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Silica Microparticles as a Solid Support for Gadolinium Phosphonate Magnetic Resonance Imaging Contrast Agents

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Supporting Information

ABSTRACT: Particle-based magnetic resonance imaging (MRI) contrast agents have been the focus of recent studies, primarily due to the possibility of preparing multimodal particles capable of simultaneously targeting, imaging, and treating specific biological tissues *in vivo*. In addition, particle-based MRI contrast agents often have greater sensitivity than commercially available, soluble agents due to decreased molecular tumbling rates following surface immobilization, leading to increased relaxivities. Mesoporous silica particles are particularly attractive substrates due to their large internal surface areas. In this study, we immobilized a unique phosphonate-containing ligand onto mesoporous silica particles with a range of pore diameters, pore volumes, and surface areas, and Gd(III) ions were then chelated to the particles. Per-Gd(III) ionic relaxivities ranged from ∼2 to 10 mM^{-1} s^{-1} (37 °C, 60 MHz), compared to 3.0−3.5 mM^{-1} s^{-1} for commercial agents. The large surface areas allowed many Gd(III) ions to be chelated, leading to per-particle relaxivities of 3.3 × 10^7 mM^{-1} s^{-1}, which is the largest value measured for a biologically suitable particle.

Magnetic resonance imaging (MRI) is a noninvasive diagnostic technique that provides high-resolution, three-dimensional images of anatomic soft tissue.[1,2] Gd(III) complexes are widely used to increase the sensitivity of the technique, allowing images of early stages of disease to be collected.[3−6] The large number of unpaired electrons in the Gd(III) ion leads to increased proton relaxivities, with most commercial Gd(III) complexes producing values of ∼3.0 mM^{-1} s^{-1} at 37 °C and 60 MHz. Interest in improved resolution and diagnosis[7,8] has motivated research into new ligands and hybrid materials as components in new contrast agents. Gd(III) chelators have been conjugated to a variety of scaffolds,[9] including nanoparticles,[10−12] dendrimers,[13,14] proteins,[15,16] viral capsids,[17] silica particles,[18−21] and zeolites,[35] as well as other hybrid materials.[22] Recent reports have shown that grafting Gd(III) chelates onto a solid support can provide a highly efficient imaging agent with a large per-Gd(III) relaxivity and also delivers large quantities of the contrast agent to a single location through surface modification with tissue-targeting biomolecules.[19−21,23] Mesoporous materials have shown much promise in the field, as their large surface areas allow many Gd(III) chelators to be functionalized onto each particle. Mesoporous materials have also been shown to increase the sensitivity of MR probes *in vivo*.[19−21] An important feature of these materials is the ability to control water exchange to the metal center, due to the porous nature of the support. Control of water exchange is important because it is directly related to relaxivity. The relaxivities of commercial complexes are often restricted by slow water exchange.

Lanthanide ions readily complex with carboxylates,[24a] hydroxypyridinones,[24] and alkoxides.[25] Many studies have illustrated the use of phosphorus-containing ligands to sequester Ln(III) ions,[26−32] and diphosphonate ligands have been shown to be efficient materials for the removal of lanthanide ions over a range of pH values.[29−32] The ligand imidodi(methanediphosphate) (NDP_2) has been shown to be a highly efficient Gd(III) chelator, even compared to the diethylenetriamine pentaacetate (DTPA) ligand used in the commercial agent Magnevist.[29] In designing a solid-based contrast agent, an additional benefit of using NDP_2 as a Gd(III) ligand is that it can easily be appended onto silica supports.

Our procedure for modifying silica particles was similar to that described by Shkrob et al., who described modifying the surface through reaction of surface silanols with aminopropyl triethoxysilane followed by reaction with methylene bis(phosphonic dichloride) in the presence of triethylamine (Scheme 1).[29] We compared three types of mesoporous silica particles, called acid-prepared mesoporous silica (APMS), as supports for the NDP_2 ligand. APMS has previously been shown to be nontoxic and nonimmunogenic.[22] The synthesis is straightforward, requires less than 2 h, and can be scaled up to 800 g in a single batch and 3 kg in multiple batches. Sample **1**

Scheme 1. Synthesis of NDP_2−Gd Complexes within Porous Silica Particles

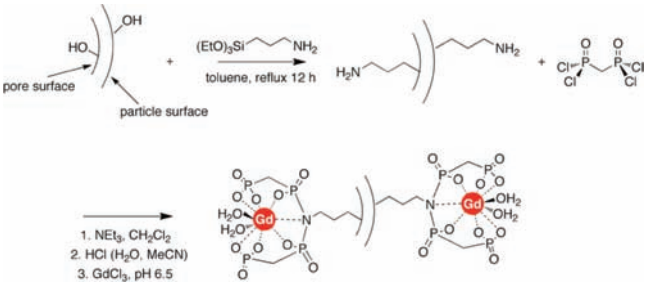

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Table 1. Physical Properties and Relaxivities of Porous Silica Particles

	as-made			functionalized			relaxivity ($mM^{-1}s^{-1}$) per-Gd		relaxivity ($mM^{-1}s^{-1}$) per-particle	
sample	d_{pore} (Å)	SA_{BET} (m^2/g)	V_{pore} (cm^3/g)	d_{pore} (Å)	SA_{BET} (m^2/g)	V_{pore} (cm^3/g)	r_1	r_2	r_1 ($\times 10^7$)	r_2 ($\times 10^7$)
1	36	790	0.87	33	380	0.41	4.6	15	3.3	11
2	39	780	0.65	34	350	0.35	6.0	17	1.7	5.0
3	55	690	0.97	45	380	0.56	10	23	2.4	5.4
Gd-DOTA[2]							3.0	3.3		
Gd-DTPA[2]							3.5	3.9		

was prepared as previously described,[19,23,33] leading to a material with an average pore diameter of 36 Å (Table 1 and Figure 1). Sample **2** was prepared by resuspending calcined **1** in

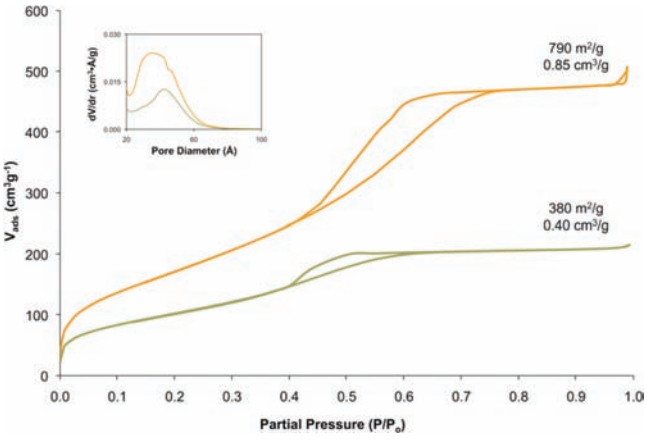

Figure 1. N_2 physisorption isotherm and pore size distribution (inset) for unmodified and modified versions of **1**.

water overnight, which increases the number of silanol groups on the surface. Based on water vapor physisorption isotherms, rehydroxylation can cause as much as a 4-fold increase in the number of silanol groups.[34]

Although the physical properties of **1** and **2** were similar, the larger number of silanols should increase the number of NDP_2 ligands on the surface and ultimately also increase the per-particle Gd relaxivity. To prepare sample **3**, the synthesis of the silica particles was altered by heating the reaction mixture for a slightly longer time than for sample **1** and by letting the reaction mixture cool slowly to room temperature rather than quenching it in an ice bath. These changes led to a material with a smaller surface area than sample **1** (690 vs 790 m^2/g) but a significantly larger average pore diameter (55 vs 36 Å) (Table 1).

After modifying the three samples, ion exchange with $GdCl_3$ led to the immobilization of Gd(III). Upon functionalization, the surface area, pore volume, and average pore diameter of all three samples decreased, indicating that the ligand had been successfully attached to the pore surfaces. Although the synthesis method makes characterization of the Gd(III) coordination environment difficult, Scheme 1 shows a proposed structure consistent with similar literature syntheses. In this structure, 1−2 water molecules are able to interact with the paramagnetic Gd(III) ion. As shown in Table 1, the per-Gd relaxivities of all three samples were higher than those of the commercially available complexes Gd-DOTA and Gd-DTPA, which are currently used as MRI contrast agents. This lends support to the influence of the phosphonate ligand on relaxivity, as well as the idea that an increased number of water molecules may be coordinated to the metal ion. However, the relaxivity values for **3** were especially notable, with r_1 ~3 times higher and r_2 ~5−7 times higher than the corresponding values for commercial agents.

As reports have demonstrated,[18c,d] relaxivity values for mesoporous silica materials are dependent on pore diameter. Water molecules are able to move more freely within larger pore materials, increasing both inner and outer sphere relaxivity. Thus, for porous solids with comparable levels of ligand and Gd modification, the material with a larger average pore diameter should show higher relaxivity values. The difference in per-Gd and per-particle relaxivities between **1** and **2** is less obvious, because they have similar measured pore diameters after functionalization and should have approximately the same water diffusion values. In sample **2**, rehydroxylation did lead to increased surface functionalization with the NDP_2 ligand; however, the amount of Gd(III) captured in this sample was less than in **1**. Thus the per-particle relaxivity of **2** is slightly less than for **1** (1.7×10^7 vs 3.3×10^7 mM^{-1} s^{-1}), while the decreased local motion of the Gd complexes resulting from molecular crowding led to a slightly increased per-Gd relaxivity (6.0 vs 4.6 mM^{-1} s^{-1}). Finally, a comparison of the per-particle relaxivities measured in this study to a variety of types of materials and complexes found in the literature (Figure 2) shows that while the per-Gd relaxivities are similar, the larger particle diameter and surface area of APMS allow more Gd to be immobilized, producing higher per-particle relaxivities.

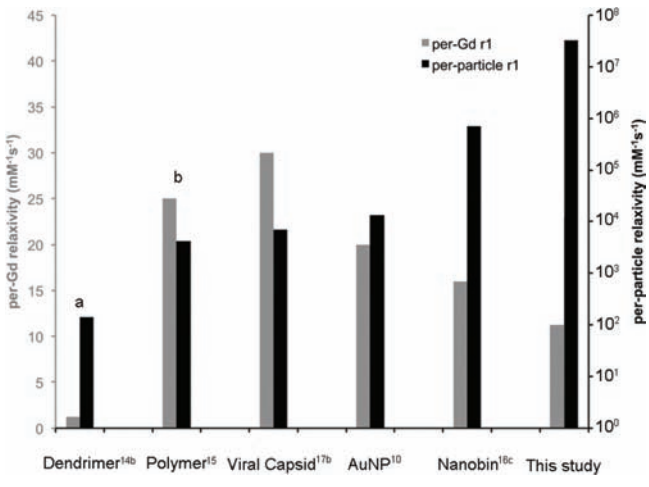

Figure 2. Comparison of relaxivities values obtained in this study to literature values. Note the linear axis for per-Gd values and the logarithmic axis for per-particle values. All data are taken at 1.41 T except (a) 3 T and (b) 0.73 T.

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In conclusion, we have developed a new class of biologically suitable MRI contrast agents that capitalize on the strong binding affinity of the NDP_2 ligand and the large internal surface area (exceeding 600 m^2/g) of mesoporous silica. The per-Gd relaxivities were significantly higher than those of commercially available contrast agents, and the per-particle relaxivities were among the highest values measured in the literature. Because APMS is nontoxic, nonimmunogenic, and excreted over time, these are exciting new materials for future *in vivo* studies.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details, additional characterization including scanning electron micrographs, and relaxometric data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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