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Paramagnetic Gold Nanostructures for Dual Modal Bioimaging and Phototherapy of Cancer Cells

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Hollow Gold Nanoparticle Synthesis. Silver nanoparticles were synthesized using a polyol process.[1] In a typical example, silver nitrate ($AgNO_3$, 0.04 g) and polyvinylpyrrolidone (PVP, 1 g) were mixed with 7.5 mL ethylene glycol (EG). After vigorous mixing, the solutions were refluxed at 120°C for 4 h and then cooled to room temperature. Silver nanoparticles for subsequent use were obtained by filtration through a 0.2 μm filter. For the synthesis of hollow gold nanoparticles, 1 mL of silver nanoparticles suspension was diluted with 50 mL of trisodium citrate solution (0.4 mM in water). The diluted suspension was refluxed in a three-neck round flask at 100°C for 10 min. Two mL of chloroauric acid ($HAuCl_4$, 10 mM) was then injected into the suspension using a micropump (Harvard apparatus) operating at 45 mL h^{-1}. Next, the suspension was stirred vigorously for 20 min. The white precipitate resulting from the addition of NaCl was removed, and the final hollow gold nanoparticles were obtained

after washing and centrifugation (more than three repetitions of the procedures are recommended). Finally, the gold nanoparticles were filtered through a 0.2 μm filter.

Conjugation of Hollow Gold Nanoparticles with anti-HER2[Gd-DPTA]. One mL of anti-HER2 (200 μg mL^{-1}, Santa Cruz Biotechnology, Inc.) was reacted with 43.33 μL DTPA (p-SCN-Bn-DTPA, MW 649.9 g mol^{-1}, Macrocyclics) at room temperature for 1 h (anti-HER2:DTPA = 1:50 molar ratio). Next, 43.33 μL $GdCl_3$ (13 mg mL^{-1}) was added and the reaction continued at room temperature for 30 min. Anti-HER2 labeled with Gd-DTPA (denoted as anti-HER2[Gd-DTPA]) was purified by dialysis. The hollow gold nanoparticles were dispersed in 1 mL PBS (pH = 7.4) and the final concentration was adjusted to an optical absorption density (O.D.) of 2.8 at 808 nm. One hundred μL of cysteine-protein G (10-15 μg mL^{-1}) was added and the reaction proceeded at 4°C for 12 h with mild stirring. Next, 80 μL of thiol-PEG (20 μg mL^{-1}) was added and the reaction continued at 4°C for 12 h. Finally, anti-HER2[Gd-DTPA] was mixed with hollow gold nanoparticles with protein G surfaces to which antibodies could be bound. After the reactions were complete, the supernatant was removed and the nanoparticles were washed three times. After centrifugation, we have checked that no MR signal was detected in the supernatant solution. We checked that there was no nanoparticle aggregation for several months in the refrigerator storage. Dynamic light scattering analysis also indicated that the hydrodynamic radius of gold nanoparticles was changed from 67+/-5 nm to 79+/-6 nm after conjugated of anti-HER2[Gd-DTPA].

UV-Vis-NIR Spectroscopy and TEM. Spectroscopic observations were performed by UV-Vis-NIR spectroscopy (Beckman Coulter, DU 800 spectrophotometer). The TEM

images were obtained with an EF-TEM (EM 912 Omega, Carl Zeiss Co., Germany). T_1-weighted image and relaxation time of paramagnetic gold nanoparticles conjugated with anti-HER2[Gd-DTPA] were acquired using a Bruker Biospin 4.7 T scanner (Bruker BioSpin, Ettlingen, Germany) with a 200 mT m^{-1} gradient system equipped with a home-made surface coil.

Cell imaging. SKBR3, a breast carcinoma cell line, was obtained from the ATCC (Manassas, VA). Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Gibco) supplemented with 2 mM glutamine, antibiotics (100 μg ml^{-1} penicillin and 50 μg ml^{-1} streptomycin), and 10% (v/v) fetal bovine serum (FBS, Gibco) at 37°C under 5% (v/v) CO_2. SKBR3 cells were plated at 2×10^5 cells/well into 48-well culture plates and incubated for 1 day. The SKBR3 cells (2.5×10^5/0.5 ml) were also prepared in 24-well plates. Five hundred μL of DPBS (Dulbecco's Phosphate Buffered Saline, 1×, Gibco) was used to wash SKBR3 cells before experiments. Gold nanoparticles (350 μL) conjugated with anti-HER2[Gd-DTPA] (O.D. = 2.0) were then added to cells and the mixture were incubated at 37°C for 1h. After washing with 500 μL DPBS three times to remove unattached nanoparticles, 300 μL McCoy's 5A medium (containing 10% [v/v] FBS and antibiotics, Gibco) was added. Light-scattering images of SKBR3 cells targeted with anti-HER2[Gd-DTPA] were captured using an inverted microscope (Nikon) under conditions where a narrow beam of light from a tungsten source was delivered with a dark-field condenser. Only scattered light from the samples was collected using an A.100×/1.35 oil Iris objective (Uplanapo). An MRI scanner (1.5 T, Philips Medical System, Best, Netherlands) was used for MRI imaging. The T1

weighted image parameters were as follows: TR = 500 ms, TE = 13 ms; Section thickness = 2 mm; Matrix = 192 × 256; number of acquisitions = 6, FOV = 100 × 100.

Photothermal Therapy of Targeted Cells. Cultured SKBR3 cell lines (with or without nanoparticle targeting) were irradiated using an NIR laser (Unique Mode GmbH, Diode Laser Module, 808 nm, optical cable designed for 200 μm, N.A.= 0.2, fiber). The SKBR3 cells then remained in the culture medium at 37°C for 2 h. After the illumination of targeted SKBR3 cells using the NIR laser (808 nm, 4.34 W/cm^2) for 3 min, the viabilities of the NIR-treated cells were assessed using a fluorescence microscope after the staining with 0.1 mM of calcein-AM (Invitrogen Inc, Hayward).

Cell Death Analysis. Apoptotic and necrotic cell death of the NIR-treated SKBR3 cells were measured by double staining with fluorescein(isothiocyanate) (FITC)-conjugated Annexin V and propidium iodide (PI). The Annexin V were bound to the apoptotic cells with exposed phosphatidylserine, whereas PI labeled necrotic cells with membrane damage. The analysis was conducted with a PI/Annexin V staining kit (BD PharMingen, San Diego, CA) according to the instruction of the manufacturer. The NIR-treated SKBR3 cells were incubated with 5 μL Annexin V-FITC and 10 μL PI solution (2 μg mL^{-1}) in 100 μL binding buffer (10 mM HEPES (pH=7.4), 140 mM NaCl, and 25 μM $CaCl_2$) for 15 min at room temperature in the dark. The NIR-treated SKBR3 cells were diluted in 400 μL binding buffer and were immediately analyzed without fixation using a FACSCalibur instrument (Becton Dickinson, Mountain View, CA) (10,000 events/sample). The percentages of viable (Annexin V$^-$/PI$^-$), early apoptotic (Annexin

V^+/PI^-), necrotic (Annexin V^-/PI^+), and late apoptotic or secondary necrotic (Annexin V^+/PI^+) cells were determined with CellQuest Pro software (Becton Dickinson).

1 I. Pastoriza-Santos, L. M. Liz-Marzarn, *Nano Lett.,* 2002, **2**, 903.

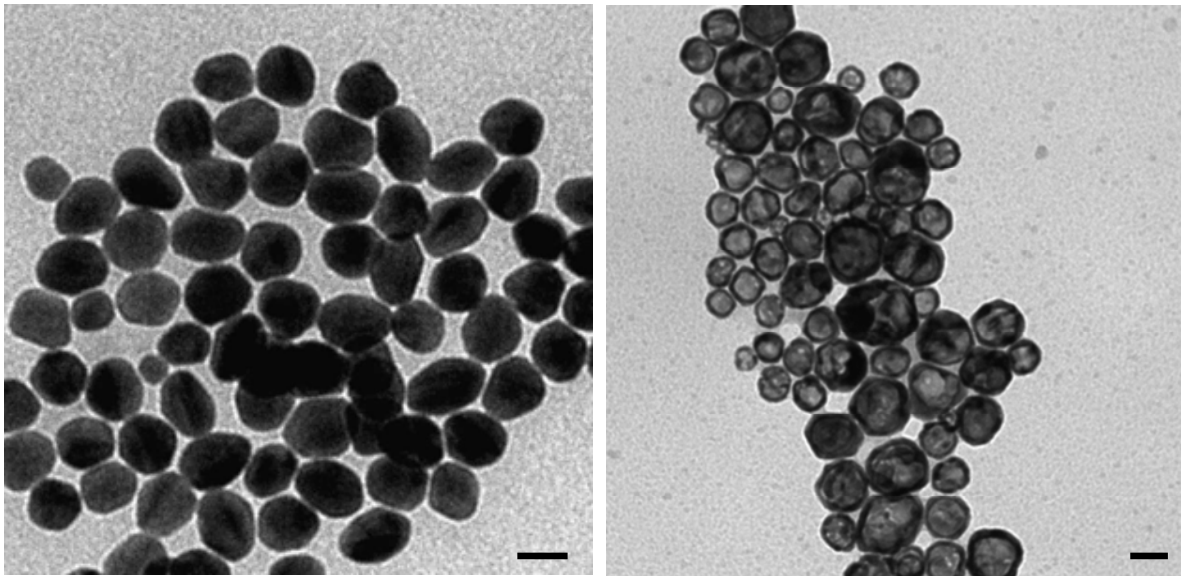

Figure S1. TEM images of silver nanoparticles (left) and gold hollow-type gold nanoparticles (right).The average size of silver and hollow-type gold nanoparticles was 65 nm and 62 nm, respectively. Scale bars represent 50 nm.

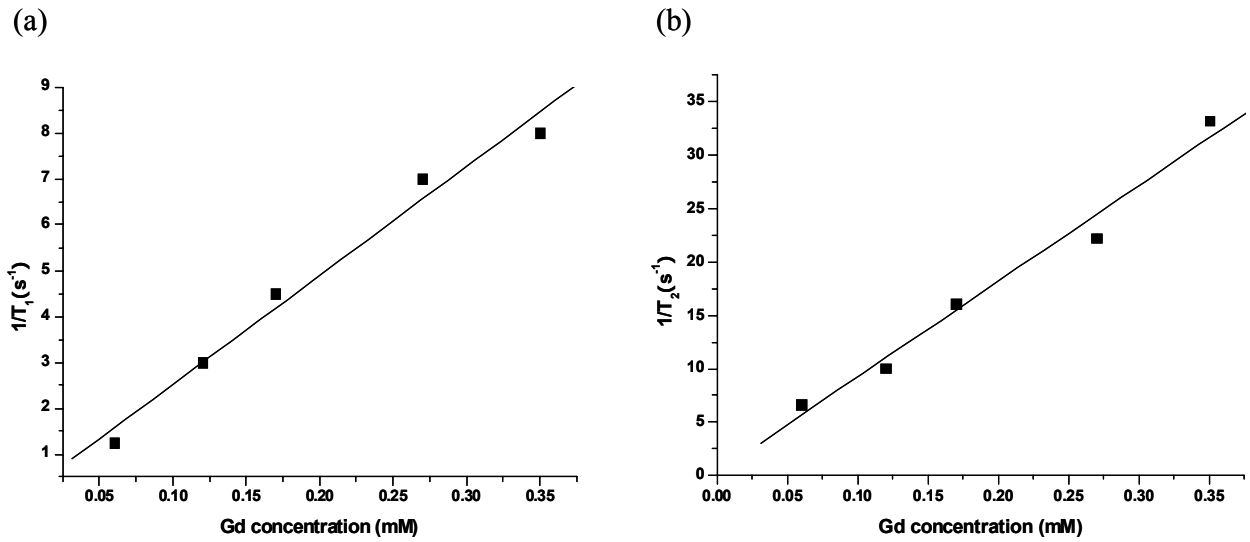

Figure S2. Relaxivity values for paramagnetic gold nanoparticles. The relaxivity values of r_1 (the slope of $1/T_1 = f[Gd]$) and r_2 (the slope of $1/T_2 = f[Gd]$) for paramagnetic gold nanoparticles were 23.7 mM^{-1} s^{-1} and 89.5 mM^{-1} s^{-1} , respectively.