Available online at www.sciencedirect.com
ScienceDirect
Nanomedicine: Nanotechnology, Biology, and Medicine 5 (2009) 225–231
nanomedicine
www.nanomedjournal.com

Original Articles: Clinical Nanomedicine, Cancer Radiation Protection

# Protection from radiation-induced pneumonitis using cerium oxide nanoparticles

Jimmie Colon, MS,[a] Luis Herrera, MD,[b] Joshua Smith, BS,[a] Swanand Patil, PhD,[d] Chris Komanski, BS,[a] Patrick Kupelian, MD,[c] Sudipta Seal, PhD,[d,e] D. Wayne Jenkins, MD,[c] Cheryl H. Baker, PhD[a,f,*]

[a]*Cancer Research Institute, M.D. Anderson Cancer Center Orlando, Orlando, Florida, USA*
[b]*Department of Thoracic Surgery, M.D. Anderson Cancer Center Orlando, Orlando, Florida, USA*
[c]*Department of Radiation Oncology, M.D. Anderson Cancer Center Orlando, Orlando, Florida, USA*
[d]*Department of Mechanical, Materials and Aerospace Engineering, Advanced Materials Processing and Analysis Center (AMPAC), University of Central Florida, Orlando, Florida, USA*
[e]*Nanoscience and Technology Center, University of Central Florida, Orlando, Florida, USA*
[f]*Burnett School of Biomedical Sciences, University of Central Florida, Orlando, Florida, USA*

**Abstract** In an effort to combat the harmful effects of radiation exposure, we propose that rare-earth cerium oxide ($CeO_2$) nanoparticles (free-radical scavengers) protect normal tissue from radiation-induced damage. Preliminary studies suggest that these nanoparticles may be a therapeutic regenerative nanomedicine that will scavenge reactive oxygen species, which are responsible for radiation-induced cell damage. The effectiveness of $CeO_2$ nanoparticles in radiation protection in murine models during high-dose radiation exposure is investigated, with the ultimate goal of offering a new approach to radiation protection, using nanotechnology. We show that $CeO_2$ nanoparticles are well tolerated by live animals, and they prevent the onset of radiation-induced pneumonitis when delivered to live animals exposed to high doses of radiation. In the end, these studies provide a tremendous potential for radioprotection and can lead to significant benefits for the preservation of human health and the quality of life for humans receiving radiation therapy.


*Key words:* Radioprotection; Nanoparticles; Pneumonitis; Quality of life

At the onset of radiation exposure, free radicals are formed through ionizing reactions, such as the photoelectric, Compton, and Auger effects. These free radicals react with DNA and RNA, causing molecular alterations, improper segregation of chromosomes during mitosis, and radiation-induced mitotic death (mitotic catastrophe).[1,2] Furthermore, radiation-induced cellular oxidative damage is initiated by the generation of reactive oxygen species (ROS), which are known to change the oxidative status of cells, resulting in changes in mitochondrial function and activation/inactivation of various proteins involved in the apoptotic (cell death) process.[3] When healthy (normal) cells are exposed to radiation, they ameliorate the damaging effect of free radicals by the release of innate protective molecules such as superoxide dismutase (SOD), glutathione, and metallothionine, which increase and intensify DNA repair mechanisms.[3] Nonetheless, although these protective and repair mechanisms for cells are efficient, they are not capable of blocking all of the damage, which ultimately leads to the death of normal tissue.

Received 24 July 2008; accepted 4 October 2008.
This collaboration was made possible through the support of the M.D. Anderson Cancer Center Orlando, University of Central Florida, and the National Science Foundation and the CCMI.
*Corresponding author. Cancer Research Institute, M.D. Anderson Cancer Center, Orlando, 110 Bonnie Loch Court, Orlando, FL 32806, USA.
*E-mail address:* cheryl.baker@orhs.org (C.H. Baker).

1549-9634/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.nano.2008.10.003

In an effort to combat the harmful effects of radiation, various free-radical scavengers have been tested for their ability to protect normal cells and tissues. Free-radical scavengers such as amifostine (Ethyol; MedImmune, Gaithersburg, Maryland), vitamin E, ascorbate, carotenes, melatonin, and lipoic acid derivatives are the subject of many recent reviews.[4-8] However, many of these free-radical scavengers were found to have limited success due to short half-lives (hours or even minutes), lack of penetration to the site of radical production, and daily dosing requirements. In this report we have identified and are investigating a novel approach for the protection of normal cells against radiation-induced cell damage by using cerium oxide ($CeO_2$) nanoparticles.

Most recently, $CeO_2$ nanoparticles have been tested for their ability to serve as free-radical scavengers[9-14] to provide protection against chemical, biological, and radiological insults that promote the production of free radicals. The chemistry of engineered $CeO_2$ nanoparticles supports a potential role as a biological free-radical scavenger or antioxidant. It was suggested that the unique structure of $CeO_2$ nanoparticles, with respect to valence and oxygen defects, promotes cell longevity and decreases toxic insults by virtue of its antioxidant effects that occur when the nanoparticles enter the cells,[15] preventing the accumulation of ROS and thereby preventing the activation of the apoptotic response and death of the cells.[9] In previous reports $CeO_2$ nanoparticles showed no toxic effect on normal breast epithelial CRL 8798 cells and only a slight effect on breast cancer MCF-7 cells at concentrations greater than 50 nM.[13] Furthermore, they selectively conferred radioprotection to normal cells (CRL 8798) compared with the tumor cells (MCF-7).[13]

In this report we hypothesized that $CeO_2$ nanoparticles represent a novel approach to the protection of normal cells from radiation-induced cell damage and tested their efficacy as a new radioprotective compound in vitro on normal lung fibroblast (CCL 135) cells and then in vivo on athymic nude mice.

## Methods

### *$CeO_2$ Nanoparticles Synthesis*

The $CeO_2$ nanoparticles were synthesized using microemulsion process consisting of surfactant sodium bis(2-ethylhexyl) sulfosuccinate (AOT), toluene, and water. All the chemicals were obtained from Aldrich Chemicals Company Inc. (St. Louis, Missouri) and used without further purification. AOT was dissolved in 50 mL of toluene, and 2.5 mL of 0.1 M aqueous cerium nitrate solution were added. The mixture was stirred for 45 minutes, and 5 mL of 30% hydrogen peroxide ($H_2O_2$) solution was then added dropwise. The reaction was carried out for 1 hour, and then the reaction mixture was allowed to separate into two layers. The upper layer was toluene containing nonagglomerated ceria nanoparticles, and the lower layer was aqueous phase. The $CeO_2$ nanoparticles were precipitated by addition of 30% ammonia solution and washed several times with acetone and water for complete removal of the surfactant. The obtained particles were then resuspended in deionized water to the required concentration.

### *Nanoparticles Characterization*

The synthesized $CeO_2$ nanoparticles were characterized using x-ray photoelectron spectroscopy (XPS) and high-resolution transmission electron microscopy (HRTEM). The ceria particles were deposited on the carbon-coated copper grid for HRTEM analysis by dip-coating method. The HRTEM images of the prepared particles were obtained with Philips (Tecnai Series; The Netherlands) transmission electron microscope operating at 300 keV. The XPS data were obtained using a 5400 PHI ESCA (XPS) spectrometer. The base pressure during XPS analysis was $10^{-8}$ torr, and Mg-$K_\alpha$ x-radiation (1253.6 eV) at a power of 300 W was used. The high-resolution narrow spectra were recorded with electron pass energy of 35.75 eV, to achieve the maximum spectral resolution. The binding energy of the Au $4f_{7/2}$ at $84.0 \pm 0.1$ eV was used to calibrate the binding energy scale of the spectrometer. Any charging shift produced by the samples was carefully removed by using a binding energy scale referred to that of 284.6 eV of C (1s) of the hydrocarbon part of the adventitious carbon line.

### *In Vitro Cell Culture Studies*

The cells were trypsinized with a brief exposure to 0.25% trypsin and 0.02% ethylenediamene tetraacetate, and 20,000 cells were delivered to 96-well plates in Dulbecco's Minimal Essential Medium supplemented with 10% fetal bovine serum. In the first set of studies the cells were exposed to 0, 5, 10, 15, 20, 25, 30 Gy of radiation for 48 zhours. Irradiation was performed on the 160-kV cell culture and small animal irradiator (radiation machine) from Kimtron Inc. (Woodbury, Connecticut). Cell viability was determined by measuring the amount of ATP present, which signals the presence of metabolically active cells. The ATP was measured using the Cell Titer-Glo Luminescent Cell Viability Assay (Promega, Madison, Wisconsin). A direct relationship exists between luminescence measured with the Cell Titer-Glo Assay and the number of cells in culture; therefore, the amount of ATP is directly proportional to the number of cells present. The detection of luminescence (reported in relative luminescence units, RLU) was measured by our own Optima Fluor Star Luminometer (BMG Lab Tech, Durham, NC). In the next set of experiments cells were treated with a predetermined optimal concentration of 10 nM (0.0017 μg/mL) of $CeO_2$ and exposed to a single dose of radiation (20 Gy).[13] Forty-eight hours later, cell viability was determined by measuring the amount of ATP present using the Cell Titer-Glo Luminescent Cell Viability Assay (Promega). In

addition, the amount of caspase 3/7 activity was measured by the Caspase-Glo 3/7 Assay (Promega), and the amount of luminescence is proportional to the caspase 3/7 activity.

### *In Vivo Studies*

Animals are and were maintained in facilities approved by the American Association for Accreditation of Laboratory Animal Care (AAALAC) and in accordance with current regulations and standards of the US Department of Agriculture, Department of Health and Human Services, and the National Institutes of Health. Athymic nude mice were and are maintained in the Cancer Research Institute AAALAC-accredited animal facility, which exceeds the national requirements for animal care, with two conventional mouse rooms, two nude mouse rooms, and one quarantine room. The use of animals for this study was and is approved by the M.D. Anderson Cancer Center Orlando's Institutional Animal Care and Use Committee (IACUC) under the IACUC protocol number 06.07.01 (due for continuing review on 22 June 2009). Radiation was administered using an IC160 x-ray cell culture and small animal irradiation system (Kimtron Inc.) located inside the animal facility. In the first set of in vivo experiments a single dose of radiation (12, 15, or 18 Gy) was administered to the thoracic ventral area of non-tumor-bearing athymic nude mice. Nine weeks after irradiation the mice were killed and the lungs were harvested and processed for hematoxylin and eosin (H&E) staining. For immunohistochemistry and H&E staining procedures, one part of the tumor tissue was formalin-fixed and paraffin-embedded and another part embedded in OCT compound (Miles, Inc., Elkhart, Indiana), rapidly frozen in liquid nitrogen, and stored at –80°C for sectioning. Immunofluorescence microscopy was performed using a 20× objective on an epifluorescence microscope equipped with narrow-bandpass excitation filters mounted in a filter wheel (Ludl Electronic Products, Hawthorne, New York). Routine procedures were used to capture images and process on Adobe Photoshop. Histological analysis was performed in collaboration with the pathology team of M.D. Anderson Orlando.

In another set of in vivo experiments, athymic nude mice (25 g) were randomized into the following groups: (1) weekly intraperitoneal (IP) injections of saline ($n$ = 10, control group); (2) thrice-weekly administrations of 5 Gy radiation ($n$ = 10); (3) twice-weekly IP injections of 15 nM (0.00001 mg/kg) $CeO_2$ nanoparticles ($n$ = 5); (4) thrice-weekly IP injections of 150 mg/kg amifostine ($n$ = 5); (5) administration of radiation combined with twice-weekly IP injections of $CeO_2$ ($n$ = 10); and (6) administration of radiation combined with an IP injection of amifostine 30 minutes before irradiation ($n$ = 10). Treatments continued for 2 weeks for a total dose of 30 Gy radiation. The mice were killed and necropsied only when they became moribund or the experiment was terminated. The weight of each mouse was measured and mortality recorded throughout the experiment, and median and percentage survival was determined.

To further investigate the in vivo toxicity of nanoceria, athymic nude mice received daily IP injections of $CeO_2$ nanoparticles (3–5 nm diameter) for 4 days. Each injection consisted of $CeO_2$ nanoparticles diluted in 100 μL deionized water ($diH_2O$). Twenty-five mice were divided equally into five groups. Each group received a total $CeO_2$ nanoparticle dose of 0 ($diH_2O$), 0.135 mg/kg, 1.35 mg/kg, 13.5 mg/kg, or 135 mg/kg. Mice were monitored and each mouse's mortality recorded over a 3-week period.

## Results

To investigate the effects of radiation exposure on cell viability, normal lung fibroblast (CCL 135) cells were exposed to increasing doses of radiation. The cell viability was measured by the quantification of ATP present, which signals the presence of metabolically active cells. As expected, normal cell viability decreased with increasing radiation doses (Figure 1, *A*).

The next set of experiments involved measurement of the extent to which $CeO_2$ nanoparticles could protect normal cells against radiation-induced cell damage. Normal lung fibroblast CCL 135 cells were treated with a predetermined optimal concentration of 0.0017 μg/mL of $CeO_2$ and exposed to a single dose of radiation (20 Gy).[13] Forty-eight hours later, cell viability was measured. Results show that when radiation was administered as a single dose the number of viable cells in culture, as measured by Cell Titer-Glo Luminescent Cell Viability Assay (which signals the presence of metabolically active cells), was significantly decreased. However, when $CeO_2$ was administered 24 hours before irradiation the $CeO_2$ nanoparticles significantly protected the normal lung fibroblast cells from radiation-induced cell death (Figure 1, *B*).

In subsequent experiments normal lung fibroblast CCL 135 cells were treated with a concentration of 0.0017 μg/mL $CeO_2$ nanoparticles and exposed to a single dose of radiation (20 Gy). Forty-eight hours later, caspase 3/7 activity (which signals the presence of apoptosis) was measured. When radiation (20 Gy) was administered as a single dose the levels of caspase 3/7 activity significantly increased as compared with that in control cells (no radiation). However, in the presence of $CeO_2$ nanoparticles the normal cells exposed to radiation were significantly protected and the activity of caspase 3/7 was significantly decreased compared with control cells, and with cells exposed to $CeO_2$ nanoparticles alone or radiation alone (Figure 1, *C*).

Radiation pneumonitis and subsequent pulmonary fibrosis can significantly decrease the quality of life of humans exposed to radiation. Therefore, in another set of experiments a murine model of radiation-induced pneumonitis was established. A single dose of radiation was administered to the thoracic ventral area of non-tumor-bearing athymic nude

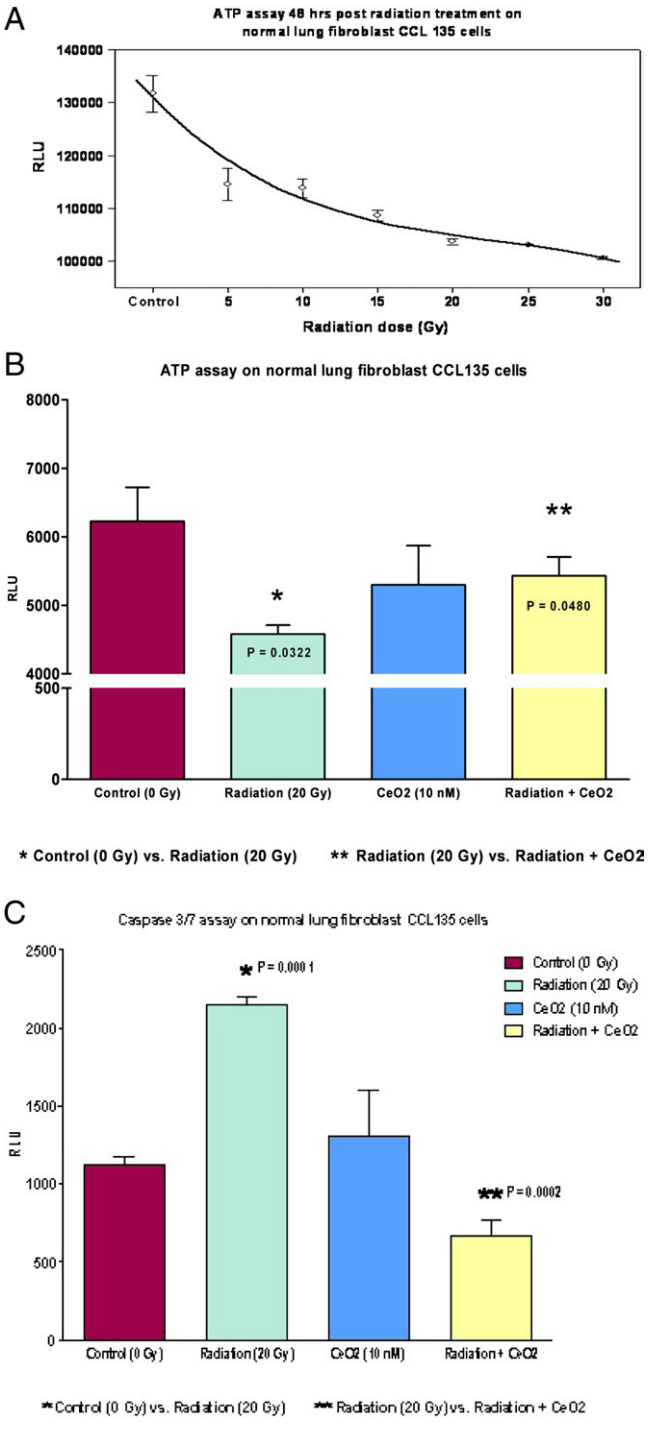


Figure 1. Cerium oxide nanoparticles protect normal lung fibroblasts against radiation-induced cell damage. **(A)** Cell viability of normal lung fibroblasts (CCL 135) 48 hours after exposure to varying doses (Gy) of radiation indicates decreased viability with increased radiation dose. **(B)** CCL 135 cells were exposed to 20 Gy radiation in the absence or presence of 10 nM (0.0017 μg/mL) $CeO_2$, and 48 hours after exposure cell viability was measured by Cell Titer-Glo Luminescent Cell Viability Assay (cell number correlates with luminescent output (RLU). **(C)** Caspase 3/7 activity (measurement of cell apoptosis) was measured by the Caspase-Glo 3/7 Assay (luminescence output in RLU is proportional to the amount of caspase activity present).

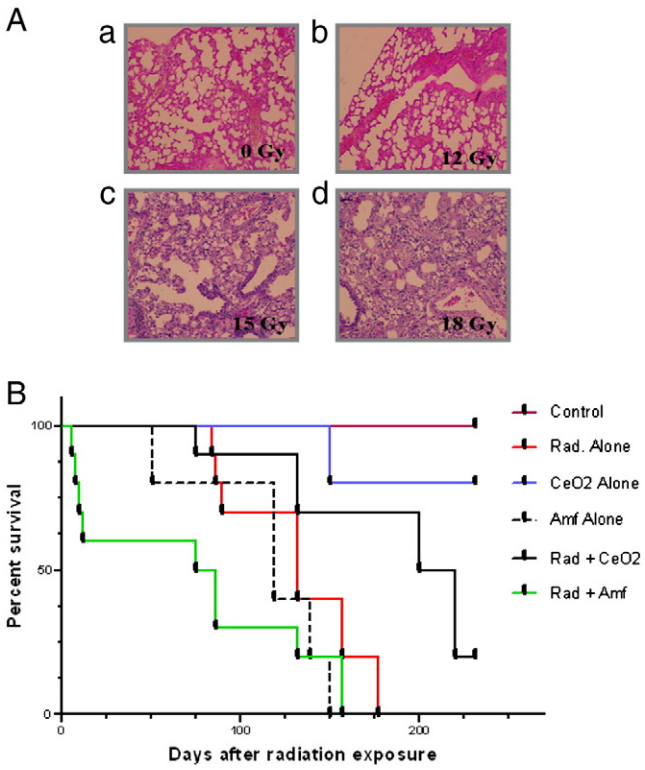


Figure 2. Radiation-induced pneumonitis and tolerability of cerium oxide nanoparticles in mice. **(A)** H&E stains of murine lungs 9 weeks after a single dose of radiation (12, 15, or 18 Gy) administered to the thoracic ventral area of non-tumor-bearing athymic nude mice (panels b–d). Control mice were not radiated (panel a). Results indicate that a successful murine model of radiation-induced pneumonitis was developed, and histology analyses show established pneumonitis (with extensive macrophage invasion) in the lungs of those mice receiving 15 (panel c) and 18 Gy (panel d) of radiation. **(B)** $CeO_2$ nanoparticles were well tolerated by mice, and the median survival of irradiated mice was significantly increased in mice pretreated with 15 nM (0.00001 mg/kg) $CeO_2$ (50% alive on day 225) as compared with mice treated with radiation alone (50% alive on day 132) or pretreated with 150 mg/kg amifostine (Amf) before radiation (50% alive on day 81). Note that 20% of mice treated with $CeO_2$ alone were terminated on day 150 for histology analysis.

mice. Results indicate that 9 weeks after irradiation a successful murine model of radiation-induced pneumonitis was developed. Histology analyses show established pneumonitis in the lungs of those mice receiving 15 and 18 Gy of radiation (Figure 2, *A*).

In an attempt to administer nanoparticles to live animals and to evaluate the radiation protection activity of $CeO_2$ nanoparticles, the survival of non-tumor-bearing athymic nude mice was measured. Non-tumor-bearing athymic nude mice were exposed to fractionated doses of 30 Gy radiation (weekly administration of 5 Gy) in the presence or absence of twice weekly IV injections of $CeO_2$ nanoparticles or IP injections of amifostine 30 minutes before radiation. Results show (Figure 2, *B*) that $CeO_2$ nanoparticles are well tolerated by athymic nude mice and protect mice from radiation-associated death. All control mice lived until termination date of 231 days. In mice treated with $CeO_2$ nanoparticles

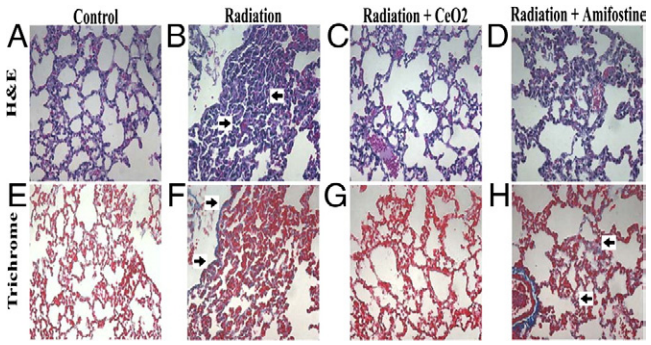


Figure 3. $CeO_2$ nanoparticles protect lungs from radiation-induced pneumonitis. H&E stains to assess lung damage in normal lungs **(A)**, lungs from mice treated with radiation alone **(B)**, lungs from mice treated with radiation plus $CeO_2$ **(C)**, and lungs from mice treated with radiation plus amifostine **(D)**. The H&E stains show significant lung damage in mice treated with radiation **(B)**. Radiation-induced cell damage is minimized in lungs of mice treated with radiation in combination with $CeO_2$ **(C)**, and these lungs appear normal as shown in control **(A)**. The amount of fibrosis and collagen deposition (indicative of chronic lung conditions) was measured by using Masson's Trichrome stain. Results show that fibrosis and collagen deposition (indicated by arrows) were common in the lungs of those mice receiving radiation alone **(F)** and in lungs of those mice receiving a pretreatment of amifostine **(H)**. The amount of fibrosis and collagen deposition in lungs of mice treated with radiation in combination with $CeO_2$ **(G)** was minimal, and these lungs appeared normal **(E)**.

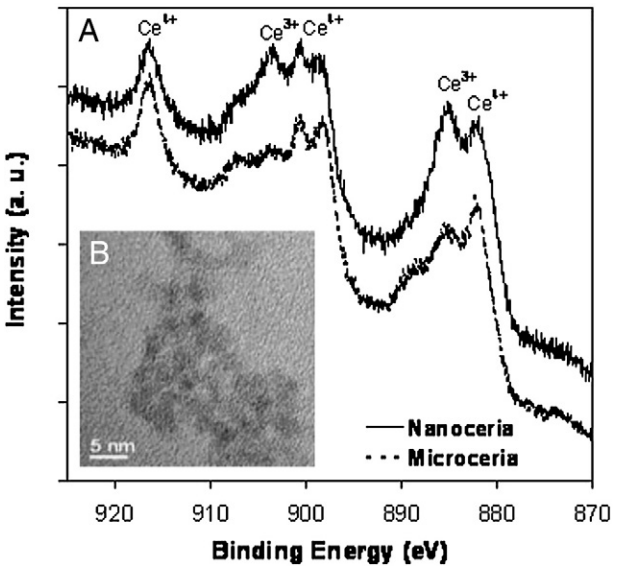


Figure 4. Characterization of $CeO_2$ nanoparticles (nanoceria). **(A)** Comparative high-resolution Ce 3d XPS spectra of micro- and nanoceria indicates high concentration of $Ce^{3+}$ in nanoceria compared with microceria particles. Peaks at 882.1 and 886 eV correspond to $Ce^{4+}$ and $Ce^{3+}$ peaks. Peaks at 918 eV correspond to satellite peaks indicating the presence of $Ce^{4+}$ peak (see Methods for details of data collection). **(B)** HRTEM image of the synthesized nanoceria particles indicating the particle size of 3–5 nm with fluorite lattice structure.

alone, 20% were killed on day 150 for histology analysis. The remaining 80% were alive until the termination date of 231 days. After treatment with radiation alone, amifostine alone, and a combination of radiation and $CeO_2$ nanoparticles, or radiation and amifostine, the median survival times were 132, 119, 225, and 81 days, respectively (control versus radiation, $P < .019$; control versus $CeO_2$, $P < .66$; control versus amifostine, $P < .0370$; radiation versus radiation and $CeO_2$, $P < .0041$; radiation versus radiation and amifostine, $P < .0432$). In contrast, amifostine was highly toxic, as shown by the significant difference in median survival time (as compared with control mice). In summary, these results suggest that $CeO_2$ nanoparticles are well tolerated by mice and have a considerable advantage over the clinically used amifostine.

To determine the degree of radiation-induced pneumonitis, the lungs were harvested and processed for histology and H&E staining. The lungs from mice in the control group (radiation alone) showed visible pneumonitis with extensive macrophage invasion, whereas the lungs from irradiated mice receiving $CeO_2$ nanoparticles showed no visible pneumonitis and appeared normal (Figure 3). In addition, the amount of fibrosis and collagen deposition (indicative of chronic lung conditions) was measured in the lungs of control mice (no radiation, normal lungs) or in lungs of those mice treated with radiation alone, radiation plus $CeO_2$, or radiation plus amifostine, using Masson's Trichrome stain. The histology analyses show that fibrosis and collagen deposition were common in the irradiated lungs of those mice given radiation alone and of those mice given a pretreatment of amifostine (Figure 3). Furthermore, the faint blue stain indicated that collagen deposits were relatively recent, as compared with the dark blue staining of older, more cross-linked collagen seen in human chronic lung diseases. In sharp contrast, no significant Trichrome staining was observed in normal lungs (control) or in those irradiated lungs of mice treated with $CeO_2$.

The in vivo toxicity was monitored by injecting mice with increasing doses of nanoceria (0–135 mg/kg). No mice died or experienced notable side effects from the doses injected, and on day 21 the mice were necropsied and no abnormal pathologies were observed (data not shown). Currently there are very few reports regarding the biological effects of $CeO_2$ nanoparticles. Recent studies have shown that $CeO_2$ nanoparticles increase neuronal life span in culture. The biological activity of the $CeO_2$ nanoparticles was evaluated in a tissue culture model of rat cells and was shown to prolong brain cell longevity in culture by two- to threefold.[14] Furthermore, these nanoparticles reduced $H_2O_2$ and ultraviolet light–induced cell injury by over 60%. It has been proposed that $CeO_2$ nanoparticles act as regenerative free-radical scavengers to give these beneficial effects in biological systems.[9,12,13] The mechanism is based on the dynamic valence state of Ce ($Ce^{3+} \leftrightarrow Ce^{4+}$) in the $CeO_2$ nanocrystals. The comparative Ce 3d XPS spectra of micro- and synthesized nano-$CeO_2$ particles are shown in Figure 4, *A* and *B*, respectively. At nanoscale, the large surface area of $CeO_2$ leads to formation of more $Ce^{3+}$ as a result of ease of formation of oxygen vacancies.

Such high concentration of oxygen vacancies and associated $Ce^{3+}$ cations gives high catalytic properties to $CeO_2$ nanoparticles.[16] The free-radical scavenging property of $CeO_2$ nanoparticles has been also attributed to the presence of oxygen vacancies. It is also suggested that there is an autoregenerative reaction cycle continuing on the surface of $CeO_2$ nanoparticles that provides an unprecedented antioxidant property.[12]

## Discussion

In this report $CeO_2$ nanoparticles were shown to confer protection against radiation-induced cell damage in vitro (normal lung fibroblast CCL 135 cells) and in vivo (athymic nude mice), suggesting that $CeO_2$ nanoparticles are an effective radioprotectant for normal tissues. Therapeutic applications of cerium oxide nanoparticles are not expected to be limited by in vivo toxicity, because athymic nude mice tolerated nanoceria at doses 3.4 million times greater than the effective therapeutic dose. At the onset of radiation exposure, free radicals are formed through ionizing reactions that are then capable of destroying normal tissues. Although cells release a certain number of protective molecules, such as glutathione and metallothionine, their incapability to block all damage results in the death of normal tissues. In an effort to combat the harmful effects of radiation, various free-radical scavengers have been tested for their ability to protect normal cells and tissues. The most effective free-radical scavenger thus far is amifostine, whose active free thiol metabolite WR-1065 has been shown to prevent both radiation-induced cell death and mutagenesis while facilitating the repair of normal cells. Although amifostine is the only clinically relevant compound, this drug has a short half-life in serum and causes serious side effects that make it difficult and costly to administer.

We have engineered nonagglomerated $CeO_2$ nanoparticles, (3–5 nm diameter), by a novel microemulsion process. The present study proposes the use of these engineered nanoparticles for protection against radiation-induced pneumonitis. Our initial results on normal breast cells show almost complete protection from irradiation of 10 Gy.[13] We hypothesize that the mixed-valence state ($Ce^{3+}$ + $Ce^{4+}$) present in the engineered nanoparticles of $CeO_2$ act as free-radical scavengers to protect the cells from radiation damage.

It is important to acknowledge that conflicting results have been published pertaining to the toxicity of $CeO_2$ nanoparticles.[17,18] However, these conflicting results can be resolved by accounting for variations in the nanoparticles' size, crystal structure, and surface chemistry. Furthermore, the synthetic method, storage duration, and re-dispersion technique seem to have a major role in the biological application of $CeO_2$ nanoparticles. The culmination of these variations seems to determine the toxicity of $CeO_2$ nanoparticles. Therefore, although the $CeO_2$ nanoparticles used in this study were nontoxic at doses up to 135 mg/kg in athymic nude mice, $CeO_2$ nanoparticles produced by different synthetic methods that are a different size and shape would be expected to have different degrees of toxicity. More experimentation is required to elucidate the specific function of each of these characteristics.

Free-radical scavengers such as amifostine, vitamin E, ascorbate, carotenes, melatonin, and lipoic acid derivatives possess few active sites per molecule. A more recently investigated antioxidant, C60, may be able to scavenge a comparatively higher number of radicals than the currently available antioxidants,[19] but the limited number of free-radical scavenging sites means that repeated dosing is required to replace molecular species that were used in free-radical reduction. However, $CeO_2$ nanoparticles offer many active sites for free-radical scavenging because of their large surface-to-volume ratio and, more importantly, their mixed-valence states for unique redox chemistry. A recent article reports SOD mimetic activity of $CeO_2$ (ref. 20). Additionally, as indicated in our previous work, the free-radical scavenging property of $CeO_2$ nanoparticles is regenerative,[12] which is not the case for other antioxidants. We believe that the chemical nature of $CeO_2$ nanoparticles, in which an autoregenerative reaction cycle ($Ce^{3+} \rightarrow Ce^{4+} \rightarrow Ce^{3+}$) continues on their surface, is probably the mechanism by which the material gains an unprecedented free-radical scavenging ability.

Nanotechnology is a multidisciplinary field that involves the design and engineering of objects smaller than 100 nm in diameter. Nanoparticles constitute a new generation of free-radical scavengers. The role of nanoparticles as radioprotectants is a cutting-edge development addressing decades of scientific interest regarding the protection of normal cells and tissues from radiation. The chemistry of engineered $CeO_2$ nanoparticles supports a potential role as a biological free-radical scavenger or antioxidant. Our study suggests that these nanoparticles may be a therapeutic regenerative material that will scavenge ROS that are responsible for radiation-induced cell damage.

When biological systems are under high-energy exposure, such as astronauts in long-duration space exploration, astronauts are exposed to numerous sources of oxidative stress, including radiation, elevated oxygen exposure, and physical and psychological stress. When ROS are produced at high levels, cellular components can be damaged. These ROS can be used by biological systems as a defense mechanism against microorganisms and can act as signal transduction and transcription agents in development, stress responses, and programmed cell death. Oxidative stress arises from the strong cellular oxidizing potential of excess ROS, or free radicals. In addition, elevated levels of oxidative damage are related to increased risks for cataracts, cardiovascular disease, and cancer.

Therefore, the potential benefit of radioprotection using $CeO_2$ nanoparticles is of great importance on multiple levels—most importantly its potential impact on human life. This research is relevant to the health and quality of life

of humans worldwide who are exposed to radiation environments such as those listed below:

- Patients receiving radiation treatments for cancer
- Astronauts exposed to particle radiation
- Military and civilians potentially exposed to radiation in battle, terrorism, or occupational exposure

Verification of the effectiveness of nanoparticles as radioprotectors opens the field for future studies that would examine, in depth, the mechanism, tissue distribution, and safety of $CeO_2$ nanoparticles, before their use in phase I clinical trials. In the end, we hope that this study can lead to faster recovery and improved quality of life for the individuals suffering from radiation damage.

## Acknowledgments

We thank Donna Schade (M.D. Anderson Cancer Center Orlando) for helping in preparing this manuscript.

## References

1. Cohen-Jonathan E, Bernhard EJ, McKenna WG. How does radiation kill cells? Curr Opin Chem Biol 1999;3:77-83.
2. Nair CKK, Parida DK, Nomura T. Radioprotectors in radiotherapy. J Radiat Res (Tokyo) 2001;42:21-37.
3. Pradhan DS, Nair CKK, Sreenivasan A. Radiation injury repair and sensitization of microorganisms. Proc Ind Natl Sci Acad B 1973;39: 516-30.
4. Beckman KB, Ames BN. The free radical theory of aging matures. Physiol Rev 1998;78:547-81.
5. Marcholi R, Schweiger C, Levantesi G, Tavazzi L, Valagussa F. Antioxidant vitamins and prevention of cardiovascular disease: epidemiological and clinical trial data. Lipids 2001;36:S53-63.
6. Kaul N, Devaraj S, Jialal I. Alpha-tocopherol and atherosclerosis. Exp Biol Med 2001;226:5-12.
7. Meldrum BS. Implications of neuroprotective treatments. Prog Brain Res 2002;135:487-95.
8. Maxwell AJ. Mechanisms of dysfunction of the nitric oxide pathway in vascular diseases. Nitric Oxide 2002;6:101-24.
9. Chen J, Patil S, Seal S, McGinnis JF. Rare earth nanoparticles prevent retinal degeneration induced by intracellular peroxides. Nat Nanotechnol 2006;1:142-50.
10. Schubert D, Dargusch R, Raitano J, Chan SW. Cerium and yttrium oxide nanoparticles are neuroprotective. Biochem Biophys Res Commun 2006;342:86-91.
11. Brunner TJ, Wick P, Manser P, Spohn P, Grass RN, Limbach LK, et al. In vitro cytotoxicity of oxide nanoparticles: comparison to asbestos, silica, and the effect of particle solubility. Environ Sci Technol 2006;40:4374-81.
12. Das M, Patil S, Bhargava N, Kang JF, Riedel JM, Seal S, et al. Autocatalytic ceria nanoparticles offer neuroprotection to adult rat spinal cord neurons. Biomaterials 2007;28:1918-25.
13. Tarnuzzer RW, Colon J, Patil S, Seal S. Vacancy engineered ceria nanostructures for protection from radiation-induced cellular damage. Nano Lett 2005;5:2573-7.
14. Rzigalinski BA, Bailey D, Chow L, Kuiry SC, Patil S, Merchant S, et al. Cerium oxide nanoparticles increase the lifespan of cultured brain cells and protect against free radical and mechanical trauma. FASEB J 2003; 17:A606.
15. Patil S, Sandberg A, Heckert E, Self WT, Seal S. Protein absorption and cellular uptake of cerium oxide nanoparticles as a function of zeta potential. Biomaterials 2007;28:4600-7.
16. Rodriguez JA, Ma S, Liu P, Hrbek J, Evans J, Perez M. Activity of $CeO_x$ and $TiO_x$ nanoparticles grown on Au(111) in the water-gas shift reaction. Science 2007;318:1757-60.
17. Park E, Choi J, Park Y, Park K. Oxidative stress induced by cerium oxide nanoparticles in cultured BEAS-2B cells. Toxicology 2008;245:90-100.
18. National Toxicology Program, National Institute of Environmental Health Sciences. National Institutes of Health, U.S. Department of Health and Human Sciences, Research Triangle Park, NC. www.ntp.niehs.nih.gov.
19. Gharbi N, Pressac M, Hadchouel M, Szwarc H, Wilson SR, Moussa F. [60] Fullerene is a powerful antioxidant in vivo with no acute or subacute toxicity. Nano Lett 2005;5:2578-85.
20. Heckert EG, Karakoti AS, Seal S, Self WT. The role of cerium redox state in the SOD mimetic activity of nanoceria. Biomaterials 2008;18: 2705-9.