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Emerging Approaches and Perception of Toxicity Assessment in Nanomaterials

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Abstract

In the 21st century, nanotechnology, an interdisciplinary research has become an innovative field and made a new revolution in science and technology. Its unique properties has led an extensive research interest among the researchers and utilized in various fields including biomedical applications. Increased use of nanomaterials in health sciences and medicine aroused a global concern on the biological response, effectiveness, and toxicity of these materials. Therefore, it has become imperative in studying the toxicity of nanomaterial (Nanotoxicology) in therapeutic applications. The main aim of nanotoxicological studies is to determine the toxic/hazardous effects of nanomaterials on humans and to the environment. The toxicity of the nanomaterials depends on various physicochemical properties such as size, shape, surface area, surface chemistry, concentration and several others parameters. Nanomaterials have shown higher toxicity particularly in inhalation studies, hence stringent regulations are made for nanotechnology products to ensure the safety of the products. There are few approaches to overcome these toxicities and improve its therapeutic efficacy and safety. Hence development of nanotechnology should occur on par with risk assessment to identify and subsequently avoid possible dangers in the near future. This article highlights on the different nanomaterials, their unique properties and frameworks for assessing the toxicity of nanomaterials.

Keywords: Nanotoxicity; Nanomaterial; Nanoparticle; Risk Assessment; Drug Delivery; Nanotechnology

Introduction

Nanotechnology is the branch of science and technology in fabricating/synthesizing the materials at the atomic scale size ranges from 1 to 100 nm. Past few decades this nanotechnology has expanded rapidly and influences in different aspects of life. These nanotechnology products are utilized extensive in human beings for various applications [1]. The widespread entry of nanomaterials into the manifolds of life has elevated concerns regarding its safety on human health subsequently its impact on the environment. Several studies are highlighted that during absorption or inhalation can enter into various tissues and organs that lead to produce toxicity in human beings [2].

Nanotoxicology is the field of study which deals with the assessment of toxicological properties of nanomaterials and its risks associated in human beings. The concept of Nanotoxicology was established to reflect the potential uniqueness of the physicochemical properties of nanomaterials suggesting that their interactions with cells and tissues may cause health treats. Nanomaterials physical

and chemical properties such as size, shape, surface area, surface charge, pH, crystal structure, coating, solubility/dissolution and chemical composition can influence/produce the toxicity. Hence, it is necessary to assess its property and the toxicity of nanomaterials in both *in vitro* as well as *in vivo* methods [3].

Nanomaterials

Based on the size, shape and surface characteristics, the nanomaterials can be developed into smart systems, encasing therapeutic and imaging agents as well as bearing stealth property. Further, these systems can deliver drug to specific tissues and provide controlled release therapy. The size of materials is reduced to Nano-scale range (<100 nm) through nanotechnology; this is more beneficial in biomedical and clinical field [4].

The noteworthy benefits of Nanomaterial in drug delivery systems are

- Aptitude to focus on certain targets in the body.
- To reach the target spot, a less quantity of medication is required.
- Accumulation of reduced concentrations in non-target locations and other organs, resulting in fewer side effects [5].

Classification and Properties

Nanomaterials are produced by manipulation in its atomic levels with at least one dimension having size of <100 nm. Whereas these nanomaterials are in a variety of shapes and sizes, including single, fused, aggregated, and agglomerated forms with spherical, tubular, and irregular geometries. Nanotubes, dendrimers, quantum dots, and fullerence are examples of nanomaterials which are generally used [6].

Based on the dimension Nanomaterials are classified into

- Zero dimensional – The entire dimension is measured within the nanoscale. Example – Graphene quantum dots, CQDs
- One dimensional – One dimension is outside the nanoscale. Example - Thin films
- Two dimensional – Two dimensions are outside the nanoscale. Example - Carbon nanotubes.
- Three dimensional – Bulk materials. Examples - dendrimers, quantum dots, fullerenes [7].

Nanomaterials Unique Properties

The properties of microstructure materials are similar to those of bulk materials; however nanoscale materials have a different ratio of surface area to volume. This different ratio of surface area to volume can cause the surface or interfacial atoms to be larger, resulting in more "surface" dependent material properties. The surface to volume ratios of nanomaterials vary depending on their shape, demonstrating their diversity and unique features. The huge fraction of surface atoms, greater surface energy and spatial confinement are significant differences between nanomaterials and bulk materials. Unique properties of nanomaterials are [8].

- Optical properties
- Electrical properties
- Mechanical properties
- Magnetic properties

Drug Delivery System- Nanoparticles

Delivering the drugs at the specified target site has long been considered as a major goal in the drug delivery system. This nanotechnology product's advancement and adaptability has considered as a wonderful alternative one in targeted drug delivery at the proper time.

Nanoparticles, which can increase the pharmacokinetic features of medications, also avoid the body's defensive mechanism. Drug delivery nanomaterials come in a variety of sizes and shapes [5].

The size and shape of nanomaterials have an important impact in delivery of drug to the target site. Also the form of the nanoparticle can affect cellular absorption, biocompatibility, and retention of drugs in different organs and tissues. The medicine can be delivered efficiently to the target site by selecting the suitable preparation technique, appropriate polymer as a carrier, and nanoparticles of desired size and form [9].

As a result, making nanoparticles from drugs and polymers in the range of 1-100 nm has been considered as a difficult task. In drug delivery system, nanoparticle in the 1-100 nm can be prepared by

- Dispersion of preformed polymers - Solvent diffusion method, solvent evaporation method, Salting out method and Nanoprecipitation method
- Polymerization of monomers
- Ionic gelation method for hydrophilic polymers.

Nanotoxicity

Nanotoxicology is the branch of science in nanotechnology to study the toxicity of nanomaterials in human health. This nanotoxicology study is a multidisciplinary approach involving different branch of science like physics, chemistry, biology, toxicology, geology, material science, medicine,

and pharmacokinetics and exposure assessment [10]. On exposure to the nanomaterials, the biochemical and biomolecules are either directly or indirectly affected. Earlier literatures on toxicological study of nanoparticles showed higher toxicity risk than larger particle owing to its imperative properties, size, shape, and large surface area. This higher toxicity risk is due to its size, physicochemical property, chemical reactivity and biological activity [11]. In addition, some nanomaterials are localized into the mitochondria leading to the production of reactive oxygen species (ROS) and apoptosis. Few of the nanomaterials are absorbed in blood circulation which induces hemolysis [12]. Hence, recently more research works have focused towards the toxicity of nanoparticles. Presently, no specific standard method is available for assessing the toxicity of nanoparticles, hence it has become a challenging task [13,14].

Several *in vitro* methods are frequently utilized to assess the toxicity of nanoparticle to predict the response in animal and human and the observed results are found reliable.

In vitro methods are

- Cytotoxicity
- Hemolysis assay
- Lactate dehydrogenase Leakage tests

Cytotoxicity

Cytotoxicity for nanomaterials is assessed by XTT and MTT assay methods [15].

XTT Assay: XTT assay is performed to assess the mitochondrial function. XTT (sodium (2,3-bis(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide)) assay, which is a colorimetric techniques for non-radioactive quantification of cells viability, proliferation and cytotoxicity. The principle behind of this assay procedure is the reduction of XTT from yellow to an orange formazan dye by metabolically active cells. This formed formazan dye is soluble in water, which can be quantified directly using an ELISA reader [16,17].

Depending upon the number of living cells, there is an increase in the quantity of orange formazan formation which shows the increased activity of mitochondrial dehydrogenases.

MTT assay: The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is a colorimetric technique to determine the cell viability, proliferation and cytotoxicity. The principle behind this assay procedure is the reduction of MTT from yellow to purple color formazan dye by metabolically active cells contains NAD(P)H-dependent oxidoreductase enzymes largely in the cytosolic compartment of the cell. The formed formazan dye is dissolved in a solubilizing solution and the colored solution can be quantified directly using a multi-well spectrophotometer. The dark color of the solution is directly related to number of viable metabolically active cells [18].

Hemolysis Assay

The impact of nanoparticle porosity, geometry, and surface functionality on human red blood cells (RBCs) are evaluated by a hemolysis assay. The hemolytic activity provides primary information on the cytotoxicity of the nanomaterials on the healthy cells. Erythrocytes have long been used to test haemolytic activity, and are thought to be the most important target, influenced by the concentration of nanomaterials. The simple availability and isolation of cells from blood are the two advantages of employing erythrocyte as a model for haemolytic action. This feature is alike to other cell membranes.

Hemolysis assay is based on properties of the reactive constituents to elicit hemoglobin leads to release of erythrocytes (red blood cells) and their contents into the surrounding fluid (cytoplasm) by membrane damage. The hemolytic activity of the nanomaterials can be evaluated using human erythrocytes, by comparing the optical density of the nanomaterials [Nanomaterial + human erythrocytes suspended in Phosphate Buffered Saline (PBS)] with the maximal hemolytic control (Positive control + Human erythrocytes suspended in PBS) and minimal hemolytic control (PBS + Human erythrocytes). Assessment of *in vitro* hemolytic activity by UV spectrophotometric method is an easy and effective technique to assess the cytotoxicity of the bioactive molecules [19,20].

Lactate Dehydrogenase Leakage Tests

Lactate dehydrogenase (LDH) is another method for the determination of cytotoxicity colorimetrically by assessing the neuronal necrosis LDH, a cytoplasmic enzyme found stable extensively in body tissues, such as blood cells and heart muscle. The principle behind this test procedure is the release of LDH into the cell culture from the plasma membrane when there is any damage like necrosis, apoptosis, and cellular damage. The release of LDH can be determined using NADH produced during the conversion to pyruvate from lactate. This conversion can be monitored by reduction of yellow tetrazolium salt into a red formazan dye. The formed formazan dye is soluble in water, which can be quantified directly in a spectrophotometer at 492 nm. The intensity of formazan color is directly proportion to the dead or damaged cells [21].

Conclusion

In this review, three major toxicity determination assays of nanomaterials have been described. Nanotoxicology is an emerging field and it has become crucial to study nanotoxicity to understand its impact on human and environment. Nanotoxicities can be overcome if certain parameters viz. while developing new method, stable and purified model nanoparticles with less toxicity are prepared, characterizing the size of individual nanoparticles using real-time imaging tools and performing effective *in-vivo* assays for screening and probing the biocompatibility toxicity of model nanoparticles are considered. Nanotoxicity can be avoided by utilizing a biocompatible, non-toxic covering substance. Antioxidants are vital in avoiding or minimizing the damage produced by reactive oxygen species (ROS). As a result, antioxidants can help to minimize toxicity. The accumulation of NPs in various tissues and induction of toxicity are the most common forms of toxicity. At present, the cytotoxicity of nanoparticles is being used in a beneficial way for curing cancer by targeting cancerous and tumor cells. Nowadays, researchers are trying to produce safe nanomaterials employing techniques of green chemistry to reduce any toxicity. Since nanomaterials have potential wider applications in many fields, the ruptures pertinent to the investigations on toxic effects of NPs should be made targeting the biogenetic systems. Hence in the future, nanomaterials should be developed in such a way to improve its safety increasing its use as it has several applications in medicine and other fields.

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