Silica-coated Iron-oxide Nanoparticles Synthesized as a $T_2$ Contrast Agent for Magnetic Resonance Imaging by Using the Reverse Micelle Method

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We synthesized iron-oxide ($Fe_3O_4$) nanoparticles by using the reverse micelle method and coated them with biocompatible silica. The coated nanoparticles were found to be spherical in the TEM images and showed a uniform size distribution with an average diameter of 10 nm. The $T_1$ and the $T_2$ relaxation times of hydrogen protons in aqueous solutions with various concentrations of silica-coated nanoparticles were determined by using a magnetic resonance (MR) scanner. We found that the $T_2$ relaxivity was much larger than the $T_1$ relaxivity for the nanoparticle contrast agent, which reflected the fact that the $T_2$ relaxation was mainly influenced by outer sphere processes. The $T_2$ relaxivity was found to be 15 times larger than that for the commercial Gd-DTPA-BMA contrast agent. This result demonstrates that silica-coated iron oxide nanoparticles are applicable as a $T_2$ agent in magnetic resonance imaging.

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I. INTRODUCTION

Recently, magnetic nanoparticles have received considerable attention because of their potential applications in biomedical fields, such as cell labeling [1] and cell separation [2], targeted drug delivery [3,4], and magnetic ferrofluids hyperthermia [5,6]. The main medical application of magnetic nanoparticles is in the field of diagnostic magnetic resonance imaging, where the superparamagnetic properties of nanoparticles can provide effective contrast in imaging [7-9]. Nanoparticle contrast agents alter the magnetic relaxation time of hydrogen protons in tissues [10].

Silica plays a very important and promising role in the development of coatings for magnetic nanoparticles, in regard to both basic research and technological applications. Silica-coated magnetic nanoparticles are more easily dispersed in liquid media by screening the magnetic dipolar attraction among the nanoparticles. The silica coating also protects the nanoparticles from leaching in an acidic environment. Silica-coated magnetic nanoparticles are also easier to activate, creating a surface with various functional groups. This is due to the existence of abundant silanol groups on the silica layer. The most important role of the silica coating is to provide a chemically inert surface for magnetic nanoparticles in biological systems [11,12].

In general, two different approaches have been employed to synthesize silica coatings on magnetite nanoparticles. The first method relies on the well-known Stöber process [13], which is comprised of hydrolysis and polycondensation of tetraethoxysilane under alkaline conditions in ethanol. The other method is based on microemulsion synthesis, in which micelles or reverse micelles are used as mini-reactors to control the nanoparticle size and the silica coating on the magnetic nanoparticles [14,15]. Smaller and more uniform particles can be synthesized, with good control over the amount of iron oxide and the resultant magnetic properties, by using the microemulsion approach [16].

In this paper, we report the synthesis of silica coatings on iron oxide ($Fe_3O_4$) nanoparticles fabricated by using the reverse micelle method, and we evaluate these
coated particles as a potential T2 contrast agent in magnetic resonance imaging. We studied the T1 and the T2 relaxations of hydrogen protons in water molecules in an aqueous solution of silica-coated iron oxide nanoparticles. We found that the T2 relaxivity for an aqueous solution of silica-coated oxide nanoparticles was 15 times larger than that of the commercial Gd-DTPA-BMA contrast agent. This result demonstrates that silica-coated iron-oxide nanoparticles are suitable as a T2 agent in magnetic resonance imaging.

II. EXPERIMENTAL

Silica-coated iron-oxide (Fe3O4) nanoparticles were synthesized by using the reverse micelle method [17]. The emulsion was prepared by adding 3.5 g of sodium dodecylbenzenesulfonate (NaDBS, Aldrich) to 30 ml of a xylene (isomers plus ethylbenzene, 98.5+%) solution and mixing well with sonication for 30 min. Under vigorous stirring at 500 rpm, an iron salt solution, composed of 0.1 M FeCl2·4H2O (99% Aldrich), 0.2 M Fe(NO3)3·9H2O (98%, Aldrich), and 1.8 ml water (ACS, reagent, Aldrich), was added to the emulsion solution. For stabilization of the reverse micelle solution (water-in-oil phase), the emulsion was stirred continuously for 16 hours at room temperature. Then, after the solution had been stirred for its homogeneity at 500 rpm under continuous argon flow for an hour, the micelle solution was slowly heated to 90 °C, and 1 ml of a hydrazine solution (34 wt-% water solution) was injected into the solution. The resulting solution was aged for 3 hours and cooled down to 40 °C within 90 min. After cooling, 4 ml of TEOS was injected into the emulsion. While stirring at 500 rpm for 6 hours, silica shells were formed on the surfaces of the iron-oxide (Fe3O4) nanoparticles by hydrolysis of TEOS molecules in the water region of reverse micelles. The coated nanoparticles were separated by using acetone and subsequent centrifugations. The collected nanoparticles were redispersed in water for the relaxivity measurements.

The particle size distribution and the structure of the silica-coated nanoparticles were checked with a TEM (transmission electron microscope, H-7600, Hitachi Ltd.). For the relaxivity measurements, aqueous solutions of various nanoparticle concentrations were prepared. The concentration of nanoparticles in the aqueous solution was measured with an ICP (inductively coupled plasma) spectrophotometer (Thermo Jarrell Ash IRIS-AP). The T1 and the T2 relaxation times of hydrogen protons in the aqueous solution of the coated nanoparticles were measured using an MR scanner (1.5T Scanner, GE Medical System).

III. RESULTS AND DISCUSSION

Figure 1 shows TEM images of the silica-coated iron-oxide (Fe3O4) nanoparticles. The coated nanoparticles are spherical, with an average diameter of 10 nm and a standard deviation of 5 nm. MR images for the aqueous solutions of various nanoparticle concentrations were obtained. We used five different samples with varying concentrations of nanoparticles: 70, 140, 280, 420, and 696 ppm.

For the T1 measurements, the inversion recovery pulse sequence was used. MR images for 35 different TI's
The relaxivity is a measure of the ability of MRI contrast agents to increase the relaxation of the surrounding nuclear spins (hydrogen protons), which can then be used to improve the contrast in MR images. The relaxivity is nuclear spins, which can then be used as contrast agents to increase the relaxation of the surrounding samples of nanoparticles, 70 and 696 ppm, respectively.

Figure 4 shows the T$_2$ relaxation time. Figure 3 shows the plot for two different samples of nanoparticles at 70 and 696 ppm.

The CPMG (Carr-Purcell-Meiboom-Gill) pulse sequence with multiple spin echo was used for the T$_2$ measurements. MR images for 22 different TE’s (times of echo) ranging from 10 to 850 msec were obtained. The signal intensity function for T$_2$ relaxation, $I \sim M_0 e^{-t/T_{2i}}$, was used to determine the T$_2$ relaxation times. Figure 4 shows the T$_2$ relaxation times for two samples of nanoparticles, 70 and 696 ppm, respectively.

The relaxivity is a measure of the ability of MRI contrast agents to increase the relaxation of the surrounding nuclear spins (hydrogen protons), which can then be used to improve the contrast in MR images. The relaxivity is expressed in units of s$^{-1}$ per ppm of nanoparticles. The contribution of paramagnetic contrast agents to the relaxation of the nuclear spins is due to both the inner and the outer sphere processes. The inner sphere process is from the chemical interchange interaction between the bound water of the paramagnetic agents and the surrounding free water, which eventually increases the relaxation (larger effect on T$_1$) of nuclear spins. On the other hand, the outer sphere process occurs when the paramagnetic agents diffuse through free water. In this process, random fluctuations of paramagnetic agents create an inhomogeneity of the local magnetic field, thus increasing the relaxation (larger effect on T$_2$) of nuclear spins [18].

In the clinically-used gadolinium-based contrast agents, gadolinium ions are formed as chelates. Thus, the bound water of the chelates can continuously interact with the surrounding free water and increase the T$_1$ relaxation of nuclear spins. Most gadolinium chelate agents have an inner sphere effect that is larger than the outer sphere effect; therefore, they are used as T$_1$ contrast agents. On the other hand, coated ferrite nanoparticle agents are completely surrounded by their coating material, and the chemical interchange interaction (inner sphere process) does not occur. However, the ferrite nanoparticles have a much larger magnetic moment than gadolinium ions and produce larger magnetic field fluctuations (inhomogeneity). Due to this property of magnetic nanoparticles, they are considered to be ideal T$_2$ contrast agents.

The relaxivities of nuclear spins in an aqueous solution of magnetic nanoparticles can be expressed as [19]

$$\frac{1}{T_{1m}} = \frac{1}{T_i} + R_iC,$$

where $i = 1$ or 2, and $1/T_i$ represents the relaxivity of nuclear spins with no nanoparticle contrast agent. $R_i$ is the relaxivity of nuclear spins per ppm of nanoparticles, and $C$ represents the concentration of nanoparticles in the aqueous solution.

Figure 5 is a plot of 1/T$_1$ versus particle concentration for silica-coated iron-oxide nanoparticles. The same plot for Gd-DTPA-BMA is shown here for reference.

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Figure 5 is a plot of 1/T$_1$ against particle concentration for aqueous solutions of silica-coated iron oxide nanoparticles and the commercial Gd-DTPA-BMA contrast agent [20]. The slopes of the lines were 0.003 and 0.0068 ppm$^{-1}$sec$^{-1}$, respectively. The T$_1$ relaxivity for the coated iron-oxide sample was 2.3 times smaller than that for the Gd-DTPA-BMA sample. This result demonstrates that for the nanoparticle contrast agent the T$_1$ relaxation was more influenced by the inner sphere process than by the outer sphere process.

Figure 6 shows 1/T$_2$ as a function of the particle concentration for the silica-coated iron-oxide sample and the commercial Gd-DTPA-BMA contrast agent. The slopes of the lines for these samples were 0.15 and 0.0097 ppm$^{-1}$sec$^{-1}$, respectively. The T$_2$ relaxivity for the nanoparticle sample was 15 times larger than that for the Gd-DTPA-BMA sample, which was expected. This result shows that the iron-oxide nanoparticles are more effective as a T$_2$ agent than the gadolinium-based con-
Fig. 6. Plot of $1/T_2$ versus particle concentration for silica-coated iron-oxide nanoparticles. The same plot for Gd-DTPA-BMA is shown here for reference. We can see that the $T_2$ relaxivity for the coated iron-oxide sample was 15-fold larger than that for the Gd-DTPA-BMA sample. See the text for details.

Fig. 7. $T_2$-weighted MR images of the abdomen of a mouse (a) before and (b) 20 minutes after the injection of 0.21 mg of iron-oxide nanoparticle agents into the mouse vein. The ratio of signal intensities at the marked position (○) before (a) and after (b) injection was measured as $I_a/I_b = 0.53$. In other words, the presence of the particles resulted in a 47% decrease in the signal intensity.

IV. CONCLUSION

We synthesized iron-oxide ($\text{Fe}_3\text{O}_4$) nanoparticles by using the reverse micelle method and coated them with biocompatible silica. The coated nanoparticles were found to be spherical in the TEM images and showed a uniform size distribution with an average diameter of 10 nm. The $T_1$ and the $T_2$ relaxation times of the hydrogen protons in aqueous solutions of varying concentrations were determined by using a magnetic resonance scanner. We found that the $T_2$ relaxivity was much larger than the $T_1$ relaxivity for the nanoparticle contrast agent, which reflected the fact that the $T_2$ relaxation was mainly influenced by outer sphere processes. The $T_2$ relaxivity was found to be 15 times larger than that for the commercial Gd-DTPA-BMA contrast agent. This result shows that silica-coated iron-oxide nanoparticles are applicable as a potential $T_2$ agent in magnetic resonance imaging.

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