Direct Immobilization of Gadolinium Complex on Silica Particles and Their MRI Properties

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This paper proposes a method for fabricating gadolinium diethylenetriamine pentaacetic acid-immobilized silica particles (SiO$_2$/Gd-DTPA). Preparation of a colloid solution of spherical silica particles with an average size of 101.7±11.7 nm was performed by a sol-gel method at 35°C using 0.2 M tetraethylorthosilicate, 25 M H$_2$O and 0.01 M NaOH in ethanol. Amino groups were introduced on the silica particles with 6×10$^{-3}$ M (3-aminopropyl)triethoxysilane (APES) at 35°C (SiO$_2$−NH$_2$), which resulted in their average particle of 80.5±9.7 nm. Gd-DTPA was immobilized on the SiO$_2$−NH$_2$ particle surface with 5×10$^{-4}$ M Gd-DTPA in 50/50 (v/v) water/dimethylformamide solution at 35°C, which provided their average particle of 101.6±12.3 nm. The APES-introduction and the Gd-DTPA-immobilization did not change the spherical structure, and shifted an iso-electric point of particles to higher pH for the APES-introduction and then to lower pH for the Gd-DTPA-immobilization, which indicated that APES and Gd-DTPA were successfully attached on the particle surface with no chemical damage. A relaxivity value for T$_1$-weighted imaging of the SiO$_2$/Gd-DTPA particle colloid solution was 2.7 mM$^{-1}$ s$^{-1}$, that was 64% of that for Magnevist.

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I. INTRODUCTION

Gadolinium (Gd) ions increase contrast of images taken by a magnetic resonance imaging (MRI) technique [1-3], because they resonate magnetically due to their paramagnetism. Commercially-available Gd ion-related MRI contrast agents are solutions dissolving the Gd complexes (GdC) homogeneously at molecular level. The contrast agents injected into living bodies flow in living bodies, and finally are discharged out of the living bodies. A velocity for the GdC molecules may be large compared to nano- or submicron particles, since they are smaller than the particles, which results in small drag force given from fluid. Thus, the discharge for the GdC molecules is quick, which makes it difficult to take high contrast images for a long period.

Not GdC molecules but particles containing GdC are promising as a new MRI contrast agent. The particles are dragged from fluid to a direction opposite to the fluid due to their projected area larger than molecules, so that they can be expected to stay in living bodies for a long period due to controlled flow of the particles in liquid. Consequently, formation of the particles containing GdC will increase residence time of GdC in the living bodies, which makes it possible to take clear MRI images for a long term. The particles are required to unaggregate in living bodies, because aggregation of the particles increases their apparent size, which prevents them from flowing in body fluid. Since surface of silica (SiO$_2$) particles prepared by a sol-gel method has a feature of colloidal stability [4-7], SiO$_2$ particles containing GdC may function as the MRI contrast agent that shows long residence time and non-aggregation in living bodies.

Two structures are mainly possible for SiO$_2$ particles containing GdC. One is SiO$_2$ particles containing GdC inside them. Their colloid solution is not expected to have excellent MRI properties. Strong interaction between water protons and GdC molecules promotes MRI properties of GdC [8-10]. Because GdC present inside them can not contact water protons, such interaction is not significant, which spoils original MRI properties of GdC. Another is SiO$_2$ particles, on which GdC molecules are immobilized. Several researchers have studied on developments of various methods for immobilizing GdCs on particles with SiO$_2$ surface [11-14]. In our previous work [15], a method for the GdCs-immobilizing on SiO$_2$ particles was proposed (SiO$_2$/GdC). The proposed method is composed of three steps. The first is surface-modification of SiO$_2$ particles with amino groups by using a silane coupling reagent with amino group (SiO$_2$−NH$_2$). The second is introduction of ethylenediaminetetraacetic acid on the SiO$_2$−NH$_2$ particle surface. The third is immobilization of Gd$^{3+}$ ions on the particle surface through formation of GdCs. This method works well but requires no less than three steps.

An alternative method for the GdCs-immobilizing is proposed in the present work. GdC is directly immobilization on SiO$_2$ particles in the proposed method, which will decrease number of steps required for production of SiO$_2$/GdC particles. The present work also studied on MRI properties of the colloid solution of SiO$_2$/GdC particles.
II. EXPERIMENTAL

A. Chemicals

A starting reagent for producing SiO$_2$ particles was tetraethylorthosilicate (TEOS) (95%). Solvent and catalyst for the sol-gel reaction of TEOS were ethanol (99.5%) and sodium hydroxide (NaOH) solution (1 M), respectively. (3-Aminopropyl)triethoxysilane (APES) (Tokyo Chemical Industry Co., Ltd., 98%) was used for surface-modification of SiO$_2$ particles. GdC used was Magnevist, i.e., Gd diethylenetriamine pentaacetic acid (Gd–DTPA) (Bayer Co., Ltd., 0.5 M Gd), which is one of representative MRI contrast agents, and dimethylformamide (DMF) (> 99.5%) was used as a solvent of Magnevist in GdC-immobilization. Hydrochloric acid (HCl) (35.0-37.0%) and the NaOH aqueous solution were used for adjustment of pH of solution in electrophoretic light scattering (ELS) measurements. Aqua regia composed of nitric acid (60-61%) and the HCl were used for dissolving the particles in inductively coupled plasma (ICP) measurements. All the chemicals except for APES and Gd–DTPA were purchased from Kanto Chemical Co., Inc., and used as received. Water that was ion-exchanged and distilled with Shimadzu SWAC-500 was used in all the preparations.

B. Preparation of materials

1. SiO$_2$ particles

SiO$_2$ particles were prepared by a sol-gel method using TEOS, water and NaOH in ethanol. Ethanol solution of the TEOS was added to a mixture of water, ethanol and NaOH aqueous solution. Initial concentrations of TEOS, H$_2$O and NaOH were 0.2, 25 and 0.01 M, respectively. The reaction temperature and time were 35°C and 24 h, respectively. The particles were washed by repeating centrifugation, removal of supernatant, addition of water and sonication over three times. Final volume of the suspension was adjusted to the volume of initial solution with the addition of water.

2. Immobilization of Gd–DTPA on SiO$_2$ particles

Amino groups in APES were first introduced on SiO$_2$ particle surface, i.e., SiO$_2$–NH$_2$ particles were fabricated, by a reaction of alkoxide groups of APES and OH groups on silica surface of the SiO$_2$ particles. APES was added to the SiO$_2$ particle colloid solution at 35°C, and then the mixture was stirred for 24 h. An initial APES concentration was adjusted to $6 \times 10^{-3}$ M. The as-fabricated SiO$_2$–NH$_2$ particles were washed by repeating a process composed of centrifugation, removal of supernatant with decantation, addition of DMF, and shake with a vortex mixer three times. The final colloid solution of SiO$_2$–NH$_2$ particles was concentrated with 2-fold by reducing the amount of DMF at the last process.

Next, Gd–DTPA was immobilized on SiO$_2$–NH$_2$ particle surface, i.e., SiO$_2$/Gd–DTPA particles were fabricated, through formation of amide bonds between carboxyl groups of Gd–DTPA and amino groups on the SiO$_2$–NH$_2$ particles. Gd–DTPA/DMF mixture was added to the SiO$_2$–NH$_2$ particle colloid solution in a volume ratio of 1:1 at 35°C. The reaction time was 24 h, and an initial Gd concentration was $5 \times 10^{-4}$ M.

C. Characterization

Morphology of the particles was investigated by transmission electron microscopy (TEM). TEM was performed with a JEOL JEM-2000FX II microscope operating at 200 kV. Samples for TEM were prepared by dropping and evaporating the nanoparticle suspensions on a collodion-coated copper grid. Dozens of particle diameters in TEM images were measured to determine volume-averaged particle size and standard deviation of particle size distribution. Introduction of amino groups and immobilization of Gd–DTPA on particle surface were qualitatively confirmed by ζ-potential measurements, though the measurements possess limited usefulness quantitatively in surface analysis. To measure ζ-potential of particles, ELS measurements were performed with a Malvern ZS90 particle size and zeta potential analyzer. HCl aqueous solution or NaOH aqueous solution was added to solution to vary pH of solution for the ELS measurement. An actual Gd concentration in particle colloid solution was measured by ICP emission spectroscopy. ICP measurement was performed with a Shimadzu ICPS-7510 atom emission spectrometer. Emission was detected at a wavelength of 342.247 nm. Samples for ICP were prepared by dissolving the particles with aqua regia and then diluting the obtained solution with water. For investigation on MRI properties, T$_1$ (longitudinal relaxation time)-weighted images of samples and T$_2$ values were obtained with a Bruker AVANCE III 400WB magnetic resonance imaging system at a static magnetic field of 9.4 T. Saturation recovery sequence was used for measuring the T$_1$ values. Echo time and repetition time were 8.5 and 1500 ms, respectively. For the imaging, the SiO$_2$/Gd–DTPA nanoparticles were washed by a process composed of centrifugation, removal of supernatant, addition of the water and sonication.

III. RESULTS AND DISCUSSION

A. Morphology of particles

1. SiO$_2$ nanoparticles

Figure 1 (a) shows a photograph of colloidal suspension of the SiO$_2$ particles. An opaque and colloidally-stable suspension was produced with the conditions. Figure 1 (b) shows a TEM image of the SiO$_2$ particles. Their average particle size was 101.7±11.7 nm. Figure 2 (a) shows their ζ-potential as a function of pH. ζ-potentials of the SiO$_2$ particles were negative in a range of pH examined, that is, the ζ-potential did not become positive even at the low pHs, though an absolute value of the ζ-potential was lowered with a decrease in pH. SiO$_2$ particles show such tendency of ζ-potential for pH [16,17]. Since the
FIG. 1. Photograph of colloid solution of SiO$_2$ particles (a) and their TEM image (b).

FIG. 2. ζ-potentials of (a) SiO$_2$ particles, (b) SiO$_2$–NH$_2$ particles and (c) SiO$_2$/Gd–DTPA particles vs. pH. ζ-potential approached to zero at pH below 2.0, the obtained SiO$_2$ particles had an iso-electric point in a range of 1.5-2.

2. SiO$_2$–NH$_2$ nanoparticles

Figure 3 (a) shows a photograph of SiO$_2$–NH$_2$ nanoparticle colloid solution. The solution was opaque, and neither precipitation nor flocculation took place in the solution similarly to the SiO$_2$ particle colloid solution. Accordingly, the particle colloid solution was colloidally stable even after the procedure of APES-introduction. Figure 3 (b) shows a TEM image of the SiO$_2$–NH$_2$ particles. The particles were spherical, and their spherical structure was almost the same as that of the SiO$_2$ particles. This indicated that the procedure of APES-introduction did not damage their structure. Their average particle size was 80.5±9.7 nm. Figure 2 (b) shows their ζ-potential as a function of pH. The SiO$_2$–NH$_2$ particles had an iso-electric point of 7.5, which was higher than that of the SiO$_2$ particles. This indicated that the APES-introduction shifted the iso-electric point of SiO$_2$ particles to high pH. Since pKα values of many kinds of amino groups are in a range of 9-10, this pH shift was presumably derived from amino groups on surface of the particles. Consequently, this result supported successful APES-introduction on the particle surface.

3. SiO$_2$/Gd–DTPA nanoparticles

Figure 4 (a) shows a photograph of the SiO$_2$/Gd–DTPA nanoparticle colloid solution. Though the solution was opaque, neither precipitation nor flocculation was observed in the solutions similarly to the colloid solutions of SiO$_2$ particles and SiO$_2$–NH$_2$ particles, which indicated that the procedure of Gd–DTPA-immobilization did not have an effect on colloidal stability of the particle colloid solution. The particle colloid solution had an actual Gd concentration of 0.105 mM. Figure 4 (b) shows a TEM image of the SiO$_2$/Gd–DTPA particles. The particles did not aggregate but were dispersed, which supported the high colloidal stability of the particle colloid solution revealed in Fig. 4 (a). Their average size was 101.6±12.3 nm. The particles had a spherical structure with, which was similar to those of the SiO$_2$ particles and the SiO$_2$–
NH\textsubscript{2} particles. This observation indicated that the SiO\textsubscript{2}/Gd–DTPA particles were structurally stable even after the procedure of Gd–DTPA-immobilization. Figure 2 (c) shows \(\zeta\)-potential of SiO\textsubscript{2}/Gd–DTPA nanoparticles as a function of pH. Their iso-electric point was 5.6, which meant that the iso-electric point of SiO\textsubscript{2}/NH\textsubscript{2} particles shifted to low pH with the Gd–DTPA-immobilization. Introduction of carboxyl groups derived from Gd–DTPA on particle surface would make the particle surface negative because of anionic carboxyl groups. Accordingly, this pH shift implied that the Gd–DTPA was successfully immobilized on the particle surface.

**B. MRI property**

Figure 5 shows \(T_1\)-weighted images of the SiO\textsubscript{2}/Gd–DTPA particle colloid solutions with various actual Gd concentrations. For \(T_1\)-weighted images, strong magnetic resonance gives positive images with light contrast. All the solutions examined were clearly imaged against a black background. Figure 6 shows a plot of relaxation rate (1/\(T_1\)) of the SiO\textsubscript{2}/Gd–DTPA particle colloid solution as a function of the actual Gd concentration. The relaxation rate increased linearly with the increase in actual Gd concentration. Active magnetic dipolar coupling occurring between protons of water molecules (solvent) and paramagnetic contrast agent, i.e. active exchange of the protons with the contrast agent shortens \(T_1\) [8-10]. A value of relaxivity (\(r_1\)), which is defined as a slope of relaxation rate with respect to actual Gd concentration, is commonly used as a guideline on the performance of positive GdC-related contrast agents. A \(r_1\) value of the SiO\textsubscript{2}/Gd–DTPA particle colloid solution was calculated by linear fitting, was 2.7 mM\textsuperscript{-1} s\textsuperscript{-1}, which was smaller than 4.23 mM\textsuperscript{-1} s\textsuperscript{-1} for Magnevist revealed with the measurement in our previous work [18]. Since the Gd–DTPA molecules on particle surface lay close to the water protons, interaction between them was expected to be significant. However, the smaller \(r_1\) value was detected in the present work. The Gd–DTPA molecules could not contact with the water protons on the side which they touched the particle surface. Consequently, such interaction was probably controlled, which provided the smaller \(r_1\) value. Since the \(r_1\) value of the SiO\textsubscript{2}/Gd–DTPA particle colloid solution amounted to 64% for that of Magnevist, the particle colloid solution was considered to be available for MRI, with the respect to \(r_1\) value.

**IV. CONCLUSIONS**

A method for producing SiO\textsubscript{2}/Gd–DTPA particles was proposed. The colloid solution of spherical SiO\textsubscript{2} particles with the size of 101.7±11.7 nm was prepared at 35\(^\circ\)C by means of a sol-gel method using ethanol solution containing 0.2 M TEOS, 25 M H\textsubscript{2}O and 0.01 M NaOH. The SiO\textsubscript{2}/NH\textsubscript{2} particles with the average size of 80.5±9.7 nm and the SiO\textsubscript{2}/Gd–DTPA particles with the average size of 101.6±12.3 nm were fabricated by reacting 6 x 10\textsuperscript{-4} M APES on the silica particles and by reacting 5 x 10\textsuperscript{-4} M Gd–DTPA on the SiO\textsubscript{2}/NH\textsubscript{2} particles, respectively. The successful APES-introduction and Magnevist-immobilization were confirmed by the ELS measurements, and the particles were chemically stable throughout the APES-introduction and the Gd–DTPA-immobilization. The SiO\textsubscript{2}/Gd–DTPA particles were colloidally stable, even after washing with centrifugation. The \(T_1\)-weighted magnetic resonance images were successfully taken for the concentrated SiO\textsubscript{2}/Gd–DTPA particle colloid solution. Its relaxivity value was 2.7 mM\textsuperscript{-1} s\textsuperscript{-1}, that was 64% of that for Magnevist. These results obtained in the present work expect that the SiO\textsubscript{2}/Gd–DTPA particle colloid solution can be used as a new MRI contrast agent.

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