Toxicity of Metallic Nanoparticles in Microorganisms- a Review

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Abstract Recent advances in the synthesis and development of nanoparticles (NPs) for wide applications has lead to a serious threat to both human and environmental health. NPs are highly reactive and catalytic in nature compared to their ions or bulk counterparts and thus applicable in various fields including drug delivery, electronics, optics, and therapeutics. Due to these applications, many varieties of NPs in massive amounts are being industrially produced. These NPs are discharged in to the environment and thus providing a path to enter into food chain via microorganisms and eventually disturbs the ecological balance. The NPs exhibit toxicity to living organisms mainly because of their small size (>100 nm), large surface-to-volume ratio and highly reactive facets. The microorganisms including bacteria present in the natural ecosystem are the primary targets that get exposed to NPs. Before these NPs enter into the food chain, it is imperative to evaluate the toxicity associated with NPs in microorganisms. The most convenient and rapid way is to perform toxicity analysis using microorganisms such as bacteria. Toxicity of nanomaterials using microorganisms such as *E.coli, Pseudomonas, Bacillus* as models for prokaryotes gives an insight into the toxic impacts of NPs. Toxicities associated with NPs in microorganisms is mainly related to their nano-size that cause membrane disorganization, generation of reactive oxygen species (ROS) and in some cases, oxidative DNA damage. In this review article we describe the toxicity of various nanoparticles in bacteria and provide a rationale for assessing nanotoxicity and discuss the current status on toxicity impacts on microorganisms.

Keywords Nanoparticles · Nanotoxicity · Membrane damage · Reactive oxygen species · Oxidative toxicity

1 Introduction

Metallic nanomaterials are among the most important catalysts, the smaller the metal particles (<100 nm), the larger the fraction of the metal atoms that are exposed at surfaces, where they are accessible to reactant molecules and available for catalysis. Due to this the nanoparticles have unusual physical and chemical properties that differ substantially from those conventional bulk materials of the same composition. The unique characteristics of metallic nanoparticles (NPs) have drawn a lot of attention for their promising applications in optical, electrical, mechanical, chemical and medical uses. However, it is not currently clear whether these nanostructures present harmful effects on the human health and environment. Therefore, exploitation of the full potential of the nanotechnologies requires close attention to the toxicities of nanoparticles on the living cells.

Currently, nanomaterials that have been found to be toxic can be classified into four types: (i) carbon based nanomaterials that are mostly made of carbon in the form of hollow spheres, ellipsoids (fullerenes), or tubes (nanotubes). These are found to accumulate in living cells and cause cytotoxicity and pulmonary toxicity (Lam et al. 2004; Magrez et al. 2006; Porter et al. 2006; Wei et al. 2007); (ii) the metal based nanomaterials

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including quantum dots, various metallic NPs, such as Au, Ag, Pt, and FePt NPs (Lengke et al. 2006; Maenosono et al. 2007; Morones et al. 2005), and metal oxides, such as TiO_2, ZnO_2, Fe_3O_4, Al_2O_3, CrO_3, and SiO_2. These have adverse effects mainly causing oxidative stress, apoptosis, inflammation of endothelial cells, and ecotoxicity (Borm et al. 2006; Gojova et al. 2007; Heinlaan et al. 2008; Jeng and Swanson 2006); (iii) the nanomaterials based on metallodendrimers are composed of nanosized, metal conjugated with organic polymers, used in molecular electronics and catalysis. For example, water-soluble star-shaped organo-iron redox catalysts have been used for nitrate and nitrite cathodic reduction in water, and electron-reservoir serving as molecular batteries and the example of C_{60} (Astruc et al. 2003; Caminade and Majoral 2004; Partha et al. 2007). Lastly, (iv) metal NPs composites that combine two different NPs, which form bulk-type materials such as Fe-Pt NPs. Nanomaterials composites, such as nanosized clays were used to enhance mechanical, thermal, barrier, and flame-retardant properties (Subramanian et al. 2003). Such composite metal NPs (Fe-Pt) causes mutagenicity in bacteria (Maenosono et al. 2007). Both metallodendrimers and metallic NP composites present toxicities that are associated with metal NPs as seen in above two cases (i and ii).

Nanomaterials, such as fullerenes (C_{60}) and carbon nanotubes have many interesting and unique properties potentially useful in a variety of biological and biomedical systems and devices and finally find their way into the environment (Seetharam and Sridhar 2007). The insoluble carbon nanotubes in aqueous phase have buoyancy and therefore float on top of the aqueous layer and are mistaken for food and ingested by aquatic organisms. There have been attempts to modify carbon nanotubes for improved bioapplications, especially for the aqueous solubility and biocompatibility of carbon nanotubes. As a result, various methodologies for the aqueous dispersion and solubilization of carbon nanotubes, such as modification by biofunctionalization, functionalization with hydrophilic polymers, and non-covalent stabilization have been reported (Reviewed by Lin et al. 2004). Carbon based nanoparticles including fullerene (C60) and single-walled carbon nanotubes (SWCNT) are taken up by aquatic organisms and that these are known to induce changes in biochemical or gene expression levels (Oberdorster 2004; Zhu et al. 2006).

Despite of the vast applications of these nanomaterials for the benefit of human, there is increasing concern that NPs affect human and environmental health. Studies have shown that NPs lead to an increase of bioavailability and toxicity (Nel et al. 2006). Currently, a complete understanding of the size, shape, composition and aggregation-dependent interactions of nanostructures with biological systems is lacking (Fischer and Chan 2007). Only, few studies have investigated the toxicological and environmental effects of direct and indirect exposure to nanomaterials and no clear guidelines exist to quantify these effects (Colvin 2003). Hence, an area of nanotechnology called nanotoxicology has emerged (Oberdorster et al. 2005b, 2007). There is a keen interest in nanotoxicology research because the processing of nanosturctures in biological systems could lead to unpredictable effects because of their distinct properties compared with their ions or bulk counterparts (Fischer and Chan 2007).

The potential hazard of manufactured NPs, their release into the aquatic environment and their harmful effects remain largely unknown (Moore 2006). Existing reports on nanoparticles show that they may conjugate with biological molecules in natural aqueous environments making them gain soluble properties that may have adverse effect on bacteria and other aquatic organisms. Interaction of carbon nanotubes or fullurenes with biological systems is well documented, especially with biological macromolecules, such as DNA, RNA, proteins as well as lysophospholipids (reviewed in Ke and Qiao 2007). A first evidence of direct contact with purified SWNT aggregates induces damage to bacterial cell membrane and thus cell death indicating the strong antimicrobial activity of SWNTs (Kang et al. 2007). Similarly, *E. coli* undergoes severe membrane damage and subsequent loss of viability due to SWNTs. However, very little information is currently available with regard to the cytotoxic mechanisms of SWNTs (Kang et al. 2007). Studies on toxicity of carbon nanotubes using *Staphylococcus aureus* and *Staphylococcus warneri* showed antimicrobial activity and inhibition of microorganism attachment and biofilm formation (Narayan et al. 2005). It is assumed that SWNTs induce significant morphological changes, that included elongation and similar changes that have been shown in bacterial cells under extreme conditions, such as that under high temperature (Rasanen et al. 2001), pressure (Ritz et al. 2001), and changes in surface-to-volume ratio and exposure to

chemical agents (Veeranagouda et al. 2006). Recently, Ghafari et al. reported that SWNTs are internalized by a protozoan *T. thermophilia* and acquire inability to ingest and digest their prey bacteria species allowing nanotubes to move up the food chain (Ghafari et al. 2008). This suggests that presence of carbon nanotubes as contaminant in the aquatic environment may have deleterious effects, which eventually lead to ecological imbalance.

Thus toxicity testing of NPs should be performed in an environmentally relevant mode to avoid misleading information on toxicity of NPs (Oberdorster et al. 2006). The effect of nanoparticles on microorganisms is much more extensive and diverse than for plants, invertebrates and vertebrates (Oberdorster et al. 2007). Nanoparticles including TiO_2 and silver have been used as antibacterial agents regardless of the particle size, but this activity is enhanced when delivered in a nanoparticulate form. One material which is not inherently antimicrobial is carbon, however C_{60} fullerens have recently been found to inhibit the growth of *Escherichia coli* and *Bacillus subtilis* (Fortner et al. 2005). However, there is insufficient evidence to suggest that all nanoparticles have antimicrobial effects, or in fact that all nanoparticles are toxic to any organism exposed in an environment. The impact of nanoparticles with respect to toxicity on microorganisms is still in its infancy stage. Unicellular microorganisms, such as bacteria and yeast can serve as the model organisms to study the toxicology of NPs. Before the toxicity of any nanomaterial to be tested on living organisms, it is imperative to understand the physicochemical properties of a given nanomaterial.

2 Physical and Chemical Properties of Nanoparticles

Nanoparticles exhibit unique physical and chemical properties compared to same material without nanoscale features. It is mainly because of following reasons; (a) their size in nanoscale measuring 100 nm or less with one or more dimensions, surface characteristics and morphology of their sub-structure, (b) their properties differ significantly from those of larger size as a result of manipulation at atomic, molecular and macromolecular scales, (c) several nanoparticles have nanostructures at the nanoscale levels. Nanomaterials have complex interrelation between the structure and the composition of the materials. Thus they acquire novel properties derived from atomic and molecular origin in a complex way along with features of its native bulk counterpart.

Nanomaterials exist in various shapes and structures such as spheres, needle, tubes, plates, sheets etc. The size and shape of nanomaterials contributes to onset of cytotoxicty, for example, single-wall nanotubes are more toxic than multi-wall nanotubes (Jia et al. 2005; Kang et al. 2007). Understanding of important physicochemical properties of nanoparticles in order for characterizing nanoparticle's toxicity to biological systems comprise (a) size distribution, (b) nature of agglomeration/aggregation, (c) shape, (d) structure of nanomaterial, (e) surface area, (f) surface chemistry, (h) surface charge, and (i) porosity (Oberdorster et al. 2005a). Various physicochemical methods have been employed to characterize the nanomaterials, including Transmission and/or Scanning Electron Microscopy (TEM or SEM), X-Ray Diffraction (XRD), Dynamic Light Scattering (DLS), Zeta potential, Isothermal adsorption, and Spectroscopic techniques (UV vis, IR, Raman, NMR) (Oberdorster et al. 2005a).

Under ambient conditions, some nanoparticles form aggregates or agglomerates. Nanoparticles also tend to aggregate by fusing and deposition that form bulk components. Nanoparticles suspended in gas tend to stick to each other more rapidly than in liquids. The primary free nanoparticle may form agglomerated primary particles (agglomerates) by interparticle interaction, which forms a collection of particles that are attached together by both weak and strong forces, including van der Waals, electrostatic forces and sintered bonds (Oberdorster et al. 2005a). The particle-particle interaction at the nanoscale level is governed by weak van der Waals forces, stronger polar and electrostatic or covalent interactions. The interparticle interaction is also influenced by viscosity and polarisability of the aqueous environment in order to form nanoparticle's aggregation. The forces involved in nanoparticle – nanoparticle interaction and nanoparticle – aqueous solution interactions are the basis for physical and chemical processes. The attractive or repulsive forces of nanoparticles crucially determine the fate of individual and collective nanoparticles. This interaction between nanoparticles results in aggregates

and/or agglomerates, which greatly influences on their physical and chemical nature.

Most of nanoparticles belonging to this category are modified chemically or engineered by surface modification to avoid agglomeration. The nanoparticles in the presence of chemical agents (surface active agents), the surface and interfacial properties may be modified and such agents can inderctly stabilize against coagulation (agglomeration) by preserving charge on the nanoparticles. The properties of nanoparticles can be significantly altered by surface modification and the distribution of nanoparticle that mainly depends upon the surface characteristics. Engineering nanoparticles by surface modification, addition or modification of surface functional groups and chemical composition to maintain the characteristics of nanoparticles that are often stable and prevent agglomeration or aggregation (reviewed in Oberdorster et al. 2005a, 2007). The behavior of nanoparticles will be dependent on their solubility and susceptibility to degradation and that neither the chemical composition nor particle size is to remain constant over time. This makes increasingly difficult to study and understand the cytotoxicity of any nanoparticle on biological systems. Therefore, the current review will provide some highlights and conclusions based on the existing information on the toxicological studies of nanosized particles, specific mechanisms underlying NPs' effects particularly focusing on the microorganisms with special attention to metallic nanoparticles as models.

3 Nanoparticles Pose Potential Threat to Bacteria

A majority of toxicity concerns that has been so far addressed is related to human cells or human health. Nevertheless, it is important to test the impact of NPs on other living organisms that exist in the natural environment including prokaryotes, such as bacteria, and other unicellular microorganisms. These unicellular microorganisms can also serve as model organisms for NP-toxicity analysis. It is most interesting that the bacteria are more sensitive than human fibroblast (Brunner et al. 2006; Limbach et al. 2005). The microorganisms are the primary targets for being exposed to the man made NPs after they are discharged into the environment. As a result, the microbial interactions and uptake of NPs would lead to entering of persistent NPs into the food chain, which eventually disturbs the ecological balance. NPs gain entry into the living cells through various means including physical rupturing of cell membrane or wall or endocytosis and cause cellular toxicities at various levels. Studies have confirmed that the metallic NPs can pass through or remain attached to the cell membrane (Borm and Kreyling 2004; Kashiwada 2006). A number of studies have examined the uptake and effects of NPs at a cellular level to evaluate their impact on humans. It may not be extrapolated to other species, such as unicellular microorganisms (bacteria or yeast) based on the conclusions of these studies, but more research is needed to confirm this assumption. Therefore, there is a need to assess the toxic impacts of various types of NPs not only on human or higher organisms but also on the microorganisms. It is important to investigating effect of NPs on bacteria because of the potential impact on microorganisms that serve as the basis of the food chain and as primary agents for biogeochemical cycles.

NPs exhibit different toxicities, which is dependent on the two major factors: (i) nature of NPs, such as size, morphology, and chemical nature; (ii) interaction with different microbial species and underlying potential mechanisms that should be investigated, which include cell wall damage and the role of NPs in disruption of membrane integrity, oxidative stress via reactive oxygen species (ROS) formation, organic radicals generated in the absence of light, and possible genotoxicities exhibited. A summary of a range of nanoparticles, their size, effective concentrations, and potential toxicity mechanisms currently available for Gram-negative and Gram-positive bacteria is summarized in Tables 1 and 2.

4 Nanoparticles Disrupt the Integrity of Cell Membrane

Nanoparticles interact with the bacterial cell membrane by adsorption or electrostatic interactions (Thill et al. 2006). Large thickness in outer membranes of some bacteria such as *E. coli* certainly plays a crucial

Table 1 Summary of different metallic and metal oxide nanoparticles and their toxic effects in Gram-positive bacteria

Bacteria	NPs	Size (nm)	Effective conc.	Toxicity action	Reference
Escherichia coli	SiO_2, TiO_2, ZnO,	205–480	10–1000 mg/L	Light induced ROS generation, cellular internalization; oxidative toxicity, antibacterial activity, membrane disorganization	(Adams et al. 2006a, b; Brayner et al. 2006; Fu et al. 2006; Reddy et al. 2007; Rengifo-Herrera et al. 2007; Tsuang et al. 2008)
	Ag	1–40	25–100 mg/L	Increased membrane permeability, cellular internalization, perforation of membrane; membrane damage and cell death	(Baker et al. 2005; Gogoi et al. 2006; Morones et al. 2005; Pal et al. 2007; Ruparelia et al. 2007; Sondi and Salopek-Sondi 2004)
	C60	25–500	0.4–4 mg/L	Decreased CO2 production; cytotoxicity, mechanical stress on the cell wall or membrane	(Fortner et al. 2005; Lyon et al. 2005; Tang et al. 2007)
	FePt	9	2.5 mg/ plate	Mutagenicity; DNA damage	(Maenosono et al. 2007)
	MgO	4	ND	Damage cell membrane; cell wall leakage	(Stoimenov et al. 2002)
	CeO_2	7	1.2–37 mg/L	Interacts outer membrane and cell-membrane damage	(Thill et al. 2006)
Pseudomonas putida	Fullerene/C60	50–200	0.09–0.5 mg/L	Oxidative stress (ROS generation), decrease levels of unsaturated fatty acids, increase cyclopropane fatty acids, altering membrane lipid composition, Phase transition temperature (by ROS), membrane fluidity	(Fang et al. 2007)
Pseudomonas aeruginosa	Ag	1–10	25–100 mg/L	Interact with cell membrane and sulfur- and phosphorous containing compunds such as DNA; Damage cell membrane and DNA	(Morones et al. 2005)
	TiO_2	20	10 mg/mL	Photoactivation of TiO2 induced loss of viability; Bactericidal	(Tsuang et al. 2008)
Salmonella typhimurium	C60	50–200	300 mg/L	Generate single oxygen, ROS generation and mutagenicity Oxidative toxicity, oxidative DNA damage, mutagenicity	(Sera et al. 1996)
	FePt	9	2.5 mg/ plate	Mutagenicity; DNA damage	(Maenosono et al. 2007)
	Ag	1–10	25–75 mg/L	Interact with cell membrane and sulfur- and phosphorous containing compunds such as DNA; Damage cell membrane and DNA	(Morones et al. 2005)
Shewanella oneidensis	Pd(0)	1:1 to 1:10 size of cell to NP	~50 mg/L	Bioreduction; Cytotoxicity	(De Windt et al. 2006)
	C60	ND	0–80 mg/L	Mechanical stress on the cell wall or membrane	(Tang et al. 2007)
Vibrio fischeri	TiO_2, CuO, ZnO	50–70	1.1–79 mg/L	Oxidative stress (extracellular ROS generation); acute toxicity, membrane damage, impaired growth,	(Heinlaan et al. 2008)
Vibrio cholera	Ag	1–10	25–75 mg/L	Interact with cell membrane and sulfur- and phosphorous containing compounds such as DNA; Damage cell membrane and DNA	(Morones et al. 2005)
Bacteroides fragilis	TiO_2	20	10 mg/mL	Photoactivation of TiO2 induced loss of viability; bactericidal	(Tsuang et al. 2008)

Table 2 Summary of different metallic and metal oxide nanoparticles and their toxic effects in Gram-negative bacteria

Bacteria	NPs	Size (nm)	Effective conc.	Toxicity action	Reference
Bacillus subtilis	C60	25–500	0.01–0.75 and 0.4–4 mg/L	Oxidative stress (ROS generation); increased iso- and anteiso-branched fatty acids, altering membrane lipid composition, Phase transition temperature (by ROS), membrane fluidity	(Fang et al. 2007; Fortner et al. 2005; Kai et al. 2003; Lyon et al. 2006, 2005)
	SiO_2, TiO_2, ZnO	205–480	10–5000 mg/L	Light induced ROS generation; oxidative toxicity, antibacterial activity	(Adams et al. 2006a)
	MgO	4	ND	Bactericidal effects, cell wall damage; cell wall disruption, desiccation	(Stoimenov et al. 2002)
	Ag	3	ND	Bactericidal effects, cell wall damage	(Ruparelia et al. 2007)
	CuO	9	ND	Bactericidal effects, cell wall damage	(Ruparelia et al. 2007)
Bacillus megaterium	MgO	4	ND	Bactericidal effects, cell wall damage; cell wall disruption, desiccation	(Stoimenov et al. 2002)
Streptococcus agalactiae	ZnO	60–150	<837 mg/L	Cellular internalization, membrane disorganization, increase membrane permeability; cytotoxic, bactericidal	(Huang et al. 2008)
Streptococcus pyogenes	Fe_3O_4-TiO_2	>100	2.57 mg/mL	Photokilling of bacteria	(Chen et al. 2008)
Staphylococcus aureus	ZnO	60–150	<837 mg/L	Cellular internalization, membrane disorganization, increase membrane permeability; cytotoxic, bactericidal	(Huang et al. 2008; Reddy et al. 2007)
	Ag	3	ND	Bactericidal effects, cell wall damage	(Ruparelia et al. 2007)
	CuO	9	ND	Bactericidal effects, cell wall damage	(Ruparelia et al. 2007)
	TiO_2	20	10 mg/mL	Photoactivation of TiO2 induced loss of viability; bactericidal	(Tsuang et al. 2008)
	Fe_3O_4-TiO_2	>100	2.57 mg/mL	Photokilling of bacteria	(Chen et al. 2008)
Staphylococcus saprophyticus	Fe_3O_4-TiO_2	>100	2.57 mg/mL	Photokilling of bacteria	(Chen et al. 2008)
Enterococcus hirae	TiO_2	20	10 mg/mL	Photoactivation of TiO2 induced loss of viability; bactericidal	(Tsuang et al. 2008)

role in the very high level of adsorption which is observed as already suspected by researchers (Chatellier et al. 2001). No clear evidence of the NPs passage inside the cells can be obtained by techniques such as transmission electron microscopy (TEM). This is possibly because of strong electrostatic interaction between the NPs and the membrane that might block them at the surface for very long time. However, it was found that NPs are found to be mainly located on the surface of the bacteria using adsorption isotherms and TEM images (Morones et al. 2005; Thill et al. 2006). Further, this adsorption onto the surface is linked to an oxidative stress for the bacteria.

Interaction of nanoparticles with cell membrane was found to be different in Gram positive and Gram negative bacteria because of their distinct membrane compositions. Exposure of nC_{60} with *Pseudomonas putida* (Gram-negative) and *Bacillus subtilis* (Gram-positive) result in altering membrane lipid composition, phase transition temperature, and membrane fluidity (Fang et al. 2007). It is suspected that lipid peroxidation is an important toxicity mechanism in bacteria, since bacterial lipids are mainly monounsaturated and thus unreactive to the lipid peroxidation reaction (Bielski et al. 1983; Imlay 2003). However, bacteria also tend to adapt physiologically by altering the membrane fatty acid compositions to cope up with the damage caused by the nanoparticles. It was found that Gram-positive bacteria exposed to nC_{60} nanoparticles increased the levels of iso- and anteiso- membrane fatty acids by 5–32%. Whereas, Gram-negative bacteria decreased the levels of unsaturated fatty acids and increased the cyclopropane fatty acids proportions (Fang et al. 2007). The distinct response by the different bacteria explains the differential responses associated with cell membrane integrity with respect to the toxicity of nC_{60}. However, it is to be noted that the nanoparticles also exist in a variety of different size, morphology, chemical nature that also contribute to the different ways of inducing cell-membrane damage. So far no detailed mechanisms of adaptation to the damage caused by NPs have been reported except for C_{60} (Fang et al. 2007). Only physical disruption of cell membrane with a range of metallic and metal oxide nanoparticles is evident from the literature. Recently size dependent silver nanoparticles were found to be located in the cell membrane as a result of direct interaction leading to bactericidal effects (Morones et al. 2005; Pal et al. 2007).

Smaller particles with a larger surface to volume ratio provide a more efficient means for antibacterial activity (Baker et al. 2005). *E. coli* cells exposed to ZnO NPs showed increase of membrane permeability leading to accumulation of ZnO NPs in the bacterial membrane and also cellular internalization of these NPs (Brayner et al. 2006). A substantial loss of cell viability/membrane integrity (∼30%) was also observed in the *E. coli* following treatment with ZnO NPs (Reddy et al. 2007). A range of metal oxide NPs including ZnO, SiO2, TiO2, and MgO have shown to cause membrane disorganization, increased membrane permeability as a result of perforation, and finally leading to cell death (Adams et al. 2006a; Brayner et al. 2006; Reddy et al. 2007; Stoimenov et al. 2002; Tsuang et al. 2008). Large amount of CeO_2 NPs measuring 7 nm sizes has been shown to be adsorbed on the *E. coli* outer membrane and undergo reduction bringing significant bacterial cytotoxicity. The toxicity effect of CeO_2 NPs is brought on by interaction with *E. coli* via adsorption followed by oxidoreduction (Thill et al. 2006). Metallic nanoparticles such as nanosilver (Ag NPs), NPs of FePt, Pd, and C_{60} also found to cause membrane disruption in both Gram-positive and -negative bacteria (De Windt et al. 2006; Gogoi et al. 2006; Maenosono et al. 2007; Morones et al. 2005; Ruparelia et al. 2007; Sondi and Salopek-Sondi 2004).

Recently, studies have shown that the silver nanoparticles caused toxicity via protein/membrane and oxidative damage, but do not result in DNA damage. However, gold nanoparticles do not cause any damage to *E. coli* (Hwang et al. 2008). In addition, these findings and that of other groups, the silver nanoparticles appear to disrupt the cell membrane, which results in a synergistic toxicity effect to the cells (Lok et al. 2006; Sondi and Salopek-Sondi 2004).

5 Nanoparticles Induce Oxidative Toxicity by Generating Reactive Oxygen Species (ROS)

The mechanism by which NPs induce toxicity is thought to be via oxidative stress that damages lipids, carbohydrates, proteins and DNA (Fang et al. 2007; Kohen and Nyska 2002). Lipid peroxidation is

considered most deleterious that leads to alterations in cell membrane properties which in turn disrupt vital cellular functions (Rikans and Hornbrook 1997; Sera et al. 1996). ROS production has been found to be with NPs as diverse as C_{60}, fullerenes, single walled nanotubes (SWNTs), quantum dots, and ultrafine particles (UFPs). These nanomaterials have shown to generate ROS especially under concomitant exposure to light, UV, or transition metals (Brown et al. 2001; Derfus et al. 2004; Fang et al. 2007; Joo et al. 2004; Li et al. 2003; Lu et al. 2004; Oberdorster et al. 2005b; Yamakoshi et al. 2003). An antibacterial activity of metal oxide nanoparticles is associated with light induced oxidative stress. For example, TiO2 and SiO2 was toxic to both *E. coli* and *B. subtilis* under both light and dark conditions, and cell growth inhibition appeared higher in the presence of light (Adams et al. 2006a, b). Oxidative stress mechanisms leading to membrane damage and antibacterial properties has been demonstrated for ZnO in *E. coli* (Zhang et al. 2007). Potential mechanisms of oxidative stress via ROS formation, organic radicals generated in the absence of light, and the role of other nanomaterials in disruption of membrane integrity have been investigated using fullerene in *Pseudomonas putida* (Fang et al. 2007), TiO_2 in *Psuedomonas aeruginosa* (Tsuang et al. 2008). Metal oxide NPs, such as SiO2, TiO2, ZnO dependent on light for inducing ROS generation in *Bacillus subtilis* and *E. coli* that eventually lead to oxidative toxicity, membrane disorganization, and antibacterial activities (Adams et al. 2006a, b; Brayner et al. 2006; Fu et al. 2006; Reddy et al. 2007; Tsuang et al. 2008). The antibacterial properties of silver is long been known for over the decades. However, the mechanism of bactericidal actions of silver is still not well understood. The action of silver nanoparticles is thought to be broadly similar to that of silver ion (Pal et al. 2007). It is speculated that a bacterial cell exposed to silver nanoparticles takes in silver ions, which inhibit a respiratory enzyme(s), facilitating the generation of reactive oxygen species and consequently damage the cell (Hwang et al. 2008).

Metal oxides are highly reactive to light because the particulate metal oxides such as MnO, WO_3, $SrTiO_3$, Fe_2O_3, ZnS, ZnO and TiO_2 absorb sufficient light/UV energy and result in the formation of electron-hole pairs through a process of electronic excitation between the valence and conduction band (Beydoun et al. 1999; Hoffmann et al. 1995). Photogenerated electrons and holes undergo reaction with dissolved molecular oxygen, surface hydroxyl groups, and adsorbed water molecules to form hydroxyl (•OH) and superoxide (O_2•$^-$) radicals, as shown in equations (1), (2), (3), and (4):

$$\xrightarrow{\text{ZnO}} \; h\nu \quad h_{vb}{}^{+} + e_{cb}{}^{-} \tag{1}$$

$$h_{vb}{}^{+} + e_{cb}{}^{-} \longrightarrow \text{ZnO} + h\nu \text{ (or heat)} \tag{2}$$

$$O_2 + e^{-} \longrightarrow O_2\bullet^{-} \tag{3}$$

$$H_2O + h^{+} \longrightarrow \bullet OH + H^{+} \tag{4}$$

where, $h_{vb}{}^{+}$ is the valence-band hole, and $e_{cb}{}^{-}$ is a conducting band electron. It is proposed that this type of reactions occur when metal oxide NPs come in contact with bacteria and exposed to light source. Studies in recent years on light induced oxidative toxicity by NPs, such as C_{60} or fullerene, TiO_2, SiO2, ZnO, and MgO in bacteria have surfaced, and the toxicity is thought to be associated with ROS generation (Adams et al. 2006a; Brayner et al. 2006; Fu et al. 2006; Tsuang et al. 2008). Recent studies showed that composite irradiated Fe_3O_4@TiO_2 nanoparticles induce photokilling in bacterial species such as *Streptococcus pyogenes, Staphylococcus saprophyticus*, and *Staphylococcus aureus*, (Chen et al. 2008). For example, induction of oxidative stress by ROS generation by fullerene exposure to *Pseudomonas putida* and *Bacillus subtilis* following alterations in membrane composition as a defense mechanism (Fang et al. 2007). *E. coli* exposed to C_{60} has been shown to decrease CO_2 production, membrane damage, and cytotoxicity is associated with oxidative toxicity (Fortner et al. 2005; Lyon et al. 2005; Tang et al. 2007). C_{60} oxidative toxicity in other bacteria, such as *Salmonella, Shewanella* sp. has also shown by inducing singlet oxygen, and oxidative DNA damage related mutagenicity (Sera et al. 1996; Tang et al. 2007). NPs interaction is likely to be unique to Gram-positive and –negative bacteria that may have different potential to induce toxicities because of the differing compositions in their cell membranes (Fang et al. 2007).

ROS and other radicals are involved in a variety of biological phenomena, such as mutation, and carcinogenesis (Kohen and Nyska 2002). It is not entirely surprising that the ROS generation by NPs can also lead to oxidative DNA damage or mutagenicity. FePt and fullerene C_{60} nanoparticles were mutagenic to bacteria

belonging to *Salmonella* sp. (Maenosono et al. 2007; Sera et al. 1996). The mutagenicity of C_{60} NPs is thought to be due to the indirect action of singlet oxygen and lipid peroxidation of linoleate that causes oxidative DNA damage (Sera et al. 1996). Silver nanoparticles interact with cell membrane and sulfur- and phosphorous containing compounds such as DNA and induce DNA damage in *V. cholera* and *S. typhus* (Morones et al. 2005). Although no clear evidence has been reported regarding the toxicity mechanisms of silver nanoparticles by generating ROS in bacteria. The ROS generation through singlet molecular oxygen production was seen by interaction between bacteria (*E. coli*) and the photo-catalytic TiO_2 nanoparticles (Adams et al. 2006b; Rengifo-Herrera et al. 2007). Light induced ROS generation appear to be common in metal oxide nanoparticles, such as TiO_2, SiO_2, and ZnO mediated cytoxicities, and thus these NPs are known to possess effective bactericidal effects (Adams et al. 2006a; Brayner et al. 2006; Fu et al. 2006; Reddy et al. 2007; Tsuang et al. 2008). A previous study demonstrated that metallic silver nanoparticles led to the production of silver ions and, subsequently, superoxide radicals. This damage is linked with the size of the particles because larger silver particles, i.e., micro-sized particles showed no toxicity to *E. coli* (Hwang et al. 2008).

6 Current Understanding on Toxicity of Nano-Sized Particles to Bacteria

Few microorganisms grow in the presence of high metal concentrations that might result from specific mechanisms of resistance. Such mechanisms include efflux systems, alteration of solubility and toxicity by changes in the redox state of the metal ions, extracellular complexation or precipitation of metals, the lack of specific metal transport systems, and the changes in membrane composition (Beveridge et al. 1997; Fang et al. 2007; Silver 1996). Recently, Fang et al. (2007) have demonstrated that Gram-positive and Gram-negative bacteria have separate ways of adaptation to fullerene nanoparticles toxicity, but both by changing membrane composition in order to cope up with the toxicity, a first ever evidence for adaptation to metal NPs by aerobic bacteria, although bacteria were not resistant to NPs. However, more research is needed to explain the similar mechanisms underlying adaptation to other nanoparticles in aerobic microorganisms. A number of researchers reported the cytotoxicity of a range of NPs in both Gram-positive and –negative bacteria. A majority of these findings conclude that NPs induce oxidative toxicity by generation of ROS, and in some instances this ROS was triggered by the light exposure on NPs (Tables 1 and 2). A most common toxicity effect of NPs is associated with physical membrane damage leading to fatal effects as a result of perforation and membrane fluidity and/or disorganization. Metal or metal oxide NPs also release soluble ions that also contribute to the chemical toxicity to bacteria (Heinlaan et al. 2008). Studies have shown that some bacteria belonging to *Pseudomonas* sp. can solubilize bulk NPs, such as ZnO NPs, into Zn ions that exhibited bactericidal effects (Fasim et al. 2002).

The fact that presence of metal/metal oxide nanoparticles is toxic to aerobic bacteria, which is most certainly due to the reactivity of metal/metal oxide NPs with molecular oxygen and/or light, followed by ROS generation. However, there are no reports on the toxicity of NPs in absence of oxygen, or under anaerobic conditions. It is assumed that light-induced metal/metal oxide NPs toxicity may have detrimental effects on anaerobic bacteria. Nevertheless, thorough experimental evidence is required on these lines to confirm the hypothesis. But, it is well documented that anaerobic bacteria (i.e., metal reducing bacteria) unlike aerobic bacteria adapt to excess metal ions by reduction of metal ions and produce metal/metal oxide nanoparticles (Mandal et al. 2006). Anaerobic bacteria tend to change the environment of their outer membrane in presence of metal ions, creating electrochemical conditions favorable for metal ion precipitation, which is most likely be associated with an organic matrix and produce a broad size-distributed nanoparticles (Frankel 1987). For example, magnetite particles with a narrow size distribution around 40–50 nm are produced by iron-reducing bacteria and these particles are enveloped by bacterial membranes (Balkwill et al. 1980; Gorby et al. 1988). Synthesis of metal/metal oxide nanoparticles from external high metal ion concentrations is an adaptation process of anaerobic bacteria to cope up with the metal ion toxicity (reviewed in Mandal et al. 2006; Nies 2003). It is unclear that the nano-sized particles produced by anaerobes exhibit

toxicity to themselves or to the co-existing microorganisms. The ability of metal-reducing bacteria to produce copious amounts of extra-cellular nanoparticles is a process of biogeochemical cycling of metal, carbon, nitrogen, phosphate, and sulfur in natural and contaminated subsurface environments which is well documented in the literature (Fredrickson et al. 2001; Liu et al. 1997; Lovely et al. 1987; Lovley 1995). It is also now important to assess the toxicity of nanomaterials on anaerobic bacteria that may have distinct cytotoxic mechanism and gives an insight into the impact of nanomaterials on both aerobic and anaerobic mesocosms.

7 Toxicity Assays of NPs Using Bacteria as Models

Toxicity assays using specific microorganisms can be used to assess the detrimental effects of various NPs on living organisms and understand its impact, mode of action or mechanism. As evidenced from the literature that NPs exhibit toxicity to bacteria (Tables 1 and 2). The detailed toxicity action of NPs in the cells or interaction with cellular proteins/enzymes and other components seems to be overlooked in most cases. Researchers have found only two major effects of NPs in bacteria; (i) NPs induce oxidative toxicity and (ii) cell-membrane/wall damage. A detailed study on a particular NP, impact of its size, chemical nature would allow us to understand the mode of NP action to cause cytotoxicity to bacteria. The outcome of this study also gives an insight into the toxicity action of NPs to other living organisms including effects on humans and environment. Only little information is currently available from the literature explaining the mechanism of toxicity, interaction with biological systems and environment (Nel et al. 2006).

Assessing toxicity of NPs using bacteria as model organism have many advantageous, including the fact that bacterial assays are faster, sensitive, less expensive and easy to handle when compared to the cells of mammalian origin. Recently, toxicity of silver nanoparticles in bacteria has been studied using recombinant bacterial biosensors and elucidated the potential mode of toxic action by silver nanoparticles (Hwang et al. 2008). Similarly, a quite a few number of studies have shown the toxicity modes of few nanomaterials, although not in detail, for example, toxicity fullerene, metal oxide NPs including ZnO, CuO, SiO_2, and TiO_2 (Fang et al. 2007; Heinlaan et al. 2008; reviewed in Oberdorster et al. 2005b, 2007). However, the toxicity mode of action deduced using these nanoparticles may not be the same with the other nanomaterials. It may be because of their variable size, surface chemistry, or chemical nature of nanomaterials. Likewise, the NPs may also have different effect on different types of cells, which depends on cell-wall composition (Fang et al. 2007). Therefore the scientific committee on emerging and newly identified health risk (SCENIHR) of Europe has concluded that there is insufficient data available at the present time to allow the identification of any systematic rules that govern the toxicological characteristics of all products of nanotechnology (SCENIHR 2006). Further, a guideline has been proposed that the risk assessment needs to be made on a case by case basis.

8 Summary and Future Outlook

It is most probable that production of nanomaterals and use for the benefit of human will lead to its entering in the environment as a result of disposal. So far there is no clear consent among the regulatory bodies and the manufacturers to examine ecotoxicological impacts of NPs. Until recently, toxicities of most nanomaterials have only focused on human cells and it is still continued to do so in future. However, very little is known about their potential adverse effects on aerobic or anaerobic microorganisms. Developing resistance properties to NPs by these microorganisms in the environment can be an evolutionary process which might take decades or centuries. It is important to assess these NPs for their toxicity on different microorganisms which provides a means for possible measures needs to be taken for safety.

Despite of the preliminary knowledge regarding NPs toxicity on humans and microorganisms, their detailed toxic effects still remained unknown at large. Limited information available on toxicity of NPs either in human cells or bacteria consistently points out that the greater surface to volume ratio or small size of

NPs is a main cause for their biological activity than larger-sized particles of the same composition. Secondly, NPs tend to induce membrane disorganization as a result of adsorption by electrostatic interaction and oxidative stress by generation of ROS in aerobic bacteria. Most importantly, the metal oxide NPs in particular are highly susceptible to light and oxygen in order for production of ROS and thus oxidative toxicity. ROS generation by the NPs is also likely to induce mutagenicity by oxidative DNA damage. This has been implicated to be occurring with few NPs, such as C_{60} and FePt in bacteria. However, no detailed mechanism for mutagenicity has yet been elucidated. It is well known that ROS generation in cells is also linked to indirect oxidative DNA damage and it is not surprising that ROS generation by NPs can also lead to oxidative DNA damage. In addition, the impact of NPs on anaerobic microorganisms is an important area to explore. So far no reports have yet found that address the effects of manmade NPs on anerobes. Therefore, more research is required to unveil the toxicity mechanisms associated with different types of NPs and sizes on aerobes and anaerobes.

A new field of nanosciences has now been emerged as a diversion to NPs which is focused on the synthesis of engineered NPs by modifying or coating with different functional groups for various applications, for example, quantum dots that have tremendous optical properties. This has raised new concerns about human and environmental health. The toxic effect of engineered NPs on microorganisms has yet to be studied in greater detail. Recently numerous engineered NPs have been industrially manufactured without the knowledge of their impact on living microorganisms. However, many of these engineered NPs have been used as fluorescent labels/markers to trace or locate the cancer or tumor cells in mice and are suspected to have cytotoxic effects, though it is still unclear. Nevertheless, it is also important to test these engineered NP's toxicity to microorganisms. Hence, there is a growing concern regarding the regulations on the synthesis and production of novel nanostructures because of their potential toxicity on microorganisms and other living systems. There seems to be lack of a model to predict toxicity on living organisms based on the physicochemical characteristics and microbial toxicity of new nanomaterials that can be used for risk assessment or for safe product design.

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