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Effects and Mechanism of Nanotoxicity:An Overview

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Abstract:

Nanotechnology is a rapidly developing field of 21st century, which has provided groundbreaking discoveries in many areas, including the medical, industrial, and consumer sectors. The unique characteristics of engineered nanoparticles (NPs) have paved their way in a variety of applications. However, these novel properties of NPs are filled with concerns for their potential toxicity. At present, more than a hundred different types of NPs are known, but there is no well-defined guideline to estimate their potential risk. Information concerning their safety is urgently needed and that is why for the past few decades, there has been a remarkable increase in this research area of the field of nanotoxicology. NPs have been studied intensively for their cell toxicity, genotoxicity, and immunotoxicity. One of the widely accepted mechanism of nanotoxicity is the generation of reactive oxygen species (ROS). Present review selectively focuses on the harmful effects and mechanisms of NP toxicity.

Key words

Nanotoxicity; Reactive Oxygen Species; Genotoxicity; Metal Nanoparticles

1. Introduction

Engineered nanoparticles are commercially produced materials which have at least one dimension less than 100 nm. Due to their small size and greater surface-to-volume ratio, they have not only gained the interest from pharmaceuticals but it contributes to almost every field of science, including physics, materials science, chemistry, biology, computer science, and engineering. Notably, in recent years nanotechnologies have also been applied to human health with promising results[1]. The majority of nanomaterials (NMs) in use comprises of carbon, silver, iron oxide, zinc oxide, and titanium oxide. Today, it is estimated that over 800 NPs incorporated products are available for consumer use[2,3]. By the year 2024, the impressive growth of global nanotechnology industry is expected to increase to US$ 125 billion[4]. The rate at which nanoindustries are growing and new types of NPs are developing far exceeds the rate of scientific studies which has been conducted to express the potential of NPs toxicity and their effects.

The “Amara’s law” states that “We tend to overestimate the effect of a technology in the short run and underestimate the effect in the long run”[5]. Meanwhile, there’s no denying the advantages nanotechnology offers us however we also can’t disregard the fact that it contributes to the production of a new type of waste. There is little to no literature available on how to handle discarded NMs and right now all NMs are regulated as non-hazardous waste[6]. Toxic NPs already occur in the environment as a by-product of the combustion of fuels, volcanic activity, photochemical reactions, and forest fires and with chemically engineered NPs we are adding more to the problem[7]. Inhalation of NPs can cause oxidative stress and pulmonary inflammation. Occupational exposure to titanium dioxide (TiO_2), silicates dust particles, asbestos fibers, and carbon black NPs can lead to cytotoxicity, inflammation, fibrosis, and oxidative injury[8,9,10,11]. NMs also possess carcinogenic risk although the potential of their toxicity may depend on the type of NP, their reactivity, retention time, and distribution in the body. This article gives an overview of some applications of different NPs, their potential side effects, and the mechanism of their toxicity.

2. Applications of Nanoparticles

Nanotechnologies are increasingly used in health care industries for diagnosing, monitoring and treating central nervous system diseases in humans. They’re also used as nanodrug delivery systems, nanoelectromechnical systems, and NPs in CNS and molecular imaging. Nanomedicines helps in limitating untoward side effects by delivering low molecular mass compounds, recombinant DNAs, and proteins to focal areas and tumors, thereby maximizing the benefit. NPs are also extensively used in cosmetic industry due to their anti-oxidant properties and ability to penetrate skin layers. Silver, titanium, and zinc NPs are used in producing wide range of products including deodorant, soap, toothpaste, shampoo, hair conditioner, sunscreen, cosmetic cream, foundation, face powder, lipstick, blush, eye shadow, nail polish, perfume, and aftershave lotion[7].Among these, silver nanoparticles (AgNPs) have been studied extensively for their anti-microbial activities .

AgNPs are used in wide variety of products including detergents, shampoo, soaps, toothpastes, sprays, air sanitizers, wet wipes, face masks, air filters, vacuum cleaners, washing machines, cellular phones, coatings of refrigerators, food storage containers, and even in liquid condoms[7,12,13]. AgNPs are also been studied for potential anti diabetic and anti-cancer drug candidate[14]. Magnetic nanoparticles, such as iron and iron oxide, have unique electrical, magnetic, and optical properties which are being tested in different biomedical applications like gene cloning, biosensors, drug delivery, DNA separation, and magnetic resonance imaging[15,16]. Titanium oxide (TiO_2) and zinc oxide (ZnO) nanoparticles are used in cosmetic and textile industries for their UV blocking, self-cleaning, and anti-bacterial properties[17,18,19]. Sulfur nanoparticles are used to enhance the electrochemical activity of lithium batteries. They have also shown antibacterial and anti-cancer activity[20]. Industrial applications of copper nanoparticles (CuNPs) include gas sensors, catalytic processes, high-temperature superconductors, solar cells, et cetera. Furthermorein medical, CuNPs are used in wound dressing[21,22].

Passivated gold nanoparticles (AuNPs) are used in bioimaging, drug delivery, and surface-enhanced Raman spectroscopy (SERS)[23]. Metallic NPs especially AuNPs are used in photodynamic therapy (PDT) of cancer[24]. NPs are also used in vaccines where core and antigen are attached to them. Aluminum nanoparticles are used as

adjuvants for tetanus, diphtheria, and influenza type b vaccines[25]. Despite aluminum NPs, gold NPs, silicon NPs, and calcium NPs are also promising candidates for vaccine applications. Carbon nanoparticles are used in bioimaging and as potent photocatalysts in energy conservation applications[26]. Due to the chemical and thermal stability of inorganic NPs, they are used as contrast agents in magnetic resonance imaging (MRI).

3. Toxic Effects of Nanoparticles

Engineered NMs are already been used by the medicine, cosmetic, electronics, and clothing industries, and with more than 800 nanotechnology based products available in the market, the global production of nanoparticles was expected to reach 58,000 tons by the year 2020[27,28]. And with their ability to penetrate living cells, translocate within the body and affect the function of major organs, NPs seem to be more toxic than microparticles[7]. Due to their small size and high surface area, many NPs are able to bypass the blood-brain barrier[29]. Chemically synthesized NPs can induce genotoxicity and increase oxidative stress in plants[30]. They can also enter human and animal's lungs, liver, spleen, and brain through gastrointestinal, pulmonary, or skin exposure[31,32,33].

Human lung alveolar epithelial cells (A-549) when exposed to iron oxide nanoparticles (Fe_3O_4-NPs) for 24 hours have shown decreased cell viability and altered cell morphology[34]. In vivo studies have shown injuries to mice kidney, spleen, and liver when exposed to 23.5 nm of copper[35]. Undergoing studies have shown toxicity of NPs depends on various factors like their size, concentration, duration of interaction, and stability. Inhalation of zinc and copper NPs can lead to myalgia, cough, dyspnea, and leukocytosis[36]. TiO_2 NPs, which are used in cosmetics are very harmful when they're long, wire-shaped, and fibrous. Another NP used in cosmetics is silver which is proved to be harmful to animals and plants. Cytotoxicity of AgNPs is proved to be higher in murine macrophage cell line than that of asbestos[37]. Their inhalation can lead to their migration to the olfactory bulb as well as their translocation to the heart, lungs, and kidney[33,38]. AgNPs have also been found in the colon of patients suffering from colon cancer and in the blood of patients suffering from blood cancer[39].

Superparamagnetic iron oxide nanoparticles (SPIONs) are used for MRI. Due to their paramagnetic properties and low toxicity below 100 mg/mL, they are the only clinically approved metal oxide NP[40]. Despite their use in tissue engineering, chelation therapy, and targeted delivery of genes or drugs in MRI, they seem to be cytotoxic[41]. They can impair the functions of DNA, nucleus, and mitochondria[28,42,43]. Their toxicity depends on various factors like the oxidation state of iron, chemical composition of the cell medium, type of surface coating, and its breakdown product. SPIONs show cytotoxicity when exposed to high levels (>100 mg/mL) and Fe^{3+} ions are more potent in inducing DNA damage than Fe^{2+}.[44]. The presence of SPIONs can cause inflammation, impaired mitochondrial function, and increase of micronuclei which is a measure of genotoxicity[45]. Some NPs used for MRI were also found to have teratogenic effects in rats and rabbits.

Zirconium dioxide nanoparticles (ZrO2 NPs) can intensify the expression of viral receptors and makes macrophages hyper-reactive to infections[46]. Small nanoparticles can cause epithelial cell proliferation, fibrosis, emphysema, and inflammation which can ultimately lead to tumors of the respiratory tract[47,48,49]. NP toxicity can cause oxidative stress, inflammation, inhibition of cell growth and cell death[50,51,52].NPs don't only accumulate in organs, cells and

tissue but also enter cell organelles. Due to the small size of NPs, they can enter the brain, and cause neurological diseases. Metallic NPs which can be oxidized, reduced, or dissolved can lead to cytotoxicity and genotoxicity. Their chemical destabilization can cause the direct release of metals and can produce free radicals that can further induce oxidation stress[53,54].

Till now there is only a little research on the toxicity of NPs. Despite their potential and promising applications in various fields, their detrimental effects have become the topic of concern. Both engineered and incidentally, unintentional release of NPs in the human body have shown severe effects on various cells and organs. But to overcome the harmful effects of nanoparticles, we must first understand the mechanism of their toxicity.

4. Mechanism of Nanotoxicity

NPs can enter the human body through inhalation, skin contact, or ingestion. Particles ranging from 10-20 nm in size are easily captured by the nasopharyngeal tract[55]. Some NPs are able to penetrate the upper layer of the epidermis at hair follicles and broken skin[38,56,57]. Gastrointestinal barrier is not effective against the dietary consumption of particles smaller than 20 μm[58]. NPs which enter the body through digestive tract, sensory, or olfactory nerves can translocate into different organs and can harm the central nervous system (CNS) and induce neurotoxic effects[59]. The intake of NPs by cells is done through the active mechanisms of internalization; also known as the Trojan horse effect. The taken-up metal and metal oxide NPs release ions to cause intracellular toxicity in the cells. The acidic environment of lysosomes sets off the release of toxic ions like Ag^{+}, Cd^{2+}, $Fe^{2+/3+}$, and $Au^{1+/3+}$ and thus this mechanism is called the "lysosome-enhanced Trojan horse effect" (LETH)[60].

The toxicity of NPs also depends on their size, shape, and surface chemistry. For example, NPs smaller in size can enter and exit a cell more easily. NPs which are spherical in shape are more certainly to be internalized in the cells than NPs which are cylindrical. Endocytosis shown by positively charged NPs is way higher than neutrally or negatively charged NPs[61].NPs toxicity has been shown to induce oxidative stress in tissues by the production of reactive oxygen species (ROS). During the process of ATP synthesis in mitochondria of cells, some amount of oxygen is not reduced completely, thus resulting in the formation of oxygen-containing radicles. NPs deposited in the organs can generate ROS which can induce oxidative stress and make cells incompetent to perform physiological redox-regulated functions[62]. Excessive ROS production causes oxidative modification of proteins, DNA damage, activate inflammatory signals which result in apoptosis, necrosis, and genotoxic effects[63,64]. When free radicals and oxidants both are present at the reactive surface of NPs, it can produce reactive oxygen species. Metals or metal oxide NPs can also produce ROS by redox cycling on the metal NPs surface or through serving as catalysts in Fenton-type and Haber-Weiss reactions[65,66,67].

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \bullet OH + :OH^{-}$

Fenton reaction

$Cu^{+} + H_2O_2 \rightarrow Cu^{2+} + \bullet OH + OH^{-}$

$Ag + H_2O_2 \rightarrow Ag^+ + \bullet OH + OH^-$

Fenton-like reaction

$Fe^{3+} + \bullet O_2^- \rightarrow Fe^{2+} + O_2$

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \bullet OH + OH^-$

Haber-Weiss cycle reaction

Along with the formation of ROS and free radicals in the cells, NP cytotoxicity can also be induced by damaging the mitochondria and its metabolism, perforating the cell membrane, and activating the synthesis of inflammatory mediators. NPs present in mitochondria can induce depolarization of the mitochondrial membrane; impair electron transport chain, and activateother NADPH- like enzyme system. Moreover, activation of immune cells like macrophages and neutrophils can lead to the generation of reactive oxygen and reactive nitrogen species[68].

Another course of action of NPs to cause inflammation, cell dysfunction, and cytotoxicity is through the release of pro-inflammatory factors by activation of microglial cells[69]. NPs are also considered as an unconventional source of autophagy. It can be caused in two ways: NP-mediated ROS-dependent autophagy and NP-mediated lysosome-dependent autophagy. Exposure to ZnO NPs can give rise to oxidative stress in macrophages and can cause autophagy and apoptosis[70].NPs are also responsible for disturbing intracellular transport and cell division by damaging the components of the cytoskeleton. Other mechanisms of NP cytotoxicity include accelerating mutagenesis through DNA damage and disturbing the in and out flow of substances in the cell by changing the structures of proteins.

The physical and chemical properties of NPs also play a major role in NP toxicity. The intrinsic properties of nanomaterials like shape, molecular size, surface area, surface coating, solubility, surface charge, and oxidation status leads to cell toxicity. Size is inversely related to the toxicity of NPs. Smaller the size, greater the surface area, and large surface-to-volume ratio means high catalytic activity. Studies have shown how the small size of NPs allows them to easily enter cells and penetrate biological barriers. For example, AuNPs of size up to 6 nm were found in the cell nucleus whereas NPs sized between 10-16 nm were only able to penetrate the cell membrane[71]. AuNPs 1.4 nm in size were found to be 60 times more toxic than AuNPs 15 nm in size. De Jong *et al.* have demonstrated that after intravenous administration in rats, the NPs sized 50-250 nm were found only in major organs like the liver and spleen but smaller sized NPs (10 nm) were widely distributed in different organs[72].

The shape also plays a great role in NP toxicity. Spheres, cylinders, sheets, cubes, ellipsoids, and rods are typical shapes of NPs. Needle-shaped and short rod-shaped hydroxyapatite nanoparticles (HAP NPs) are more likely to inhibit the growth in primary rat osteoblasts and induce apoptosis than spherical and long-rod-like NPs[69]. But shape is not always a contributing factor in NP toxicity. Zhao *et al.* compared different shaped HAP NPs (needle-like, rod-

like, plate-like, and spherical) on cultured BEAS-2B cells and showed that needle-shaped and plate-like NPs were responsible for more cell death than spherical and rod-like NPs[73].

The chemical composition is yet another factor that influences NP toxicity. After the degradation of NPs, their toxicity depends on environmental conditions like Ph or ionic strength. Particle dissolution of nanomaterials in biological system results in ionic species generation, which can cause toxicity[74]. Some metal ions are highly toxic (Ag and Cd) while some are only toxic when present at high concentrations. For example, iron and zinc are biologically useful but can damage cellular pathways at higher concentrations. Zhu et al. compared the toxicity of nano-CdO, nano-CuO, and nano-TiO_2 and found CuO nanoparticles to be the most likely to cause DNA damage followed by nano-CdO and nano-TiO showed very low cytotoxicity[75].

Apart from the size, shape, and chemical composition of the NPs, surface charge also plays an important role in their toxicity. For example, positively charged NPs can easily enter cells as compared to negatively charged and neutral NPs, resulting in high toxicity[76,77]. To decrease the toxicity of NPs, shell is often used. Shell onto the surface of NPs changes their optical, electrical, and magnetic properties. It also improves the biocompatibility of NPs and their solubility in biological fluids by increasing stability and decreasing aggregation capacity[78,79].

5. Conclusion

Nanotechnology is an emerging field and has enormous potential and use in variety of sectors. NPs may be considered as the 21st century equivalent of fire; as they both possess helpful as well as dangerous properties at the same time. As the production of engineered NPs has been increasing exponentially, so does the possible unpredictable negative consequences of their toxicity. Proper design of nanomaterials is needed to establish the fact that they are safe for human body and leave minimal carbon footprint. A number of approaches of NPs designing with decreased levels of toxicity are already available. Green synthesis of NPs is one such method, which is sustainable, inexpensive, and mostly free of contaminants. Although many methods have emerged for studying the NP toxicity and its mechanism, we need more precise techniques which can overcome the existing limitations. A relevant experimental model to evaluate their impact on biological systems and advanced research should be done to design NPs, which have little to no negative effects. A full understanding of nanotoxicity will help us ensure that NPs are made and disposed safely and the products that utilize NPs are exploited to their full potential.

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