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Nano-shape varied cerium oxide nanomaterials rescue human dental stem cells from oxidative insult through intracellular or extracellular actions

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ABSTRACT

Cerium oxide nanomaterials (CeNMs), due to their excellent scavenging properties of reactive oxygen species (ROS), have gained great promise for therapeutic applications. A high level of ROS often degrades the potential of stem cells in terms of survivability, maintenance and lineage differentiation. Here we hypothesize the CeNMs may play an important role in protecting the capacity of stem cells against the oxidative insult, and the suppression mechanism of ROS level may depend on the internalization of CeNMs. We synthesized CeNMs with different directional shapes (aspect ratios) by a pH-controlled hydrothermal method, and treated them to stem cells derived from human dental pulp at various doses. The short CeNMs (nanoparticles and nanorods) were internalized rapidly to cells whereas long CeNMs (nanowires) were slowly internalized, which led to different distributions of CeNMs and suppressed the ROS levels either intracellularly or extracellularly under the H_2O_2-exposed conditions. Resultantly, the stem cells, when dosed with the CeNMs, were rescued to have excellent cell survivability; the damages in intracellular components including DNA fragmentation, lipid rupture and protein degradation were significantly alleviated. The findings imply that the ROS-scavenging events of CeNMs need special consideration of aspect ratio-dependent cellular internalization, and also suggest the promising use of CeNMs to protect stem cells from the ROS-insult environments, which can ultimately improve the stem cell potential for tissue engineering and regenerative medicine uses.

Statement of Significance

Oxidative stress governs many stem cell functions like self-renewal and lineage differentiation, and the biological conditions involving tissue repair and disease cure where stem cell therapy is often needed. Here we demonstrate the unique role of cerium oxide nanomaterials (CeNMs) in rescuing stem cell survivability, migration ability, and intracellular components from oxidative stress. In particular, we deliver a novel finding that nano-morphologically varied CeNMs show different mechanisms in their scavenging reactive oxygen species either intracellularly or extracellularly, and this is related with their different cellular internalizations. We used human dental pulp stem cells for the model study and proved the CeNMs were effective in controlling ROS level by means of scavenging intracellularly or extracellularly, which ultimately led to improving the intact therapeutic potential of stem cells. This work touches an important biological issue of nanomaterial interactions with stem cells under the conditions related with oxidative stress and the resultant damage. The correlation of shape factor in therapeutic nanomaterials with stem cell interaction and the oxidative stress-related functions will provide informative ideas in the design of CeNMs for cellular therapy.

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1. Introduction

Stem cells, including those multipotent and pluripotent are venerable to reactive oxygen species (ROS), and often significantly damaged under the ex vivo culture expansion environments and in the in vivo pathological conditions [1–3]. Preservation of the stem cell characteristics and the survivability under the ROS insult conditions is thus considered an important issue to gaining appropriate therapeutic potential of the stem cells for use in regenerative medicine.

Treatment of biomaterials particularly nanomaterials to stem cells has been a promising approach to modulate the cell functions, including the maintenance of cell proliferation and stimulation of differentiation into specific lineages [4–6]. For example, neural stem cells cultured with nanoparticles with different modifications (such as size and surface chemistry) alter their phenotypes in differentiation stage [7–10]. Moreover, mesenchymal stem cells have often been stimulated by the treatment of bioactive inorganic nanoparticles toward an osteogenic lineage [11,12],. Not only stem cells but many other tissue specific cells have also shown to be affected by the nanoparticles in their proliferation, migration, and maturation [13–15].

Therefore, the regulation of stem cell capacity, i.e., protection of the stemness and survivability from the ROS-inducing environments is considered possible through the action of nanomaterials. Among other nanomaterials, cerium oxide is considered one of the most intriguing compositions. The nanoparticle form of cerium oxide has the ability to scavenge ROS, and thus has shown significant cellular and sub-cellular effects [16,17],. Cerium oxide belongs to rare earth metal element in lanthanide series, and has variable oxidation state, i.e., Ce^{3+} and Ce^{4+}, which combined with lattice structure defects, allows its action as oxidation and reduction catalyst in a continuous fashion (fenton-like reaction) [18], exhibiting unique biological behaviors [19,20],. For instance, cerium oxide nanoparticles treated to bone marrow stromal cells had substantial influences on cell migration and differentiation, and this was related with the reduced oxidative stress [21]. Neural cells, in response to cerium oxide nanoparticles, increased their survival, proliferation and differentiation due to the protection against endogenous peroxynitrite and Aβ-induced mitochondrial fragmentation [22]. Moreover, cardiac progenitor cells when dosed with cerium oxide nanoparticles preserved their proliferative capacity and cellular activity benefited from the reduction of oxidative stress [23].

Based on these studies, we hypothesize the cerium oxide nanomaterials (CeNMs) may be potentially used to improve the stem cell capacity such as proliferation and migration ability, and even protect their survivability particularly under the ROS insult environments. For this purpose, here we use human stem cells derived from dental pulp, as this cell source has been implicated to hold excellent therapeutic capacity in terms of high proliferative rate and profound differentiation to different cell lineages including neural cells [24–27]. We particularly develop the CeNMs with different sizes, namely different aspect ratios, and this is implemented by the approach of a surfactant- and organic template-free pH-controlled hydrothermal method. The CeNMs developed with a range of shapes are treated to the human dental pulp stem cells at various doses. We aim to examine the effects of the treatment of CeNMs on the cell behaviors, including viability, migration, and the salvaging of subcellular components, and then to correlate the events with the cellular uptake and the ROS scavenging role of the nanomaterials. We consider this information will advance future development of CeNMs and their therapeutic uses for stem cell treatment in tissue engineering and regenerative medicine, where the need for ROS control and scavenge is considered critical.

2. Materials and methods

2.1. Hydrothermal synthesis of various shaped cerium oxide nanomaterials

Different shaped CeNMs were prepared using a hydrothermal synthesis method, with a slight modification of a previous work [28]. In a typical cerium oxide nanoparticle synthesis, 0.1 mM of hexadecyltrimethylammonium (CTAB) and 1 mM of cerium (IV) ammonium nitrate ($Ce(NH_4)_2(NO_3)_6$, 99.9% purity) were dissolved in 60 mL of deionized water (DW) for 30 min under pH adjusted to 8.0 with ammonium hydroxide solution. After additional agitation for 60 min, the as-obtained mixing solution was transferred into a bottle held in a stainless steel autoclave, sealed, and maintained at 140 °C for 24 h. As the autoclave cooled to a room temperature naturally, the precipitates were separated by centrifugation, washed with deionized water and ethanol in sequence, and then dried in air at 80 °C for 24 h to obtain the cerium oxide nanoparticle sample.

For the preparation of nanorod- and nanowire-shaped cerium oxide samples [29], supersaturated solutions were first prepared by mixing cerium (III) chloride ($CeCl_3{\cdot}7H_2O$, 99.9%) in various molar concentrations (0.60, 0.35, and 0.12 M). Small amounts of NH_4OH (28%) or HCl, used to adjust pH between 4.0 to 2.0, were dissolved in 50 mL of DW to form a solution 'A'. Then, 0.04 mM of trisodium phosphate hexahydrate ($Na_3PO_4{\cdot}6H_2O$) was dissolved in 10 ml DW to form a solution 'B'. The solution 'B' was introduced to the solution 'A', while vigorously stirring for 10 min. After additional agitation for 60 min, the as-obtained mixing solution was transferred into a bottle held in a stainless steel autoclave, sealed, and maintained at 140 °C for 24 h. After cooling, the precipitates were separated by centrifugation, washed with deionized water and ethanol in sequence, and then dried in air at 80 °C for 24 h to obtain the cerium oxide nanorods and nanowires. CTAB was removed from the synthesized CeNMs according to the method previously established [30]. The CeNMs were dispersed in 60 mL of pure ethanol containing 500 mg of ammonium nitrate and stirred at 60 °C overnight. The CeNMs were collected by a repeated process (x3) of centrifugation at 12,000 rpm for 5 min and washing in deionized water, and then dried at 45 °C for 24 h for further uses.

2.2. Characterizations of CeNMs

The crystal structure was determined by X-ray diffraction (XRD; Ragaku). The samples were scanned in the range of diffraction angle $2\theta = 10–60°$ at a rate of $2°\ min^{-1}$ with a step width of 0.02° 2θ using Cu Kα1 radiation at 40 kV and 40 mA. The nanomorphology of the samples was characterized by scanning electron microscopy (SEM, Hitachi S-3000H) and transmission electron microscope (TEM, JEOL-7100). For the TEM observation, the aqueous suspension of the nanoparticles was drop-cast onto a carbon-coated copper grid, and the grid was air-dried at room temperature. Based on the TEM images, the size and aspect ratio of nanoparticles were calculated and averaged (n = 20 to 30). Zeta (ζ) potential of the samples was measured using a Malvern Zetasizer (ZEN3600; Malvern), at 25 °C in deionized water in triplicate. The size of samples was analyzed by a particle size analyzer (Zetasizer Nano ZS, Malvern Instruments, UK) based on a dynamic light scattering (DLS) measurement. Specific surface area were determined by nitrogen gas adsorption/desorption isotherm at 77 K using a Quadrasorb SI automated surface area and pore size analyzer (2SI-MP-9 Quantachrome). Samples were degassed under vacuum prior to analysis, and the degassing temperature was 100 °C with an outgas time of 4 h. The X-ray photoelectron spectroscopy (XPS; AES-XPS ESCA 2000, Thermo Fisher Scientific Inc.,