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# $Eu^{3+}$:$Y_2O_3$@CNTs —— a Rare Earth Filled Carbon Nanotubes Nanomaterial with Low Toxicity and Good Photoluminescence Property

Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

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Red-emission phosphor europium-doped yttria ($Eu^{3+}$:$Y_2O_3$) nanoparticles have been successfully filled into the nanocavity of carbon nanotubes (CNTs) via supercritical reaction and supercritical fluids followed by calcination. The existence of $Eu^{3+}$:$Y_2O_3$ nanoparticles inside in CNTs were characterized by TEM, EDS and XRD. The as-prepared nanomaterials ($Eu^{3+}$:$Y_2O_3$@CNTs) exhibited strong red emission at 610 nm, which corresponded to $^5D_0 \rightarrow {}^7F_2$ transition within $Eu^{3+}$ ions. It showed that the existence of the walls of CNTs did not quench the luminescence of $Eu^{3+}$:$Y_2O_3$. By the surface modification with Tween 80, $Eu^{3+}$:$Y_2O_3$@CNTs had good water solubility. In-vitro cytotoxicity studies showed that the as-prepared nanomaterials had low toxicity on HeLa cells at concentrations of 10-1000 μg/mL. And their use as luminescence probes for live cell imaging was demonstrated by using inverted fluorescence microscope. With the advantages of the easy dispersion in water, low toxicity, and good photoluminescence (PL) property, the as-prepared $Eu^{3+}$:$Y_2O_3$@CNTs could potentially be used as nanophosphors in bio-imaging.

## Introduction

Benefited from their unique electronic, magnetic, and chemical properties arising from the 4f electrons, [1] rare-earth compounds have drawn great research attention in recent years.[2, 3] Among the family of rare earth compounds, yttrium oxide ($Y_2O_3$) is a common used luminescent host material,[4] and $Eu^{3+}$:$Y_2O_3$ is considered to be one of the most promising red phosphors owing to its excellent luminescent performance,[5, 6] which has practical application in high quality fluorescent lamps,[7] emissive displays,[8] vivo biological imaging[9] and so on. In the application for luminescence probes, the water-solubility and biocompatibility of nanoparticles are important.

Unfortunately, despite recent advances in synthetic methods for controlling the size and shape of $Eu^{3+}$:$Y_2O_3$ nanoparticles,[5, 10-20] many $Eu^{3+}$:$Y_2O_3$ nanoparticles synthesized by solution method are not directly used as biolabels due to their hydrophobic surface ligands leading to their very low water-solubility. Additionally, the biological safety of the rare-earth nanomaterials remains controversial.[21-24] For instance, T. Andelman et al. demonstrated that the $Y_2O_3$ nanoparticles could cause cytotoxicity by increasing the amount of reactive oxygen species (ROS) .[23] Sungho LEE et al. reported that the development of actin filament and cell proliferation could be inhibited by $Y_2O_3$ nanoparticles and the cytotoxicity of $Y_2O_3$ nanoparticles relied on their size.[22] Therefore, it is necessary to develop a new and effective method to synthesize water-soluble or easily water-soluble and highly biocompatible $Eu^{3+}$:$Y_2O_3$ nanoparticles for their application as biological fluorescent probes.

Carbon nanotubes (CNTs), which have hollow structures, can load functional materials inside the walls and combine the properties of CNTs with those of their guest components so as to be used as delivery vehicles for drugs,[25-28] bioimaging[29-31] and so on. The water-solubility of CNTs has been improved by sidewall covalent functionalization or noncovalent modification.[32, 33] Moreover, in recent years, researchers have done many works on cytotoxicity of nanotubes[34-49] and nanowires[50-57] and found that water-soluble functionalized carbon nanotubes had low toxicity.[48, 49] Thus, CNTs can be used as ideal carrier for rare-earth materials.

In this article, CNTs filled with $Eu^{3+}$:$Y_2O_3$ nanoparticles were synthesized by a supercritical technique since this technique shows intriguing advantages for the synthesis of CNT-based composites, especially in filling materials into the nanocavity of CNTs.[58, 59] The property and structure of the as-synthesized nanomaterials were characterized by TEM, EDS, XRD, and fluorescence spectroscopy. In order to demonstrate the potential application of the synthesized nanomaterials in bioimaging, we have further functionalized the nanomaterials with Tween 80 so as to render them water-soluble. In vitro cytotoxicity studies of the synthesized $Eu^{3+}$:$Y_2O_3$@CNTs ware also undertaken.

## Materials and methods

**Materials**

Y(III) nitrate hexahydrate (99.9%) and Eu(III) nitrate hexahydrate (99.9%) were purchased from Aldrich Chemical Co., CCK-8 Cell Counting Kit and MTT cell proliferation and cytotoxicity assay kit were purchased from Beyotime Biological Co. Ltd.，Dulbecco's Modified Eagle's Medium (DMEM) cell culture mediumand and Fetal bovine serum (FBS) were purchased from Gibico Biologicals Inc.. All of other chemicals were analytical grade and purchased from Sinopharm Chemical Reagent Co. Ltd. and were used without further purification.

**Materials synthesis**

**Functionalization of carbon nanotubes**

CNTs used in this work were purchased from Chengdu Organic Chemicals Co. Ltd. The outer diameter was 10-20 nm, and their average length was 0.5-2 μm. CNTs were oxidized and cut by refluxing in 60% $HNO_3$ for 12 h. After this reaction, the ends of CNTs were opened and defects were formed on the side walls, resulting in carboxyl-contained CNTs.

**Synthesis of $Eu^{3+}$:$Y_2O_3$ @ CNTs Nanomaterials**

$Eu^{3+}$:$Y_2O_3$@CNTs was synthesized by a supercritical method, similar to the synthesis of $Eu^{3+}$:$Y_2O_3$ nanoparticles,[15] except that opening CNTs were added as the container of the rare-earth compounds. In a typical synthesis, 0.85 mmol of $Y(NO_3)_3 \cdot 6H_2O$ and 0.15 mmol of $Eu(NO_3)_3 \cdot 6H_2O$ were dissolved in 20 mL water and then mixed with 15mL methanol. The mixed solution was put in beaker with vigorous stirring for 10 min, and then the solution pH was adjusted to 7 by using 5 mL KOH solution. Finally, 40 mL of the above prepared mixture (volume ratio, water/methanol=25/15) was transferred into a 100 mL stainless steel high-pressure reactor and mixed with 2 mg CNTs and heated at 400 ºC for 10 min (the pressure was increased to 30 MPa). The filled samples were washed with distilled water for several times. The products were sonicated in 10 ml 65% $HNO_3$ solution several times to remove the rare-earth compounds attached on the surface of CNTs and washed with distilled water and ethanol for three times and dried in air at 60 $^0$C for 6 h. The final products were obtained through a heat treatment at 1000 ºC in Ar for 2 h. For comparison, $Eu^{3+}$:$Y_2O_3$ nanoparticles were also synthesized by a similar method.

**Noncovalent Modification of $Eu^{3+}$:$Y_2O_3$@CNTs with Tween 80**

In order to obtain better water solubility, Tween 80 was used to modify as-synthesized nanomaterials. Noncovalent modification of $Eu^{3+}$:$Y_2O_3$@CNTs was performed as follows: as-prepared $Eu^{3+}$:$Y_2O_3$@CNTs nanomaterials were sonicated in aqueous solution with 1% (v/v) Tween 80 for 0.5 h, followed by centrifugation to remove large aggregates and bundled nanotubes. The free Tween 80 was then thoroughly removed by repeatedly filtrating through 8-14kDa filters (Millipore). The Tween 80-functionalized $Eu^{3+}$:$Y_2O_3$@CNTs was finally re-suspended in phosphate-buffered saline (PBS). The as-prepared $Eu^{3+}$:$Y_2O_3$ nanoparticles and pristine CNTs were also modified with Tween 80 by the same method.

**In vitro cytotoxicity studies of as-prepared nanomaterials in water**

The in vitro cytotoxicity of the as-synthesized nanomaterials was evaluated using two methods: the Cell Counting Kit-8 (CCK-8, Dojindo) and the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide] assay. In the CCK-8 assay, HeLa cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. The cells were seeded in 96-well plates ($1\times10^4$ cells per well). After 12 h of incubation, the medium was replaced with Tween 80 functionalized $Eu^{3+}$:$Y_2O_3$@CNTs, $Eu^{3+}$:$Y_2O_3$, and pristine CNTs, respectively, with different test concentrations (10, 100, 1000 μg/mL) in culture medium. After these materials were added, the incubations were carried out at 37 ºC and in 5% $CO_2$ atmosphere for another 12 h. Then the cell medium was removed and the dishes were washed by D-Hanks buffer solution for three times. One hundred microliters of CCK-8 solution was added to each well and incubated for an additional 2 h at 37 ºC. The optical density (OD) of each well was recorded at 450nm on a Microplate Reader (Infinite M200 Pro, A52769).

In the MTT assay, 20 μL MTT was added to each well after the cells were exposed with the nanomaterials and incubated at 37 ºC for 4h. The formazan product was then dissolved in DMSO and the absorbance of each well was recorded at a 570 nm wavelength.

The cell viability (% of control) is expressed as the percentage of (ODtest - ODblank)/( ODcontrol - ODblank), where ODtest is the optical density of the cells exposed to the nanomaterials, ODcontrol is the optical density of the cells and ODblank is the optical density of the wells without HeLa cells.

**Cellular imaging**

HeLa cells ($1\times10^4$ cells) were seeded on glass coverslips that had been placed at the bottom of a culture dish. HeLa cells were incubated for 12 h at 37 ºC, and then the media was replaced by media that contained the $Eu^{3+}$:$Y_2O_3$@CNTs (200μL, 100μg/mL). After 6 h incubation, the cells were washed three times with an excess amount of PBS and directly imaged in inverted fluorescence microscope (AMG EVOS f1).

**Characterization**

Morphology of the samples was observed using a transmission electron microscope (TEM, Tecnai G2 F20 U-TWIN) operating on 200 kV. The crystal structure of the sample was determined by X-ray diffraction analysis (XRD, Bruker D8 Advance) using graphite-monochromized Cu Kα radiation (λ=1.5406A). Energy dispersive X-ray spectroscopy (EDS) spectra were collected on Tecnai G2 F20 with an accelerating voltage of 200 kV. The contents of Eu and Y were determined by inductively coupled plasma mass spectrometry (ICP–MS) (PerkinElmer, Nexlon 300D).The photoluminescence spectra were measured by a spectra fluorophotometer using 450W xenon lamp (Fluorolog-3 FL3-21) at room temperature. Cellular imaging was obtained in inverted fluorescence microscope (AMG EVOS f1).

## Results and discussion



 

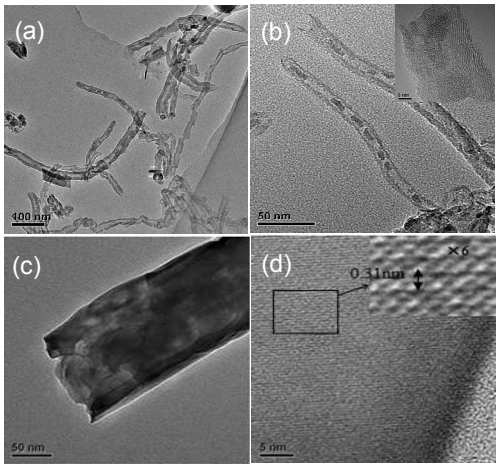


**Fig.1** TEM and HRTEM images of as-synthesized $Eu^{3+}$:$Y_2O_3$@CNTs (a, b) and $Eu^{3+}$:$Y_2O_3$ (c, d)

Fig. 1 shows the TEM and high-resolution TEM (HRTEM) images of the as-synthesized $Eu^{3+}$:$Y_2O_3$@CNTs (Fig.1a, 1b) and $Eu^{3+}$:$Y_2O_3$ (Fig.1c, 1d). It can be seen from the TEM image that $Eu^{3+}$:$Y_2O_3$ nanoparticles have been successfully inserted into the nanocavity of CNTs through supercritical synthesis process. The size and aggregation of the $Eu^{3+}$:$Y_2O_3$ nanoparticles filled in CNTs were confined by the nanocavity of CNTs compared to the free $Eu^{3+}$:$Y_2O_3$ prepared under the same condition (Fig.1b, 1c). HRTEM image (inset of Fig. 1b) shows that there was no existence of $Eu^{3+}$:$Y_2O_3$ nanoparticles on outside walls of the CNTs. The restricted inner nanocavity of CNTs prevents encapsulated $Eu^{3+}$:$Y_2O_3$ nanoparticles from forming large scale crystal (Fig. 1d).

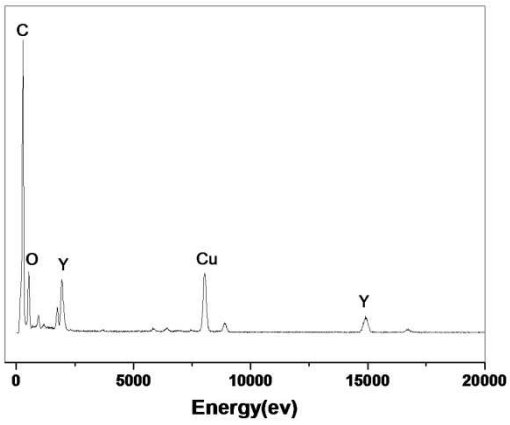


**Fig. 2** EDS analysis of the as-synthesized $Eu^{3+}$:$Y_2O_3$@CNTs

The $Eu^{3+}$:$Y_2O_3$ encapsulated CNTs were also analysed by energy dispersive X-ray spectroscopy (Fig. 2). The presence of Y and O elements attributed to $Eu^{3+}$:$Y_2O_3$. Since the $Eu^{3+}$:$Y_2O_3$ nanoparticles attached to the outer walls of CNTs have been carefully removed with acid, the existence of Y elements further confirmed that the $Eu^{3+}$:$Y_2O_3$ had been filled into the cavity of the CNTs. The peak of Eu element was not appearing due to the low content of Eu in as-prepared nanomaterials. The accurate content of Eu and Y elements was detected by ICP-MS (Eu 9 wt%, Y 42 wt%), and the atom ratio of Eu and Y was found out to be 1:8.

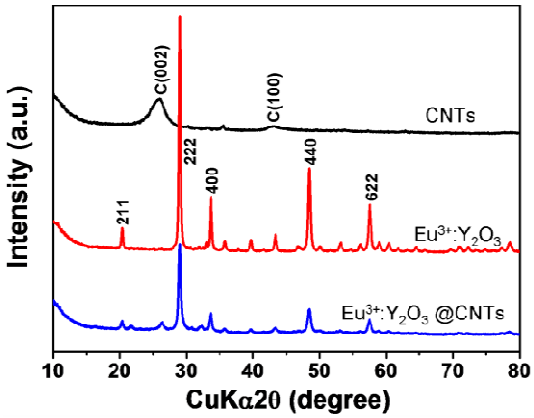


**Fig. 3** XRD patterns of pristine CNTs, $Eu^{3+}$:$Y_2O_3$, and $Eu^{3+}$:$Y_2O_3$@CNTs, respectively.

In order to further investigate the crystal structure of as-synthesized nanomaterials, XRD was employed. Fig. 3 shows the XRD patterns of the pristine CNTs, $Eu^{3+}$:$Y_2O_3$@CNTs and $Eu^{3+}$:$Y_2O_3$, respectively. The XRD diffraction peaks confirmed the crystallinity of as-synthesized nanomaterials. The peaks located at 2θ values 25.7 and 43.2 can be assigned to (002) and (100) diffractions of CNTs, whereas the strong peaks located at 2θ values 20.4, 29.0, 33.7, 48.4 and 57.6 are considered to be the (211), (222), (400), (440) and (622) diffractions of cubic $Y_2O_3$ (JCPDS FILE 70-0603), respectively. The XRD pattern of as-synthesized $Eu^{3+}$:$Y_2O_3$@CNTs nanomaterials exhibits both diffractions of CNTs and $Y_2O_3$ confirms the successful synthesis of filling $Eu^{3+}$:$Y_2O_3$ nanoparticles into the nanocavity of CNTs.

The room-temperature photoluminescence (PL) emission spectra of as-prepared $Eu^{3+}$:$Y_2O_3$@CNTs nanomaterials and pristine CNTs are shown in Fig. 4. The strongest peak of $Eu^{3+}$:$Y_2O_3$@CNTs at 610nm excited by 254 nm of UV-light corresponds to the forced electric dipole transition ($^5D_0 \rightarrow {}^7F_2$) within $Eu^{3+}$ ions, which is the typical spectral property as that reported for $Eu^{3+}$:$Y_2O_3$ nanoparticles[24]. The peaks at 587, 592 and 599 nm are due to $^5D_0 \rightarrow {}^7F_1$ transition, which are similar to that of $Eu^{3+}$:$Y_2O_3$ nanoparticles synthesized by supercritical method[15]. The pristine CNTs show no peak between 570 nm to 670 nm under the same excitation, so it can be concluded that the PL emission of $Eu^{3+}$:$Y_2O_3$ nanoparticles encapsulated in the hollow space of CNTs is not quenched by the walls of CNTs.

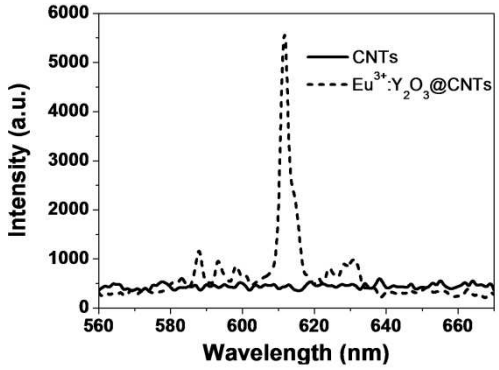


**Fig. 4** Photoluminescence spectra of $Eu^{3+}$:$Y_2O_3$@ CNTs and pristine CNTs excited at 254 nm.

For the potential applications as probes for bio-imaging, the cytotoxicity of the as-synthesized nanomaterials was investigated. The cytotoxicity of Tween 80 functionalized $Eu^{3+}$:$Y_2O_3$@CNTs was undertaken in HeLa cells using CCK-8 and MTT assay. The viability of untreated cells was chosen as the control. Fig. 5 shows the viability of HeLa cell treated by functionalized $Eu^{3+}$:$Y_2O_3$@CNTs, $Eu^{3+}$:$Y_2O_3$ and pristine CNTs at concentrations in the range of 10~1000 μg/mL. The results show a dose dependent decrease in their relative cell viability with the concentrations of the composite increased. The $Eu^{3+}$:$Y_2O_3$@CNTs show low toxicity to HeLa cells even when the cells were exposed to a high concentration of 1000 μg/mL for a period of 12h while $Eu^{3+}$:$Y_2O_3$ shows rather higher cytotoxicity in the same concentration, which indicate that after encapsulated by CNTs the cytotoxicity of $Eu^{3+}$:$Y_2O_3$ nanoparticles reduced. The above results suggest that the as-synthesized $Eu^{3+}$:$Y_2O_3$@CNTs can be used as potential probes for bio-imaging. The results also indicated that pristine CNTs are more toxic to HeLa cells than $Eu^{3+}$:$Y_2O_3$ filled CNTs in the same weight concentration for according to the results of ICP-MS, the mass ratio of Eu and Y in $Eu^{3+}$:$Y_2O_3$@CNTs was around 50%, the number of CNTs in pristine CNTs was much more than in $Eu^{3+}$:$Y_2O_3$@CNTs in the same weight.

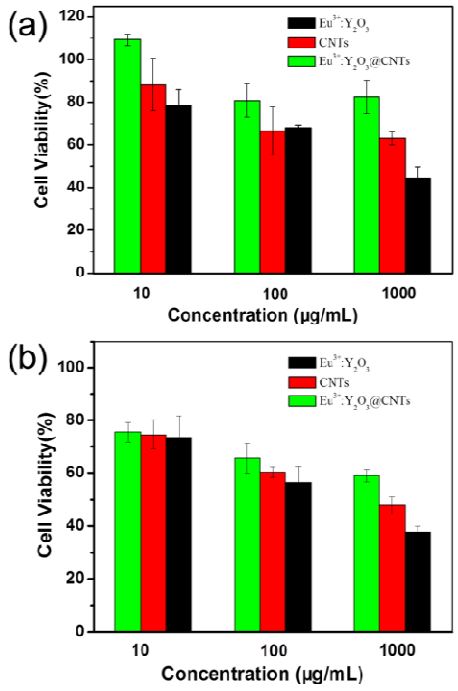


**Fig. 5** Cellular viabilities of HeLa cells exposed to functionalized $Eu^{3+}$:$Y_2O_3$@CNTs, CNTs, and $Eu^{3+}$:$Y_2O_3$ nanoparticles with different concentrations determined by CCK-8 assay (a) and MTT assay (b)

To demonstrate the potential of as-prepared $Eu^{3+}$:$Y_2O_3$@CNTs as probes for biological imaging, we incubated amphiphilic tween 80 functionalized $Eu^{3+}$:$Y_2O_3$@CNTs with HaLa cells. Fluorescence image (Fig. 6) shows the $Eu^{3+}$:$Y_2O_3$@CNTs exhibit good photoluminescence property inside the cells.

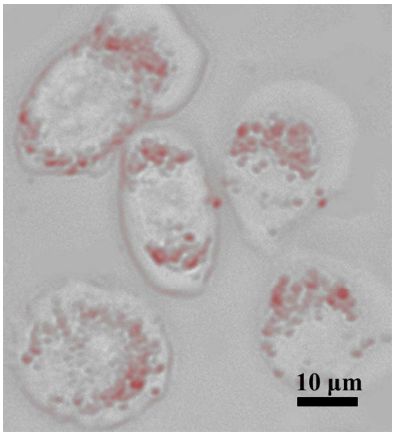


**Fig.6** Overlay image of live HeLa cells after incubated with $Eu^{3+}$:$Y_2O_3$@CNTs nanomaterials (100 μg/mL) (red represents emission from $Eu^{3+}$:$Y_2O_3$@CNTs).

## Conclusions

In summary, $Eu^{3+}$:$Y_2O_3$ filled CNTs nanomaterials have been successfully synthesized by a supercritical technique, which is the first case that supercritical synthesis proceeds in the nanocavity of nanotubes. The as-synthesized $Eu^{3+}$:$Y_2O_3$@CNTs was characterized by TEM, EDS, XRD and PL spectroscopy. With the excitation by 254 nm of UV-light, the special red emission at 610nm has been observed, which was from the $Eu^{3+}$:$Y_2O_3$ nanoparticles encapsulated in the CNTs. Surface functionalization with Tween 80 enabled the synthesized materials to be dissolved in water and to have the possibility of further functionalization by other biomolecules. The $Eu^{3+}$:$Y_2O_3$@CNTs nanomaterials show low cytotoxicity in HeLa cells at concentrations of 10~1000 μg/mL. By use of inverted fluorescence microscope, we have demonstrated the application of $Eu^{3+}$:$Y_2O_3$@CNTs as luminescence probes for live cells. With the properties of fluoresce emission, easily water-soluble and low cytotoxicity $Eu^{3+}$:$Y_2O_3$@CNTs nanomaterials have potential applications in bio-imaging. The supercritical synthesis method in filling the inner space of the CNTs could be applied to other rare-earth materials such as up-conversion materials, which have greater potential in deep-tissue imaging.

## Acknowledgements

The research was supported by National Basic Research Program of China (973 Program) (2012CB932601), National Natural Science Foundation of China (21271174).

## Notes and references

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