used for r_1 and r_2 calculations, respectively. We derived specific relaxivities based on the molar concentration of gadolinium atom measured by ICP-AES.

Investigation of cell labeling with the SiO_2-PEG.

Cell culture. A human breast cancer cell line, MDA-MB-435s cells were maintained in growth media (L-15 with L-glutamin, 1% antibiotic-antimycotic, 10% fetal bovine serum) at 37 °C in a humidified atmosphere of 0 % of CO_2. Cells were regularly passaged and reseeded 24 h before cellular uptake experiments.

Confocal laser scanning microscopy (CLSM). MDA-MB-435s cells were plated in chamber slides 24 h before cellular uptake, at a density of 5 × 10^3 cells per well. The cells were cultured with the spheres of **SiO_2-PEG** for 2 h at 37 ^oC. The concentration of gadolinium atoms was 1 μM. Then the cells were washed with cold PBS three times. The cells were fixed in 4 % paraformaldehyde solution, after cell washed with cold PBS three times. The slides were covered with a DAPI mounting medium. The cells were imaged with the 544 nm excitation source by using Bio-rad Radiance 2100 Confocal Microscope System.

In vitro MRI of cells. MDA-MB-435s cells incubated with the spheres of **SiO_2-PEG** at 50 μg/ml, 250 μg/ml, 1 mg/ml of Gd concentrations were centrifuged, and the resulting cell pellets were mixed with 1 % agarose solution. The mixture was transferred into Eppendorf-tube for MRI. T_1- and T_2-weighted MRI of the cells mixed with agarose were performed with

a 3.0 T whole body MRI system (Philips, Achieva ver. 1.2, Philips Medical Systems, Best, The Netherlands) equipped with 80 mT/m gradient amplitude and 200 ms/m slew rate.

Cytotoxicity evaluation of the SiO_2-PEG. Cell viability was determined in MCF-7 cells maintained in grow media (RPMI 1640 and Leibovitz's L-15 media with L-glutamin, 1% antibiotic-antimycotic, 10% fetal bovine serum) at 37 °C in a humidified atmosphere of 5 % of CO_2. Cytotoxicity of the spheres of **SiO_2-PEG** was evaluated by measuring the inhibition of cell growth using the MTT assay.[S3] Briefly, cells were plated at a density of 5×10^3 cells/ml in 96-well plates and treated with the above compounds for 72 h. After treatment, the cells were washed and incubated for an additional 0.5mg of MTT for 3 h. Cell viabilities were presented as the ratio of the number of cells treated to the number of non-treated control cells. Cell viability graphs were plotted as concentration of gadolinium atom.

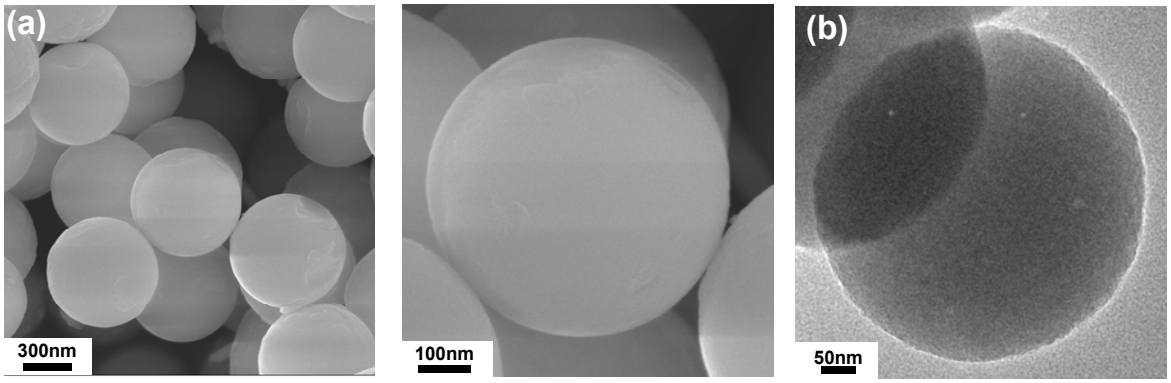

Figure S1. (a) SEM and (b) TEM images of the reaction adduct obtained from the control experiment with LGdH:Eu and unmodified SiO_2 spheres.

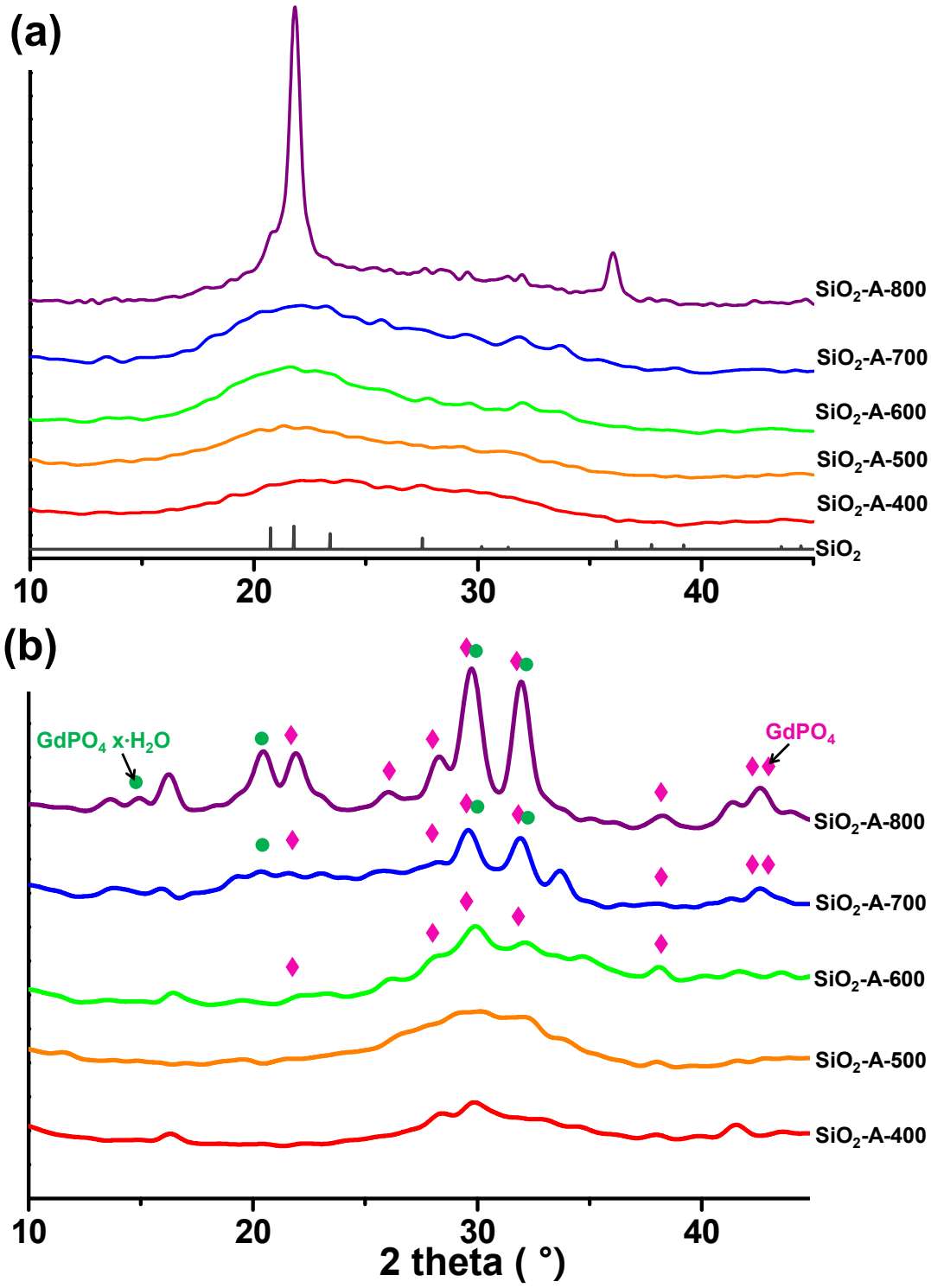

Figure S2. XRD patterns of (a) the as-prepared **SiO_2-A**s and (b) the solids obtained by etching **SiO_2-A**s with a NaOH solution. The crystalline phases of $GdPO_4$ and $GdPO_4{\cdot}xH_2O$ were assigned by comparing with JCPDS Cards (No. 84-0920 and 39-0232), respectively. Two XRD peaks positioned at 16 º and 41 º were due to the formation of $Gd(OH)_3$ (JCPDS Card No. 83-2037) during the silica etching process.

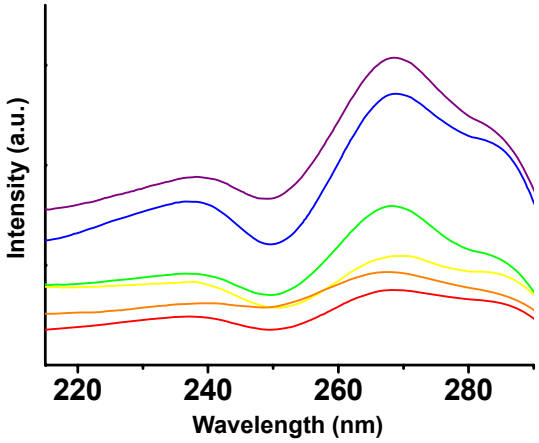

Figure S3. Excitation spectra of the powders of **SiO_2-L** and **SiO_2-A**s for the emission at 615 nm.

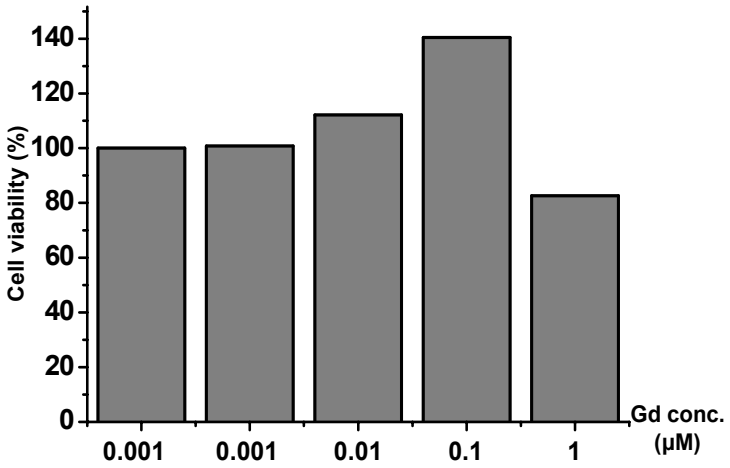

Figure S4. The cytotoxicity of **SiO_2-PEG** to MCF-7 cells as a function of the Gd concentration. The results are presented as the mean of four experiments with standard deviations.

References for the Supporting Information

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[S2] R. P. Bagwe, L. R. Hilliard and W. Tan, *Langmuir* 2006, **22**, 4357.

[S3] (a) T. Mosmann, *J. Immunol. Methods* 1983, **65**, 55. (b) D. Gerlier and N. Thomasset, *J. Immunol. Methods* 1986, **94**, 57.