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Review Article

The Behaviour and Toxicity of Nanoparticles

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ABSTRACT

Nanotechnology and the production of nano particles are growing exponentially, research into the toxicological impact and possible hazard of nano particles to human health and the environment is still in its infancy. For hazard assessment of nano particles quantitative nanoecotoxicological data are required. Current nanomaterial research is focused on the medical applications of nanotechnology, whereas side effects associated with nanotechnology use, especially the environmental impacts, are not taken into consideration during the engineering process.Nanomedical users and developers are faced with the challenge of balancing the medical and societal benefits and risks associated with nanotechnology.Current advances in nanotechnology have led to the development of the new field of nanomedicine, which includes many applications of nanomaterials and nanodevices for diagnostic and therapeutic purposes. The same unique physical and chemical properties that make nanomaterials so attractive may be associated with their potentially calamitous effects on cells and tissues.

Keywords: Nano particles, Nano technology, Nano devices, Nano medicine.

INTRODUCTION

Toxicology traditionally addresses adverse poisoning effects of chemicals to humans, animals and the environment. Nano toxicology has emerged only recently, years after the first boom of nanotechnology, when various nano materials had already been introduced into a number of industrial processes and products.Nano materials have the potential to revolutionize medicine because of their ability to affect organs and tissues at the molecular and cellular levels. Incorporating environmental concerns into nano material engineering and nano medicine development of nano materials have been promoted as a revolutionary technology for cell and tissue engineering, medical device development, and the encapsulation and delivery of drugs, diagnostics, and genes, important but it greatly increases decision complexity. The extraordinary sensitivity of the physical characteristics of nano particle complexes with bio-molecules has initiated a significant interest in the design of new sensors making use of a strong dependence of electron transfer andenergy transfer on donor-acceptor distances.

Reactive oxygen species

For some types of particles, the smaller they are, the greater their surface area to volume ratio and the higher their chemical reactivity and biological activity. The greater chemical reactivity of nanomaterials can result in increased production of reactive oxygen species (ROS), including free radicals[1]. ROS production has been found in a diverse range of nanomaterials including carbon fullerenes, carbon nanotubes and nanoparticle metal oxides. ROS and free radical production is one of the primary mechanisms of nanoparticle toxicity; it may result in oxidative stress, inflammation, and consequent damage to proteins, membranes and DNA.

Biodistribution

The extremely small size of nanomaterials also means that they much more readily gain entry into the human body than larger sized particles. How these nanoparticles behave inside the body is still a major question that needs to be resolved. The

behavior of nanoparticles is a function of their size, shape and surface reactivity with the surrounding tissue. In principle, a large number of particles could overload the body's phagocytes, cells that ingest and destroy foreign matter, thereby triggering stress reactions that lead to inflammation and weaken the body's defense against other pathogens. In addition to questions about what happens if non-degradable or slowly degradable nanoparticles accumulate in bodily organs, another concern is their potential interaction or interference with biological processes inside the body. Because of their large surface area, nanoparticles will, on exposure to tissue and fluids, immediately adsorb onto their surface some of the macromolecules they encounter. This may, for instance, affect the regulatory mechanisms of enzymes and other proteins.

Nanomaterials are able to cross biological membranes and access cells, tissues and organs that larger-sized particles normally cannot[2]. Nanomaterials can gain access to the blood stream via inhalation[3] or ingestion[4] At least some nanomaterials can penetrate the skin[5] even larger microparticles may penetrate skin when it is flexed[6]. Broken skin is an ineffective particle barrier sugges that acne, eczema, shaving wounds or severe sunburn may accelerate skin uptake of nanomaterials. Then, once in the blood stream, nanomaterials can be transported around the body and be taken up by organs and tissues, including the brain, heart, liver, kidneys, spleen, bone marrow and nervous system[7] .Nanomaterials have proved toxic to human tissue and cell cultures, resulting in increased oxidativestress, inflammatory cytokine production and cell death.Unlike larger particles, nanomaterials may be taken up by cell mitochondria and the cell nucleus[8] Studies demonstrate the potential for nanomaterials to cause DNA mutation[9] and induce major structural damage to mitochondria, even resulting in cell death[10,11].

NANOMATERIALS

Material having at least one dimension 100 nanometres or less, up to10,000 could fit across a human hair. Nano materials can be nano scale in one dimension (e.g. surface films), two dimensions (e.g. strands or fibres), or three dimensions (e.g. particles). They can exist in single, fused, aggregated or agglomerated forms with spherical, tubular, and irregular shspes.

Types of Nano Materials

i) Nano Gold

Suspension (or colloid) of sub-micrometre-sized particles of gold in a fluid — usually water

Usually either an intense red colour (for particles less than 100 nm), or a dirty yellowish colour (for larger particles).Properties and applications of colloidal gold nanoparticles depends upon shape. For example, rod like particles have both transverse and longitudinal absorption peak, and anisotropy of the shape affects their self assembly.

Uses of Nano Gold

1. Colloidal gold and various derivatives have long been among the most widely-used contrast agents for biological electron microscopy.
2. Colloidal gold particles can be attached to many traditional biological probes such as antibodies, lectins, superantigens, glycans, nucleic acids and receptors.
3. Particles of different sizes are easily distinguishable in electron micrographs, allowing simultaneous multiple-labelling experiments.

ii). Silver Nano Particles

1. Nano silver particles of between 1 nm and 100 nm in size.
2. While frequently described as being 'silver' some are composed of a large percentage of silver oxide due to their large ratio of surface to bulk silver atoms.
3. Many different synthetic routes to silver nano particles. They can be divided into three broad categories: physical vapour deposition, ion implantation, or wet chemistry.

Uses of Silver Nano Particles

1. Over the last decades silver nano particles have found applications in catalysis, optics, electronics and other areas due to their unique size-dependent optical, electrical and magnetic properties.
2. Currently most of the applications of silver nano particles are in antibacterial/antifungal agents in biotechnology and bioengineering, textile engineering, water treatment, and silver-based consumer products.
3. There is also an effort to incorporate silver nano particles into a wide range of medical devices, including but not limited to
    - bone cement
    - surgical instruments

- surgical masks
- wound dressings
- treatment of HIV-1

iii) Fullerence Nanomaterial

1. Fullerenes are a class of allotropes of carbon which conceptually are graph sheets rolled into tubes or spheres. These include the carbon nano tubes (or silicon nano tubes) which are of interest both because of their mechanical strength and electrical properties.
2. Fullerenes were under study for potential medicinal use: binding specific antibiotics to the structure of resistant bacteria and even target certain types of cancer cells such as melanoma.

iv) Carbon Nano Tubes

1. Carbon nano tubes are molecular-scale tubes of graphitic carbon with outstanding properties. They are among the stiffest and strongest fibres known, and have remarkable electronic properties and many other unique characteristics. Commercial applications have been rather slow to develop, however, primarily because of the high production costs of the best quality nano tubes.
2. These cylindrical carbon molecules have novel properties that make them potentially useful in many applications in nanotechnology, electronics, optics and other fields of materials science, as well as potential uses in architectural fields.
3. Nano carbon tubes exhibit extraordinary strength and unique electrical properties, and are efficient thermal conductors.

TOXICITY OF NANO PARTICLES

This schema describes the major pathways involved in interactions of nano particles (unrefined SWCNTs) with macrophages. These interactions include oxidative burst due to activation of NADPH oxidase, catalytic reactions of transition metals with oxygen radicals, and possible interactions of nano particles with microbial pathogens. NADPH oxidase complex is activated in macrophages during inflammation and acts as the major source for generation of reactive oxygen species, such as superoxide O2–d radicals that disproportionate to form hydrogen peroxide (H2O2). Transition metals, through their interactions with O2 –d and H2O2, act as catalysts for the formation of highly reactive hydroxyl (OH.) radicals. Oxidatively modified lipids generated by cyclooxygenase (COX-2) and lipooxygenase (LOX) participate in amplification of the inflammatory response via recruitment of new inflammatory cells.

Nano medicine: Nanotechnology, Biology, and Medicine 1 (2005) 313–316production and release of proinflammatory (tumor necrosis factor-a, interleukin-1h) to anti-inflammatory profibrogenic cytokines (transforming growth factor-h, interleukin-10).

The inflammatory and fibrogenic responses were accompanied by a detrimental decline in pulmonary function and enhanced susceptibility to infection (Listeria monocytogenes) [12].Transition metals and oxidative stress induced by nano particles.

Pulmonary Toxicity of Nanoparticles

Although several laboratories have reported potential toxic effects of nanoparticles on different types of cells invitro [12-18], there are only a few publications on in vivo toxicity of nanomaterials [19,20]Our recent study demonstrated that aspiration of SWCNTs elicited an unusual inflammatory response in the lungs of exposed mice [21]. The initial acute inflammatory reaction is probably triggered by damage to pulmonary epithelial type I cells. The response includes a robust neutrophilic pneumonia followed by recruitment and activation of macrophages. The unusual feature of the response is a very early switch from the acute phase of the response to fibrogenic events resulting in significant pulmonary deposition of collagen and elastin.n This is accompanied by a characteristic change in the lung, the initial target for cytotoxicity by SWCNTs is probably type I epithelial cells whose necrotic death stimulates a proinflammatory response and recruitment of inflammatory cells. This schema describes the major pathways involved in interactions of nanoparticles (unrefined SWCNTs) with macrophages. These interactions include oxidative burst due to activation of NADPH oxidase, catalytic reactions of transition metals with oxygen radicals, and possible interactions of nanoparticles with microbial pathogens. NADPH oxidase complex is activated in macrophages during inflammation and acts as the major source for generation of reactive oxygen species, such as superoxide O2–d radicals that disproportionate to form hydrogen peroxide (H_2O_2). Transition metals, through their interactions with O_2 and H_2O_2.

ASSESSMENT OF NANO TOXICITY

a. Nano particle characterisation—size, shape, charge

1. In order to measure toxicological endpoints, the starting point, here the nano material, needs to be fully understood and characterised[22]. Otherwise, possible toxic

effects cannot be easily attributed to a certain property of the nano material or even the nano material itself because, for example, impurities and other components could be held responsible [23]. Therefore it is absolutely critical to know the starting material and its properties. In the past this has not always been straightforward for industrially produced nano particles due to crude production processes and therefore huge variations in material properties e.g. size, shape, etc. [24]. The more nanotechnology advances, more refined manufacturing processes are developed leading to nano materials with uniform and consistent properties. Nano = Ultrafine = < 100 nm (Conventional)

a. Nano = <10 nm (suggested by unique quantum and surface-specific functions)
b. Fine = 100 nm - 3 μm
c. Respirable (rat) = < 3 μm (max = 5 μm)
d. Respirable (human) = < 5 μm (max = 10 μm)
e. Inhalable (human) = ~ 10 - 50 μm

2. These differences seem to be a result of their size. Nano particles have much larger surface area to unit mass ratios which in some cases may lead to greater pro-inflammatory effects (in, for example, lung tissue). In addition
3. Nano particles seem to be able to trans locate from their site of deposition to distant sites such as the blood and the brain.
4. Resulted in a sea-change in how particle toxicology is viewed- instead of being confined to the lungs, nano particle toxicologists study the brain, blood, liver, skin and gut. Nano toxicology has revolutionised particle toxicology.

b. Nano particle "dose" and dose metric

As outlined above, dose is one of the key parameters in toxicology. In nano toxicology it is important to evaluate relevant and realistic dose regimes in order to draw meaningful conclusions from in-vitro and in-vivo experiments for public health risk assessment. This means that nano toxicologists should test nano particle toxicity based on real-world doses rather than unrealistically high doses in order to achieve a biological response. The latter [25] using instillation can be useful in elucidating mechanisms but is unlikely to be predictive of human pathology from environmental exposure. On the other hand, low dose inhalation experiments [26] are likely to be much more predictive of human harm.

C. Nano particles and the environment

Nano particles released into the environment interact with air, water and soil. This often changes the surface properties of the particles which can result in particle aggregation or changes in particle charge and other surface properties [27]. These effects have been studied in water ecosystems and soil [28,29] and show the importance of understanding nanoparticles and their environmental setting as a "complex" that needs to be looked at in its entirety in order to understand particle behaviour in the environment [30]. A current debate addresses whether nano particles can cause toxicity as a contaminant in, for example, soil or water, via "piggyback" mechanism on natural organic matter[31] .

***I n vivo* toxicity**

Recapitulating known mechanisms of nano particle toxicity at the cellular level, allows predictions regarding what damage nano particles could cause in certain parts of the body. Protein mis-folding and protein fibrillation[32] induced by nano particles could cause major problems in the brain [33], however studies so far on the issue are based on in-vitro experiments and in-vivo confirmation needs to be performed.

NANO TOXICOLOGICAL RESEARCH

Currently, assessing the safety of synthetic nano particles has become a worldwide issue. The eco-toxicological research on nano particles is also supported and promoted by EC science policy. On the 7th June 2005, the Action Plan "Nano sciences and nanotechnologies: An Action Plan for Europe 2005–2009" was adopted (European Commission,2004) for the "immediate implementation of a safe, integrated and responsible strategy for nano sciences and nano technologies of a safe, integrated and responsible strategy for nano-sciences and nanotechnologies".

From eco-toxicology to nano eco-toxicology

Eco-toxicology is a relatively new science concerned with contaminants in the biosphere and their effects on constituents of the biosphere, including humans [34]. The term eco-toxicology was defined it as "the branch of toxicology concerned with the study of toxic effects, caused by natural or synthetic pollutants, to the constituents of ecosystems, animal (including human), vegetable and microbial, in an integral context" [35]. Eco-toxicological research was rapidly developing due to the pollution of the environment induced by the rapid industrial

development. Eco-toxicological tests were mostly developed for aquatic test organisms and water-soluble chemical compounds. Thus, aquatic toxicity testing of nano particles is definitely a challenge. However, whatever the apparent route of exposure and the mechanisms of toxicity, bioavailability remains a key factor for the hazard evaluation of synthetic nano particles.

Bioavailability is a dynamic concept that considers physical, chemical, and biological processes of contaminant exposure and dose. Bioavailability incorporates concepts of environmental chemistry and eco-toxicology, integrating contaminant concentration, fate, and an organism's behavior in the given environment. Bioavailability of nano particles depends on the: (i) physicochemical properties of the particles (aggregation, solubility), (ii) on nano particle-organism contact environment, but also (iii) on the target organism (particle-ingesting or not). Thus, environmental risk assessment of synthetic nano particles requires thorough characterization of nano particles before, during and after exposure. Many methods still need development and optimization, especially for new types of nano particles, but extensive experience can be gained from environmental chemistry.

CHALLENGES IN NANO-ECO-TOXICOLOGICAL RESEARCH

The "Nature Nanotechnology" section "News and Views" indicates three main problems that should be solved within the next few years: (i) the choice of nano particles to use in biological experiments, and the tests (analysis of physico-chemical properties, aggregation, sedimentation, etc.) needed to characterize them before, during and after these experiments, need to be determined; (ii) the need to examine the route of uptake of synthetic NPs by organisms in different environments (important for the behavior of synthetic NPs in the food-chain); (iii) the choice of organisms and endpoints measured. The above mentioned challenges and what has been already done to solve these problems will be discussed below.

a) Representative manufactured nano materials for testing

In 2005, the International Life Sciences Institute Research Foundation/Risk Science Institute convened an expert working group to develop a screening strategy for the hazard identification of engineered nano materials. The expert groups outlined three key elements of the toxicity screening strategy: physicochemical characteristics, in vitro assays (cellular and non-cellular), and in vivo assays. This list includes 8 inorganic nano materials: silver NPs, iron nano particles, titanium dioxide, aluminium oxide, cerium oxide, zinc oxide, silicon-dioxide, nano clays and 6 organic nano materials: carbon black, fullerenes (C60), single-walled carbon nano tubes(SWCNTs), multi walled carbon nano tubes (MWCNTs), polystyrene, dendrimers.

b) Bio-availability of synthetic NPs in different environment

Eco-toxicological tests were mostly developed for aquatic test organisms and water-soluble chemical compounds. Thus, aquatic toxicity testing of NPs is definitely a challenge. Bioavailability of NPs depends on the: (i) physicochemical properties of the particles (aggregation, solubility), (ii) on nano particle-organism contact environment, but also (iii) on the target organism (particle-ingesting or not). Thus, environmental risk assessment of synthetic NPs requires thorough characterization of NPs before, during and after exposure. Many methods still need development and optimization, especially for new types of NPs, but extensive experience can be gained from environmental chemistry.

c) Physico-chemical properties determining the bioavailability and toxicity of NPs

It is well known that at nano size range, the properties of materials differ substantially from bulk materials of the same composition, mostly due to the increased specific surface area and reactivity, which may lead to increased bioavailability and toxicity.TiO_2 and Al_2O_3 NPs were about twice more toxic than their respective bulk formulations towards nematodes. However, none of the above mentioned authors did observe significant differences in toxicity of nano andbulk ZnO and that will be discussed below. Silver nano particles, their antibacterial effect has been shown to depend not only on size but also on shape .

d) Modulation of bioavailability by environmental factors

As discussed above, the environmental fate of synthetic NPs and thus the bioavailability of the toxic "component" will also depend on the interactions of synthetic NPs with aquatic colloids
that may strongly influence their behaviour in surface waters.

Human health and safety risks associated with nanotechnology

Carbon nanotubes – characterized by their microscopic size and incredible tensile strength – are

frequently likened to asbestos, due to their needle-like fiber shape. In a recent study that introduced carbon nanotubes into the abdominal cavity of mice, results demonstrated that long thin carbon nanotubes showed the same effects as long thin asbestos fibers, raising concerns exposure to carbon nanotubes may lead to pleural abnormalities such as mesothelioma (cancer of the lining of the lungs caused by exposure to asbestos)[36].Given these risks, effective and rigorous regulation has been called for to determine if, and under what circumstances, carbon nanotubes are manufactured, as well as ensuring their safe handling and disposal[37].

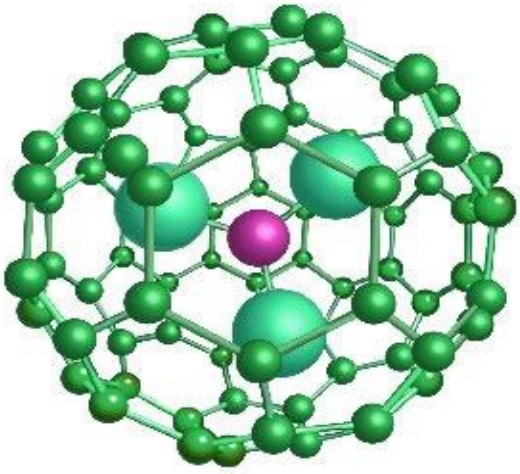

Fig. 1: Nano Materials

Fig. 2: Nano Gold

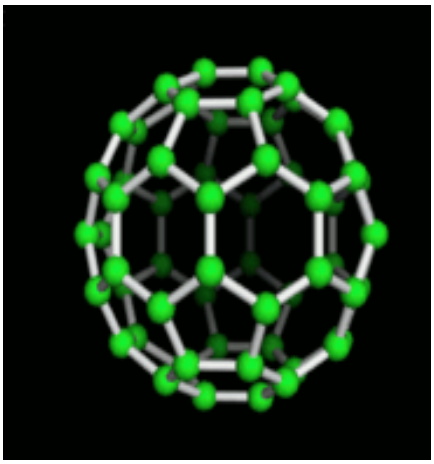

Fig. 3: Fullerence Nanomaterials

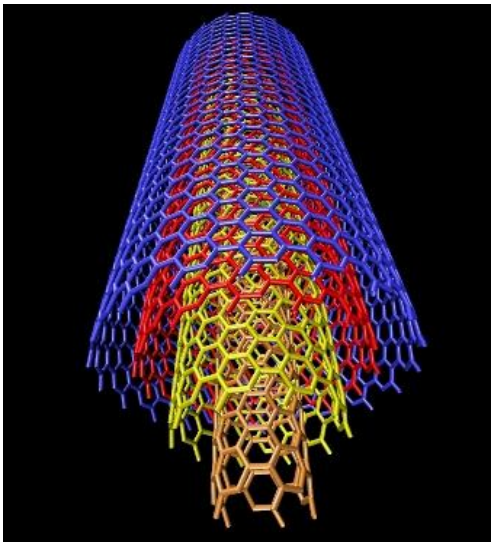

Fig. 4: Carbon Nanotubes

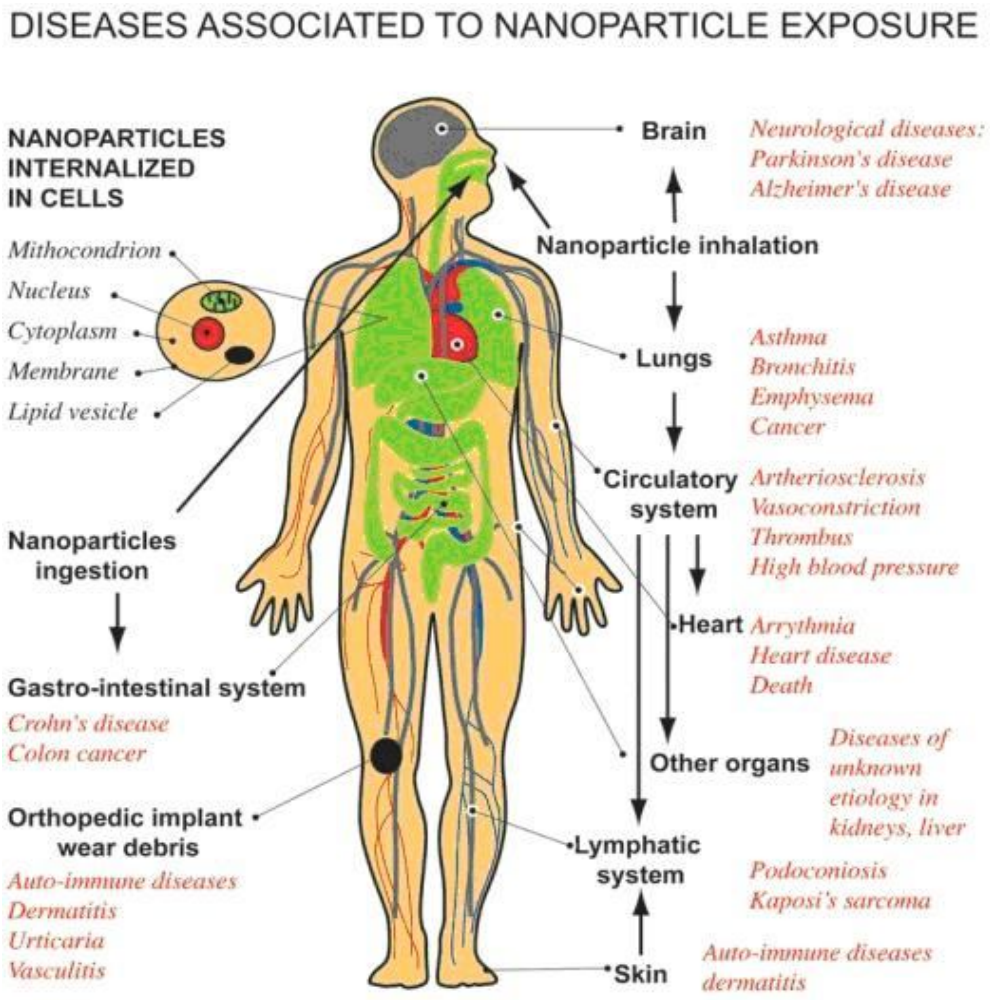

Fig. 5: Disease Associated to Nano Particles Exposure

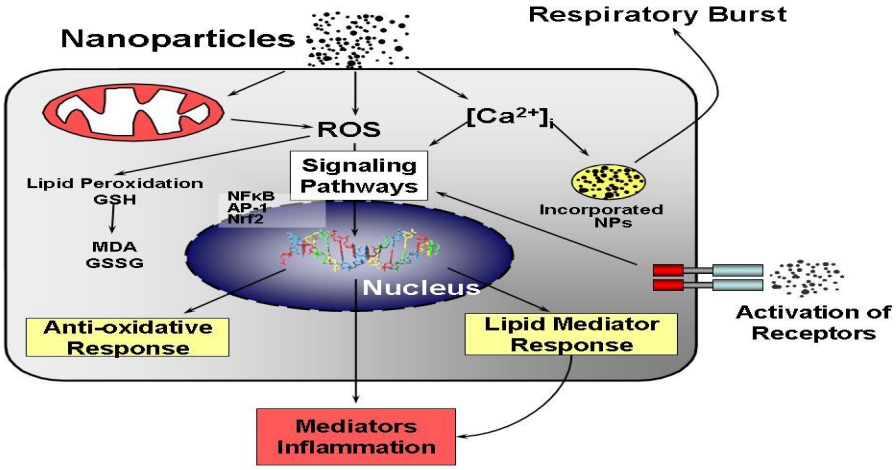

Fig. 6: Nanoparticles Effect

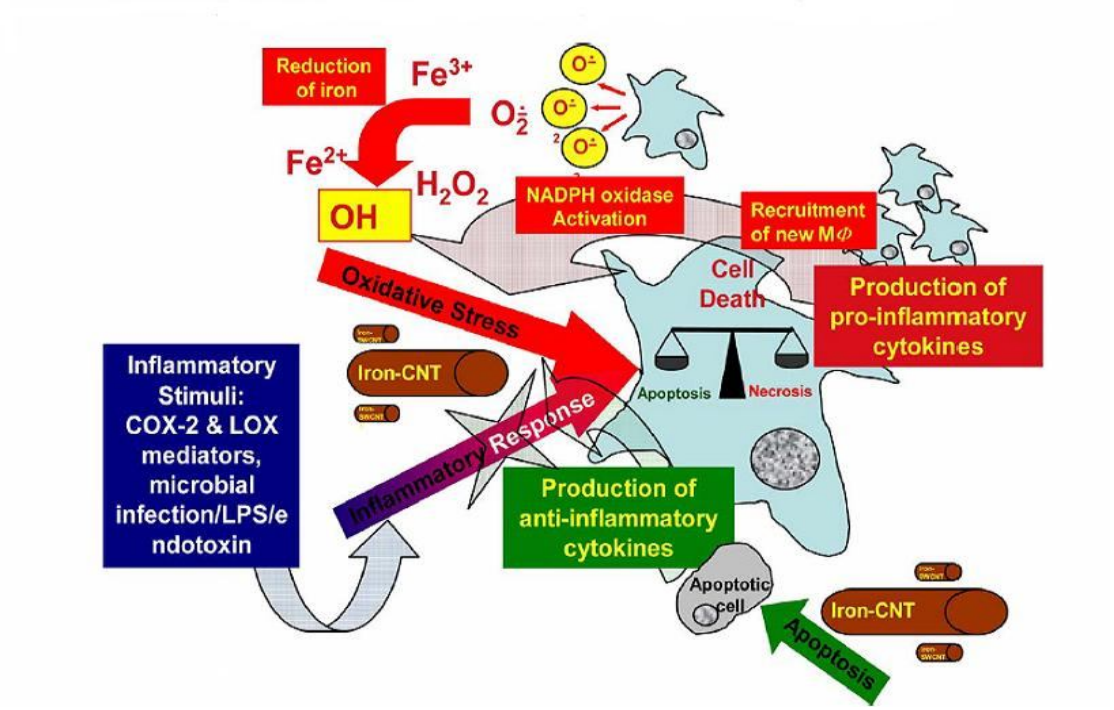

Fig. 7: Nanomedicine, Nanotechnology, Biology and Medicine

CONCLUSIONS

Nano materials are widely used in research, industry, and medicine. The same unique physical and chemical properties that make nano materials so attractive may be associated with their potentially calamitous effects on cells and tissues. Our results indicate that inhalation of SWCNTs elicits robust and unusual inflammatory response in the lungs of exposed mice. These findings call for further toxicological studies and assessment of risk associated with the manufacturing and use of nano particles. Nanotoxicology is a sub-specialty of particle toxicology. It addresses the toxicology of nanoparticles (particles <100 nm diameter) which appear to have toxicity effects that are unusual and not see with larger. Nanoparticles can be divided into combustion-derived nanoparticles (like diesel soot), manufactured nanoparticles like carbon nanotubes and naturally occurring nanoparticles from volcanic eruptions, atmospheric chemistry etc. Quantitative data on toxicological effects of NPs are still scarce even at the single organism ievel.The most sensitive test organisms towards NPs werw aigae and crust aceans revealing the vulnerability of these organism groups in the acquatic food chain.The later was true not only for the seven different types of nanoparticles but also for the seven different chemicals.the currently existing quantitative nano ecotoxicological data on single model organisms would classify nanoparticles from" to"extremely toxic "harmful".

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