Advanced Pharmaceutical Bulletin

Adv Pharm Bull, 2015, 5(4), 447-454
doi: 10.15171/apb.2015.061
http://apb.tbzmed.ac.ir

Review Article

A Review of Molecular Mechanisms Involved in Toxicity of Nanoparticles

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Article info
Article History:
Received: 4 February 2015
Revised: 20 August 2015
Accepted: 25 August 2015
ePublished: 30 November 2015

Keywords:
· Oxidative stress
· Reactivity oxygen species
· Cytotoxicity
· Nanoparticles
· Mechanism
· Prooxidant effects

Abstract
In recent decades, the use of nanomaterials has received much attention in industrial and medical fields. However, some reports have mentioned adverse effects of these materials on the biological systems and cellular components. There are several major mechanisms for cytotoxicity of nanoparticles (NPs) such as physicochemical properties, contamination with toxic element, fibrous structure, high surface charge and radical species generation. In this review, a brief key mechanisms involved in toxic effect of NPs are given, followed by the in vitro toxicity assays of NPs and prooxidant effects of several NPs such as carbon nanotubes, titanium dioxide NPs, quantum dots, gold NPs and silver NPs.

Introduction

Nanotechnology advancement in medical sciences led to the design and synthesis of nanostructures for biomedical applications. Due to unique properties of NPs such as small size (1-100 nm in diameter) and the greater surface area to volume ratio as well as different electronic, magnetic, optical and mechanical properties and also particle shape, these particles hold great interests in the various fields.[1-6]

It may seem that NPs do not have toxic effects. However, the greater surface area to volume ratio of these particles causes their higher chemical reactivity and results in increased production of reactive oxygen species (ROS). Indeed, the NPs surface area is a key factor in their intrinsic toxicity because of the interaction of their surfaces with biological system.[7-10]

ROS formation is one of the mechanisms of NPs toxicity which could cause oxidative stress, inflammation and consequent damages to the proteins, cell membrane and DNA. Therefore, assessment of nanoparticles toxicity is necessary in biomedical applications including drug delivery systems, gene delivery and therapeutic applications.[11-14]

Prooxidants are chemicals that induce oxidative stress through either creating reactive oxygen species or inhibiting antioxidants. NPs react with cells and induce their prooxidant effects via intracellular ROS generation involving mitochondrial respiration and activation of NADPH-dependent enzyme systems.[15-17]

NPs can activate the cellular redox system specifically in the lungs where the immune cells including alveolar macrophages (AM) and neutrophils act as direct ROS inducers. Professional phagocytic cells of the immune system including neutrophils and AMs induce remarkable ROS upon internalization of NPs via the NADPH oxidase enzyme system.[16,18]

In this review, we have focused on introducing in vitro toxicity assays for cytotoxicity assessment of nanoparticles. We have also reviewed toxic effect of several nanoparticles such as carbon nanotubes, titanium dioxide NPs, quantum dots, gold NPs and silver NPs.

Cytotoxicity assays of nanoparticles

Cytotoxicity assays are classified as in vivo and in vitro tests. In vivo toxicity assays (cell-based assay) are time-consuming and expensive and involve ethical issues but in vitro toxicity tests (cell cultured-based assay) are faster, convenient, less expensive and devoid of any ethical issues. Due to these advantages, in vitro assays are the first choice for toxicity assessment of most nanomaterials.[19]

In vitro methods include approaches for assessment of integrity of the cell membrane and the metabolic activity

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of viable cells. Evaluation of cell membrane integrity is one of the most common approaches to measure cell viability. It is based on the leakage of substances such as lactate dehydrogenase (LDH) that normally reside inside cells to the external environment and the measurement of LDH activity in the extracellular media. Alternatively, membrane integrity can be determined by penetration of dyes such as trypan blue and neutral red into the damaged cells and staining intracellular components. These dyes cannot enter living cells. Metabolic activity of viable cells could be determined through colorimetric assays, such as the MTT and MTS assays.[20-23]

Bioluminescent methods including methods using luciferase, which catalyzes the formation of light from adenosine triphosphate (ATP) are also commonly used as cell viability assays in which the number of surviving cells is determined by measuring the uptake and accumulation of neutral red dye and trypan blue after exposure to the toxicant.[24-26] Among in vitro methods, LDH, MTT and MTS assay are most widely used for assessment of nanoparticles cytotoxicity (Table 1).[27]

LDH test

In general, LDH test is a colorimetric assay that quantitatively measures LDH, a marker of cell membrane integrity, released from damaged cells into the culture media. This assay is a fast, simple and reliable method for determining cellular toxicity.[28]

MTT assay

MTT assay is another candidate assay for measurement of cytotoxicity of NPs. 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide, (MTT), is a yellow substance which reduces to purple insoluble formazan crystals by mitochondrial succinate dehydrogenases in viable cells. This method is directly related to the number of viable cells.[29]

MTS assay

In the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay, viable cells will convert tetrazolium salt into a colored soluble formazan product by mitochondrial dehydrogenase enzymes. Indeed, in MTS assay, similar to MTT assay, a colorimetric product is formed. The formazan produced is directly proportional to the number of living cells in the culture.[30]

Toxicity mechanisms of nanoparticles

Physicochemical reactivity of NPs lead to the formation of free radicals or ROS including superoxide radical anions and hydroxyl radicals direct or indirect through activation of oxidative enzymatic pathways result in oxidative stress (Figure 1).[31-36] In general, there are several sources for oxidative stress:

- Oxidant-generating properties of particles themselves as well as their ability to stimulate generation of ROS as a part of cellular response to nanoparticles
- Transition metal-based nanoparticles or transition metal contaminants used as catalysts during the production of non-metal nanoparticles.
- Relatively stable free radical intermediates present on reactive surfaces of particles.
- Redox active groups resulting from functionalization of nanoparticles

The following briefly introduces cytotoxicity of some of nanoparticles such as carbon nanotubes, titanium dioxide NPs, quantum dots, gold NPs and silver NPs.

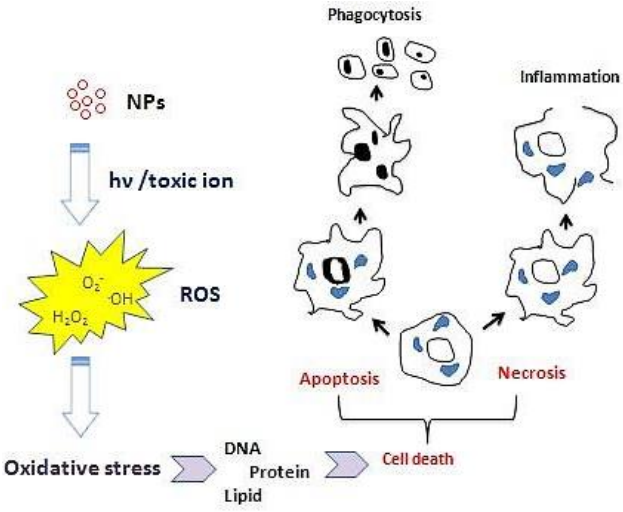

Figure 1. ROS generation induced by NPs and their cytotoxicity mechanism.

Cytotoxicity of carbon nanotubes

Carbon nanotubes (CNTs), fiber shaped nanostructures, are allotropes of carbon which are categorized as single wall carbon nanotubes (SWCNT) and multi wall carbon nanotubes (MWCNT). In addition to industrial uses, carbon nanotubes, due to their unique electrical, physical and thermal qualities hold great interest in biomedical applications.[37-39]

Numerous reports have shown that CNT could induce the ROS generation in
multitudes of cell lines and activation of ROS-associated intracellular signaling pathways in a dose-dependent manner such as mitogen activated protein kinase (MAPK), activator protein-1 (AP-1) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) in mesothelial cells.[40-43]

It has been reported that MWCNT are able to stimulate the release of the cytokines, IL-1β, TNF-α, IL-6 and IL-8 from mesothelial cells and macrophages. Murphy et al. demonstrated that direct exposure to MWCNT causes to length-dependent cytokine release from macrophages but not mesothelial cells. However, treatment of the mesothelial cells with conditioned medium from CNT-treated macrophages led to increased secretion of cytokines. In another study, MWCNT were revealed to trigger the macrophages to produce TGF-β1 and platelet-derived growth factor (PDGF) that promoted the

transformation of lung fibroblasts to myofibroblasts, a major factor in development of fibrosis.[44]

Cytotoxicity of TiO_2 nanoparticles

Widespread applications of titanium dioxide nanoparticles (TiO_2 NPs) in consumer products including cosmetic, paints, pharmaceutical preparations, food additives and so on is a result of their ability to confer opacity and whiteness.[45,46] In recent years, the photocatalytic killing effect of TiO_2 NPs on cancerous cells has received great attention.[47-49]

The potential mechanism of cytotoxicity induced by these non-soluble metal oxide NPs are still controversial. In some literature, these NPs are even considered as a natural nanomaterial.[50] Conversely, some reports have pointed out the potential toxicity of TiO_2 nanoparticles, including their ability to induce oxidative stress, genotoxicity and immunotoxicity.[51,52] However, the mechanisms of these toxic effects are still blurred but cytotoxicity evaluation of these metal oxide NPs is important for in vivo and in vitro studies. Despite other NPs such as ZnO, quantum dots and so, TiO_2 NPs do not release toxic ions hence toxicity of these particles could be attributed to the size-dependent interaction between nanoparticles and intracellular biomolecules adsorbed onto nanoparticles.[53-55] These interactions result in generation of ROS, mitochondrial depolarization, plasma membrane leakage, intracellular calcium influx and cytokine release.[56-59]

In a study, Xiong et al. investigated size influence of TiO_2 NPs on their phototoxicity. Results showed that there was a converse relationship between phototoxicity and the size of these particles; as, the mortality of the cells treated with 10 nm TiO_2 NPs after photoactivation by UV light was significantly higher than that of the cells treated with larger particles (20 and 100 nm particles). Furthermore, cytotoxicity of non-photoacivated 10, 20 and 100 nm NPs was not inconsiderable for cells treated with them. In addition, the treated cells with 10 nm photoactivated particles demonstrated a higher generation of mitochondrial superoxide in comparison to 20 and 100 nm particles.

Indeed, the higher cytotoxicity induced by smaller particles is related to their higher surface area and hence contain a larger number of surface-exposed TiO_2 molecules. Phototoxicity of these NPs could be decreased via surface coating with chitosan or PEMA because of the prevention of biomolecule adsorption and hydroxyl radicals (·OH) production in the photoactivation process.[54]

In another study, size-dependent toxicity of both TiO_2 and PLGA was investigated. Findings revealed that biomedically used PLGA nanoparticles did not show strong cytotoxic effect in comparison to TiO_2 nanoparticles. However, the smaller PLGA nanoparticles have the potential to trigger the release of TNF-α. 200 nm PLGA nanoparticles could not trigger any negative response from cells. Higher cytotoxic effect was observed in cells treated with TiO_2 nanoparticles, especially at concentrations higher than 100μg/ml. The size-dependent cytotoxicity of both PLGA and TiO_2 nanoparticles could be attributed to the smaller size and larger specific surface area and thus exposure of more molecules on the surface that led to the adsorption of more biomolecules such as proteins in the environment.[60]

Cytotoxicity of quantum dots

Quantum dots (QDs), colloidal semiconductor nanoparticles, are a promising type of NPs which possess exceptional optical properties including high fluorescent quantum yield, broad absorption, narrow emission and high photostability. These properties make QDs an attractive candidate for in vivo imaging instead of fluorescent dyes.[61]

Similar to other NPs, cytotoxicity of QDs depends on parameters including size, shape, concentration, charge, redox activity, surface coatings and mechanical stability of these particles. Toxicity of uncoated core CdSe or CdTe-QDs have been investigated in some literature. Two major mechanisms are involved in the toxicity effects of these inorganic nanoparticles are as follows:[62-65]

1) Cd^{+2} ions existing in QDs structure:
   These toxic metal ions cause toxic effects through several routes such as interference with DNA repair and substitution for physiologic Zn. Cd^{+2} ions increase oxidative stress but they cannot directly generate free radicals.
2) Free radical formation:
   QDs of CdSe and CdTe are highly reactive, thus, photoactivation of these QDs via visible or UV light leads to their oxidation. Indeed, a photon of light could excite the QD and consequently generates an excited electron that transfers to molecular oxygen, forming singlet oxygen. Reaction of singlet oxygen with water/other biological molecules results in production of free radicals.

Kauffer et al. recently demonstrated that variation in core compositions and surface chemistries of QDs, CdSe QDs vs. CdS QDs, lead to their different cytotoxicity. The former produced •OH radicals immediately after light activation, while the latter required extensive irradiation to generate an equivalent amount of radicals. Therefore, the toxicity observed for CdSe QDs could be directly related to •OH radicals produced. Indeed, cytotoxicity of colloidal NPs can be controlled and relieved by choosing appropriate materials for QD core and suitable irradiation condition.[66]

Cytotoxicity of gold nanoparticles

Gold nanoparticles (GNPs), are one of the promising inorganic (NPs) that have attracted scientific and technological interests due to their ease of synthesis, chemical stability and excellent optical properties.[67-69] These unique properties of GNPs, make them appealing tools for cancer diagnosis and treatment.[70-72]

Most of in vitro studies have indicated that these NPs are nontoxic for cells. Evaluation of GNPs cytotoxicity is essential because of broad spectrum application of GNPs

in biomedical sciences. In the most of literature investigations have demonstrated that these inorganic nanoparticles are nontoxic. Cytotoxicity of GNPs depends on their size, shape and surrounding ligands.[73,74] Anisotropic GNPs have more potential oxidation than the isotropic ones due to their highly exposed surface areas and defects. Also, in some literature investigations exhibited that spherical GNPs are suitable for biomedical application.[75-77]

Recently, the cytotoxicity effects of 5 and/or 15 nm GNPs 5 and 15 nm in vitro on Balb/3T3 mouse fibroblasts have been investigated. In order to understand the observed differences in cytotoxicity of two sizes of GNPs, Gioria et al. examined the uptake and the intracellular distribution of the NPs. The results indicated cytotoxicity effects only for the cells treated with 5 nm GNPs but no toxicity was revealed on Balb/3T3 for 15 nm GNPs. This observation is due to high number of 5 nm GNPs taken-up by cells in comparison to the larger particles (15 nm particles).[78]

Cytotoxicity of silver nanoparticles

Antimicrobial properties of silver nanoparticles (AgNPs) cause to the use of these NPs in a broad spectrum of consumer products including cosmetics, electronics, household appliances, textiles, and food products.[79,80] In the recent decade, AgNPs have been used in medical fields such as drug delivery, designing biosensors, and imaging contrast agents etc.[81-83] Thus, toxicity assay is an important factor to be considered in their application for biomedical purposes. Cytotoxicity of these NPs is related to comfortable oxidation AgNPs to Ag+ ions which are very toxic for biological systems and cellular components.[84-87]

Compton and coworkers in a study showed that AgNPs in aqueous system are more toxic compared to the bulk Ag is more toxic due to the presence of dissolved oxygen, its reduction on NPs and then the release of H2O2 from AgNPs. Also, results demonstrated that ROS generation from nanoparticulated Ag are greater than that of macro (bulk) silver.[88]

Recently, in a report the size- and coating-dependent toxicity of thoroughly characterized AgNPs was investigated following exposure to human lung cells. The results revealed that only the cytotoxicity of the 10 nm particles was independent of surface coating. In contrast, all AgNPs tested caused an increase in overall DNA damage after 24 h which suggests independent mechanisms for the cytotoxicity and DNA damage. However, there was no increased production of intracellular ROS; therefore, the toxicity observed was related to the rate of intracellular Ag release. Interaction with thiol and amino groups of biomolecules and appearance of the toxicity effect on cellular components were a result of sliver release. Thus, AgNPs with higher Ag release are more toxic.[89]

Table 1. Some in vitro assays with type of NPs and cell types.

Assay	Type of NPs	Type of cells (system)	References
MTT assay	QDs	Human embryonic kidney cells	90
	TiO_2	Human erythrocyte/ lymphocyte cells	59
Natural red	TiO_2 NPs	Zebrafish embryos	91
LDH test	TiO_2 NPs	Human kidney cells	92
	CNTs	human pneumocytes cells	
MTS assay	Ag NPs	mouse embryonic fibroblasts	93
	Gold NPs	Mammalian cells	94
Trypan blue	Gold NPs	mouse fibroblast	78
	TiO_2 NPs	human lung epithelial cells	95

Conclusion

Despite the wide spread applications of nano-sized materials in various sciences areas, there are numerous reports about side effects of these materials on biological systems and cellular compartments. In addition to physicochemical properties, the production of toxic ions, fibrous structure, high surface charge and generation of radical species result in cytotoxicity by NPs including carbon nanotubes, titanium dioxide NPs, quantum dots, gold NPs and silver NPs. Both in vivo and in vitro assays are used for toxicity assessment of NPs. In vitro assays have received more attentions compared to in vivo assays due to being faster, convenient, less expensive, and devoid lacking any ethical issues.

Ethical Issues

Not applicable.

Conflict of Interest

The authors declare that they have no conflict of interest.

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