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Research Article

In Vitro Cytotoxicity of Mesoporous SiO_2@$Eu(OH)_3$ Core-Shell Nanospheres in MCF-7

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Initially, the sample was synthesized by a modified sol-gel process. Morphological analysis of growth SiO_2@$Eu(OH)_3$ was confirmed by applying field emission transmission electron microscopy (high and low resolution FETEM). The images confirmed the average diameter of mesoporous SiO_2@$Eu(OH)_3$ core-shell nanospheres (~392–400 nm) with a silica core of ~230 nm in diameter and a shell composed of europium hydroxide ~162 nm (thickness). Moreover, an absorption band at 280 nm was confirmed which initiates from the europium hydroxide. The photoluminescence spectrum of the nanosphere was also recorded at ambient temperature under the excitation of 3.82 eV. Cytotoxic studies in vitro were performed by applying MTT, NR assays, and morphological analysis. Morphological changes and % loss in cellular viability was assessed in human breast cancer cells (MCF-7) labeled with mesoporous SiO_2@$Eu(OH)_3$ core-shell nanospheres at different concentrations ranging from 10 μg/mL to 200 μg/mL. Current study demonstrates the quite rational strategy which might be useful in future clinical approach/applications.

1. Introduction

In the recent past, mesoporous nanoparticles (MNPs) have been proved as excellent nanomaterial in diverse field of biomedical applications. Due to versatile characteristics said nanoparticles (NPs) were suitable candidate for multiple purposes applications and can be developed as per desire due to their unique properties (optical, electro optical, electrical, catalytic, magnetic, mechanical, and thermal properties) [1]. The MNPs are robust and inexpensive material, have specific size and physiochemical properties, and have attracted researchers as well as biomedical scientists. In reported data, it is investigated that the above-mentioned nanomaterials are useful for purpose of drug delivery, diagnostic, biomarkers, and fluorescence as well [2, 3]. Therefore, it is worth mentioning that the mesoporous nanomaterials (MNs) should be manufactured with modified and controlled properties (surface properties, dimensions, composition, etc.). Preparation of the nanoparticles of the desired properties has become a subject of great interest for researchers, especially, in biomedical and clinical research studies to attain the biocompatibility, biosafety, and significant localization of drug to the cells [4, 5].

In current era, mesoporous nanoparticles based drug delivery platforms have proven valuable solubilizing tonic cargos due to development of appropriate vehicles for overcoming the multidrug resistance via suitable intracellular pharmacokinetics of drugs which was associated with traditional therapy system. High possibility of drug accumulation and long-term retention release profile in targeted/tumorous

area is one of the advantageous factors of said mesoporous nanoparticles. It has been experienced that nanoparticles possess instabilities like oxidation, agglomeration, and coalescence. Additionally, nanomaterials, such as quantum dots, which are synthesized by using organic solvents, are not soluble in water and are not biocompatible as well. Also they do not have function groups required for bioconjugation. However, they can be made water soluble by suitable techniques such as ligand exchange [6, 7]. Here the hydrophobic ligand molecule that works as a capping agent in organic based quantum dots is replaced by a suitable biofriendly hydrophilic ligands. This issue can be overcome by coating SiO_2 shells with nanoparticles as well [8–11]. We can provide surface functionalities for bioconjugation by incorporating functional organosilane molecules into the shell. Nanomaterials should be highly water dispersed especially for biomedical applications. Core-shell silica nanoparticles (SiO_2@$Ln(OH)_3$) are suitable material and useful for making the surface biocompatible as well as increasing the photostability of the nanomaterials [12]. Another approach is to coat an amphiphilic polymer or phospholipids to the quantum dots which enclose with the hydrophobic ligand molecule through hydrophobic attraction to provide a hydrophilic exterior and hence ensure water solubility. In this case, the hydrophobic ligands are still maintained on the quantum dot surface and thus retain the high emission quantum yield of QDs [13]. This also protects the QD surface from deterioration in biological medium. However, the modified, larger size of QDs may limit some of their biological applications.

In the present work, desired mesoporous SiO_2@$Eu(OH)_3$ core-shell nanospheres have been synthesized by modified sol-gel method [12]. The present mesoporous core-shell nanospheres are almost spherical having diameter of ~392–400 nm. The core of the nanosphere is made of silica and the diameter of the core is ~230 nm while the shell is ~162 nm in thickness which is composed of europium hydroxide. After successful growth and characterization of the nanoparticles, the author is ambitious to trace the actual nature of fabricated nanoparticles regarding biocompatibility of these nanospheres by using human breast cancer cells as biological model. This type of core-shell nanoparticle, for example, SiO_2@$Ln(OH)_3$, not only provides increased stabilization but also permits fine tuning of the surface biocompatibility and luminescence properties such as sharp absorption and emission lines, highly significant quantum yields, long lifetimes, and high photostability [13].

Hence, the present work demonstrated that the said nanosphere nanomaterials may induce cell death via loss in mitochondrial membrane potential, p53, and caspase pathways. In addition to this, the current study offers scientific data for biomedical applications of mesoporous SiO_2@$Eu(OH)_3$ core-shell nanosphere. Human breast cancer cell line named MCF-7 was for the first time derived from pleural effusion developed by Dr. Soule [14–16]. In this research article, MCF-7 cells viability was investigated in the mesoporous SiO_2@$Eu(OH)_3$ core-shell nanospheres. The biological model for the current study is MCF-7 and its cell viability was assessed by using MTT assay and neutral red assay (NRA). Additionally, morphological changes in the cells were also examined.

Consequently, over the past three decades, this characteristic of solid tumors has been a major stimulus for widespread research struggles aimed at applying nanoparticles to innovative therapy techniques.

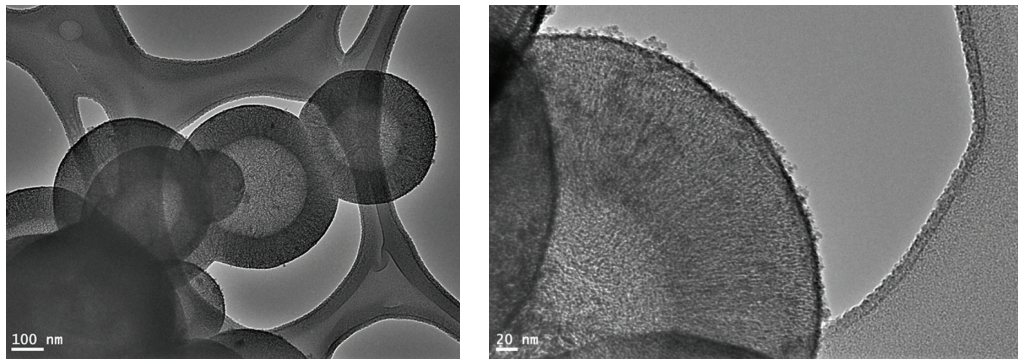
2. Experimental

2.1. Materials and Methods. Tetraethyl orthosilicate [$Si(OC_2H_5)_4$, called TEOS (99 wt.% analytical reagent, AR)], Europium oxide (Eu_2O_3, 99.99%, Alfa Aesar, Germany), nitric acid (HNO_3), ammonium hydroxide (NH_4OH), and ethanol (C_2H_5OH) were used as main precursors. The precursors were used without any further purification and nanopure water was used as a solvent to make solutions. Eu_2O_3 was dissolved in diluted HNO_3 resulting in europium nitrate [$Eu(NO_3)_3 6H_2O$]. Milli-Q system (Millipore, Bedford, MA, USA) was applied to prepare ultrapure deionized water.

2.2. Synthesis of Mesoporous SiO_2@$Eu(OH)_3$ Core-Shell Nanospheres. The core-shell nanospheres have been prepared using modified sol-gel method [12]. In a typical procedure, 0.5 g CTAB and 3.5 mL solution of 1 N of sodium hydroxide (NaOH) were dissolved into 100 mL double-distilled water and heated up to 70°C. After addition of TEOS (3 mL), the solution was stirred vigorously resulting in white precipitates. Then 2 mL solution of $Eu(NO_3)_3 6H_2O$ (1 m mol) and CTAB (0.1 mg) in ethanol was added into the foregoing solution. The reaction was continued for 2 hours under constant stirring and mesoporous SiO_2@$Eu(OH)_3$ core-shell nanospheres were formed. The resulting nanospheres were centrifuged and washed in C_2H_5OH and hot water in order to eliminate the surfactant molecules.

2.3. Material Characterization. X-ray diffraction (XRD) analysis of mesoporous SiO_2@$Eu(OH)_3$ core-shell nanospheres was evaluated using a PANalytical X'Pert X-ray diffractometer at room temperature and CuK_α (λ = 1.54056 Å) was used as X-ray source. The operating voltage and operating current were kept as 40 kV and 30 mA, respectively. Field emission transmission electron microscope (FETEM, JEM-2100F, JEOL, Japan) was applied for morphological analysis (size and shape) of the samples. The operating voltage was kept as 200 kV. Perkin-Elmer Lambda 40 spectrophotometer was used to measure the UV/Vis absorption spectrum. The sample contained 1 cm^3 stoppered quartz cell of 1 cm path length, in the range 190–600 nm.

2.4. MTT Assay. Loss in cellular viability (% age) was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and the same analysis was adopted by researchers in their published data [13]. Initially, 96-well plates were used and cells were cultured and incubated for 24 hours in an incubator having CO_2. Each well consists of 1×10^4 cells and incubated at 37°C temperature. In the second step, after incubation of 24 hours, 5 mg/mL of MTT (stock in PBS) was added. The concentration was kept



(a) (b)

Figure 1: (a) Low resolution and (b) high resolution FE-TEM image of mesoporous SiO_2@$Eu(OH)_3$ core-shell nanospheres.

as 10 μL in 100 μL of cell suspension in each well and plates and incubated at 37°C. After incubation for 4 hours in CO_2 incubator, the supernatant was discarded and 200 μL/well of DMSO was added. The solution was mixed gently and the final color was observed at 550 nm. In parallel, control cells were also undergoing the same conditions without treatment.

2.5. Neutral Red Assay. Siddiqui et al. reported a procedure to measure the loss percentage in cells viability by using NRA [17] and the same protocol is used in the present cytotoxicity studies. After exposure, the cells were removed from the medium and washed two times using phosphate buffer saline (PBS). Furthermore, the cells were kept for incubation for three hours in a medium having neutral red (concentration = 50 μg/mL). A solution of calcium chloride and formaldehyde (1% and 0.5%, resp.) was used to aspirate the medium and cells were washed out with ethanol (50%) and acetic acid (1%) and incubated for 30 minutes at 37°C. The final color was observed at 540 nm and results were compared with the untreated control cells.

2.6. Morphological Assay. Morphological changes were observed to describe the alterations induced by NPs in MCF-7 cells. All the cells were exposed to different concentrations ranging from 10 μg/mL to 200 μg/mL of NPs for 24 h. The images were recorded using an inverted phase contrast microscope at 20x magnification.

3. Results and Discussion

Figures 1(a) and 1(b) show the high resolution and low resolution FETEM images of the core-shell nanospheres which determine the diameter of the particles about ~392–400 nm. These figures confirm the spherical shape of the nonagglomerated particles with a silica core having a diameter around ~230 nm and the thickness of the core was observed to be varied as ~10 nm. Moreover, thickness of the shell was confirmed to be about ~162 nm. It is clear from FETEM image that every particle has a clear thickness of shell and core as well. However, the morphology of the resulting core-shell nanospheres was not affected by the deposited layer of europium hydroxide. Europium hydroxide shell was deposited onto the silica core nanospheres which results in SiO_2@$Eu(OH)_3$ core-shell nanospheres. The deposited layer of europium hydroxide helps to promote the dispersion of mesoporous SiO_2@$Eu(OH)_3$ core-shell nanospheres.

Figure 2(a) shows the absorption spectra of the as-prepared core-shell nanospheres dispersed in ethanol. The spectrum showed a strong band at 211 nm with a shoulder at 280 nm. As mentioned above (Figure 1), the nanospheres have a core of silica and a shell composed of europium hydroxide. Figure 2(a) describes a strong band for silica core (at 211 nm) [18] while a low intensity band for europium hydroxide (at 280 nm) results from the transition between the ground state ($^7F_0$) and the charge-transfer state of the Eu–O bond ($^4f_7 \rightarrow {}^4f_7 2p^{-1}$). The results are in good agreement with the previous reported data [19–21]. The absorption spectrum of core-shell nanospheres confirmed the formation of $Eu(OH)_3$ shell on the surface of silica core. It is obvious that the silica core nanospheres have prominent absorption spectra resulting after the surface modifications by europium hydroxide shell. Furthermore, this indicates that the nanospheres become more stable showing colloidal behaviour which is more important thing for biological studies. Figure 2(b) shows photoluminescence spectrum of the nanosphere at ambient temperature under the excitation of 3.82 eV. The spectrum shows all characteristics transitions of europium ions. The spectrum shows that the typical emission peaks of Eu^{3+} ions at 1.78, 1.89, 2.00, 2.1, and 2.14 eV can be attributed to transitions from $^5D_0$ level to sublevel of $^7F_0$, $^7F_1$, $^7F_2$, $^7F_3$, and $^7F_4$, respectively.

In our study, MCF-7 human breast cancer cells were used for studying the histology of tumor biology and hormone mechanism of action under labeling condition of SiO_2@$Eu(OH)_3$ core-shell nanospheres materials. Significant toxic effects were recorded by applying MTT assay and NR assay. But conflicting results were found in previous conducted study [12]. Osborne et al. conducted experiment on four different types of MCF-7 breast cancer cell lines and reported that some structural chromosomes alterations and estrogen receptor (ER) and progesterone receptor (PgR) were recorded in different types which can affect the growth of breast cancer cell line [16]. In Asia and developing countries, breast cancer is the major issue/problem in women aged

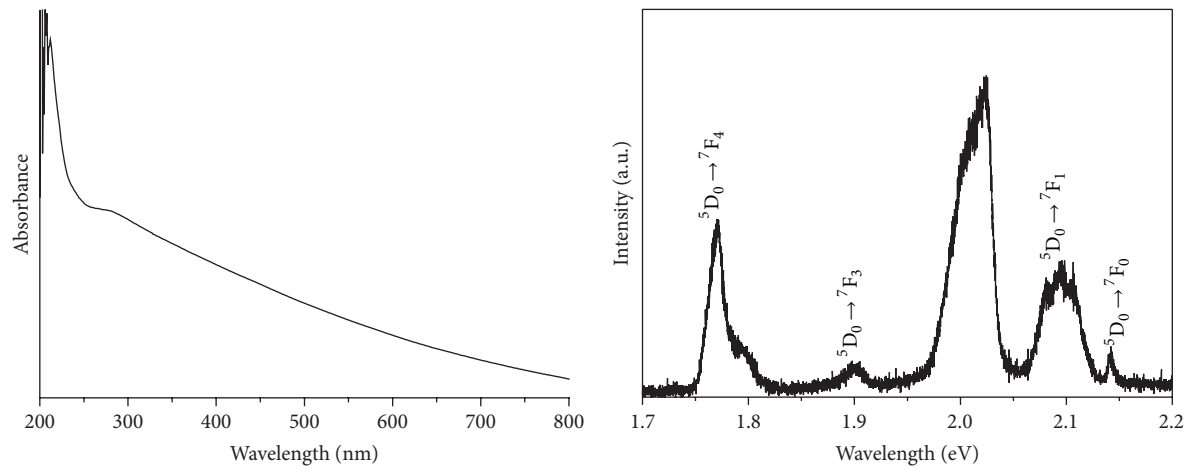


FIGURE 2: (a) UV/Vis absorption spectra of mesoporous SiO_2@$Eu(OH)_3$ core-shell nanospheres in ethanol. (b) Photoluminescence spectrum of mesoporous SiO_2@$Eu(OH)_3$ core-shell nanospheres.

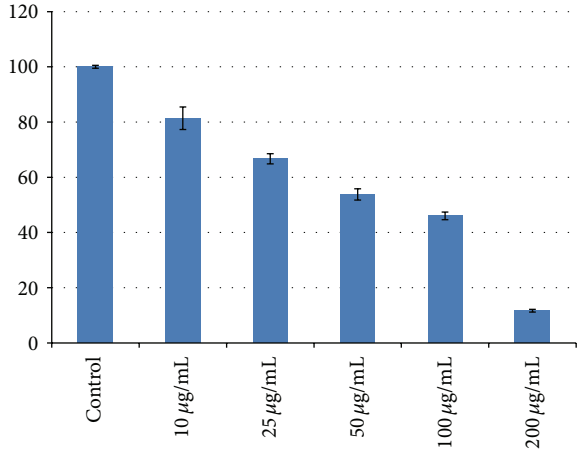


FIGURE 3: Percent cell viability by MTT assay in MCF-7 cells exposed to various concentrations of NPs.

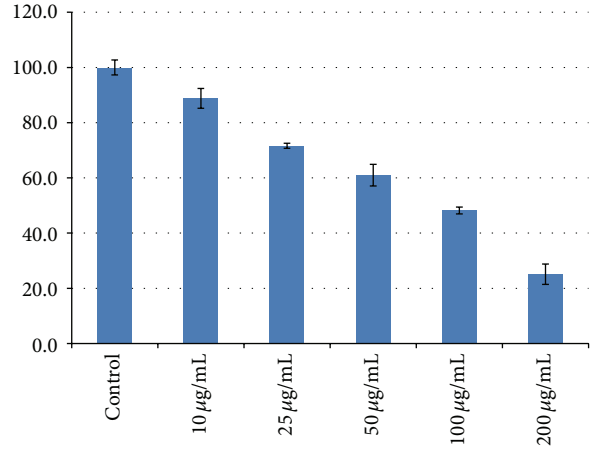


FIGURE 4: Percent cell viability by neutral red assay (NRA) in MCF-7 cells exposed to various concentrations of NPs.

≈25 years and above. The writer used the suggested cell line (MCF-7) as an experimental biological model. After TEM and elemental analysis of SiO_2@$Eu(OH)_3$ core-shell nanospheres, the phototoxicity of given nanomaterials was tested in human breast cancerous cell line. To determine the optimal concentration of nanoparticles, range was selected from 10 μg/mL to 200 μg/mL for checking of localization of dispersed solution and loss in cell viability in the absence of any light dose as shown in Figure 3. In parallel controlled/untreated MCF-7 cells were grown in 96-well plates for assessment of loss in cell viability under nanoparticles exposure. The same nature of study was performed by Fatima et al. using α-Fe_2O_3 and SiO_2 nanoparticles regarding toxicity in human muscle carcinoma [22]. It was observed that under exposure of 10 μg/mL only 10% cell viability loss was recorded which is insignificant as compared to previous published data [22]. But convincing results were observed under exposure of 200 μg/mL of SiO_2@$Eu(OH)_3$ core-shell nanomaterials disperse solution once labeled with MCF-7 cell line. About 75% loss in cell viability was examined with 200 μg/mL of nanoparticles.

However, some other possibilities might be involved for cell killing process. The $Eu(OH)_3$ destabilizes the cell membrane and can damage the mitochondrial membrane which leads to liposomal loss, vascular shutdown process, or Eu^{3+} which contributes to depolarize the cells by affecting the electron transport chain. It is clear from Figure 4 that loss in cell viability follows the dose/concentration dependent manners of SiO_2@$Eu(OH)_3$ core-shell nanomaterials. Morphological changes were observed when MCF-7 cells were labeled with 50 μg/mL to 200 μg/mL; in parallel controlled (without any nanomaterials SiO_2@$Eu(OH)_3$ core-shell localization) MCF-7 cells were examined. Figure 5(a) shows the control without any labeling of nanomaterials. Significant dead cell clusters were visualized when cells were exposed to SiO_2@$Eu(OH)_3$ coreshell having concentration of 50 μg/mL to 150 μg/mL (as shown in Figures 5(b) and 5(c)). It has been

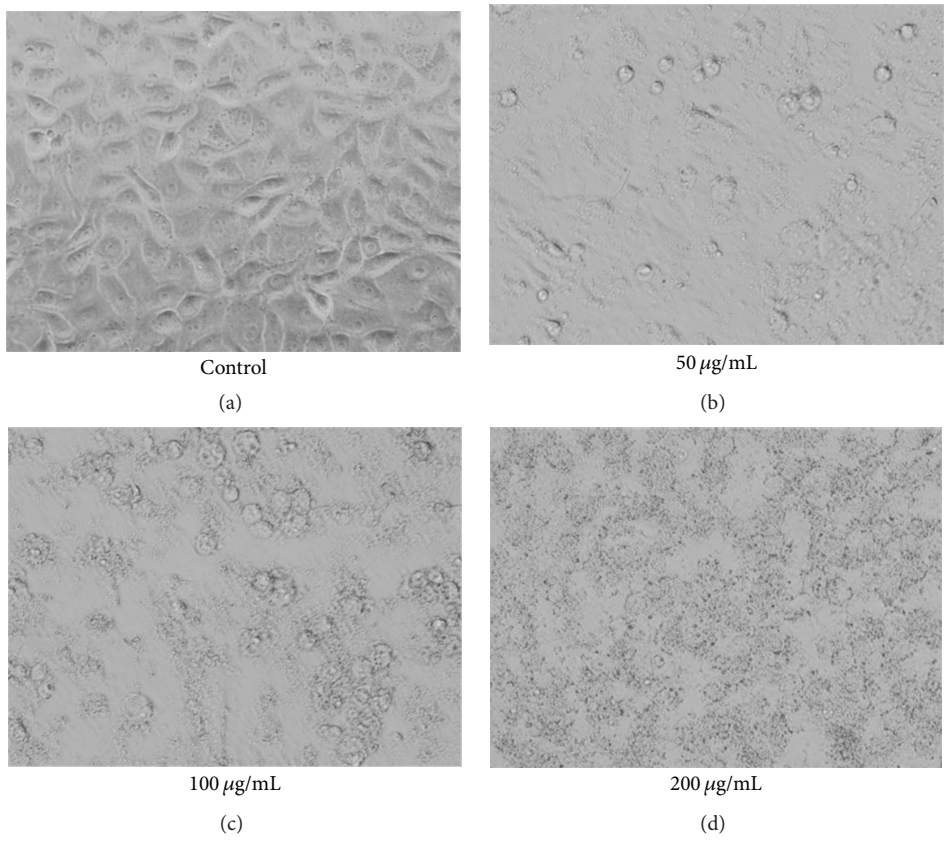


Figure 5: Morphological changes in MCF-7 cells exposed to various concentrations of NPs.

reported by many researchers in their previously published data that cellular uptake and accumulation of nanospheres within the biological cells is time and dose dependent [23]. It was surprising when 200 μg/mL of SiO_2@Eu$(OH)_3$ was labeled with MCF-7 cell line (as depicted in Figure 5(d)). No presence of cells (viable/dead) was observed. After cell apoptosis each dead cell was sticked with well plate bottom and about 75% loss in cell viability was confirmed by NR analysis. Similar cytotoxic results were evaluated by Lin et al. on HepG2 cells labeled with mesoporous SiO_2@Eu$(OH)_3$ core-shell nanospheres using MTT assay [12]. In summary, the current experimental results are in good agreement with the previous reported studies of different authors [2–5].

4. Conclusion

A modified sol-gel process has been established to prepare highly uniform mesoporous SiO_2@Eu$(OH)_3$ core-shell nanosphere. After successful growth of the particles, high resolution and low resolution FETEM analysis were performed in order to confirm the morphology of nanospheres. The absorption spectrum at 280 nm indicates the presence of europium hydroxide while the photoluminescence spectrum also confirms all the features of europium ions. Cytotoxicity of grown mesoporous materials was determined by using MCF-7 cells. Experimental results at a concentration of 200 μg/mL of nanoparticles showed that core-shell nanospheres produce significant toxic effects. A 75% loss in cell viability was found by analysis of MTT and NR assay. Cell debris was also confirmed at 200 μg/mL from image (5D) taken using an inverted phase contrast microscope at 20x magnification. Our current study of high level induction of apoptotic response by mesoporous SiO_2@Eu$(OH)_3$ core-shell in MCF-7 breast cancer cell line suggested that the explored scientific data needs further investigation for long-term exposure results, which will be useful for future medical applications.

Competing Interests

The authors declare that they have no competing interests.

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