Preliminary application of cerium-based hollow nanospheres as drug carrier

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Abstract. As more and more chemical activity and biological activity are found, cerium, one of the largest reserves of cheap rare earth lanthanides, is widely used in many fields such as flint, decolorizer, abrasive polish, glass additives, reducing agents, catalysts, harmful metal substitutes and other special functional materials. Cerium nanoparticles can be used in cardiovascular diseases, neurodegenerative diseases and radiation-induced tissue damage, also be used as biomimetic enzymes, biocatalysis, biomedicine, biological scaffolding and other biological fields. However, cerium-based hollow nanospheres have never been properly tested as a drug carrier. In this report, we explored the potential of cerium-based materials as a drug carrier which is a brand new attempt to expand the scope of application of cerium-based nanomaterials in the field of biomedicine.

1. Introduction
Due to its oxygen storage capacity via facile Ce4+/Ce3+ redox cycles, high mechanical strength, and unique optical properties, the cerium elemental oxygen compound (ceria) as one of the most important functional rare earth oxides is a promising material widely be used in many fields such as catalysts[1], corrosion prevention[2], fuel cells[3, 4], oxygen sensors[5], magnetic materials[6], ultraviolet blocks[7], and optical materials[8]. In addition to these traditional applications, more and more studies have shown that cerium oxide compound has many biological activities and can be applied in the biomedical field. Ceria can mimic the activity of key antioxidant enzymes, for instance, superoxide dismutase (SOD) and catalase (CAT) mimetic activity. The mechanism potentially abates noxious intracellular reactive oxygen species (ROS) via the Ce4+/Ce3+ self-regenerating redox cycles which might fight chronic inflammation and the pathologies associated with oxidative stress including cancer and neurodegeneration[9-13]. The status of cerium-based nanomaterials in the biomedicine field will be more and more important. Like other inorganic hollow nanospheres, cerium-based hollow nanospheres also have the unique chemical and physical properties, which make them have obtained the most attention for medicinal applications[14,15]. The large void space in hollow structures have been successfully used for adsorption, enrichment, protection and release of sensitive materials and biologically active species[16-20]. So they can be developed into potential intelligent drug carriers which can maintain
the concentration of drugs in the precise sites of the body, improve the therapeutic efficacy and reduce toxicity[21-23]. However, cerium-based hollow nanospheres have never been properly tested as a drug carrier. Here in our study, cerium-based hollow nanospheres were synthesized with a typical method[24] and then have been tried to carry a model medicine, protoporphyrin, as a fluorescent indicator into the cell and reached very good repercussion. The carrier can be encapsulated by chitosan which to obtain pH response function for tumor targeting delivery in vivo and release from the acidic microenvironment around tumor. We explored the potential of cerium-based nanospheres as drug carrier for the first time which is a brand new attempt to expand the scope of application of cerium-based nanomaterials in the field of biomedicine.

2. Experimental section
The cerium hollow nanospheres were synthesized by the previously reported method[24] and characterized by electron microscopy. A rapid colorimetric assay for cellular growth and survival, the tetrazolium-based colorimetric assay (MTT), was applied to measure proliferation and cytotoxicity for evaluation of cytotoxicity in vitro. Erythrocyte protoporphyrin was used as an indicator drug for preliminary testing and evaluation of drug delivery ability of cerium hollow nanospheres by microscopy to observe fluorescence. The specific experimental procedure has shown in the schematic (Figure 1).

![Figure 1](image1.png)

Figure 1. Schematic illustration of the experimental procedure

3. Results and discussion

3.1. Characterization

![Figure 2](image2.png)

Figure 2. Characterization by electron microscopy: A. SEM image B. TEM image
The morphology and internal structure of the cerium hollow nanospheres were characterized by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The SEM and TEM images in figure 2 revealed that these hollow-structured nanospheres had the mesoporous shells that were composed of a large amount of uniform nanoparticles. Nanoshell structure fragments microphotograph in the figure 2A indicated that the shell of cerium hollow nanospheres were formed by the aggregation of many small spherical cerium nanoparticles and filled with a lot of mesoporous between them. The TEM microphotograph (Figure 2B) showed the obvious hollow structure inside cerium nanospheres. The disordered mesostructure of the surface and the internal hollow structure of make the nanospheres have excellent mass-storage properties for loading and adsorption capacity.

3.2. The safety evaluation in vitro

![Figure 3](image-url)

**Figure 3.** Cell viability after treat with different cerium hollow nanospheres concentrations for 24 hours

The 3-(4, 5-dimethyl-2 thiazoyl) -2, 5-diphenyl-2H-tetrazolium bromide (MTT) assay was used to assess cell viability and evaluate the cytotoxicity in vitro. The MTT results (Figure 3) showed that the cells maintained a high level of viability even at high concentrations. This suggests cerium hollow nanospheres have no inhibition on L02 cells which can provide basis for the further biomedical application.

3.3. Preliminary evaluation as a drug carrier

Erythrocyte protoporphyrin was used as an indicator drug for preliminary testing and evaluation of drug loading and delivery properties of cerium hollow nanospheres by microscopy to observe the fluorescence. Laser confocal scanning microscopy (LCSM) confirmed that the cerium hollow nanospheres and protoporphyrin were uptake by cells (Figure 4). The red fluorescence emitted by protoporphyrin was clearly visible and full of cytoplasm. It was also clearly be seen that cerium hollow nanospheres loading with protoporphyrin also emitted red fluorescence around the cell and in the cytoplasm. All these have reflected the assessment that there was a potential of cerium hollow nanospheres for fluorescence imaging and drug carrier applications in vitro.
4. Conclusions
Cerium element has good biological safety and biological activity. The ceria has many excellent properties that it can absorb and block UV, mimic enzymatic activity (superoxide dismutase (SOD) and catalase (CAT)), which may be the main reason makes it possible as a potential drug and might use to fight chronic inflammation and lesions associated with oxidative stress including cancer and neurodegeneration. In this report, cerium hollow nanospheres were used as an exploratory study of drug carriers and as a satisfactory consequence to expand their application in the biomedical field. With the discovery of more biological activity, the scope of application for cerium-based nanomaterials will be greatly expanded in the future, especially in the medical and health care fields.

5. Acknowledgments
This work was financially supported by the National Science Fund for Distinguished Young Scholars of China (No. 81325011).

6. References
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![Figure 4](image-url) Figure 4. Laser confocal scanning microscopy image: A. Bright field image; B. Fluorescence field image
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