Materials Science and Engineering C 50 (2015) 151–159

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Cerium fluoride nanoparticles protect cells against oxidative stress

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ARTICLE INFO

Article history:
Received 12 September 2014
Received in revised form 12 January 2015
Accepted 30 January 2015
Available online 2 February 2015

Keywords:
Cerium fluoride nanoparticles
Terbium doped CeF_3
Luminescence
Living cells
Virus
Oxidative stress
Toxicity

ABSTRACT

A novel facile method of non-doped and fluorescent terbium-doped cerium fluoride stable aqueous sols synthesis is proposed. Intense green luminescence of CeF_3:Tb nanoparticles can be used to visualize these nanoparticles' accumulation in cells using confocal laser scanning microscopy. Cerium fluoride nanoparticles are shown for the first time to protect both organic molecules and living cells from the oxidative action of hydrogen peroxide. Both non-doped and terbium-doped CeF_3 nanoparticles are shown to provide noteworthy protection to cells against the vesicular stomatitis virus.

1. Introduction

Biomaterials engineering is one of today's most promising areas of materials science. Substantial progress is observed in this field due to the widespread use of artificial nanomaterials [1]. Particular success has been achieved in the development of nanoparticle-based theranostic systems, including drug delivery platforms, luminescent and magnetic biomarkers, and sensoric and diagnostic devices. [2,3].

In very recent years, nanocrystalline ceria has been shown to possess an enormous biological activity, which originates from its ability to participate readily in the redox processes under biologically relevant conditions, as well as its relatively low toxicity, making biological application of ceria-based materials relatively safe [3–6]. Ceria-based nanomaterials belong to a new class of artificial enzymes (nanozymes) [7] and are able to scavenge various reactive oxygen species (ROS) deleterious to living cells [8–10]. For instance, nano-ceria exhibits a superoxide dismutase-mimetic activity [8,9] and catalase-mimetic activity [10], protecting living cells against superoxide anion and hydrogen peroxide. Nano-ceria is expected to be useful in the therapy of aging-related diseases, including various types of cancer, diabetes, ischemic stroke, and Alzheimer's disease [3–16].

From a chemical point of view, the mechanism of the nano-ceria protective action is not quite clear yet. According to a common opinion, ceria becomes strongly non-stoichiometric in a nanocrystalline state and thus can participate in various redox processes [1–14]. However, the role of cerium valence states in ROS inactivation is still under question. Celardo et al. [15] demonstrated that it is Ce^{3+} ions which determine the redox-dependent anti-apoptotic effect of cerium oxide nanoparticles. On the other hand, Das et al. [16] stated that the catalase mimetic property of nano-ceria (unlike the SOD mimetic one) depends essentially on the content of Ce^{4+} in ceria nanoparticles. We can assume that both valence states of cerium actually play an important role in the scavenging of hydrogen peroxide. While performing the cell protection function, Ce^{3+} is oxidized by hydrogen peroxide to the tetravalent state. Simultaneously, H_2O_2 is bonded strongly to Ce^{4+} ions on the surface of nano-ceria [17], forming cerium perhydroxide, which is further decomposed to form non-toxic water and oxygen.

Until now, the question is open as to whether other cerium-containing nanomaterials can protect living cells from hydrogen peroxide or other reactive oxygen species when the cerium ion would be initially in a trivalent state only. A good candidate for such examination is cerium fluoride (CeF_3). This compound would presumably be non-toxic, because the toxicity of fluoride-containing inorganic substances

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http://dx.doi.org/10.1016/j.msec.2015.01.094
0928-4931/

generally depends on their solubility in the water. Similar to cerium dioxide, cerium fluoride has low solubility in water and biological fluids [18]. For instance, the CeF_3 solubility product constant ($K_{sp} = 8 * 10^{-16}$) is substantially lower than that of fluorspar CaF_2 ($K_{sp} = 4 * 10^{-11}$), which is classified as "not dangerous" [19]. Another good reason to study CeF_3 is the prominent luminescence properties of Ce^{3+} species (unlike the Ce^{4+} ones), especially when doped with some other rare earth elements (terbium, europium etc. [20]). The luminescence of Ce^{3+} is strongly quenched when it is oxidized by oxygen or ROS to Ce^{4+} [21]. Thus the luminescence of cerium fluoride (or doped cerium fluoride) could allow simultaneous monitoring of CeF_3 nanoparticles' behavior in cells and their redox state in a microenvironment.

There are plenty of synthetic methods for obtaining cerium fluoride dispersions and sols: thermolysis of fluorine-containing precursors, co-precipitation from aqueous solutions, reversed micelle and micro-emulsion precipitation, hydrothermal synthesis, precipitation from non-aqueous solutions and solvothermal synthesis, and the sol–gel method, among others [22]. For example, monodisperse photoluminescent CeF_3, CeF_3:Tb^{3+}, and CeF_3:Tb^{3+}@LaF_3 core/shell nanoparticles of small size (<15 nm) have been synthesized by thermolysis of rare earth oleate complexes in a high boiling mixture of oleic acid and 1-octadecene [23]. Cerium fluoride nanoparticles (15–30 nm) have been successfully prepared from water-in-oil microemulsions [24]. Re-dispersible CeF_3 nanoparticles were synthesized by a polyol route, using ethylene glycol [25] or diethylene glycol [26] as a solvent. CeF_3 nanocrystals with plate-like and perforated morphologies were synthesized via a hydrothermal route, using polyvinylpyrrolidone as a stabilizer [27]. Large-sized nanoparticles of cerium fluoride were prepared in water under sonication without any surfactants, using cerium nitrate and ammonium hydrofluoride as the starting materials [28]. A Triton X-100 surfactant was used for aqueous synthesis of TbF_3@CeF_3 and TbF_3@CeF_3@SiO_2 complex nanostructures [29]. Unfortunately, only a small part of the methods listed above are suitable for biological applications. Toxic solvents (ethylene or diethylene glycols, octadecene) or toxic capping agents (oleylamine, ethoxylated phenols) are typically used in the syntheses, so the products demand thorough purification; in turn, surfactant-free syntheses in aqueous media (hydrothermal, microwave etc.) often lead to the coarse-grained and/or agglomerated specimens that can't be peptized to form stable transparent sols.

Here we report on the synthesis of stable transparent aqueous sols containing nearly monodisperse luminescent CeF_3 or CeF_3:Tb nanoparticles by a novel facile surfactant-free low-temperature technique. These materials are shown to be non-toxic and capable of protecting organic molecules and living cells from the oxidation by hydrogen peroxide. Finally, the antiviral activity of CeF_3 and CeF_3:Tb nanoparticles against the vesicular stomatitis virus in vitro is demonstrated for the first time.

2. Materials and methods

2.1. Synthesis of CeF_3 and Tb-doped CeF_3 nanoparticles

We have elaborated a novel method, allowing CeF_3 and CeF_3:Tb nanoparticles synthesis via facile precipitation in alcoholic media at room temperature. Briefly, 1.86 g of cerium(III) chloride heptahydrate (5 mmol) (Aldrich, #228931) was dissolved in 15 ml of distilled water and added to 150 ml of isopropyl alcohol (Aldrich, W292907). Hydrofluoric acid (20 mmol) (Sigma-Aldrich, #30107), dissolved in 50 ml of isopropyl alcohol, was added drop-wise to a cerium salt solution under vigorous stirring. The resulting white sediment was filtered and washed carefully by pure isopropyl alcohol, several times. Then the suspension was slightly dried to form a paste-like substance and dispersed in 110 ml of distilled water, using an ultrasonic bath. The resulting transparent colloid solution was boiled for 5 min to remove residual alcohol. Tb-doped CeF_3 nanoparticles were synthesized by the same protocol, using a mixture of 4.25 mmol of cerium chloride and 0.75 mmol (15%) of terbium chloride (Aldrich, #212903) as a starting material.

2.2. Synthesis of cerium oxide nanoparticles

A non-stabilized ceria aqueous sol (ceria sol #1) was synthesized by hydrothermal-microwave treatment of the colloid solution, formed upon anionite treatment of a cerium(III) nitrate aqueous solution [30]. Briefly, ion-exchange resin Amberlite IRA 410 CL (Aldrich, #216569), preliminarily converted to the OH-form, was gradually added to a 0.01 M cerium(III) nitrate (Aldrich, #238538) solution until pH reached 10.0. Sols formed in this way were separated from the resin by filtering, immediately transferred to 100 ml polytetrafluoroethylene autoclaves (filled to 50%) and subjected to microwave-hydrothermal treatment in a Berghof Speedwave MWS-3 + setup at 190 °C for 3 h. Upon completion of the synthesis the autoclaves were withdrawn from the microwave oven and cooled down to room temperature in air.

A citrate-stabilized ceria aqueous sol (ceria sol #2) was synthesized by the previously reported procedure [31]. Briefly, 2.0 g of citric acid (Sigma-Aldrich, #251275) was mixed with 25 ml of a 0.4 M aqueous cerium(III) chloride (Aldrich, #228931) solution. The resulting solution was rapidly poured under stirring into 100 ml of a 3 M ammonia (analytic grade, Chimmed, Russia) solution, and then exposed for 2 h at ambient conditions and further boiled for 4 h. Then the solution was cooled to room temperature and purified from precursors and by-products by sedimentation and further re-dispersion.

2.3. Characterization of nanoparticles

Transmission electron microscopy was performed using a Leo 912 AB Omega electron microscope operating at 100 kV. Before the analysis sols were brought onto the copper grids using micropipette without any specific pretreatment and dried in ambient air. Particle size measurements by dynamic light scattering were carried out on a Malvern Zetasizer Nano ZS analyzer. The light source used was a helium-neon laser (the radiation wavelength was 632.8 nm). Local elemental analysis (with ca. 0.5 μm resolution) of CeF_3 and CeF_3:Tb nanoparticles pre-deposited on a conductive carbon tape was performed using a Carl Zeiss NVision 40 scanning electron microscope, equipped with an Oxford Instruments X-MAX energy-dispersive X-ray analyzer, operating at a 20 kV acceleration voltage. The overall Ce:Tb atomic ratio in the CeF_3:Tb sample was additionally checked by total reflection X-ray fluorescence (TXRF) spectrometry, using a Bruker PICOFOX S2 laboratory spectrometer equipped with an air-cooled Mo-anode X-ray tube. UV–vis absorption spectra of CeF_3 and CeF_3:Tb colloid solutions were recorded using standard quartz cells for liquid samples, on an Agilent Technologies Cary 5000 UV–Vis spectrophotometer. Photoluminescence spectra of CeF_3 and CeF_3:Tb colloid solutions were recorded using a Perkin Elmer LS55 spectrometer at room temperature (resolution: 0.5 nm; slit width: 3–8 nm). The laser scanning microscopy investigations were carried out using a Carl Zeiss LSM-710-NLO microscope equipped with a pulse femtosecond Chameleon Ultra II laser system (Coherent Inc., USA), tunable in the 690–1060 nm range. The luminescence spectra of CeF_3:Tb nanoparticles was registered by the 32 channel GaAsP detector under excitation by a 488 nm CW laser or 735 nm 80 MHz pulse laser, with a pulse width of 140 fs. Scanning was performed at the lowest speed (177 μs/pixel) because of the prolonged luminescence lifetime of Tb^{3+}.

2.4. Hydrogen peroxide inactivation

Indigoid dyes are easily decomposed by ROS [32,33], the discoloration of indigo by hydrogen peroxide being used in biochemical assays