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Phosphate-dependent modulation of antibacterial strategy: a redox state-controlled toxicity of cerium oxide nanoparticles

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Abstract. Specific reactivity of cerium oxide nanoparticles with phosphate ions was used to design a novel antibacterial system. The redox sensitivity of cerium oxide nanoparticles (CeNPs) was used to irreversibly scavenge phosphate ions from the microbial growth media resulting in nutrient starvation in microbes. Cerium oxide nanoparticles surface was engineered with different ratios of (Ce (+3)/Ce (+4)) cerium oxidation states and the effect of surface oxidation states was evaluated on the antibacterial activity. The nutrient depletion-based antibacterial activity is demonstrated selectively by CeNPs with higher Ce (+3)/Ce (+4) ratio on the surface. The surface chemistry of Ce (+3) is altered in the presence of phosphate, resulting in the irreversible formation of surface cerium phosphates leading to the loss of its intrinsic superoxide dismutase (SOD) activity. It is hypothesized that nutrient starvation by Ce (+3) leads to oxidative stress in microbes which is not neutralized by the altered surface chemistry of CeNPs with high (Ce (+3)/Ce (+4)) ratio. On the contrary, CeNPs with higher (Ce (+4)/Ce (+3)) ratio did not show any reactivity towards phosphate, thus depicted no antibacterial activity, confirming the hypothesis that surface chemistry, rather than size or morphology-dependent toxicity is the main reason for the observed antibacterial activity of CeNPs.

Keywords. Redox active nanomaterials; cerium oxide nanoparticles; antibacterial; superoxide dismutase; catalase.

1. Introduction

Recent progress in nanoscience offers great advances and potential applications in several fields such as biomedical technology and therapies [1–3]. Nanomaterials exhibit shape- and size-dependent unique electronic and optical properties at nanodimensions which are central to myriad of applications [4,5]. Among these, cerium oxide nanoparticles (CeNPs) are of interest as a biocatalyst that catalyse antioxidant reactions in biological model systems [6–8]. In last two decades, CeNPs have emerged as an excellent antioxidant nanocatalyst, exhibiting biological enzyme-like properties such as superoxide dismutase (SOD), catalase, oxidase and peroxidase-like activities [9–13]. Based on these properties, CeNPs have established several applications in wide areas such as treatment of oxidative stress-based neurodegenerative disorders, oxygen ion conductor in commercial fields and as anode in low-temperature solid oxide fuel cells [14,15]. The interconversion of Ce^{+3} and Ce^{+4} oxidation states is central to wide range applications of CeNPs [16]. The redox active CeNPs demonstrate SOD and catalase enzyme-like activity due to the high +3/+4 and +4/+3 ratio of 'Ce' surface atoms, respectively [9]. It is well-documented that due to high surface to volume ratio, nanomaterials tend to react with the components of suspension media. Singh and co-workers [17] have shown that CeNPs with surface 'Ce' atoms predominantly in +3 oxidation state show strong affinity and binding with phosphate anions, however, do not interact with other physiologically relevant anions, such as carbonates and sulphates, found in excess in cytoplasm. It was also shown that interaction of CeNPs (+3) with phosphate leads to the loss of intrinsic SOD activity. Conversely, CeNPs having surface 'Ce' atoms in +4 oxidation state do not interact with phosphate or other similar anions, therefore, retain their intrinsic catalase-like activity [18]. These studies suggest that the interaction of CeNPs with components of biological media or buffers such as amino acids, sugars, salts and other molecules, is redox state-dependent. Owing to this property, the activity of pristine CeNPs can be tuned from antioxidant to pro-oxidant [19].

Phosphate and its salts are essential for optimal growth, fertility and bone development in all the organisms as it is the fundamental building block for biomolecules and energy supplement [20]. Although, in environment, phosphates stimulate the growth of plankton which leads to the growth of fish

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population in an aquatic system, high concentration of phosphate can imbalance the nutrient and material cycling process and offer a serious threat to the stability of the ecosystem through eutrophication [21,22].

Recently, there has been rise in bacterial strains resistant to conventional antimicrobials resulting in complicated and prolonged treatment of infections which has increased the risk of mortality worldwide [23]. Additionally, some antimicrobials are unable to cross either the cell wall of microbe or the plasma membrane of cell, thus incapable of treating intracellular pathogens. Therefore, broad spectrum antimicrobial agents are needed that can unlock the restrictions experienced by conventional agents. Owing to the vast physiochemical and functionalization properties, nanomaterials can be promising and broad-spectrum antimicrobial agents. Among nanomaterial-based antimicrobials, silver nanoparticles (AgNPs) are the predominant nanoparticle type as an antimicrobial [24,25]. However, the mechanism of action lies in the slow release of Ag+ ions, which interact largely with 'S' (proteins) and 'P' (nucleic acid)-based biomolecules in the microbe [26,27]. It has also been reported that the thick microbial cell wall is permeable to ions only and not to nanoparticles, thus, the direct participation of nanomaterials is limited to the antimicrobial activity [27]. Taking a cue from our earlier work of strong affinity of CeNPs (+3) with phosphate, herein, we investigate the antibacterial potential of CeNPs (+3) through a non-contact phosphate starvation mechanism. We also demonstrate that how the antimicrobial activity of such materials can be controlled.

2. Materials and methods

Ferricytochrome-C, xanthine oxidase, catalase, 2′,7′-dichlorodihydrofluorescein diacetate (DCFDA), ammonium cerium nitrate and cerium nitrate hexahydrate were purchased from Sigma Aldrich (St. Louis, MO, USA). Hypoxanthine and Dulbecco's phosphate buffered saline were purchased from Hi-Media Pvt. Ltd. (Mumbai, India). Luria-Bertani (LB) broth was purchased from Merck (Mumbai, India). Hydrogen peroxide (H_2O_2) was purchased from SD Finechem. Ltd. (Mumbai, India).

2.1 *CeNPs (+3) synthesis*

CeNPs (+3) were prepared by dissolving cerium nitrate hexahydrate in water, followed by drop-wise addition of hydrogen peroxide until yellow colour appears due to the formation of CeNPs (+4). Solution was kept for 10–15 days at room temperature until it gets colourless, due to the conversion of Ce (+4) to Ce (+3), which indicates the formation of CeNPs in +3 oxidation state.

2.2 *CeNPs (+4) synthesis*

Ammonium cerium nitrate was dissolved in water followed by drop-wise addition of ammonium hydroxide until the white precipitate disappears from the solution. The pale yellow colour solution of CeNPs was obtained, which indicates the formation of CeNPs in +4 oxidation state.

2.3 *Characterization of CeNPs*

CeNPs (+3) and (+4) were characterized by UV–visible spectra which was acquired using a spectrophotometer (Synergy HT Biotek spectrophotometer) at a room temperature in quartz cuvette of 1 cm path length. CeNPs (+3) exhibit characteristic absorbance at 250 nm, whereas CeNPs (+4) at ∼300 nm. The particle size of CeNPs (+3) and (+4) was analysed by transmission electron microscope (TEM) (JEOL-JEM 1400). The images are shown supplementary figure S1.

2.4 *Preparation of phosphate buffer saline*

Phosphate buffer was prepared by dissolving 9.6 g of Dulbecco's phosphate buffered saline powder in 1 l of water to obtain 1× phosphate buffer saline.

2.5 *Reaction of CeNPs with phosphate*

Different concentrations of CeNPs (+3) and (+4) were incubated with phosphate (varying concentrations) overnight followed by the measurement of absorbance by UV–visible spectra.

2.6 *SOD mimetic activity*

SOD mimetic activity of CeNPs and CeNPs with phosphate, was measured by the method described by Korsvik *et al* [9]. In summary, reduction of ferricytochrome-C by superoxide was measured spectrophotometrically at 550 nm for 20 min. Hypoxanthine/xanthine oxidase was used to generate superoxide radicals in the system and the reaction was buffered with 10 mM Tris pH 7.5. Catalase was added to remove any trace of hydrogen peroxide produced as a side reaction. Reactions were done in triplicate in a 96 well plate with total volume of 100 µl.

2.7 *Catalase mimetic activity*

Catalase mimetic activity of CeNPs (+4) was determined with the change in concentration of H_2O_2, followed by its absorbance at 240 nm using spectrophotometer [11]. Reaction was buffered with 50 mM Tris, pH 7.5 and performed in total volume of 1 ml. 1 mM DTPA (diethylene triamine pentaacetic acid) was used in the reaction to avoid any potential interference by metals.

2.8 *Antibacterial activity*

Monocolony of *E. coli* was cultured in a shaking incubator in 10 ml amyl nutrient media (13 g powder in 1 litre of

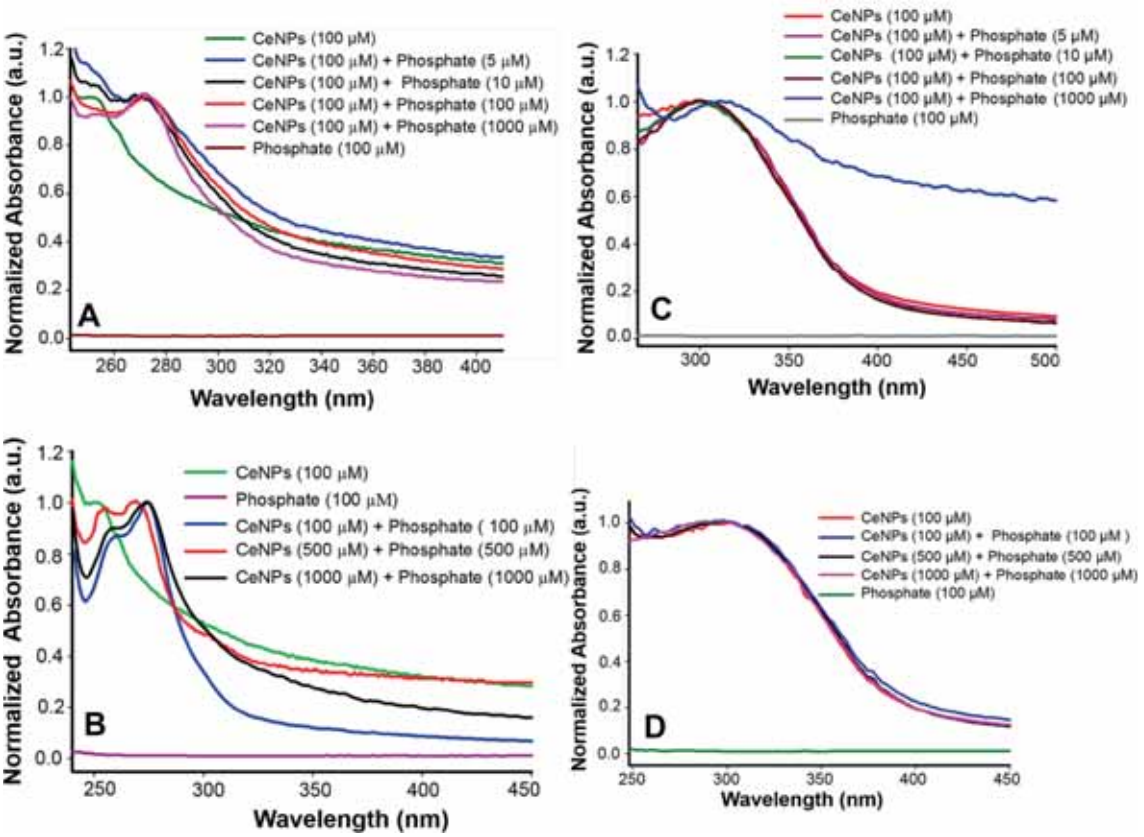

Figure 1. Interaction of CeNPs (+3) and (+4) with phosphate. CeNPs (+3) were incubated with varying (**a**) and equimolar (**b**) concentrations of phosphate. Similarly, CeNPs (+4) were also incubated with varying (**c**) and equimolar (**d**) concentrations of phosphate. These mixtures were incubated for 24 h and subsequently analysed by UV–visible spectroscopy measurements from the resultant suspensions.

deionized water) at 37°C, 100 rpm for overnight. The cell density of the overnight grown bacteria was adjusted to 0.5 optical density (OD) by recording OD at 600 nm (OD_{600}) using a UV–visible spectrophotometer. A measured volume of 1 ml was transferred to 100 ml of fresh media for a further conditioning at 37°C, 250 rpm for 3 h. Finally, bacterial cell density was adjusted to 0.1 OD $\approx 1 \times 10^8$ colony forming units (CFU) ml^{-1} for antibacterial experiments. The antibacterial activity of phosphate, CeNPs (+3 and +4) and mixture of CeNPs (+3 and +4) and phosphate towards *E. coli* was determined by recording OD_{600} using microplate reader at two time-points, 3 and 6 h. In a typical experiment, 1×10^8 CFU ml^{-1} of *E. coli* was exposed to 100 μM CeNPs (+3), 100 μM phosphate and 100 μM CeNPs(+3) + 100 μM phosphate in minimal media. The bacterial growth kinetics was followed for 3 and 6 h by recording OD_{600}. Similarly, another set containing 1×10^8 CFU ml^{-1} of *E. coli* was exposed to 100 μM CeNPs (+4), 100 μM phosphate and 100 μM CeNPs (+4) + 100 μM phosphate in minimal media and the growth kinetics was followed for 3 and 6 h by recording OD_{600}. The experiments were performed in triplicate and repeated three times.

2.9 *ROS generation E. coli*

0.1 OD at 600 nm were incubated with minimal media CeNPs (500 μM), phosphate (500 μM) and CeNPs (500 μM) + phosphate (500 μM). After 6 h of treatment, cells were centrifuged (6000 rpm, 10 min) and washed with PBS. 20 μM of DCFDA was added to the above bacterial suspension and incubated for 30 min at 37°C. Next, 100 μl of DCFDA exposed bacterial sample was transferred to 96 well plate and fluorescence emission intensity was measured at 528 nm, whereas fluorescence excitation was done at 485 nm using Synergy HT Biotek spectrophotometer.

3. Results and discussion

Phosphate is one of the important components in typical biological buffers and culture media, required for the growth of bacterial to mammalian cells. Due to high surface area and surface energy, nanomaterials interact with the culture media components, which often results in the alteration of intrinsic properties of nanomaterials [28]. The widespread

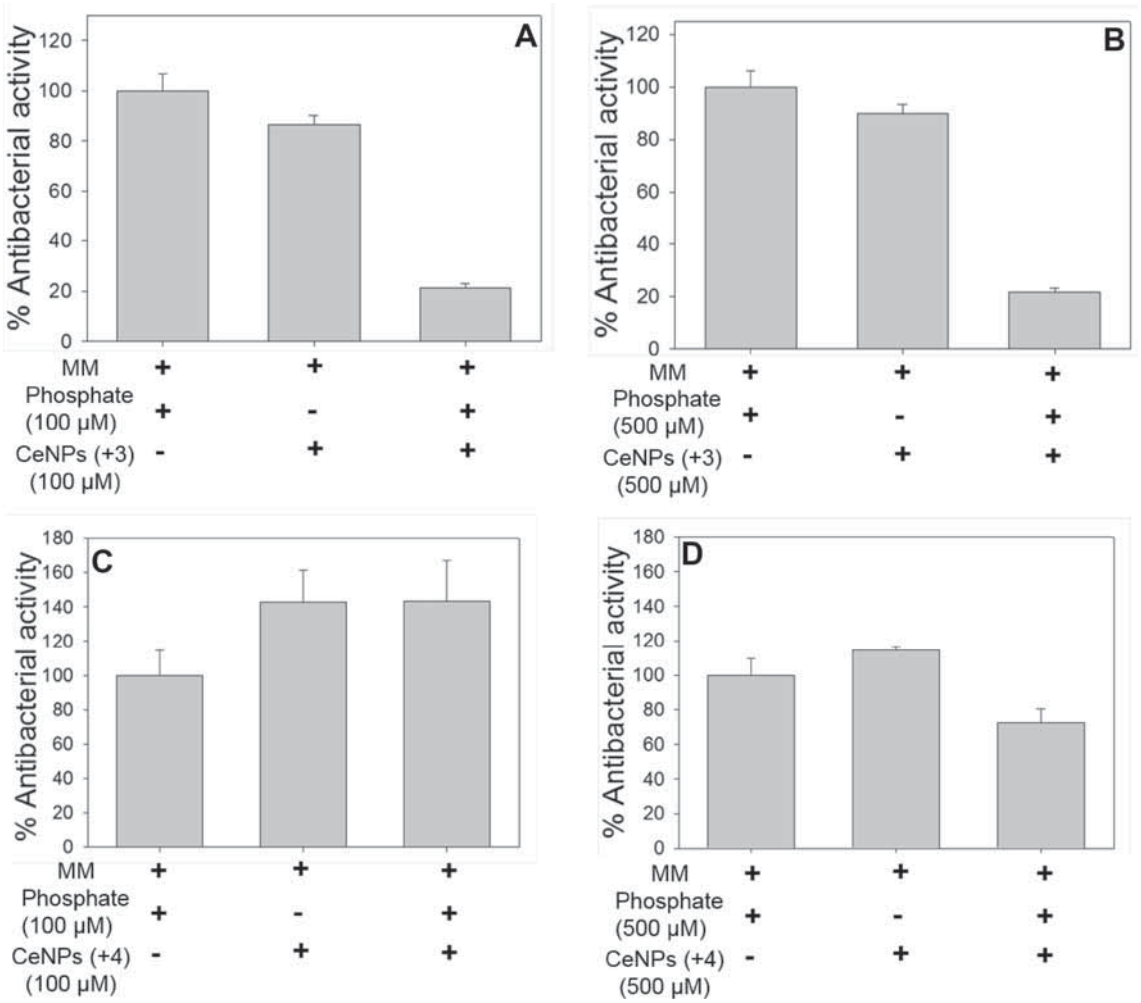

Figure 2. Comparison of antibacterial activity of CeNPs (+3) and (+4) followed up to 3 h. *E. coli* was grown in minimal media with and without phosphate and CeNPs for 3 h followed by absorbance measurement at 600 nm. With (**a**) 100 and (**b**) 500 µM CeNPs (+3) in 100 and 500 µM phosphate, respectively, killed >80% of bacteria. However, *E. coli* exposed to (**c**) 100 and (**d**) 500 µM CeNPs (+4) in 100 and 500 µM phosphate, respectively, did not alter the viability. Only phosphate and pristine CeNPs are not toxic to *E. coli*.

use of phosphate in several biomedicines and their strong affinity with known nanoparticles could lead to the toxicity of the organisms [29,30]. To test the affinity of CeNPs (3–5 nm in diameter and quasi-spherical in shape, supplementary figure S1) towards phosphate, 100 µM CeNPs (+3) was incubated with varying concentrations of phosphate (5, 10, 100 and 1000 µM) for ∼12 h followed by UV–visible spectra measurement. Figure 1a clearly depicts that CeNPs (+3) show an absorbance at ∼250 nm, which is the characteristic feature of CeNPs with high number of +3/+4 oxidation state. This absorbance peak of CeNPs (+3) is red-shifted to 275 nm upon exposure to higher phosphate concentrations (100 and 1000 µM) due to the transformation of cerium oxide to cerium phosphate [17]. Spectra recorded from CeNPs (+3) exposed to lower concentrations of phosphate (5 and 10 µM) exhibit two absorbance peaks at 250 and 275 nm, due to the presence of both CeNPs (+3) and cerium phosphate, respectively. Since the concentration of phosphate is lower than CeNPs (+3), it is assumed that these concentrations are not enough to interact with entire surface Ce atoms present in CeNPs, resulting in the absorbance signature at 250 nm. From UV–visible spectra, it is evident that 100 µM CeNPs exposed to equimolar concentration of phosphate is enough for the formation of cerium phosphate. To investigate this further, we mixed three equimolar concentration ratios of CeNPs (+3) and phosphate (100:100 µM, 500:500 µM, 1000:1000 µM) and measured the UV–visible spectra. Figure 1b clearly shows the presence of absorbance maxima at ∼275 nm in all the three conditions. Similarly, CeNPs with high +4/+3 oxidation state, was also exposed to different concentrations (5, 10, 100 and 1000 µM) of phosphate (figure 1c). As reported by Singh *et al* [18], CeNPs (+4) do not interact efficiently with phosphate, therefore, the characteristic absorbance maxima at ∼310 nm was

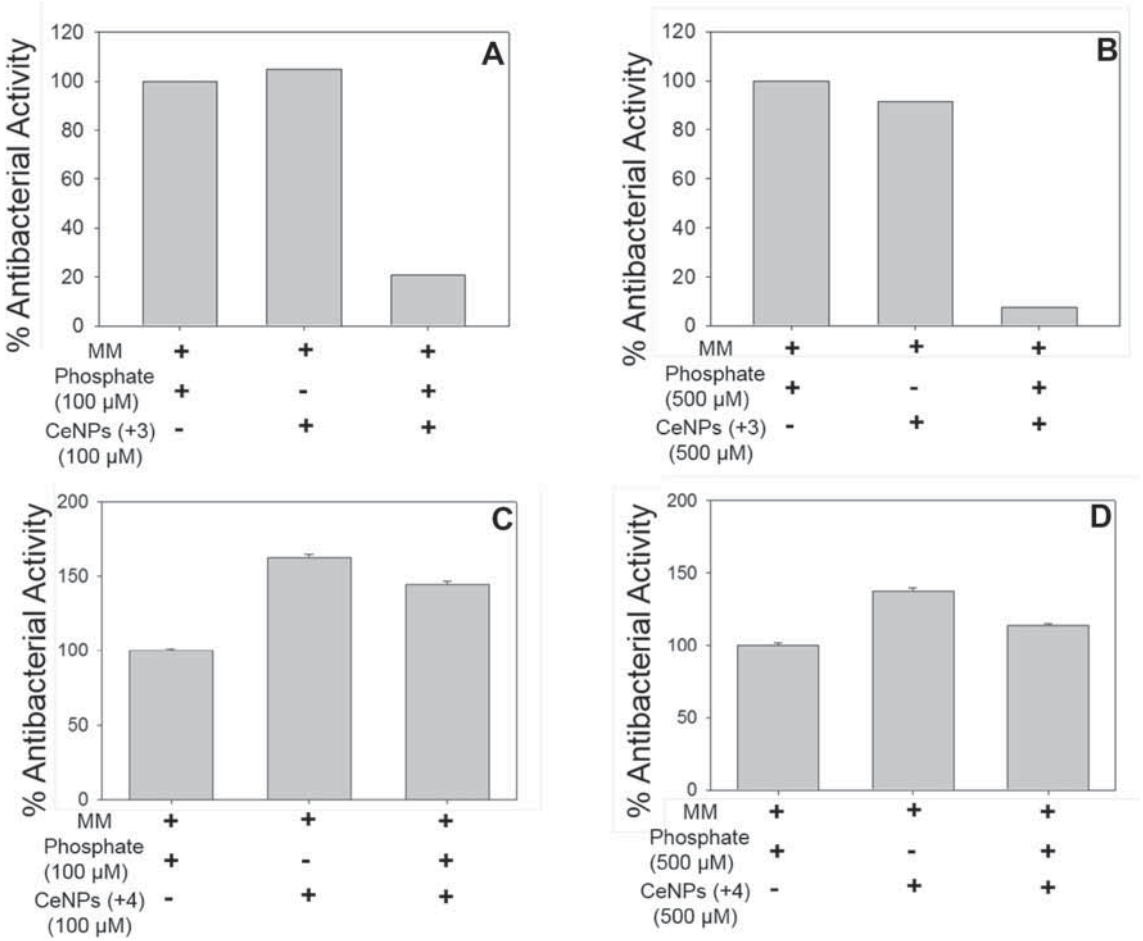

Figure 3. Comparison of antibacterial activity of CeNPs (+3) and (+4) followed up to 6 h. *E. coli* was grown in minimal media with and without phosphate and CeNPs for 6 h followed by absorbance measurement at 600 nm. With (**a**) 100 and (**b**) 500 µM CeNPs (+3) in 100 and 500 µM phosphate, respectively, killed >90% of bacteria. However, *E. coli* exposed to (**c**) 100 and (**d**) 500 µM CeNPs (+4) in 100 and 500 µM phosphate, respectively, did not alter the viability, significantly. Only phosphate and pristine CeNPs are not toxic to *E. coli*.

unaltered (figure 1c). Subsequently, the interaction between equimolar concentrations of CeNPs (+4) and phosphate (100:100 µM, 500:500 µM, 1000:1000 µM) was also investigated by UV–visible spectroscopy, which further revealed that there was no appreciable interaction between phosphate and CeNPs (+4), as no shift in absorbance maxima was observed at ∼310 nm (figure 1d).

Based on these analyses, we next investigated the effect of limiting the availability of phosphate in medium on the growth of *Escherichia coli* (*E. coli*) in presence of CeNPs. *E. coli* was grown (up to 6 h) in minimal media supplemented with different phosphate concentrations (100 and 500 µM) in presence of equimolar concentration of CeNPs (figures 2 and 3). Exposure of only CeNPs (+3) (100 and 500 µM) to *E. coli* did not alter the viability significantly. Similarly, only phosphate exposure also did not affect the viability of *E. coli*. However, exposure of *E. coli* to the equimolar mixture of CeNPs (+3) and phosphate for 3 h (figure 2a and b) resulted in significant cell death (∼90%) at both the concentrations. However, *E. coli* exposed to 100 and 500 µM CeNPs (+4) in presence of equimolar phosphate concentration (100 and 500 µM), did not affect the cell viability significantly (figure 2c and d). To estimate the long-term antibacterial effect, we exposed *E. coli* with CeNPs in presence of phosphate for 6 h (figure 3). As evident from figure 3a and b, the viability of exposed *E. coli* did not improve upon longer exposure time. Further, CeNPs (+4) did not affect the *E. coli* viability at the tested concentrations of 100 and 500 µM, in presence of 100 and 500 µM phosphate ions even upon exposure up to 6 h (figure 3c and d).

Although, it has been reported that nanomaterials of ∼100 nm are rapidly internalized by mammalian cells, however, bacterial cells do not internalize particles >20 nm. Even though, the CeNPs used in this study are <20 nm,

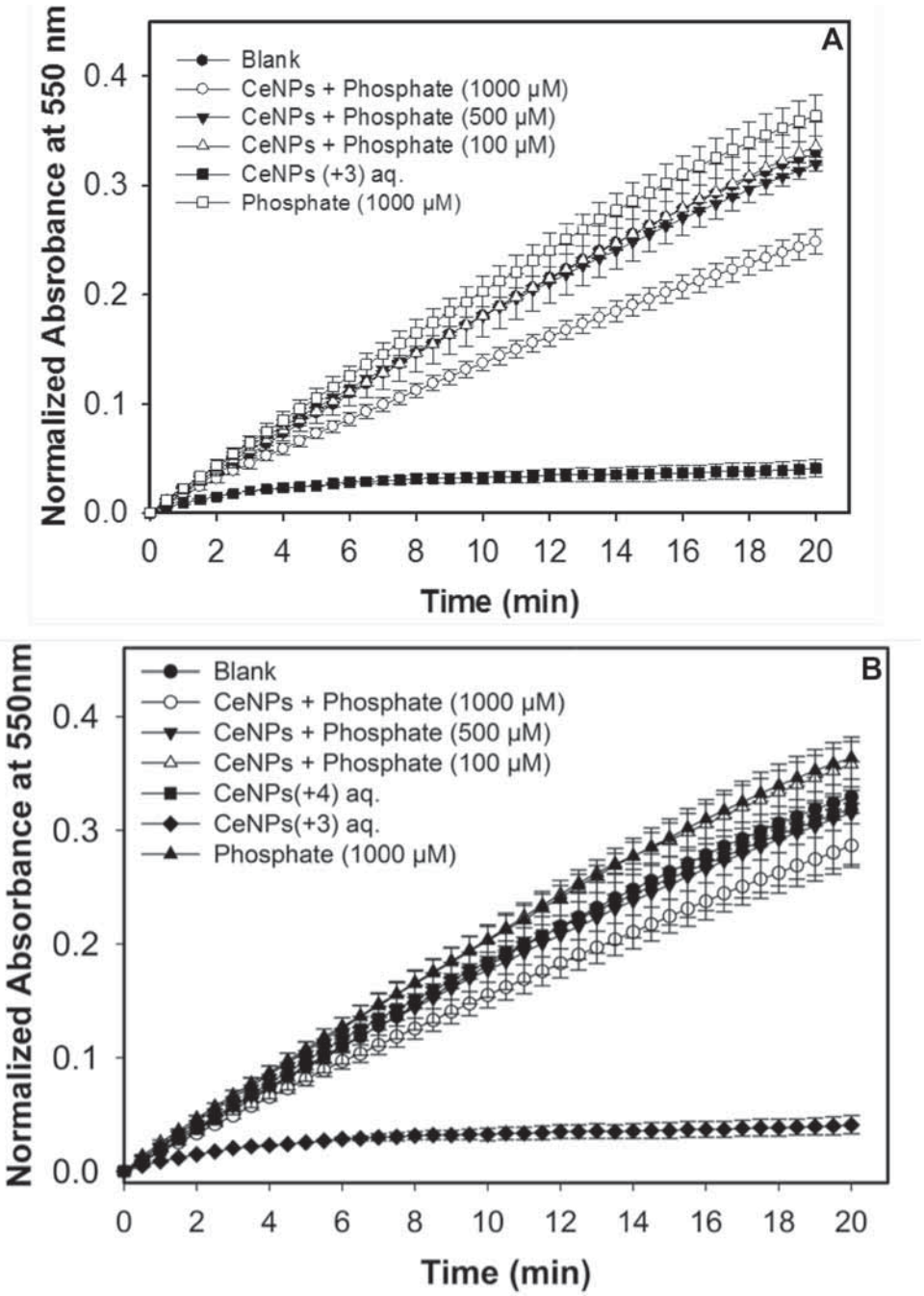

Figure 4. Comparison of SOD activity of CeNPs after incubation with phosphate. CeNPs (**a**) (+3) and (**b**) (+4) were incubated in equimolar concentrations of phosphate for 24 h and subsequently analyzed for change in SOD-like activity pattern by UV–visible spectroscopy measurements from the resultant suspensions of a concentration of 100 µM concentration. CeNPs (+3) has been taken as (**b**) positive control. Data expressed as SD calculated from three ($n = 3$) independent experiments

but they do not cause any toxicity to *E. coli* when exposed at concentrations in the range of 100–500 µM as observed in figure 2, suggesting the biocompatibility of CeNPs in both the oxidation states. This could be due to the SOD-like and catalase-like activity of CeNPs (+3) and CeNPs (+4), respectively. This observation also excludes the probability of direct toxicity exerted by CeNPs, even after internalization within the bacteria and therefore, the observed antibacterial action of CeNPs in presence of phosphate occurs without the physical contact of CeNPs with *E. coli*. As suggested by the current study that the strong affinity of CeNPs (+3) towards phosphate could result in the observed toxicity to

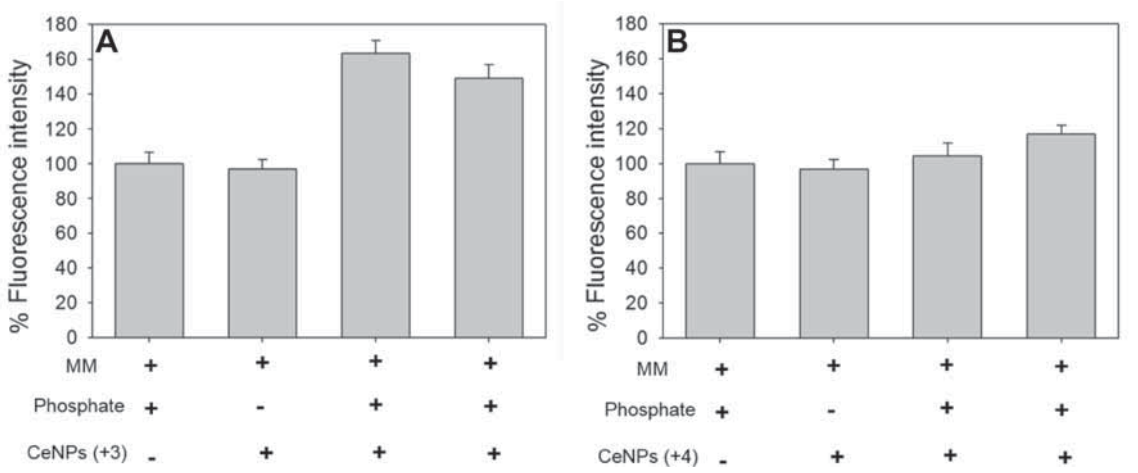

Figure 5. ROS generation in *E. coli*. *E. coli* was grown in a minimal media with and without phosphate and CeNPs for 3 h followed by emission intensity measurement of DCFDA at 520 nm. With 100 and 500 μM of CeNPs (+3) in 100 and 500 μM phosphate, respectively, (**a**) induced ∼1.6 fold increase in ROS in *E. coli*. However, *E. coli* exposed to 100 and 500 μM of CeNPs (+4) in 100 and 500 μM phosphate, respectively, (**b**) did not increase the ROS level, significantly. Data expressed as SD calculated from three ($n = 3$) independent experiments.

E. coli. Mechanistically, it can also be suggested that due the limited availability of phosphate in the medium, there is a severe competition between CeNPs (+3) and bacteria for the available phosphate. As cerium oxide reacts very rapidly with phosphate to form cerium phosphate, it will consume all the phosphate from the medium as compared to the slower uptake of phosphate by the bacteria. It is clearly evident from UV–visible absorbance results that the lower concentration of phosphate than CeNPs (+3) is not sufficient to bind all the phosphate in solution. Equimolar concentration of CeNPs (+3) and phosphate is needed to completely scavenge the phosphate by CeNPs (+3) Inversely, CeNPs (+4) did not affect the *E. coli* viability in phosphate in the medium. This observation is in concurrence with UV–visible spectroscopy data, suggesting that CeNPs (+4) show no affinity towards phosphate and therefore, did not scavenge it from the medium. The free availability of phosphate in the medium in presence of CeNPs (+4) helped *E. coli* to grow and divide normally.

E. coli is known to overexpress phosphate binding proteins during phosphate starvation condition, which helps to transport the phosphate from surrounding medium to the periplasm [31]. However, the strong affinity of CeNPs (+3) with phosphate probably leaves no free phosphate in the medium. Cerium phosphate is a highly stable compound as depicted by its very low solubility constant in water. In addition, the natural occurrence of cerium as cerium phosphate (mineral monazite) suggests a high stability of cerium phosphate in naturally occurring conditions. Recently, it was also shown that the solubility of cerium oxide as free cerium ions in highly acidic solution is also reduced in the presence of phosphate [32]. This is also ascribed to high reactivity of reactive cerium sites on its surface with available phosphate in the medium. Thus, even though, the dissociation

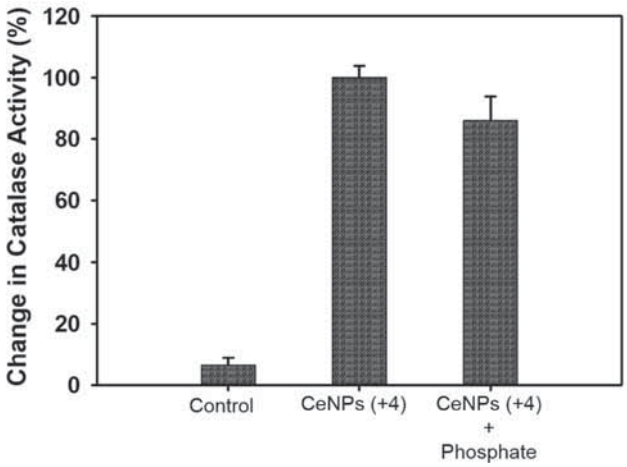

Figure 6. Alteration in catalase-like activity of CeNPs (+4) in presence of phosphate. 100 μM CeNPs (+4) were incubated in equimolar concentration of phosphate for 24 h and subsequently analysed for change in catalase mimetic activity by following the absorbance pattern at 240 nm by UV–visible spectroscopy. Activity exhibited by CeNPs (+4) was considered as 100%. Data expressed as SD calculated from three ($n = 3$) independent experiments.

constant of phosphate binding proteins is of the order of 10^{-7} M, it is still not enough to prevent the reaction of cerium oxide with phosphate. Additionally, the dissociation constant of phosphate binding proteins ($K_d \sim 10^{-7}$ M) is several order of magnitude lower than the solubility constant of cerium phosphate [33]. Therefore, *E. coli* would not be able to assimilate substantial amount of phosphate in the presence of CeNPs (+3), even with the help of phosphate binding proteins. Additionally, it is also reported that if the

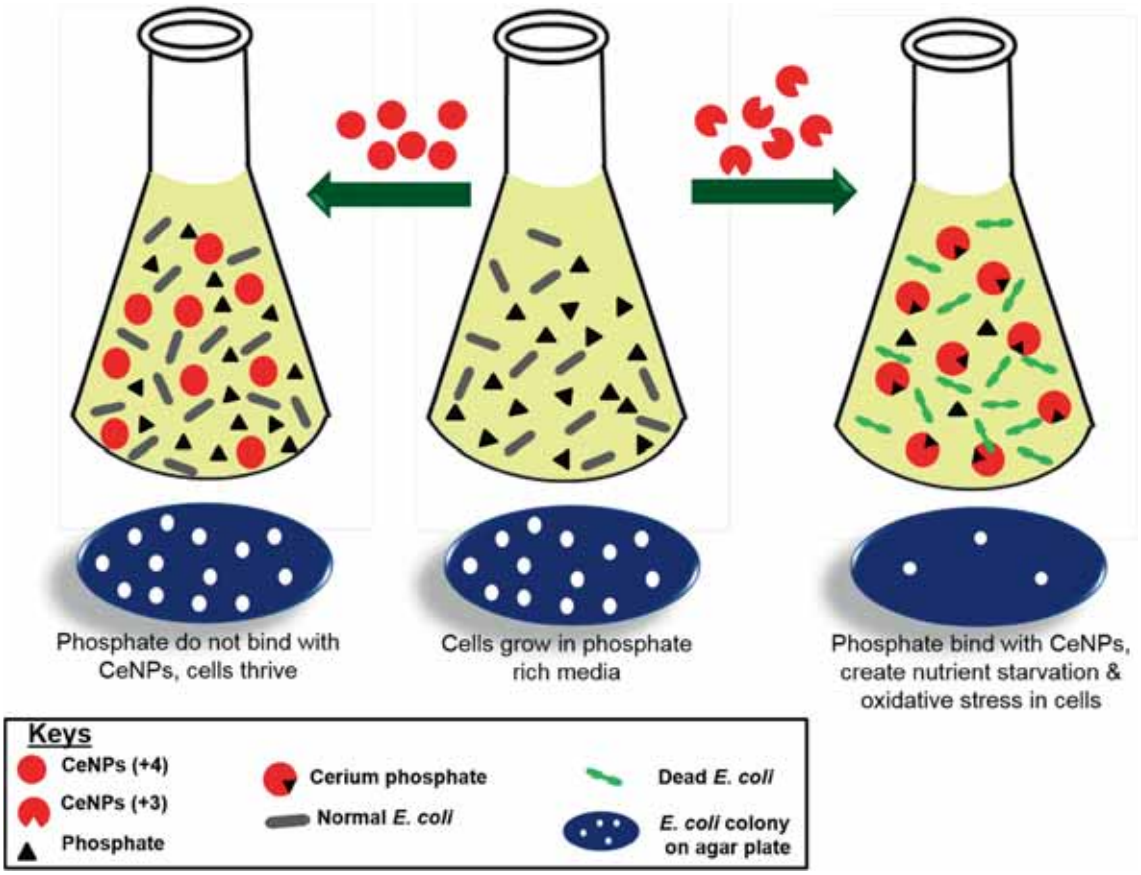

Figure 7. Schematic representation showing the mechanism of antibacterial activity of CeNPs. High affinity of Ce (+3) with phosphate leads to the formation of cerium phosphate, thus create a phosphate-deficient nutrient media, leading to starvation and oxidative stress in *E. coli*, ultimately cell death. However, exposed Ce (+4) do not react with phosphate, phosphate starvation condition is not created in culture media, and thus, cells thrive.

phosphate concentration in the external environment drops below the threshold concentration, bacteria will efflux phosphate from the cell to medium. This exchange rate is very rapid and *E. coli* can exchange ∼90% of its internal phosphate with external media in 20 min [34]. Therefore, in this context, it is expected that under the influence of low phosphate concentration in external media, *E. coli* would efflux out its internal phosphate to external media, which thereby would react with extremely high affinity CeNPs (+3) and form cerium phosphate. This process would further make *E. coli* more phosphate-deficient.

To understand the mechanism of antibacterial action, we studied the alteration in the enzyme-like properties of CeNPs. As evident from figure 4a, pristine CeNPs (+3) show excellent superoxide radical scavenging activity, however, when exposed to phosphate, its SOD activity was significantly diminished. Further, three equimolar concentrations (1000, 500 and 100 μM) of phosphate and CeNPs (+3) were mixed, followed by SOD activity measurement. As expected, none of these samples showed any SOD activity. Pristine CeNPs (+4) alone or after reaction with equimolar concentration (1000, 500 and 100 μM) of phosphate did not show any SOD activity (figure 4b). This observation is in agreement with UV–visible spectra of CeNPs (+4) and phosphate (figure 1c and d), suggesting that CeNPs (+4) do not show affinity with phosphate, therefore, the activity is not altered, however, high affinity of CeNPs (+3) with phosphate leads to the formation of cerium phosphate species and exhibit concomitant loss in SOD activity. Additionally, we also investigated the effect on SOD activity of CeNPs dispersed in minimal media (supplementary figure S2). Results clearly reveal that there was slight but nonsignificant decrease in SOD activity of CeNPs (+3) after dispersion in minimal media (supplementary figure S2a). As expected, CeNPs (+4) also did not show any SOD activity (supplementary figure S2b). Further, it is also suggested, at least in part, that due to the phosphate starvation, free radicals are generated in *E. coli* and since abundant cerium phosphate cannot scavenge these radicals, bacterial cells are destroyed. To explore it further, we estimated the free radical generation in *E. coli* exposed to CeNPs (+3) and (+4) in presence of phosphate (figure 5). As evident from figure 5a that exposure of 100 μM CeNPs (+3) to *E. coli* in presence of

100 μM phosphate, results in ∼60% increase in free radicals. Similarly, when 500 μM of CeNPs (+3) was exposed to 500 μM of phosphate, ∼50% increase in free radical level was observed in cells. The *E. coli* cells exposed to 500 μM CeNPs (+3) or 500 μM phosphate alone were considered as controls. On contrary to this, exposure of similar concentrations of CeNPs (100 and 500 μM) (+4) to *E. coli* in presence of equimolar concentration of phosphate (100 and 500 μM) did not induce any significant increase in free radical level (figure 5b). This observation suggests that *E. coli* produces excess of free radicals in the phosphate starvation condition. It may also be reasoned that high affinity of Ce (+3) with phosphate results in the loss of free radical scavenging ability of CeNPs (+3), leading to increase in oxidative stress in cells. However, since Ce (+4) does not show much affinity with phosphate, CeNPs (+4) might have retained its catalase enzyme-like activity, thus, avoiding any increase in free radical level in cells. Concomitantly, *E. coli* exposed to CeNPs (+4) did not face phosphate starvation condition, therefore, free radicals level was not enhanced. Additionally, the catalase-like activity of phosphate-exposed CeNPs (+4) were also investigated (figure 6) and results reveal that since CeNPs (+4) do not interact with phosphate and therefore retain >90% catalase activity when compared with pristine CeNPs (+4). This small drop in activity could be ascribed to the transient physiosorption of phosphate on CeNPs (+4) surface. Thus, on the basis of the above results, it can be summarized that high affinity of Ce (+3) with phosphate leads to the formation of cerium phosphate, which creates a phosphate-deficient condition, leading to starvation and oxidative stress in *E. coli*, and ultimately results in cell death (figure 7). However, exposed Ce (+4) do not react with phosphate, phosphate starvation condition is not created in culture media, and thus cells thrive.

4. Conclusion

In contrast to a plethora or antibacterial compounds that kill the microbes through their direct contact mechanism or through the release of toxic ions, we have demonstrated a novel strategy to kill bacterial cells through noncontact phosphate starvation method using CeNPs (+3). The study clearly shows that redox active CeNPs (+3) can act as excellent antibacterial agents by competing against *E. coli* for available phosphate, thereby limiting the available nutrient for bacterial cell growth and division. Phosphate starvation results in oxidative stress in bacterial cells. In general, CeNPs (+3) are regarded as good antioxidant materials that could potentially prevent the bacterial cells from this oxidative stress. However, the free radical scavenging activity of CeNPs (+3) is lost in presence of phosphate that further augments the antibacterial activity. Redox state-dependent affinity of CeNPs with phosphate further provides the tunability to the antibacterial activity. Additionally, the antibacterial strategy shown here is of particular interest because this method can bypass the leaching of toxic material and provide an antibacterial strategy with small ecological footprint. Nutrient starvation by nanoparticles also opens up the possibility of evaluating the toxicity of nanoparticles in a new paradigm wherein their surface reactivity could scavenge essential micronutrients from the medium, resulting in toxicity. This possibility could then be extended to design a broad spectrum antimicrobial from reactive nanomaterials that can sterilize a compartment by vigorously removing phosphate and other essential micronutrients from living medium and thereby inhibiting the growth of microbes.

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