Synthesis and characterization of ultrasmall gadolinium oxide nanoparticles for advance T1 MRI contrast agent

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Introduction
We reported the synthesis and characterization of ultrasmall gadolinium oxide nanoparticles. In this work, the ultrasmall gadolinium oxide nanoparticles with an average d of 1 nm having r$_1$ of 9.9 $s^{-1} mM^{-1}$, which is much larger than those of Gd(III)-chelates. It seems that surface Gd(III) ions in gadolinium oxide nanoparticles cooperatively induce the longitudinal relaxation of the water proton. As a result, this cooperative induction effect accelerates the longitudinal relaxation of the water proton, providing a larger r$_1$ than Gd(III)-chelates. We address this by carefully examining the d dependence of r$_1$. We finally took in vivo $T_1$ MR images of a rat with a brain tumor by using D-glucuronic acid coated ultrasmall gadolinium oxide nanoparticles and observed a clear contrast enhancement in $T_1$ MR images of the tumor after injection.

Material and Methods
In order to synthesize ultrasmall gadolinium oxide nanoparticles, 5 mmol of a Gd(III) ion precursor i.e gadolinium chloride hydrate was mixed with 50 mL of tripropylene glycol and magnetically stirred at 100 °C until the precursor was completely dissolved into solution. Reaction temperature was increased to 250-260 °C and refluxed for 24 hours while air was passed through the solvent. After the reaction, the reaction solution was cooled to room temperature, and the precipitate was washed with distilled water three times. For this, 400 mL of distilled water was added to the reaction solution, and the top solution was decanted after the reaction product was settled down in a few days. This procedure was repeated three times. A powder sample was obtained by drying the reaction product in air and then used for characterization. The same procedure was used for all three precursors.

For the synthesis of D-glucuronic acid coated ultrasmall gadolinium oxide nanoparticles, the temperature of above reaction mixture was lowered to 150 °C and 5 mmol of D-glucuronic acid was added to the reaction solution. The reaction was continued for 24 hours at this temperature. The reaction solution was cooled down to room temperature. D-Glucuronic acid coated ultrasmall gadolinium oxide nanoparticles were washed with distilled water three times to remove unreacted Gd(III) ions from solution by using the same procedure described above. For further characterization, the powder sample was obtained by drying the D-glucuronic acid coated ultrasmall gadolinium oxide nanoparticles in air. The remaining part was dispersed in distilled water (total 40 mL), and then, 2 mmol of sodium citrate was added to the solution to increase the colloidal stability of the D-glucuronic acid coated ultrasmall gadolinium oxide nanoparticles in solution. However, potential measurement showed that the colloidal stability only slightly increased after the sodium citrate was added. For this, the solution was heat-treated at 120 °C for 5 min and then cooled to room temperature. This solution was used as a sample solution for both relaxivity and in vivo $T_1$ MR image measurements.

Results and Discussion
The longitudinal ($T_1$) and transverse ($T_2$) relaxation times were measured at various solutions of different Gd(III) ion concentrations. The $r_1$ and $r_2$ were then estimated to be 9.9 and 10.5 $s^{-1} mM^{-1}$ from the slopes of the 1/$T_1$ = $r_1$ and 1/$T_2$ = $r_2$ plots versus Gd(III) ion concentration, respectively (fig.1a). The $r_2/r_1$ ratio is estimated to be 1.06. The $R_1$ and $R_2$ map images were also measured (fig.1b,c, respectively). They show a clear dose-dependent color change which is due to the relaxation increase of the water proton as the concentration of the sample is increased. This suggests a high sensitivity of ultrasmall gadolinium oxide nanoparticles as $T_1$ MRI contrast agent. In general, the $r_1$ should be as large as possible, and the $r_2/r_1$ ratio should be as close to 1 as possible in order for a chemical to be used as a highly sensitive $T_1$ MRI contrast agent. The ultrasmall gadolinium oxide nanoparticles seem to satisfy both conditions to a great extent. For clinical application, the sample solution should be excreted through kidney and bladder. Figure 2a,b shows a series of in vivo 3 tesla $T_1$ MR images of both kidney and bladder after vein injection of the sample solution. These MR images show a renal excretion of the sample solution. We also observed a blood pool effect of the sample solution. Figure 2c clearly shows a contrast enhancement of an aorta for a prolonged time after the vein injection. Although not quantitative, the brain tumor, kidney, bladder, and aorta MR images all together qualitatively show a wide biodistribution of the sample solution in a rat with a brain tumor. This is likely because of ultrasmall dimension of the D-glucuronic acid coated ultrasmall gadolinium oxide nanoparticles. Furthermore, it is expected that the D-glucuronic acid coated ultrasmall gadolinium oxide nanoparticle will be extremely valuable for target specific cancer detection, which we plan to do in the near future.

Conclusion
Paramagnetic ultrasmall gadolinium oxide (Gd$_2$O$_3$) nanoparticles with particle diameters (d) of ~1 nm were synthesized by using Gd(III) ion precursor and by refluxing in tripropylene glycol under an O$_2$ flow. A large longitudinal relaxivity ($r_1$) of water proton of 9.9 $s^{-1} mM^{-1}$ was estimated. As a result, high contrast in vivo $T_1$ MRI images of the brain tumor of a rat were observed. This large $r_1$ is discussed in terms of the huge surface to volume ratio (S/V) of the ultrasmall gadolinium oxide nanoparticles coupled with the cooperative induction of surface Gd(III) ions for the longitudinal relaxation of a water proton. In conclusion, the resulting data suggest that the paramagnetic ultrasmall gadolinium oxide nanoparticles can be used as an advanced $T_1$ MRI contrast agent.

Figure 1. (a) Plots of the $R_1$ and $R_2$ as a function of Gd(III) ion concentration. Slopes provide the $r_1$ and $r_2$. (b) $R_1$ and (c) $R_2$ map images as a function of Gd(III) ion concentration.

Figure 2. In vivo $T_1$ MR images of a rat with a brain tumor. (a) Kidney and (b) bladder contrast enhancement after injection of the sample solution. These MR images clearly show that the sample solution was excreted by the renal pathway. (c) In addition, the sample solution shows a blood pool effect: a long blood circulation time with a prolonged abdominal aorta contrast enhancement.