Surface Coated Eu(OH)₃ Nanorods: A Facile Synthesis, Characterization, MR Relaxivities and In Vitro Cytotoxicity

Krishna Katte
Ja Young Park
Wenlong Xu
Badrul Alam Bony
Woo Cheol Heo, et al.

Available at: https://works.bepress.com/wasiahmad/9/
Surface Coated Eu(OH)₃ Nanorods: A Facile Synthesis, Characterization, MR Relaxivities and In Vitro Cytotoxicity

Krishna Kattel¹, Ja Young Park¹, Wenlong Xu¹, Badrul Alam Bony¹, Woo Cheol Heo¹, Tirusew Tegafaw¹, Cho Rong Kim¹, Md. Wasi Ahmad¹, Seonguk Jin², Jong Su Baeck², Yongmin Chang², *, Tae Jeong Kim³, Ji Eun Bae⁴, Kwon Seok Chae⁵, Ji Yun Jeong⁶, and Gang Ho Lee¹, *

¹Department of Chemistry, College of Natural Sciences, Kyungpook National University, Taegu 702-701, South Korea
²Department of Molecular Medicine and Medical and Biological Engineering, School of Medicine, Kyungpook National University, Taegu 702-701, South Korea
³Department of Applied Chemistry, College of Engineering, Kyungpook National University, Taegu 702-701, South Korea
⁴Department of Nanoscience and Nanotechnology, Kyungpook National University, Taegu 702-701, South Korea
⁵Department of Biology Education, Teacher's College, Kyungpook National University, Taegu 702-701, South Korea
⁶Department of Endocrinology and Metabolism, School of Medicine, Kyungpook National University, Taegu 702-701, South Korea

The water-soluble and biocompatible D-glucuronic acid coated Eu(OH)₃ nanorods (average thickness x average length = 9.0 x 118.3 nm) have been prepared in one-pot synthesis. The D-glucuronic acid coated Eu(OH)₃ nanorods showed a strong fluorescence at ∼600 nm with a narrow emission band width. A cytotoxicity test by using DU145 cells showed that D-glucuronic acid coated Eu(OH)₃ nanorods are not toxic up to 100 μM, making them a promising candidate for biomedical applications such as fluorescent imaging. The minimum Eu concentration needed for a conventional confocal imaging was estimated to be ∼0.1 mM. Therefore, D-glucuronic acid coated Eu(OH)₃ nanorods can be applied to fluorescent imaging. However, a very tiny magnetization of ∼1.2 emu/g at room temperature and at an applied field of 5 tesla was observed. As a result, very small r₁ and r₂ water proton relaxivities were estimated, implying that surface coated Eu(OH)₃ nanorods are not sufficient for MRI contrast agents.

Keywords: Eu(OH)₃ Nanorod, Fluorescent Properties, Cytotoxicity, Water Proton Relaxivities.

1. INTRODUCTION

One dimensional nanostructures such as nanorods, nanowires and nanotubes have attracted considerable academic and industrial interest due to their unique optical, electronic and magnetic properties.¹⁻³ Lanthanide nanomaterials have been widely applied to various biomedical areas such as magnetic resonance imaging (MRI)⁴⁻⁹ and fluorescent imaging (FI).¹⁰⁻¹⁵ In addition to lanthanide nanomaterials, potential applications of lanthanide-doped and lanthanide based nanomaterials have also been actively explored.¹⁶⁻¹⁸ This is due to their optical and magnetic properties arising from 4f-electrons.¹⁹ In this work, we investigated the possibility of application of Eu(OH)₃ nanorod to MRI and FI. So far studies of europium nanomaterials on FI are somewhat rich because of their strong emission at ∼600 nm (red color),¹⁰⁻¹⁴ whereas those on MRI are very rare probably because of a nearly zero magnetization of Eu(III) ion at room temperature.

Various large europium oxide and hydroxide nanoparticles (d > 9 nm) and hydroxide nanorods (20 x 50 nm) had been investigated as FI agents.¹⁰⁻¹⁴ They displayed an intense red fluorescence due to transitions of Eu(III) ion from ⁵D₀ to ⁷F_j (j = 0–6), among which the j = 2...
transition is most intense. The radiation lifetime of Eu(III) ion is so large (∼9.7 ms) that the fluorescence microscopy is also possible. Furthermore, they are nearly non-toxic as proved in the previous and present works.

Various organic molecules with carboxylic groups had been used as coating molecules for metal oxide nanoparticles. The D-glucuronic acid is used in this work. Due to the presence of highly polar –OH and –COOH groups, they are highly soluble in water and thus, both nanorod synthesis and in-situ ligand coating of nanorods can be done in one-pot. The D-glucuronic acid is also biocompatible and non-toxic. The carboxylic group chemically binds to surface Eu(III) ions of a nanorod.

In the present work, we synthesized D-glucuronic acid coated Eu(OH)₃ nanorods and characterized their physical, optical, magnetic properties, cytotoxicities, water proton relaxivities, and fluorescent confocal imaging properties by using various methods. From these, we find that D-glucuronic acid coated Eu(OH)₃ nanorods are suitable for an in vitro fluorescent imaging with a strong fluorescence in the red region and a low cytotoxicity. In vivo applications will not be easy because of large rod sizes. However, they are not suitable for MRI contrast agents because of small water proton relaxivities arising from tiny magnetizations at room temperature.

2. EXPERIMENTAL DETAILS

Eu(NO₃)₃·5H₂O (99.9%), NaOH (> 99.9%), ethanol (> 99.5%), and D-glucuronic acid (99.99%) were purchased from Sigma-Aldrich and used as received. Triply distilled water was used throughout the experiment for preparing both washing and preparing a sample solution. 5 mmol of Eu(NO₃)₃·5H₂O was dissolved into 40 mL of triply distilled water at 60 °C with magnetic stirring for ~10 minutes. Then, 15 mmol of NaOH pellets was added to the reaction mixture, which was magnetically stirred at 80 °C for 24 hours. For surface coating, 5 mmol of D-glucuronic acid was added to the reaction mixture and the reaction continued at 80 °C for additional 24 hours. After this, the solution cooled to room temperature and then, transferred into a beaker filled with 400 mL of triply distilled water. It was magnetically stirred for 30 minutes and then, stored for a couple of days until the D-glucuronic acid coated Eu(OH)₃ nanorods were completely settled down to the bottom of a beaker. The top transparent solution was decanted and the remaining sample was washed with triply distilled water. This process was repeated three times to remove impurities from sample such as solvent, ligand, and precursor. Finally, the sample was divided into two parts for various characterizations: one half was dispersed in triply distilled water and remaining half was dried in air to make a powder sample.

The core rod size was measured by using a high resolution transmission electron microscope (HRTEM) (JEOL, JEM-2100F, 200 kV). A drop of an aqueous sample solution was dropped onto a carbon film supported on a 200 mesh copper grid by using a micropipette (Eppendorf, 2–20 μL) and allowed to dry in air at room temperature. A multi-purpose X-ray diffractometer (MP-XRD) (Philips, X’PERT PRO MRD) with an unfiltered CuKα (λ = 0.154184 nm) radiation was used to measure the crystal structure of nanorods. Inductively coupled plasma atomic emission spectrometer (ICP-AES, Thermo Jarrell Ash Co., IRIS/AP) was used to determine the Eu concentration of nanorods in an aqueous sample solution. The attachment of D-glucuronic acids to Eu(OH)₃ nanorods was characterized by using a Fourier transform infrared (FT-IR) absorption spectrometer (Mattson Instruments, Inc., Galaxy 7020A). A pellet was made by pressing a mixture of KBr and powder sample. The coating amount of ligand in surface coated nanorods was estimated by using a thermogravimetric analyzer (TA Instruments, SDT-Q600). The TGA curve was recorded between room temperature and 700 °C. From the mass drop, the coating amount was estimated. Magnetic properties were measured by using a superconducting quantum interference device (SQUID) magnetometer (MPMS-7, Quantum Design). Both magnetization (M) versus applied field (H) (i.e., M–H) curves (−5 ≤ H ≤ 5 tesla) at temperatures, T = 5 and 300 K and zero-field-cooled (ZFC) M–T curves (3 ≤ T ≤ 330 K) at H = 100 oersted (Oe) were recorded. The PL spectrometer (JASCO, FP-6500) at a high resolution mode was used to record a PL spectrum of surface coated nanorods dispersed in ethanol at the excitation wavelength (λex) of 390 nm. A quartz cuvette with four optically clear sides was filled with a sample solution. A MRI instrument (GE 1.5 T Signa Advantage, GE medical system) equipped with the knee coil (EXTREM) was used to measure both T₁ and T₂ relaxation times. The parameters used are: number of acquisition = 1, temperature = 22 °C, FOV = 16 cm, percentage phase FOV = 0.5, matrix size = 256 × 128, slice thickness = 5 mm, pixel spacing = 0.625, pixel band width = 122.10, repetition time (TR) = 2000 ms and time to echo (TE) = 9 ms. Five different concentrations of Eu were used to measure both T₁ and T₂ relaxation times. Finally, r₁ and r₂ water proton relaxivities of surface coated Eu(OH)₃ nanorods were obtained from linear curve fittings to T₁ and T₂ relaxation times versus Eu concentration. The cellular toxicity of surface coated Eu(OH)₃ nanorods was measured by using a human prostate cancer (DU145) cell line and a CellTiter-Glo Luminescent Cell Viability Assay (Promega, WI, USA). In this assay, the intracellular ATP was quantified by using a luminometer (Victor 3, Perkin-Elmer). For a fluorescent confocal imaging, ~2 μL of each sample solution was dropped onto a glass slip (Marienfeld, Germany) and the confocal fluorescence microscopy was carried out by using a laser scanning confocal microscope (Carl Zeiss LSM 700, Germany) at λex of 488 nm and a laser power of 8 W.
3. RESULTS AND DISCUSSION

Figure 1 shows an XRD pattern of a powder sample of D-glucuronic acid coated Eu(OH)₃ nanorods. The XRD pattern indicates a single phase attributed to the Eu(OH)₃ with a hexagonal structure. The cell constants were estimated to be $a = 6.35$ and $c = 3.62$ Å, which are consistent with the reported values.²³

Figures 2(a)–(c) show HRTEM images of D-glucuronic acid coated Eu(OH)₃ nanorods at different magnifications which exhibit clear lattice fringes parallel to the rod axis. The lattice spacing was estimated to be 0.30 nm which is consistent with an interplanar spacing of (101) of hexagonal Eu(OH)₃.²³ The D-glucuronic acid coated Eu(OH)₃ nanorods have a diameter of 8–10 nm and a length of 115–128 nm. A log-normal function fit to the observed nanorod distribution showed an average rod length of 118.3 nm (Fig. 2(d)). The high density of nanorods in HRTEM images implies that the nanorods can be prepared on a large scale by means of this facile one-pot synthesis.

The FT-IR absorption spectroscopy was employed to prove the surface coating of Eu(OH)₃ nanorods by D-glucuronic acid. The FT-IR absorption spectrum of D-glucuronic acid coated Eu(OH)₃ nanorods as well as that of a free D-glucuronic acid are shown in Figure 3(a). An intense and sharp band at $\sim 3607$ cm$^{-1}$ is assigned as due to the Eu-O-H stretch of Eu(OH)₃.¹³ The stretches of functional groups of a D-glucuronic acid such as C=O at $\sim 1618$ cm$^{-1}$, C–H at $\sim 2920$ cm$^{-1}$, and C–O at $\sim 1051$ cm$^{-1}$ confirmed the surface coating by D-glucuronic acid. The peak at 3410 cm$^{-1}$ is assigned as due to water stretch. The peak at 1384 cm$^{-1}$ is likely

Fig. 1. An XRD pattern of a powder sample of D-glucuronic acid coated Eu(OH)₃ nanorods. The assignments are the Miller indices (hkl).

Fig. 2. HRTEM images of (a)–(c) D-glucuronic acid coated Eu(OH)₃ nanorods at different magnifications, and (d) a log-normal function fit to the observed lengths of D-glucuronic acid coated Eu(OH)₃ nanorods.
due to stretch of CO$_2^-$, which was formed from reaction between absorbed water and CO$_2$ in air. The surface coating amount by D-glucuronic acids in a D-glucuronic acid coated Eu(OH)$_3$ nanorod was estimated by recording a TGA curve as shown in Figure 3(b). The weight loss is due to both oxidation of surface coated D-glucuronic acids and dehydration of Eu(OH)$_3$ (i.e., $2\text{Eu(OH)}_3 \rightarrow \text{Eu}_2\text{O}_3 + 3\text{H}_2\text{O}$). The net weight percent of surface coated D-glucuronic acids was estimated to be 16.4%, after taking into account the weight loss due to dehydration.

Both mass corrected $M$–$H$ and ZFC $M$–$T$ curves are shown in Figures 4(a) and (b), respectively. Here, the mass corrected $M$ corresponds to the net $M$ of Eu(OH)$_3$ nanorods in D-glucuronic acid coated Eu(OH)$_3$ nanorods. The $M$–$H$ curves at $T = 5$ and 300 K show that both coercivity and remanence are zero. This lack of hysteresis as well as no magnetic transition down to $T = 3$ K in the ZFC $M$–$T$ curve show that the powder sample is paramagnetic, which is consistent with previous experiments. From the $M$–$H$ curves, small $M_r$ at 5 and 300 K were estimated to be 1.8137 and 1.1963 emu/g, respectively. These small $M_r$ are due to the magnetic moment ($J$) of Eu(III) ion which is nearly zero.

Both inverse longitudinal ($1/T_1$) and transverse ($1/T_2$) relaxation times were plotted as a function of Eu concentration and the corresponding $r_1$ and $r_2$ relaxivities were obtained from the corresponding slopes (Fig. 5). It is known that only the electron spin magnetic moment can efficiently induce $r_1$ because a slow electron spin relaxation is closely in tune with a slow water proton relaxation. However, a fast electron orbital motion is quite far from the water proton relaxation. Therefore, $r_1$ will be high if $J$ of Ln(III) solely arises from electron spin but it will be small if $J$ has a contribution from an electron orbital. For Eu(III), $J = 0$ with an orbital contribution to $J$. This is the reason that Eu(OH)$_3$ sample has a low $r_1$. On the other hand, $r_2$ is proportional to the square of $M$ of a sample at room temperature. Since the observed $M$ at $T = 300$ K is quite small (i.e., $\sim 1.2$ emu/g), the observed $r_2$ is also low accordingly. These small $r_1$ and $r_2$ are similar to those of Eu$_2$O$_3$ nanoparticles. Therefore, Eu nanomaterials are not suitable for $T_1$ and $T_2$ MRI contrast agents.

Figure 6 shows a PL spectrum of D-glucuronic acid coated Eu(OH)$_3$ nanorods dispersed in ethanol at room temperature, together with that (i.e., insert) of Eu(III) ions. The emission peaks are composed of characteristic emissions of Eu(III) ion, corresponding to $^5\text{D}_{0}^0\rightarrow{^7\text{F}_J}$ ($J = 0, 1, 2, \text{and } 3$). The transitions were observed at 580, 593, and...
Fig. 5. Plots of $1/T_1$ and $1/T_2$ inverse relaxation times of aqueous sample solutions of D-glucuronic acid coated Eu(OH)$_3$ nanorods as a function of Eu concentration. The corresponding slopes correspond to $r_1$ and $r_2$ water proton relaxivities, respectively.

618 nm for $J = 0, 1, 2,$ and 3, respectively. These transitions are red-shifted by $\sim 4$ nm from those of a free Eu(III) ion, respectively. This is likely related to the quantum size effect. Figure 7 illustrates the confocal images of D-glucuronic acid coated Eu(OH)$_3$ nanorods dispersed in ethanol for sample solutions with concentration of 1, 0.5, 0.25, and 0.125 mM Eu. They show a red fluorescence arising from Eu(OH)$_3$ nanorods. Here, these fluorescent confocal images as a function of Eu concentration suggest that the minimum Eu concentration needed for a conventional confocal fluorescent imaging is $\sim 0.125$ mM. A repeated experiment showed a consistent result.

Finally, Figure 8 shows the in vitro cytotoxicity result of D-glucuronic acid coated Eu(OH)$_3$ nanorods at Eu concentration ranging from 0 to 100 $\mu$M by using a DU145 cell line. The D-glucuronic acid coated Eu(OH)$_3$ aqueous sample solution was found to be nontoxic for the tested concentration range up to 100 $\mu$M, which is consistent with the previously reported cytotoxicity of europium nanomaterials. This low toxicity suggests that europium nanomaterials can be safely used for biomedical applications.

4. CONCLUSIONS

We accomplished a facile one-pot synthesis of D-glucuronic acid coated Eu(OH)$_3$ nanorods and characterized their crystal structure, rod size, surface coating, magnetic properties, fluorescent properties, water proton relaxivities, cytotoxicities, and fluorescent confocal imaging properties by using XRD, HRTEM, FT-IR absorption spectrometer, TGA, MPMS, PL spectrometer, MRI instrument, and a laser confocal microscope, respectively. The sample solution in ethanol exhibited a strong red fluorescence at $\sim 600$ nm. The minimum Eu concentration needed to record conventional fluorescent confocal images is estimated to be $\sim 0.1$ mM. The aqueous sample solution
showed nearly non-toxicity up to 100 μM, implying that surface coated Eu(OH)₃ nanorods will be safely used for biomedical applications. However, magnetization of D-glucuronic acid coated Eu(OH)₃ nanorods at 300 K was very small (i.e., ~ 1.2 emu/g) and as a result, small r₁ and r₂ water proton relaxivities were observed accordingly, implying that Eu nanomaterials are not suitable for MRI contrast agents.

Acknowledgments: This work was supported by the Basic Science Research Program through the National Research Foundation (NRF) funded by the Ministry of Education, Science and Technology (Grant No’s. 2013R1A1A4A03004511 to GHL, 2011-0015353 to YC, and 2012R1A1B3004241 to KSC), and the R&D program of MK/EKIT (10040393, development and commercialization of molecular diagnostic technologies for lung cancer through clinical validation). This work is also partially supported by Kyungpook National University Research Fund (2013) and a grant from the Korea Health Technology R&D Project, Ministry of Health and Welfare, Republic of Korea (A111345). We thank the Korea Basic Science Institute for allowing us to use their XRD, DLS, and HRTEM.

References and Notes

23. JCPDS-International Centre for Diffraction Data, Card no. 07-0616.

Received: 1 May 2012. Accepted: 20 December 2012.