ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Nanotoxicology and Its Implications.

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ABSTRACT

Nanotechnology, which is the one of the most rapidly emerging fields of the present day, has shown many promising applications in the field of electronics, communication, environmental remediation, medicine, agriculture and health care among others. However, as is seen with any technological advancement and innovations, there are several drawbacks associated with this technology, of which the most apparent aspect of concern is the toxicity associated with the several types of nanomaterials and nanoparticles which are being indiscriminately used in several fields and industries today. This is felt more so in areas involving nanomedicine, pharmaceuticals, cosmoceuticals, etc. which involve direct interaction of these particles with human and animal cells, tissues and organs. In case of their use in applications which do not directly affect human health and welfare, their disposal after use presents a more severe problem. There are no regulatory authorities which look into the toxicological aspects of these materials currently and several of the statutory bodies which deal with toxicity assessment issues do not consider a material as different unless their composition differs. Hence is a bulk material of a particular composition has been traditionally established as non toxic, then the corresponding nanomaterial is also considered safe today. It is yet to be strongly realized that reduction in particle size lead to alteration in properties of many materials, and this may have many adverse impacts compared to bulk material of the same composition.

Keywords: Nanomaterials, Environmental remediation, nanomedicine, cosmoceuticals

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ISSN: 0975-8585

INTRODUCTION

The origin of the term ‘‘nanotechnology’’ is associated with the Greek word ‘‘nano,’’ which means ‘dwarf.’ There has been a dramatic growth of the nanotechnology industry in the recent times and nanomaterials are being developed at a rapid rate for use in innumerable industries. From the physical, chemical and material science perspective, the improvement of new as well as established products, with the aid of nanomaterials is very exciting for a given particle-type, as one scales down to the 10^{-9} level, the properties change miraculously, sometimes for better and at times for worse. For the same mass / volume of a material, when the number of particles increases, the surface area proportionately increases and quantum effects also start dominating. These, in turn, are largely responsible for the modified properties. For example, titanium dioxide particles lose their white color, seen in their bulk form and convert to colorless at size ranges below 50 nm [1]. The large surface area of nanomaterials bestows them with unusual catalytic properties. This promises to improve their propellant and fuel catalysis properties, their applications in automotive catalytic converters and also help in environmental remediation, where they react with pollutants, for easy detoxification. However at the same time, the properties of high reactivity may cause unwanted reactions and hence risks of production of toxic chemicals or gases which could have serious repercussions on the environment. Thus may lead to increased concentration of harmful chemicals causing profound health issues and leading also to genotoxicity [2].

Nanoparticles are reported to be toxic in general to aquatic life. However coating them with natural and effluent organic matter may decrease their toxicity. This has been confirmed with quantum dots. The hydrophobic nature of these organic matter may be responsible for mitigation of the toxicity of these materials [3].

Nanoparticles and nano devices are being formed intentionally, accidentally and manufactured by different methods and many times, released into the environment directly or indirectly without any safety tests. These materials are known to induce cytotoxicity by initiating stress and eliciting inflammatory responses at the molecular and atomic level. Nanomaterials can travel more freely and easily as compared to their larger counterparts and thereby can be more toxic than larger materials. They can enter the human body through water, air and skin contact. They can then access the internal body tissues and organs causing damages which can be even fatal [4]. It has been reported that the smallest nanoparticles show profound reactivity and exhibit the high levels of vascular thrombosis as well as the low extravasation. This is observed in a study wherein a lactic acid based nanoparticle encapsulated a drug (meso-tetra (carboxyphenyl) porphyrin) with size range between 121 to 343 nm. The research suggested that large nanoparticles are more rapidly eliminated from the bloodstream and are thus effective within a short period of time and with less harmful effects [5].

Metal based nanoparticles (Ag, Au, and Cu) are also known to induce toxicity in mammalian cells by interacting with proteins and enzymes after entering the body. This imbalances the natural antioxidant defense mechanism, leading to the generation of reactive oxygen species (ROS). The consequence is the initiation of an inflammatory responses, along with the perturbation and sometimes even destruction of the

mitochondria. This may in turn lead to cell apoptosis and / or necrosis [6]. Nanoparticles, which are added to sunscreens to protect against the UV light, may accumulate on the surface of the skin and in the stratum corneum among keratinized cells, over a period of time. This may normally not lead to toxicity, but if the skin is compromised due to burns and injury, there are chances of these particles entering the body and causing unfavorable effects [7]. Sunscreens and other aesthetic products contain either titanium dioxide or zinc oxide nanoparticles. The photoactive surfaces of the nanoparticles are susceptible to the formation of ROS. Smaller particles with their greater surface area to volume ratio have high chemical reactivity as well as biological activity. The greater chemical reactivity results in large production of ROS which poses several health issues. ROS production has been reported to occur in a different and diverse range of nanomaterials which include carbon nanotubes, fullerenes or bucky balls and nano sized metal oxides. ROS and free radical production is one of the primary mechanisms of nanoparticle toxicity which leads to oxidative stress, inflammation, cell injury and subsequent damage to membranes, protein, cell organelles and DNA. The latter culminates in different types of mutations which in turn causes production of structural damage to the protein encoded by it. The protein thus produced can either malfunction, or be non-functional, causing metabolic blocks in important metabolic pathways. This catabolic or anabolic block, can lead to the accumulation of a by-product or an intermediate which again may lead to certain consequences with varying degree of effects depending on the type of substance that gets accumulated. For example, if a nanoparticle reacts with or damages an enzyme associated with degradation of Phenylalanine making it non-functional, then there may be an accumulation of a specific intermediate and the deficiency of a reactant for the next reaction in the chain, hence affecting the entire Phenylalanine metabolism, leading to several metabolic disorders.

A NEED FOR NANOTOXICOLOGY

The branch of nanotoxicology deals with the study relating to the toxicity of the nano materials, as it is imperative to know that how toxic a nano material is before using it for various applications. The effects and impacts on human health also needs to be assessed accordingly. The field of nanotoxicology has been growing fast, and literature reviews show that the results are not only numerous but also exciting. The International Council on Nanotechnology (ICON) has formed a database of all the publications of several nanomaterials along with their impact on environmental health and safety. This emphasizes on the interesting trends associated with the field of nanotoxicology[8].

Two consecutive articles were published in 1990, in the Journal of Aerosol Science analyzing whether inhaled particles, which are lesser than 100 nm in dia elicits a greater pulmonary response than the one which is normally expected. When studied on a mass for mass basis, titanium di oxide and aluminium oxide particles, which were of the nanometer scale, did elicit a significantly greater inflammatory response in the lungs of rats when compared with larger particles of the same chemical composition. These two studies challenged the long held presumption that response to a particular type of particle was dependent only on the chemical composition and not on the size. The unusual biological activity related with nanometer-scale materials was noticed for the first time. It was concluded here that phagocytosis of particles in alveoli, may impede translocation of

particles into the interstitial space. However if the alveolar macrophages die or dysfunction the translocation from alveoli into interstitium may be facilitated. 0.02-0.03 nm dia particles may penetrate more easily than particles of 0.2-0.5 nm. Small particles may form aggregates. Their aerodynamic size is a factor which controls the deposition in the airways. Deagglomeration may take place after deposition. For primary particle sizes raging from 0.02- 0.03 nm, deagglomeration may affect translocation of the particles more than for aggregates which may consist of larger particles. The ever increasing manufacture and use of nanomaterial, specifically in the form of nanoparticles (NPs) of spherical and fibre-like shapes, for diverse industrial and biomedical applications and other useful products is on a proportional rise. But we have concerns about their safety for human health and the environment [9].

TOXICITY TESTING OF NANOMATERIALS

The greatest challenge faced in the field of Nano toxicology today is the identification as well as the evaluation of the deleterious effect of various engineered nanomaterials with their diverse physiochemical properties, which are constantly being produced and introduced for versatile applications. It is not very easy to find the hazard denominations of nanoparticles due to various reasons.

In vitro test systems have a lot of limitations in hazard identification of nanoparticles because of their highly diverse and versatile physiochemical properties. On the other hand, conducting *in vivo* studies on animal models for every new nanomaterial manufactured is highly impractical. Thus, short term *in vivo* studies in rodents, and *in vivo* and *in vitro* investigation, including high throughput proteomics and genomics studies, to identify toxic pathways of well characterized reference materials of some class or subclass of nanomaterials are carried out. This data can be now used as a reference for any newly manufactured nanomaterial. The hazard potential of the new nanomaterial can be assessed by only conducting *in vitro* high throughput studies and this data can be compared with reference material of related class or subclass of nanomaterial[10].

IMMUNOTOXICITY

The pharmaceutical industry is researching a number of drugs and diagnostic procedures based on nanoparticles. Efforts are on to develop target based drugs which work on the nanoscale and affect only the cells/organs associated with the disease. Understanding and evaluating the immune response of nanoscale drugs is posing a major challenge for scientists. Assessing the immunotoxicity of nanoparticles, functionalized with small molecules is regulated by International Conference for Harmonization (ICH)-S8 guidelines. During the evaluation process, two parameters are given highest priority First is the data of standard toxicity testing evaluated in the organ of immune system in specific histopathology specimens and also the changes in white blood cell population. The second factor is the pharmacological action of the drug. The nanoparticles are tested for their biodegradable nature and their routes of elimination from the body [11].

NANOPARTICLES AND THEIR TOXICITY

CARBON NANOTUBES (CNT)

These are hollow nanostructures derived by rolling graphene sheets. Research CNT has exploded during the last two decades after Ijima discovered them in 1991. It can be said safely that CNT is one of the most widely researched and used nanomaterial today in electronic and semiconductor industries. Their health care uses are also being aggressively explored. However, several workers have reported 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/m^3. This stated value was put down as no-observed-adverse -effect-level [12]. 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 [13]. 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. In such instances, the dispersing agent may also contribute to the toxic effects seen in live systems. The effects of such agents like THF, Triton -X, SDS, etc has been studied on model organisms like *Pseudokirchneriellasubcapitata*and *Ceriodaphniadubia*. 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 [14].

BUCKY BALLS OR FULLERENES

Traditionally, the structure of this nanomaterial has revolved around a 60 carbon atom spherical closed cage. However to suit different applications, its molecular makeup has been modified and surface chemistry has been altered to provide versatility to its structure. Further, other small molecules have also been physically entrapped in its cage to serve diverse purposes in nanomedicine and other non health care applications. Its physicochemical characteristics as well the biological mechanisms which drive the toxicity of fullerenes has been extensively discussed in the literature and it has been reported that fullerene toxicity involves genotoxic, oxidative and cytotoxic responses at cellular level[15].

METAL NANOPARTICLES

Nobel and other transition metal nanoparticles have been extensively employed in medical, medicinal, pharmaceutical, cosmoceutical and electronic fields. Silver, gold, platinum, aluminium, zinc, copper and iron nanoparticles, to name a few, have been used widely applied for diverse applications. The toxicity associated with these has been reported by several authors. Aluminium 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 [16]. Aluminium nanoparticles are also implicated in development of Alzheimer's disease although this aspect has not been proved as yet. It would be relevant to state here that aluminum oxide nanoparticles are used widely in the manufacture of antiperspirant and deodorants by several cosmetic manufacturing companies. 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 [17].

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. Lei *et al* have proposed an integrated metabolomic pathway which facilitates *in vivo* screening for nanotoxicity of nanomaterials. 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[18]. Nanocopper 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 [19]. It has been proposed that the size dependent high reactivity of nanocopper, compared to its ionic form, may be responsible for inducing high toxicity as evidenced by *in vivo* and *in vitro* tests. The pathological examination of tissues and biochemical assays attest this fact. 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 [20]. 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 nanocopper levels are kept well below toxicity standards in products which are released into the market.

Gold nanoparticles may prove to be ideal for studying the size-dependent biological response of systems to nanoparticles due to their excellent biocompatibility. Their size can be controlled accurately during the process of chemical synthesis. The toxicity of gold nanoparticles *in vivo*has been reported. When naked particles, ranging from 3 to 100 nm intraperitoneally injected into mice at a dose of 8 mg/kg/week, the particles in the sizes of 3, 5, 50, and 100 nm did not elicit any damaging effects. However, the particles in the size range of 8 to 37 nm elicited a severe sickness response in mice. They also exhibited fatigue and weight less along with loss of appetite and change of fur color. A number of mice died also within 21 days [21]. 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 [22].Potential lung and cumulative toxicity of iron nanoparticles have also been reported by Zhu *et al* [23].

Toxic response of nickel nanoparticles (Ni NPs) has been observed in lung epithelial A549 cells when treated at concentrations of 0, 1, 2, 5, 10 and 25 µg/ml for 24h and 48h. Some of the toxicity end points which were studied included membrane leakage of lactate dehydrogenase (LDH assay), mitochondrial function (MTT assay), production of reactive oxygen species (ROS), levels of reduced glutathione (GSH), caspase-3 activity and peroxidation of membrane lipids (LPO). Reduction of mitochondrial function and LDH leakage were seen along with induction of oxidative stress in a manner which was dose and time-dependent. This was indicated through the production of ROS and LPO simultaneously with the depletion of GSH. The activity of the enzyme caspase-3 which is taken as an indicator of apoptosis was also measured as appreciably high with time in treated cells and with Ni NPs dosage[24]. 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 [25]. 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 anatase structure induces necrosis and the rutile structure leads to the production of ROS which in turn initiates apoptosis [26]. Titanium di oxide particles have been found to be toxic to erythrocytes and when the latter is treated with nano TiO_2, it is found to undergo abnormal sedimentation. Haemagglutination was also seen along with dose dependent haemolysis. These changes were not seen when the cells were treated with micro TiO_2 [27].

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[28].

NANOTOXICITY RELATED TO BLOOD

The developments in nanotechnology have lead to the indiscriminate production of nanoparticles which accumulates in the air, water and soil to endanger human and animal health. Certain methods have been developed to assess the genotoxicity of such nanoparticles. For example, a study carried out to assess the toxicity of Aluminum dioxide particles of different sizes, *viz.,* 30nm and 40nm. The characterization was of these particles was carried out using modern analytical techniques like TEM, Doppler velocimetry and light scattering methods. The micronucleus test and comet assay suggests micronuclei and tail migration respectively in rat peripheral blood cells and exposure to the given nanoparticles at varying concentrations. Genotoxic assay was done in both the male as well as female mice. The micronuclear test revealed a dose dependent increase in the frequency of micronuclei, Similarly, the tail DNA showed an increase with increase in the dosage. The plasma mass spectrometry studies showed that the penetration of the nanoparticles into the different organs depended on the size of nanoparticles as the urine and feces samples where analyzed. It was concluded that the genotoxicity*in-vivo* was dose and size dependent [16].

EFFECTS ON EMBRYONIC BLOOD CELLS

Nanotoxicology correlates the risks of nanoparticle exposure to the organisms. The focus and concern needs to be is directed towards embryonic blood vessels as they are more vulnerable as compared to the mature blood vessels. For this reason the study of nanoparticles needs to be carried out at *in-situ* level, as such properties are wholly dependent on the environmental factors. One such study has examined the dynamics of quantum dots and polystyrene nanospheres when introduced into the blood vessels of chicken embryos chorioallantoic membrane. Subsequently, fluorescence correlation spectroscopy is used to determine the concentrations and hydrodynamic radii of the injected nanoparticles[29].

Nanomaterials such as nanotubes, nanowires, fullerene derivatives and quantum dots are currently being used as new types of tools in life sciences and also in health technology. But the geno toxic effect of these have not been clearly established. A study on the cytotoxic effect of these materials on the male germ line *in vitro* shows no significant effect for the nanomaterials tested except for silver and molybdenum trioxide nanoparticles. Here too the toxicity was found to be very less [30].

Nano materials like carbon nanotubes also cause cancer related to lungs. Experiments performed on mice shows that CNT can aggravate the inflammation caused by bacterial lipopolysaccharide (LPS) in rats and lead to the development of pulmonary fibrosis [31].

Some studies also indicate that Multi Wall CNT (MWCNT) produces inflammation in the pre-existing allergic asthma because it can aggravate ovalbumin-induced allergic airway inflammation in mice. It has been proved in an experiment wherein injection of long MWCNT into the peritoneal cavity of mice, resulted in inflammation, suggesting MWCNT to have asbestos-like pathogenicity[32].

Impurities in the nanoparticles also affect the cytotoxicity of these particles this effect has been studied by Akhtar *et al* wherein silica nanoparticles with size 10 nm and 80 nm (amorphous silica) and 15 nm and 46 nm (silica nanoparticle). Due to impurities of trace metals 73.67% viability for 10 nm and 71.60% for 80 nm has been observed. They are least toxic to the human lung cell line because they induce less oxidative stress in the cell which is responsible for the cytotoxicity[33].

Nanoparticles are also engineered for special purposes like targeted drug delivery, imaging and diagnostic applications etc. and these may have toxic effects too as they tend to accumulate in the pulmonary arteries and exert adverse effects on pulmonary structure and function. The pulmonary applications and lung toxicity related to engineered nanoparticles has been reviewed [34].It has been shown that pulmonary nanoparticle exposure impairs dilation of systemic arterioles. It also affects other endothelium depended responses because nanoparticle exposure enhances microvascular oxidative stress by~60%, and also causes a fourfold increase in nitrosative stress. It decreases the NO production too. In combination with microvascular dysfunction, nanoparticle exposure reduces NO bioavailability [35].

Agglomeration of nanoparticles plays an important role in case of pulmonary toxicity. It has been reported that upon inhalation of 10-40nm calcined aluminum oxyhydroxides (AlOOH) for 4 weeks by rats, the particles tend to agglomerate and the resultant pulmonary inflammatory response is due to this agglomeration and not due to the primary particle size [36].

CONCLUSION

Nanotechnology may be useful in the development of a large number of tools for the advancement in the understanding of the various chemical, physical and biological phenomenon. However, its use needs to be scrutinized for deleterious effects thoroughly before applying them for the service of mankind.

ACKNOWLEDGEMENT

The authors acknowledge VIT University, Vellore, India for providing the facilities required for the preparation of this review.

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