

TOXICITIES ASSOCIATED WITH ENGINEERED NANOPARTICLES**Jadhav A.S.^{1*}, Phalke P.L.², Gadhave M.V.¹, Thorat R.M.³ and Gaikwad D.D.¹**¹Vishal Institute of Pharmaceutical Education & Research, Ale.²Institute of Pharmacy for Women Ale.³Institute of Pharmacy, Ale.Article Received on
23 April 2015,Revised on 17 May 2015,
Accepted on 08 June 2015***Correspondence for****Author****Jadhav A.S.**Vishal Institute of
Pharmaceutical Education
& Research, Ale.**ABSTRACT**

This Review shows that humans have always been exposed to tiny particles via dust storms, volcanic ash, and other natural processes to cause hazardous effects on body. By the development of nanoscience and nanotechnology, knowledge of interactions between engineered nanomaterials and cells, tissues and organisms has become very important, especially in relation to possible hazards to human health. This review give an overview of current research on nano-biointeractions, with a focus on the effects of ENP and their interactions with live cells. We summarize the Types of ENPs And Also in vivo and in vitro studies of ENPs. Cytotoxic effects are also

discussed. Animal and human studies show that inhaled nanoparticles are less efficiently removed than larger particles by the macrophage clearance mechanisms in the lung, causing lung damage, and that nanoparticles can translocate through the circulatory, lymphatic, and nervous systems to many tissues and organs, including the brain, causing abnormal function or cell death

KEYWORDS: Engineered Nanoparticle, Endocytosis, Red blood cell, Silanols, Poration, Cytotoxicity.

INTRODUCTION**Nanomaterials**

Nanomaterials are defined as materials with at least one external dimension in the size range from approximately 1-100 nanometers. Nanoparticles are objects with all three external dimensions at the nanoscale.^[1] Nanoparticles that are naturally occurring (e.g., volcanic ash, soot from forest fires) or are the incidental byproducts of combustion processes (e.g.,

welding, diesel engines) are usually physically and chemically heterogeneous and often termed ultrafine particles.

Engineered nanoparticles^[2]

ENPs are intentionally produced and designed with very specific properties related to shape, size, surface properties and chemistry. These properties are reflected in aerosols, colloids, or powders. Often, the behavior of nanomaterials may depend more on surface area than particle composition itself. Relative-surface area is one of the principal factors that enhance its reactivity, strength and electrical properties.

Engineered nanoparticles may be bought from commercial vendors or generated via experimental procedures by researchers in the laboratory (e.g., CNTs can be produced by laser ablation, HiPCO (high-pressure carbon monoxide, arc discharge, and chemical vapor deposition (CVD).

Hazard

United States Environmental Protection Agency (EPA) which defines hazard 'Inherent toxicity of a compound.^[2,3] According to this definition, if a chemical substance has the property of being toxic, it is therefore hazardous. Any exposure to a hazardous substance may lead to adverse health effects in individuals or even death.

Risk

EPA defines Risk_ a measure of the probability that damage to life, health, property, and/or the environment will occur as a result of a given hazard³. According to this definition, if the probability of an exposure to a hazardous material is high and the consequences for the health or environment are significant, then the risk is considered to be high. It is important to consider both the frequency of the event and the degree of the hazard to estimate risk.^[4]

Hazard Identification

Hazard identification (HI) is defined as the Identification of the adverse effects, which a substance has an inherent capacity to cause. Until recently, much of the discussion about the environmental and health risks of ENPs was considered to be rather speculative than realistic. In the last few years, however, a number of experimental studies found that exposure to certain ENPs can lead to adverse health effects in living organisms.

Nanotoxicity-it is the degree to which nanomaterials can damage an organism, Because of quantum size effects and large surface area to volume ratio, nanomaterials have unique properties compared with their larger counterparts.^[5]

Two types of nanoparticles (NPs) can be distinguished

(1) Naturally occurring NPs

(e.g., produced naturally in volcanoes, forest fires or as combustion by-products)

(2) Engineered nanoparticles (ENPs)

deliberately developed to be used in application (e.g., carbon black, fumed silica, titanium dioxide (TiO₂), iron oxide (FeO_x), quantum dots (QDs), fullerenes, carbon nanotubes (CNTs), Naturally occurring NPs do NOT fall in the scope of this article.^[6]

The studies are divided into two categories—*in vivo* and *in vitro* studies.

In vivo studies

Carbon nanotubes (CNTs)

A study, performed by Lam *et al.*^[7], demonstrated that single-walled carbon nanotubes (SWCNTs) are able to cause dose-dependent effects of interstitial inflammation and lesions in *mice* and *rats* (0–0.5 mg·kg⁻¹ for 7 to 90 days). Warheit *et al.* observed pulmonary granulomas in *rats* after exposure to SWCNT soot (1 and 5 mg·kg⁻¹ for 24 hours to 3 months). In contrast to Lam *et al.*^[7], however, the effects, observed by Warheit *et al.*^[8] were not dependent on dose. Smith *et al.*^[9] tested the ecotoxicity of SWCNTs, dissolved in sodium dodecyl sulphate (SDS) and sonication on *juvenile rainbow trout* (0.1, 0.25 and 0.5 mg·L⁻¹ for 24 hours to 10 days) and they observed a dose-dependent rise in ventilation rate, gill pathologies (oedema, altered mucocytes, hyperplasia), and mucus secretion with SWCNT precipitation on the gill mucus. They also observed a significant dose-dependent decrease in thiobarbituric acid reactive substances (TBARS), especially in the gill, brain and liver, which is an indication of oxidative stress.

Multi-walled carbon nanotubes (MWCNTs) were shown by Carrero-Sanchez *et al.*^[10], to exhibit acute toxicity in *rats* with LD₉₀ of 5 mg·kg⁻¹. Long MWCNTs were shown by Poland *et al.*^[11] to cause significant inflammation and tissue damage in *mice*, while shorter MWCNTs caused less inflammation, which suggests that CNT toxicity is influenced by the

particle morphology. In addition, they concluded that water-soluble components of MWCNT do not produce strong inflammatory effects in *mice*.

C60 fullerenes

Most studies on the toxicological effects of C60 fullerenes suggest that these materials tend to induce oxidative stress in living organisms.^[12-15] Lai *et al.*^[12] observed a significant increase in lipid peroxidation (LP) products (a sign of oxidative stress) after intravenous administration of 1 mg kg⁻¹ C60 (OH)₁₈ in male *mongrel dogs*. Oberdörster^[13,14] studied the effects of C60 fullerenes in the brain of *juvenile largemouth bass* and observed high LP levels (0.5 and 1 ppm for 48 h). Elevated LP was also observed by Zhu *et al.*^[15] in the brain and gills of *daphnia magna* after exposure to hydroxylated C60 fullerenes (C60 (OH)₂₄) and tetrahydrofuran (THF)-dissolved C60, as it was shown that THF did not contribute to the effect. Sayes *et al.*^[16] detected an increase in the numbers of bronchoalveolar lavage (BAL)-recovered neutrophils (*i.e.*, white blood cells) after intratracheal instillation of C60 and C60 (OH)₂₄ in *rats*, 1 day after the exposure. They also observed a significant increase in LP values 1 week after the exposure. Accute effects of functionalized C60 were also reported. Zhu *et al.*^[15] estimated LC₁₀₀ in *fathead minnow* after exposure to 0.5 ppm of THF-dissolved C60 for 6–18 hours. Chen *et al.*^[17] observed a LD₅₀ of 600 mg·kg⁻¹ polyalkylsulfonated C60 in female *rats* after intraperitoneal administration (0–2,500 mg·kg⁻¹ for up to two weeks). Oberdörster^[18]

Metal and metal oxide ENPs

Li *et al.*^[19] found that metal ENPs induce more severe lung toxicity in *mice* than bulk particles from the same materials. Gordon *et al.*^[20] tested the effects on *humans* of exposure to zinc (Zn) ENPs. After 2 hours of exposure to 5 mg·m⁻³ of Zn ENPs, the exposed individuals started feeling sore throat, chest tightness, headache, fever and chills. Beckett *et al.*^[21] repeated that test in three trials, 2 hours each, but at lower concentration (*i.e.*, 500 µg·m⁻³), and found no indication of adverse effects. The latter two studies suggest that Zn ENPs toxicity is concentration-dependent and the most probable uptake path is through the respiratory system. A study of Sayes *et al.*^[17] concluded that environmental exposure to Zn ENPs causes pulmonary (lung) inflammatory response in *mice*. Wang *et al.*^[22] found that Zn ENPs can cause severe symptoms of lethargy, anorexia, vomiting, diarrhea, loss of body weight and even death in *mice* when gastrointestinally administered, whereas they observed limited effect for micro-scale Zn at equal concentrations. Yang and Watts^[23] tested the effect

of Aluminium (Al) ENPs on the relative root growth (RRG) in *Zea mays* (corn), *Glycine max* (soybean), *Brassica oleracea* (cabbage), and *Daucus carota* (carrot). The study found that the ENPs significantly inhibited the growth of the plants after administration of 2 mg·mL⁻¹ for 24 h.

Oberdörster^[24] and Oberdörster *et al.*^[25] observed that smaller TiO₂ ENPs tend to cause more severe pulmonary damage in *mice* than larger particles. In addition, Warheit *et al.*^[26] found that smaller silicon dioxide (SiO₂) particles cause stronger lung inflammation in *rats* than larger ones. Wang *et al.*,^[27] noticed that the smaller the TiO₂ particle size is, the greater the concentration in the liver of *mice* is. Bourrinet *et al.*^[28] reported hypoactivity, ataxia, emesis, exophthalmos, salivation, lacrimation, discolored and mucoid feces, injected sclera, and yellow eyes in *dogs* after single-dose intravenous bolus administration of 20 and 200 mg·kg⁻¹ FeO ENPs and a significant increase in fetal skeletal malformations in *rats* and *rabbits*.

***In vitro* studies**

Carbon nanotubes (CNTs)

A number of cytotoxicity studies with SWCNTs were reported in the literature. Shvedova *et al.*^[29] observed oxidative stress and cellular toxicity in human epidermal keratinocytes, after 2 to 18 hours exposure to unrefined (iron containing) SWCNTs in concentrations, ranging from 0.6 to 0.24 mg·mL⁻¹. Cui *et al.*^[30] observed dose- and time-dependent inhibition of cell proliferation and a decrease in cell adhesive ability in human embryo kidney cells after exposure to SWCNTs in concentrations between 0.8 and 200 µg·mL⁻¹. Sayes *et al.*^[31] found that the surface functionalization of SWCNTs plays an important role in their cytotoxicity towards human dermal fibroblasts. Bottini *et al.*^[32] noticed that MWCNTs were more cytotoxic when oxidized towards Jurkat T leukemia cells, whereas Monteriro-Riviere *et al.*^[33] observed a decrease of the viability of human osteoblastic lines and human epidermal keratinocytes after exposures to 0.1, 0.2, and 0.4 mg·mL⁻¹ of MWCNTs for 1 to 48 hours. Kang *et al.*^[34] compared the cytotoxicity of commercially obtained MWCNTs in bacterial systems before and after physicochemical modification and they observed highest toxicity when the nanotubes were uncapped, debundled, short, and dispersed in solution. Kang *et al.*^[34] concluded that there is need for careful documentation of the physical and chemical characteristics of CNTs, when reporting their toxicity.

C60 fullerenes

Adelman *et al.*^[35] observed a reduction of the viability of bovine alveolar macrophages after exposure to sonicated C60 and increased levels of cytokine mediators of inflammation (*i.e.*, IL-6, IL-8 and TNF), while Porter *et al.*^[36] found that C60 and raw soot were not toxic towards bovine-and human alveolar macrophages. The reason behind the discrepancy between the results of Adelman *et al.* and Porter *et al.* can be attributed to the fact that they used very different methods. Porter *et al.* used transmission electron microscopy (TEM) to image the distributions of the fullerenes within the macrophages, while Adelman *et al.* used a viability assay, based on metabolic activity as primary parameter.

Studies on the effects of ENPs on alveolar macrophages are very important because the alveolar macrophages are the first line of cellular defense against respiratory pathogens^[37,38] Yamawaki and Iwai^[39] observed dose-dependent cytotoxicity of C60 (OH)₂₄ (1–100 µg·mL⁻¹ for 24 hours), resulting in decreased cell density and lactate dehydrogenase (LDH) release in human umbilical vein endothelial cells cavity (a sign of increase in non-viable cell numbers). Rouse *et al.*^[40] observed a dose-dependent decrease in the viability of human epidermal keratinocytes after exposure to C60-phenylalanine, as no contribution to the effect was attributed to the phenylalanine groups.

Quantum dots (QDs)

The toxicity of QDs was found to be *influenced by several factors: (1) composition, (2) size, (3) surface charge and (4) coating of the QDs*^[41],. Jaiswal *et al.*^[42] found that CdSe/ZnS QDs (*i.e.*, CdSe QDs in a zinc sulfide (ZnS) matrix), coated with dihydrolipoic acid (DHLLA) had no effect on mammalian cells, while Hoshino *et al.*^[43] reported adverse effects on *mouse* lymphocytes after exposure to CdSe/ZnS QDs, coated with albumin. In addition, Lovrić *et al.*^[44] observed that smaller (2.2 ± 0.1 nm), positively charged QDs exhibit stronger cytotoxicity than larger (5.2 ± 0.1 nm), equally charged QDs under the same conditions. It was also found that the cytotoxicity of QDs is influenced by the exposure to light and by temperature.^[45,46] Green and Howman^[45] observed 56% damaged DNA after exposure to CdSe/ZnS together with UV light versus only 29% after exposure to CdSe/Zn in the absence of UV light. Chang *et al.*^[46] found that CdSe/CdS (*i.e.*, CdSe QDs in a cadmium sulfide (CdS) matrix) were toxic to cancer cells at 37 °C, but at 4 °C they were not toxic at all.

Metal and metal oxide ENPs

Sayes *et al.*^[47] found that anatase TiO₂ ENPs are able to kill human dermal fibroblast (HDF) cells at LC₅₀ of 3.6 µg·mL⁻¹, while Wang *et al.*^[48] observed decrease in the viability of human lymphoblastoid cells due to exposure to TiO₂ ENPs (0–130 µg·mL⁻¹ for 6–48 h). Chen & Mikecz^[49] found that SiO₂ ENPs do significantly inhibit replication and transcription in human epithelial HEp-2 cells (25 µg·mL⁻¹ for 24 h). Muller *et al.*^[50] observed that Fe₃O₄ ENPs, coated with dextran, decrease the viability of human monocyte macrophages. Alt *et al.*^[51] found that nano-particulate silver (Ag) is an effective bactericide against *S. epidermidis*, while Baker *et al.*^[52] noticed that it effectively kills *E. coli* bacteria too. Sayes *et al.*^[53] observed an increase in the production of LDH levels (an indicator of inflammation) in immortalized rat lung epithelial cells after 1 hour exposure to Zn ENPs at 520 µg·cm⁻².

Limitations to hazard identification of ENPs

A lot of studies, relevant for HI, have been carried out with different ENPs, but most of them were obviously not meant to facilitate risk assessment; they use non-standardized tests, differing greatly from each other in regard to endpoints, tested species, methods of administration, dose ranges and exposure periods.^[41] The lack of standardized testing results in non-reproducible results and makes the univocal HI of ENPs impossible.

Another significant drawback for the HI of ENPs is the serious lack of characterization data, which makes it difficult to identify which physical and/or chemical characteristics (or combinations of characteristics) determine the hazards, documented in the (eco)toxicological studies.^[54,55,56]

Red blood cells (RBCs)

Using confocal laser scanning microscopy (CLSM) in combination with digital data restoration, conventional TEM, and energy filtering TEM to investigate passive NP uptake, a quantitative analysis revealed that only the size determined the uptake efficiency. They confirmed that particles <200 nm enter RBCs. Small (~100 nm) adsorbed to the surface of RBCs without disturbing the membrane or the cell morphology (Figure 1). In contrast, adsorption of large MSNs (~600 nm) induced strong local membrane deformations, followed by internalization of the particles and, eventually, hemolysis.

The interactions of MSNs with the RBC membranes apparently depended on the presence of silanol groups on the particle surface because blocking these silanols with organic groups reduced their interactions with the RBC membranes. Recently,

Wang et al.^[57] studied the interactions between 8-nm QDs coated with the small, zwitterionic amino acid ligand D-penicillamine (DPA) and RBCs. At neutral pH, the charges on the amino and carboxylic acid groups of the surface ligands are balanced. After incubation with 10 nM DPA-QDs in PBS solution for different time periods and separation of free DPA-QDs by centrifugation, the RBC cells were transferred to a microscope sample cell and imaged using confocal fluorescence microscopy. The data clearly showed that the DPA-QDs adhered to the RBC membranes, and the number of fluorescence spots, either close to the cell membranes or inside the cells, increased with exposure time (Figure 1). Moreover, the adsorbed DPA-QDs did not induce strong local membrane deformations. In fact, the RBC membranes remained largely intact during NP penetration of the bilayer, as evidenced by confocal microscopy images taken in the presence of calcein violet AM. This cell membrane-permeant dye becomes impermeant after entering the cell because of hydrolysis by intracellular esterases.^[58]

Surface-enhanced infrared absorption spectroscopy (SEIRAS) measurements carried out on model membrane preparations resembling RBC membranes revealed that the bilayer structure was softened in the presence of DPA-QDs, which may facilitate penetration of DPA-QDs into the lipid bilayer without causing poration. The interaction of the NP with the membrane is arguably the most critical step in passive membrane penetration.

Van Lehn et al.^[59] proposed that, to avoid pore formation, the interaction should lead to fusion of the NP with the membrane. They suggested that fusion is highly favored when the ligand layer on the NP is able to easily fluctuate to adjust to the membrane, allowing surface charges to rearrange so that the NP appears locally hydrophobic.

As the ligand layer around smaller particles contains a large amount of free volume because of the high curvature, ligand fluctuations are maximized so that small NPs should more easily penetrate a membrane. Certain small peptides^[60,61] and synthetic nanomaterials such as carbon nanotubes^[62] were found to be capable of crossing membranes without poration. The DPA monolayer of the QDs used by Wang et al.^[57] resembles the pattern of hydrophobic and charged residues found in cell-penetrating peptides. Charged particles such as cationic QDs,

however, typically induce transient poration of the cell membranes, which may result in cytotoxic effects.^[58]

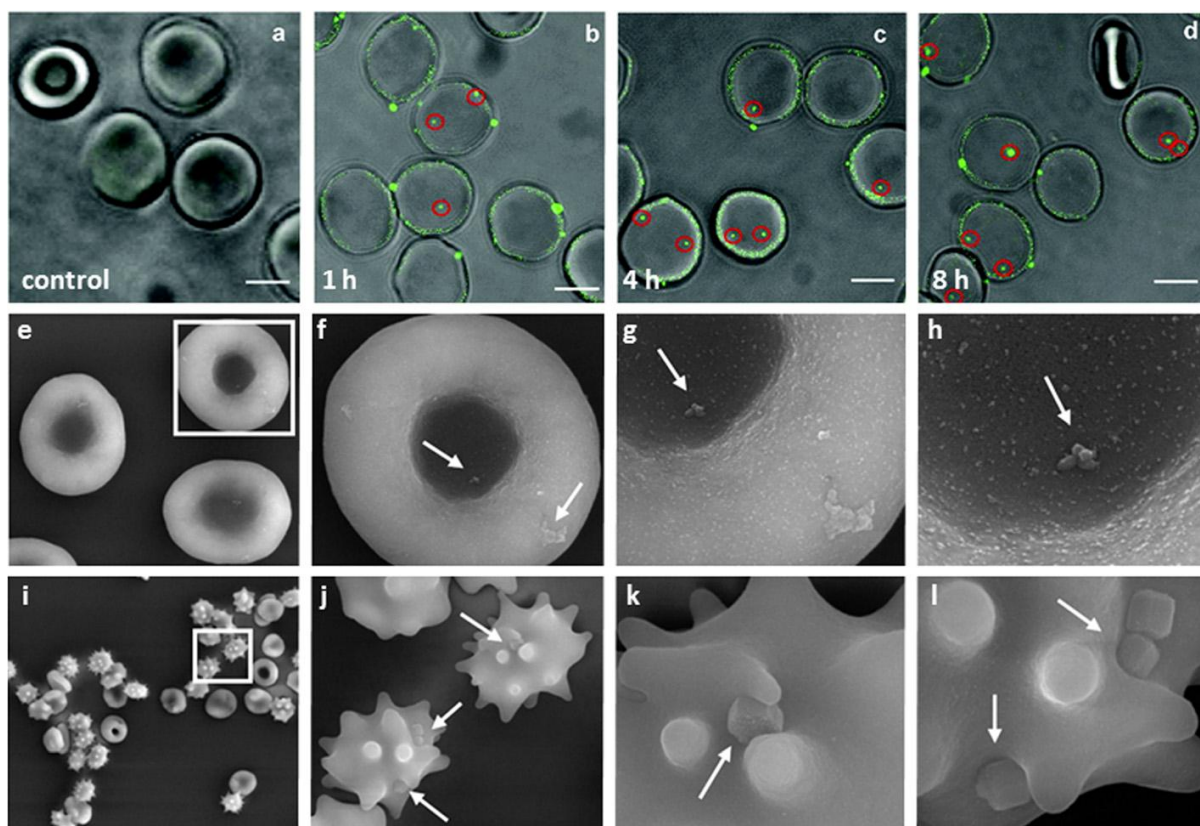


Figure 1: Passive NP uptake by red blood cells. (a – d) Internalization of DPA-QDs (8 nm).^[57] (e – l) Scanning electron micrographs (SEM) of RBCs (5% hematocrit) incubated with 100 µg mL⁻¹ of (e – h) small (~100 nm) and (i – l) large (~600 nm) mesoporous silica particles (MSN).^[58] Reproduced with permission from the American Chemical Society.

Cytotoxic effects of NPs

A protein adsorption layer on the surface confers a new biological identity to the NP, which may completely modify the subsequent cellular and tissue responses, e.g., the distribution to various organs, tissues, and cells. Once inside a cell or tissue, the surface layer, including the adsorbed biomolecules, and also the NP core material will likely be metabolized. Subsequently, the (remnants of the) NPs may be excreted by the organism. All these interactions with the biological environment are again dependent on the physicochemical properties of NPs including their size^[64] (Figure 2). To evaluate the toxicity profile of NPs, two main approaches have been established: (i) functional assays assess the effects of NPs on cellular processes, (ii) viability assays probe whether the NPs cause death in a cell or a

system of cells^[65] Although some aspects of size dependent NP toxicity may be reasonably well predicted by in vitro techniques, it remains difficult to judge whether the observed cytotoxicity is clinically relevant. As can be inferred from the studies, smaller NPs appear to be more toxic than larger ones. Small NPs possess a high surface area relative to their total mass, which increases the chance to interact with surrounding biomolecules and, as a consequence, to trigger adverse responses. Pan et al.^[66] observed that small AuNPs (1.4 nm) were highly toxic and caused predominately rapid cell death by necrosis within 12 h, while larger, 15-nm AuNPs displayed low toxicity, irrespective of cell type and surface ligands. The toxic effects in mice were less pronounced after coating the NP surface with peptides that induced an enhanced immune response. cationic NPs are considered more toxic than neutral or anionic ones, possibly due to their high affinity towards the negatively charged plasma membrane. Therefore, NP toxicity must be evaluated by changing NP properties systematically, one at a time.

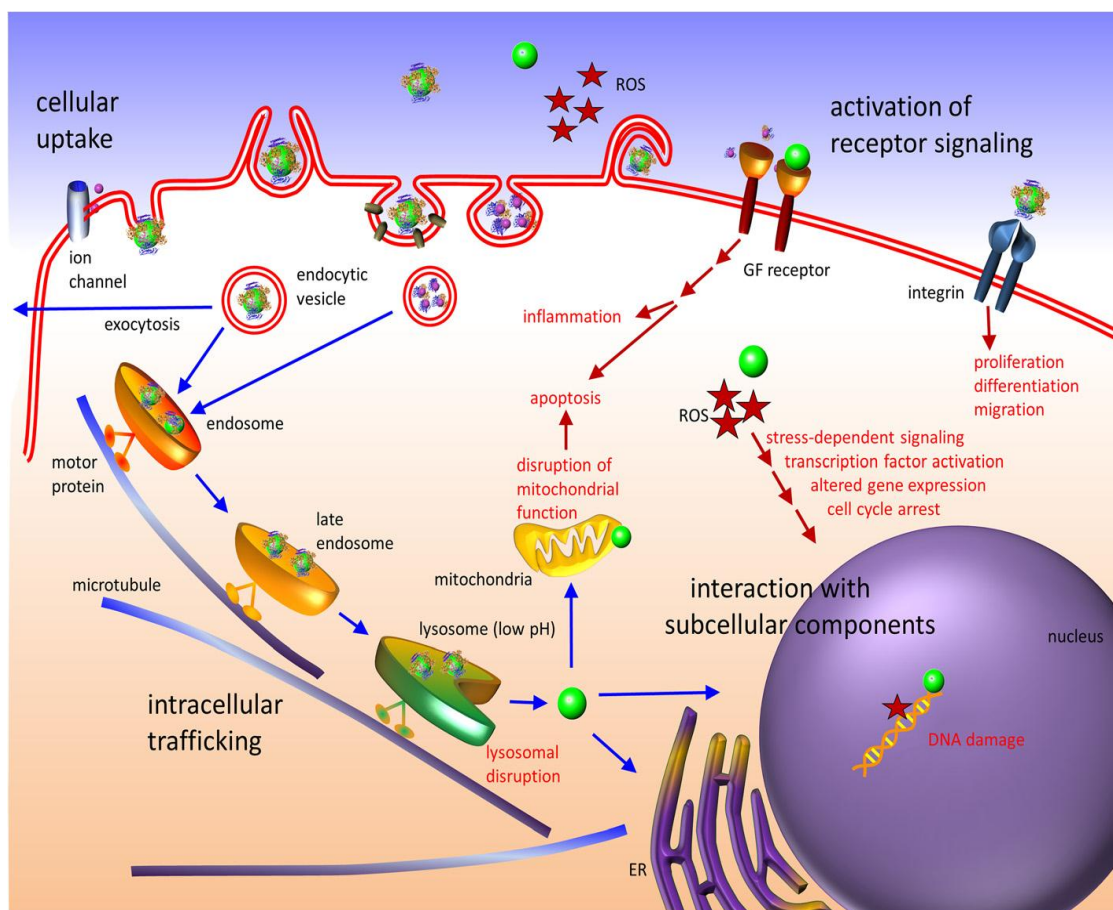


Figure 2: Cytotoxic effects of NPs. In the biological environment, NPs may trigger the production of reactive oxygen species (ROS). Elevated ROS levels may lead to (i) activation of cellular stress-dependent signaling pathways, (ii) direct damage of subcellular organelles such as mitochondria and (iii) DNA fragmentation in the nucleus, resulting in cell cycle arrest, apoptosis, and inflammatory response. NPs may interact

with membrane-bound cellular receptors, e.g., growth factor (GF) receptors and integrins, inducing cellular phenotypes such as proliferation, apoptosis, differentiation, and migration. After internalization via endocytic pathways, NPs are trafficked along the endolysosomal network within vesicles with the help of motor proteins and cytoskeletal structures. To access cytoplasmic or nuclear targets, NPs must escape from the endolysosomal network and traverse through the crowded cytoplasm.

CONCLUSION

The smaller partical size of ENPs offers more surface area And show more harmful effects on body. Interactions between engineered nanomaterials and cells, tissues and organisms has become very important, especially in relation to possible hazards to human health. current research on nano-biointeractions, with a focus on the effects of ENP and their interactions with live cells. Also in vivo and in vitro studies of ENPs showed Toxicities Induced by ENPs .Hazard identification to facilitate risk assessment due to lack of Standardised Testing And Characterisation data results in Nonreproducible Results And makes the Univocal HI of ENPs impossible.

ACKNOWLEDGMENT

The authors gratefully acknowledge the contributions of Prof. Gadhave M.V.HOD M. Pharm., Mrs. Thorat R.M.,Principal, Instt.of Pharmacy , Dr.Gaikwad D.D. Principal, Vishal Institute of Pharmaceutical Education & Research, Ale. for constant motivation and encouragement.

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