Retraction

Retraction: A Review on Development of Rare Earth Based Contrast Agents for Dual Modal Imaging of Cancer Cells (J. Phys.: Conf. Ser. 1916 012219)

Published 23 February 2022

This article (and all articles in the proceedings volume relating to the same conference) has been retracted by IOP Publishing following an extensive investigation in line with the COPE guidelines. This investigation has uncovered evidence of systematic manipulation of the publication process and considerable citation manipulation.

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IOP Publishing regrets that our usual quality checks did not identify these issues before publication, and have since put additional measures in place to try to prevent these issues from reoccurring. IOP Publishing wishes to credit anonymous whistleblowers and the Problematic Paper Screener [1] for bringing some of the above issues to our attention, prompting us to investigate further.

[1] Cabanac G, Labbé C and Magazinov A 2021 arXiv:2107.06751v1
Retraction published: 23 February 2022
A Review on Development of Rare Earth Based Contrast Agents for Dual Modal Imaging of Cancer Cells

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Abstract. The risk of developing cancer is becoming higher due to the genetic and environmental factors. Diagnosing cancer at an early stage is a very big challenge to clinicians and researchers. Several imaging modalities are being used in hospitals for diagnostic purposes. But each imaging modality has some limitations to identify the cancer cells at their early stage. Magnetic resonance imaging can be combined with optical imaging for better diagnosis of cancer. This concept of combining two imaging modalities is termed as ‘dual modal imaging’. In dual modal imaging, the limitation of one technique becomes the advantage of other. This review article focuses on the dual modal imaging which is achieved by using rare earth doped gadolinium oxide nanoparticles. By doping the rare earth ions into the gadolinium oxide matrix, both the optical and magnetic properties of the material are shared.

1. Introduction
Cancer incidence is a big threat to the people all over the world. The mortality rate due to cancer is alarming. Cancer can occur due to the uncontrolled and sudden multiplication of cells, which grow beyond their boundaries, invade other organs and metastasize. According to World Health Organization (WHO) report, it is the leading cause for death worldwide. Nearly 8.2 million people die every year due to cancer. Lung, breast, stomach, prostate and liver cancer are commonly occurring types of cancer. Therapy process for cancer includes surgery, chemotherapy, radiation therapy and palliative care to some extent [1 - 2]. Early diagnosis of cancer is essential for providing proper therapy. More number of deaths occurs due to the inefficiency in diagnosing cancer at the proper time [3].

Diagnostic techniques play an essential role in identifying the cancer cells and help the clinicians for providing therapy. Cancer diagnosis is possible with the imaging techniques such as computed tomography (CT), magnetic resonance imaging (MRI), single photon emission computed tomography (SPECT), positron emission tomography (PET), ultrasound and optical imaging [4]. Nanotechnology provide a solution to the clinicians and biomedical researchers to identify/imagine the cancer cells at an initial stage. Cancer cells have diameters in the range of a few microns. These imaging techniques can only detect the cancer cells if they are few centimetres in size [5]. Nanoparticles in the range of 1 to 100 nm are employed to detect the cancer cells as they can penetrate through the cells easily. Therefore, imaging modalities can help in the detection of cancer cells at an early stage with the help of nanoparticles [6]. Nanoparticles are used as imaging agents which alter the tissue property and helps in the visualization of abnormal structures. Iron oxide, gadolinium oxide and manganese oxide nanoparticles possess better contrasting ability [7]. This article focuses on the rare earth doped
gadolinium oxide nanoparticles and their applications in MRI/optical dual modal imaging of cancer cells.

1.1. Dual modal imaging
In the dual modal imaging, any two imaging techniques are combined so that the advantages of both the techniques are shared. Some examples for dual modal imaging are MRI/optical imaging, MRI/PET imaging, CT/SPECT imaging, MRI/CT imaging and optical/ultrasound imaging [8]. While combining the two techniques, the limitation of one technique is overcome by the other. For example, MRI has the problem of low sensitivity and it can be combined with optical imaging modality which has higher sensitivity than MRI [9]. Figure 1 depicts the advantages and limitations of MRI/optical imaging techniques.

1.2. Magnetic resonance imaging
MRI is a non-invasive imaging modality which helps to know the anatomical, physiological and metabolic in vivo tissue information. MRI helps to obtain three dimensional images of soft tissues with higher spatial resolution and higher depth of penetration. The resolution of clinical MRI is around 1 mm. But for research purposes, the resolution is about 1 μm at higher field strength. The limitation of this technique is its sensitivity. The sensitivity can be improved with the help of contrast agents [10, 11].

The principle of MRI relies on the interaction of spinning motion of nucleus and the external magnetic field. Figure 2 depicts the schematic diagram for explaining the principle of MRI. Protons which are present in the water molecules have an intrinsic spin associated with them. They are randomly arranged in the absence of an external magnetic field. When these protons are subjected to a magnetic field, they are aligned either parallel or anti-parallel to the direction of the magnetic field.

Figure 1. Schematic diagram showing the advantages and limitations of MRI/optical dual imaging modality

Figure 2. Principle of MRI a) Protons are randomly aligned in the absence of the magnetic field b) Under the influence of external magnetic field (B₀), the protons are aligned in the direction of the field c) When RF pulse is applied, the protons flips to the transverse plane due to the absorption of energy d) Upon removal of RF waves, the protons releases energy and comes back to the original position.
Mostly the protons are aligned with the direction of the magnetic field since they do not have enough energy to oppose the field. The applied magnetic field produces an additional spin called precession. The precessional frequency of the protons is given by the Larmor equation, \( \omega_0 = \gamma B_0 \) where \( \omega_0 \) is the precessional frequency (MHz), \( B_0 \) is the applied magnetic field strength (Tesla), and \( \gamma \) is the gyromagnetic ratio (MHz/Tesla).

The gyromagnetic ratio of hydrogen is 42.57 MHz. During the application of the radio frequency (RF) pulses, the parallel aligned protons gain energy and become anti-parallel. When the RF pulses are turned off, the protons lose their energy and return back to their equilibrium state (relaxation) by releasing energy. The process of relaxation can be classified into T1 and T2 relaxation processes. In T1 relaxation, there is a recovery of the net magnetization vector in the longitudinal plane. Here, the energy is transferred to the surrounding tissue and are known as spin-lattice relaxation. T2 relaxation represents the decay of net magnetization vector along the transverse plane. The energy exchange is due to the nuclei interacting with the neighbour one and they are termed as spin-spin relaxation. The released energy is useful for constructing the image in MRI [12, 13].

### 1.3. MRI contrast agents

MRI contrast agents increases the relaxation time of protons in the surrounding tissues. They differentiate various types of tissues by producing higher or lower signal intensities based on the number of protons present in them [14]. Contrast agents used for imaging purposes should (i) differentiate the normal and cancer cells, (ii) have reasonable clearing period, (iii) exhibit low toxicity (iv) have good stability, and (v) have high relaxivity [15, 16, 17]. Two types of contrast agents exist based on their relaxation times: (1) T1 contrast agents, and (2) T2 contrast agents. T1 contrast agents mainly comprise of complexes of gadolinium or manganese. They have the ability to produce bright images by shortening the T1 relaxation time. Superparamagnetic iron oxide nanoparticles are used as T2 contrasts. Dark images are acquired with the help of them by shortening the T2 relaxation time [18]. Several contrast agents are approved by the Food and Drug Administration (FDA) and are being used in clinical practice. The FDA approved contrast agents with their molecular structure and respective manufacturer are listed in Table 1. Contrast agents can be administered either by oral or intravenous route [19, 20].

| Commercial name | Structure | Classification | Manufacturer |
|-----------------|-----------|----------------|--------------|
| Magnavist       | Gd-DTPA-BMA | T1 contrast agent | Bayer Healthcare, Germany |
| Omniscan        | Gd-DTPA-BMA | T1 contrast agent | GE Healthcare, USA |
| Optimark        | Gd-DTPA-BMEA | T1 contrast agent | Coviden, Ireland |
| Dotarem         | Gd-DOTA (Gd-DTPA-BMA) | T1 contrast agent | Guerbet, France |
| Gadavist        | Gd-DTPA-BMA | T1 contrast agent | Bayer Healthcare, Germany |
| Millennium      | Gd-DTPA-BMA | T1 contrast agent | Bracco Diagnostics, Italy |
| ProHance        | Gd-DTPA-BMA | T1 contrast agent | Bracco Diagnostics, Italy |
| Vasovist        | Gd-DTPA-BMA | T1 contrast agent | Lanthemn medical imaging, USA |
| Abahance        | Gd-DTPA-BMA | T1 contrast agent | Bayer Healthcare, Germany |
| Fersavist       | Gd-DTPA-BMA | T1 contrast agent | Amersham, Great Britain |
| Telsavist       | Gd-DTPA-BMA | T1 contrast agent | Berlex, USA |

### 1.4. Optical imaging

Optical imaging allows non-invasive, invivo and real time imaging of cancer cells at low cost. The molecular and physiological functioning of cells or tissues can be found with higher sensitivity [21,
The interaction of light with the cells is the principle behind the working of optical imaging which is shown in Figure 3. When light interacts with the biological cells/tissues, the phenomenon such as absorption, scattering and emission occurs [23]. Many applications utilize these phenomena to find the hemoglobin concentration, oxygenation status of the heme molecules and cytochrome oxidation changes. The normal and abnormal cells can be found by monitoring the changes in the scattered light. Cell swelling, changes in tissue components, blood volume and flow rates can also be detected by the optical imaging technique. Generally cancer cells are hypoxic, where the cells have low oxygen concentration than the normal healthy cells. Due to this condition, the optical absorbance of oxygenated and deoxygenated hemoglobin differs. This difference is used to identify the cancer cells from the normal cells [24].

![Figure 3. Schematic diagram depicting the principle of optical imaging.](image)

1.5. **Optical contrast agents**

When the fluorescent molecules or particles are excited with incident light in the visible or near infrared (NIR) region, emission of light occurs. The emitted light, usually have low energy compared to the incident light. Quantum dots, dye doped silica nanoparticles, rare earth nanoparticles and carbon nanomaterials are used as contrasts in optical (fluorescent) imaging [25]. Rare earth oxides have superior properties compared to other materials and are used as dopants in imaging applications. When doped into a suitable host matrix, they act as effective emitters and exhibit sharp absorption and emission lines [26]. Rare earth oxides possess the following properties: (i) higher photostability, (ii) low cytotoxicity, (iii) absence of blinking, (iv) large Stokes shift and (v) long lifetime [27, 28]. Europium, dysprosium, terbium, ytterbium, holmium and erbium are a few rare earth ions which can be doped for optical imaging applications [29 - 31].

1.6. **Rare earth doped gadolinium oxide nanoparticles**

There are some limitations in the usage of iron oxide contrast agent which produces dark images. Iron oxide particles have large magnetic susceptibility and can magnetize large volume of cells. This affects the anatomical information of the untargeted cells, which have impact in the signal intensity. Moreover, the voids generated by the contrast agents and other sources such as artifacts cannot be distinguished by MRI. Since void detection depends on the resolution of the image, T1 contrast agents have higher resolution than T2 contrast agents which are efficient in detecting these voids. This is the main reason for using positive T1 contrast agents in MRI [32]. Gadolinium oxides (Gd₂O₃) have higher relaxivity (r₁) value and are capable of producing T₁ (bright) contrast images [33]. Gd₂O₃ has seven unpaired electrons in the 4f orbital with the electron spin of 7/2. They have large magnetic moment (7.94 μB) and longer electron spin relaxation time (10⁻⁸ to 10⁻⁹ s) [34]. The cubic and monoclinic gadolinium oxide possesses paramagnetic property at room temperature [35]. The commonly occurring oxidation state of gadolinium is +3. Gd³⁺ ions have ionic radii (0.99 Å) equal to that of Ca²⁺ ions. Because of similar ionic radii, the Gd³⁺ ions replace the Ca²⁺ ions easily. This is the main reason for the toxicity of gadolinium in the invivo applications [36]. The toxicity associated with the gadolinium can be rectified by surface coating with some biocompatible
materials, which is discussed in the next section. Gd$_2$O$_3$ is a good choice of host material for doping with other rare earth materials. They have large band gap (5.4 eV), good thermal stability, high refractive index (> 1.9) and low phonon energy (phonon cutoff \( \sim 600 \text{ cm}^{-1} \)). Gd$_2$O$_3$ can be easily doped with other rare earth ions because of their similar atomic radii [37, 38].

Few papers of rare earth doped Gd$_2$O$_3$ nanoparticles for MRI applications are available in literature. Nabil M Maalej et al synthesized Eu$^{3+}$ doped Gd$_2$O$_3$ nanoplatelets for MRI and fluorescent imaging [39]. Polyol method was adopted to synthesize Eu$^{3+}$ doped Gd$_2$O$_3$ nanoplatelets with different concentrations of europium (2, 5 and 10%). From the field emission scanning electron microscopy (FESEM) and transmission electron microscopy (TEM) analysis, the nanoplatelet structure was confirmed. A sharp peak at 612 nm corresponds to the radiative transitions of europium. MRI result proves the contrast enhancement when the concentration of gadolinium ions is increased from 0.05 to 0.2 mM.

Ultrasmall PEGylated Tb$^{3+}$ doped Gd$_2$O$_3$ nanocrystals capped with organic acid in the size range of \( \sim 4 \text{ nm} \) were prepared [40]. A strong green emission at 544 nm with the transition line $^5D_4 - ^7F_5$ was observed. CLSM studies indicate that the nanoparticles taken by THP-1 cells and fibroblasts shows fluorescent signals throughout the cytoplasm. MR images shows bright contrast images when the concentration is increased. Fluorescent and MRI studies suggests that the nanoparticle acts as contrast agent for both fluorescent and MR imaging.

1.7. Surface coating

Rare earth nanoparticles used in the development of MRI contrasts are highly toxic in nature. To reduce the toxicity of these nanoparticles, their surfaces are modified by coating with a layer of biocompatible materials. The surface coatings are done to prevent the aggregation of nanoparticles, improve the biocompatibility, increase the stability, reduce the toxicity and improve the water solubility. Biocompatible materials such as polyethylene glycol (PEG), lactobionic acid, D-glucuronic acid, silica and citric acid are used for surface coating. Combined MRI and fluorescent imaging by using gadolinium-europium oxide nanoparticles. Lactobionic acid was coated to increase the water solubility and biocompatibility of the nanoparticles. Invitro MRI studies reveal higher $r_1$ and $r_2$ values of 11.9 and 38.7 s$^{-1}$mM$^{-1}$ respectively were observed at 1.5 Tesla. Positive contrast enhancements were noted in the invivo MRI mouse studies at 3 Tesla. Red fluorescence was observed due to the presence of europium ions in both invitro and invivo fluorescent imaging.

2. Materials and Methods

Several methodologies have been studied extensively for the preparation of rare earth oxide nanoparticles. Polyol method, hydrothermal method, microwave-assisted synthesis, laser ablation in liquid and co-precipitation method are widely employed for the synthesis of rare earth oxide nanoparticles.

2.1. Polyol method

Spherical oxide nanoparticles of 30-200 nm can be prepared by polyol method. This method requires high boiling alcohol to control the size of the nanoparticles. The alcohol acts as a stabilizing agent, prevents aggregation and limits the particle growth. The metal precursors are heated in the alcohol at high temperatures (> 150 °C) to yield crystalline oxide particles. synthesized ultrasmall Tb$^{3+}$ doped gadolinium oxide nanoparticles by polyol method for fluorescent imaging and MRI applications. Chloride precursors and diethylene glycol were used to prepare the nanoparticles. The average size of the synthesized nanoparticles was found to be \( \sim 4 \text{ nm} \) [30]. synthesized ultrasmall mixed gadolinium-dysprosium oxide (GDO) nanoparticles by polyol method with triethylene glycol as solvent. 1 mmol of gadolinium chloride and dysprosium chloride were added to 40 ml triethylene glycol and stirred magnetically until the precursor dissolves. Then 6 mmol of NaOH was dissolved in 10 ml methanol and added drop-wise to the precursor solution. The reaction mixture was stirred for 24 h at 200 °C to
yield gadolinium-dysprosium oxide nanoparticles. prepared ultrasmall lanthanide oxide particles (lanthanide = Eu, Gd, Dy, Ho, and Er) by low temperature polyol method. The reaction was carried out at 80 °C, which is comparatively lower than the traditional method of synthesis. Hydrogen peroxide, a strong oxidizing agent is used in the reaction to convert the hydroxides formed to oxides. Finally, the lanthanide oxides were surface coated with D-glucuronic acid to improve the biocompatibility.

2.2. Hydrothermal method
In the hydrothermal method, metal oxide nanoparticles that are insoluble in atmospheric pressure and temperature can be prepared by pressurizing the precursors at high temperature. High purity and good homogeneity nanorods can be obtained by this technique. Hydrothermal method does not require any templates or catalyst to prepare the particles. The rare earth hydroxides are produced which can converted into oxides by the calcination process. Zhen Liu et al reported the preparation of Yb$^{3+}$, Er$^{3+}$ doped Gd$_2$O$_3$ nanorods by hydrothermal route for multi-modality imaging. In this method, appropriate moles of rare earth nitrates were dissolved in de-ionized water to form a homogenous solution. Aqueous sodium hydroxide solution was added to the precursor solution with vigorous stirring until the pH reaches 13. The final solution was transferred to a Teflon-lined stainless steel autoclave and was made to react at 180 °C for 24 h. The resulting white powder was washed with water and ethanol and dried in vacuum at 60 °C. The hydroxides obtained were calcined at 800 °C for 4 h to produce Yb$^{3+}$, Er$^{3+}$ doped Gd$_2$O$_3$ nanorods.

2.3. Microwave-assisted synthesis
Microwave-assisted synthesis is a simple and fast method to produce nanoparticles. During this process, an increase in the internal temperature due to the rapid transfer of energy directly to the reactant occurs. Decomposition of precursors takes place and creates a highly supersaturated solution where the nucleation and growth occurs to produce nanocrystals. Shafquat Majeed and Shivashankar S.A. prepared Eu doped Gd$_2$O$_3$ nanoparticles using microwave-assisted technique. Gadolinium trisacetylactone hydrate and benzyl alcohol were taken in a round bottom flask and was magnetically stirred. The clear solution was transferred to a microwave accelerated reaction system (2.45 GHz) with a water-cooled condenser and a fibre-optic temperature sensor. The solution was then irradiated for 10 minutes with 800 W microwave power, to yield a white precipitate. Finally, the precipitates are centrifuged, washed with water and ethanol and are dried at 75 °C for 12 h in a hot air oven.

2.4. Laser ablation in liquid
Laser ablation in liquid is a simple top-down approach to prepare nanoparticles. In this technique, laser pulse is focused onto a target immersed in liquid. This method does not require any reducing agent as in the case of wet chemical synthesis. High purity nanoparticles are produced and the size of the nanoparticles can be controlled by varying the laser parameters such as fluence, pulse duration, beam focus and repetition rate. Fei Chen and his co-workers prepared Tb$^{3+}$ doped Gd$_2$O$_3$ nanoparticles by a two-step process: solid state reaction followed by laser ablation in liquid. First step is the preparation of Tb$^{3+}$ doped Gd$_2$O$_3$ targets by solid-state reaction. Tb$^{3+}$ doped Gd$_2$O$_3$ targets were immersed in a container filled with deionized water and the targets were made to ablate with the Nd:YAG microsecond laser. Laser pulse of 1064 nm wavelength with 6 ms pulse duration, 100 Hz repetition and 70 mJ per pulse power were focused on the target for 30 min. The ablated colloids were aged for 24 h and the clear solutions were collected for use.

2.5. Co-precipitation method
Co-precipitation is a simple and cost-effective method to produce rare earth oxide nanoparticles. Moreover, it is an environmental friendly technique which aids in mass production of samples. In this method, the nitrates of rare earth are made to react with a base (ammonium hydroxide). This method does not involve the use of any catalysts, templates, organic solvents, surfactants or membranes. Eu$^{3+}$
doped Gd$_2$O$_3$ nanorods were synthesized by mixing appropriate mole percentage of europium nitrate and gadolinium nitrate in 25 ml of de-ionized water. Then the solution was stirred for few minutes and the pH was adjusted to 10 by the addition of 25 wt% of ammonium hydroxide. The mixture solution was heated to 75 °C and magnetically stirred for 18 h. The resulting precipitate was washed with water and dried in air to obtain Eu$^{3+}$ doped gadolinium hydroxide (Gd(OH)$_3$) nanorods. Eu$^{3+}$ doped Gd(OH)$_3$ was converted to Eu$^{3+}$ doped Gd$_2$O$_3$ nanorods by calcining at 750 °C for 1 h. Similar procedure was followed to prepare Er$^{3+}$ doped Gd$_2$O$_3$ nanorods and Eu$^{3+}$ doped Gd$_2$O$_3$ nanotubes respectively.

3. Characterization

3.1. Structural and morphological analysis

X-ray diffraction (XRD) analysis is done to find the crystal structure, phase, average grain size and strain defects. Gan Tian et al prepared rare earth doped Gd$_2$O$_3$ hollow spheres and performed XRD analysis using a Japan Rigaku D/max-2500 X-ray powder diffractometer with Cu Kα radiation ($\lambda = 1.54 \text{ Å}$). XRD patterns of as-prepared, pure Gd$_2$O$_3$ and Yb/Er-codoped Gd$_2$O$_3$ after calcination at 800 °C for 2 h are shown in the figure 4. In the as-prepared sample (Figure 4(a)), amorphous peaks are noted. After calcination at 800 °C for 2 h, the peaks of pure and Yb/Er-codoped Gd$_2$O$_3$ can be indexed with the cubic phase of Gd$_2$O$_3$ (JCPDS No. 12-0797) as shown in the figure 4(b) and (c). Peak shift towards the higher angle was noted in the Yb/Er-codoped Gd$_2$O$_3$ by comparing with the pure Gd$_2$O$_3$. The probable reason can be the smaller ionic radius of the doped rare earth ions.

![Figure 4](image)

Figure 4. XRD patterns of (a) as-formed core-shell precursor, (b) pure Gd$_2$O$_3$, and (c) Yb/Er-codoped Gd$_2$O$_3$. Reproduced with permission from ACS Publications © 2011.

Transmission electron microscopy (TEM) images help to study about the size and morphology of the synthesized nanoparticles. In our earlier work, we have prepared Eu$^{3+}$ doped Gd$_2$O$_3$ nanorods with the dimensions of around 600X40 nm as shown in the figure 5(a). The surfaces of the nanorods were coated with silica of around 15 nm thickness to improve the biocompatibility (Figure 5(b)). In figure 5(c), the HRTEM image of Eu$^{3+}$ doped Gd$_2$O$_3$ shows crystalline phase with the lattice spacing of 0.31nm in the (222) plane. Both the crystalline and amorphous phases are noted after coating with silica (Figure 5(d)). Selected area electron diffraction (SAED) pattern in the figure 5(e) shows ring pattern of the Eu$^{3+}$ doped Gd$_2$O$_3$ sample. After silica coating, no ring structures are formed due to the amorphous nature of silica.

![Figure 5](image)

Figure 5. TEM images of (a) uncoated nanorods (Scale bar: 200 nm), and (b) Silica-coated nanorods (Scale bar: 20 nm); HRTEM images of (c) uncoated nanorods (Scale bar: 5 nm) and (d) Silica-coated nanorods (Scale bar: 5 nm); SAED pattern of (e) uncoated nanorods and (f) Silica-coated nanorods. Reproduced with permission from © 2017.

3.2. Optical measurements
The photoluminescence studies of mesoporous Eu\textsuperscript{3+} doped Gd\textsubscript{2}O\textsubscript{3} nanorods were studied. The excitation spectrum was taken by monitoring the emission wavelength at 612 nm. A broad peak at 254 nm in the excitation spectrum corresponds to the charge transfer band of europium which is shown in the figure 6(a). Emission spectrum was obtained by exciting at a wavelength of 254 nm. The peaks in the longer wavelength region correspond to the f-f transitions of Gd\textsuperscript{3+} and Eu\textsuperscript{3+} ions. Figure 6(b) shows a sharp peak in the emission spectrum at 612 nm which lies in the red region. This is due to the hypersensitive electric-dipole-allowed transition $^5D_0—^7F_2$ of the europium ion.

**Figure 6.** (a) Excitation spectrum and (b) Emission spectrum of mesoporous Eu\textsuperscript{3+} doped Gd\textsubscript{2}O\textsubscript{3} nanorods. Reproduced with permission from John Wiley and Sons © 2015.

3.3. *In vitro* cytotoxicity

MTT assay is used to determine the cytotoxicity and cell viability of the nanoparticle. Bipin Kumar Gupta et al evaluated the biocompatibility of europium doped Gd\textsubscript{2}O\textsubscript{3} (Gd\textsubscript{2}−xEu\textsubscript{x}O\textsubscript{3}, x=0.15) by two human breast cancer cell lines T47D and MDA-MB-231 using MTT (3-(4,5-dimethyl-2-yl)-2,5-diphenyltetrazolium bromide) assay. The cells were cultured and maintained in DMEM glucose medium (Invitrogen) with the composition of 4.5 g L\textsuperscript{−1} D-glucose, 4mM L-glutamine, and 110 mg L\textsuperscript{−1} sodium pyruvate, supplemented with 10% fetal bovine serum (FBS), 100 IU mL\textsuperscript{−1} penicillin and 100μg mL\textsuperscript{−1} streptomycin in a humidified incubator at 37 °C with 5% CO\textsubscript{2}. 4×10\textsuperscript{3} cells were cultured in each well of 96 cells culture plate with 100 μL culture medium and incubated overnight. The synthesized nanoparticles were added to each well in the concentration range from 0.01–10 μg mL\textsuperscript{−1}. DMEM medium was added to the control cells also. The cells were washed with 500 μL phosphate buffer solution (PBS) after 24 hrs and 48 hrs of incubation. Then, 200 μL MTT reagent was added to each well and incubated for 4 hrs to allow the formation of formazan dye. After incubation, medium with MTT reagent was replaced with 200 μL of DMSO. After incubating the cells for 5 minutes, the optical density of solubilized formazan salts was assessed at optical absorbance of 570 nm in microplate reader. The cytotoxicity of Gd\textsubscript{1.85}Eu\textsubscript{0.15}O\textsubscript{3} nanorods were analyzed at two different time intervals (24 and 48 h). DMEM treated cells were used as positive control for cell growth and Taxol (anticancer drug) was used as positive control for cytotoxicity. From the figure 7, it is obvious that no cytotoxicity was noted up to the concentration of 5μg mL\textsuperscript{−1} of Gd\textsubscript{1.85}Eu\textsubscript{0.15}O\textsubscript{3} nanorods in both cancer cells.

**Figure 7.** Cell viability assay with human breast cancer cell lines, (a) T47D and (b) MDA-MB-231 incubated with different concentrations of Gd\textsubscript{1.85}Eu\textsubscript{0.15}O\textsubscript{3} nanorods. Reproduced with permission from Nature Publishing Group © 2016.

3.4. Magnetic resonance imaging
In vitro and in vivo magnetic resonance imaging of the rare earth doped Gd$_2$O$_3$ nanoparticle is discussed in this section. Wenlong Xu et al prepared lactobionic acid coated ultrasmall mixed gadolinium–europium oxide nanoparticles for MRI and fluorescent imaging. To measure the $T_1$ and $T_2$ relaxation time, 1.5 Tesla MRI instrument (GE 1.5 T Signa Advantage, GE Medical Systems) equipped with a knee coil (EXTREM) was used. Different concentrations of sample solution such as 1, 0.5, 0.125, 0.0625, and 0 mM were prepared for calculating the relaxation time. The following parameters were used for image acquisition: Magnetic field ($H$) = 1.5 Tesla, temperature = 22 °C, number of acquisitions (NEX) = 1, field of view (FOV) = 16 cm, phase FOV = 1, matrix size = 512 x 512, slice thickness = 5 mm, spacing gap = 0, pixel bandwidth = 61.0547, repetition time ($TR$) = 2009 ms and echo time ($TE$) = 9 ms. From the plot of ($1/T_1$) and ($1/T_2$) as a function of concentration, the relaxivity values $r_1$ and $r_2$ were found. From the figure 8(a), the $r_1$ and $r_2$ were calculated to be $11.9 \pm 0.1$ and $38.7 \pm 0.1$ s$^{-1}$mM$^{-1}$ respectively. The corresponding $R_1$ and $R_2$ map images are shown in the figure 8(b).

**Figure 8.** (a) Plots of $1/T_1$ and $1/T_2$ and (b) $R_1$ and $R_2$ map images of an aqueous solution of lactobionic acid coated ultrasmall mixed gadolinium–europium oxide nanoparticles as a function of Gd concentration. The slopes in (a) correspond to $r_1$ and $r_2$ relaxivities, respectively. Reproduced with permission from Royal Society of Chemistry © 2012.

In vivo $T_1$ and $T_2$ MR images were taken by 3 Tesla MRI instrument (SIEMENS 3.0 T MAGNETOM Trio a Tim). A five-week SD mouse of weight $\sim 111$ g was used. The mouse was anesthetized with 1.5% isoflurane in oxygen. Measurements were made before and after injecting a sample solution into a tail vein. The injection dose was 486 mL of 23mM Gd ($= 0.1$ mmol Gd kg$^{-1}$). During measurement, the mouse was maintained at $\sim 37$ °C by using a warm water blanket. The imaging parameters for $T_1$ 3D fast SPGR (spoiled GRASS) and $T_2$ TSE (turbo spin echo) images were as follows: $H = 3$ Tesla, temperature = 37 °C, NEX = 3–4, FOV = 100 mm, phase FOV = 0.5, matrix size = 320x290, slice thickness = 1–2 mm, spacing gap = 0–0.5 mm, pixel bandwidth = 15.63, TR = 11 and 3200 ms for $T_1$ and $T_2$ images, respectively, and the TE = 3.2 and 40 ms for $T_1$ and $T_2$ images, respectively. $T_1$ and $T_2$ MRIs were acquired in vivo before and after injecting an aqueous solution of lactobionic acid coated ultrasmall mixed gadolinium–europium oxide nanoparticles into a mouse tail vein, respectively. Positive contrast enhancements were clearly noted in the $T_1$ images of Figure 9(a). Figure 9(b) proves that gadolinium produced negative contrast images ($T_2$) in the axial $T_2$ images of liver. This is due to the longer clearance time taken by the nanoparticles through the liver.

**Figure 9.** (a) Coronal $T_1$ MR images of the kidney (indicated as K) and aorta (indicated as A), and (b) Axial $T_2$ MR images of the liver (indicated as L) before and after injecting an aqueous solution of lactobionic acid coated ultrasmall mixed gadolinium–europium oxide nanoparticles into a mouse tail vein.
vein (dose = 0.1 mmol Gd kg⁻¹). Reproduced with permission from Royal Society of Chemistry © 2012.

3.5. Fluorescent imaging

Emission from the rare earth ions can be studied in vivo by fluorescent imaging. Shafquat Majeed and Shivashankar S.A. revealed the fluorescence of Eu³⁺ ions in HEK293 (human embryonic kidney 293) cell line. HEK293 cells (1x10⁴) were grown on cover slips in a 6-well plate, and were allowed to adhere and gain morphology overnight. The cells were then treated with 400 mg mL⁻¹ of Eu:Gd₂O₃ nanoparticles and incubated for another 24 h. Cells were fixed using 4% paraformaldehyde for 5 min. Cells were permeabilised and blocked for nonspecific sites with 2% bovine serum albumin in PBS supplemented with 0.2% Triton-X-100. Cells were counter-stained using 1mgmL⁻¹ DAPI (pseudo color blue) for 5 min. After washing, coverslips were mounted on slides with an anti-fade solution (Sigma-Aldrich, USA) and photographed with a confocal Zeiss LSM 510META microscope. No fluorescence was observed in untreated cells (control) and in cells treated with undoped Gd₂O₃ nanocrystals. Red emission was observed in cells treated with Eu:Gd₂O₃ nanocrystals (Figure 10).

![Figure 10](image_url) Eu:Gd₂O₃ nanocrystals can permeate the HEK293 cells and fluorescence red as shown by white arrows. Blue represents DAPI stained nuclei. (a) Luminescence, (b) bright field, (c) counter stained and (d) superimposed bright field, and luminescence images of untreated (control) and Eu:Gd₂O₃ nanoparticle-treated HEK293 cells. Reproduced with permission from Royal Society of Chemistry © 2014.

4. Conclusion

Cancer cells can be identified at an early stage by the combination of two imaging modalities i.e., dual modal imaging. By developing suitable contrast agent for dual modal imaging applications, the mortality rate can also be decreased. Many researchers attempted to synthesize contrast agents for MRI/optical imaging, but the factors such as the size of the nanoparticles, biocompatibility and toxicity should be considered. Higher relaxivity value with bright emission is ideal for the development of MRI/optical contrast agents.

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