Applied Biological Research 19 (3): 241-247; (2017) **MINI-REVIEW**
DOI: 10.5958/0974-4517.2017.00036.2

RARE-EARTH NANOMATERIALS FOR BIO-PROBE APPLICATIONS

Saima Wani[1] and Shafquat Majeed[2]*

[1]*Department of Biochemistry, Indian Institute of Science, Bangalore - 560 012, Karnataka (India)*
[3]*Department of Nanotechnology, University of Kashmir, Srinagar - 190 006, Jammu & Kashmir (India)*
***e-**mail: smshah@uok.edu.in*

(Received 9 August, 2017; accepted 12 October, 2017)

ABSTRACT

Rare-earth compounds are of immense significance due to the presence of *f*-orbital electrons and the resultant magnetic, electronic, catalytic, chemical and optical qualities. This leads to their practical application in diverse fields, such as luminescent devices or phosphors (component in LEDs, household compact fluorescent lamps, high-resolution flat panel displays), medical diagnostics and imaging, biochemical probes, powerful magnets, sensors, and in the catalysis of some technically important chemical processes. However, much attention has recently been devoted to the synthesis of rare-earth-based compounds in nanometric dimensions that can perform multiple functions, such as fluorescence imaging, drug delivery, and so on. In this mini review, a short summary of the applications of rare-earth nanomaterials in the fluorescent labelling of cells, MRI contrast imaging, and dual imaging modalities have been discussed.

Key words: Fluorescent labelling, MRI imaging, nanomaterials, rare-earths

1. INTRODUCTION

The name 'rare-earth' has its source in the history of the discovery of these elements. The very scarcity of these rare-earth minerals (previously called "earths") led to the term "rare earth". These elements have never been found as free metals in the Earth's crust and minerals of individual rare-earth elements do not exist. Rare-earth element (REE) or rare-earth metal as per the definition of IUPAC (*International Union of Pure and Applied Chemistry)* is one of a set of seventeen chemical elements in the periodic table, specifically the 15 *lanthanoids* (or *lanthanides*) with atomic numbers 57 through 71, from *lanthanum* (La) through *lutetium* (Lu) plus *scandium* and *yttrium* (Damhus *et al.*, 2005). *Scandium* and *Yttrium* are considered rare-earth elements because they tend to occur in the same ore deposits as the *lanthanides* and also exhibit similar chemical properties. Rare-earth elements are used extensively as phosphors in fluorescent lighting, medical imaging, lasers, display phosphors etc.; in capacitors, sensors, scintillators, etc. in ceramic industry; as catalysts in petroleum refining, catalytic converters, chemical processes, etc. In addition, they are used in nuclear technology, defense applications, metallurgical alloys (NimH batteries, fuel cells, steel etc.), glass and polishing industry and numerous other applications. In short, at present it is difficult to imagine technology without rare-earth elements. Apart from the above applications, the availability of these rare-earth-based/doped materials in nano form enables their use in specific applications in the biomedical field because, in such applications, their bulk counterparts cannot be used due to the limitations stemming from the particle size. From the nanotechnology perspective, some of the applications of rare-earth compounds in the biomedical field are discussed.

2. NANOPHOSPHORS IN CELL LABELLING OR IMAGING

Considerable attention has recently been paid to the development of rare-earth-based luminescent nanomaterials for fluorescent cell labelling or bioimaging (Louis *et al.*, 2005; Resch-Genger and Grabolle, 2008; Eliseeva and Bünzli, 2010). Rare-earth-based fluorescent nanomaterials are presently being investigated as emerging new materials, and proposed as a new generation of biological luminescent labels and as suitable alternatives (Shen *et al.*, 2008; Gai *et al.*, 2013; Chen *et al.*, 2014). They have several advantages over other conventional luminescent labels like organic dyes and quantum dots (QDs) {Goldys *et al.*, 2006} [Table 1]. However, the exploration of biological application of rare-earth-based nanomaterials is still in its early stages, and further research and understanding are needed. Although a number of synthetic strategies on rare-earth nanomaterials have been described, it still remains a challenge to tune their size and morphology, which have a pronounced effect on their properties. Some of the soft chemical routes for the synthesis of rare-earth nanomaterials are shown in Fig. 1. These routes are based on solution-phase chemistry, wherein the reactant molecules (salts, additive molecules etc.) are homogeneously mixed at the molecular or ion level in the solution

Table 1: Advantages and disadvantages of rare-earth based/doped nanomaterials over quantum dots and organic dyes used in bioprobe applications

Advantages	Disadvantages
▪ In contrast to QDs, the emission wavelength of *lanthanide* ions is not sensitive to particle size and thus rigorous control over synthesis is obviated. ▪ Much longer fluorescence lifetime (upto several ms) than QDs and organic species, which makes it possible to substantially diminish the spontaneous background fluorescence of the biological specimen via time-gated detection. ▪ In contrast to organic dyes and QDs, multicolour luminescence is easily realized by varying the Ln dopant and host matrix. ▪ A large Stokes shift (a few hundred nanometres) makes it possible to easily remove any interference from the excitation wavelength. ▪ High chemical and photochemical stability than organic dyes and quantum dots. ▪ Flexibility of excitation wave-lengths in contrast to organic dyes. ▪ Low toxicity and reduced photobleaching than organic dyes and quantum dots.	▪ While organic dyes and QDs are easily available, systematic design of synthetic strategies needed for controlled synthesis and surface modification of rare-earth nanomaterials. ▪ Organic dyes and QDs have been studied extensively, where as, for rare-earth nanomaterials toxicological studies are in their early stage and long term clearance and toxicity studies in larger animal models are still necessary. ▪ Appropriate surface modifications needed to make them biocompatible, however, this also leads to reduced quantum yield.

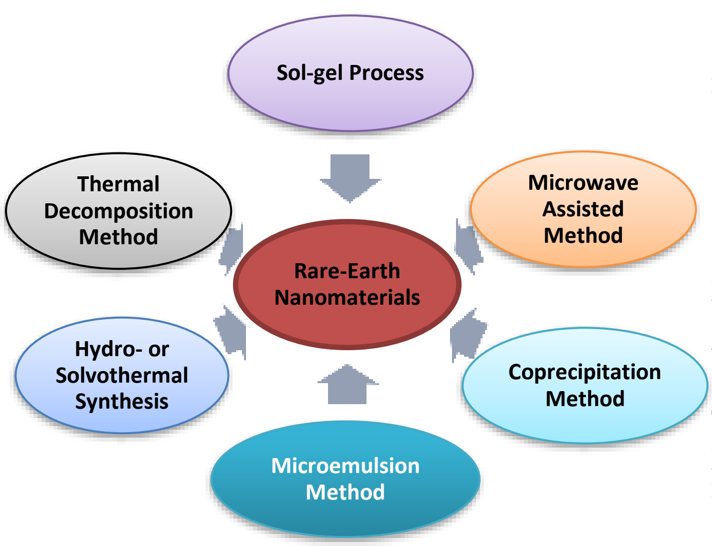

Fig. 1: Soft chemical routes for the synthesis of rare-earth-based nanomaterials

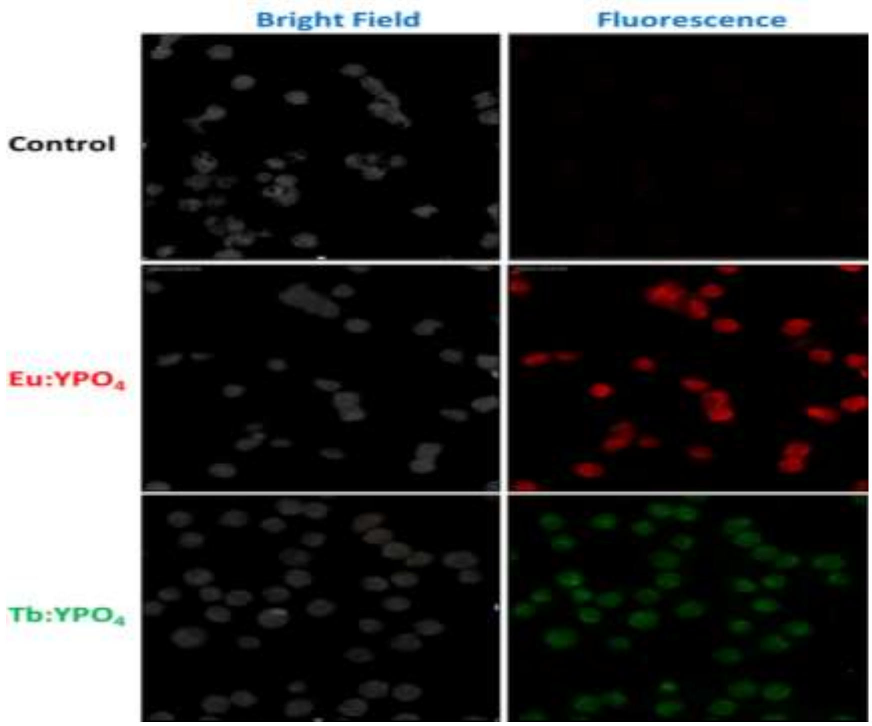

Fig. 2: Fluorescence and bright-field optical micro-scopy images of HeLa cells alone (control) and HeLa cells incubated for 10 h with 400 µg mL^{-1} of Eu- and Tb-doped YPO_4 nano-bundles. [Reproduced from Majeed *et al.* (2015) with permission from Springer]

medium. This process allows one to tune the products precisely in terms of size, shape and composition at the nanometer scale. As an example, Ln^{3+} (Eu and Tb)-doped crystalline nano-bundles of YPO_4 were prepared by Majeed *et al.* (2015) through a facile microwave-assisted route with water as solvent, and without using any surfactant. HeLa cells labelled with Eu- and Tb-doped YPO_4 nano-bundles show bright red (Eu) and green (Tb) intracellular luminescence under a confocal microscope (Fig. 2). Furthermore, the photo-stability of doped YPO_4 nano-powders was further investigated by time-sequenced measurement of photoluminescence emission intensity. The doped YPO_4 samples were continuously irradiated with a laser beam (325 nm wavelength, He-Cd laser) for 1 h and the emission intensity was measured at various time intervals. It has been shown that there is no appreciable reduction in PL emission even after 1 h of continuous laser irradiation. This experiment further demonstrates the high photo-stability of rare-earth-based bio-labels and shows their great promise for bio-labelling applications.

3. MRI CONTRAST AGENTS

During recent decades, magnetic resonance imaging (MRI) has become a well-established and very important technique for clinical diagnosis. The use of contrast agents to obtain improved imaging data has played a key role in increasing the utility of MRI. Emerging investigations of rare-earth oxide nano-particles synthesized through different routes have been explored as potential MRI contrast agents. Contrast in MRI is governed by longitudinal (T_1) and transverse (T_2) relaxation times of water protons. Relaxivity (r_i) is the proportionality constant between 1/Ti (i = 1, 2) and the concentration of the contrast agent. Enhancement of the contrast in MR images is dependent on the relaxivity of the contrast agents. Negative contrast agents like superparamagnetic iron oxides SPIOs (e.g., Resovist) have high transverse relaxivity (r_2); whereas paramagnetic Gd-chelates (e.g., Gadavist, a Gd-DTPA chelate) are considered positive contrast agents possessing high longitudinal relaxivity (r_1) (Caravan *et al.*, 1999; Na *et al.*, 2009; Ahrén *et al.*, 2012).

Gadolinium-based rare-earth nanomaterials, in addition to their potential in cell labeling, have also recently been studied as potential MRI contrast agents (Reynolds *et al.*, 2000; Engström *et al.*, 2006; Huang *et al.*, 2008). The main advantage of a nano-particle-based contrast agent over a chelate complex is that the former is expected to yield considerably higher relaxivity values due to a higher density of magnetic ions (Fortin *et al.*, 2007; Park *et al.*, 2009; Johnson *et al.*, 2011). Thus, each nanoparticle contains a higher payload of metal ions, which ensures high local contrast (Sakai *et al.*, 2012; Nuñez *et al.*, 2013; Li *et al.*, 2014). Furthermore, the dissociation and the consequent leakage of Gd^{3+} ions are minimized because they are positioned in a rigid inorganic crystal structure that holds them together. Small nanoparticles of Gd_2O_3 having size between 20-40 nm were investigated by McDonald and Watkin (2006) who showed that the relaxivity of these nanoparticles is comparable to that of Gd-DTPA chelates, whereas Engström *et al.* (2006) reported that ultra-small particles of Gd_2O_3 (USPGO) of size 3-10 nm have double the relaxivity of the Gd-DTPA complex. Ultra-small nanocrystals of undoped and Eu-doped gadolinium oxide (Gd_2O_3) were synthesized by Majeed and Shivashankar (2014) through a simple, rapid, microwave-assisted route, using benzyl alcohol as the reaction solvent. The nanoparticles were paramagnetic and showed higher relaxation rate than the standard Gadavist or Gd-DTPA (Gadopentetic acid or Gd-diethylenetriamine-pentaacetic acid) used in MRI imaging, as shown in Fig. 3a. Detailed analysis also shows that nanocrystalline Gd_2O_3 is biologically compatible with very low toxicity towards cells, which they permeate (Fig. 3b).

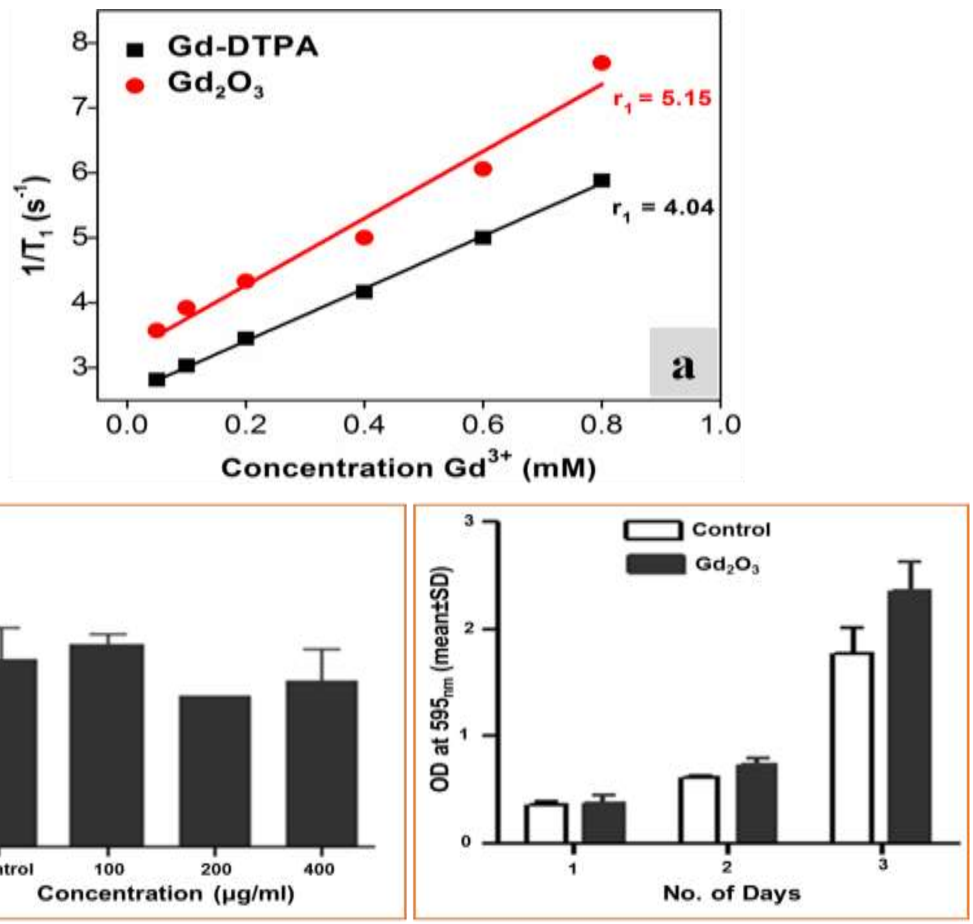

Fig. 3: a) Variation of experimentally measured longitudional relaxation rate R_1 ($1/T_1$) with the concentration of Gd^{3+} ions in Gd_2O_3 nanocrystals and Gd-DTPA. A larger reduction in the relaxation time of water protons for Gd_2O_3 nanocrystals is observed than for the standard contrast agent Gd-DTPA. b) Effect of Gd_2O_3 nanocrystals on viability of HEK293 cells. The viability of HEK293 cells is not affected by treatment with different concentrations of Gd_2O_3 nanocrystals or even after 3 days of treatment with Gd_2O_3 nanocrystals when compared to control cells. The results are an average of three experimental observations. [Reproduced from Majeed and Shivashankar (2014) with permission from the Royal Society of Chemistry].

4. MULTI-MODAL IMAGING PROBES

Different molecular imaging techniques like MRI, CT and optical imaging have their own advantages and disadvantages with respect to various parameters like spatial resolution, depth of penetration, etc. For instance, MRI shows high spatial resolution and penetration depth though, at the same time, it suffers from insufficient sensitivity and poor planar resolution. Similarly, optical imaging provides the highest sensitivity and excellent planar resolution; however, it suffers from poor penetration depth (Shen *et al.*, 2008; Gai *et al.*, 2013). Dual-mode or multi-modal imaging probes based on Ln-doped Gd-based nanomaterials are attractive, promising, single-phase multifunctional bio-probes that are being presently investigated (Petoral Jr *et al.*, 2009; Das *et al.*, 2010; Cheng *et al.*, 2011). The combination of magnetic resonance and optical imaging modalities in multifunctional nanostructured rare-earths is the most popular multimodal imaging probe presently being investigated (Zhou *et al.*, 2012). Optical imaging is responsible for high sensitivity and spatial resolution for *in vitro* imaging, whereas MRI provides excellent spatial resolution and depth for *in vivo* imaging. By combining the advantages of both these techniques, it is possible to obtain multiple imaging data that result in deeper understanding, diagnosis and, ultimately, the prevention and cure of various clinical conditions. Therefore, a combination of imaging probes and contrast agents constitutes an important research field in the biomedical sciences that involves the analysis of biological information and disease diagnostics.

5. GAPS, CONSTRAINTS AND FUTURE PERSPECTIVE

Currently, bio-probe applications of rare-earth nanomaterials are being developed steadily. Although a wide variety of high-quality nanomaterials with tailored architectures have been prepared through the facile adjustment of various reaction parameters, current synthetic strategies depend strongly on experimental trial-and-error approaches. Due to the lack of proper measurement techniques and supporting data, most of the synthetic mechanisms are collections of hypotheses, thus needing development of advanced techniques to properly understand the reaction mechanism. One of the primary requirements for these materials to be used in the biomedical field is the suitability of their morphology/size and surface properties. Simple synthetic strategies or generalized protocols must be devised for the preparation of monodisperse, uniform, spherical nanoparticles having size less than 50 nm, which would be perfect for bio-probe applications, since they make biomolecules more accessible and are easier to disperse.

To realize the application of rare-earth-based materials in biomedicine, hydrophilic surface modification and bio-conjugation are critical to enhance the permeability of these nanomaterials through cell membranes. Further investigations are needed to examine their cellular cytotoxicity, cell permeability (nanoparticle surface modification/dispersibility), surface charge and the optimum nanoparticle size for cellular intake and clearance from the body, before these materials can be used in biomedical field. Finally, prior to their clinical use in human system further studies involving distribution, metabolism and removal, pharmacokinetics and pharmacodynamics in larger animals need to be undertaken.

REFERENCES

Ahrén, M., Selegård, L., Söderlind, F., Linares, M., Kauczor, J., Norman, Kall, P.O. and Uvdal, K. 2012. A simple polyol-free synthesis route to Gd2O3 nanoparticles for MRI applications: An experimental and theoretical study. *Journal of Nanoparticle Research*, **14:** 1-17.

Caravan, P., Ellison, J.J., McMurry, T.J. and Lauffer, R.B. 1999. Gadolinium (III) chelates as MRI contrast agents: Structure, dynamics and applications. *Chemical Reviews*, **99:** 2293-2352.

Chen, G., Qiu, H., Prasad, P.N. and Chen, X. 2014. Upconversion nanoparticles: Design, nanochemistry, and applications in Theranostics. *Chemical Reviews,* **114:** 5161-5214.

Cheng, L., Yang, K., Li, Y., Chen, J., Wang, C., Shao, S.T., Lee, M. and Liu, Z. 2011. Facile preparation of multifunctional upconversion nanoprobes for multimodal imaging and dual-targeted photothermal therapy. *Angewandte Chemie*, **50:** 7385-7390.

Damhus, T., Hartshorn, R.M. and Hutton, A.T. 2005. *Nomenclature of inorganic chemistry: IUPAC recommendations*. Royal Society of Chemistry, Cambridge, United Kingdom.

Das, G., Heng, B., Ng, S., White, T. and Loo, J. 2010. Gadolinium oxide ultranarrow nanorods as multimodal contrast agents for optical and magnetic resonance imaging. *Langmuir*, **26:** 8959-8965.

Eliseeva, S.V. and Bünzli, J.C.G. 2010. Lanthanide luminescence for functional materials and bio-sciences. *Chemical Society Reviews,* **39:** 189-227.

Engström, M., Klasson, A., Pedersen, H., Vahlberg, C., Käll, P.O. and Uvdal, K. 2006. High proton relaxivity for gadolinium oxide nanoparticles. *Magma (New York),* **19:** 180-186.

Fortin, M.A., Petoral Jr, R.M., Söderlind, F., Klasson, A., Engström, M., Veres, T. and Uvdal, K. 2007. Polyethylene glycol-covered ultra-small Gd_2O_3 nanoparticles for positive contrast at 1.5T magnetic resonance clinical scanning. *Nanotechnology,* **18:** 395501-1-395501-9.

Gai, S., Li, C., Yang, P. and Lin, J. 2013. Recent progress in rare earth micro/nanocrystals: Soft chemical synthesis, luminescent properties and biomedical applications. *Chemical Reviews*, **114:** 2343-2389.

Goldys, E.M., Drozdowicz-Tomsia, K., Jinjun, S., Dosev, D., Kennedy, I.M., Yatsunenko, S. and Godlewski, M. 2006. Optical characterization of Eu-doped and undoped Gd_2O_3 nanoparticles synthesized by the hydrogen flame pyrolysis method. *Journal of the American Chemical Society,* **128:** 14498-14505.

Huang, C., Liu, T., Su, C., Lo, Y., Chen, J. and Yeh, C. 2008. Superparamagnetic hollow and paramagnetic porous Gd_2O_3 particles. *Chemistry of Materials*, **20:** 3840-3848.

Johnson, N.J.J., Oakden, W., Stanisz, G.J., Scott Prosser, R. and van Veggel, F.C.J.M. 2011. Size-tunable, ultrasmall $NaGdF_4$ nanoparticles: Insights into their T_1 MRI contrast enhancement. *Chemistry of Materials*, **23:** 3714-3722.

Li, Y., Chen, T., Tan, W. and Talham, D.R. 2014. Size-dependent MRI relaxivity and dual imaging with $Eu_{0.2}Gd_{0.8}PO_4 \cdot H_2O$ nanoparticles. *Langmuir,* **30:** 5873-5879.

Louis, C., Bazzi, R., Marquette, C.A., Bridot, J.L., Roux, S., Ledoux, G. and Tillement, O. 2005. Nanosized hybrid particles with double luminescence for biological labeling. *Chemistry of Materials*, **17:** 1673-1682.

Majeed, S., Bashir, M. and Shivashankar, S.A. 2015. Dispersible crystalline nanobundles of YPO_4 and Ln (Eu, Tb)-doped YPO_4: Rapid synthesis, optical properties and bio-probe applications. *Journal of Nanoparticle Research,* **17:** 1-15.

Majeed, S. and Shivashankar, S.A. 2014. Rapid, microwave-assisted synthesis of Gd_2O_3 and Eu:Gd_2O_3 nanocrystals: Characterization, magnetic, optical and biological studies. *Journal of Materials Chemistry B*, **2:** 5585-5593.

McDonald, M.A. and Watkin, K.L. 2006. Investigations into the physicochemical properties of dextran small particulate gadolinium oxide nanoparticles. *Academic Radiology*, **13:** 421-427.

Na, H. Bin, Song, I.C. and Hyeon, T. 2009. Inorganic nanoparticles for MRI contrast agents. *Advanced Materials*, **21:** 2133-2148.

Nuñez, N.O., Rivera, S., Alcantara, D., Jesus, M., García-Sevillano, J. and Ocaña, M. 2013. Surface modified Eu: $GdVO_4$ nanocrystals for optical and MRI imaging. *Dalton Transactions*, **42:** 10725-10734.

Park, J.Y., Baek, M.J., Choi, E.S., Woo, S., Kim, J.H., Kim, T.J. and Lee, G.H. 2009. Paramagnetic ultrasmall gadolinium oxide nanoparticles as advanced T_1 MRI contrast agent: Account for large longitudinal relaxivity, optimal particle diameter and *in vivo* T_1 MR images. *ACS Nano*, **3:** 3663-3669.

Petoral, Jr, R., Söderlind, F., Klasson, A., Suska, A., Fortin, M.A. and Abrikossova, N. 2009. Synthesis and characterization of Tb^{3+}-doped Gd_2O_3 nanocrystals: A bifunctional material with combined fluorescent labeling and MRI contrast agent properties. *The Journal of Physical Chemistry C*, **113:** 6913-6920.

Resch-Genger, U. and Grabolle, M. 2008. Quantum dots versus organic dyes as fluorescent labels. *Nature Methods*, **5:** 763-775.

Reynolds, C.H., Annan, N., Beshah, K., Huber, J.H., Shaber, S.H., Lenkinski, R.E. and Wortman, J.A. 2000. Gadolinium-loaded nanoparticles: new contrast agents for magnetic resonance imaging. *Journal of the American Chemical Society*, **122:** 8940-8945.

Sakai, N., Zhu, L., Kurokawa, A., Takeuchi, H., Yano, S., Yanoh, T. and Ichiyanagi, Y. 2012. Synthesis of Gd_2O_3 nanoparticles for MRI contrast agents. *Journal of Physics: Conference Series*, **352:** 12008.

Shen, J., Sun, L. and Yan, C.H. 2008. Luminescent rare earth nanomaterials for bioprobe applications. *Dalton Transactions*, **9226:** 5687-5697.

Zhou, L., Gu, Z., Liu, X., Yin, W., Tian, G., Yan, L. and Zhao, Y. 2012. Size-tunable synthesis of lanthanide-doped Gd_2O_3 nanoparticles and their applications for optical and magnetic resonance imaging. *Journal of Materials Chemistry*, **22:** 966-974.