Supporting Information

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Hybrid Silica Nanoparticles for Multimodal Imaging

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1. Materials. Triton X-100, GdCl₃·6H₂O, 1-hexanol, hexanes, cyclohexane, tetraethyl orthosilicate (TEOS), pyridine, diethylenetriamine pentaacetic acid dianhydride, methanol, and aqueous NH₄⁺OH⁻ were purchased from Aldrich and used without further purification. 3-aminopropyl triethoxysilane (APS) and 3-(trimethoxysilylpropyl)diethylene triamine were purchased from Gelest. Thermogravimetric analysis (TGA) was performed using a Shimadzu TGA-50 equipped with a platinum pan and heated at a rate of 3 °C / min under air. A Hitachi 4700 field emission scanning electron microscope (SEM) and a JEM 100CX-II transmission electron microscope were used to determine particle size and morphology. SEM images of the nanoparticles were taken on glass substrate. A Cressington 108 Auto Sputter Coater equipped with a Au/Pd (80/20) target and MTM-10 thickness monitor was used to coat the sample with approximately 5 nm of conductive layer before taking SEM images. Gd³⁺ ion concentration was measured on an Applied Research Laboratories (ARL) SpectraSpan7 Direct Current Plasma (DCP) Spectrometer. Emission and excitation data were collected on a Shimadzu RF-5301PC Spectrofluorophotometer. T1 and T2 values were determined on a Bruker 3.0 Tesla full body Magnetic Resonance Imaging (MRI) scanner. Confocal laser scanning microscope images were taken with a Zeiss LSM5 Pascal Confocal Laser Scanning Microscope or a Leica SP2 Laser Scanning Confocal Microscope with 488 nm excitation and a 530 LP emission filter. Fluorescence microscope images were taken with a Zeiss Axiovert 100 TV Fluorescence Microscope using a FITC filter.

2. Ligand and Gd-Complex Synthesis.

3-aminopropyl(trimethoxysilyl)diethylenetriamine tetraacetic acid (Si-DTTA) (Scheme S1). Bromoacetic acid (0.5558 g, 4.00 mmol) and 3-(trimethoxysilylpropyl)diethylene triamine (0.2654 g, 1.00 mmol) were dissolved in 1.0 mL of distilled H₂O and 2.0 mL 2M NaOH (4.00 mmol) with magnetic stirring. The reaction solution was subsequently heated to 50°C, and an additional 3.0 mL of 2M NaOH were added

dropwise over approximately 30 minutes. After stirring for an additional 2 h at 50°C, the solvent was removed under reduced pressure to yield a viscous yellow oil. An off-white hygroscopic powder was isolated from the oil in high yield (>90%) by precipitation with EtOH, and subsequent drying in vacuo. MS (ESI negative ion): m/z 542.2 [M-H]⁻ for the silanetriol from a basic solution. NMR: ¹H (D₂O, 300 MHz, ppm): 0.47 (2H), 1.55 (2H), 2.62-2.78 (10H), 3.14-3.21 (8H).

Scheme S1.

Synthesis of Gd-Si-DTTA Complex. The gadolinium complex was prepared by dissolving the isolated Si-DTTA product (108.6 mg, 0.2 mmol) in 4 mL H₂O with magnetic stirring at room temperature. GdCl₃ (380 μ L of a 0.50 M solution, 0.19 mmol) was slowly titrated into the solution until the formed precipitate would no longer dissolve back into solution, while maintaining a pH of ~9 with the dropwise addition of 2M NaOH. After stirring the above reaction for 2 h, Chelex 100 (Na⁺ form) was added to remove excess Gd³⁺, which was removed via filtration after 30 min. The resultant solution was then concentrated to 1 mL to yield a ~0.20 M solution of the mono-silyl derived Gd complex (Gd-Si-DTTA).

Bis(3-aminopropyl triethoxysilyl)diethylenetriamine pentaacetic acid (Si-DTPA) (Scheme S2). Diethylenetriamine pentaacetic acid dianhydride (5.000 g, 13.995 mmol) was dissolved in 110 mL of anhydrous pyridine under a steady flow of nitrogen. Using standard Schlenk line techniques 3-aminopropyl triethoxysilane (6.85 g, 31.00 mmol) was added and the resultant reaction mixture was magnetically stirred under nitrogen for 24 hours. The product was then precipitated with copious amounts of hexane, isolated via centrifuge, washed with additional aliquots of hexanes, and dried to yield 10.436 g (93.2 %) of the desired compound (Si-DTPA). MS (ESI negative ion): m/z 631.3 [M-H]⁻ for the silanetriol from a basic solution. NMR: ¹H (DMSO, ppm): 0.52 (t, 4H), 1.14 (t, 18H), 1.44(p, 4H), 2.81 (t, 4H), 2.92 (t, 4H), 3.04 (q, 4H), 3.22 (s, 6H), 3.34 (s, 4H), 3.73 (q, 12H), 8.06 (t, 2H). ¹³C{¹H} (DMSO, ppm): 8.0 (2C), 18.8 (18C), 23.4 (2C), 41.8 (2C), 51.2 (2C), 52.8 (2C), 55.9 (2C), 56.7 (2C), 58.3 (1C), 58.4 (6C), 170.7 (2C), 173.4 (3C).

Scheme S2.

Synthesis of Gd-Si-DTPA Complex. To prepare the gadolinium complex, Si-DTPA (1.77 g, 2.22 mmol) was dissolved in ~3 equivalents of NaOH (6.0 mL of a 1.0 M solution) with magnetic stirring for 30 minutes. To this solution was added 0.90 equivalent of GdCl₃ (4.0 mL of a 0.5 M solution, 0.002 mol) and the mixture was magnetically stirred at room temperature for several hours, the volume of the solution was adjusted to 10 mL to yield a visibly clear yellow 0.20 M solution of the modified gadodiamide complex.

3. Synthesis and Characterization of Silica Nanoparticles.

Silica nanoparticles (SNPs) were synthesized via the neutral Triton X-100/1-hexanol/cyclohexane microemulsion system. Initially, Triton X-100 (15.625 g, 0.075 mol) and 1-hexanol (38.318 g, 0.375 mol) were dissolved in cyclohexane and diluted to 250 mL to make a 0.3 M Triton X-100 stock microemulsion solution with 5 molar equivalents of the co-surfactant 1-hexanol. A typical synthesis using a $w \sim 15$ ($w = [\text{H}_2\text{O}]/[\text{surfactant}]$) microemulsion system consisted of adding 3.05 mL distilled H₂O and 500 μL TEOS to 50 mL of a 0.3 M Triton X-100/1.5 M 1-hexanol/cyclohexane stock solution while vigorously stirring at room temperature. After 10 min of vigorous stirring, or until the microemulsion mixture became optically transparent, 1 mL of aqueous NH₄⁺OH⁻ was added to initiate hydrolysis, and the resultant visibly clear microemulsion mixture was stirred for another 24 hrs before workup, which consisted of precipitating the nanoparticles with an equivalent volume (with respect to the total microemulsion volume) of methanol, isolating the nanoparticles via centrifuge at 12500 rpm, and subsequently washing them with methanol and H₂O before redispersing them in H₂O.

Ru(bipy)₃²⁺-Doped Gd-Si-DTTA Functionalized SNPs. Ru(bpy)₃²⁺-doped SNPs were prepared by adding 2.28 mL distilled H₂O, 160 μL of a 0.1 M Ru(bipy)₃²⁺ aqueous solution, and 400 μL TEOS to 40 mL of a 0.3 M Triton X-100/1.5 M 1-hexanol/cyclohexane stock solution while vigorously stirring at room temperature. After 10 min of vigorous stirring at room temperature, 0.8 mL of aqueous NH₄⁺OH⁻ was added to initiate hydrolysis, and the resultant optically transparent red microemulsion mixture was stirred for another 20 hrs before adding 1.0 mL of a 0.12 M Gd-Si-DTTA aqueous solution to the reaction mixture and stirring for an additional 24 hrs. The functionalized SNPs were then precipitated with an equivalent volume of methanol and isolated via centrifuge at 12500 rpm for 30 min. The SNPs were subsequently washed twice with MeOH and twice with H₂O by re-dispersing via sonication and isolating via centrifugation before re-dispersing them in water. Approximately 150 mg of functionalized SNPs were isolated from this procedure.

Ru(bipy)₃²⁺ Doped Gd-Si-DTPA Surface Functionalized SNPs. Ru(bpy)₃²⁺-doped SNPs were prepared by adding 2.85 mL distilled H₂O, 200 μL of a 0.1 M Ru(bipy)₃²⁺ aqueous solution, and 500 μL TEOS to 50 mL of a 0.3 M Triton X-100/1.5 M 1-hexanol/cyclohexane stock solution while vigorously stirring at room temperature. After 10 min of vigorous stirring at room temperature, 1 mL of aqueous NH₄⁺OH⁻ was

added to initiate hydrolysis, and the resultant optically transparent red microemulsion mixture was stirred for another 24 hrs at room temperature. To a 10 mL aliquot of the above reaction mixture was added 385 μL of a 0.2 M bis(aminopropyltriethoxysilyl)diethylenetriamine pentaacetate gadodiamide (Gd-Si-DTPA) solution and the reaction mixture was stirred for an additional 12 hrs. The Gd-Si-DTPA functionalized SNPs were then precipitated with an equivalent volume of methanol and isolated via centrifuge at 12500 rpm for 30 min. The SNPs were subsequently washed twice with MeOH by re-dispersing via sonication and twice with H_2O before re-dispersing them in 5 mL of water. Approximately 55 mg of functionalized SNPs were isolated from this procedure (using 10 mL of the above microemulsion reaction). Results suggest that when care is taken in isolating and washing the SNPs, >300 mg of nanomaterial can be isolated from a ~54 mL microemulsion reaction. TGA analysis of **2** showed an initial weight loss of 13.5% from r.t. to 180 $^\circ\text{C}$ for the adsorbed solvent species and a further weight loss of 33.2% from 280 – 450 $^\circ\text{C}$ for the organic components of Gd-Si-DTPA.

The average diameter is 63 nm and 22 nm for a w value of 10 and 20, respectively. The inverse dependence of particle size on the w value is likely a result of enhanced nucleation of silica particles at the reverse micelle oil-water interface since the number of reverse micelles typically increases as the w value increases.

4. Determinations of Gd-loadings on Each Particle of **1** or **2**.

Silica nanoparticles of **1**:

Concentration: 7.6 mg / mL, 2.086 mM
 Mass % Gd: 4.3%
 Diameter: 37 nm

$$\begin{aligned}\text{Mass silica NP: } (4/3) \times \pi \times (37/2)^3 &= 26500 \text{ nm}^3 \times 1 \times 10^{-21} \text{ cm}^3 \text{ nm}^{-3} = 2.65 \times 10^{-17} \text{ cm}^3 \\ 2.65 \times 10^{-17} \text{ cm}^3 \times 2.0 \text{ g cm}^{-3} &= 5.30 \times 10^{-17} \text{ g SiNP}^{-1}\end{aligned}$$

$$\begin{aligned}\# \text{ Gd per NP: } (Y \times m_{\text{Gd}}) / (m_{\text{SiNP}} + Y \times m_{\text{Gd-DTPA}}) &= \text{mass \% Gd} \\ (Y \times 157.25 \text{ g mol}^{-1}) / [(5.30 \times 10^{-17} \times 6.022 \times 10^{23} \text{ SiNP mol}^{-1}) + (Y \times 530 \text{ g mol}^{-1})] &= 4.3 \% \\ Y \times 157.25 &= 1.37 \times 10^6 + Y \times 22.8 \\ Y \times 134.5 &= 1.37 \times 10^6 \\ Y &= 10200 \text{ Gd SiNP}^{-1}\end{aligned}$$

Silica nanoparticles of **2**:

Concentration: 9.5 mg / mL, 8.37 mM
 Mass % Gd: 13.9%
 Diameter: 37 nm (conservative estimate)

$$\begin{aligned}\text{Mass silica NP: } (4/3) \times \pi \times (37/2)^3 &= 26500 (\text{nm}^3) \times 1 \times 10^{-21} (\text{cm}^3 \text{ nm}^{-3}) = 2.65 \times 10^{-17} \text{ cm}^3 \\ 2.65 \times 10^{-17} (\text{cm}^3) \times 2.0 \text{ g (cm}^{-3}) &= 5.30 \times 10^{-17} \text{ g SiNP}^{-1}\end{aligned}$$

Gd per NP:
$$(Y \times m_{\text{Gd}}) / (m_{\text{SiNP}} + Y \times m_{\text{Gd-DTPA}}) = \text{mass \% Gd}$$

$$(Y \times 157.25 \text{ g mol}^{-1}) / [(5.30 \times 10^{-17} \times 6.022 \times 10^{23} \text{ SiNP mol}^{-1}) + (Y \times 628 \text{ g mol}^{-1})] = 0.139$$

$$Y \times 157.25 = 4.43 \times 10^6 + Y \times 87.3$$

$$Y \times 70.0 = 4.43 \times 10^6$$

$$Y = 63200 \text{ Gd SiNP}^{-1}$$

5. Cell Culture Studies.

Monocyte immortalized lines were generated using the previously described methods of Monner (1) and Walker (2) with minor modifications described by Lorenz et al. (3). Briefly, bone marrow progenitor cells from C57Bl/6 mice were harvested and grown in conditioned medium containing 10% heat-inactivated fetal calf serum, 1% l-glutamine, and 20% LADMAC (catalog no. CRL 2420; American Type Culture Collection) supernatant in Minimal Essential Medium. Once immortalized, cells were grown in the aforementioned conditioned medium, which provides the isolated monocytes with colony-stimulating factor-1. Cell lines were matured over 9 months to achieve a homogeneous population expressing the macrophage/monocyte marker MOMA-2 (data not shown) with phagocytic capacity.

6. MRI Image Acquisition.

Monocyte cells were trypsinized for 5 minutes at 37 °C and 5% CO₂ before collection by low speed centrifugation. Cell concentration was determined by the trypan blue exclusion assay. Approximately 18.1×10^6 monocytes were placed in a culture dish with 1 mL of media and 0.433 mL of nanoparticle solution (24.6 mg/mL). After 1 hour of incubation, the cells were washed with fresh media twice and pelleted. A final layer of PBS (200 µL) was added on top, careful not to disturb the pellet, for MR imaging of the cells. Upon completion of MR imaging, the cells were digested in 1.0 M HNO₃ for DCP measurements of the total Gd³⁺ taken in by the cells.

References:

- (1) Monner DA, Denker B. Characterization of clonally derived, spontaneously transformed bone marrow macrophage cell lines from lipopolysaccharide hyporesponsive LPS(d) and normal LPS(n) mice. *J Leukoc Biol* 1997; 61(4):469-480.
- (2) Walker WS. Establishment of mononuclear phagocyte cell lines. *J Immunol Methods* 1994; 174(1-2):25-31.
- (3) Lorenz E, Patel DD, Hartung T, Schwartz DA. Toll-like receptor 4 (TLR4)-deficient murine macrophage cell line as an in vitro assay system to show TLR4-independent signaling of *Bacteroides fragilis* lipopolysaccharide. *Infect Immun* 2002; 70(9):4892-4896.

Figure S1. TEM micrographs of silica nanoparticles synthesized with the following w values: (left) $w=10$, (middle) $w=15$, and (right) $w=20$.

Figure S2. SEM images of nanoparticles of 1.

Figure S3. SEM image of nanoparticles of 2.

Figure S4. Thermogravimetric (TGA) plots of silica nanoparticles of **1** (left) and **2** (right).

Figure S5. T1-weighted and T2-weighted phantom MR images of SNPs of **1** and **2** dispersed in H₂O. An OmniScan standard was used for comparison.

Figure S6. Absorbance (dashed) and emission (solid) spectra for aqueous [Ru(bpy)₃]Cl₂ (blue) and SNPs of **1** in water (red). An excitation wavelength of 488 nm was used to collect the emission spectra.

Figure S7. Flow cytometric data for the monocyte cells under different incubation conditions. The insets show the purity of the cell populations. (a) 0 mg of $1/1 \times 10^6$ cells in mL media; R1 = 95.5% of total events; (b) 0.004 mg of $1/1 \times 10^6$ in 2 mL media; 0.6% NP labeling efficiency, R2 = 94.0% of total; (c) 0.042 mg of $1/1 \times 10^6$ in 2 mL media; 10.8% NP labeling efficiency, R2 = 94.2% of total events; (d) 0.418 mg of $1/1 \times 10^6$ in 2 mL media; 98.0% NP labeling efficiency, R2 = 90.9% of total events; (e) 2.140 mg of $1/1 \times 10^6$ in 2 mL media; 99.4% NP labeling efficiency, R2 = 91.3% of total events.