Toxicity and Cytotoxicity Effects of Selected Nanoparticles: A Review

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Toxicity and Cytotoxicity Effects of Selected Nanoparticles: A Review

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Abstract. The appreciable development in nanotechnology has drawn the attention of several researchers cutting across different fields. However, some nanoparticles have been identified to possess harmful effects on humans and the environment. Hence, putting these cause and effect patterns into context is highly required for future research and discussions about nanotechnology. This study reviewed existing literature on the toxicity and cytotoxicity effects of some nanoparticles to compare reaction patterns. Many kinds of research used different cell cultures, including cancer cell lines, human endothelial cells, hepatic cells, which were tested both *in vitro* and *in vivo* to check the mechanism of the possible toxicity effects. Adverse effects of nanoparticles identified involved damaged DNA leading to mutations and generation of reactive oxygen species (ROS). The prominent identified common toxicity responses in nanoparticle-cell interaction were lysosomes formation interference, necrosis and apoptosis, nanoparticles and protein interaction, and agglomerate formation in other body parts. Some reports showed that the causes of these responses might be due to the physicochemical properties of the interrogated particles, such as particle size, shape, surface functionalisation, surface charge. Furthermore, nanoparticles' toxicity effects are both concentration-dependent and time-dependent, highly pronounced in chemical or physical-based synthetic routes. Cytotoxic effects of nanoparticles were mainly linked to their synthetic method, nature of the reducing agent, and culture media.

Keywords: Nanoparticles, cytotoxicity, ROS generation, mitochondria damage

1. Introduction

Nanotechnology is technology on a nanoscale with many applications in the real world; nanoparticles are the building blocks of nanotechnology. The word "nanoparticle" is from the word's particle and nano, which describes particles with physical lengths or structural dimensions that are a billionth of a meter (10^{-9} or <100 nm) [1]. It is a well-known research area in science and technology. Development in this area has drawn the attention of several researchers cutting across different fields such as bio-medical and biotechnological fields [2], engineering, agriculture and food technology and connecting other branches of sciences, including chemistry, biology, and physics. Over the years, the enormous applications of nanotechnology have led to concerns about its safety. Several studies have been carried out to give accurate answers to the toxicity concerns relating to nanoparticles. So far, an appreciable number of studies reveal that nanoparticles pose some level of toxicities that can lead to mild or fatal damages in the biological environment. From reports, the toxicity effect of NPs has been related to their shape, size, stabilisation/functionalisation technique, synthetic routes, reducing agent, capping agent, the media in which they were cultivated, and the chemicals used. The toxicity and cytotoxicity of nanoparticles are assessed via in *vitro* and in *vivo* measurements and observed via dose-response, exposure assessment, and hazard observation [3].

The unique properties and behaviours of particles at the nanoscale have made their study particularly fascinating. Compared to bulk particles, these properties include increased surface area to volume ratio, ease in manipulation, variety in shapes and sizes. These factors dictate the behaviour of

nanoparticles, giving a resultant change in their mechanical, thermal, electronic, and catalytic activities. Nanomaterials exhibit physicochemical properties which are also distinctly unique [4]. Copper appears transparent at the nanoscale. On exposure to UV light, the catalytic property of titanium dioxide nanoparticles (TiO_2-NPs) is used to inactivate chemicals when introduced in water treatment [5]. Metal nanoparticles generally adopt more advanced optical/optoelectrical properties. The application of nanoparticles spreads across various consumer products, such as UV protection fillers and coatings, primarily on sunscreens, windows, and lenses. Silver and copper are recognised for their antimicrobial properties; hence, they are more effective at the nanoscale and gain application in food packaging. Another application is in the textile industry, where they are incorporated to reduce odour issues. Gold nanoparticle (Au-NPs) is widely used in nanomedicine as a targeted drug delivery agent and for cancer detection. Nanoparticles are also more efficient as catalysts than bulk material due to decreased particle size and increased surface area to volume ratio. For instance, carbon graphene nanotubes make up solid composite structures used in energy production and storage. The unique optical properties known as surface plasmons used in surface-enhanced Raman spectroscopy makes NPs exploited in plasmonics. Nanopowders are applied in batteries. Further applications include nanomembrane filtration, nanomedicine, etc.

Particles at the nanoscale are synthesised by applying two methods of approach - (Top-down and bottom-up approach (Figure 1a). The techniques are further divided into subclasses and classes. The techniques revolve around chemical processes (sol-gel, colloidal, and spray pyrolysis), physical approach (sputtering, laser ablation, laser pyrolysis,) or biological approach (green synthesis, using plant stems, leaves, seeds, or microbes such as bacterial and yeast). These approaches are used under the scope of each research and the procedures that have been established [4-8].

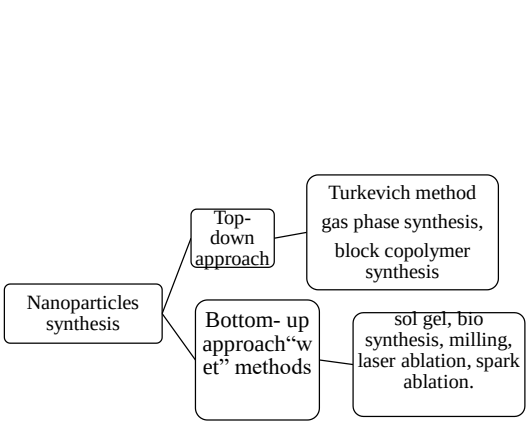
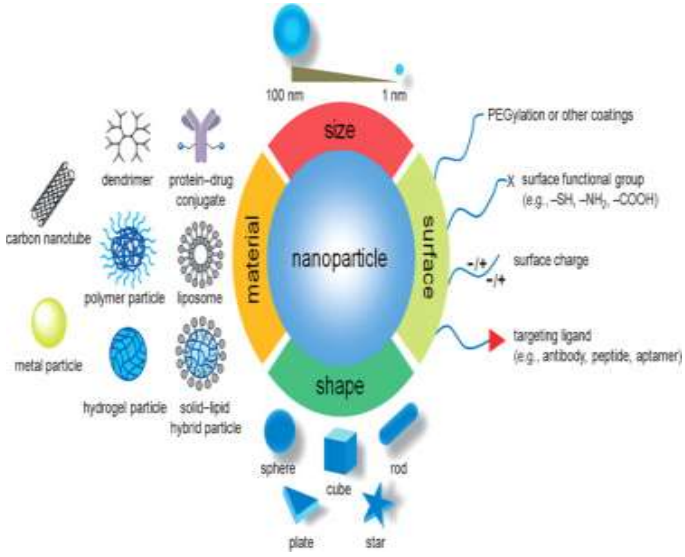


Figure 1: (a) Methods of synthesising nanoparticles



(b) Surface functionalisation of nanoparticles

Source: [9]

2. Classification of Nanoparticles

Nanoparticles may be classified based on their dimensions (0D, 1D, 2D, 3D), chemical property/composition, shell or core component, physical characteristics, electrical charge, origin, and laboratory synthesised nanoparticles. Tables 1 and 2 show classification based on chemical, organic or inorganic components. Other categories include dendrimers, ceramic, lipid-based, composite-based, and semiconductor nanoparticles [10].

This review focuses on the toxicity and cytotoxicity of selected nanoparticles, details about causes of nanoparticles' toxicity, effect, and updates on their mechanism of action. It also encompasses *in vivo* and *in vitro* studies of nanoparticle toxicity observation, operations patterns, and reaction mechanisms.

Table 1: Classification of Nanoparticles Based on their Chemical Property/Composition

Category	Nanoparticles	Applications	References
Metal nanoparticles	Ag, Au, Cu, Ti Fe, Pt, Al NPs	Medical diagnostics, fuel cells, medical testing, water treatment, and other applications.	[10, 11]
Metal oxides-based NPs	TiO_2, ZnO, nanosilica, CeO_2	Antibacterial, drug delivery agents, UV blocking agents	[12]
Carbon-based nanoparticles	Made up of carbon such as Fullerenes, graphene, carbon nanofibers and carbon nanotubes	Solar cells, improved coatings, water purification.	[13]
Polymeric nanoparticles	Thermosets, elastomers, and thermoplastics	Nanofibers and nanoporous membranes for water filtering.	[14, 15]

Table 2: Classification of Nanoparticles Based on Organic and Inorganic Properties

Nanoparticles	Advantages	Disadvantages	Examples	References
Organic	Less hazardous synthetic methods include long blood circulation, precise targeting, biocompatibility, larger surface area	Dose-dependent disruption of cell metabolism, immune response, non-scalable synthesis.	Polymeric nanoparticles, polymeric micelles, liposomes and dendrimers	[14,16,17]
Inorganic	Bioinertness, unique optical properties: SERS AND SPR, radioactive labelling manipulation by physical stimuli	Immune response, inflammation, accumulation in MPS organ, e.g., quantum dots, magnetic nano property, carbon nanotubes, gold NPs, silica nanoparticles.	Gold, carbon nanotubes, nanographene, silica, quantum dots, magnetic nanoparticles.	[18-20]

3. Applications of Nanoparticles

Nanoparticles (NPs) have substantially impacted technology, and research into novel techniques to maximise effectiveness is still ongoing. The improved dynamic anisotropy of nanohybrids suggests that they could be used as an optical material in colourimetric metal nanoparticle-mediated sensors [24]. In agriculture, iron (iii) (Fe_2O_3) nanoparticles exhibit a significant impact on the enzyme activity of microbes and the degradation of organic matter during composting of agricultural waste. The nanoparticles' surface, size, and shape can be modified to achieve better monodispersity, leading to their exploration in medical fields. For instance, well-dispersed gold nanoparticles (Au-NPs) widely used in nanomedicine can absorb a reasonable number of different molecules, such as medications, and then disperse these absorbed molecules throughout the bloodstream effectively.

The various applications of NPs have led to their mass production alongside their direct or indirect release into exposed body systems and environment, which according to literature, poses some level of toxicity. Nanoparticles are transported into biological systems by inhalation, ingestion, dermal

absorption, injection, and implantation. The study of different toxicity effects of nanoparticles spores from the need to answer the frequently asked questions; how far can these particles go, in a biological system, and in what concentration, if they successfully settle in the system, and how destructive are they capable of being? Researchers are putting in an appreciable amount of work to contextualise specific answers to these questions. There is still a lot to be tested on this aspect of nanotechnology due to the wide range of available particles, their properties, functionalisation, and varying behaviour in different environments.

4. Cytotoxicity of Nanoparticles

NPs infiltrate the environment as they settle on the water, soil, and air, exposing humans to them. In some cases, engineered or laboratory synthesised NPs are purposely injected or dumped into the ground or aquatic systems for treatment's sake [58]. As a result, questions are being raised concerning the safety of this process. Nanoparticles with sizes from 1 -100 nm are small enough to enter cells via endocytosis. Studies show that NPs are either ingested, inhaled, or enter a system through skin contact and translocated to other vital body organs and tissues, where they can cause disturbance to some biological processes. The respiratory system is prone to be easily affected by the potential toxicity of NPs since it's the path of entry for inhaled particles [26] [15, 27].

The physicochemical property, surface charge, and surface chemistry of NPs all influence their cytotoxicity. Reports [28] revealed that long, thin, and multi-walled nanotubes (NTs) elicited a greater inflammatory response than single-walled nanotubes (NTs). Also, the packed nature of the latter's responses is attributed to their geometrical structure and size. Similar observations were seen in other research [29-31]. The cytotoxic effect of NPs is also linked to their synthetic route, nature of the reducing agent, capping agent, the media in which they were cultivated and the chemicals being used [32] compared the effect of synthesising gold nanoparticles using citrate- and 11-mercaptoundecanoic acid (MUA). They discovered that citrate media showed a higher level of toxicity compared to MUA under similar conditions. However, some studies have shown that the toxicological effects of NPs are limited to some nanoparticles. The green synthetic approach of synthesising nanoparticles should always be considered as they involve fewer chemicals that influence toxicity in chemically synthesised nanoparticles. Studies also show regularities and irregularities in the toxicity pattern and mechanism of toxicity of nanoparticles. Below are recorded causes of nanoparticle toxicity effects, cause and patterns of their mechanism.

4.1. Size and Shape of nanoparticles and their effects

Synthesised NPs can be modulated into different shapes and sizes for various purposes, such as influencing biocompatibility, uptake, and retention in body tissues and organs (Table 3). Several studies were carried out on the causes of nanotoxicity; size and shape-dependent toxicity were also studied. The smaller the size of the particles, the more toxic they would be [33]. It is undoubtedly due to the larger surface area, which may lead to excess release of ions that can cause oxidative stress on cells. Smaller NPs have been localised in the kidney and lungs, while bigger particles are more likely to accumulate in the liver and spleen. This paradigm was observed in an experiment conducted by Wang *et al.* [34], who conducted an *in vivo* test by ingestion. They discovered more accumulation of 40 nm-sized gold nanoparticles (Au-NPs) in the liver than 20 nm-sized gold nanoparticles (Au-NPs) localised in the lungs.

Similarly, some nanoparticle shapes have been recorded to have more tendency to be toxic than others with the same composition. An experiment on the toxicity of nanorods and nanoflower showed greater cytotoxicity of nanorods, and this observation suggested that it could result from higher internal exposure to cells. Records on nanotubes showed length and diameter dependent toxicity and are less biocompatible with the cells than nanoflakes. Zhao *et al.* [35] also determined the length and diameter dependent toxicity of 6 multi-walled carbon nanotubes MWCNTs with different diameters and lengths. They reported increased toxicity on human endothelial cells with decreased diameters and increased particle length on similar experimental conditions such as concentration. Similarly, Magesky and Pelletier [36] observed that multi-walled nanotubes possed more toxic effects than single-walled nanotubes.

A comparative study on the shape and size-dependent toxicity of NPs was carried out by Enea *et al.* [37], using nano-stars and nanosphere gold NPs (Au-NPs) sizes 15 nm and 60 nm. They observed there is a higher internalisation of the star-shaped Au-NPs than the sphere-shaped Au-NPs. Production

of nanocrystals by solvothermal methods for particles such as metal oxides, carbon nanoparticles and quantum dots involve forming crystal structures that could be anatase, rutile, or amorphous. This crystallinity was also recorded to be a cause of toxicity. Amorphous (TiO_2) was observed to generate more reactive oxygen species (ROS) than anatase or rutile with comparable size, with rutile TiO_2 causing the least ROS. Amorphous TiO_2 was more likely to have surface imperfections and thus active sites that can generate ROS. Even though the particles possess similar size and composition, the anatase form of TiO was more toxic to PC12 cells than the rutile form. In a murine macrophage cell line (RAW 264.7), rod-shaped Fe_2O_3 nanoparticles elicited substantially stronger cytotoxic reactions than sphere-shaped Fe_2O_3 nanoparticles, including higher lactate dehydrogenase leakage levels (LDH), inflammatory response, ROS generation, and necrosis. Finally, rod-shaped CeO_2 nanoparticles were more cytotoxic than octahedron or cubic particles in RAW 264.7 (macrophage cell line). In RAW 264.7 cells, rod-shaped CeO^2 nanoparticles caused considerable lactate dehydrogenase LDH release and tumour necrosis factor-alpha (TNF), but neither octahedron nor cubic nanoparticles did. The report showed that the crystalline nature of the particles might be the cause of toxicity [38].

Table 3: Geometrical Structures of Nanoparticles

Nanoshapes	Characteristics	References
Nanorod (nanocylinders or nanotubes)	Single-walled or multi-walled for controlled drug delivery, they form more substantial composite structures and can be toxic to cells	[39]
Nanospheres	Most available shapes for purchase and literature and are primarily synthesised from organic or inorganic precursors	[40-41]
Nanostars (nanoflowers)	Mainly investigated with DNA and mostly theoretical in manipulation	[19, 37]

4.2. Surface Charge of nanoparticles and their effects

In reduction methods of synthesising NPs such as turkevich method/ citrate reduction, an electric double layer on the particle's surface occurred and was described as electrostatically stabilised. This stabilisation is necessary for cellular uptake, but research shows that inaccurate stabilisation methods will lead to aggregation formation when added to the testing media [42]. From Figure 2, using gold NPs as an example, when AuNPs was produced by citrate reduction using hydroxylamine (seeding growth), it led to electrostatic stabilisation. Low endocytosis occurs when a negatively charged cell membrane interacts with a negatively charged particle due to low affinity. Still, positively charged particles with a high relationship with the cell membrane charge will improve cellular uptake [43]. Both were influencing more prolonged circulation in the blood and intercellular uptake, respectively. This concept is essential to note when dealing with the accumulation of particles in the cell.

This concept is also essential to note when dealing with the accumulation of particles in the cell. Excess uptake of either negatively or positively charged particles will lead to accumulation in lysosomes by endocytosis, which has been observed to damage lysosome, leading to disturbance of cell cytoplasm pH level and even cell death [44]. Furthermore, solubility factors such as oxidation state will affect metal ion speciation in the cellular environment, as well as their interactions with biological targets. Ion release has also been connected to situations where thermal activity favours NP disintegration in a biological context. The organic matter or other natural particles (colloids) found in freshwater substantially influences how NPs combine in hard water and saltwater. In some situations, toxicity

associated with metal ion release has been recorded as time and pH-dependent [11]. Hence, when dealing with metal NPs that can produce cytotoxic responses, metal ions release is the most often documented mechanism of toxicity. For instance, [27] recorded the transformation of silver nanoparticles to other silver species. Ag NPs were synthesised and then sulfidized to create $Ag@Ag_2S$ NPs, a core-shell system. When diluted in water, the particle produced stable dispersions with diameters of less than 100 nm. Still, it formed agglomerates when exposed to RTgutGC cells cultured on a permeable membrane to mimic the intestinal barrier. Despite the decreased cytotoxicity of the sulfidised Ag NP form, the particles can enter a fish system and act as a long-term source Ag^+ release and cytotoxicity source.

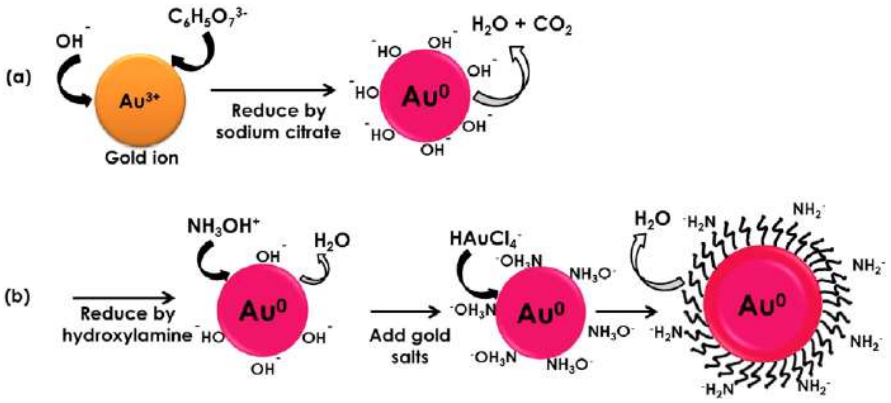


Figure 2: Synthesis of Au-NPs by (a) citrate reduction (b) seeding growth [27]

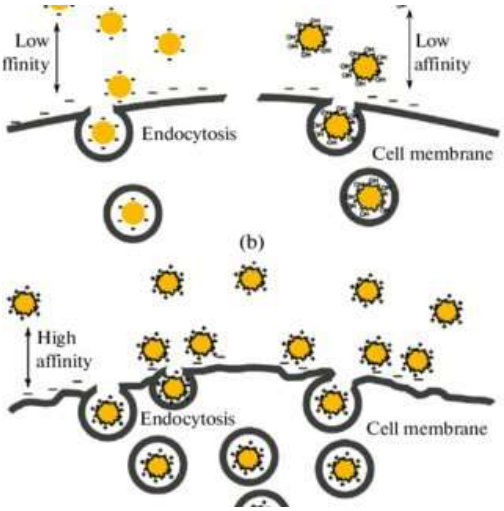


Figure 3: Surface charged nanoparticles and cell interaction [27]

4.3. Zeta potential (ζ-Potential) of nanoparticles and their effects

The zeta potential is included in the characterisation or estimation of the physical stability of the charged particles, with the primary purpose of determining the physical strength of nanosuspensions, due to the electrostatic repulsion of individual particles [45-46]. A zeta potential below 30 mV to +30 mV, low zeta potential can lead to particle aggregation and flocculation due to weak forces acting upon them and resulting in physical instability.

Aggregation was recorded as independent of stabilisation when introduced to a culture media containing high ionic strength. More so, it possesses monovalent and divalent ions such as potassium ion (K^+), sodium ion (Na^+) magnesium ion (Mg^{2+}), which can cause a decrease in electrostatic stabilisation leading to unintended aggregation. Hence, aggregated and non-aggregated particles have a level of toxicity. However, aggregated particles will strongly interact with the cell membrane, affect

their structural integrity, and lead to fatal damages. Methods such as laser ablation may lead to uneven dispersion of particles and may form aggregation.

Positively charged ZnO nanoparticles had a higher cytotoxic effect in A549 cells than negatively charged particles of the same shape and size. Negatively charged nanoparticles interacting with positively charged DNA caused DNA damage in the same way [13]. Periods 4 transition metal oxides (Zn, Cr, Ni, Cu, Mn, Fe and Ti) were investigated for the accessible binding site on the particle's surface, the dissolution of metal NPs, surface charge, and the band-gap energy as features of nanomaterials. They determined the charge of the particle using PZC. XPS was used to measure the accessible binding site of the particle surface. Inductively coupled plasma mass spectrometry was used to investigate metal ions emitted from oxides (ICP-MS). Finally, spectroscopic analysis was utilised to calculate band-gap energy in insulators and semiconductors, the energy difference between the top of the valence band and the bottom of the conduction band. They discovered that (1) cytotoxicity increases with the element's atomic number and (2) particle surface charge, accessible binding sites on a particle surface, and particle metal dissolution affect cell viability, but not band-gap energy.

4.4. Surface functionalisation of nanoparticles and their effects

Functionalisation strategies aim to improve stability and add extra functions to the particles to meet a specific purpose. Different functionalisation strategies include introducing antibodies, drugs, carbohydrates, DNA, dendrimers, aminosilanes, thio-carboxylic acids, -COOH, -OH, -C = O, and pegylation (polyethylene glycol). Surface functionalisation of engineered nanoparticles significantly impacts their applications by changing the hydrophilicity, hydrophobicity, charge, and chiral nature. The essence is to achieve particle stability and enhance shielding before application. For instance, PEGylating is a popular and dependable functionalisation technique used to stabilise NPs and aids in reducing protein absorption. However, an increase in poly ethylene glycol (PEG) length on particle surface causes them to assemble in a mushroom-like manner, which reduces the packing density and allows the protein to bind to exposed areas of the particle surface, increasing the amount of protein absorbed. The proteins on the surface of the nanoparticles increase the cellular uptake of these patterned NPs in most cases and induce toxicity.

Enea *et al*. [32] experimented using PEG with citrate- or 11-mercaptoundecanoic acid (MUA) capping. The sample cells of rats' primary hepatocytes (PRH) and the HepaRG human cells were cultivated with foetal bovine serum (FBS)-supplemented media and serum-free media cultivation, respectively. Their result showed that though the toxicity appeared low in the hepatic cells, the smaller sized (15 nm) and spherical AuNPs nanoparticle capped with PEG showed more toxicity than MUA. Protein corona is a kind of natural functionalisation. Particles injected into an organism are coated in proteins as found in the blood, such as albumins or globulins, affecting the cellular uptake of this corona [44]. Some of the protein will absorb on the particles' surface and enter the cell, carrying along with protein nanoparticles, thereby subjecting the cell to protein overdose or proteins that are not naturally occurring in the cytoplasm, causing protein overdose, which can lead to cell death [47]. In the absence of proper protein coatings, the nanoparticles interact directly with cell membranes and cause damage.

5. Common Cell Responses to Nanoparticle Toxicity

5.1. ROS Generation

Cellular responses to toxicity have been recorded to include ROS generation, cell viability, DNA damage, cytoskeleton damage, mitochondria damage and autophagy trigger (Figure 4). According to Carcy, ROS generation is the most common response of NPs cytotoxicity. Mitochondria sites for ATP synthesis reduced molecular oxygen to water through electron and proton transfer reactions. In the reaction mechanism, some of the oxygen did not wholly reduce, resulting in the formation of superoxides; hence, ROS are an oxidative metabolism by-product of cells. The reaction led to free radicals, which will readily interact with antibodies and cause harm to the system. As reported in some studies, the observed generation of free radicals or the excessive generation of ROS results from the

physicochemical reactivity of nanoparticles. Excess production of ROS would induce oxidative stress, which results in cells failing to maintain their normal redox-regulated functions [48]. These species include superoxide radical anions and hydroxyl radicals. Free radicals lead to oxidative stress indirectly or directly by activation of oxidative enzymatic pathways. Generally, there are several triggers of oxidative stress [49-50]. A study on the interaction of TiO_2 NPs *in vitro* mice showed that after long exposures to TiO_2 NPs, superoxide (O_2^{-}.) were generated alongside H_2O_2, which led to lipid peroxidation.

Furthermore, cell oxidation and oxidative stress formed by ROS generation on nanoparticles may be accounted for ageing, diabetes, neurodegeneration diseases and possibly cancer. Cytoskeleton's components were damaged on exposure to CNTs leading to disturbance of intracellular transport and cell division [52, 53]. From scheme 1, (A) is the OH generation at the nano-bio interface of Fe_2O_3 NPs; (B) represents the process of generating OH by Fe_2O_3 NPs; (C) involves the acidic lysosomal microenvironment, free radical generation of intracellular OH. In situ, the dissolution or reductive dissolution of magnetic iron oxide NPs is induced by surface hydroxylation and the surface Fe oxidation state of iron oxide NPs. Free Fe ions or NPs can react with hydrogen peroxide and superoxide in mitochondria and cytoplasm, resulting in high production of reactive OH. Considering the existing applications of nanoparticles and potentials in several fields, the apprehension of cytotoxic and toxicity possibilities, potential environmental and health risks associated with these particles cannot be overemphasised. Awareness of the importance of the green synthetic approach is required to reduce the possible toxicity effect of nanoparticles. Also, legitimate risk assessments are to be carried out to shape discussions related to nanoparticles in the future. Research shows that nanoparticle pollution is most likely growing to become a prominent cause of pollution in the future. On comparing the size ranges associated with ROS production by TiO_2, particles whose sizes are below 10 nm and slightly above 30 nm were investigated, and an appreciable increase in ROS as surface area increased [29].

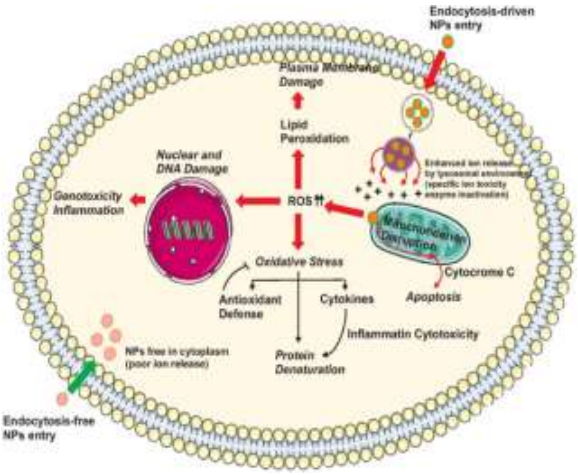


Figure 4: Cytotoxicity of nanoparticles and ion interaction with cell [51]

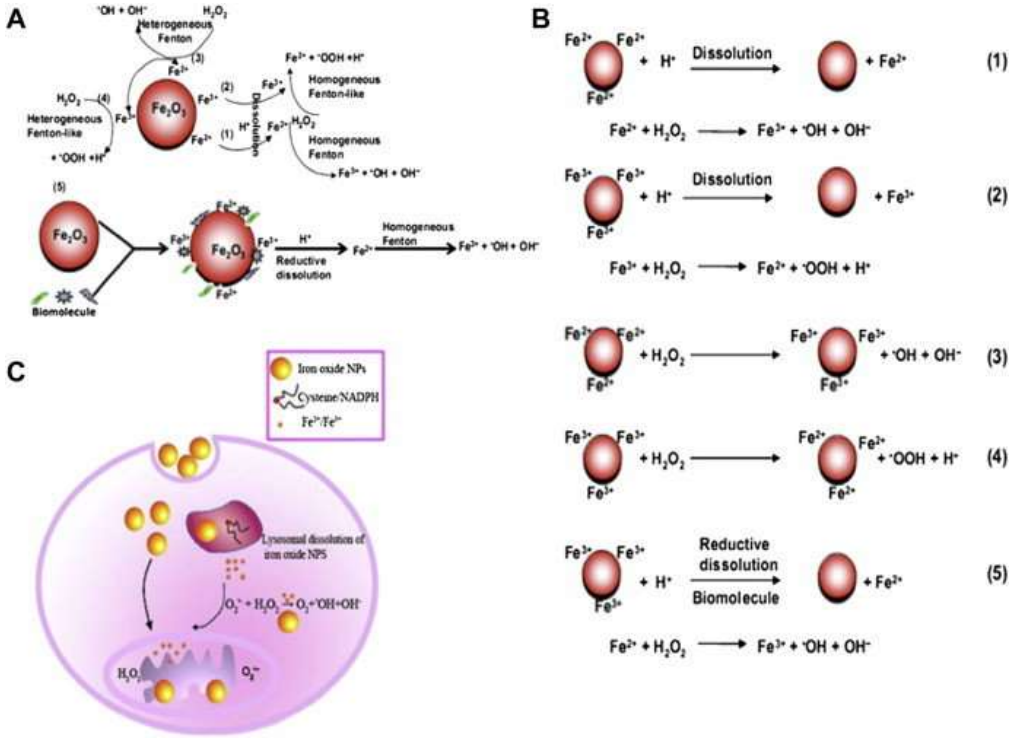


Figure 5: Generation of OH free radical from cell and Fe_2O_3 NP interaction [53]

5.2. Protein-nanoparticle interaction

Nanoparticles had been proved to produce an unfavourable interaction with proteins due to wrong surface functionalisation, free energy, curvature, bigger particle shape, and size. Interaction with protein folding processes has become an issue in a biological system [29]. This interaction may lead to conformational changes, exposure to a new epitope, and affinity and function changes. The regular biological activity or operation of the misfolded protein may lead to injuries in the living cells [54]. Kermani [45] showed that neuronal microtubules were stabilised under normal conditions by tau protein. However, tau undergoing modifications by NPs were recorded, and this reaction may lead to cytotoxicity. In addition, the observed tau bounded to Al_2O_3 led to more packed structures responsible for membrane leakage, activations, and cell apoptosis and necrosis.

More so, the effects of NPs on the endoplasmic reticulum (ER) generated ER stress, which disrupts protein folding processes and alters normal biological processes. Suppose typical cell structure and function are disrupted, chronic critical organ events and subsequent whole-organ reactions such as cancer, cardiovascular disease, pulmonary fibrosis, neurotoxicity, and inflammation. Protein unfolding, fibrillation, cross-linking of thiols, and loss of enzyme activity are adverse biological outcomes of this unfavourable nanoparticle-protein interaction. Bhargava *et al.* [47] elaborated on this folding pattern of corona protein during interaction with silver nanoparticles under serum rich conditions. They suggested it to be the deciding factor of the fate of cellular toxicity.

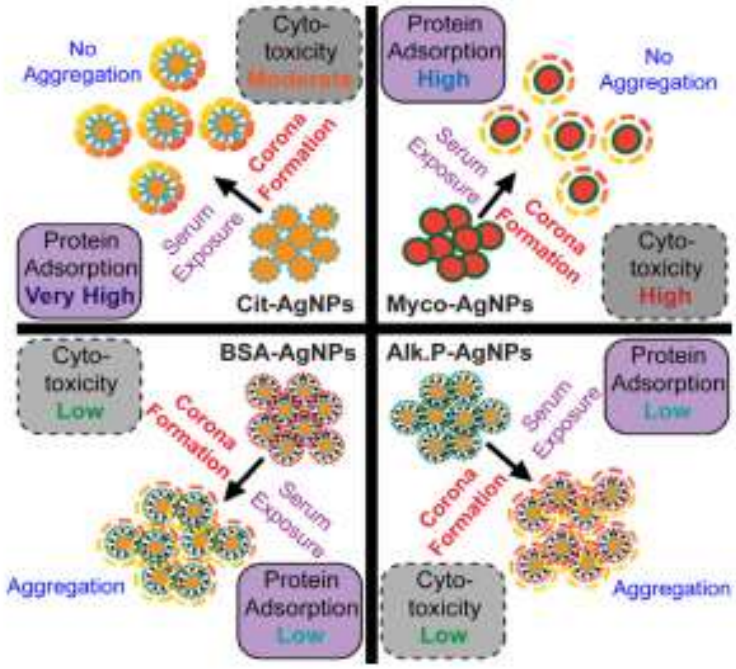


Figure 6: Protein and NP interaction [47]

6. Toxicity and Cytotoxicity of Some Nanoparticles

6.1. Aluminium-based nanoparticles (Al NPs)

Aluminium nanoparticles (Al NPs) are well-utilised nanoparticles in biological fields. However, they are known to exhibit toxicity potentials. The interaction between Al_2O_3 NPs and tau protein using cd spectroscopic and fluorescence methods was investigated at low 10 um/mg concentrations [45]. The result showed that a static complex formed from tau folding towards a packed structure. Also, the NPs bound to the hydrophilic residues of the tau segments and stimulate specific marginal structural folding of the part after molecular docking and dynamic molecular examination. The cellular experiments revealed that Al_2O_3 NPs could cause cell death by causing membrane leakage, activating caspase-9/-3, and inducing apoptosis and necrosis. At a higher concentration of 200 - 400 µm/mg recorded under different laboratory conditions, Radziun [46] tested the effect of Aluminium nanoparticles (Al NPs) after exposing the cell culture to Al_2O_3 for 24 hours. Their result showed no increase in apoptosis or decrease in cell viability, but Al_2O_3 easily penetrated the L929 and BJ cells. More so, according to Balasubramanyam, aluminium oxide (Al_2O_3) NPs (30-40 nm) displayed dose-dependent genotoxic effects after genotoxicity testing. Rat blood cells were used to perform a comet assay and a micronucleus test to determine genotoxicity. Another study utilising a mouse lymphoma cell line found that aluminium oxide (Al_2O_3) nanoparticles (50 nm) generate genotoxic effects in the form of DNA damage without being mutagenic. There are only a few *in vivo* studies that have considered this aspect of NPs.

6.2. Silver Nanoparticles (Ag NPs)

Silver nanoparticle is classified as one of the most utilised nanoparticles. A report showed that Zebrafish embryos exposed to silver nanoparticles (AgNPs) by injection resulted in shorter bodies, slower heartbeats, and abnormal motions [54]. Ag NPs are used in various consumer products, releasing them in an aquatic and terrestrial environment. Dissolved Ag is deposited, causing harmful effects on marine creatures such as bacteria, algae, and fish. Ag NP was recorded to elevate the cellular levels of toxic Ag^+ ions, causing overproduction of reactive oxygen species (ROS) and inducing caspase-dependent apoptosis via direct effects on mitochondrion [13, 55]. Qusan *et al.* [15] confirmed this when they found

that Ag NP caused ER stress response in retinal cells by increasing transmembrane ER stress sensors and cleaved ATF6 of ROS, and these species caused oxidative stress. According to in vivo investigations, silver nanoparticles with 10 to 100 nm and a concentration of 5 to 10 mg/mL are more hazardous to mitochondrial activities.

6.3. Carbon-based Nanoparticles

Carbon nanotubes are used for various purposes, including catalyst support, coatings, nano-porous filters, solar collectives. They have also been recorded to combine with some polymers in the biological field to regenerate tissues and more. CNTs are used in agriculture to control fish disease. However, concerns have been raised about how safe this system is for fish. Gao *et al.* [56] studied the toxicity effect of a single-walled carbon nanotube functionalised with amine (NH2 f - SWCNT) deposited in water and fish nutrient. After the test, changes in fish histology and the concentration-dependent oxidative stress were observed. The experiment was time-dependent and dose-dependent; tissue injuries resulted in inflammation and cell apoptosis. Furthermore, Fang *et al.* [57] proposed that the presence of two different NPs in an environment would most likely affect the properties of the other.

Again, human alveolar cancer cell lines, conventional human bronchial epithelial cell lines, and human keratinocytes cell lines were used to study the effect of carbon nanotube. The result showed that the toxicity effect of carbon nanotube was size-dependent. Furthermore, compared to single-walled carbon nanotubes, which were readily taken up by macrophages, multi-walled carbon nanotubes produced carcinogenic effects like asbestos after injection into the peritoneal cavity in rats. The toxicity and biological response of cells in the case of carbon NPs are determined by their size, mode of synthesis, and presence of trace metals. Carbon-based nanomaterials known as fullerenes are a form of carbon-based nanomaterial. They are widely distributed in our surroundings because of fuel burning. Non-functionalised fullerenes C60 are widely dispersed throughout the body, with long-term accumulation in the liver, kidney, bones, and spleen. After incubating fullerenes (1 ng/mL) with Chinese hamster ovary cells, human epidermoid-like carcinoma cells, and human embryonic kidney cells (HEK293) for 80 days, in vitro experiments revealed that fullerenes cause DNA strand breaking, chromosomal damage, and micronucleus production. According to a different study, fullerenes do not affect DNA strand breakage as measured by the comet assay. These discrepancies in results could be due to the various experimental settings used [58].

Table 4 describes the toxicity patterns, concentration, and particle size-dependent toxicity. The bigger the particle, the more likely they are to form agglomerates and accumulate. In contrast, smaller particles interact with the biological system by penetrating the membrane, leading to apoptosis.

Table 4: Cytotoxicity of some Nanoparticles

NPs	Precursor material	Method of synthesis and stabilisation	Morphology Size (nm)	Concentration (mg/mL)	specimen	Effect	Method of detection	References
Al_2O_3	$Al(NO_3)_3.9H_2O$	Sol -Gel method	Cyrystalline Size- 92.73 ± 8.95	0,5,10, 20	*In vitro*	Tau folding and neuronal cell death.	fluorescence and CD spectroscopic methods	[45]
Al_2O_3	Et3Al + Aluminium alcoholate (iPrO)3Al + air	Thermal decomposition	Nanosphere Shape Size- 50–80	10, 50 and 0.4	*In Vitro*	Penetrate through membranes of L929 And BJ Cells.	EZ4U assay	[46]
MWCNTs, with a CNT content >90%,	purchased	Unidentified	Nanotubes 10-20 height	0.033.8 and 0.169.2	*In vivo*	Oxidative stress and cell membrane damage	SEM TEM	[57]
MWCNTs XFM4 X7FM22 XF8M34	purchased	Unidentified Fbs	Nanotubes 4, 22 And 34 diameter	32	*In vitro* (human endothelial cells)	Cell viability, er stress	TEM	[35]
Gold NPs	AuNPs@citrate	Turkevich synthesis	Crystalline, monodispersed and nanospheres size- 45 - 55	0.2	*In vivo*	Cell proliferation affected by observed changes in nucleic	CyQuant assay MTS assay	[59]
AgNPs	purchased	Unidentified antibodies	Nanospheres 6.3	0.005	*In vitro* (human cells)	Er stress and chromatin condensation	TEM	[15]
Gold	Sigma-Aldrich	Turkevich method polyethyleneimine (PEI)	20 And 50	0.1	*In vitro*	accumulation of particles agglomerate formation	MTT and NR	[32]

Gold NPs	$AuNP_{20}$	Seeding-growth method polyethylene glycol (PEG)	Monodisperse 50	0.5	*In vivo*	Accumulation in the digestive tract		[49]
Silver NPs Ag@Ag	Diamine silver nitrate complex ($[Ag(NH_3)_2]NO_3$)	Turkevich synthesis	Spherical, well dispersed height-46, width-51	0.0625 – 32 (for translocation)	*In Vitro*	Accumulation, persistent settling of nanoparticles on the intestine oxidative stress	MTT and NR assays	[49]
AgNPs	purchased	Unidentified ultrasonication	1.06 – 10	1e-4	*In vivo*	Gill histopathology observed high mortality rate on juvenile carps	TEM	[60]
AgNPs	purchased	Unidentified Fetal Calf Serum	nanospheres, about 100	10	*In vitro* (mice)	also impaired the release of the ovarian steroid hormone progesterone	RT-PCR	[61]
TiO_2	Purchased	Unidentified ultrasonication	Anatase/rutile, 99þ%, 20	0.01	*In vivo* (carp)	low mortality rate observation on juvenile carps weight loss on exposure to Tio_2-	TEM	[60]
TiO_2	Purchased	Unidentified Fetal calf serum	Rutile and anatase, 102	0.01, 0.1, 1 or 10	*In vitro* (mice)	Suppress ovarian cell proliferation and apoptosis.	RT-PCR	[61]
Fe NPs (SPIONs)	$Fe(acac)_3$	Thermal decomposition DHP	30.0	0.08	*In vitro* (cancer cell)	ROS generation, leading to cell death	Fluorescence method	[57]

7. Conclusion and Recommendation

This review highlighted the majorly recorded causes of cytotoxicity and toxicity effect of some nanoparticles. It was observed that regular biological responses such as cell apoptosis, protein and particle interactions could lead to DNA disturbance, which, if left unchecked, would lead to a genetic mutation. Still, many kinds of research have been carried out on these interactions to contextualise the possible effects of exposure and how they can aid in the fight against medical-related cases such as cancer. The observed leading causes of nanoparticle toxicity are their most potent characteristics, such as size, shape, surface functionalisation, surface charge, and synthetic methods from literature so far. Also, the negative nanoparticle-protein interactions may be due to wrong surface functionalisation, free energy, curvature, bigger particle shape and size, which interacts with protein folding processes and causes them to lose structural integrity. The observed ROS generation was indicated as one of the most recorded responses to nanoparticle – cell interaction. Hence, contextualisation of the patterns of toxicity and cytotoxicity of nanoparticles would aid future research.

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