International Journal of Current Biotechnology
ISSN: 2321 - 8371
Journal Homepage : http://ijcb.mainspringer.com

Molecular Mechanism of Nanotoxicity - A Critical Review

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ARTICLE INFO

Article History:
Received 04 May 2018
Received in revised form 14 May 2018
Accepted 28 May 2018
Available online 30 May 2018

Key words:
Nanoparticles, Nano-biointerphase, Nanotoxicity, ROS, NF-κB, IKK, Inflammation, DNA, RHD, TNF-α.

ABSTRACT

In the past decades, nanoparticles have been widely used in industry and pharmaceutical fields for drug delivery, anti pathogen and diagnostic imaging purposes because of their unique physicochemical characteristics such as special ultrastructure, dispersity and effective cellular uptake properties. Hence, bio-nanotechnology has been receiving immense attention in recent years but the nanotoxicity has been raised over the extensive application of nanoparticles and despite the extensive use of nanoparticles today there is still a limited understanding of nanoparticle mediated toxicity. So, there is a dire need for a full understanding of the mechanisms by which nanoparticles bioaccumulate, intrude the skin barrier as well as interact with biomacromolecules such as protein, lipid and DNA. Literature review over the past decade reveals very scanty, sporadic and confusing molecular level descriptions of such mechanism. In this review, an attempt has been made through an exhaustive study to explore the possible molecular and cellular events responsible for nanotoxicity.

Introduction

Growing exploration of nanotechnology has resulted in the discovery of many distinctive properties of nanomaterials such as superior catalytic, optical, magnetic, mechanical and electrical properties when compared to conventional formulations of the same material (Viswanath and Kim, 2016). Inorganic ceramic nanoparticles such as silica, titania and alumina are also commonly being used for drug administration for cancer therapy due to their porous nature, although their applications are limited due to their non-biodegradable nature. On the other hand, metallic nanoparticles, including super paramagnetic iron oxide nanoparticles (Mahmoudi *et al.*, 2011), gold shell nanoparticles and titanium dioxide (TiO_2) nanoparticles, are routinely used for magnetic resonance imaging contrast enhancement and as cancer drug carrier systems (Peng *et al.,* 2015), whereas silver nanoparticles (AgNP) are being explored as antibacterial agents for treatment of infectious diseases (Franci *et al.,* 2015), due to their ability to stabilize nanoparticles and favourable optical/chemical properties. Notably, carbon nanoparticles, which are comprised of fullerenes and nanotubes, are the most widely used materials for drug delivery purposes due to the fact that fullerenes contain multiple attachment points responsible for tissue binding (Bosi *et al.,* 2003), and nanotubes offer high electrical conductivity and strength. Polymers such as polysaccharide chitosan nanoparticles (CS-NPs) function in drug delivery due to their ability to facilitate both protein and drug conjugation (Agnihotri *et al.*, 2004). Many of these applications involve new materials which provide radically different properties through functioning at the nanoscale, where new phenomena are associated with the very large surface area to volume ratios experienced at these dimensions and with quantum effects that are not seen with larger sizes. However, just as phenomena taking place at the nanoscale may be quite different to those occurring at larger dimensions and may be exploitable for the benefit of mankind. So, these newly identified processes and their products may expose the same humans and the environment in general, to new health risks, possibly involving quite different mechanisms of interference with the physiology of human and environmental species. Humans may be exposed to nanomaterials through inhalation (respiratory tract), skin contact, ingestion, and injection (Oberdo *et al.,* 2005). The tiny size of nanomaterials allows them to pass more easily through cell membranes and other biological barriers, therefore, nanomaterials can be easily taken up into living organisms and cause cellular dysfunction (Nel *et al.,* 2006). The exposure to nanoparticles having

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characteristics not previously encountered may well challenge the normal defence mechanisms associated with, for example, immune and inflammatory systems. Molecular mechanisms underlying nanotoxicity includes (a) Oxidative stress and DNA damage and (b) Inflammation-mediated nanotoxicity. A variety of nanomaterials can generate reactive oxygen species (ROS) under certain experimental conditions. Among various toxic responses, nanomaterial induced oxidative stress mediated by ROS has been studied most extensively. Studies specifically dealing with the toxicity of nanoparticles have only appeared recently and, although now emerging in the literature, are still rare. Data concerning the behaviour and mechanism of toxicity of nanoparticles mainly comes from study entitled "Mechanism of nanotoxicity-Generation of Reactive Oxygen Species" (Fu *et al.,* 2014) discusses the mechanisms and decisive determinants of the generation of ROS by nanomaterials.

Toxicity of nanomaterials

Nanotoxicology is an emerging discipline attempting to characterize and categorize the health effects caused by engineered nanomaterials in order to determine structure-function relationships between nanoparticles and toxicity in order to formulate a set of design rules for the design of safe nanomaterials. The range of nanotechnology products is wide and they can be classified into several different compound categories, including metals, metal oxides, carbon, silica, ceramic and semiconductor nanomaterials. The increased prevalence of these materials in consumer goods has brought nanotoxicology to the forefront and has called attention to the gap in toxicological information regarding these materials. The accumulation of nanoparticles in various organs and adverse side effects has hindered their use in the field of nanomedicine. So, the mechanisms underlying the toxicity of nanomaterials may provide clues for circumventing the toxicological effects of nanoparticles which has never been studied intensively so far. An important mechanism of nanotoxicity is the generation of reactive oxygen species (ROS), resulting in the subsequent formation of oxidative stress in tissues. Interestingly, oxidative stress also results in the release of pro-inflammatory mediators through the principal cascades such as the NF-κB (Nuclear Factor-κB), mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3-K) pathways (Poljak *et al.,* 2010, Li *et al.,* 2010), suggesting that oxidative stress is linked to inflammation reciprocally. The toxicity of nanomaterials has been studied in different biological systems, both in cell line systems and different organisms, which include rodents, humans, and aquatic species, such as zebrafish (Ozel *et al.,* 2013), catfish (Wang Y *et al.,* 2011), algae (Wang Z *et al.,* 2011), and macrophages (Sohaebuddin *et al.,* 2010). Carbon and metallic nanomaterials are among the most widely used types of engineered nanomaterials. Engineered carbon nanoparticles and nanotubes were found to induce the aggregation of platelets *in vitro*, and thus enhance vascular thrombosis in rat carotid artery (Radomski *et al.,* 2005). Furthermore, the effect of single walled carbon nanotubules (SWCNTs) was studied in cellular models of human kidney and bronchi, where they were observed to induce cell apoptosis and decrease cell adhesion via either upregulating genes involved in cell death or downregulating genes associated with cell proliferation and survival (Alazzam *et al.,* 2010). The skin can be exposed to solid nanoparticles through the application of lotions or creams that contain nano-TiO_2 or nano-ZnO as a sunscreen component or fibrous materials coated with nanoscale substances for water or stain repellent properties. The accumulation of TiO_2, Al_2O_3, ZnO nanoparticles in the brain can cause auxiliary toxicity, disrupting normal metabolism of neurotransmitters and ultimately leading to brain damage (Poli *et al.,* 2004). While comparing the toxicity of three nano-metal oxides, nano-CuO, nano-CdO, and nano-TiO_2, nano-CuO was determined to be the most potent in cytotoxicity and DNA damage, leading to 8-hydroxy-20-deoxysuanosine (8-OHdG) formation, while nano-TiO2 was the least, without inducing a significant level of 8-OHdG (Zhu et al., 2013).

Molecular mechanisms underlying nanotoxicity

Overproduction of ROS and cell damage

While studying the ROS mediated development of oxidative stress at first we have to know why mitochondria is the main domain for the overproduction of ROS as well as the thermodynamics of ROS formation and biochemical characteristics of the mitochondrial electron transport chain. Although oxygen [O_2 (g)] is a very strong oxidant, there are some restrictions in its chemistry that are strongly relevant for the production of reactive oxygen species like $^{o}O_2^-$ etc. Since diatomic oxygen in its triplet ground state has parallel spins, due to Pauli principle, two electron reductions of oxygen are only possible if both the added electrons have same spin (Beckman and Ames, 1998). This is usually not the case in free solution, and anti-ferromagnetic coupling with metal ions is required to allow this to happen. One electron reductions of oxygen on the other hand are possible without violating any spin conservation rules. Hence, only one electron transfer reaction in the electron transport chain can potentially be short circuited by molecular oxygen, provided that it is accessible. This criteriation explains why mitochondrial electron transport chain produces ROS while the other redox chain reactions, such as glycolysis and Krebs cycle which are occurring at cytoplasm do not (Muller, 2000).

Figure 1 shows the orbital electronic configuration of various ROS including singlet oxygen, superoxide anion radical and peroxide anion. The bond order (BO) of ground state oxygen (O_2)=2, super oxide anion ($^{o}O_2^-$)=1.5 and that of peroxide ion ($^{o}O_2^{2-}$) is 1, where, BO=0.5(N_b-N_a); N_b is the number of bonding orbital and N_a is the number of anti-bonding orbital. Since, the bond order reduces as we go from molecular oxygen to superoxide and finally peroxide, the bond energy decreases which in turn results in lower stability and higher reactivity. This is why ROS are highly reactive and immediately removes electrons from any molecule in its path, turning that molecule into a free radical and thus propagating chain reaction. However, peroxide is the most reactive among all the ROS which is damaging the DNA and can travel into the nucleus of the cell and oxidizes cys-62 of p50 unit present within NF-kB. As a result, it loses its DNA binding power followed by the inhibition of transcription of pro-inflammatory genes. This hinders the immune response which gives rise to inflammation and ultimate cell death (Evans *et al.,* 2004).

In the mitochondria of cells, ATP is synthesized by the reduction of molecular oxygen(O_2) to water(H_2O) through a sequence of coupled proton and electron transfer reaction.

Step1: The reduction of molecular oxygen (O_2) produces super oxide ($^{o}O_2^-$) and this is the precursor of most other reactive oxygen species as illustrated by reaction 1.

Figure - 1: Orbital electronic configuration of various reactive oxygen species.

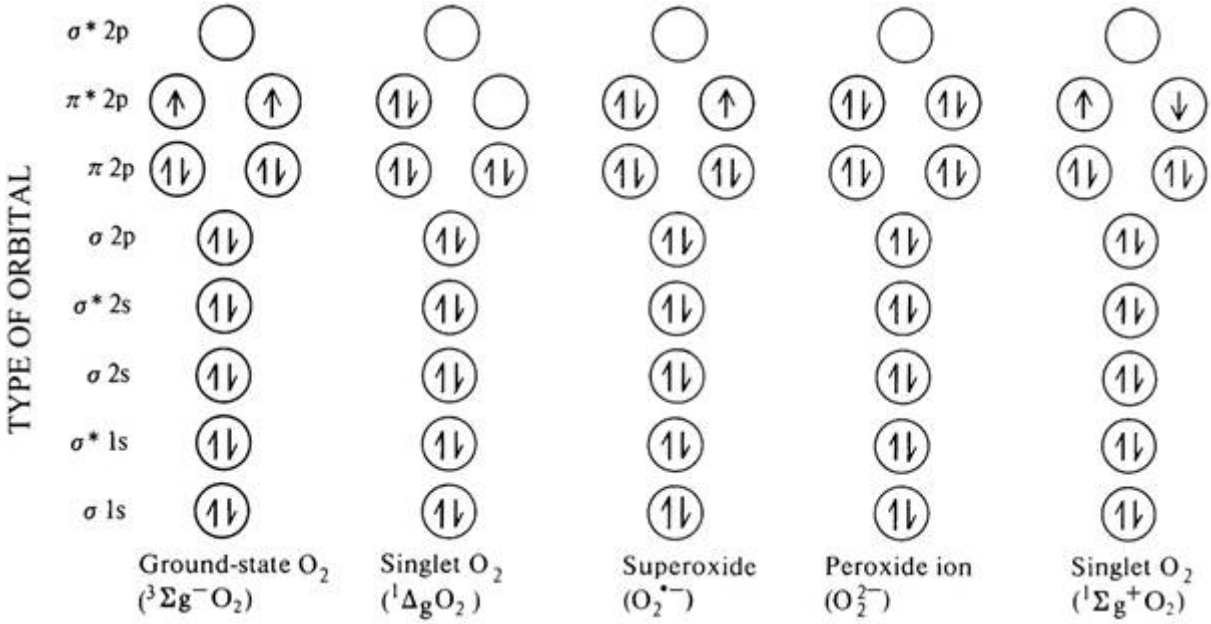


Figure – 2a: Role of reactive oxygen species in the oxidation of protein backbone.

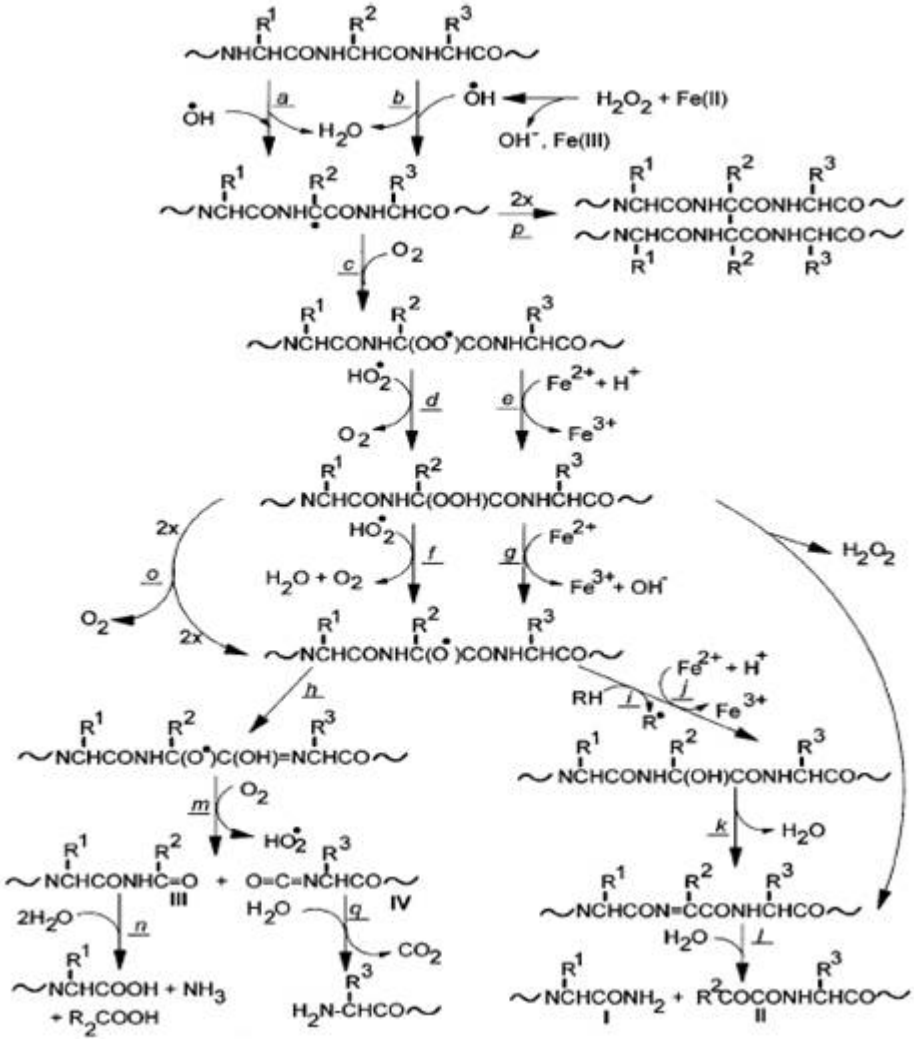


Figure – 2b: Role of reactive oxygen species in the oxidation of protein backbone (Simplified view).

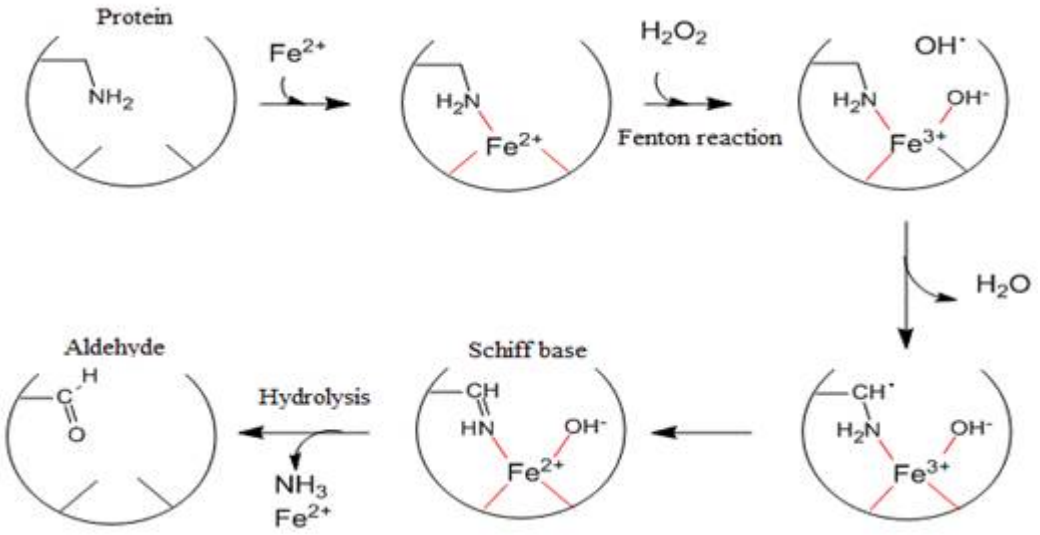


Figure – 3: Sequence of reactions involved in lipid peroxidation and deactivation of G6PDH.

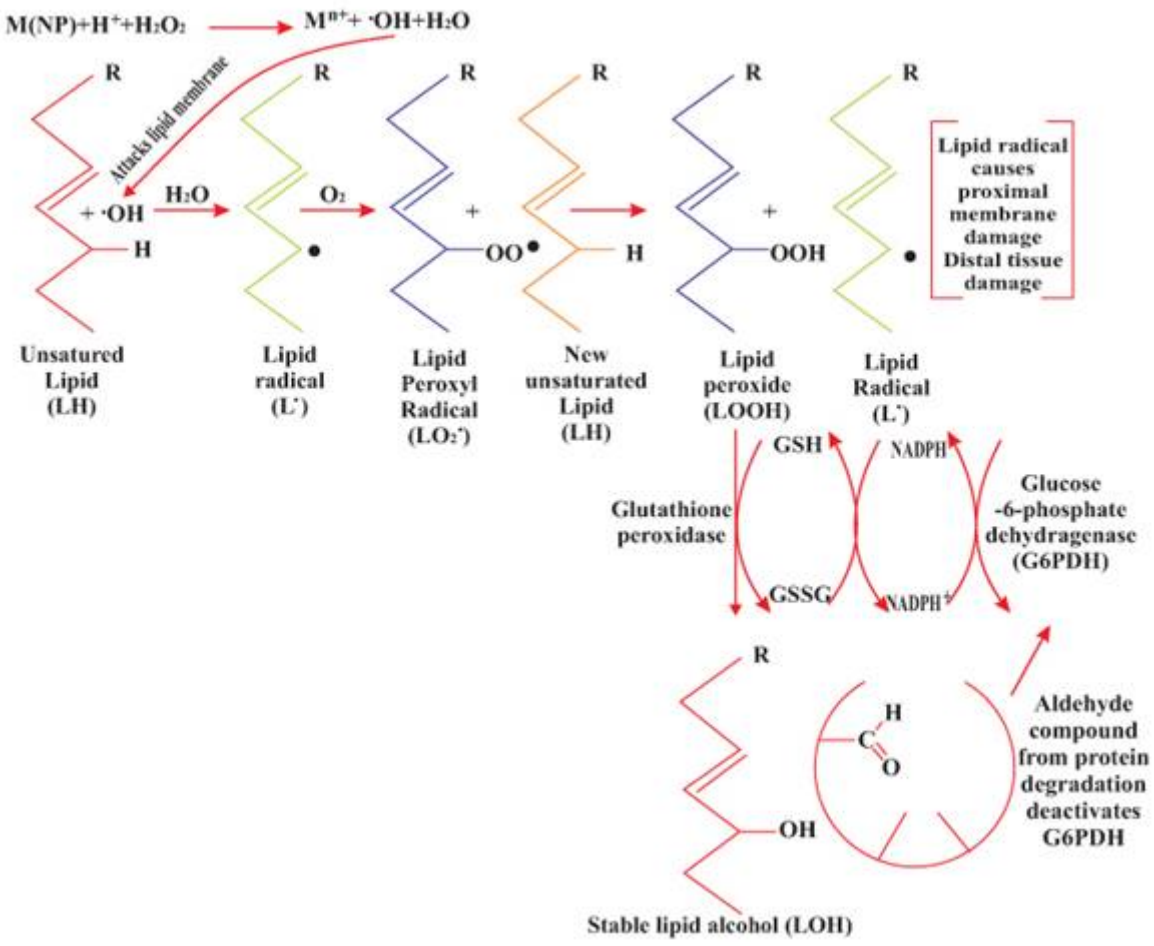


Figure – 4: Antioxidant activity of vit E (Tocopherol) and overconsumption leads to its deficiency.

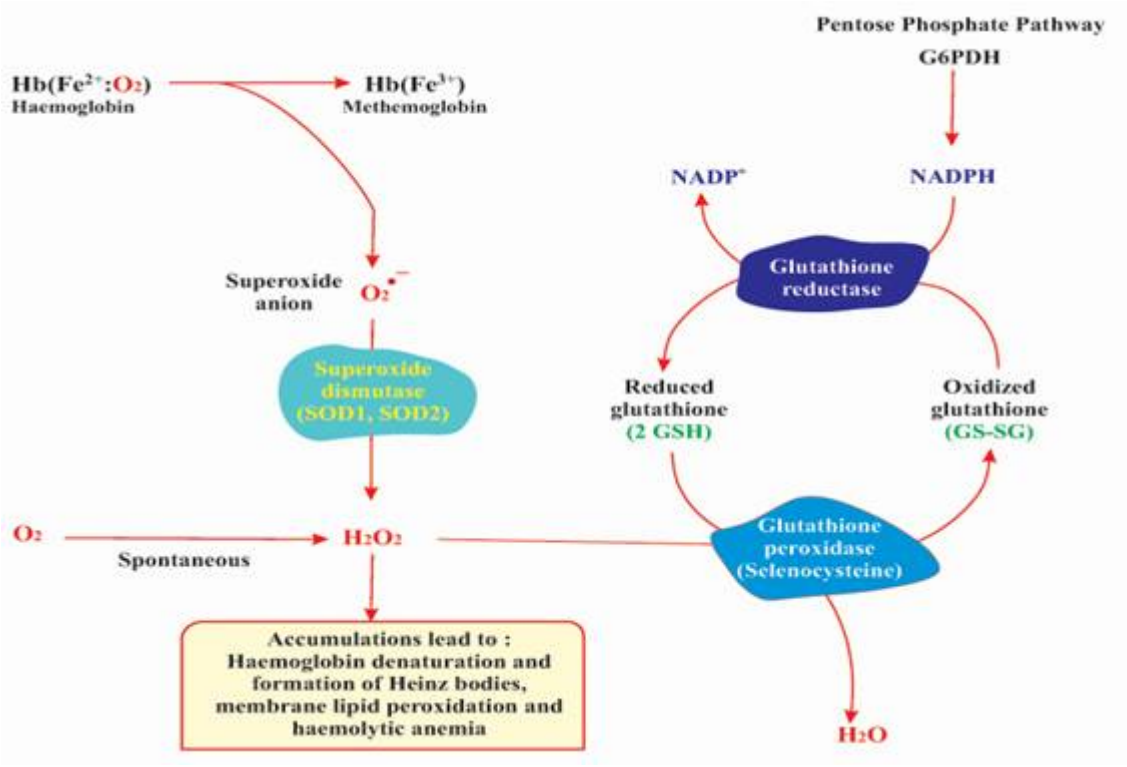
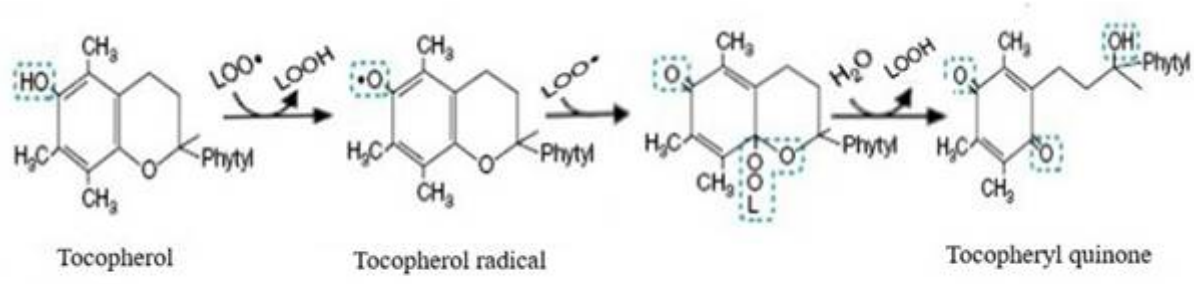


Figure - 5: Pathway for reactive oxygen species removal in erythrocytes.



$O_2 + e^- \rightarrow {}^{o}O_2^-$ (1)

Step2: Reaction 2 shows dismutation of superoxide that produces hydrogen peroxide (H_2O_2).

$2H^+ + {}^{o}O_2^- + {}^{o}O_2^- \rightarrow H_2O_2$ (2)

Step3: This hydrogen peroxide in turn may be partially reduced to hydroxyl radical (^{o}OH) (Reaction 3) or fully reduced to water (H_2O) (Reaction 4).

$H_2O_2 + e^- \rightarrow OH^- + {}^{o}OH$ (3)

$H_2O_2 + 2e^- + 2H^+ \rightarrow 2H_2O$ (4)

During this process, presence of small percentage of unreduced oxygen may result in the formation of $^{o}O_2^-$, ^{o}OH, H_2O_2 and $^1O_2$. Superoxide is a poor oxidant and has a low reactivity towards most biological molecules. Many deleterious effects of superoxide are due to the conversion of superoxide to a more reactive radical, particularly the hydroxyl radical. The hydroxyl radical possesses the highest one-electron reduction potential of all the physiologically relevant ROS, and is extremely reactive with almost every type of bio-molecule, including proteins and nucleic acids (Lubec, 1996). There is no known enzymatic reaction that can scavenge the hydroxyl radical in vivo. The only known defence against hydroxyl radicals comes from antioxidants. Antioxidants are essentially reducing agents; they participate in redox reactions by donating electrons or hydrogen atoms. This action helps cell to function normally and avoid the consequence of oxidation of structural and other vital components. ROS are the by-products of cellular oxidative metabolism, much of which occurs in mitochondria as described earlier. Oxidative stress occurs when the generation of ROS exceeds the capacity of the cellular antioxidant defence system. Owing to the quantum size effect, nanomaterials possess unique physiological and chemical properties that are different from those in either macroscopic (bulk) or atomic form; these unique properties may lead to the nanomaterial induced toxicity. A variety of metal nanoparticles (NPs) have been reported to exhibit intrinsic activity in generating ROS including Ag, Au, Pt, Cu, Fe, Co and Ni (He *et al.,* 2014). Ag (Carlson *et al.,* 2008), Au (Oo *et al.,* 2012), Cu (Shi *et al.,* 2012), Fe (Xu *et al.,* 2009), Ni (Ahmed *et al.,* 2013) and Co NPs have been reported to their ability to induce the generation of ROS under certain experimental conditions. Au NPs generate ROS including $^{o}O_2^-$, ^{o}OH and $^1O_2$, whereas Ag NPs enable the production of $^{o}O_2^-$ and ^{o}OH (Vankayala *et al.,* 2013). Cu NPs generates ^{o}OH and $^1O_2$ in presence of hydrogen peroxide and phosphate-buffered saline respectively (Jose *et al.,* 2011). ROS production occurs mainly through two mechanisms:

Figure – 6: Inhibition to DNA binding of proinflammatory genes in NF-kB pathway due to overproduction of ROS.

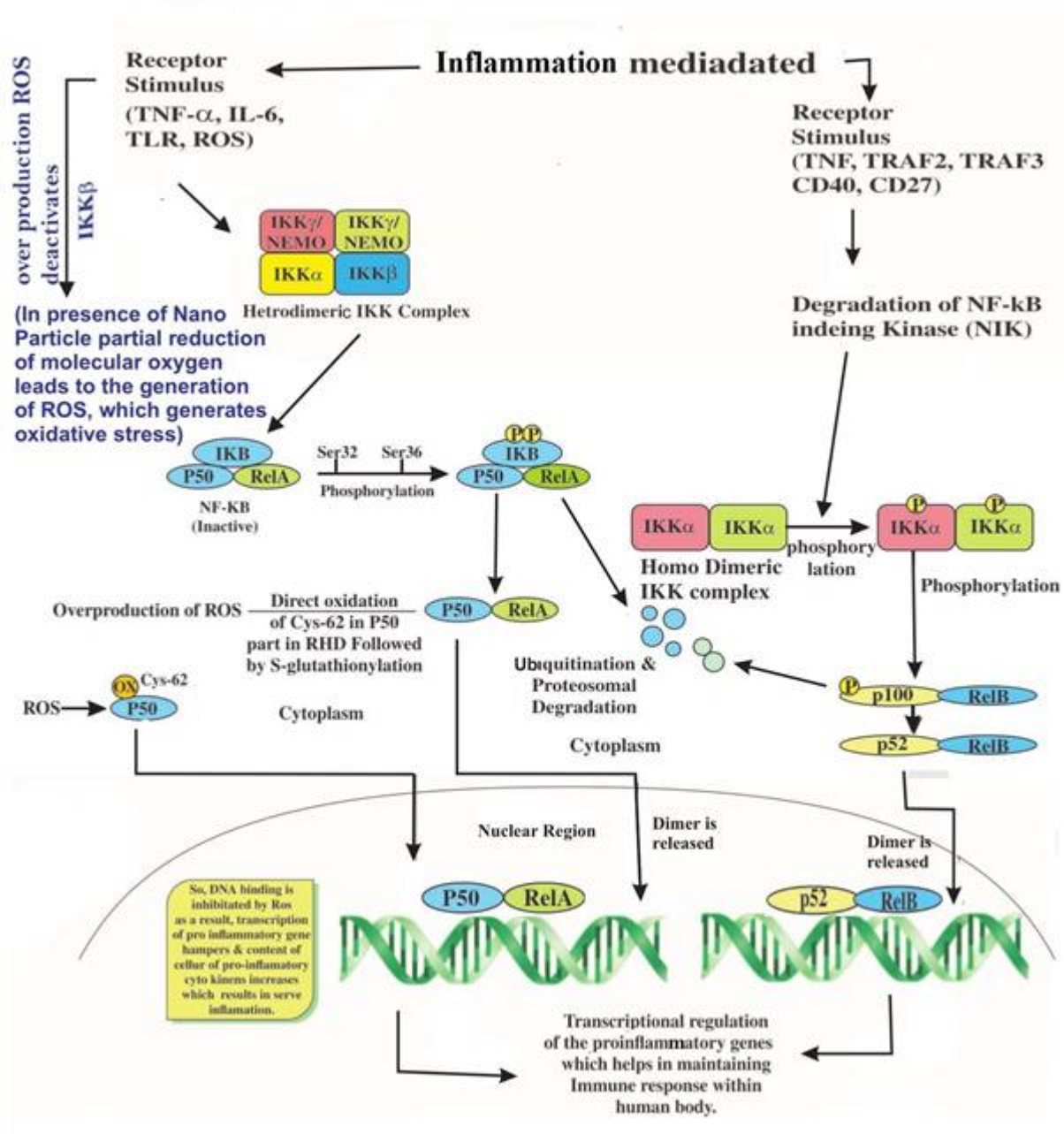
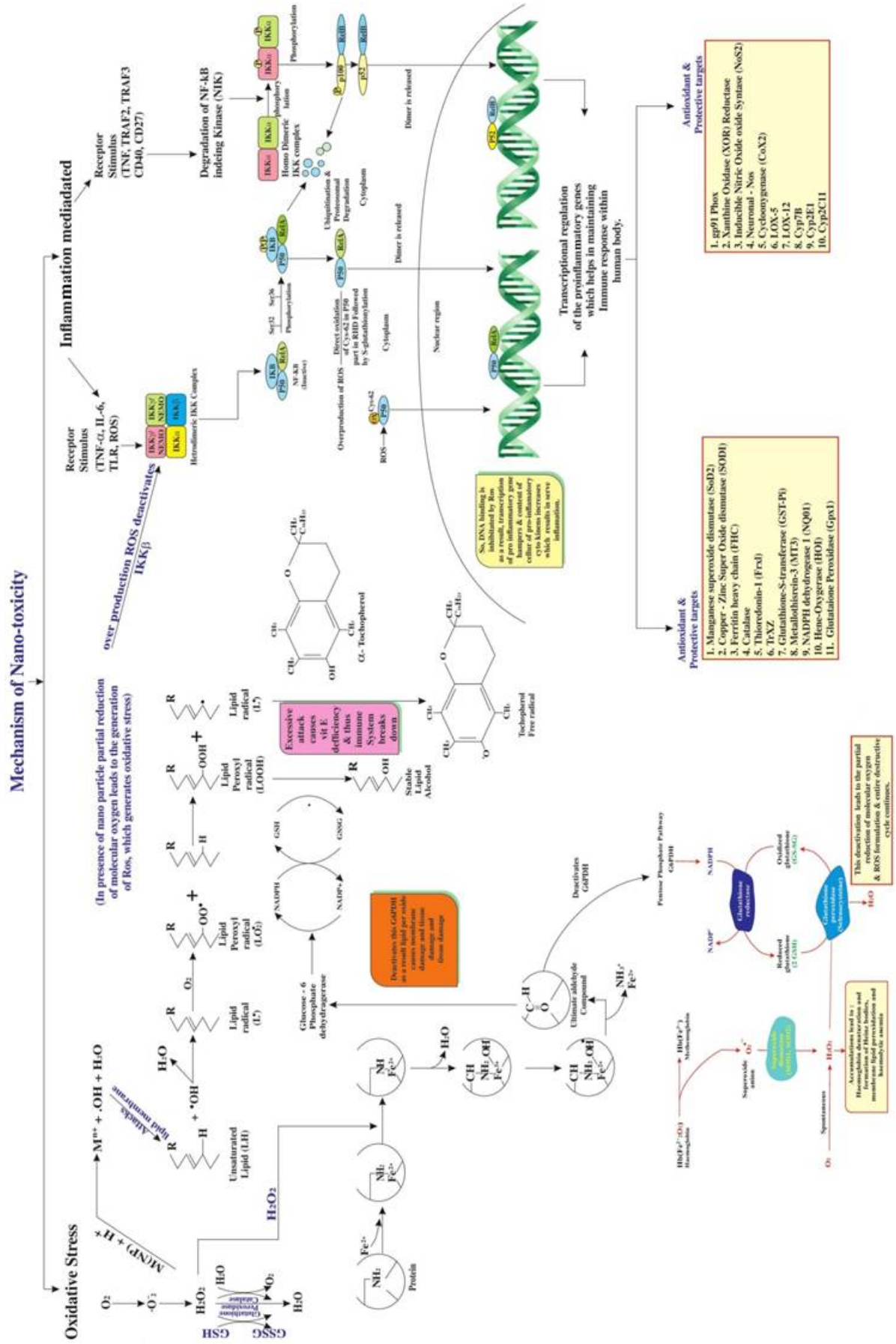


Figure – 7: Complete mechanism of nanotoxicity due to overproduction of ROS.



(a) Fenton-like reaction and (b) Surface plasmon resonance enhancement.

ROS generation via Fenton reaction

A Fenton or Fenton like reaction is a process that leads to the generation of hydroxyl radicals (Ï%OH), which can be best exemplified by the reaction between H_2O_2 and Fenton like reagents involving transition metal ions such as Fe^{2+} and Cu^{+} zero-valent metal NPs with relatively low redox potential, such as Ag, Cu and Fe NPs, can be viewed as Fenton-like NPs. They all have redox potentials less than that of H_2O_2/H_2O (1.77V). For example, elemental silver ($E_{Ag+/Ag}$ = +0.7996 V), copper ($E_{Cu+/Cu}$ = +0.52 V) and iron ($E_{Fe2+/Fe}$ = -0.44 V) are thermodynamically favourable to trigger the Fenton reaction in presence of H_2O_2, as displayed in reaction (6).

$$H_2O_2 + M^{n+} \rightarrow M^{n+1} + {}^{o}OH + OH^{-} \quad (5)$$

$$M\,(NP) + H_2O_2 + nH^{+} \rightarrow M^{n+} + {}^{o}OH + H_2O \quad (6)$$

Therefore, a Fenton reaction is always accompanied by oxidation and dissolution of metal nanoparticles. Dissolved metal ions such as Fe^{2+} and Cu^{+} can further promote the Fenton reaction as shown in reaction (5). Thus, in presence of NPs, H_2O_2 reacts with it in presence of transport protons (H^{+}) as shown in reaction (6) which leads to the production of hydroxyl free radicals (^{o}OH). In this way, the cascade ROS generation starts. Ag and Cu NPs are reported to produce hydroxyl radical via Fenton reaction as reported by (He *et al.,* 2014).

Surface plasmon resonance enhancement

Since the metal NPs contain free electron, they can be easily affected by the electromagnetic wave which results in collective oscillation of electrons. A resonance phenomenon known as surface plasmon resonance occurs when the frequency of the incident light photons equals to that of electron. A local enhanced electromagnetic field formed on the surface of metal NPs helps in the generation of ROS. Ag NPs generate superoxide and hydroxyl radicals under UV radiation (Zhang *et al.,* 2013). Au, Ni NPs also generate ROS via this pathway.

The production of ROS by nanomaterials may proceed through a variety of mechanisms. The ROS formation from a particular nanomaterial is dependent on the physical and chemical properties of the nanomaterials as well as the testing systems, such as different cell types (Shaligram *et al.,* 2013). The critical chemical and physical structural determinants of the nanomaterial that lead to the generation of ROS and toxicity include molecular size, shape, oxidation status, surface area, bonded surface species, surface coating, solubility, and degree of aggregation and agglomeration (Wang *et al.,* 2008, Lu *et al.,* 2010 and Nel *et al.,* 2006). Silver nanoparticles (AgNPs) of different sizes (4.7 and 42 nm) showed the induction of ROS, glutathione depletion, as well as a slight inhibition of superoxide dismutase (Avalos *et al.,* 2014). Studies with gold nanoparticles (AuNPs) of sizes ranging from 5 to 250 nm have also revealed that smaller diameter nanoparticles with larger surface area produce higher amounts of ROS, thus establishing an inverse relationship between these two parameters (Misawa and Takahashi, 2011).

Mechanism of nanotoxicity influenced by excessive ROS generation

Nanoparticles are known to induce reactive oxygen species (ROS) production, leading to an oxidative stress when redox state of the cell is imbalanced. ROS induction by nanoparticles is considered the primary cause of nanotoxicity, and has been attributed to the presence of pro-oxidant functional groups on their reactive surface or due to nanoparticle-cell interaction. ROS production is a normal cellular process which is involved in varied aspects of cellular signalling, as well as in the defence mechanism of the immune system. The over-production of ROS can induce oxidative stress, resulting in cells falling to maintain normal physiological redox regulated functions. The damage in cell function and development includes oxidative modification of proteins to generate protein radicals, initiation of lipid peroxidation, DNA strands break, modification of nucleic acids, modulation of inflammatory responses through signal transduction leading to cell death.

Oxidative modification of protein

Most of our knowledge about the modification of proteins by ROS comes from the pioneering studies of (Garrison *et al.,* 1987, Swallow *et al.,* 1960) which demonstrate that the oxidation of protein by ROS can lead to oxidation of amino acid residue side chains, cleavage of peptide bonds and formation of covalent protein-protein cross linked derivatives. Peptide bond cleavage, as shown in figure 2 is mainly done by hydroxyl free radical (reaction 6) or metal catalyzed cleavage of H_2O_2 (reaction 5) can abstract hydrogen atoms from the R-CH(R) - group of the polypeptide backbone (Figure 2a, reactions a, b). The alkyl radical thus formed may react with oxygen to form the alkylperoxy radical (reaction c) or with another alkyl radical to form inter- or intra-protein cross-linkages (reaction p). The protein peroxy radical can be converted to the alkyl peroxide by either reaction with free peroxy radical (reaction d), reaction with Fe^{2+} (reaction e), or abstraction of a hydrogen from another source (not shown). Irrespective of how it is formed, the protein alkyl peroxide can be converted to the alkoxy protein derivative by either dismutation (reaction o), reaction with free peroxy radical (reaction f), or reaction with Fe2+ (reaction g). Finally, the alkoxy radical may undergo conversion to the hydroxy derivative (reactions i, j), which will undergo peptide bond scission by the so-called R-amidation pathway (reactions k, l). Alternatively, the alkoxy radical may undergo peptide bond cleavage by the so-called diamide pathway (reaction m). It is noteworthy that the N-terminal amino acid residue of the peptide fragment derived from the C-terminal portion of the protein by the R-amidation pathway exists as the R-ketoacyl derivative. Diseases associated with premature aging (Werner's syndrome) show very high levels of oxidized proteins at an early age (Stadtman and Berlett, 1997). End products of free radical action aldehydes (Figure 2b) inhibit the activity of membrane enzymes like Glucose-6-phosphate dehydragenase, adenylate cyclase. These aldehydes react selectively with proteins or enzymes containing SH groups and cause tissue damage.

Lipid peroxidation

Lipid peroxidation is the oxidative degradation of lipids. It is a process in which free radicals steals electron from the lipids present within the cell membrane, resulting in cell damage. In the initiation stage a fatty acid radical/ Lipid radical (L^{o}) is produced. The most notable initiators in living cell are ROS such as hydroxyl free radical, which combines with a hydrogen atom to make water and fatty acid radical (L^{o}). This fatty acid radical (L^{o}) is not a very stable molecule, so it reacts readily with molecular oxygen

(O_2) and produces peroxy fatty acid radical (LO_2^o), this being unstable reacts with another unaffected fatty acid, producing a new fatty acid radical (L^o) and lipid peroxide (LOOH) which is shown sequentially in figure 3. The cycle continues as the newly unaffected fatty acid reacts in the same way. Any free radical reaction stops when two radicals react and produces a non-radical species. This happens only when the concentration of radical species is high enough and for this there to be a high probability of collision of two radicals. Living organisms have different molecules that speed up termination by neutralizing free radicals thus protects the cell membrane. One important such antioxidant is vitamin E, being a lipid soluble one, it neutralizes lipid free radicals (L^o) but in case of oxidative stress, due to the generation of higher amount of free radicals it cannot be neutralized by vitamin E which results in the deficiency of vitamin E, the ultimate effect of which is the breakdown of immune system which is shown in figure 4 and as a result, the lipid radicals causes proximal membrane and distal tissue damage. ROS generation decreases mitochondrial membrane potential, increases levels of lipid peroxide and decreases enzymatic activities of antioxidants which are found to be induced by single-walled carbon nanotubes (Wang *et al.,* 2011).

DNA damage

DNA is a critical cellular target of ROS. Toxicity of nanoparticles is attributed to oxidative stress, followed by DNA damage and apoptosis. Nanoparticles can cause a wide variety of DNA damage, ranging from chromosomal fragmentation, DNA strand breakages and the induction of gene mutations (Singh *et al.,* 2009). AuNPs (20 nm size) at 1 nM concentration have been shown to exhibit DNA damage in the form of 8-hydroxydoxyguanosine (8OHdG) adducts formation in embryonic lung fibroblasts with a decreased expression of DNA repair and the cell cycle checkpoint genes *MAD2*, *cyclin B1* and *cyclin B2* (Li *et al.,* 2008). Various studies have also confirmed the occurrence of DNA fragmentation and formation of oxidation-induced DNA adducts on exposure to metal oxide nanoparticles. In response to this DNA damage, the cells either initiate DNA repair mechanisms or invoke cell cycle arrest and apoptosis. One of the major effect or molecules activated in response to DNA damage is p53. It plays a central role in DNA repair and cell cycle arrest, thereby preventing mutagenic events favouring the process of carcinogenesis (Lane, 1992). Metal oxide nanoparticles including TiO_2, ZnO, Fe_3O_4, Al_2O_3, and CrO_3 of particle sizes ranging from 30 to 45 nm were found to induce apoptosis (Jeng and Swanson, 2006). Cadmium-telluride quantum dots were found to significantly increase p53 levels and upregulate the p53-downstream effectors Bax, Puma and Noxa in human breast carcinoma cells (Choi *et al.,* 2008).

Formation of the reactive oxygen species (ROS), H_2O_2, can occur spontaneously from O_2 or is generated via superoxide dismutase (SOD) action on superoxide anion produced as a result of O_2 oxidation of the ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}) in haemoglobin. The consequence of the latter reaction is the formation of methemoglobin. Since haemoglobin is a heterotetramer, and each subunit contains Fe^{2+} iron in its heme there is the potential for multiple Fe^{3+} irons to be present in methemoglobin. The Fe^{3+} form of iron does not bind O_2, however, the presence of at least one Fe^{3+} in the haemoglobin tetramer results in enhanced binding of the O_2 to the remaining Fe^{2+} irons causing reduced delivery of the O_2 to the tissues with potential for cyanosis. Normal daily levels of methemoglobin range from 0.5%–3%. The ferric iron in methemoglobin is reduced to ferrous via the action of the NADH-requiring enzyme, methemoglobin reductase (cytochrome b5 reductase 3: CYB5R3). The NADPH produced in the Pentoser phosphate pathway (PPP), and the antioxidant glutathione (GSH), are both necessary for the continual removal of ROS from the erythrocyte (red blood cell, RBC). The enzyme, glutathione peroxidase (GPx) utilizes reduced glutathione (GSH) as the electron donor in the process of reducing H_2O_2 to H_2O while simultaneously generating oxidized glutathione (GS-SG). GPx is one of several selenocysteine-containing redox enzymes. For continuous use of GSH, the oxidized molecule needs to be reduced via action of the NADPH-requiring enzyme, Glutathione reductase. But, the ultimate aldehydes product from oxidative modification of proteins deactivate these enzymes thus the pathway for ROS removal in erythrocytes is no longer spontaneous as shown in figure 5.

Inflammation mediated nanotoxicity

Oxidative stress also results in the release of pro-inflammatory mediators through the principal cascades such as the NF-κB (Nuclear Factor-κB), mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3-K) pathways, suggesting that oxidative stress is linked to inflammation reciprocally (Huang *et al.,* 2010) i.e. allow level of oxidative stress can stimulate NF-κB activation, while higher levels may lead to the inhibition of activation. NF-κB proteins are a family of transcription factors and are central importance in inflammation and immunity (Vallabhapurapu and Karin, 2009). The mammalian NF-κB protein family consists of five members: NF-κB1 (p50/p105), NF-κB2 (p52/p100), RelA (p65), RelB, and c-Rel. These proteins share an evolutionary conserved domain called Rel-homology domain (RHD) or Rel-homology region (RHR). The RHD comprises domains for dimerization, DNA binding, and nuclear localization (Basak and Hoffmann, 2008, Gloire and Piette, 2009). NF-κB dimers bind to the promoters of a diversity of genes at sequences known as κB elements, whose consensus was defined as 5'GGGRNWYYCC3' (N: any base; R: purine; W: adenine or thymine; and Y: pyrimidine). Three Rel members of the family RelA, RelB and c-Rel have a C-terminal transcription activation domain (TAD) that serves to positively regulated gene expression (Siomek, 2012).

In unstimulated cells, the NF-κB dimers are sequestered in the cytoplasm by a family of inhibitors, called IκB (Inhibitor of κB), which are proteins that contain multiple copies of a sequence called ankyrin repeats. By virtue of their ankyrin repeat domains, the IκB proteins masks the nuclear localization signals (NLS) of NF-κB proteins and keep them sequestered in an inactive state in cytoplasm.NF-κB pathway may be activated via at least two distinct routes namely, canonical and the non-canonical pathway. In case of canonical pathway (Oliver *et al.,* 2009), activation of NF-κB is initiated by signal induced degradation of IκB proteins. This occurs primarily via activation of a kinase called IκB kinase (IKK). IKK is composed of a heterodimer of the catalytic IKKα and IKKβ subunits and a master regulatory protein termed NEMO (NF-κB Essential modulator) or IKKγ. IKKα and IKKβ have catalytic properties while IKKγ has a regulatory nature. The IκB kinase phosphorylates IκB-α on serine 32 and serine 36 located in an IκB regulatory domain. When phosphorylated on these serines, the IκB proteins are modified by a process called ubiquitination which then leads to be degraded by a cell structure called proteosome. With the degradation of IκB, the NF-κB

complex is then freed to enter the nucleus where it can turn on the expression of specific genes that have DNA binding sites for NF-κB nearby. The DNA+ NF-κB complex then recruits other proteins such as co-activators and RNA polymerase which transcribe downstream DNA to m-RNA. In turn, m-RNA is translated into protein resulting a change in cell function (Morgan and Liu, 2011). Activation of NF-κB/Rel transcription family by neuclear translocation of cytoplasmic complexes plays a central role in inflammation through its ability to induce transcription of proinflammatory genes (Bonizzi and Karin, 2004).

The classical pathway is activated by a diverse set of stimuli including proinflammatory cytokines such as tumor necrosis factor α(TNF α), interleukins (IL-1, IL-6), TLR and ROS (Senftleben *et al.,* 2001). A proinflammatory cytokine is a type of signalling molecule that is excreted from immune cells like helper T cells, macrophages and some other cell types that promote inflammation. Excessive chronic production of inflammatory cytokines due to the deactivation of NF-κB pathway contributes to the inflammatory diseases that have linked to different diseases such as atherosclerosis and cancer.

Overproduction of ROS induces direct oxidation of cys-62 in p50 part in RHD followed by S-glutathionylation. This results in inhibition of DNA binding which is represented in figure 6. Thus, transcription of proinflammatory gene is hampered and as a result the content of proinflammtory cytokines increases which results in severe inflammation. Both *in vitro* and *in vivo* studies show that nanoparticle-induced lung injury and pulmonary fibrosis lead to the ROS-mediated activation of NF-κB and production of pro-inflammatory mediators such as TNF-α, IL-8, IL-2 and IL-6 (Bryne and Baugh, 2008). Several metal oxide nanoparticles including zinc, cadmium, silica, and iron have also been shown to exert their toxicity via the production of inflammatory cytokines induced by NF-κB.

The MAPK pathway regulates a diverse range of cellular responses, including cell proliferation, differentiation, mitosis, cell survival and apoptosis. They are a family of serine/threonine protein kinases that include growth factor-regulated extracellular signal-related kinases (ERK) and the stress-activated MAPK, c-Jun NH2-terminal kinases (JNK) and p38 MAPK. The ERKs are mainly associated with cell proliferation and differentiation, whereas the JNKs and p38 MAPKs are known to regulate responses to cellular stresses (Torres and Forman, 2003). IL-8 production via the p38 MAPK and/or ERK pathway is shown to mediate toxicity in human bronchial epithelial cell line upon treatment with titanium dioxide nanoparticles. The NF-κB, MAPK and PI3-K pathways facilitate nanoparticle-induced inflammation, and release of several pro-inflammatory cytokines and chemokines including TNF-α, IL-6 and IL-8, leading to cytotoxicity and cell death. The nanoparticles induce ROS production, causing an imbalance in redox state and subsequently leading to an oxidative stress in cell is illustrated in Figure 7.

Possible ways to circumvent nanotoxicity

Since oxidative stress is the main reason for nanotoxicity as it creates imbalance in the redox state of the cell which induces 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, a complete decrease in ROS production in the cells is noticed. Concomitantly, ascorbic acid also led to a decrease in AgNP-induced mitochondria damage, apoptosis and DNA damage, reducing the toxic effects induced by AgNPs (Guo *et al.,* 2013).

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 Nrf2 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 circumvented by treatment with quercetin (Gonzalez *et al.,* 2015).

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 is 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 (Stevanovic *et al.,* 2014).

Conclusion

As the use of nanomaterials for both commercial goods and novel applications has been increasing exponentially due to their favourable physiochemical properties, the need for relevant, accurate and predictive nanotoxicological assessment also grows which has become an issue of interest to public. Due to the complication of free radical formation and it's interaction with cellular components i.e. the biochemical mechanism of nanomaterial induced ROS generation followed by inflammation and cell damage is also very challenging. In all, the physiologically relevant ROS, the hydroxyl radical possesses the highest one electron reduction potential and is reactive towards all types of biomolecules including lipids, proteins and nucleic acid. Enzymatic defences have evolved to protect against harmful biological oxidants. SODs, peroxidases and catalases are some of the prominent and extensively studied antioxidant enzymes. Antioxidants also play an important role in preventing/limiting the damage caused by ROS. The hydroxyl radical is the most powerful ROS in causing biological damage. Although, there is no antioxidant enzymes that can destroy hydroxyl radical, some endogenous and dietary antioxidants are effective in scavenging hydroxyl radicals.

In order to meet overarching goal of determining a set of design rules for safe nanomaterial use based on predictive nanoparticle toxicity, standard protocols needs to be developed. These protocols will require accurate characterization of nanomaterials, high-throughput in vitro screening to identify potentially problematic nanomaterials, followed by in vivo toxicity studies based on these materials and mechanistic studies using relevant models revealed by in vivo toxicity assessment. The output from this cycle can then be used to further refine future nanotoxicity studies and inform safe nanomaterial design.

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