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Health hazards associated with nanomaterials

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Abstract

Nanotechnology is a major scientific and economic growth area and presents a variety of hazards for human health and environment. It is widely believed that engineered nanomaterials will be increasingly used in biomedical applications (as therapeutics and as diagnostic tools). However, before these novel materials can be safely applied in a clinical setting, their toxicity needs to be carefully assessed. Nanoscale materials often behave different from the materials with a larger structure, even when the basic material is same. Many mammals get exposed to these nanomaterials, which can reach almost every cell of the mammalian body, causing the cells to respond against nanoparticles (NPs) resulting in cytotoxicity and/or genotoxicity. The important key to understand the toxicity of nanomaterials is that their minute size, smaller than cellular organelles, allows them to penetrate the basic biological structures, disrupting their normal function. There is a wealth of evidence for the noxious and harmful effects of engineered NPs as well as other nanomaterials. The rapid commercialization of nanotechnology field requires thoughtful, attentive environmental, animal and human health safety research and should be an open discussion for broader societal impacts and urgent toxicological oversight action. While 'nanotoxicity' is a relatively new concept to science, this comprehensive review focuses on the nanomaterials exposure through the skin, respiratory tract, and gastrointestinal tract and their mechanism of toxicity and effect on various organs of the body.

Keywords

Nanoparticle, nanotubes, toxicity, oxidative stress

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Introduction

Nanotechnology has been defined by the US National Nanotechnology Initiative (NNI) as 'understanding and control of mater at dimensions of roughly 1 to 100 nm (nanomaterials) where unique phenomena
AQ2 enable novel applications' (NNI, 2007). Nanomaterials include nanoparticles (NPs), nanofibres, and nanotubes, composite materials, and nanostructured surfaces. Examples of NPs are gold (Au) NPs, carbon NPs, europium oxide (Eu_2O_3) NPs, titanium NPs,
AQ3 magnetic NPs (MNPs), biodegradable NPs (PLGA), carbon nanotubes (CNTs; singled-walled and multi-walled (SWCNT and MWCNT, respectively)), nanowires, fullerene derivatives, quantum dots (QDs), and so on. Research on toxicologically relevant properties of these engineered nanomaterials has increased tremendously during the last few years. Nanomaterials may have different properties like chemical, optical, magnetic, and structural properties; and hence consequently they have differential toxicity profiles (Lanone and Boczkowski, 2006; Studart et al., 2007). Engineered nanomaterials are the nanomaterials with specific physicochemical characteristics manufactured intentionally by humans. Nanomaterials hold great promise in a range of biomedical applications, including medical imaging and diagnostics and for targeted delivery of therapeutic compounds, or the simultaneous monitoring of disease processes and therapeutics (theranostics) (Farokhzad and Langer, 2009; Riehemann et al., 2009). Engineered NPs are intentionally designed, which have application in nanomedicine, are

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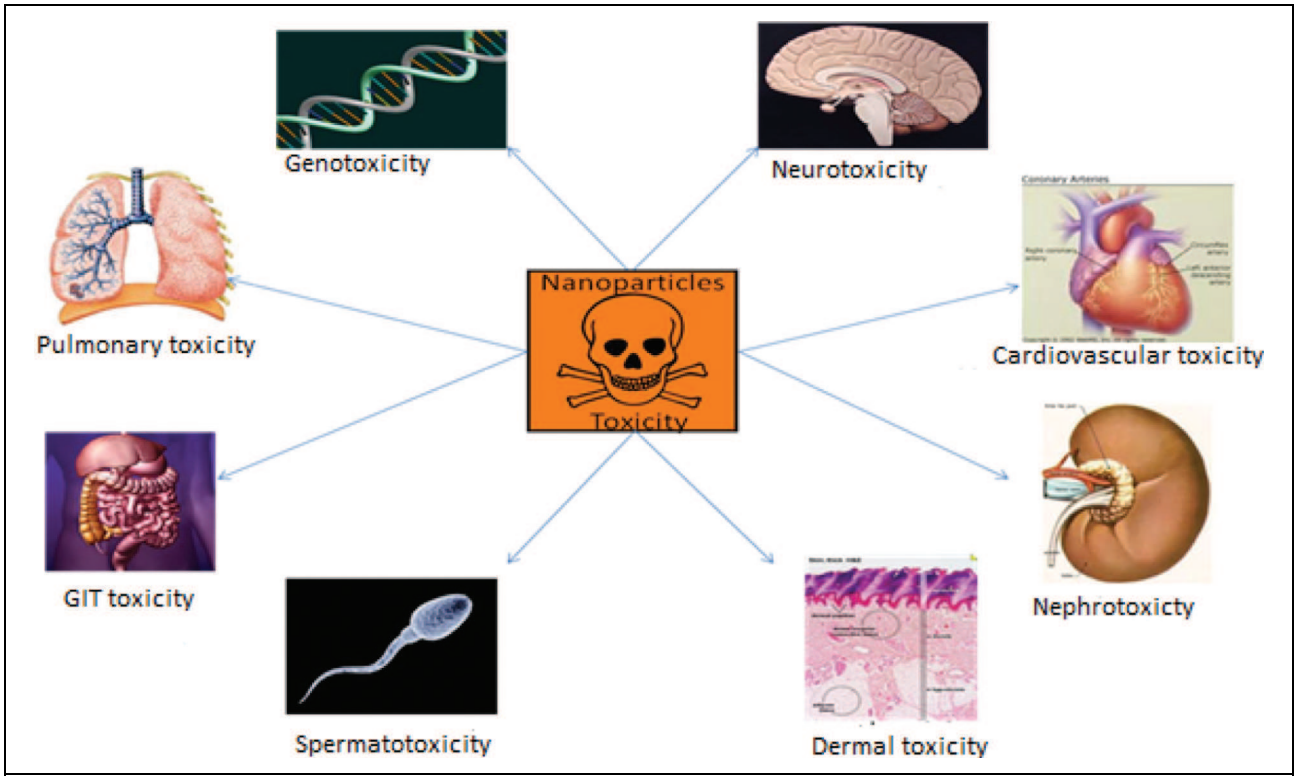

Figure 1. An interdisciplinary-science: nanotoxicology. An overview of the potential toxic effects associated with nanomaterials, *in vivo* and *in vitro*. Figure showing different toxicities due to nanomaterials like genotoxicity, neurotoxicty, pulmonary toxicity, cardiovascular toxicity, gastrointestinal toxicity, nephrotoxicity, spermatotoxicty and dermal toxicity (modified from El-Ansary and Al-Daihan, 2009).

monodispersed, and are in solid form, while unintentional nanosized particles are polydispersed and chemically complex (Moghimi et al., 2005; Oberdorster et al., 2005). However, the same toxicological principles apply to unintentionally and intentionally designed NPs (Oberdorster et al., 2005).

Nanotoxicology refers to the study of the interactions of nanostructures with biological systems with an emphasis on elucidating the relationship between the physical and chemical properties of nanostructures with induction of toxic biological responses (Oberdorster et al., 2005). Since past few years, researchers had investigated the toxicity of the nanomaterials in various organs as shown in the Figure 1.

Entry of NPs into living system

Possible routes of entry into the body include inhalation, absorption through the skin or digestive tract, injection, and absorption or implantation for drug delivery systems. In particular, inhalation and ingestion are likely to be the major routes of NP uptake in terrestrial organisms (Brigger et al., 2002).

Respiratory tract

The respiratory tract can be divided into three regions: nasopharyngeal, trachea–bronchial, and alveolar regions. Significant amounts of certain particle size ranges can deposit in each region, for example, about 50% of NPs of 20 nm in diameter deposit in the alveolar region and remaining 15% in the nasopharyngeal region and 15% in the trachea–bronchial region. In comparison, NPs of 1 nm size does not reach the alveolar region and about 90% deposit in nasopharyngeal region and 10% in the trachea–bronchial region (Moghimi et al., 2005). Inhalation NPs are deposited in all regions of the respiratory tract, but only smaller particles reach distal airways and larger particles may be filtered out in the upper airways (Curtis et al., 2006; Hagens et al., 2007). The NPs are absorbed across the lung epithelium and enter into the blood and lymph to reach cells in the bone marrow, lymph nodes, spleen, and heart (Hagens et al., 2007; Oberdorster et al., 2005). Diesel exhaust (DE) and DE particles (DEP) are one of the major compounds responsible for air pollution. These compounds consist of nanopaticles that induce adverse health effects.

Several studies reported that the effects of NPs on the human body (mammals) have shown that NPs exacerbate lung injury (Inoue et al., 2006). When the NPs are administered through the nasal mucosa, they accumulate in the brain via the olfactory nerve and exacerbated inflammatory reactions (Elder et al., 2006), and these NPs affect the circulatory system by altering heart rate (Chalupa et al., 2004).

Gastrointestinal tract

NPs can reach the gastrointestinal tract through the food, water, cosmetics, drugs, and drug delivery devices (Hagens et al., 2007; Maynard and Michelson, 2005; Medina et al., 2007; Oberdorster et al., 2005). Acute toxicity of copper particles and nanocopper was measured in mice; median lethal dose (LD_{50}) for nanocopper is 413 mg/kg compared with >5000 mg/kg for copper (Riehemann et al., 2009). Nanocopper was also reported to cause pathological damage to the liver, kidney, and spleen. Some studies have glanced at the toxicity of NPs following oral ingestion (Riehemann et al., 2009). Further, more research on gastrointestinal lymphatic uptake and transport and direct toxicological effects on the gastrointestinal tract are required.

Skin

The dermal exposure is hypothesized as one of the most significant route of exposures for NPs (Riehemann et al., 2009), but even few literature reports are available that refer to the absorption and effects of NPs in the skin. Dermal absorption and skin penetration of NPs need a better evaluation because few and contradictory data are present in literature, mainly on titanium dioxide (TiO_2) (Curtis et al., 2006; Hagens et al., 2007; Lanone and Boczkowski, 2006; Oberdorster et al., 2005).

General mechanism of NP toxicity

Entry of NPs into a cell is largely governed by biological mechanisms of endocytosis (Porter et al., 1992). The receptor-mediated entry is the prominent route of endocytosis, which requires recognition of some ligands (surface molecule or epitope) by specific biological receptor. The best known of these are clathrin-mediated endocytosis, caveolin-mediated endocytosis (Panyam and Labhasetwar, 2003; Song et al., 2010), and so on. All these endocytic routes of uptake involve delivery of NP into a subcellular compartment, that is, the endosome. Most of these endocytic routes also end up in a degradative compartment of the cell, that is, the lysosome, where materials are exposed to high concentrations of a wide variety of hydrolytic enzymes. NPs also gain entry through the passive diffusion, pinocytosis, and other clathrin and caveolin-independent endocytosis. Upon internalization, the NPs may presumably be degraded into ions in the lysosomes. This 'free ions' can potentially pass through the nuclear or mitochondrial membrane, and in the latter case, the free ion can react with hydrogen peroxide (H_2O_2), and oxygen produced by the mitochondria to produce highly reactive hydroxyl radicals. AQ5 Hydoxyl radicals (^{-}OH) generated could indirectly damage DNA, proteins, and lipids (8-hydroxydeoxyguanosine (8OHdG), malondialdehyde (MDA), 4-hydroxy-2-nonenal (HNE)) (Singh et al., 2010). This leads to more oxidative stress on the body, which have a role in the induction or the enhancement of inflammation through the upregulation of redox sensitive transcription factors (e.g. nuclear factor-κB (NF-κB)), activator protein-1, and kinases involved in inflammation (Italia et al., 2007; Lanone and Boczkowski, 2006; Moghimi et al., 2005). The organs like liver and spleen are the main targets of oxidative stress because of slow clearance and accumulation (storage) of potential free radical producing nanomaterials as well as prevalence of numerous phagocytic cells in the organs of the reticuloendothelial system. Additionally, organs of high blood flow such as the kidneys and lungs, exposed to nanomaterials can also be affected. Nanomaterials such as silver (Ag)-coated gold NPs, fullerenes, block copolymer micelles, and CNTs may be capable of localizing mitochondria and inducing apoptosis and reactive oxygen species (ROS) formation; nanomaterial-induced nuclear DNA damage, cell-cycle arrest, mutagenesis, and apoptosis are a possible sources of toxicity (Unfried et al., 2007).

Effect of nanomaterials on different organs

The toxicity of the nanomaterials depends upon physicochemical properties of nanomaterials and genetic component of an individual; hence, not all the nanomaterials produce adverse health effects. Diseases associated with nanomaterials are asthma, emphysema, bronchitis, lung cancer, arteriosclerosis, arrhythmia, heart disease, Crohn's disease, liver damage, kidney damage, and so on (reviewed by Buzea et al. (2007)).

Cells of respiratory system

Effect of NPs on lung epithelial cells. Barlow et al. (2005) evaluated the effect of carbon black (CB) NPs (size: 14.3 nm, surface area: 253.9 m2/g) and TiO_2 NPs (size: 29 nm, surface area: 49.78 m^2/g) on type II alveolar epithelial cells (line L-2) of rat. However, only the CB NPs showed effect in stimulating the release of chemotaxins. Additionally, a dose-dependent increase in production of lactate dehydrogenase (LDH) after exposure to the NPs was observed. Hence, it is concluded that the carbon NPs are very likely responsible for the production of chemoattractants by type II cells because of high surface free radical reactivity.

Effect of NPs on bronchial cells. The effect of ultrafine anatase-sized (10 and 20 nm) TiO_2 particles on human bronchial epithelial cells (BEAS-2B) in the absence of photoactivation was studied by Gurr et al. (2005). Results showed oxidative DNA damage, lipid peroxidation, formation of micronuclei, and increased H_2O_2 and nitric oxide (NO) production in the cells (anatase particles of 10 and 20 nm (10 μg/mL)). This unveiled that the smaller the size of the particles, the higher their potential to induce oxidative stress in the absence of photoactivation.

Effect of different NPs on lung tissues

Carbon nanotubes. Three types of SWCNTs toxicities were investigated in mice by Lam et al. 2004. Mice were intratracheally instilled with 0, 0.1, or 0.5 mg of CNTs, a CB negative control, or a quartz positive control and were euthanized 7 or 90 days after the single treatment to carry out histopathological study of the lungs. The results showed that regardless of the amount of metal impurities, dose-dependent lung lesions were characterized chiefly by interstitial granulomas and SWCNTs were taken up by alveolar macrophages. In macrophages, SWCNTs clustered to form granulomas in centrilobular locations. Similar study was carried by Muller et al. (2005), where they compared the pulmonary toxicity of ground (0.7 ± 0.07) and unground (5.9 ± 0.05) MWCNTs by intratracheal instillation of 0.5, 2, or 5 mg dose to Sprague–Dawley rats, using asbestos (Rhodesian chrysotile) and CB as references. They observed higher degree of pulmonary inflammation with ground MWCNTs than that with intact MWCNT-treated animals after 60 days. They also noticed that the adverse effects of MWCNTs depend on the length of the material used *in vivo*. Moreover, pulmonary exposures to SWCNT (1 or 5 mg/kg) in rats produced a non-dose-dependent series of multifocal granulomas (Warheit et al., 2004). Scanning electron microscopic (SEM) images of MWCNT scaffolds prepared in our laboratory on polyethyleneimine-coated glass surface at different magnifications and different views are shown in Figure 2(a) and (b). The topological features of nanonetwork assembly and the surface modification by protein adsorption served to convert CNTs into a bioactive material with pronounced cell growth and functional activities (Rafeeqi and Kaul, 2010a, 2010b).

TiO_2 NPs. Li et al. (2007) conducted a comparative study and through the measurement of selected biochemical parameters in bronchoalveolar lavage (BAL) fluid found that the acute pulmonary toxicity is induced by 3 and 20 nm TiO_2. At 3-day postexposure, the 3-nm TiO_2-induced significant increase in albumin, alkaline phosphatase (ALP) and acid phosphatase (ACP) concentrations in high-dose group (40 mg/kg) and also induced significant increase in ALP and ACP activities in mid-dose group (4 mg/kg), but did not induce significant increase in total protein and LDH concentrations in any dose group. On the other hand, 20 nm TiO_2 induced significant increase in all biochemical parameters in high- and mid-dose groups. At 3-day postexposure, TiO_2 particles did not induce obvious pulmonary toxicity in their low-dose (0.4 mg/kg) groups as an evidence of no significant increase in all biochemical parameters. They reported that the pH value of TiO_2 particles in medium, other than particle size, surface area, and aggregation, plays important role in affecting TiO_2 NPs pulmonary toxicity.

Cells of nervous system

Effect of NPs on brain

TiO_2 NPs. The female mice were intranasally instilled with 500 μg of TiO_2 NPs suspension every other day for 30 days. Maternal exposure of mice to TiO_2 NPs (80 nm, rutile and 155 nm, anatase; purity >99%) affected the expression of genes related to the development and function of the central nervous system (CNS), like gene expression levels associated with apoptosis were altered in the brain of newborn pups, and those associated with brain development were altered in early age. Nano-TiO_2 has also been shown to induce an increase in glial fibrillary acidic

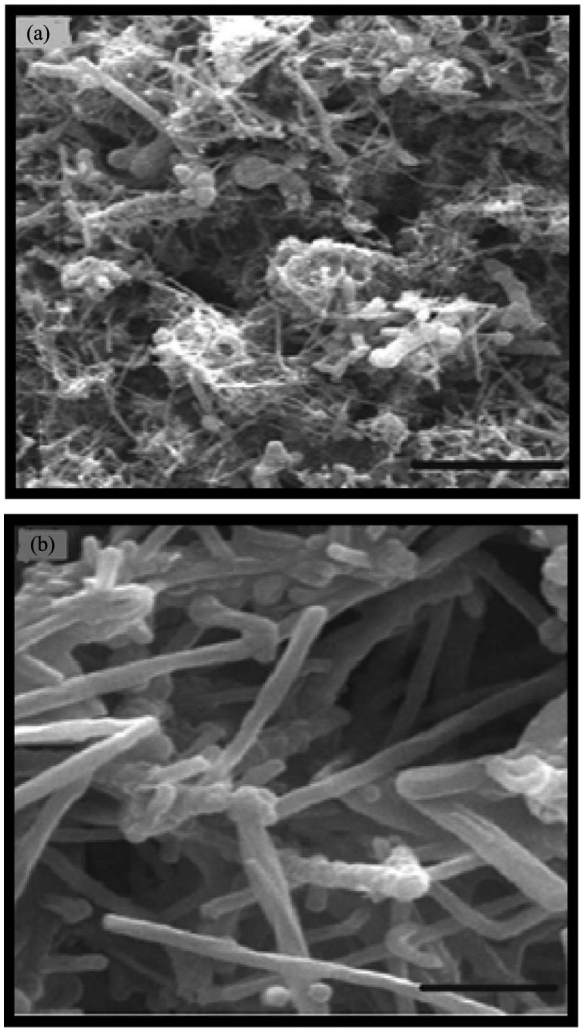

Figure 2. ((a) and (b)) Scanning electron microscopic images of MWNTs scaffold. When observed by scanning electron microscope at different magnifications and different views, these scaffolds with compact structure were composed of many thousands of highly entangled nanotubes with diameters ranging from few nanometers to several micrometers in length. Scanning electron micrographs show MWNTs distributed all over the surface. Scale bars represent (a) 5 μm and (b) 1 μm (our laboratory: Rafeeqi and Kaul, 2010b). MWNTs: multiwalled nanotubes.

protein (GFAP), producing positive astrocytes in the CA4 region. This resulted in lipid peroxidation, protein oxidation, and increased activities of catalase as well as the excessive release of glutamic acid and NO, thus contributing to oxidative stress in the brain of exposed mice. These findings indicate that anatase TiO_2 NPs exhibited higher concern on tested biological effects. The results provided the preliminary evidence that intranasally instilled TiO_2 NPs could be translocated into the CNS and cause potential lesion of brain (Chai, 2008).

Zinc, iron, and silicon NPs. Cha et al. (2007) exposed zinc (300 nm), iron (100 nm), and silicon (10–20, 40–50, and 90–110 nm) NPs to glioma cell line. Results showed that the NPs did not alter the membrane permeability and the *in vitro* cytotoxicity was low. Moreover, it was not dependent on the types and the sizes of NPs (Zhao et al., 2009), and thus, here the toxicity was inferred to be due to material chemistry rather than size (Cha and Myung, 2007). AQ6

Fe_2O_3 MNPs. The temporary exposure to anionic iron (III) oxide (Fe_2O_3) MNPs having the size in the range between 5 and 12 nm with a final dose of 0.15–15 mM results in a dose-dependent reduced ability of rat pheochromocytoma (growing neuron cell line, PC12) to respond to nerve growth factor (NGF). PC12 cells exposed to AMNPs show reduced viabilities, increased cytoskeletal disruption, decreased intracellular contact, and diminished ability to form mature neuritis in response to NGF exposure when compared with control cells (Pisanic et al., 2007). AQ7

Magnetic NPs. The effect of MNPs on the adhesion and cell viability concerned to astrocytes was assessed by Au et al. (2007). The astrocytes were treated for 6 h with or without 10 μg of MNPs per mL of HEPES buffer. They observed that NPs impede the attachment of astrocytes to the substratum. However, once astrocytes attach to the substratum and grow to confluence, NPs may cause mitochondrial stress. The lack of a significant difference between the control and NP-treated groups strongly suggests that the addition of NPs to astrocytes does not disturb membrane integrity. AQ8

Effect of NP on spinal cord. When chicken embryonic spinal cord or dorsal root ganglia are exposed to SWCNTs (30 μg/mL), the DNA content is significantly decreased. This effect was more pronounced when cells were exposed to highly agglomerated SWCNTs (100 nm or even more) than when they were exposed to better dispersed SWCNT (20 nm) bundles. This showed that the toxicity also depends upon agglomeration property (Belyanskaya et al., 2009).

Cardiovascular system

Effect of NP on cardiac tissue

Zinc NPs. Wang et al. (2006) assessed the effect of zinc NPs on the cardiovascular cells of the cardiac

tissues. The two size particles, nanoscale zinc and microsize zinc powder, at a dose of 5 mg/kg body weight were administered gastrointestinally to the healthy adult male and female mice, while one group of mice treated with sodium carboxy methyl cellulose was used as the control. They detected the fatty degeneration, which can be caused by anemia. In mice exposed to NPs lead to cardiac impairment, which was indicated by increase in certain biochemical blood parameters like creatinine kinase, aspartate aminotransferase, LDH, and hydroxybutyrate dehydrogenase. Hence, the size of the particle will play major role in the toxicity pattern.

Effect of NPs on endothelial cells (ECs)

CB NPs. Yamawaki and Iwai (2006) evaluated the effect of CB on the human umbilical vein endothelial cells (HUVECs). HUVECs were treated with CB, a component of DEPs (mean diameter $\pm$ SD of 248.2 $\pm$ 161.4 nm) for 24 h. Outcome of this study showed that CB induced cytotoxic morphological changes such as cytosolic vacuole formation, cell disorientation, and decreased density, because CB has minimal metallic components (Lam et al., 2004), and it seems unlikely that the effects were mediated by chemical reactions. CB also induced cytotoxic injury in both the cells and plasma membranes. HUVECs growth was inhibited in a dose-dependent manner. The effect of CB on the expression of PCNA, which is specifically expressed in the S phase of the cell cycle, was significantly suppressed by 100 μg/mL CB compared with controls. The expression of genes-related vascular inflammation is unregulated by CB. CB reduced the expressions of connexin 37 and endothelial NO synthase. Authors suggested that ECs injury/inflammation and membrane disintegration are related to the initiation of atherosclerosis, and NO are antiatherogenic and antithrombogenic; the direct effects of NPs on ECs may represent one mechanism behind environmental air pollution-mediated atherosclerosis and ischemic heart disease. There is also a report that examined the toxic effects (Table 1) of several nanomaterials, including metals (TiO_2 particles size: 20 and 160 nm with mean size 70 nm; silica (SiO_2) particle size: 4 and 40 nm with mean size 14 nm; cobalt (Co) particles size: 50 and 200 nm with mean size 120 nm; Ni particle size: 50 nm; polyvinylechloride particles size: 60 and 170 nm with mean size 130 nm), on ECs, and it showed that only Co particles had a cytotoxic effect on ECs. Thus, it seems likely that there are variations in the effects of nanoparticles Peters et al. (2004).

Effect of different NPs on RBCs

Magnetite NPs. Creanga et al. (2009) carried out an *in vitro* test on magnetite colloidal NP (11.44 nm) effects upon animal red blood cell (RBC). Aliquots of aqueous magnetic fluid were brought in direct contact with the blood samples, the concentration of magnetite NPs were being of 10^{-12} to 10^{-13} per mL. They found significant increase in hemolysis. The hemolysis extent was found increased up to 2.75 and 2.81 in horse and dog, respectively. The magnetite NPs influenced the heme energetic levels by two possible ways – either electronic or vibrational – by interacting with heme from released hemoglobin (Hb) molecules or by exerting chronic magnetic exposure on the heme iron following their addition to the RBC membranes, respectively.

Gold NPs. Wiwanitkit et al. (2008) evaluated the effect of gold NPs on human RBC *in vitro*. Mixture of gold NP solution and blood sample was analyzed and observed the accumulation of gold NPs in the RBC, but showed no significant destruction of the RBC.

CNTs, ZnO, and Fe_2O_3 NPs. Loeb et al. investigated the toxic effect of MWCNT, zinc (II) oxide (ZnO), and Fe_2O_3 nanomaterials (concentrations of 25, 50, 100, 200, and 400 mg/mL) on human RBC. As hemolysis of erythrocytes is a useful method to examine the effects of particles on the cell membrane. The interaction of RBC and NPs was studied with the help of ultrahigh resolution imaging systems. This unveiled attachment of NPs to RBC and their cross-linking effects. MWCNT were able to induce only hemolysis (at 400 μg/mL), where as Fe_2O_3 displayed only hemagglutination (at 50 μg/mL), and ZnO nanorods showed both hemolysis as well as hemagglutination (at 400 μg/mL). It showed that the MWCNT, ZnO, and Fe_2O_3 are toxic to human RBC, irrespective of the blood group. This showed that the toxicity of nanomaterials on RBCs was due to the result of a combination of shape and composition.

Cells of digestive system

Effect of NPs on gastrointestines

Selenium. Zhang et al. (2005) investigated the effect of nanoselenium (nano-Se) after ingestion (at an oral

Table 1. Recent spotlight research work on nanoparticles and their *in vitro* and *in vivo* toxic effects

Nanoparticles	Toxic effects: *in vivo*	Toxic effects: *in vitro*	References
Single-walled carbon nanotubes (SWCNTs)	Mice: uphold allergic response; genotoxicity and lung inflammation; 8-oxo-dG increase in lung and liver Rat: Platelet activation and thrombus formation	Inflammatory mediator response suppression in human lung epithelium; disruption of actin filament integrity and vein endothelial-cadherin (vascular endothelial-cadherin) distribution in human aortic endothelial cells; oxidative stress, DNA damage, micronuclei formation, oxidative stress, apoptosis, cytotoxicity in various cells.	Nygard et al., 2009; Bihari et al., 2010; Folkmann et al., 2009; Yang et al., 2008; Walker et al., 2009; Herzog et al., 2009; Lindberg et al., 2009; Migliore et al., 2010; Murray et al., 2009; Wang et al., 2010; Witasp et al., 2009; Zhang et al., 2010.
Multiwalled carbon nanotubes (MWCNTs)	Mice: induce inflammation but decrease production of reactive oxygen species in lung; induce apoptosis; pulmonary toxicity; granuloma formation; cytotoxicity and fibrosis in lungs.	Disruption of actin filament integrity and vein endothelial-cadherin distribution in human aortic endothelial cells; induce micronuclei and double strand breaks in DNA; induce apoptosis; oxidative stress and cytotoxicity.	Crouzier et al., 2010; Elgrabli et al., 2008; Han et al., 2010; Ma-Hock et al., 2009; Porter et al., 2010; Poland et al., 2008; Walker et al., 2009; Reddy et al., 2010; Cveticanin et al., 2010; Ravichandran et al., 2009.
Gold NPs	Mice: bioaccumulation in important body organs, cross blood brain barrier; adverse effect on human sperm motility; apoptosis and acute inflammation in liver.	Autophagy, oxidative stress; mitochondrial damage which triggers necrosis; affects cellular motility.	Li et al., 2010; Pan et al., 2009; Tarantola et al., 2009; Cho et al., 2009; Lasanga-Reeves et al., 2010; Wiwanitkit et al., 2009.
Silver NPs	Rats: blood-brain barrier destruction, astrocyte swelling, and neuronal degeneration; brain edema formation Mice: expression of genes related to oxidative stress in brain	Chromosome instability and mitotic arrest cytoskeleton deformations in human cells; mitochondrial disruption, decreased metabolism; apoptosis, DNA damage, cytotoxicity, JNK activation in mammalian cells.	Asharani et al., 2009a; Asharani et al., 2009b; Foldbjerg et al., 2010; Miura and Shinhoray., 2009; Hsin et al., 2008; Tang et al., 2009; Rahman et al., 2009; Sharma et al., 2010.
Fullerens	Rats: genotoxicity, elevated level of 8-oxo-dG in lung and liver. Increase in proinflammatory cytokines and Th1 cytokines in BAL fluid	Oxidative stress, DNA damage, cytotoxicity in mammalian cells.	Folkmann et al., 2009; Dhawan et al., 2006; Zhang et al., 2009; Jacobsen et al., 2008.
Metal oxide NPs, Copper oxide, ZnO, TiO_2, nickel oxide.	Rats: infilteration of macrophages, alveolities, inflammation of lung, collagen deposition.	Cytotoxicity, oxidative stress; alteration of gene expression and calcium homeostasis; DNA damage, apoptosis, disturbance of physiological functions and ionic homeostasis rat hippocampal CA3 pyramidal neurons by ZnO NPs	Ogami et al., 2009; Haung et al., 2010; Falck et al., 2009; Hussain et al., 2010; Zhao et al., 2009; Fahmy and Cormier, 2009.

NP: nanoparticle; ZnO: zinc (II) oxide.

dose of 6 mg/kg body weight per day administered for 12 days consecutively). The results showed a lower incidence of retardation growth, pronounced oxidative stress, and liver injury in mice compared with animals receiving non-nanoparticulate sodium selenite. Nano-Se ranging from 5 to 200 nm had no size-dependent effect in upregulating seleno-enzymes both in cultured cells and mice liver (Zhang et al., AQ 18 2004). Together it concludes that the selenium has less toxic effect in the nano form.

Zinc NPs. Wang et al. (2006) assessed the acute toxicity of zinc powder NPs by gastrointestinal administration at a dose of 5 mg/kg body weight in AQ 19 CD-ICR mice of both sexes and they exhibited severe symptoms of lethargy, anorexia, vomiting, and diarrhoea. Furthermore, during the initial 3 days, a 22% reduction in weight gain in mice exposed to NPs was observed when compared with the control group.

Copper NPs. Chen et al. (2006) evaluated the effect of metallic copper NPs (23.5 nm) in the mice. Mice showed gastrointestinal disorders like loss of appetite, diarrhea, vomiting in contrary to those that received microparticles (in the same mass concentrations of AQ 20 17 μm). The LD_{50} for the nano- and microcopper particles and cupric ions exposed to mice via oral gavage were 413, >5000, and 110 mg/kg body weight, respectively. The toxicity class for both nano- and ionic-copper particles was class 3 (moderately toxic) and for micro-copper was class 5 (practically nontoxic) of Hodge and Sterner scale. They also noticed tremors or hypopnea in some mice that received NPs. The parameters like blood urea nitrogen, creatinine, total bile acid, and ALP were significantly higher than in the controls. Results indicate a gender-dependent feature of nanotoxicity. Moreover, nanotoxicity depends upon several factors such as huge specific surface area, ultrahigh reactivity, and so on.

CdSe QD. Wang et al. (2008) evaluated the possible AQ 21 toxicity of CdSe QD exposure via ingestion, on enterocyte-like Caco-2 cells as a small intestine epithelial model. Cells were incubated in Cd^{2+} (2–200 nmol/mL) containing medium for 24 h. A Cd^{2+} concentration of 200 nmol/mL resulted in a drop in the relative viability of Caco-2 to 0.62, which is significantly lower than control. Moreover, it also showed that in cultured, intestinal cell detachment, AQ 22 and death. However, cytotoxicity depended largely on the QD coating and treatment (e.g. acid treatment and dialysis). This concluded that Caco-2 cell viability correlated with the concentration of free Cd^{2+} ions present in cell culture medium. Exposure to low (gastric) pH affected cytotoxicity of CdSe QDs, indicating that the route of exposure may be an important factor in QD cytotoxicity.

Effect of NPs on liver

Silver NPs. Hussain et al. (2005) observed that the circulatory Ag NPs (15 and 100 nm) are going to accumulate in liver. The results in *in vitro* BRL 3A liver cell showed a decrease in mitochondrial function, LDH leakage, and abnormal cell morphologies. The depletion in glutathione (GSH) level and increased ROS in association with mitochondrial perturbation, suggesting that the oxidative stress might mediate the cytotoxicity of Ag NPs. Based on these findings, a preliminary impression can be formed that Ag NPs may interact with proteins and enzymes with thiol groups within mammalian cells (Takenaka et al., 2001).

Zinc NPs. Wang et al. (2006) assessed the effect of zinc NPs and microparticles in the mouse liver at a dose of 5 mg/kg body weight. The mice exhibited edema, hydropic degeneration, and a slight necrosis of the hepatocytes around the central vein. Microparticles and NPs induced liver damage that was indicated by an increase in certain biological parameters like alanine aminotransferase, aspartate aminotransferase, ALP, and LDH. In general, zinc microparticles induced more severe liver damage than NPs.

Selenium NPs. The study conducted by Zhang et al. (2005) showed less hepatic function alterations in mice that ingested selenium NPs (Nano-Se: 20–60 nm; 2 and 4 mg/kg for 15 days) when compared with those to which non-nanoparticulate sodium selenite had been administered.

Effect of NP on spleen

The study by Chen et al. (2006), which involved use of 23.5 nm metallic copper NPs on mice, showed a severe atrophy and change in spleen color. They also noticed an atrophy of the splenic units, lymphocyte reduction, and fibrosis in the interstitium of the spleen. This concluded that spleen is one of the target organ for metallic copper NPs. A black coloration of the gall bladder in certain mice exposed to NPs was also observed.

Effect of NPs on skin

Silver NPs. Cultured keratinocytes are exposed to the extracts of several types of Ag containing dressings like Acticoat, Aquacel-Ag, Aquacel, Algisite M, Avance, Comfeel Plus transparent, Contreet-H, Hydrasorb, and SeaSorb for 40 h (Paddle-Ledinek et al., 2006). The results showed that Ag NPs are most toxic. Extracts of Ag-containing dressings like Acticoat, Aquacel-Ag, Contreet-H, and Avance were most cytotoxic. Hence, they concluded that the Ag-based dressings are cytotoxic and should not be used in the absence of infection.

TiO_2 NPs. The skin penetration of four different types of rutile TiO_2 (T-35, 35 nm, noncoating; TC-35, 35 nm, with almina/SiO_2/silicon coating; T-disp, 10 × 100 nm, mixture of alumina-coated and silicon-coated particles, dispersed in cyclopentasiloxan; T-250, 250 nm, noncoating) with a suspension of 2 μl/cm^2 was studied by Senzui et al. (2010). This test was determined with *in vitro* intact, stripped, and hair removed skin of Yuca-tan micropig to study the dispersion and skin condition. The outcome of this study showed that there was no penetration regardless of TiO_2 in intact and stripped skin. Electron microscopy and energy dispersive x-ray spectroscopy analysis showed titanium penetration in vacant hair follicles (>1 mm below the skin surface); however, it did not penetrate into dermis and viable epidermis.

Maghemite (g-Fe_2O_3) and iron (Fe) NPs. The impact of 5.9 nm metallic maghemite (g-Fe_2O_3) and 4.9 nm iron (Fe) NPs in human full-thickness skin was studied by Baroli et al. (2007). They examined that the NPs were able to passively penetrate the skin through the subcutaneous (SC) lipidic matrix and hair follicle orifices, reaching the deepest layers of the SC, the stratum granulosum, and hair follicles. However, in some exceptional cases, NPs were also found in the viable epidermis. Hence, the NPs <10 nm have the ability to penetrate the skin and cause the toxicity.

Cells of excretory system

Copper NPs. A substantial change in the color of kidney in mice exposed to metallic copper NPs (23.5 nm; 1080 mg/kg) was observed by Chen et al. (2006). They detected the signs of glomerulonephrities, like swelling of the glomeruli and damage to the proximal tubule cells. They also observed degeneration of the epithelial cells of the proximal convoluted tubule and massive irreversible necrobiosis of the cells. The nuclei of the epithelial cells of the renal tubule became less and less visible (starting at 341 mg/kg) and nearly disappeared at 1080 mg/kg. In the renal tubules of mice exposed to the doses ranging from 341 to 1080 mg/kg lead to a purple deposition of protein fluid indicating a disorder of the protein metabolism.

Zinc NPs. A slight swelling of the glomeruli in the kidneys of mice exposed to zinc NPs or microparticles (at a dose of 5 mg/kg body weight) was examined by Wang et al. (2006). They also detected the dilation of renal tubules and the presence of protein moulds in mice exposed to NPs. In general, NPs induced more severe kidney damage.

CB and TiO_2. The *in vitro* effects of CB (FW2–13 nm, Printex60-21 nm, and LB101-95 nm) and TiO_2 (TiO_2-15 and TiO_2-50 nm) NPs on glomerular mesangial (IP15) and epithelial proximal tubular (LLC-PK1) cells of kidney were evaluated by Azou et al. (2008). Results showed that CB was the most cytotoxic with classic dose-behavior to both cell lines because of its ability to produce ROS in both the cells. FW2 exhibited more toxicity on IP15 cells than other NPs, and IC_{50} was calculated to be 30 μg/cm^2. In contrast, P60 and LB101 had only a slight effect on mitochondrial function. When using TiO_2-15 and TiO_2- 50 NPs, there was slight or no significant differences were observed up to a concentration of 160 μg/cm^2.

Cells of reproductive system

Effect of NPs on testis. *CB NPs.* The *in utero* effect of CB on the reproductive function of male offspring was investigated by Yoshida et al. (2010). They administered CB *in utero*; approximately 0.2 mg of 14-nm carbon NPs per mouse was administered intratracheally on days 7 and 14 and observed that the daily sperm count (DSP) was significantly reduced in male offspring at all three ages (5-week-old mice: 47% ($p < 0.001$); 10-week-old mice: 34% ($p < 0.001$]; and 15-week-old mice: 32% ($p < 0.001$)). Even when CB was administered to adult mice, DSP decreased significantly (Yoshida et al., 2009). When three sizes (14, 56, and 95 nm) of CB NPs were intratracheally administered (0.1 mg/mouse for 10 times every week) to ICR male adult mice, the incidence of seminiferous tubule damage was high (vacuolation of the seminiferous tubules); however, its severity was mild

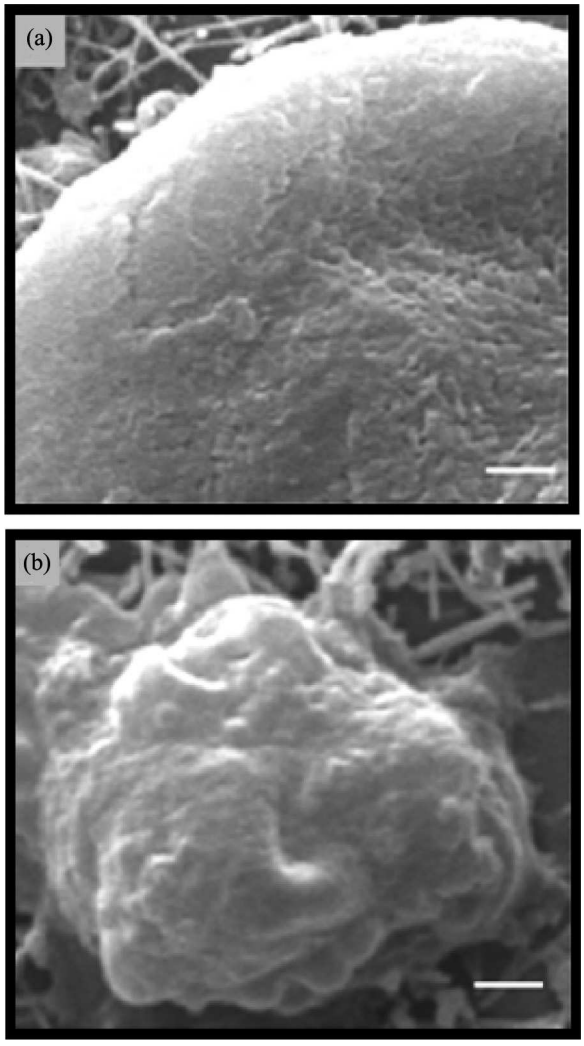

Figure 3. ((a) and (b)) Higher magnification scanning electron microscopic images of spermatogonial cells during *in vitro* culture on MWNTs and fMWNTS. Note the cell body maintaining its shape and adhering properly with substratum. Scale bars represent (a) 1 μm and (b) 2 μm. (our laboratory: Rafeeqi and Kaul, 2010c). MWNTs: multiwalled nanotubes; fMWNTS: functionalized multiwalled nanotubes.

AQ26 (Yoshida et al., 2009). The intercellular adhesions of seminiferous epithelia and seminiferous tubules damage were observed in the testis of male offspring and thus inhibited the spermatogenesis. Figure 3(a) and (b) shows the spermatogonial stem cells cultured on MWCNT and functionalized MWCNT (fMWCNTS) scaffold, preprepared on polyethyleneimine-coated glass surface. The SEM images showed that the spematogonial stem cells had adhered properly and extensions of the cell were seen in all directions on CNTs scaffolds. These results provided the degree of biocompatibility between spermatogonial cells and CNTs and the real possibility for CNTs to be used as an alternative nanomaterial for *in vitro* growth of these cells (Rafeeqi and Kaul, 2010c).

Silver NPs. The effect of silver (Ag: 15 nm), molybdenum trioxide (MoO_3: 30 nm), and aluminum (Al: 30 nm) NPs on the cells of the male mouse germ line (C18-4) was evaluated by Braydich-Stolle et al. (2005). Silver, MoO_3, and aluminum NPs were first dispersed in phosphate-buffered saline (PBS) and used at final concentrations of 5, 10, 25, 50, and 100 μg/mL culture medium. The dramatic changes induced by Ag NPs at the concentration of 10 μg/mL and above and apoptosis had occurred. The cytotoxic effect of the NPs on the mitochondrial activity increases in relation to increasing concentration. A concentration-dependent toxicity is observed for all types of particles tested, whereas the corresponding soluble salts had no significant effect. Silver NPs were the most toxic, while MoO_3 NPs were the least toxic. AQ27

Effect of different NPs on spermatozoa

Gold NPs. Wiwanitkit et al. (2007) demonstrated that the gold NPs (9 nm size produced at a concentration of 44 ppm) affect the motility of spermatozoa. They noticed that gold particles can penetrate sperm cells, which could result in fragmentation. They also found that when the semen is mixed with NP solution, 25% of sperm were not motile, where as the motility of the control sperm was 95%.

Eu_2O_3 NPs. Impact of Eu_2O_3 NPs on bovine spermatozoa motility and their acrosome reaction was studied by the Makhluf et al. (2008). These Eu_2O_3 NPs were stabilized with poly vinyl alcohol (PVA) or poly vinyl propyl (PVP) solution. The motility of the sperm cells treated with Eu_2O_3 NPs was negatively affected, that is, the sperm cells were alive, but barely moving. But the sperm cell is not affected by loading of the NPs with PVA or PVP. Also the presence of Eu3+ ions inside the bovine spermatozoa has been demonstrated in the same study.

TiO_2 and ZnO NPs. Gopalan et al. (2009) assessed the effects of ZnO and TiO_2 NPs (40–70 nm range) in the presence and absence of ultraviolet (UV) light in human sperm and human lymphocytes in the dark (D), after preirradiation (PI) with UV and simultaneous irradiation (SI) with UV. Preliminary studies

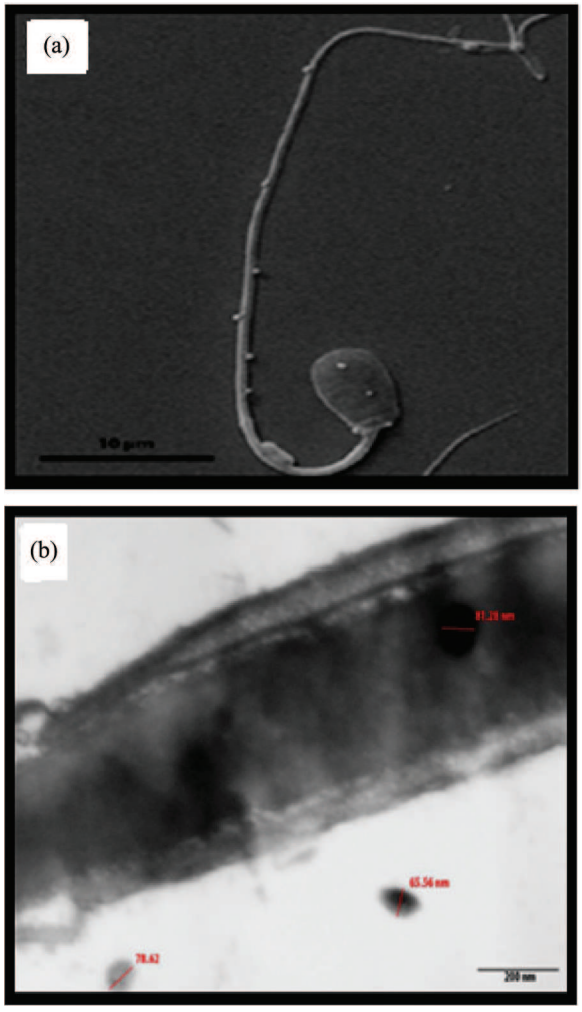

Figure 4. ((a) and (b)) Scanning electron microscopic photographs of buffalo spermatozoa. Spermatozoa mixed with nanoparticles showing nanoparticles attached on the membrane of tail and head. (b) Transmission electron microscopic photographs of buffalo spermatozoa (head region) incubated with titanium dioxide nanoparticles for 6 h. Longitudinal section of sperm head showing nanoparticles inside, at 20,000× magnification (our laboratory: courtesy, Pawar and Kaul 2010).

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in the presence and absence of UV revealed concentration-dependent induced DNA damage in both sperms and lymphocytes on exposure to ZnO and TiO_2.The effect of TiO_2 NPs showed that the percentage reduction in head DNA was greater for PI and SI samples compared with samples treated in the dark. However, with regard to photogenotoxicity, sperm exhibited no significant differences when the results for PI and SI and the dark were compared, except at the lowest concentration for SI samples in the case of ZnO and the lowest concentration for PI in the case of TiO_2. Scanning electron microscopy of spermatozoa loaded with the TiO_2 NPs revealed attached TiO_2 NPs on the surface/membrane of spermatozoa (head and tail both) and transmission electron microscopic pictures revealed the presence of NPs attached on and inside the head and tail region (Figure 4(a) and (b)).

Effect of NPs on Leydig cells

DEP, CB, and TiO_2 NPs. The effect of DEP, CB, and TiO_2 on mouse Leydig TM3 cells (the testosterone-producing cells of the testis) was investigated. They assessed that TiO_2 was more cytotoxic to Leydig cells than other NPs. The proliferation of Leydig cells was suppressed transiently by treatment with TiO_2 or DEP. When mouse Leydig TM3 cells are treated with DEP, the expression of heme oxygenease-1 (HO-1), a sensitive marker for oxidative stress, was induced remarkably. With the gene expression of the steroidogenic acute regulatory (StAR) protein, the factor that controls mitochondrial cholesterol transfer was slightly increased when exposed to CB and DEP. Hence, overall results found that DEPs, TiO_2, and CB NPs were taken up by Leydig cells and affected the viability, proliferation, and gene expression (Komatsu et al., 2008).

Effect of NPs on ovarian granulosa cells

Liu et al. (2010) investigated the effect of calcium phosphate NPs on both steroid hormone production and apoptosis in human ovarian granulosa cells. Granulosa cells were exposed during 2, 4, 24, 48, and 72 h to NPs, whose smallest size was 20–30 nm, and <10% particles were greater than 100 nm. Results showed that calcium phosphate NPs could enter into granulosa cells, and distributed in the membrane compartments, including lysosome, mitochondria, and intracellular vesicles. Treatment with calcium phosphate NPs at the concentrations of 10 and 100 μM for 48 h results in significant increase in apoptotic rate at 100 μM, but there was no significant change observed either in the progesterone or estradiol level in culture fluid and the expression levels of mRNAs. Hence, concluded that the calcium phosphate NPs interfered with the cell cycle of cultured human ovarian granulosa cells, thus increasing cell apoptosis.

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Cells of immune system

Magnetic NPs. Chen et al. (2010) investigated the effect of MNPs of Fe_3O_4 (Fe_3O_4-MNPs) on the mice

immune system. ICR mice were injected intravenously with 20 nm Fe_3O_4-MNPs having magnetization of 25.6×10^{-3} emu/mg. The doses of 5.14 mg/kg (low dose group), 20.7 mg/kg (medium dose group), and 51.4 mg/kg (high dose group) Fe3O4-MNPs were dissolved in normal saline and intravenously injected into mice once. Using flow cytometry and enzyme-linked immunosorbent assay, the peripheral T-cells and the induction of primary immune responses in mice were investigated. The result showed that the lymphocyte transformation rates in the suspension of spleen were higher in low-dose group than those in the control group, while the proliferation of splenocytes was low in the medium and high groups when compared with the control group. They also observed that in the peripheral blood, the proportion of subset $CD4^+$ and $CD8^+$ T-lymphocytes in the low-dose group were higher than those in the control group. The production of interleukin-2 (IL-2), interferon-γ, and IL-10 was enhanced by the Fe_3O_4-MNPs. They concluded that Fe_3O_4-MNPs could influence immune functions of normal ICR mice in a dose-dependent manner.

Carbon nanotubes. The effect of SWCNTs on primary immune cells *in vitro* was investigated by Zhang et al. (2008). The results showed that SWCNTs at 25 and 50 mg/mL could promote the proliferation of spleen cells but not at concentrations of 1 and 10 mg/mL. Interestingly, they can inhibit T-lymphocyte proliferation at higher concentrations but no effect on T-lymphocyte proliferation stimulated by concanavalin-A (ConA) at lower concentrations. They also observed that SWCNTs inhibited the B-lymphocyte proliferation stimulated by lipopolysaccharides at the concentrations of 1, 10, 25, and 50 mg/mL. They concluded that SWCNTs have possibly negative effects on immune cells *in vitro*.

Fe_2O_3 NPs. Park et al. (2010) treated the mice with Fe_2O_3 NPs (size: 5.3 ± 3.6 nm in PBS, surface charge: 23.14 mV) by a single intratracheal instillation (250 μg/kg, 500 μg/kg, and 1 mg/kg). At the low dose of 250 μg/kg, GSH was not changed in the cells of BAL fluid, but it was dose-dependently decreased by the treatments of 500 μg/kg and 1 mg/kg. The G1 of cell cycle was arrested, but S-phase was significantly decreased. The concentrations of proinflammatory cytokines (IL-1, TNF-α, and IL-6) were dose-dependently increased at day 1 after instillation in the BAL fluid and in the blood. Proinflammatory cytokines (IL-1, TNF-α, and IL-6), Th0 cytokine (IL-2), Th1-type cytokine (IL-12), Th2-type cytokines (IL-4 and IL-5), TGF-α, and IgE were also elevated. Expressions of many genes related with inflammation or tissue damage such as heat shock protein, matrix metalloproteinase, tissue inhibitors of metalloproteinases, and serum amyloid were significantly induced. They observed the formation of microgranuloma, which is one of the indicators of chronic inflammatory response in the alveolar space. Based on the result, Fe_2O_3 NPs may subchronically induce inflammatory responses via oxidative stress in mice by a single intratracheal instillation.

Mast cell

Gold NPs. Cells were cultured with NPs of diameter 26.5 ± 6.0 nm for 24 h (Cornell, 2006). They observed the current versus time graph for the chemical release from a mast cell, where current spikes have much lower amplitude than that of the control group. By further analysis, they conclude that NPs tend to cluster in the granules of the mast cells. Since the granules are essential to the primary function of mast cells, this location of NPs could explain the observed negative electrochemical effect of the addition of NPs. AQ30

Genotoxicity of nanomaterials

Gold NPs

Li et al. (2008) investigated the effect of gold NPs (20 nm) on embryonic lung fibroblasts. They demonstrated significant oxidative DNA damage in the form of 8OHdG adducts, at the concentrations as low as 25 mg/mL gold NP. Even it is also accompanied by decreased expression of DNA repair genes and the cell cycle checkpoint genes MAD2, cyclin B1, and cyclin B2, which is of concern as lowering the cellular DNA damage response pathways. If the cells are subjected to further shock, then it promotes genetic instability. They conclude that gold NPs are capable of inducing DNA damage indirectly through an oxidative stress response, although in a cell type or size-dependent manner.

Silver NPs

Ahamed et al. (2008) compared the effect of surface coated Ag NPs (25 nm) and uncoated Ag NPs (25 nm) in mouse embryonic stem cells and embryonic fibroblasts at a concentration of 50 μg/mL. Results showed that coated Ag NPs exhibited a more severe

DNA damage response, which was indicated by increased expression of repair proteins and H2AX phosphorylation, than uncoated Ag NPs. It is due to uniform and better distribution of the coated particles and also increased surface area, which provided greater access to the cellular components when compared with the agglomeration of the uncoated particles. Although both types of Ag NPs increased p53 expression and p53 phosphorylation, upregulated the DNA damage repair protein Rad51, and also elevated the phosphorylation of H2AX at 50 μg/mL, it indicates that exposure to Ag NP may result in genetic aberrations.

Co NPs

Colognato et al. (2008) studied the effect of Co NPs (100–500 nm) with a median value of 246 nm on human peripheral blood leukocytes. Cells were treated with the suspensions of ^{60}Co NP and solutions of $^{57}Co^{2+}$ for 24 and 48 h at the different concentration. Results showed that Co NP were capable of inducing genotoxicity *in vitro* in human peripheral blood leukocytes. They also showed a dose-dependent increase in the frequency of micronucleated lymphocytes and reduced cell viability. However, they depend upon chemical properties and their physical interactions with cellular components.

TiO_2 NPs

Nanoparticulate TiO_2 is considered safe for use in sunscreens but as sunlight-illuminated TiO_2 catalyzes, DNA damages both *in vitro* and *in vivo* (Dunford et al., 1997). TiO_2 samples were extracted from over-the-counter sunscreens by washing with organic solvents. Human cells (MRC-5 fibroblasts) were illuminated on ice with or without sunscreen TiO_2 (0.0125% w/v). Results demonstrated that sunscreen TiO_2 can catalyze oxidative damage to DNA *in vitro* and in cultured human fibroblasts. When exposed to UV light, TiO_2 catalyzes the generation of ROS-like superoxide anions, H_2O_2, free hydroxyl radicals, and singlet oxygen in aqueous media (Hirakawa et al., 2004; Konaka et al., 1999, 2001). Exposure to TiO_2 NPs may indirectly result in DNA aberration because oxidative stress and inflammation are associated with inducing genotoxicity via the damaging activity of ROS. Kang et al. (2008) and Karlsson et al. (2008) observed increased strand breakages following the exposure of TiO_2 NPs to lung epithelial cells by the comet assay.

SiO_2 NPs

Chen and Von (2005) showed that 40–80 nm SiO_2 NPs at a concentration of 25 μg/mL can enter the cell nucleus and potentially bind to the DNA phosphate backbone. The time-dependent redistribution of topo I from its homogenous location throughout the nucleoplasm to distinct clusters suggested that SiO_2-NP-induced protein aggregation of this nuclear protein. SiO_2-NP-induced protein aggregates contain nuclear proteins such as CBP that are essential in gene expression, cellular polyQ proteins, and proteasomes. Intranuclear protein aggregates leads to the inhibition of replication, transcription, and cell proliferation. Even a SiO_2 NP also induces inflammatory (NF-kB activation) and oxidative stress responses both *in vivo* and *in vitro* (Kaewamatawong, et al., 2006; Lin et al., 2006; Sayes et al., 2007). Oxidative stress can result into increased ROS levels. Hydroxyl radical is one of the ROS, which is a highly reactive molecule. The generation of (hydroxyl radical) –OH close to the DNA could readily lead to the induction of DNA strand breaks and oxidized bases (Valko et al., 2006). Wang et al. (2007) found that SiO_2 NPs do indeed induce chromosomal damage with the help of micronucleus assay. Despite these abnormalities, still there is limited evidence to suggest SiO_2 NPs are genotoxic.

Magnetite NPs

Berry et al. (2003) showed the dextran–magnetite (Fe_3O_4) NPs (7.8 nm) can result in cell death and reduced proliferation similar to that caused by uncoated Fe_2O_3 particles. This cytotoxicity was attributed to breakdown of the dextran shell exposing the cellular components to chains or aggregates of Fe_2O_3 NPs. Stroh et al. (2004) investigated the effect of citrate-coated very small superparamagnetic Fe_2O_3 NPs (VSOP) on rat macrophages. Result showed that significant increase in the levels of malonyldialdehyde and protein carbonyls leads to oxidative stress in macrophages.

Fullerenes

Fullerenes are less toxic than CNTs, CB, and DEPs. These fullerenes do have an ability to both quench and, conversely, to generate ROS (Jacobsen et al., 2008). As a result of oxidative stress mechanism, these fullerenes damage DNA. Fullerenes can induce DNA strand breakages, chromosomal damage,

mutagenicity, and form complexes with DNA (in cell-free systems). C(60) fullerenes (C(60)) and SWCNT were assessed in the FE1-Mutatrade markMouse lung epithelial cell line. None of these particles induced cell death within 24 h at doses between 0 and 200 μg/mL or during long-term subculture exposure (576 h) at 100 μg/mL. The mutant frequency in the cII gene was unaffected by 576 h of exposure to either 100 μg/mL C(60) or SWCNT when compared with control incubations. These results indicate that SWCNT and C(60) are less genotoxic *in vitro* than CB and DEPs.

NP–protein interaction: Overview

Interaction of protein with a NP surface can easily disrupt the native conformation and, therefore, the protein function which has implications for the biological impact of NPs. Park et al. (2003) showed experimentally that the CNT is effective in blocking some biological membrane ion channels due to its structural match in size and shape (Park et al., 2003). Karajanagi et al. (2004) investigated the conformational changes of α-chymotrypsin and soybean peroxidase enzymes after adsorbing onto the SWCNT using atomic force microscope and Fourier transform infrared spectroscopy (Karajanagi et al., 2004). In result, they argued that the distribution of the hydrophobic residues on the surface of the enzymes may determine the protein conformational changes when adsorbed onto the CNT. The experiments on the interactions between small peptides and CNTs also showed that the tryptophan residues play a key role in the binding of the peptides on CNTs (Wang et al., 2003; Zheng et al., 2009). Thus, CNTs can affect the function of the proteins by either disrupting the structure of active site or shielding the active site from competing ligands. Furthermore, necrosis occurs when cationic components of the carrier and cell surface proteoglycans or proteins in the cytoskeleton of the target cell interact, disestablishing the membrane, and cause the formation of pores (Curtis et al., 2006; Moghimi et al., 2005).

The interaction between bare CdS QDs and human adult Hb techniques under physiological pH 7.43 has been investigated by fluorescence, synchronous fluorescence, CD, and Raman spectroscopic techniques. Result showed that CdS QDs dramatically alter the conformation of Hb, quenching the intrinsic fluorescence of Hb and decreasing the α-helix content of the secondary structure from 72.5 to 60.8%. Raman spectra results indicated that the sulfur atoms of the cysteine residues form direct chemical bonds on the surface of the CdS QDs (Shen et al., 2007). Nowadays, to control the interaction of NPs with proteins, the functionalization of NPs surface with peptides is increasingly being used (Aubin-Tam and Hamad-Schifferli, 2005; You et al.,2008). The study of gold NPs effect on positive, negative, and neutral ligands on attached CytC structure reveals that the protein retains its structure with neutral ligands but denatures in the presence of charged species (Aubin-Tam and Hamad-Schifferli, 2005). A more recent report from Bellezza et al. (2007) suggests that the interaction of Mb with phosphate-grafted zirconia NPs induces significant rearrangements in the Mb structure, particularly loss of the secondary structure (α-helices).

NPs interact with the proteins with high concentrations and high association rate constants, which will initially occupy the NP surface; however, they may also dissociate quickly to be replaced by proteins of lower concentration, slower exchange, and higher affinity (Cedervall et al., 2007). NP–protein binding shows that the vast majority of NP types studied so far bind apolipoproteins (Cedervall et al., 2007). Moreover, there are multiple receptors for apolipoprotein complexes at cell surfaces that NPs with surface-adsorbed apolipoproteins can potentially exploit to enter cells (Kim et al., 2007). Thus, it may be hypothesized that besides size and shape, the NP–protein corona also determines the final subcellular location of a specific NP upon interaction with a cell and, thereby, the range of disease processes that the NP can access (Lynch et al., 2006).

NP–lipid interaction: Overview

Membranes, structural proteins, such as actin and microtubules, oligonucleotides (DNA and RNA), as well as larger constructions, such as mitochondria or Golgi apparatus, will be affected due to the interaction with NPs, which depend upon mainly the initial ability of the NP to breach the cell membrane. Polycationic amine-terminated poly(amidoamine) dendrimers nanomaterials are capable of inducing nanoscale hole (15–40 nm) in supported lipid bilayers (Hong et al., 2007). Moreover, this type of polyamidoamine (PAMAM) dendrimer has also been shown to induce significant permeability in lipid vesicles (Ottaviani et al., 1998; Zhang and Smith, 2000) liposomes (Karoonuthaisiri et al., 2003), and cell membranes (Fischer et al., 2003; Hong et al., 2004). Gold particles

modified to have polycationic surfaces also induce vesicle leakage and can lead to cell lysis (Goodman et al., 2004). Furthermore, the toxicity of the gold NPs is related to their interactions with the cell membrane, a feature initially mediated by their strong electrostatic attraction to the negatively charged bilayer.

Hong et al. (2007), using atomic force microscopy observed that positively charged generation 7 (G7) PAMAM dendrimers caused the formation of nanoscale holes with diameters between 15 and 40 nm in lipid bilayers. Thus, it is hypothesized that this process is driven by the electrostatic interactions and the formation of dendrimer-nucleated lipid vesicles (Mecke et al., 2004). The greatest degree of cell permeability was observed with larger, positively charged PAMAM dendrimers tested (5–7 nm). Whereas smaller, positively charged dendrimers (3 nm) charge neutral dendrimers, or negatively charged dendrimers exhibited less or no nanoscale hole formation and significantly reduced or no enzyme leakage and dye diffusion (Hong et al., 2007).

Hence, NP interacts with the lipid bilayer in the cell membrane directly and indirectly by producing ROS after entering into the cell. The presence of ROS leads to lipid peroxidation, which is the oxidative degradation of cell membranes and is most commonly measured by assaying the presence of MDA or other thiobarbituric acid reactive substances (Buege and Aust,1978; Sayes et al., 2004; Yang et al., 1997). Hence, this assay has been generally used to demonstrate the ability of a variety of nanomaterials to elicit lipid peroxidation in multiple cell types, such as: fullerenes in human dermal fibroblasts and human liver carcinoma (HepG2) cells (Sayes et al., 2004).

Conclusion

Several research were carried out with different NPs causing abiotic stress on the animal and human health. This shows that engineered NPs must be handled with care and workers exposure must be minimized, since these effects are extremely variable from one product to another. Although studies are conflicting regarding the magnitude and mechanisms of nanomaterial toxicity, it is evident that some nanomaterials that were previously considered biocompatible due to safety of the bulk material may indeed be toxic. Still the pharmacokinetic behavior of different types of NPs requires detailed investigation and a database of health risks associated with different NPs (e.g. target organs, tissue, or cells) should be created. Existing research on nanotoxicity has concentrated on empirical evaluation of the toxicity of various NPs, with less regard given to the relationship between NP properties (exact composition, crystallinity, size, size dispersion, aggregation, ageing, etc) and their toxicity in mammals. This approach gives very limited information, and should not be considered adequate for developing predictions of toxicity of seemingly similar NP materials. The studies must include, research on NPs translocation pathways, accumulation, short- and long-term toxicity, their interactions with cells, the receptors, and signaling pathways involved, cytotoxicity, and their surface functionalization for an effective phagocytosis in the mammals. Hence, there is a serious lack of information concerning the human health, animal health, and environmental implications of manufactured nanomaterials.

Understanding the interactions of these 'new age materials' with biological systems is the key to safe usage of these materials in novel biomedical fields like diagnostics and therapeutics.

Since these are relatively new particles, it requires thoughtful environmental, human health, animal health and safety research, meaningful and an open discussion of broader societal impacts, and urgent toxicological oversight action.

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