Ceria nanoparticle theranostics: harnessing antioxidant properties in biomedicine and beyond

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Abstract
Theranostic nanoparticles (NPs) which provide both therapeutic and diagnostic capabilities have potential to fundamentally change biomedical sciences and improve disease diagnostics and therapy. This review summarizes the recent advances in the development of ceria NPs (CeNPs) therapeutics with combined free radical scavenging activity and biosensing functions as a promising class of theranostic probes in biomedicine. The unique physicochemical properties of CeNPs including the antioxidant, anticancer and anti-inflammatory properties are discussed in relation to their therapeutic efficacy in disease models including neurodegenerative diseases, anti-inflammatory, hypoxic damage, ischemia-reperfusion. The potential to combine the antioxidant properties with sensing functions to achieve synergistic therapeutic and biosensing functions is highlighted with a focus on personalized medicine and next generation therapy. The current state-of-the-art, challenges and opportunities for future development of CeNPs as active theranostic probes in biomedicine are also discussed.

1. Introduction

The rapid development of nanotechnology enabling precise fabrication of functional nanoparticles (NPs) that exhibit interesting physical and chemical properties due to their small size and large surface area have provided opportunities to improve therapeutic interventions for a variety of diseases [1] revolutionizing medicine and pharmacology. This progress is illustrated by an increasing number of nano-enabled drugs and therapies developed for in-vitro, in-vivo, and ex-vivo applications, as targeted drug delivery systems, implantable sensors and bio-scaffolds, and a large body of research which has shown significant promise especially in regenerative medicine [2].

The ability to rationally design the size and functional properties of NPs provides exciting opportunities to improve targeting, prolong retention time within the body and deliver drugs and imaging reagents by doping or loading the targeting domain (e.g. imaging agents like fluorophores, antibodies for specific targeting or therapeutic agents) on their surface [1, 3]. Nanotechnology has shown the ability to develop improved drug delivery and imaging techniques by attaching or encapsulating probes of fluorescent agents in nanomaterials, imparting fluorescence, enhancing pharmacokinetic behavior and providing specific targeting to the desired tissues. Therefore, NP delivery systems with imaging or sensing capabilities are promising candidates to enhance effectiveness of diagnostics. A variety of NPs-mediated diagnostics and therapeutic strategies can be designed that have potential to surpass conventional pharmaceutical approaches. To date, a wide range of nanomaterials have been studied, including polymers, liposomes, dendritic structures, metal and metal oxide NPs. Of these, some oxide NPs such as cerium oxides (CeO2, nanoceria, or CeNPs) have intrinsic free radical scavenging properties and therefore can by themselves be used as a drug to inhibit free radical formation, preventing oxidative damage. Additionally, these particles can be engineered as tiny biosensors through doping or surface modification to perform concomitant
sensing functions. These collective functions, e.g. inhibition of free radicals and sensing, would enhance the efficacy of treatment and provide opportunities for the diagnosis and treatment of a variety of diseases.

Cerium is a rare earth element in the lanthanide series that exists in two oxidation states, Ce³⁺ and Ce⁴⁺ [4]. The chemical and physical properties of CeO₂ have been studied for over 30 years [5] and their use in industrial applications such as polishing [5], gas sensors [6], solid oxide fuel cells [2], additive in diesel fuels [7] and in various electronic equipment [8] is well documented. In the past 10 years, there has been increasing interest in studying ceria NPs (CeNPs) in biomedicine due to their more recently discovered antioxidant properties, similar to antioxidant enzymes. CeNPs can switch reversibly between Ce³⁺/Ce⁴⁺ states which exist simultaneously at their surface, while losing an oxygen in the lattice. These properties provide a unique ability to these NPs to act as a recyclable and regenerative catalyst. Therefore, CeNPs are used as catalase (CAT) and superoxide dismutase (SOD) mimetics to inactivate free radicals and mitigate oxidative stress in biological systems.

This paper provides an overview of the status of CeNP research as inorganic antioxidants and sensing probes and their potential as next generation theranostics tools for treatment and therapeutic applications in biomedicine. We first describe the physico-chemical properties that make CeNPs unique and briefly outline pharmacological approaches for therapy using these particles. Then, we highlight the biosensing functions of CeNPs, focusing on the effect of doping and surface modification to achieve specific imaging and targeting functions. We then highlight the key features of CeNPs as multifunctional theranostics systems exhibiting the ability to inactivate and scavenge reactive oxygen species (ROS), acting as an inorganic antioxidant, and visualize changes in the local microenvironment, providing an opportunity to concomitantly monitor therapeutic efficacy. Finally, we discuss potential toxicity issues related to use of these materials and highlight potential strategies for improving their efficacy in biomedicine and beyond.

2. Theranostic NPs

Theranostic NPs which provide both therapeutic and diagnostic capabilities have potential to radically move molecular sciences toward personalized medicine and improved therapeutics. Conventional theranostic systems include platforms in which the NP is a delivery vehicle loaded with a therapeutic and an imaging reagent. Commonly used NPs include supermagnetic iron oxides (SPIONs) for magnetically guided delivery, and gold nanostructures as well as quantum dots (QDs), carbon-based materials and more recently upconversion NPs for near IR imaging [9–13]. Theranostic NPs are complex biomolecular assemblies, ideally equipped with the ability to control the release of therapeutic agent, and provide concomitant imaging or sensing functions.

The desirable performance of theranostic NPs are: (a) biocompatibility, (b) stability, selectivity and rapid accumulation at the target of interest, (c) rapid response of biochemical changes or markers of disease, (d) effective therapeutic function, ideally on demand, without damaging healthy organs, (e) clearance or biodegradation into nontoxic products. Theranostic NPs can be created in several ways. The most common method involves conjugation of therapeutic agents to fluorescent NPs. Alternatively, loading imaging agents such as fluorescent dyes or various isotopes to NPs can also be performed. Incorporating both the therapeutic drug with the imaging agent into a composite NP platform is also possible. A different type is to use the NPs that have inherent therapeutic properties and modify them by conjugation with fluorescent agents or by doping with luminescent ions. Development of material systems that incorporate functional NPs possessing unique intrinsic reactivity and signal-responsive function and that are stable and functional in biological settings is an essential step in the creation of molecular theranostic probes. The primary challenge is to develop such properties and achieve control over the NP system while maintaining the useful functions in the biological environment.

Several NP systems have been explored as molecular probes for fluorescence-based measurements and MRI applications in biomedicine. Figure 1 summarizes the different types of theranostic NPs that can be used for simultaneous imaging and therapy in biomedicine. While most commonly used fluorescence detection are based on organic dyes, inorganic NPs (e.g. QDs) have been created as functional fluorophores that can be easily modified and used as fluorescent probes to improve performance (e.g. aqueous solubility, monodispersity and biocompatibility) and enable imaging and sensing applications in biological environments [14–18]. In addition to QD, systems made of a crystalline host and lanthanide (Ln⁴⁺) dopants, have been created as a new class of fluorescence nanostructures [19, 20]. Several studies have demonstrated the applicability of fluorescent rare earth doped NPs in biomedicine [21, 22]. Among the various types of NP candidates that can be used as theranostics, cerium-based oxide NPs is a relatively new class that has high reactivity, oxygen storage and release characteristics as well as the ability to scavenge free radicals. CeNPs can be engineered through doping or surface modification to provide additional targeting and measurement capabilities. Therefore, CeNPs have potential to be used as antioxidant and serve as tiny biosensors to
Figure 1. Summary of theranostic NPs and possible functionalities for dual therapeutic and diagnostics applications.

indicate changes specific to the local physiological environment and connect targeted delivery with biosensing functions by directly relating the readout of the biosensor to a therapeutic output.

3. Cerium oxides: unique properties, chemistry, and surface reactivity

CeO$_2$ is a rare earth oxide material used in catalytic convertors, fuel cells, chemical mechanical polishing and sensing [23, 24]. In biological environments, CeNPs have demonstrated unique catalytic and antioxidant properties including the ability to mimic redox enzymes like catalase, SOD, peroxidase, and oxidases, and to scavenge reactive oxygen and nitrogen species (ROS/RNS), depending on their size and surface properties [25]. Excessive production of ROS generates oxidative stress and affects cell metabolism in biological systems. High levels of ROS can induce cell death, biological aging, and disease. The capability of CeNPs to inactivate ROS [26–29] and protect cells against oxidative damage [30, 31] by preventing formation of free radicals [32] is well established suggesting potential therapeutic value in the treatment of oxidative diseases, inflammation, and aging [26, 33–35]

These unique properties are the consequence of the dual Ce$^{3+}$/Ce$^{4+}$ state [30] and O$_2$ vacancies at the CNPs surface [30, 32]. The presence of mobile lattice oxygen and the large diffusion coefficient facilitate the conversion Ce$^{4+}$ ↔ Ce$^{3+}$ and thus, allow oxygen to be released or stored in its cubic structure (figure 2) [30, 36, 37]. Important to biomedical applications is the dependence of these properties on synthetic method, surface coating, size, morphology, Ce$^{3+}$/Ce$^{4+}$ ratio, and reaction conditions [28, 38–46]; these properties also can be enhanced by doping with Zr, Pd, Ti and Pt [47, 48]. For a given morphology, their activity is related to the concentration of surface oxygen defects [49]. Therefore, controlling these parameters is critical for the utilization and practical use of these materials. Formation of oxygen vacancies is accompanied by Ce$^{4+}$/Ce$^{3+}$ reduction, which is responsible for its enzyme-mimetic activities [25, 50]. The surface and bulk Ce$^{3+}$ can be re-oxidized by oxygen or other agents [51] enabling regeneration and providing opportunities for recycling and reuse. The reduction of ceria leads to the formation of oxygen vacancies in its structure [52] as shown in the figure 2(A). This redox exchange provides opportunities for a variety of applications from an enzyme mimetic and a therapeutic agent to an environmental catalyst [53].

X-ray photoelectron spectroscopy (XPS), FTIR and UV–Vis studies [54] showed that CeNPs treated with H$_2$O$_2$ have a decreased ratio of reduced to oxidized cerium (Ce$^{3+}$/Ce$^{4+}$) and confirmed the presence of peroxyl groups on ceria [55]. The best known mechanism of ROS inactivation involves oxidation/reduction and oxygen exchange at the CeNP surface [32]. The coexistence of the Ce$^{3+}$/Ce$^{4+}$ enables CeNPs to react catalytically with both O$_2^{−}$ and H$_2$O$_2$ (oxidation of Ce$^{3+}$ and reduction of Ce$^{4+}$) [28, 29, 56] and neutralize a wide range of ROS [57, 58]. Most reported works are detailing the ROS-scavenging properties of CeNPs. The reactivity of CeNPs for H$_2$O$_2$ has been the most studied. Although essential to their function, the
Figure 2. (A) Schematic representation for charge distribution for the redox process for ceria lattice. The process results in a neutral oxygen vacancy. The Ce atoms are located at the apex of tetrahedron while the O$_2$ is centrally located for the oxidized state. The oxidation states of the individual atoms are mentioned. (B) CeNPs reactivity, Ce$^{3+}$/Ce$^{4+}$ switching and O$_2$ release mechanism and (C) CeNPs reactivity for H$_2$O$_2$ displaying characteristic color changes of a CeNPs dispersion (20 nm particles) upon addition of H$_2$O$_2$.

oxygen-transport properties and quantitation of ROS species in relevant biological environments have been less studied. It has been shown that H$_2$O$_2$ induces oxidation of the surface exposed Ce$^{3+}$ to Ce$^{4+}$ and formation of O$_2^-$ complexes at the particle surface, confirmed by FTIR and XPS spectroscopy [55]. XPS spectra revealed the presence of both Ce$^{3+}$ and Ce$^{4+}$ species, which change after addition of H$_2$O$_2$, indicating a redox mechanism. The presence of the O$_2^-$ stretching vibration (852 cm$^{-1}$) in the H$_2$O$_2$ treated sample provides evidence for adsorption of O$_2^-$ demonstrating a surface adsorption mechanism.

CeNPs, can be produced by a variety of synthetic procedures including solution precipitation, sonochemical, ball milling, hydrothermal, solvothermal, thermal hydrolysis, and sol-gel method. The most common methods (wet chemical precipitation and calcination) make use of solvents and materials that can have a negative impact on the human body, and therefore the use of biocompatible reagents and surface coatings is desirable when exploring biomedical applications of these materials. Recent studies have shown that CeNPs can be synthesized in an environmentally friendly way by making use of plant-based solvents and materials. These eco-friendly methods are efficient, have low or no impact on the environment, and are potentially safe for use in therapeutic and pharmacological applications [59]. Examples include: (a) plant- and fungal-mediated synthesis that make use of natural plant extracts as stabilizing and capping agents; (b) ovalbumin and lysozyme used as to create electrostatic interaction between the cerium ions which can help produce small and stable NPs, (c) biopolymers to form large molecular structures which can help stabilizing small and size-controlled particles.

4. CeNPs as sensing probes: tailoring surface reactivity and fluorescence properties

CeNPs with enzyme like activity have been recently explored as a new wave of molecular probes for detection of molecular targets and in situ imaging in biological environments. Several sensing platforms in which CeNPs were used as inorganic probes to replace soluble redox dyes and oxidase and peroxidase enzymes [55] for detection of H$_2$O$_2$, glucose, dopamine, glutamate and polyphenols [42, 60, 61] monitored by spectroscopic and electrochemical methods [42, 55, 62–70] have been reported in literature.

Native CeNPs have weak emission and therefore their use in fluorescence imaging is limited [71–73]. To impart fluorescence, CeNPs were doped with luminescent ions having strong fluorescence [19, 74, 75]. In particular, CeNPs doped with lanthanide ions added to the host lattice in low concentrations were shown to provide strong fluorescence [76]. Due to formation of oxygen vacancies in the lattice, doping also enhances catalytic activity [77], thus improving the overall reactivity and the enzyme-like properties of these particles. This aspect has been studied in depth theoretically as well as experimentally [78–80]. Since europium (Eu$^{3+}$) has strong red emission signal [19, 74, 75] and its radius (0.1066 nm) is in between that of Ce$^{3+}$ (0.1143 nm) and Ce$^{4+}$ (0.097 nm), Eu$^{3+}$ is one of the most promising dopants, enabling homogenous doping and formation of stable luminescent for CeNPs [81]. Another exciting application of the doped particles is the use as MRI contrast agents; most currently used are Gd (III) based agents (GBCAs) [82]. Gd possesses seven
unpaired electrons leading to high magnetic moment and a slow relaxation rate, which make it an ideal contrast agent. GBCAs deliver diagnostic information almost immediately after injection in body and has very low adverse effects, however, their retention is low [83] and can be improved by incorporation of Gd within CeNPs [82]. The ionic radius of Gd (0.105 nm) is amenable for incorporation into the CeNP matrix, making this a powerful theranostic candidate. This doping was shown to enhance the Ce$^{3+}$ concentration in the CeNPs [82]. CeNPs with fractions of up to 50% Gd have shown high MRI-contrast properties; the higher Gd concentrations reduced the size and increased the aspect ratio of the NPs. Upon H$_2$O$_2$ treatment, the Gd-doped CeNPs showed a shift in the Ce3d near edge x-ray absorption fine structure NEXAFS spectra of 3$^+$ and 4$^+$ oxidation states. Figure 3 shows the concept of Gd-doped CeNPs that incorporate antioxidant and MRI contrast enhancing properties. The theranostic capability of these particles as combined ROS scavenger and MRI contrast agent was demonstrated in human neutrophils [82].

To obtain the necessary magnetic properties while avoiding the Gd toxicity, Gd-doped CeNPs were obtained by substituting the Gd in place of the Ce$^{4+}$ ions in the lattice since the ionic radius of Gd$^{3+}$ lies in between of Ce$^{3+}$ and Ce$^{4+}$. These particles are shown to retain the magnetic properties of Gd while having better biocompatibility than that of GBCAs. Studies have also been done on capped Gd particles to improve their biocompatibility [46, 84]. Naumo et al have illustrated recently the magnetic properties of lanthanide-doped CeNPs, demonstrating the ability of these particles to serve as suitable nanoparticles that may serve a dual purpose as MRI contrast agents and antioxidants [85].

CeNPs prepared via high temperature thermal decomposition of ceria precursors [86, 87] from cerium (III) nitrate, Ce(NO$_3$)$_3$·6H$_2$O, was used as a nanoenzyme-mimetic fluorescence probe to quantitatively measure H$_2$O$_2$, dopamine, glucose, lactate, alkaline phosphatase activity and neurotransmitters [76]. Eu$^{3+}$ was used as dopant to introduce intrinsic fluorescence and enhance oxygen vacancies. Stabilization of the CeNPs in biological fluids was achieved via coating with poly(acrylic acid) octylamine copolymer (PAA-OA). High-resolution transmission electron microscopy (HRTEM), energy dispersive x-ray spectroscopy (EDX), and x-ray diffraction (XRD) (figure 4) showed uniform CeNPs with a size distribution of 4.7 ($\pm$0.14) nm. XRD pattern of Eu-CNPs shows 1.49 ($\pm$0.25) % with $\sim$10% increase in oxygen and Eu peaks at $2\theta = 28.5^\circ$, 33.0$^\circ$, 47.6$^\circ$, and 56.4$^\circ$, which correspond to (111), (200), (220), and (311) planes characteristics of a face cubic (fcc) structure. Enhancement of the diffraction peaks was observed indicating successful Eu doping in the ceria lattice [88, 89]. The absorption spectra of CeNPs and Eu-CeNPs (figure 4) showed peaks at 290 and

![Figure 3. Concept of theranostic CeNPs with synergistic antioxidant capabilities and MRI contrast properties. Reproduced from [82]. CC BY 4.0.](image-url)
288 nm, which is in agreement to those reported for CeO$_2$. These peaks are attributed to the transitions of O$^{2-} \rightarrow$ Ce$^{3+}$ and O$^{2-} \rightarrow$ Ce$^{4+}$ [19]. In the EuCeNPs, these peaks are enhanced due to the O$^{2-} \rightarrow$ Eu$^{3+}$ transition [16, 19]. The doped EuCeNPs showed increased fluorescence at excitation and emission wavelengths of 466 and 522 nm, respectively, due to increased oxygen vacancies resulted from doping with Eu$^{3+}$. These vacancies can act as donors and induce formation of new energy levels in the band gap under photoexcitation conditions [90]. It was shown that H$_2$O$_2$ enhances the fluorescence of the EuCe NPs, enabling concentration-dependent detection of H$_2$O$_2$. Using this mechanism, oxidase enzyme substrates like glucose and lactate can be determined with µM sensitivity [76], indicating potential utility of these NPs for measurements of biologically important molecules and biomarkers of relevance in biomedicine. Additionally, enzyme-generated quinone derivatives were observed to quench the fluorescence of EuCe NCs. Additional targeting capabilities can be achieved by interfacing the particles with ssDNA aptamers and measuring reactivity changes after target binding [91, 92].

The EuCeNPs have been shown to limit ROS formation and ameliorate ischemia-reperfusion (IR) injury in an intestinal mouse model of hypoxic injury [86]. An increased production of superoxide (O$_2^{-}$) radicals was detected throughout the ischemia stage and again after initiating reperfusion measured using locally implanted electrochemical probes in the intestine. The increased concentration of superoxide during ischemia and reperfusion was associated with expression of inflammatory cytokines, suggesting an inflammatory response in the intestine. Administration of EuCeNPs into the intestinal lumen during the onset of ischemia effectively blocked O$_2^{-}$ buildup and ameliorated the intestinal pathology. These results suggest that EuCeNPs are effective antioxidants against O$_2^{-}$ accumulation in intestine, providing protection against IR-induced injury [86].

Ensuring biocompatibility, stability and dispersibility in the biological environment is a prerequisite for the use of these materials in biomedicine. Enhanced stability can be obtained by ligand grafting at the NP surfaces. Ligands such as PAA-OA have been particularly effective at preventing aggregation in saline and physiological buffers over several months, while also retaining the fluorescent properties of CeNPs [76]. Due to their ROS scavenging activity, CeNPs have been studied in a variety of cellular and animal models [93]. In general, CeNPs has exhibited biocompatibility in various studies and surface modification enabled them to be chemically stable in physiological fluids. Their ability to act as antioxidants can be improved by mixing, doping or surface modification with other compounds which can improve their efficacy of ROS scavenging [94].

CeNPs have several advantages over other NP systems including: (a) high stability in aqueous solutions; (b) the ability to inactivate ROS-mediated oxidative damage; (c) availability of doped CeNP congeners with inherent fluorescence properties and stability in biological environments; (d) high catalytic activity, operating as an enzyme mimetic catalyst with oxidase, CAT and SOD-like activities; (e) versatility for use as molecular probe, enabling detection of a variety of biologically-important targets such as H$_2$O$_2$, oxidase.
enzyme substrates like glucose and lactate, phosphatase activity and catecholamine neurotransmitters. These NPs can be used as a universal multifunctional probe with combined ROS-scavenging activity, fluorescence and sensing characteristics, making them promising candidates for bioimaging, sensing, and theranostics applications. In addition, they can be used for drug delivery, cell targeting, and the activation of cellular pathways [95].

Figure 5 shows the multidimensional properties of these particles and their potential applications in biomedicine.

The following sections discuss the therapeutic properties that can be combined with the biosensing and imaging capabilities to develop theranostic CeNPs candidates. We start by discussing the mechanism of cellular uptake of the NPs. We then discuss the antioxidant, anti-inflammatory, immunological, regenerative, antimicrobial, and anticancer activity of CeNPs, and provide examples of applications of these NPs to develop new drugs and treatment approaches. We conclude with a critical discussion of the biocompatibility and toxicity issues in biological environments, and their potential for future developments as next-generation theranostic probes in biomedicine.

5. Uptake and localization of CeNPs

The uptake and localization of CeNPs is important in understanding their properties and behavior in biological systems, where they can serve as mediators for drug delivery, imaging, cancer therapy, etc. The size, shape and surface properties of the NPs are crucial to the uptake mechanisms. It is known that NPs that are smaller than 70 nm are rapidly and more easily uptaken by cells and NPs less than 10 nm are the most useful for targeted drug delivery [1]. One study reported that NPs of 25–50 nm were taken up much faster and more efficiently than smaller NPs and that cellular internalization can be improved by surface modification to prevent agglomeration [96]. Macrophages and other endocytotic vesicles quickly process and internalize NPs especially if they are attached to proteins [97]. NPs agglomeration in biological environments is strongly dependent on the NPs size, surface coating (ligand and surface charge), electrolyte concentration and the pH of the environment [98].

Extracellular particles can enter through cellular membranes by passive (non-energy-dependent) or by active (energy-dependent) transport mechanisms [99, 100]. Non-energy-dependent transport are observed for lipid-based NPs where a direct mixing or exchange of the lipid between NPs and plasma membrane are thought to facilitate transport across the membrane [98]. For the large majority of NPs, the uptake is an energy-dependent active process where localized regions of the plasma membrane engulf the extracellular material and internalize through the formation of endocytic or phagocytic vesicles [98, 99]. In a first stage, NPs attach near the pores of the cell membrane, determined by the surface charge, adsorbed protein, or targeting ligand on the particle. The second stage involves internalization by one or more of various mechanisms, some of which are clathrin-dependent endocytosis, caveolin-dependent endocytosis, clathrin- and caveolin-independent endocytosis, and macropinocytosis [97, 99, 100]. The mechanism of cellular uptake can be studied by fluorescence microscopy, where the NPs are labeled with fluorescent probes, time-lapse imaging of live cells or by radioactive isotope tagging [98]. Because CeNPs have a low intrinsic fluorescence, the NPs need to be labeled with a fluorescent probe or doped with luminescent ions in order to efficiently track their cellular uptake.
When studying the mechanism of cellular uptake and distribution of fluorophore-conjugated CeNPs in the range of 3–5 nm, Self et al showed that CeNPs co-localize with mitochondria lysosomes and endoplasmic reticulum (ER) [101]. It was observed that uptake occurred within 3 h of the particles being introduced to the cells, on both suspended and adherent cells, but suspended cells internalized the NPs faster than adherent cells. This energy-dependent process occurred through clathrin-mediated endocytosis. NPs were also seen in the nucleus and cytoplasm. The NPs were nontoxic to cells when tested at the levels used to visualize cellular update.

The potential of fluorescently labeled CeNPs as a theranostic platform has been evaluated using covalently attached rhodamine B (RhB). The RhB CeNPs were tested for their ability to simultaneously detect oxidative species and inactivate ROS, as an antioxidant [102]. The dual properties have been demonstrated in HeLa and Hep3B human cell lines. The RhB–CeNPs having a diameter of ~10 nm, were internalized by cells (figure 6) and exhibited higher cytosolic antioxidant activity than bare CNPs. The particles were able to measure oxidative stress in HeLa cells using the intrinsic fluorescence of the RhB present on the NP surface, similar to the DCFH–DA probe. This study showed the ability of the RhB-CeNPs as antioxidant agents and sensors for quantifying ROS production.

6. Therapeutic properties of CeNPs

6.1. Antioxidant properties
Reactive oxygen and nitrogen species (ROS/RNS) are highly unstable reactive molecules produced in small quantities as mitochondrial and cellular metabolism by-products [103]. These include hydrogen...
peroxide (H$_2$O$_2$), nitric oxide (NO), hydroxyl (HO$^-$) and superoxide (O$_2^{-}$) radicals. In normal physiological conditions, ROS/RNS are involved in several cellular and transduction pathways; and their generation is tightly regulated by endogenous and exogenous antioxidants inherently present in the body. Endogenous antioxidants are usually enzymes which include SOD, CAT and glutathione peroxidase. Some naturally occurring exogenous antioxidants are ascorbate (vitamin C), tocopherol (vitamin E), retinol (vitamin A), and some flavanoids [104, 105]. When ROS/RNS species are produced or accumulate in excessive amounts that cannot be efficiently inactivated by the natural antioxidant defense system, oxidative stress occurs [104], leading to oxidative damage of cell and tissues by changing the structure and function of proteins, lipids, polysaccharides, and DNA, inducing necrosis, ATP depletion, and ultimately inducing cell death by apoptosis [103, 105].

An emerging area is the use of redox NPs as free radical scavengers [57, 106]. These 'artificial' antioxidants have demonstrated the ability to inactivate ROS/RNS species, thus acting as antioxidants by themselves. The structure of CeNPs enable them to mimic the activity of oxidative enzymes, SOD and CAT [28, 54]. They have also showed mild phosphatase-, oxidase-, peroxidase-, and ATPase- mimetic activity. Their properties could further be modulated by modifying the ratio of Ce$^{3+}$ and Ce$^{4+}$, the size and the surface coating [93]. For example by changing the surface stabilizer, the antioxidant activity can be enhanced by stabilizing the Ce$^{3+}$ and the stability of the NPs in the biological media can be improved. Coatings such as PAA, polyethylene glycol (PEG), dextran, and polyethylenimine [93] have been shown to stabilize the NPs and prevent aggregation. In addition to preventing aggregation, the coating can modulate the Ce$^{3+}$/Ce$^{4+}$ ratio, which is important at controlling antioxidant properties. For example, engineered oleic acid coated CeNPs of 3 nm showed higher antioxidant properties, approximately nine times higher than commercially available antioxidants [39]. A single monodispersed layer conserved strong antioxidant activity even after 18 exposures to H$_2$O$_2$ over a 6 month period [39]. Another unique quality is the ability of CeNPs to regenerate providing opportunities for recycling and reuse [107]. Moreover, depending on the synthesis process and stabilizing agents, CeNPs are biocompatible and biologically stable.

Several studies have shown that co-localization of CeNPs within mitochondria can lead to effective treatment of mitochondria-related oxidative stress [101]. It was also found that CeNPs helped reduce oxidative stress from ROS and reduced inflammation [108]. The ROS scavenging activity of CeNPs was found to be dose dependent [26]. Another study showed that CeNPs could decrease protein and mRNA levels of inducible nitric oxide synthase (iNOS), being able to reduce the production of NO in cells [108]. CeNPs have also been hypothesized to help reduce ER related oxidative stress [101].

While the ROS scavenging of CeNPs is all documented, one study showed that CeNPs can induce oxidative stress in human lung epithelial cells but had no effect on human brain cells and rat cardiomyocytes, indicating that the NPs might have different effects on different types of cells [109]. Moreover, the ability to control the size, shape and the physico-chemical properties of the CeNPs is key to their antioxidant activity and enzyme-mimicking properties, since these directly influence how the NPs interact with cells and tissue. A slight change in these properties can significantly change their behavior, sometimes resulting in undesirable effects [93].

Although there are numerous in-vitro studies showing the apparent antioxidant activity of CeNPs, in-vivo studies are few. In one such in-vivo study, mice were administered with CeNPs one week prior to being injected with carbon tetrachloride (CCL$_4$), which is known to induce liver toxicity and oxidative stress. Interestingly the mice showed reduced lipoperoxidation, which has been linked to the antioxidant properties of CeNPs. The effects were comparable to another group, which was administered N-acetyl cysteine, a known antioxidant to CCL$_4$ [110]. Custom-made CeNPs of 2.9 nm in diameter and coated with EDTA showed the ability to confer protection in a mouse model of immunological and free-radical mediated oxidative injury [111]. The particles, administered intravenously were able to penetrate the brain, reduce ROS and alleviate clinical symptoms and motor function within a murine model of multiple sclerosis.

An exciting recent development in engineering CeNPs-based antioxidant system showed the therapeutic efficacy of CeNPs to control excessive ROS release of eye diseases when the particles were embedded within polyhydroxyethyl methacrylate (PHEMA) based contact lenses [112]. The CeNPs lenses were optically transparent and had physicochemical attributes similar to the commercial lenses but showed the ability to scavenge extracellular ROS (figure 7). The lenses had a protective effect on the eye in a mouse model upon administration of eye drops containing 3% H$_2$O$_2$.

### 6.2. Immunomodulatory properties

The immune system provides the body a defense mechanism against invading pathogens and harmful foreign substances. Therapeutic interventions in specific situations require stimulation or suppression of the immune response. The unique properties of CeNPs enable their potential application as both immunosuppressant and immunostimulatory. The immunosuppressant function of CeNPs is linked to their...
Figure 7. Concept of CeNP-based antioxidant contact lenses showing (a) CeNP-contact lenses on the surface of the eye and (b) the synthesis protocol in aqueous environment using PHEMA as the main matrix and methacrylic acid (MAA) and 1-vinyl-2-pyrrolidinone (NVP) as copolymers. Reprinted with permission from [112]. Copyright (2020) American Chemical Society.

ROS scavenging activity, which imparts an anti-inflammatory effect. CeNPs ability to inhibit inflammation was demonstrated in vitro in murine macrophage cell line [108]. Cellular uptake was observed without any signs of toxicity after treating the cells with 3–5 nm NPs, and the particles were found in both cytosol and phagosomes. This was associated with a reduction in the ROS level and the inflammatory mediator iNOS, which generates NO in the cells [108]. In a similar study, CeNP treatment of macrophages lowered ROS production along with decreased secretion of inflammatory cytokines interleukin 1 beta (IL-1β) and Tumor necrosis factor-α (TNF-α), and reduced expression of iNOS cyclooxygenase-2 (COX2) enzymes [113]. When evaluated in rat intracerebral hemorrhage (ICH) model, intravenous injection of CeNPs lowered macrophage recruitment in the perihematomal area along with signs of lowered perihematomal inflammation, suggesting potential therapeutic application of the particles for ICH [113]. CeNPs are also shown to influence the function of antigen presenting dendritic cells (DCs). NP treatment of DCs stimulated the secretion of anti-inflammatory cytokine IL-10, which helped to generate a T_{H2} biased T-helper cell response with higher secretion of IL-4, IL-5 and IL-10 [114]. Interestingly, in the same study, TiO$_2$ NPs stimulated the release of inflammatory cytokine TNF-α from the DCs and favored a T_{H1} dominated T-cell profile, suggesting that differences in surface reactivity of the NPs can determine how they interact with the immune system.

The immunomodulatory and antioxidant effects of CeNPs were observed in the in vivo studies using marine mussel Mytilus. After an exposure to ∼9 nm NPs for 96 h, several lysosomal, immune, inflammatory and antioxidant factors were assessed. Gene transcription analysis showed that NPs induced upregulation of MT20 and MT10 isoforms, which are involved in heavy metal homeostasis and antioxidant defense [115]. Additionally, a higher expression of antioxidant genes, notably for lysozyme was observed in the hemocytes, while there was a prominent decrease in lysosomal accumulation of lipofuscin in the digestive gland [115]. The redox properties of CeNPs are ascribed to be responsible for the physiological effects, including the antioxidant and immunomodulatory effects observed in this aquatic model. The lysosomal response to CeNPs were corroborated in studies involving human cell lines. CeNPs functionalized with biocompatible coatings (N-acetylglucosamie, PEG and polyvinylpyrrolidone) were shown to activate the lysosome-autophagy and enhance the autophagic clearance through activation of the transcription factor EB, a master regulator of lysosomal function and autophagy [116]. It is interesting to note that although the autophagic cell clearance pathway was activated, no cytotoxic response was observed. Since autophagy is a crucial process in the regulation of immune response [117], this research opens a new direction of using CeNPs for immunomodulation. Additionally, this property can be used in the treatment of lysosomal storage disorders where unwanted cell debris, proteolipids, and glycoprotein substrates are stored excessively [116].

While CeNPs are well known for their ROS scavenging and anti-inflammatory function, they were known to elicit inflammatory response in certain situations. Delivered intranasally, NPs were found to be extensively taken up by alveolar macrophages and neutrophils, along with significant increase of inflammatory cytokines (IL-1β, TNF-α, andIL-6) level in blood and bronchoalveolar lavage [118]. A more therapeutically important immunostimulatory property of CeNPs is its ability to increase vaccine efficacy. When administered together with Vaxigrip, a vaccine for influenza, CeNPs significantly amplified the immune response [119]. Interestingly, this enhancement of response was observed with non-stabilized CeNPs but not with citrate-stabilized CeNPs, which emphasizes the crucial role of surface chemistry for the desired biological effect. Since the CeNP concentration used with the vaccine was very low, it was concluded that the immune response was due to the vaccine itself and the NPs acted as an adjuvant [119].
6.3. Antimicrobial and antiviral properties

Ceria was known for its therapeutic properties for over 200 years and bulk ceria was used along with other metal oxides and rare earth salicylates as a component in ointments, powders, and other wound treatments due to its perceived anti-bacterial and antiseptic properties. Gram negative bacteria were thought to be more vulnerable to the effects of ceria when compared to gram positive bacteria [120]. When used in the form of CeNPs, the bactericidal properties could be enhanced due to the addition of free radical scavenging properties. Polymer coated hollow CeNPs synthesized using anionic polystyrene with/without a conductive polymer coating have shown bactericidal activity on *Escherichia coli* upon illumination [121]. Another study investigating the effects of CeNPs coated with biocompatible and nontoxic materials (e.g. dextran or PAA) showed that the coated NPs had a successful bactericidal effect on *Pseudomonas aeruginosa* [122]. Other studies have demonstrated bactericidal effects of CeNPs to *Staphylococcus aureus*, *Candida albicans*, *Bacillus subtilis*, *Streptococcus fecalis*, *Shewanella oneidensis*, and *Pseudokirchneriella supcapitata* [123, 124]. The rate of inhibition depends on the size of the NPs and the duration of incubation and most effects were attributed to the interaction of the NPs with the cell surface [123].

Bioactive glass modified with CeNPs showed increased toxicity to microbial cells, potentially due to their ability to inhibit respiration, disrupt cellular metabolism, compromise membrane integrity, and impart oxidative stress [125]. CeNPs synthesized by green synthesis using *Moringa oleifera* peel have shown bactericidal effects on *E. coli* and *S. aureus* [126] although this could also be due to the known bactericidal effects of *M. oleifera* itself. Other materials such as Zn and Zr doped CeNPs also showed promising antimicrobial activity especially on gram negative bacteria [127, 128]; gold conjugated CeNPs (Au/CeO$_2$) demonstrated antibacterial activity towards *B. subtilis*, *E. coli*, *Salmonella enteritidis* and *S. aureus* [129]. CeNPs were also shown to exhibit moderate anti-fungal effects when applied to *Aspergillus niger*, *C. albicans*, *Aspergillus terrus*, *Candida tropicalis*, *Aspergillus fumigatus* and *Aspergillus flavus* [128].

Nanomaterials can be used as drug delivery agents to target specific cells and tissues or they can be used as adjuvants in fighting disease, for example to treat viral infections such as HIV, influenza, hepatitis, and herpes [130]. When CeNPs were added to Vaxigrip influenza vaccine, a high antibody titer against virus strains were found even 2 months after vaccination. CeNPs acted as adjuvants by interacting with the M-protein in the vaccine and eliciting a stronger immune response by potentially modifying the protein conformation [119]. In another study, CeNPs stabilized with PAA demonstrated antiviral effects in animal cell culture model [131].

Understanding the mechanism how NPs interact at the microorganism interface is essential to the understanding of their antimicrobial and antiviral properties. The interaction and cellular uptake by *E. coli* is thought to involve first the adsorption of positively charged CeNPs on to the negatively charged cell wall. Thereby the NPs can affect the cell in two ways: by creating oxidative stress or by inhibiting the transport of nutrients into the cell. Oxidative stress is known to create lasting damage to microorganisms by chemical degradation. They also interfere with cell metabolism, cellular respiration, etc, by binding to mesosome and transport proteins. The CeNPs could also kill the microorganisms through physical damage of the cellular surfaces [132].

6.4. Regenerative effects of CeNPs on nerve tissue

The unique property of CeNPs is their ability to regenerate upon interaction with ROS, make them a promising candidate for tissue engineering and regenerative medicine [133]. Owing to very limited intrinsic regenerative capacity, functional recovery after nerve tissue damage is very challenging. Another obstacle comes from the blood brain barrier (BBB), which the therapeutic agents need to cross to reach the target brain tissue. The ability to enhance cell survival, modify the course of stem cell differentiation, and retain their catalytic properties over extended periods, makes CeNPs a promising candidate for next generation therapy in regenerative medicine. CeNPs can also be used to boost existing treatments due to their high functionalization, biological stability, antioxidant properties, and structural integrity, among other properties. In general, design of NPs can be tailored to support neuronal survival and growth, regulate neuronal cell differentiation, and enhance synaptic activity [134]. Furthermore, NPs can be conjugated with other molecules and used as carriers to improve the effectiveness of drug delivery [27].

Research showed that ultratine (2–5 nm) non-agglomerated auto-catalytic CeNPs enhanced the survival of adult spinal cord neurons in an *in-vitro* system even when applied at low doses [57]. The neuro-protective action was explained by the regenerative properties of CeNPs provided by the coexistence of the Ce$^{3+}$/Ce$^{4+}$ states. These regenerative autocatalytic properties indicate that CeNPs can be used as an effective and long lasting source of antioxidant molecules as compared to regular antioxidants [57]. CeNPs showed the ability to inhibit the differentiation of neural stem cells, tested in a C17.2 murine cell line. Gene expression analysis confirmed that NP treatment led to changes in the neuronal differentiation pathways relevant for neural
Figure 8. Design of a CeNPs-based planform using a CeNP core and ANG and PEG shell structure for stroke treatment.

**Conditions:** (a) formations of oxygen vacancies, cerium (III) and cerium (IV) species in ceria nanoparticles as biomimetic antioxidant enzymes; (b) main functions of each part on edaravone-carried and PEG/ANG-conjugated ceria nanoparticles (E-A/P-CeO$_2$); (c) synthetic procedure for surface modification and drug loading; (d) illustration of receptor-mediated (ANG-LRP) endocytosis of E-A/P-CeO$_2$, part of which remains inside the brain capillary endothelial cells (BCECs) for BBB protection (i) and the others cross the BBB via transcytosis for stroke treatment (ii). Reprinted with permission from [107]. Copyright (2018) American Chemical Society.

6.5. Anticancer properties

Developing successful treatment for cancer is one of the most difficult challenges for modern medicine. Many cancer therapeutic approaches are already approved for clinical use or currently under development with variable degree of success. Inhibition of angiogenesis is considered as one potential approach for cancer therapy. While angiogenesis, formation of new blood vessels, is essential for tissue growth since it enables delivery of nutrients and oxygen to these tissues, it can also promote tumor growth and metastasis [137]. Many cancers stimulate angiogenesis for their growth and increased expression of several angiogenic factors are associated with a poor prognosis [138]. Some of the well-known angiogenic factors are vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), angiopoietin, transforming growth factor,
TNF-α, and interleukins [138]. Studies have shown that ROS induces higher expression of angiogenic factors such as VEGF and FGF, leading to angiogenesis and tumorigenesis [137, 139].

The antioxidant properties of CeNPs have been utilized to reduce ROS level in endothelial cells and decrease their proliferation, potentially by lowering the expression of angiogenic growth factors [140]. Corroborating this finding, another study showed CeNP to inhibit VEGF-mediated proliferation and capillary tube formation by human umbilical vein endothelial cells [139]. When treated on ovarian cancer cell lines, these NPs inhibited ROS production and decreased cell migration and invasion. The CeNP treatment was also successful in reducing tumor growth and proliferation in mouse model of ovarian cancer and was accompanied by a lower degree of angiogenesis [139].

Apart from angiogenesis, CeNPs could exert toxicity on cancer cells through other mechanisms. Treatment of lung cancer cell line with CeNPs of 20 nm size exhibited dose and exposure time-dependent cytotoxicity though the mechanism of cell death was not fully understood [141]. In a different study, CeNP-mediated inhibition of proliferation and migration of gastric cancer cells treatment was reported both in vivo and in vitro, although the proliferation response was observed at a much higher concentration of NPs. Furthermore, it was shown that CeNP treatment was associated with an increased expression of DHX15, an RNA helicase, which further activated downstream p38 MAPK signal pathway [142]. In vitro studies also demonstrated a higher toxicity of CeNPs toward cancer cells in comparison to normal cells [143]. Additionally, the method of synthesis (hydrothermal and hydrolysis) could have a significant impact on cellular uptake and toxicity [143]. Thus, while preliminary results are promising, careful design, characterization, and extensive biological evaluation are required to successfully develop CeNP-based cancer therapeutics [144, 145].

7. Pharmaceutical and biomedical applications of CeNPs

The promising properties of the CeNPs described above provide opportunities for the development of nanotherapeutic approaches in pharmacology and biomedicine. Discussed below is a summary of a few novel, potentially effective therapies with clinical applications.

7.1. Mitigation of endometriosis

Endometriosis is a condition where there is presence of endometrial tissue in areas other than the lining of uterine cavity [146]. This condition is associated with chronic inflammation where oxidative stress is considered to be a contributory factor for the disease pathogenesis as demonstrated by a high ROS release and lipid peroxidation (LPO) [147]. Excessive angiogenesis also plays a key role in endometrial tissue growth by maintaining adequate oxygen and nutrients supply, and therefore, an anti-angiogenic treatment is thought to control the disease progress [148]. The antioxidant and anti-angiogenic properties of CeNPs have drawn attention on their potential use to treat endometriosis.

Initial promise was shown in a study where treatment with CeNPs (3–5 nm diameter) in endometriosis-induced mice resulted in a decrease in ROS, LPO, and total antioxidant capacity. CeNP treatment caused a reduction in in adrenomedullin and VEGF expression, lowered angiogenesis in the endometrial tissue, and was associated with a drastic decrease in the number of endometrial glands (figure 9(a)) [148]. In a different work, CeNP treatment was shown to increase the number of maturing oocytes in the ovarian follicles of older mice, accompanied by an increase in the number of granulosa cells and a decrease in both necrotic and apoptotic cells [150]. Thus, CeNPs demonstrate promise as a therapeutic option for challenging problems involving the female reproductive system, including endometriosis and age-related infertility.

7.2. Protection against neurodegeneration

Oxidative stress is a known contributing factor for many neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, and Huntington's disease [105]. Neurodegeneration is an irreversible process resulting in the progressive loss of neurons. Oxidative stress is thought to disrupt mitochondrial function in neurons, leading to dysfunction of the redox reactions [153–155]. The ability of CeNPs to protect cells against ROS [93, 101] has raised the possibility of its application in treating neurodegeneration. In one study, CeNPs were conjugated with triphenylphosphonium, a lipophilic cation capable of targeting mitochondria, and tested for the effects on mouse model of Alzheimer’s disease. Conjugated NPs were found to localize in mitochondria, and were able to reduce neuronal death along with the suppression of reactive gliosis (figure 9(b)) [151]. CeNPs without such surface modification were also found to be internalized by neuronal cells in culture and localize to the mitochondrial membrane [27]. Furthermore, CeNPs treatment decreased peroxynitrite and amyloid beta protein-induced mitochondrial fragmentation and reduced neuronal death [27].
The neuroprotective effects of CeNPs were observed in nerve tissue located outside the central nervous system (CNS) as well. After a single intravitreal injection in albino rats, CeNPs were found to stably localize in the outer photoreceptor segment of retina and provided protection against the damage to bright light exposure after three weeks [156]. Similar to the observation in CNS, CeNPs offered protection of retina by their ROS scavenging effect, which decreased microglial activation and inflammatory reaction, thereby reducing the neuronal death. Interestingly, when the NPs were delivered through intravenous route, the protection against light-induced damage was not observed [156]. The potent, long-lasting antioxidant properties and the ability to cross the BBB [27] suggest the therapeutic potential of CeNPs for slowing down neurodegeneration.

7.3. Radiation induced damage
Radiation therapy (RT) is one of the main modalities of cancer treatment options available currently, however, one major disadvantage of RT is the generation of excess free radicals. Accumulation of ROS can damage and even induce death of healthy tissues surrounding the tumor [157]. Amifostine is a clinically approved cytoprotective agent that is currently used to restrict radiation-induced cell injury and mutagenesis, but its application is limited due to a short half-life, requirement for daily administration, and high cost [157].

The powerful antioxidant effects of CeNPs can be utilized for use as an adjuvant in RT. Studies have shown that treatment with CeNPs preceding RT has a protective effect on normal cells with radiation-induced damage, due to their SOD-mimetic and antioxidant properties [144, 157]. When CeNP-treated Balb-C mice were subjected to 12.5 Gy of x-ray radiation, none of the animals died and had
minimal damage to the skin. In comparison, 20% of control mice receiving same dose of radiation died and many exhibited substantial skin desquamation [157]. In vitro, cultured normal lung fibroblasts and human colon cells exposed to radiation showed substantially lowered cell death upon treatment with CeNPs [152, 157]. This CeNP-mediated protective effect against radiation exposure is specific to normal cells as improved viability was not observed for lung cancer cells [157]. CeNP treatment also reduced radiation-induced apoptosis in mouse colon crypts concomitant with a sharp increase in SOD2 expression (figure 8(c)) [152]. High SOD2 expression could add to the SOD-mimetic effects of CeNP to confer protection against the oxidative stress induced by radiation.

7.4. Wound healing

Cerium based topical medications were used to treat wounds in the early nineteenth century. Cerium nitrate is still used in combination with silver sulfadiazine to treat burns to protect from bacterial infections and to maintain T-cell function [120]. Apart from secondary infections, oxidative stress is considered as one critical factor for wound healing where excess generation or inadequate removal of ROS delay the healing process of chronic wounds [158]. Thus, the antiseptic and antibacterial effects of cerium compounds, coupled with their antioxidant and anti-inflammatory properties, could make them an excellent material for wound healing. CeNP treatment increased the motility and/or proliferation of keratinocytes, fibroblasts, and vascular endothelial cells in culture and their topical application accelerated the wound healing rate in mouse model along with reduced oxidative damage to the cell membrane and proteins at the wound site [159]. Extending this concept, when CeNPs added to poly (ε-caprolactone)/gelatin film and used to treat full-thickness excisional wound, a faster wound closure with maturation of epidermis and appearance of skin appendages was observed (figure 9(d)) [149]. These results are promising and suggest that CeNPs could be utilized to design better wound-healing materials.

7.5. Anticoagulant

Anticoagulants prevent or slow down formation of blood clot in the circulation and are critical for patients who are at higher risk of thromboembolism. In the current clinical practice, commonly used anticoagulants include heparin and coumarins [160] but they suffer from the limitation of a relatively short shelf life and a short half-life. Cerium and other rare earth metals are known for their anticoagulant function partly acting through inhibition of enzymes in the coagulation cascade [161]. Although, cerium chloride was demonstrated to increase the clotting time of mouse blood plasma [162], a recent study reported no effects of CeNPs on blood coagulation [163]. However, the CeNPs used in the latter study were polydisperse in size (5–80 nm diameter) and did not have any surface modification, and therefore, calls for further investigation. Heparin functionalized CeNP [140] will be a promising candidate to study in this context, which could combine the properties of both heparin and CeNP for anticoagulation function.

7.6. Cardio-protection

Oxidative stress is known to be a contributor to cardiac dysfunctions, which eventually leads to chronic heart failure. Studies have shown an association between ROS signaling and multiple heart pathophysiology such as myocardial hypertrophy, cardiomyocyte death, contractile dysfunction, and arrhythmia [164]. Excess ROS production occurs in the cardiac tissue following ischemic events and play a key role in the reperfusion injury of myocardium [165]. However, it has been increasingly recognized that the redox signaling mechanisms underlying many of the chronic cardiac diseases are more complex than simple buildup of excess ROS, emphasizing the need for deeper investigations. Tested in vitro, CeNPs was shown to reduce H$_2$O$_2$ induced oxidative stress and cytotoxicity in cardiac progenitor cells without affecting their growth or functions [166]. When tested in isoproterenol-induced cardiac toxicity model in rat, intraperitoneally administered CeNPs demonstrated substantial ameliorative and protective effect against toxicity with effectivity comparable to captopril, used as a reference drug control [167]. This effect of CeNPs was associated with a strong increase in the tissue level of CAT and SOD. While these initial results are promising, further studies are needed to establish if CeNPs could be used as an effective cardio-protective agent.

7.7. Protection of gastrointestinal epithelium

The gastrointestinal epithelium is prone to ROS mediated injury as it is continuously exposed to ingested materials, toxins, and gut microbial population, and the response elicited against them by mucosal immune cells often causing inflammation. A number of chronic gut pathologies such gastroesophageal reflux disease, gastritis, peptic ulcer, inflammatory bowel disease, and malignancies have been associated with oxidative stress in the gut mucosal epithelium [168]. Owing to their antioxidant properties, CeNPs was tested as a treatment option against ROS-mediated gastrointestinal mucosal injury. A study showed that CeNPs administered orally to rats with ethanol-induced peptic ulcer offered protection comparable to commonly
used anti-ulcer drug ranitidine [169]. Further analysis of mucosal tissue samples showed CeNPs to increase SOD and CAT activity, which could contribute to the observed protective effect.

CeNPs has also been studied as a prebiotic candidate in combination with *Lactobacillus* and *Bifidobacterium* strains, two well-known probiotic bacteria. The administration of CeNPs along with probiotic strains was not only successful in lowering the free and bound cholesterol level after a lipid enriched diet, but also reduced the number of fungi and gram-positive cocci [170]. Since gut microbial composition is strongly linked to inflammatory cytokine production, the ability to modify the microbiome using CeNPs could provide a novel way to reduce gut inflammation and prevent gut diseases that result from persistent inflammation [171].

7.8. Rheumatoid arthritis (RA)

RA is a chronic inflammatory disorder primarily affecting the joints but can affect many other organ systems. The disease is progressive in nature and left untreated can lead to fibrosis and severe joint deformity. RA has an autoimmune origin but it is also associated with a high level of ROS generation, causing an increase in LPO, protein oxidation, and DNA damage [172]. A variety of treatment options are approved for RA ranging from anti-inflammatory agents such as non-steroidal anti-inflammatory drugs and corticosteroids to various disease modifying antirheumatic drugs (DMARDs) [173]. While many DMARDs targets the immune system to slow down the disease progress, they are highly expensive and might elicit serious side effects. The enzymatic and ROS scavenging properties of CeNPs have inspired researchers to study their potential to reduce the inflammatory response in RA. A recent work in this direction developed manganese ferrite and ceria co-decorated mesoporous silica NPs, which are capable of both ROS scavenging and oxygen generation [174]. Intra-articular injection of these particles in the knee joints of rat RA models was able to reduce inflammation and facilitated the conversion of inflammatory (M1) macrophages to noninflammatory (M2) phenotype. The therapeutic effects of these particles were further enhanced by encapsulation and delivery of the drug methotrexate. In a different work the CeNPs were used as a theranostic agent, by first generating albumin-cerium oxide NPs and then conjugating indocyanine green (ICG) dye to the particles (figure 10) [175]. Injected into mouse RA model, these particles showed preferential distribution to inflamed RA joints, allowed visualization of the particles through *in vivo* fluorescence imaging, reduced inflammation, and significantly improved the clinical score of arthritis, comparable in efficacy to methotrexate treatment used as a positive control.

8. Toxicity and biodistribution

As the potential of CeNPs in various applications emerges, concerns are raised about the toxicity of these particles. Although many studies have claimed the biocompatibility of CeNPs, there are several studies
including a list of studies that tested toxicity of CeNPs with the doses and routes of administration. Moreover, the penetration in the brain parenchyma was low, even for a small particle size of blood circulation time for the smaller sized NPs (5 nm) were significantly higher than the larger NPs (15, 30, interface will be the critical determinant of particle functionality as well as potential toxicity. Toxicity evaluation should be done with specific design of the particles and in the context of intended uses. The biodistribution and long-term toxicity studies after systemic administration are especially important showing that the innate qualities and pharmaceutical applications of CeNPs can induce cytotoxic effects. Table 1 includes a list of studies that tested toxicity of CeNPs with the doses and routes of administration.

| Particle size (duration of treatment) | Concentration | Cell type (route of administration) | Toxicity | Ref |
|--------------------------------------|---------------|-------------------------------------|----------|-----|
| 20 nm (24, 48, 72 h)                 | 3.5, 10.5, 23.3 µg ml⁻¹ | Human lung cancer | Nontoxic | [179] |
| 15–30 nm (24, 48 h and 14 d)         | 641 mg m⁻³ | Mouse lungs (in-vivo, IP and oral) | Toxic | [181] |
| 15–30 nm (72 h)                      | 125, 500 µg ml⁻¹ | Human lung carcinoma epithelial | Nontoxic | [183] |
| 630–790 nm (90 d)                    | 0.1–3 mg m⁻³ | Mouse alveoli | Toxic | [184] |
| <25 nm (24, 48 h)                    | 1–100 µg ml⁻¹ | Lung adenocarcinoma | Toxic | [177] |
| <25 nm (24)                          | 10, 20, 50, 100, 200 µg ml⁻¹ | Human neuroblastoma | Toxic | [176] |
| 20–30 nm (24, 48, 72 h)              | 0, 12.5, 25, 50, 100 µg ml⁻¹ | Human hematoma | Toxic | [185] |
| 38 nm (24, 48 h)                     | 80 µg ml⁻¹ | Human melanoma | Toxic | [186] |
| 7, 12 nm (24, 48, 72 h)              | 10, 50 µg ml⁻¹ | Human coronary artery endothelial | Nontoxic | [140] |
| 20 nm (24 h)                         | 0–25 µg ml⁻¹ | Human alveolar adenocarcinoma | Toxic | [141] |
| 100–200 nm (24, 72 h)                | 5 mg ml⁻¹ | Normal mouse fibroblast | Nontoxic | [143] |
| 3–5 nm (24 h)                        | 0–10 µM | Murine macrophages | Nontoxic | [108] |
| 3–5 nm (2, 5 wk)                     | 0.5 mg kg⁻¹ | Mice (PO, IV, IP) | Nontoxic | [110] |
| 5–8 nm (24 h)                        | 10, 24, 50 µg ml⁻¹ | Cardiac progenitor cells | Nontoxic | [166] |

The toxicity evaluation studies on CeNPs are primarily focused on exploring cellular toxicity and organ level damage due to their accumulation following systemic administration [2, 124]. A number of mechanisms have been suggested to explain cytotoxicity, which include the activation of autophagy, mitochondrial injury, DNA damage, induction of apoptosis, and generation of oxidative stress [176–179]. Although specific cell death mechanisms are associated with specific NP design and cell types, no universal mechanism is observed, and several studies reported nontoxicity of the particles as shown in table 1. Intracellular localization of particles could be a determinant for cytotoxicity as cell death was observed following lysosomal uptake of particles but not with cytoplasmic uptake [178]. CeNPs also demonstrate a selective toxicity towards cancer cells [143]. Although the reason for such selectivity is not completely understood, a slightly lower pH in the cancer cells due to the Warburg effect could be a contributing factor [129, 145]. The pH of the surrounding environment could determine whether CeNPs would demonstrate a beneficial or toxic response through antioxidant and oxidant effects, respectively [178].

The properties CeNPs such as size, shape, surface charge, and surface modifications play important roles in determining their functional properties as well as toxicity. In general, CeNPs exhibit higher toxicity than bulk ceria oxide and smaller sized NPs tend to be more toxic [180, 181]. Shape of NPs was also shown to be an important parameter as a dose-dependent increase in the toxicity macrophage cell line was observed with rod-shaped NPs but not when the shape was cubic or octahedral [182]. Surface charge (both positive and negative) of the NPs determines their intracellular localization in lysosomal compartment in specific cell types, which can trigger a cytotoxic response [178].

The biodistribution and long-term toxicity studies after systemic administration are especially important from therapeutic standpoint, especially since no clearance mechanisms for CeNP are known. CeNPs administered through intravenous and intraperitoneal routes showed highest amount of deposition in the spleen, followed by liver, lungs, and kidneys [110, 187]. More than 98% of the intravenously administered NPs were retained in liver and spleen with no significant elimination was observed in 30 days [188]. The blood circulation time for the smaller sized NPs (5 nm) were significantly higher than the larger NPs (15, 30, and 55 nm). Moreover, the penetration in the brain parenchyma was low, even for a small particle size of 5 nm, probably due to the presence of an intact BBB [188, 189]. The absorption of orally administered NPs was found to be very low with 95% of the total dose eliminated through the feces within 24 h [110]. Inhalational delivery of CeNPs revealed the penetration of particles in the alveolar space, generating a persistent inflammatory response [184]. Tissue elimination of the particles were found to be very slow with feces being the only route for elimination [110]. Even with a high retention of NPs in the tissues, signs of overt systemic toxicity were not observed [110].

Based on current findings, it will be difficult to make a general conclusion on the toxicity of CeNPs. Toxicity evaluation should be done with specific design of the particles and in the context of intended applications. Specifically, careful considerations are needed on particle physiochemical properties (e.g. size, surface modification) and the surrounding biological environment as the complex interactions at their interface will be the critical determinant of particle functionality as well as potential toxicity [190, 191].
9. Conclusion

Theranostic NPs hold significant promise to advance regenerative and personalized medicine by combining therapeutic and diagnostic functions. Due to their inherent ROS scavenging properties, biocompatibility, and the possibility to engineer sensing functions, CeNPs are promising candidates for the development of next generation theranostics. However, the development of theranostic NPs is still in the very early stages and most efforts to date have been dedicated to fundamental and pre-clinical research to engineer CeNPs and study their properties and effectiveness in *in vivo* and *in vitro* systems. In order to advance clinical translation of these technology, several challenges need to be addressed including: (a) improvement of bioconjugation, (b) the development of improved synthesis procedure that can produce uniform CeNPs in high quantity and with high reproducibility and stability in biological medium (e.g. months to years), (c) improved biodistribution, circulation and pharmacokinetics without compromising imaging and sensing functions, (d) development of strategies to produce activatable theranostic NPs to enable all-in-one sensing and on demand therapy. On demand or activatable NPs that can provide real-time monitoring of the pharmacological effectiveness of the NPs and getting information about the treatment response would be useful for the advancement of personalized medicine. Although NPs-based imaging and therapeutic approaches hold promise, their approval by the Food and Drug Administration for implementation in real world application is slow, with most examples of particles evaluated in clinical trials still being iron oxide, gold, silica, and silica-gold NPs.

Through the field of theranostic NPs is still in its infancy, future research could lead to a new wave of combined therapeutic and diagnostic treatment for many disease conditions. Given the large number of applications that can be envisioned, the potential of CeNPs is very large. In order to take advantage of the unique capabilities of these nanomaterials, a fundamental understanding of the nature and mechanism that drives their catalytic properties and sensing capabilities in relation to the physicochemical parameters is still needed. A critical understanding of the role of the surface chemistry and redox processes at the CeNPs surface is critical to rationally design NP therapeutics for therapy and diagnostics purposes. As more knowledge of structure-activity is gained, rational engineering and integration of the diagnostics and sensing features might be possible. In addition, the toxicity of the CeNPs and their biodistribution needs to be studied in greater detail before future biomedical applications with these particles can be pursued.

**Data availability statement**

No new data were created or analysed in this study.

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