Antibiofouling Polymer Coated Gold@Iron Oxide Nanoparticle (GION) as a Dual Contrast Agent for CT and MRI

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Received May 13, 2009, Accepted June 17, 2009

Key Words: Computed tomography, Magnetic resonance imaging, Contrast agent, Gold nanoparticle, Iron oxide nanoparticle

Computed tomography (CT) is one of the most widely used imaging modalities to diagnose various diseases in clinical field.[1-3] Although iodine-based compounds are widely used as current CT contrast agents,[4] several shortcomings have been reported, including short imaging times due to rapid renal clearance, renal toxicity, and vascular permeation.[5] In our previous study, we demonstrated the PEG coated gold nanoparticles can be used as a novel CT contrast agent for blood pool imaging (angiography) and for diagnosis of hepatoma, suggesting gold nanoparticle-based CT contrast agent may be able to overcome limitations of conventional iodine based contrast agents.[6] On the other hand, magnetic resonance imaging (MRI) has advantages over CT such as high spatial resolution and sensitivity. Superparamagnetic iron oxide nanoparticles (SPIONs) have been shown to be useful as MRI contrast agents and drug delivery vehicles.[7-12] Because MRI and CT imaging modalities have been used to compensate for the weakness of each modality, a dual contrast agent that works for both MRI and CT simultaneously would be useful in the diagnosis of various diseases.

Here we report a poly(ethylene glycol) (PEG) coated hybrid nanoparticle comprising an iron oxide nanoparticle core and a thin gold layered shell as a potential CT/MRI dual contrast agent. The gold@iron oxide core-shell nanoparticle (referred to as GION) was prepared by a previously reported method with slight modification (Figure 1a).[13] Gold acetate was reduced by oleylamine to form a thin gold layer onto oleylamine/oleic acid stabilized SPIONs. In the next step oleylamine/oleic acid that covers the resulting GIONs was exchanged by PEG-SH that is known to be biocompatible and to form a chemisorbed surface layer on gold surfaces. PEG coating layer on GION is necessary to ensure long circulation in blood stream as demonstrated in the PEG coated gold nanoparticle system.[6] The PEG coated GION was characterized by transmission electron microscopy (TEM), X-ray diffraction (XRD), thermal gravimetric analysis (TGA), and UV-Vis spectrophotometry.

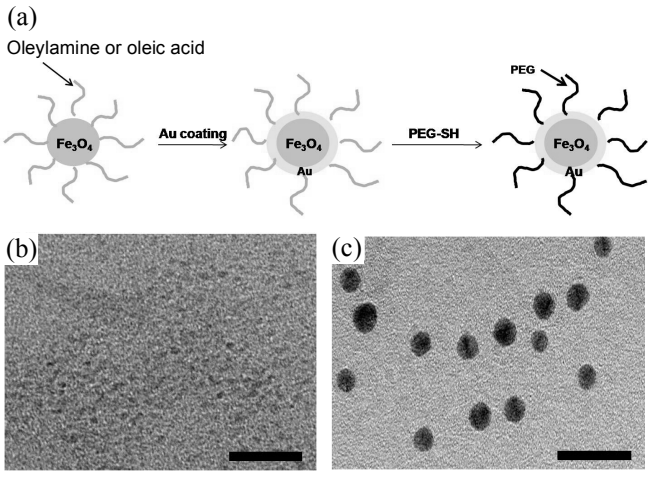

Figure 1. (a) A synthetic scheme for preparation of PEG coated GION. TEM images of (b) SPIONs (Fe_3O_4) and (c) PEG coated GIONs. The scale bar indicates 20 nm.

Figure 1b and 1c show the typical TEM images of oleylamine/oleic acid coated SPION and PEG coated GION. The average size of SPION measured by TEM (n = 100) before gold layer formation was approximately 2.8 ± 0.4 nm. After gold layer formation, the latter nanoparticles became to be more discrete, round-shaped, and darken in comparison with the former one, suggesting formation of the gold metallic layer. PEG coated GION was dispersed well in distilled water. While the average size of PEG coated GION measured by TEM (n = 100) was approximately 4.4 ± 0.5 nm, the mean hydrodynamic size in water measured by dynamic light scattering (DLS) was 47 ± 12 nm, suggesting the presence of PEG layer. Since dynamic light scattering measurements provide hydrodynamic particle size of whole clusters including polymer coating layers, the size measured by DLS was much larger than that obtained from TEM. The presence of the PEG layer on GION was further confirmed by TGA analysis, revealing that the polymer layer constitutes approximately 20% of the weight of PEG coated GION (Figure 2a).

Figure 2b shows the representative XRD spectra of PEG coated GION. The diffraction peaks at $2\theta = 38.3^{o}$, 44.56^{o}, 65.01^{o}, 78.42^{o}, and 81.43^{o} are corresponding to the FCC metallic gold diffraction. On the other hand, the diffraction peaks of iron oxide are hidden under the pattern of gold, indicating that gold shell surrounds iron oxide nanoparticle core completely. UV absorption spectrum of gold nanoparticle (5 nm), oleylamine/oleic acid coated SPION, and PEG coated GION were compared (Figure 2c). While SPION did not show any absorption band in the visible region, PEG coated

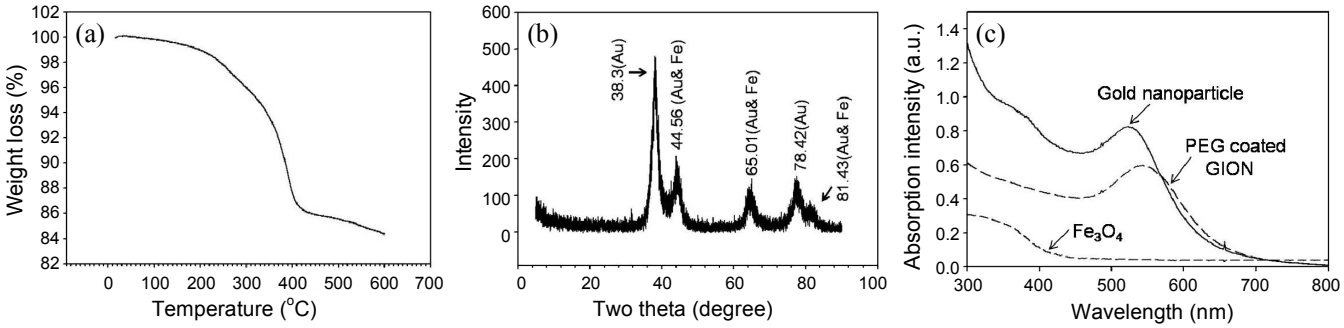

Figure 2. Characterization of PEG coated GION: (a) TGA spectrum, (b) XRD pattern, and (c) UV-Vis spectra of oleylamine/oleic acid coated Fe_3O_4 nanoparticles, gold nanoparticles (5 nm), and PEG coated GION.

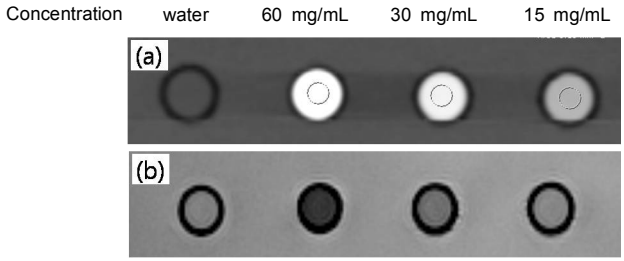

Figure 3. CT images (a), T_2-weighted MRI images (b) of PEG coated GION at various concentrations.

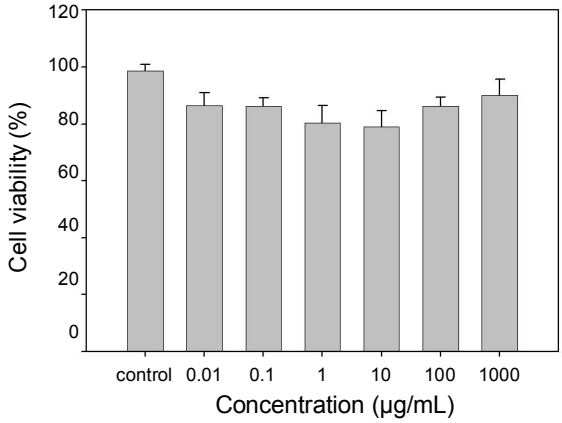

Figure 4. Cell viability of HepG2 cells measured after 24 h incubation with increasing amounts of PEG coated GION.

GION exhibited a clear surface plasmon resonance peak at 542 nm with red shift compared to that of gold nanoparticle (523 nm). All these measurements provide the evidence for the formation of PEG coated GION.

To examine the feasibility of PEG coated GION as a CT/MRI dual contrast agent, we measured X-ray absorption and T_2-weighted MRI phantom study with increasing amounts of the hybrid nanoparticles. Figure 3a shows CT images of PEG coated GION at various concentrations, which are seen in brighter image than water due to X-ray absorption of the gold layer. The intensity in CT images is proportional to concentration of the nanoparticles and is larger than conventional iodine based contrast agent when compared at the same concentration (w/v). PEG coated GION showed darkened images in T2-weighted MR images in concentration dependent manner similar to the CT result (Figure 3b). However, the T2 signal intensity of PEG coated GION was relatively weak compared to oleylamine/oleic acid coated SPION having no gold shell at the same Fe concentration. The lowered T2 signal may be attributed to the embedded structure of iron oxide nanoparticle in inner core. These in vitro phantom imaging study clearly suggests that PEG coated GION can be used as a CT/MRI dual contrast agent.

MTT assay was carried out to evaluate cytotoxicity of PEG coated GION. Figure 4 shows cell viability data of HepG2 cells after 24 h incubation with increasing amounts of PEG@GION. As expected, the nanoparticles did not show appreciable toxicity to cells even at high concentation (1 mg/ mL), suggesting the nanoparticle may be used safely in the *in vivo* use.

In this work, we synthesized PEG coated GIONs for potential use as a dual CT/MRI contrast agent. We demonstrated that the nanoparticles had high CT intensity and mild MRI signal due to the presence of gold for CT and SPION for MRI. Further the nanoparticles did not show appreciable cytotoxicity. *In vivo* animal imaging using PEG coated GIONs is currently under way to verify *in vivo* usefulness of the hybrid nanoparticles.

Experimental Section

Materials. Iron (III) acetylacetonate, 1,2-hexadecanediol, oleylamine, oleic acid, and phenyl ether were purchased from Aldrich Chemical Co. (Milwaukee, WI). Gold acetate was purchased from Alfa Aesar (Karlsruhe, Germany). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) solution was purchased from Sigma (St. Louis, MO). PEG-SH (molecular weight, 5000) was purchased from Nektar (Huntsville, AL). All organic solvents were used as received.

Measurements. Thermal gravimetric analysis (TGA) was carried out using TGA-50H (Shimadzu Co., Kyoto, Japan). The temperature of the sample increased gradually from 20 °C to 800 °C at a rate of 10 °C /min. A D/MAX uitima III X-ray diffractometer (Rigaku, Japan) was used to measure X-ray diffraction pattern. The morphology and size of nanoparticles were assessed by transmission electron microscopy (TEM) using a TECNAI F20 electron microscope (Philips Electronic Instruments Corp., Mahwah, NJ) operated at 200 kV.

Synthesis of PEGylated GION. GIONs were synthesized according to a previously reported method[13] with slight modification. 0.71 g iron (III) acetylacetone, 2 mL of oleic acid,

and 2 mL of oleylamine was mixed in 20 mL of phenyl ether under nitrogen atmosphere with vigorous stirring. To the mixture was added 1,2-hexadecanediol (2.58 g, 10 mmol) and heated to 210 °C to reflux. After refluxing for 2 h and then cooling to room temperature, 10 mL of the reaction mixture containing oleylamine/oleic acid coated Fe_3O_4 nanoparticles (~ 0.33 mmol) was transferred to another round bottomed flask containing 30 mL of phenyl ether. To this mixture was added gold acetate (0.83 g, 2.2 mmol), 1,2-hexadexanediol (3.1 g, 12 mmol), oleic acid (0.5 mL), and oleylamine (3 mL). The reaction solution was heated to 180 ~ 190 °C at a rate of 10 °C/min under nitrogen atmosphere with vigorous stirring and was kept at this temperature for 1.5 h. After cooling to room temperature, ethanol was added into the reaction mixture to precipitate nanoparticles. GIONs were collected by centrifugation at 7,000 rpm for 20 min, washed with ethanol, and redispersed in hexane. To coat GIONs with PEG-SH, hexane was evaporated and then PEG-SH dissolved in distilled water was added to the dried GIONs. This resulting suspension was applied to sonication at 200W for 15 min using an ultrasonic processor (VCX-500) of SONICS & MATERIALS INC (Newtown, CT).

CT & MR imaging: *In vitro* phantom study. Various concentration of PEG coated GION was prepared in distilled water (15 mg/mL, 30 mg/mL, and 60 mg/mL, respectively) prior to take CT and MR images. CT was performed to determine attenuation value of the GIONs using a GE Light Speed VCT 64-detector CT (GE Amersham Healthcare System, MI). Imaging parameters were as follows: slice thickness, 0.625 mm; Pitch, 0.984:1; 80 kVp, 500 mA; Field of view, 512 × 512, gantry rotation time, 0.4 s; table speed, 40 mm/rotation. CT data were analyzed using the measurement of the Hounsfield units (HU) for region of interest.

T_2-weighted MR imaging was performed with a 1.5-T MR scanner (GE Signa Exite Twin-speed, GE Health Care System, Milwaukee, USA) using an animal coil (4.3 cm Quadrature volume coil, Nova Medical System, Wilmington, DE). Imaging parameters were set as follows: TR/TE, 3,000/102 msec; flip angle, 90°; echo train length, 10; field of view, 5 cm; section thickness, 2 mm; intersection gap, 0.2 mm; matrix, 256 × 160. MR data were analyzed using the measurement of the T_2-weighted signal intensity for region of interest.

Cell culture and Preparation. HepG2 cells (American Type Culture Collection, Manassas, VA) were grown as a monolayer in a humidified incubator in a 95% air/5% CO_2 atmosphere at 37 °C in Dulbecco's Modified Eagle's Medium (GIBCO, Grand Island, NY) supplemented with 10% (v/v) heat-inactivated fetal bovine serum, 100 IU/mL penicillin, and 100 IU/mL streptomycin.

***In vitro* cell cytotoxicity analysis.** Cytotoxicity of PEG coated GIONs was determined using a MTT colorimetric assay. HepG2 cells were seeded in 96-well plates at a density of 8×10^3 cells/well in 100 μL of medium and incubated overnight at 37 °C in an atmosphere of 5% CO_2/95% air. The medium of each well was then replaced with 100 μL of fresh medium containing various concentrations of the nanoparticles. All concentrations were tested in replicates of six. After 24 h, the medium was aspirated, and the cells were washed twice with PBS to eliminate remaining particles. Next, 100 μL of fresh culture medium was added to each well, followed by 20 μL of MTT solution (2.5 mg/mL in PBS). The cells were then incubated for a further 4 h at 37 °C, and then the medium was carefully aspirated. The cells were solubilized in 200 μL of DMSO and the absorbance in each well at 570 nm was measured using a FL600 microplate reader (Bio-Tek Inc., Winooski, VT). The data are expressed as the percent of viable cells compared to the control group.

Acknowledgments. This work was supported by the Korea Research Foundation Grant (KRF-2007-314-D00326) funded by Ministry of Science and Technology of the Korean government.

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