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FEATURE ARTICLE
Pasquale Stano and Pier Luigi Luisi
Achievements and open questions in the self-reproduction of vesicles and synthetic minimal cells
Fabrication of a silica sphere with fluorescent and MR contrasting GdPO₄ nanoparticles from layered gadolinium hydroxide†

Young-su Yoon, Byung-II Lee, Kyung Sig Lee, Hyejung Heo, Jung Hee Lee,* Song-Ho Byeon* and In Su Lee*

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The delaminated gadolinium hydroxide layers doped with Eu³⁺ ions were assembled on the surface of silica spheres and annealed at high temperatures, resulting in the formation of fluorescent and MR active GdPO₄: Eu nanoparticles at the surface.

Layered hydroxides have attracted considerable attention due to their potential in wide areas such as catalysis, adsorption, nanocomposites, and drug delivery.¹ Very recently, a new series of layered rare-earth hydroxides (LRHs) with the general composition of RE₂(OH)₅X·nH₂O (RE = rare-earths, X = anions) were synthesized and generated extensive interest on account of their unique ability to combine the useful properties of rare-earth ions with the host–guest chemistry of layered double hydroxides (LDHs).² In the previous study, we demonstrated the potential utility of layered gadolinium hydroxide (LGdH) as a contrast agent for magnetic resonance (MR) imaging by taking advantage of their large water-accessible surface area and capacity to incorporate functional molecules between the layers.³ Another attractive aspect of LRH is that films with a wide range of applicability can be fabricated on several types of substrate by various techniques.⁴ In this study, it was envisioned that a coating with delaminated LGdH layers could provide colloidal particles with an MR active paramagnetic surface. Although some of the colloidal particles containing Gd³⁺ ions were recently evaluated as potent MR contrast agents, materials that operate with much lower concentrations of potentially toxic Gd³⁺ ions are still needed for further development.⁵,⁶ In this context, a thin-coating that carries a high loading of paramagnetic ions concentrated at the MR surface is an attractive source for an advanced MR contrast agent. Furthermore, this coating can exhibit photofluorescent properties by incorporating activator ions, such as Eu³⁺ and Tb³⁺, and provide a multimodal contrast agent combining optical and MR imaging.⁷

In this study, delaminated LGdH layers doped with Eu³⁺ ions (LGdH: Eu) were assembled on the surface of silica spheres and annealed at high temperatures, leading to a transformation into nano-sized granules of Eu³⁺-doped GdPO₄ (GdPO₄: Eu). Some rare-earth phosphate nanoparticles doped with activator ions were recently synthesized and reported to show photofluorescent properties.⁸ The annealed silica spheres were then modified with biocompatible poly(ethylene glycol) (PEG) groups, resulting in a stable colloidal suspension of fluorescent and paramagnetic particles, which can allow the development of a multimodal probe combining optical and MR imaging.⁹ This paper reports a novel method for coating the surface of colloidal silica with fluorescent and MR active GdPO₄: Eu nanoparticles through a process involving the assembly and transformation of delaminated LGdH layers. The effectiveness of the resulting silica spheres as dual imaging agents in labeling for both fluorescent and MR imaging was also demonstrated using an in vitro experiment with live cells.

Scheme 1 shows the procedure for fabricating the colloidal suspension of the silica spheres carrying GdPO₄: Eu nanoparticles at the surface. An aqueous colloidal suspension containing delaminated layers of 11% Eu³⁺-doped LGdH (LGdH: Eu), [Gd₁₊₄Eu₁₋₄(OH)₂H₂O]₃Cl, was synthesized according to the previous procedure.³ a SiO₂ spheres with 500 nm of average size were also prepared and their surface were anionically modified with methylphosphonate groups (SiO₂-P) by a modification of a previously reported procedure.¹⁰ The assembly of LGdH: Eu on the silica sphere was carried out by sonicating the formamide suspension containing the delaminated LGdH: Eu layers and SiO₂-P spheres followed by centrifugation and several washes with water. Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) images of the resulting silica spheres (SiO₂-L) revealed nanosheets of the LGdH: Eu layers deposited uniformly over the SiO₂-P spheres, most likely through electrostatic interactions. This resulted in the targeted silica spheres being coated with an ultrathin film of LGdH: Eu. A control reaction carried out using unmodified silica spheres did not show any binding of LGdH: Eu layers to the silica surface (ESI†). For a robust and photoluminescent coating of the surface, the SiO₂-L spheres were annealed and the solid-state transformation of the LGdH: Eu nanosheets was examined at various temperatures (Fig. 1). While no significant change in the size and shape of LGdH: Eu layers was observed at 400 °C, annealing the SiO₂-L spheres at higher temperatures caused the LGdH: Eu nanosheets to transform gradually into nano-sized granules. The TEM and SEM images of the spheres annealed at 500 °C (SiO₂-L-A-500) show the budding
of tiny protuberances from the LGdH : Eu nanosheets on the sphere surface. When annealing was performed at 600 °C, the LGdH : Eu nanosheets disappeared and instead nanoparticles with an average size of 32(±8) nm were created on the surface, resulting in a silica sphere with an embossed surface. Annealing of SiO$_2$-L at 700 °C and 800 °C resulted in the formation of nanoparticles with average sizes of 40(±20) nm and 70(±20) nm, respectively. The formation of a lower number of larger nanoparticles with an increase in the annealing temperature can be explained by the coalescence and growth of the grains at higher temperature. The X-ray diffraction (XRD) patterns of the solids isolated by etching the SiO$_2$-A spheres with NaOH showed a gradual growth of reflection peaks attributed to GdPO$_4$ with increasing the annealing temperature (ESI†). A full set of peaks representing the crystalline phase of GdPO$_4$ were observed in the XRD patterns of the solid annealed at 800 °C. These observations suggest that high-temperature annealing induced a reaction between the LGdH : Eu nanosheets and methylphosphonate groups immobilized on the silica surface, leading to the formation of silica spheres embossed with GdPO$_4$ : Eu nanoparticles. This is also supported by the energy dispersive X-ray spectroscopy (EDX) maps of the SiO$_2$-A spheres that show the diffusion and localization of P element around the as-transformed nanoparticles during the annealing process (Fig. 1). The atomic ratio of P : Gd and Eu in SiO$_2$-A-700 was determined as 1 : 1.6 by the inductive coupling plasma (ICP) analysis, indicating 63% conversion of the hydroxide into the phosphate at 700 °C. In addition, the high-temperature transformation of the LGdH : Eu to GdPO$_4$ : Eu greatly improved the photoluminescence properties because the luminescence of LGdH : Eu layers is strongly affected by the hydroxyl groups. As shown in Fig. 2a, while the SiO$_2$-L did not show any luminescence, the SiO$_2$-A powders showed red emission under UV irradiation at 254 nm. The brightness of red color was significantly enhanced with increasing the annealing temperature up to 800 °C. The photoluminescence spectra of SiO$_2$-A, measured at the excitation of 275 nm, showed typical emission bands centered at around 590 and 615 nm, which were assigned to $^5$D$_0$–$^7$F$_1$ and $^5$D$_0$–$^7$F$_2$ transitions of Eu$^{3+}$ ion, respectively (Fig. 2b). The emission intensity increased from SiO$_2$-A-400 to SiO$_2$-A-800, which is in agreement with the larger amount of conversion from LGdH : Eu to GdPO$_4$ : Eu at higher temperatures. The larger enhancement of the band at 615 nm, compared with that at 590 nm, with an increase of the annealing temperature indicates the generation of a more disordered environment around Eu$^{3+}$.11

The fluorescence and magnetic relaxation properties, which are required for biomedical imaging, were evaluated using an aqueous suspension of the SiO$_2$-A-700 spheres. In order to provide spheres with water- and bio-compatibility, the SiO$_2$-A-700 spheres were treated with 2-(methoxy(polyethyleneoxy))-propyl)trimethoxysilane (MPEOPS), which reacts to coat the silica shell of nontoxic, nonimmunogenic, nonanigenic, and protein resistant PEG chains. The PEG-modified SiO$_2$-A-700 (SiO$_2$-PEG) spheres dispersed readily in water to form a colloidal suspension. TEM showed no discernable changes in the GdPO$_4$ : Eu nanoparticles and their assembled superstructure during the PEG modification reaction (Fig. 1). Illumination of the SiO$_2$-PEG suspensions with UV excitation light produced a red fluorescence, as shown in Fig. 2c, demonstrating its utility as an optical probe. The emission spectra of SiO$_2$-PEG suspension showed similar fluorescence bands with that of the unmodified SiO$_2$-A-800 powder and the fluorescence intensity was nearly proportional to the nanosphere concentration (Fig. 2d). The magnetic relaxation properties of the SiO$_2$-PEG were examined with its aqueous suspension using a 3.0 T human clinical scanner. The SiO$_2$-PEG was found to decrease both the longitudinal relaxation time ($T_1$) and transverse relaxation time ($T_2$). Accordingly, bright and dark signal enhancement was observed in the $T_1$- and $T_2$-weighted images,
respectively (Fig. 3a). The specific relaxivity of the SiO$_2$-PEG, which was determined by measuring the relaxation rates as a function of the Gd concentration, was 1.6 (s mM)$^{-1}$ and 30.6 (s mM)$^{-1}$ for $r_1$ and $r_2$, respectively (Fig. 3b). The satisfactory relaxivities of SiO$_2$-PEG suggested that it can be used as a contrast agent for MRI.

In order to evaluate their effectiveness as a dual contrast agent for MR and fluorescent imaging, the SiO$_2$-PEG spheres were incubated with MDA-MB-435 s cells. Confocal laser scanning microscopy (CLSM) with excitation at 544 nm displayed the red-fluorescence images from the SiO$_2$-PEG spheres that were internalized intracellularly and localized in the cytoplasm region indicating their utility in cellular labeling for fluorescent imaging (Fig. 3c). The red fluorescence with the cytoplasm region indicating their utility in cellular labeling for both fluorescence and MR imaging.

Transformation, a method was developed to fabricate an aqueous colloid of a silica sphere with fluorescence and magnetic relaxation properties, which are suitable for biomedical imaging. We also demonstrated that the colloids prepared using the newly developed method have the potential utility as a multimodal contrast agent in labeling cells for fluorescence and MR imaging.

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Notes and references