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Understanding Nanotoxicology and Its Implications for Overcoming Challenges in the Development of Nanoparticles

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ABSTRACT

Nanotoxicology is an emerging new multidisciplinary field of science. This new technology deals with measures, manipulates, and manufactures at the atomic, molecular, and supramolecular levels, aimed at creating materials, devices, and systems with fundamentally new molecular organizations, properties, and functions associated with greater strength, stability, chemical and biological activity. They are used in rapidly increasing nanoproducts, nanodevices, electronics, diagnostics and drug delivery systems. They are present in a variety of consumer products such as foods, drugs, cosmetics, food colour additives, food containers, paints and surface coatings. Because of their extremely small size they are capable of entering the human body by inhalation, ingestion, skin penetration, intravenous injections and medical devices, and have the potential to interact with intracellular macromolecules. Because of their greater stability they are anticipated to remain in the body and in the environment for long periods of time. However, information on their potential adverse health effects is very limited at the present time. It is not known at what concentration or size they can exhibit toxicity. Therefore, there are obvious public safety concerns. This has led to the initiation of a new research discipline commonly known as Nanotoxicology. The current review article reveals the concept of Nanotoxicology from nanomedicine and non-medical nanoparticles.

Keywords: Nanotechnology, nanoparticles**,** nanomaterials, nanomedicine, nanotoxicity.

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INTRODUCTION

Nanotechnology is a rapidly developing, emerging branch of modern technology. This new technology deals with measures, manipulates, and manufactures at the atomic, molecular, and supramolecular levels, aimed at creating materials, devices, and systems with fundamentally new molecular organizations, properties, and functions.[1] These technologies involve utilization of man-made products no larger than 1–1000 nm (i.e., a few atoms to smaller than a single cell). A dictionary definition (Nano-pref. 1: Extremely small nanoid. 2: One-billionth [10^{-9}m] nanometer) elucidates the scale of this field and allows us to define that nanoscale particles are in the 10^{-9}m dimension range, consistent with the magnitude of most synthetic nanoparticles to date. For a real perspective, the width of a DNA molecule is 2.5 nm; cell membranes are 6–10 nm thick; and most proteins are between 5 and 20 nm in diameter.[2]

Applications of nanotechnology for treatment, diagnosis, monitoring, and control of biological systems has recently been referred to as "Nanomedicine" by the National Institutes of Health.[3] There is a huge range of Nanomedicine devices which involves medical applications of nanomaterials, nano electronic biosensors, and even more useful and practical future applications of molecular nanotechnology. In the field of medical and biological world nanomedicine has greater significance as this application has facilitated the mankind so well.[4] Artificial nanostructures being of the same size as biological entities, can readily interact with biomolecules on both the cell surface and within the cell (Figure 1).

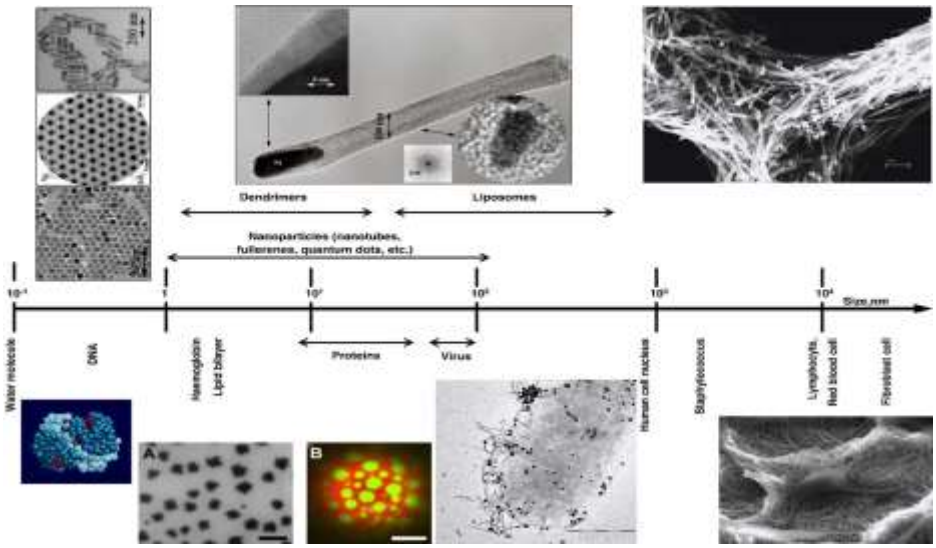

Figure 1: Nanotechnology structures that have the same size than biological systems[5]

Nanoparticles may overcome solubility or stability issues for the drug and minimize drug-induced side effects yet at the same time they have generated serious concerns about potential health and

environmental risks from exposure to engineered nanoscale materials (Figure 2)[6]. Also highly intensive, media-driven debate could be expected with the introduction of nanodevices, similar to the genetically modified food debate. The nanoparticles, because of their size and ability to pass across cellular membranes, represent a potential biohazard. The issue of toxicity becomes even more serious for intravenously injected nanoparticles, as size partly determines tissue distribution. Also the nanomaterials are rapidly increasing their use as Nano products, Nano devices, electronics and diagnostics. Their presence in a variety of consumer products such as foods, drugs, cosmetics, food colour additives, food containers, paints and surface coatings. This trend is expected to result in an ever-increasing presence of nanoparticles in the human environment. Because of their extremely small size they are capable of entering the human body by inhalation, ingestion, skin penetration, intravenous injections and medical devices, and have the potential to interact with intracellular macromolecules. Because of their greater stability they are anticipated to remain in the body and in the environment for long periods of time. However; information on their potential adverse health effects is very limited at the present time. It is not known at what concentration or size they can exhibit toxicity. Therefore, there are obvious public safety concerns. This has led to the initiation of a new research discipline commonly known as nanotoxicology.[7,8]

Figure 2: Nanotechnology; its benefits and Risks

Nanotoxicity

Nanotoxicology is a branch of bionanoscience which deals with the study and application of toxicity of nanomaterials.[9] Increases in nanotechnological applications for industrial, consumer

and medical uses promise many benefits, yet at the same time they have generated serious concerns about potential health and environmental risks from exposure to engineered nanoscale materials. For example, a review on silver nanoparticles toxicity on human health and environment has been reported recently. Nanotoxicity refers to the study of the potential toxic impacts on biological and ecological system. The toxicity of nanoparticles mostly depends on two factors: their surface area and the reactivity or intrinsic toxicity of that surface. Early nanotoxicity studies arise from aerosol studies examining size-dependent particle effects; the field continues to draw from that heritage as well as from diverse fields such as molecular toxicology, material science, molecular biology, analytical chemistry, and engineering.[10,11]

The change in the physicochemical and structural properties of manufactured nanomaterials with a decrease in size could be responsible for a number of material interactions that could lead to toxicological effects. The effect of nanomaterials on the basis for pathophysiology and toxicity as shown in Table 1;[12]

Table 1: Nanomaterials effect as the basis for pathophysiology and toxicity

Experimental Nanomaterial's effects	Possible Pathophysiological Outcomes
ROS generation	Protein, DNA and membrane injury, oxidative stress
Oxidative stress	Phase II enzyme induction, inflammation, mitochondrial perturbation
Mitochondrial perturbation	Inner membrane damage, permeability transition pore opening, energy failure,apoptosis, apo-necrosis, cytotoxicity
Inflammation	Tissue infiltration with inflammatory cells, fibrosis, granulomas, atherogenesis, acute phase protein expression (e.g., C-reactive protein)
Uptake by reticulo-endothelial system	Asymptomatic sequestration and storage in liver, spleen, lymph nodes, possible organ enlargement and dysfunction
Protein denaturation, degradation	Loss of enzyme activity, auto-antigenicity
Nuclear uptake	DNA damage, nucleoprotein clumping, autoantigens
Uptake in neuronal tissue	Brain and peripheral nervous system injury
Perturbation of phagocytic function, "particle overload," mediator release	Chronic inflammation, fibrosis, granulomas, interference in clearance of infectious agents
Endothelial dysfunction, effects on blood clotting	Atherogenesis, thrombosis, stroke, myocardial infarction
Generation of neoantigens, breakdown in immune tolerance	Autoimmunity, adjuvant effects
Altered cell cycle regulation	Proliferation, cell cycle arrest, senescence
DNA damage	Mutagenesis, metaplasia, carcinogenesis

NANOPARTICLES AND THEIR TOXICITY

Carbon Nanotubes (CNT)

CNTs are a special form of carbon, where the chemical bonds of carbon form tubes from carbonatoms. CNTs exist in two forms. Single-wall CNTs (SWCNTs) are containing only one tube in the CNT's structure. Multiwall CNTs (MWCNTs) are containing more than one concentric tube in the basic element of the CNT. CNT material contains many very small tubes (fibres) which are created from carbon atoms and which have different lengths. The lengths are generally dependent on the synthesis time but are typically in the order of tens of microns, although significantly shorter and longer nanotubes have been made. The diameters of SWCNT fibres, which varied between about 0.7 and 3 nm, are controlled by the size of the catalyst. MWCNTs are generally in the range of 10 to 200 nm in diameter

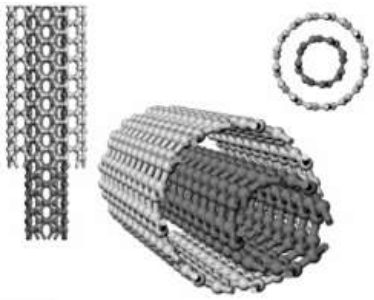

Figure 3: carbon nanotubes

Carbon nanotubes (CNTs) represent one of the most promising materials in the field of nano-science and technology. Their potential in industrial applications has brought them much attention and the wide spectrum of usage has made it imperative that the impact of CNTs on human health and the environment is investigated thoroughly. In addition to their various beneficial applications, there is a potential for hazardous effects on human health. For example, the potential hazards through inhalation of CNTs have not been sufficiently evaluated. CNTs produce reactive oxygen species (ROS) which are associated with diminishing cellular activities, such as a decrease in the mitochondrial membrane potential etc.[13]

Investigation reported that CNT to be toxic to mammalian cells. Exposing Wistar rats to multiwalled CNT by inhalation leads to lesion formation at the upper respiratory tract and inflammatory changes in the lower tract, at concentrations > 0.1mg/m3. This stated value was put down as no-observed-adverse -effect-level.[14,15] Liu et al., have reported the foreign tissue body response caused in the lungs by CNT inhalation and the distribution of these particles has been observed in vivo in the target organs along with their time and dose effects. A series of multiple lesions in the lung tissues is seen to develop in a time and dose dependent manner, suggesting

potential occupational hazard for workers handling this material. It is not only the CNT as such, which may lead to deleterious changes at the organ and cellular level, but the materials and polymers which are employed to coat and functionalize it may also in turn be hazardous. CNT, with its high aspect ratio being hydrophobic, it is quite common to use dispersing agents to overcome its water repelling character for various biological applications. The results certify the variability of the nature of these reagents, nanomaterials and concentrations in determining the final toxicity of the overall compound to the living systems.[16]

Bucky Balls or Fullerenes

Fullerenes are a very important class of carbon based nanostructured materials. The most common is a buckminsterfullerene (buckyballs), C60. C60 itself shows limited solubility in organic solvents but its solubility has been increased by chemical modification and functionalization, therefore, derivatized fullerenes have opened an avenue in the field of biological sciences including possible use in the pharmaceutical industry. For example, C60-containing bilayer lipid membranes may be useful in a biosensor.

In toxicity behavior, Yamago et al. stated when used a radio labeled fullerene with C14 and found fast migration, with liver as the major target organ. It has been reported that fullerene derivatives could even pass through the blood–brain barrier.[17]

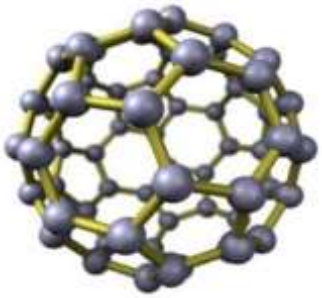

Figure 4: Bucky balls/ Fullerenes

Further, other small molecules have also been physically entrapped in its cage to serve diverse purposes in Nano medicine and other non-health care applications. Its physicochemical characteristics as well the biological mechanisms which drive the toxicity of fullerenes involves genotoxic, oxidative and cytotoxic responses at cellular level.[18]

Table 2: Cytotoxicity of fullerene-based nanostructured materials

Fullerene-Based Nanomaterials	Cytotoxic Effect
C_{60} water Suspensio	Antibacterial; Cytotoxic to Human cell lines; taken up by human keratinocytes; stabilizes proteins
C_{60} encapslated in poly(viylpyrrolidone), cyclodextrins or poly(ethylene glycol)	Damages eukaryotic cell lines; antibacterial
Hydroxylated fullerence	Oxidative eukaryotic cell damage
Carboxyfullerence (Malonic acid derivatives)	Bacteriocidal for Gram-positive bacteria, cytotoxic to human cell lines
Fullerence Derivatives with pyrrolidone groups	Antibacterial; inhibits cancer cell proliferation; cleavage plasmid DNA
Other alkane derivatives of C_{60}	Antimutagenic; cytotoxic; induces DNA damage in plasmids; inhibits protein folding; antibacterial; accumulates in rat's liver
Metallofullerence	Accumulates in rat's liver

Quantum Dots

Quantum dots have been used as a fluorescent labeling agent for both in vitro and in vivo studies for stem cell labeling, medical imaging, sensors, light-emitting diodes, in vivo imaging, 199-200 biological sensing, and multiplexing gene analysis. Recently, cytotoxicity of quantum dots (QDs) and deleterious effects of the labeling procedure on human mesenchymal stem cells has been reported. The cadmium-based quantum dots (QDs) showed cytotoxic effects. The CdTe quantum dots induce cell death by involving both Cd2+ and reactive oxygen species (ROS) accompanied by lysosomal enlargement and intracellular redistribution[19]

Metal Nanoparticles

Nobel and other transition metal nanoparticles have been extensively employed in medical, medicinal, pharmaceutical, cosmoceutical and electronic fields. Silver, gold, platinum, aluminum, zinc, copper and iron nanoparticles, to name a few, have been used widely applied for diverse applications.

Aluminum oxide nanoparticles of size 30 and 40 nm were seen to cause dose and size dependent genotoxicity in vivo as compared to bulk material of the same element, in Wistar rats. Here, micronuclear test and comet assay were used to determine the % of tail DNA and micronuclei migration in peripheral rat blood cells, which was taken as a measure of genotoxicity.

Cadmium ions form components of quantum dots which are utilized for imaging in nanomedicine. The effect of cadmium ions on caspase 3, mitochondrial membrane potential and on oxidative stress markers in murine thermocytes has proved deleterious. DNA damage and apoptogenic potential of these cells were observed by internucleosomal fragmentation on histone and their subsequent detection by ELISA.

Copper nanoparticles form part of formulations for the manufacture of lipsticks where there is a danger of ingesting them, thus facilitating their entry into the gastrointestinal tract. Excess copper in the digestive system is known to induce metabolic alkalosis. Copper nanoparticles screened this way in multiple organs for several biochemical parameters showed the induction of hepato and nephrotoxicity at dosages of about 200mg/kg/day, when rats were exposed for five days. Nano copper suspensions were also found to be toxic to aquatic biota and the toxicity operates through different mechanisms which are in turn dependent of the type of organism and prevailing concentrations. The nano copper ions may themselves per se may not be toxic but may induce copper ion overload culminating in metabolic alkalosis, as nanosized copper particles consume hydrogen ions in abdomen more rapidly than their micron sized particles. These results indicate the imperative need to relook at the compositional characteristics of cosmetic products and ensure proper in vitro and in vivo trials after suitable ethical clearance so that Nano copper levels are kept well below toxicity standards in products which are released into the market.

Iron nanoparticles are used in biomedical devices due to their magnetic property. A number of biomimetic systems have also been developed used these particles. However, it has been shown that intracellular delivery of even small concentrations of iron nanoparticles may adversely affect cell structure and function. Specifically it is seen that these particles impair the ability of PC12 cells to differentiate in response to nerve growth factor. Potential lung and cumulative toxicity of iron nanoparticles have also been reported.

Silver nanoparticles find extensive applications in the pharmaceutical industries and used in the treatment of burn injuries. However it has been reported that these particles may be toxic to organs and may induce inflammation as observed in spleen of rats. It has been inferred that in case of titanium toxicity, both crystal structure and size contribute to the cytotoxicity and the mechanism of cell death depends on the crystal structure.

The effect of particle agglomeration as well as serum protein adsorption on influencing the toxicity of amorphous silica nanoparticles has been reported on a eukaryotic cell model. It has been strongly inferred that the observed that the toxicity of silica nanoparticles here is a consequence of physiochemical properties of silica nanoparticles and not related to silica material as such.[14]

The effects of nano-TiO2 varied depending on exposure concentrations and conditions. Inhalation exposure studies observed that the toxicity of nanoTiO2 was dependent not only on concentration but also on the physical properties of the materials (e.g., specific surface area, mineral phase). Literature reporting human dermal absorption of nano-TiO2 found that the nanoparticles did not penetrate through the stratum corneum under the conditions studied.[20]

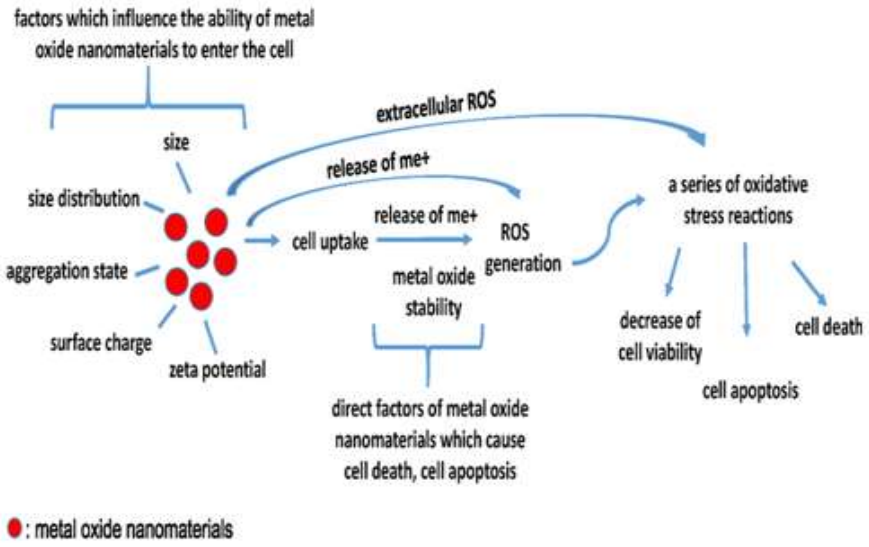

Figure 5: Effect of Metal oxide Nanomaterials cause cell death.

FACTOR AFFECTING NANOTOXICITY

Dose

Earlier the toxicological studies were governed by the saying "Dose makes the poison". Toxic effects of nanoparticles do not always appear to correlate with particle mass dose. Indeed, paradoxically, a high concentration of nanoparticles may promote particle aggregation and could therefore reduce toxic responses compared to lower concentrations of the same particles.

Surface area

The relative portion of surface atoms to bulk atoms is considerably different in nano-sized when compared to micro sized particles of same chemistry. For example, less than 1% of atoms of a micro particle occupy surface positions, while 10% of the atoms in a 10-nm particle reside on its surface. Thus, when size of the materials is reduced, it contributes to changes in surface physical and chemical properties.

Size

The comparing the various cytotoxicity studies involving different sized gold nanoparticles provides a great scope to understand the size dependent toxicity. Gold Nano clusters (1.4nm) were shown to be toxic to cells owing to their specific interaction with major grooves of DNA, whereas smaller or larger gold particles did not behave in this way. The gold nanoparticles of 35nm size were non-toxic to a murine macrophage-like cell line.

Crystalline structure

The cytotoxic properties of titanium dioxide Nanoparticles appear to correlate with their phase composition. Titania exists in a variety of crystal structures and the most researched forms are

rutile, anatase and brookite. In a study with titanium dioxide nanoparticles of size ranging between 3-10nm, demonstrated that anatase titanium dioxide was 100 times more toxic than an equivalent sample of rutile titanium dioxide. They reported that the generation of ROS under UVillumination correlated well with the observed biologicalresponses. In addition, the pulmonary toxicities of fine andultrafine (nano-sized) quartz particles appeared to correlatebetter with surface activity than with particle size and surface area. Interestingly, the crystal structure of titanium dioxide also dictates the mode of cell death. Anatase TiO2 nanoparticles, regardless of size, were reported to induce necrosis, whereas rutile TiO2 nanoparticles triggered apoptosis through the formation of reactive oxygen species.

Surface coating

The surfaces of ENPs make contact with cells and a thorough understanding of its surface composition is therefore vital to understand the interactions of nanoparticles with biological systems. The contaminants on the surface of ENPs do contribute to toxicity. For instance, the surface of CNTs when contaminated with ferrous iron can induce the production of ROS through Fenton's reactions inside biological system. The frequent problem with all biomaterials is the possible adsorption of the ubiquitous bacterial endotoxin, lipopolysaccharide which can also contribute to the cellular responses, in particular immunological responses. Hence, it is also crucial to distinguish between undesirable cellular responses to nanoparticles themselves and residual materials associated with the nanoparticle such as surfactants or transition metals as a product of the synthetic process.[21]

PATHWAYS OF EXPOSURE OF NANOPARTICLES

Nanoparticles enter the body mainly through respiratory system. Intravenous and oral administrations have a more rapid systemic effect compared to other routes and once in systemic circulation, most substances are subject to first-pass metabolism within the liver where they may accumulate or distribute via vasculature to end organs including brain. Liver is the site for first-pass metabolism, and it is particularly vulnerable to NP toxicity. The hepatotoxic potential of silica NPs could cause mononuclear inflammatory cell infiltrates at the portal area with concomitant hepatocyte necrosis.

Skin exposure to NPs can occur during the intentional application of topical creams and other drug treatments or accidental exposure. There are controversial data about the dermal absorption of NMs although stratum corneum, the outer layer of epidermis, is a good barrier for chemical exposure. The penetration of a variety of NPs in the dermis and translocation to the systemic vasculate via lymphatic system and regional lymph. In some studies, the cytotoxicity of NPs

applied to the skin was demonstrated. Cultured keratinocytes were exposed to extracts of several types of silver containing dressings. Of these, extracts of nanocrystalline silver coated dressings were most cytotoxic.

Because of its large surface area, localization/accumulation of drugs within the pulmonary tissue, lung is an attractive target for drug delivery due to the non-invasive nature of inhalation therapy. Inhaled NPs can be deposited in all regions of respiratory tract. Being different than micron sized particles that are largely trapped and cleared by upper airway mucociliary escalator system, particles less than 2.5 **u**m can get down to the alveoli. The deposition of inhaled ultrafine particles (aerodynamic-diameter < 100 nm) mainly takes place in the alveolar region. After absorption from the respiratory tract, NPs can enter blood respiratory tract, NPs can enter blood and lymph to reach cells in the bone marrow, lymph nodes, spleen and heart. In respiratory tract, alveolar macrophages engulf and process particles that are not cleared by mucociliary action and coughing. Upon phagocytosis macrophages are activated torelease substantial amounts of oxygen radicals, proteolytic enzymes, proinflammatory mediators, etc. these mediators may lead to both acute and chronic lung inflammation. Ultrafine NPs are suggested to have more toxic properties than larger particles with the same chemical identity due to their larger surface area. Ultrafine silver particles were taken up by alveolar macrophages and aggregated silver particles persisted there for up to 7 days. Aggregated silver NPs and some other Nanomaterials have been shown to be cytotoxic to alveolar macrophage cells as well as epithelial lung cells.

Nanoparticles can reach the gastrointestinal tract after mucociliary clearance from the respiratory tract through the nasal region, or can be ingested directly in food, water, cosmetics, drugs, and drug delivery devices numerous kinds of NPs can pass through the gastrointestinal tract and are rapidly eliminated in feces and urine. However some NPs can accumulate in the liver during first-pass metabolism. Recently reported the occurrence of systemic argyria after ingestion of colloidal Nano silver proves its translocation from the intestinal tract. Nano copper was reported to cause damage to liver, kidney and spleen. Injections and implants are other possible routes of exposure, primarily limited to engineered materials. Thus, nanoscale particles can end up in different parts of the body depending on size and other characteristics as well as routes of entry.[22]

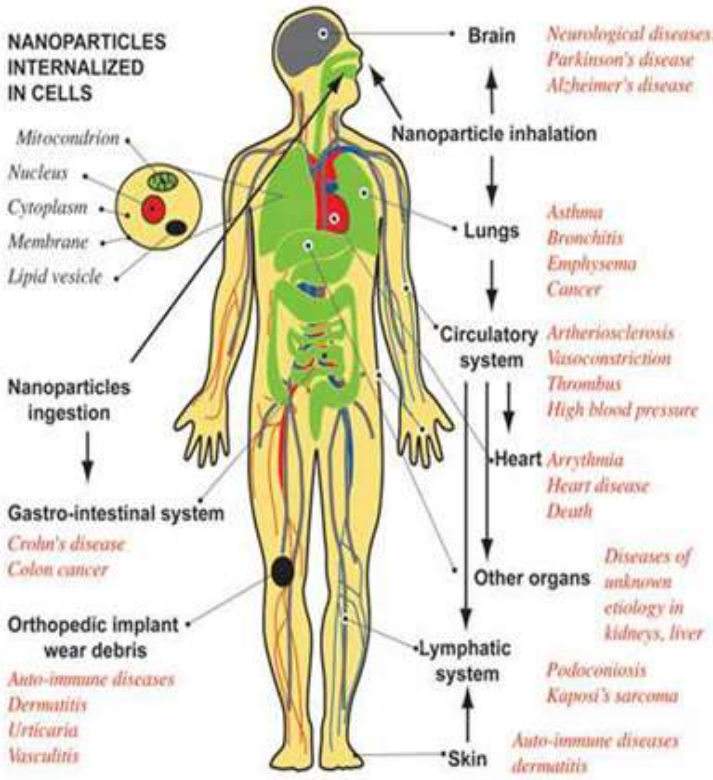

Figure 6: Pathways of exposure to nanoparticles and associated diseases[23]

NANOTOXICITY ON HUMAN BODY

A large proportion of the atoms that make up a nanoparticles because of their small size, are exposed to the exterior of the particle and may participate in many chemical processes. It may lead to the adverse consequences due to exposure in to the environment. Studies in human showed that deposition of nanoparticle in the lungs increases with decreasing particle size and the toxicity of inhaled insoluble nanomaterials increases with decreases particle size and increasing particle surface area. Certain classes of nanoparticle could be responsible for destructive inflammatory processes in the lungs eg. Carbon black nanoparticle may induce a type-II alveolar epithelial cell line to release pro-inflammatory mediators. Nanotechnology changes the properties of substance eg. Carbon as fullerens and nanotubes an attractive candidate for applications but also make them dangerous, when expose to environment. The measure for safety needs to be taken on environmental concern with the use of nanotechnology. Nanomedicine have potential to cross blood brain barrier may cause harm to the patient. The protection and maintenance of health information of the patient is the ethical issue, and while using nanotechnology in medical field ensuring privacy and confidentiality is of utmost importance.[24] The proposed toxicological mechanisms of NMs include oxidative stress, cytotoxicity, genotoxicity and inflammatory responses[25].

Oxidative stress and reactive oxygen species

NMs can induce oxidative stress, which refers to a redox imbalance within cells usually as a result of increased intracellular reactive oxygen species (ROS) and decreased antioxidants. In general, small and transient increases in ROS can be tolerated by most cell types, whereas higher levels which persist over a longer time period are more likely to result in cell damage. ROS are highly reactive molecules that can interact with cellular macromolecules such as DNA, proteins and lipids.

Composition of NMs and their high surface area are associated with the generation of ROS by NMs. Consequently, the smaller NP, the higher oxidative stress they induce. NMs have been described to possibly generate ROS by different mechanisms: direct generation of ROS as a result of exposure to an acidic environment such as the lysosomes, interaction of the NMs with cellular organelles such as mitochondria, interaction of NMs with redox active proteins such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and interaction of NMs with cell surface receptors and activation of intracellular signaling pathways. Oxidative stress induced by NPs is reported to enhance inflammation though up regulation of redox-sensitive transcription factors.[22]

Cytotoxicity

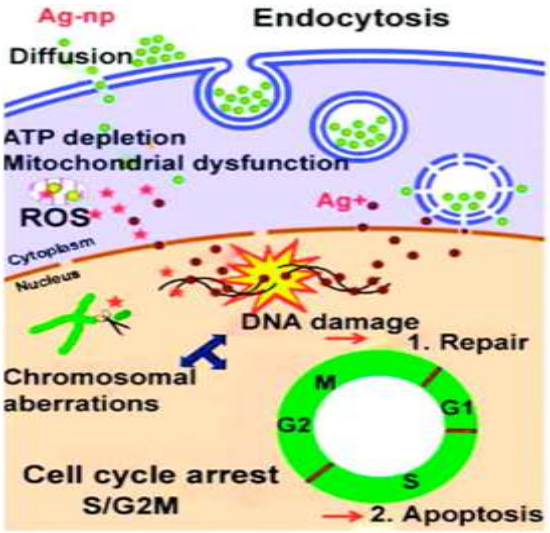

Figure 7: Hypothetical mechanism of silver nanoparticle cause cytotoxicity

The membrane stability can be affected by NPs either directly like physical damage or indirectly like oxidation which can cause cell death. Interactions of NPs with membranes are associated with surface properties of NPs. The higher surface area over volume ratio of NMs augments the surface available for interaction with cellular components.

The identification of cytotoxicity of NPs toward mammalian germ line stem cells has aroused great concern over the biosafety of NMs. The results showed that AgNPs were the most toxic with manifestations like drastic reduction of mitochondrial function, increased membrane leakage, necrosis and induction of apoptosis. Due to their small size, AuNPs have been found easily enter cells. 7 nm sized cerium oxide NPs have caused cytotoxicity with absorption on cell membrane. Several in vitro studies have demonstrated the cytotoxicity of single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) in guinea pig alveolar macrophages.[22]

Gentotoxicity

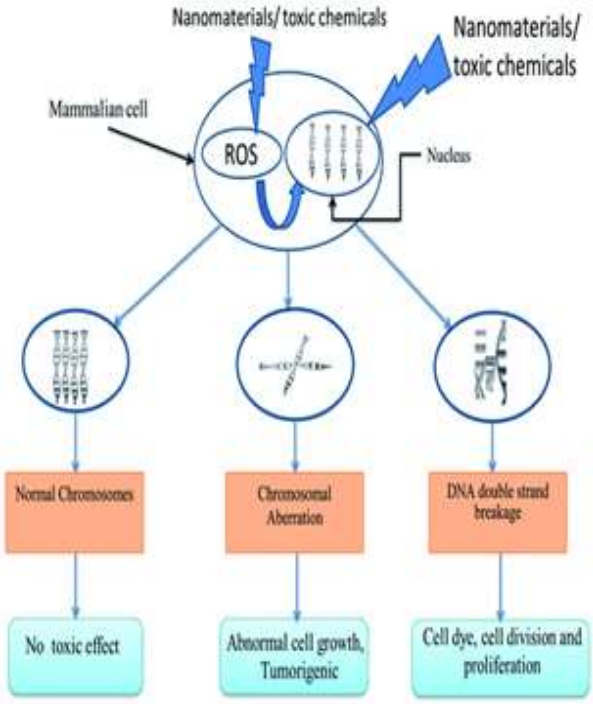

Figure 8: Mechanism by which nanoparticles entry cause gentotoxicity.

Due to their small size and large surface area, NMs may have unpredictable genotoxic effects and the most important genotoxic effect is DNA damage induction which can cause mutagenesis, and carcinogenesis. NMs are small enough, so they may pass through cellular membranes and they may interact with DNA directly. When they promote oxidative stress and inflammatory responses, they may also interact with DNA indirectly. DNA damage induced by NPs, single-strand DNA breaks, double-strand breaks, DNA deletions and genomic instability in the form of increase in 8-hydroxy-2-deoxyguanosine (8-OHdG) levels are formed. Long term exposure of cells to NPs caused genome instability, altered cell cycle kinetics and induced protein expression of p53, which have a critical role in responding to various stresses that cause damages in DNA and in DNA

repair related proteins AuNPs, AgNPs and TiO_2 NPs are important for ROS production and genotoxicity.[22]

Inflammatory responses

Inflammation is an important physiological process in response to tissue injury and is mediated by inflammatory cells that secrete a large variety of soluble factors, including cytokines, migration inhibition factors, reactive nitrogen species and ROS. These factors are important defences against infection and tissue injury. Oxidative stress induced by NPs is reported to enhance inflammation through upregulation of redox-sensitive transcription factors such as nuclear factor kappa B (NFKB), activating protein 1 (AP-1), extracellular signal regulated kinases (ERK) c-Jun, N-terminal kinases, JNK, and p38 mitogen-activated protein kinases pathways. The increase of TNF-a level can cause damage of cell membrane and apoptosis. Additionally, chronic inflammation has been strongly associated with carcinogenesis. NPs are described to be more toxic than larger particles with the same chemical entity, causing inflammation or allergic response.[22]

STRATEGIES TO AVOID NANOTOXICITY

Preventing Oxidative Stress

Oxidative stress results from imbalances in the redox state of the cell. The redox state is disturbed by ROS production in response to nanoparticle exposure and/or nanoparticle-induced inflammation cascade.

One possible way to prevent oxidative stress-mediated nanotoxicity is the introduction of ascorbic acid upon nanoparticle exposure. Ascorbic acid, also known as vitamin C, is an antioxidant capable of scavenging free radicals. By introducing ascorbic acid into AgNP-treated acute myeloid leukemia cells there is a complete decrease in ROS production in the cells. Concomitantly, ascorbic acid also led to a decrease in AgNP-induced mitochondria damage, apoptosis and DNA damage, reducing the toxic effects induced by AgNPs. Similar mitigation of ROS generation and glutathione depletion were also observed when ascorbic acid was added to human lung epithelial (A549) cells treated with nickel ferrite nanoparticles (26 nm in diameter) . An in vivo Drosophilamelanogaster study has also shown a decrease in nanotoxicity when ascorbic acid was supplemented in the diet of Drosophila exposed to AgNPs. In vivo studies with rats have further revealed that acute oxidative stress and inflammation induced by ZnO nanoparticles (of particle size 21 nm) were alleviated when 1% aqueous ascorbic acid was given as drinking water. Hence, the administration of ascorbic acid after nanomaterial exposure is a feasible strategy to overcome nanotoxicity, both in vitro and in vitro. Quercetin, a naturally occurring flavonoid in many plants and food, is an anti-oxidant having free radical scavenging ability. Quercetin has been found to

reduce Fe_2O_3 nanoparticles-induced oxidative injury and inflammation by increasing Bad phosphorylation and Nrf_2 translocation through PI3-K/Akt dependent pathways. In vivo studies have also revealed that TiO_2 NPs induced liver and kidney oxidative stress can be avoided by treatment with quercetin.[10]

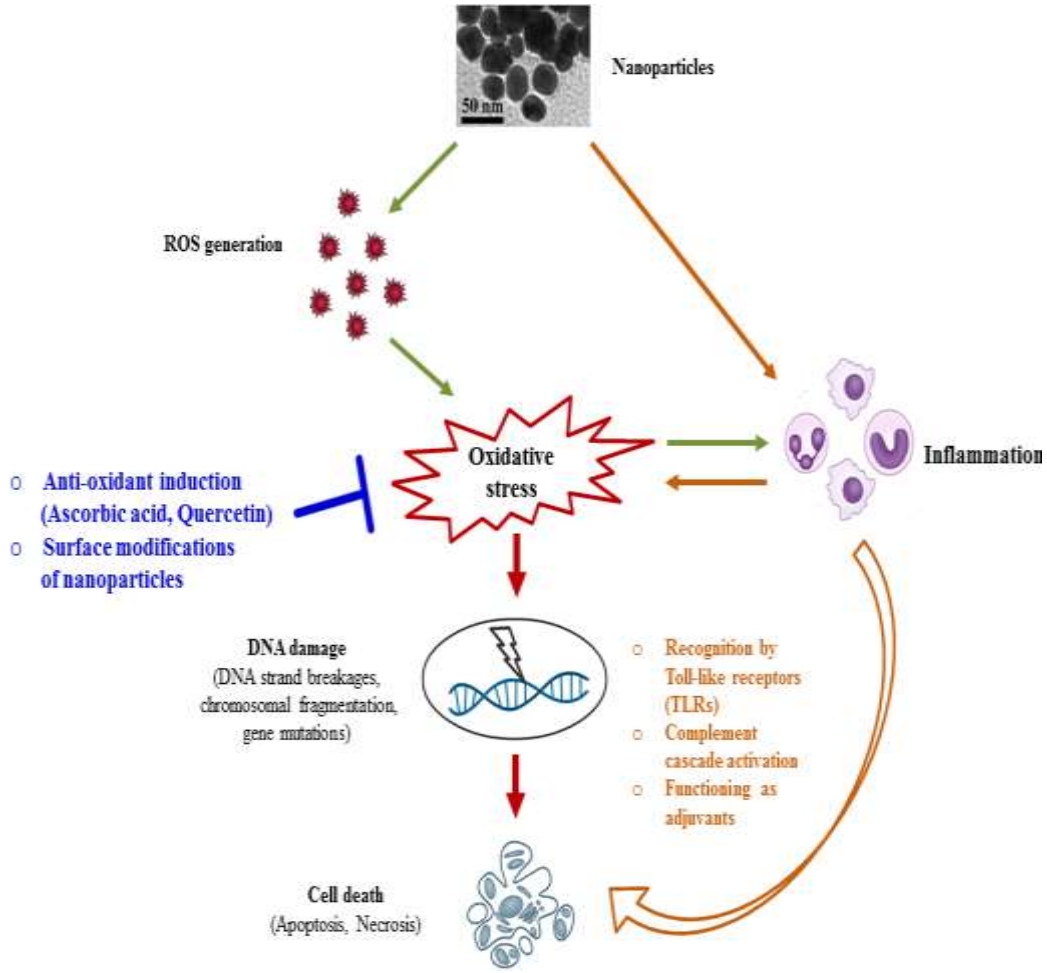

Figure 9: Nanotoxicity, and possible strategies to avoid the toxicity[10]

Surface modification of nanoparticles

Surface modification of nanoparticles can also be carried out to decrease nanotoxicity. An example would be the encapsulation of ascorbic acid with poly (L-glutamic acid)-capped silver nanoparticles (AgNpPGA) within a poly (lactide-co-glycolide) (PLGA) polymeric matrix (PLGA/AgNpPGA/ascorbic acid particles). A reduction in ROS generation was observed in HepG2 cells treated with the PLGA/AgNpPGA/ascorbic acid particles as compared to control cells, suggesting that nanoparticles encapsulated with ascorbic acid can reduce oxidative stress in cells, possibly decreasing the nanotoxic effects of nanoparticles. Copper nanoparticles coated with polysaccharides such as chitosan have also shown to decrease in vitro toxicity and ROS generation, although the modification increased inflammatory responses when administered via the

lung. Furthermore, Fe2O3 nanoparticles coated with chitosan resulted in a decrease in cellular damage and moderated ROS production, thereby, reducing the cytotoxic effects of the nanoparticles. Polymer coatings such as polyethylene glycol (PEG) on super paramagnetic iron oxide nanoparticles (SPIONs) have also effectively reduced nanoparticle cytotoxicity by reducing ROS formation. Hence, targeting these signaling molecules also holds promise as an effective tool to evade/circumvent the inflammation-mediated toxicity, thereby allowing for the development of nanoparticle-based applications in the field of medicine.[10]

HANDLING OF NANOPARTICLES

1. Some of the organisation like National Institute of Occupational Safety and Health has started an active program for studying the safe handling of nanomaterials in the workplace.
2. During manufacture and handling of these materials there may be a chance of release and exposure of nanoparticles to workers which can get inside their body through inhalation, dermal contact and ingestion routes. Only limited information on the risks of handling of these materials are available, so workers should implement strict control procedures and engineering safety features to limit exposure when working with them and not to allow them to eat or drink in the laboratory.
3. When workers handle the nanomaterials they should use laboratory safety practices such as Personnel Protective Equipment (PPE) including gloves, lab coats, safety glasses, face shields, closed-toed shoes etc avoid the skin contact with nanoparticles or nanoparticles containing solutions. If it is necessary to handle nanoparticle powders with exhaust laminar flow hood, workers should wear appropriate respiratory protection. Use of fume exhaust hoods to expel fumes from tube furnaces or chemical reaction vessels is very much crucial. Laboratory personnel should be trained with the risk associated with workplace hazards, Material Safety Data Sheets (MSDS), labeling, signage etc periodically.
4. Disposal of nanoparticles is also reflecting on the safety of the environment. It should be according to hazardous chemical waste guidelines.[26]

CONCLUSION

Nanoparticles hold great prospective in the field of nanomedicine due to their favourable physicochemical properties. But there could be significant toxicity issues associated with the nanomaterial themselves. In this paper, we have discussed the (a) various nanoparticles and their toxicity, (b) various factors affecting nanotoxicity, (c) pathways of uptake (d) various mechanisms by which nanoparticles exerts its toxicities, and (e) various strategies to avoid nanotoxicity. On the

entire, this review is expected to encourage the academic researchers to design safer nanotherapeutics and to carry out the toxicity studies to establish the safety of nanoparticles.

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