Health Implications of Nanoparticles: A Comprehensive Review

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Abstract

Nanoparticles (NPs) are extensively utilized in the diagnosis and treatment of numerous human diseases, including autoimmune disorders and cancer. However, the cytotoxic effects of NPs on normal cells and organs remain a significant limiting factor for their clinical application. The diversity of NPs and their physicochemical properties, such as particle size, shape, surface area, dispersity, and protein corona effects, are crucial determinants of their safety and toxicological behavior. Current research focuses on identifying the targets and mechanisms of NP-induced side effects, emphasizing the patterns of NP transport, accumulation, degradation, and elimination in both in vitro and in vivo models. NPs can enter the body through inhalation, dermal contact, and ingestion. Therefore, reliable information on the effects of NPs on various organs is essential to understand their efficacy and health impact. This review consolidates the existing knowledge, aiming to better equip us to address these challenges.

Keywords: Nanoparticle, Autoimmune disorders, Physicochemical, Toxicology, Health

Introduction

Nanoparticles (NPs) are widely utilized in various industries, including electronics, agriculture, textile production, and medicine. The International Organization for Standardization defines NPs as structures with dimensions ranging from 1 to 100 nm in one, two, or three dimensions (1). NPs can be classified based on their physical parameters (e.g., electrical charge), chemical characteristics (e.g., core or shell composition), shape (e.g., tubes, films, rods), and origin (natural NPs like volcanic dust and viral particles, or artificial NPs, which are the focus of this review) (2). The primary concern limiting the use of NPs in disease treatment and diagnosis is their toxicity to living organisms (3). Researchers often encounter challenges and side effects

related to NP toxicity, making the choice of appropriate experimental models for in vitro (cell lines) and in vivo (experimental animals) toxicity assessment crucial (4). NPs can enter the body through inhalation, dermal contact, and ingestion, depending on their physicochemical properties and production methods (5). The interaction of NPs with the body can occur via respiratory, digestive, dermal, or blood pathways (6). Some NPs, such as ZnO and TiO_2, are used in health products for their UV-blocking properties, raising concerns about their health, safety, and environmental risks due to environmental dispersion (7).

Studies have shown that NPs can enter the human body through various routes, reach vital organs via the bloodstream, and cause tissue and cell damage (8). Although the exact mechanisms of NP toxicity are not fully understood, factors such as particle shape, size, dispersity, surface charge, and protein corona effects are believed to play significant roles (9). Research indicates that NPs can induce oxidative stress and activate genes involved in inflammation (10). NPs can accumulate in different tissues and organs, and some can even cross the blood-brain barrier (BBB) by interacting with CXCR6 chemokine receptors. The passage, performance, and metabolism of NPs within cells are still under investigation.

This review aims to elucidate whether NPs have destructive and toxic effects on organs or if they are safe for use. The development of safe, biocompatible NPs for diagnosing and treating human diseases requires a comprehensive understanding of the interactions and mechanisms underlying NP toxicity.

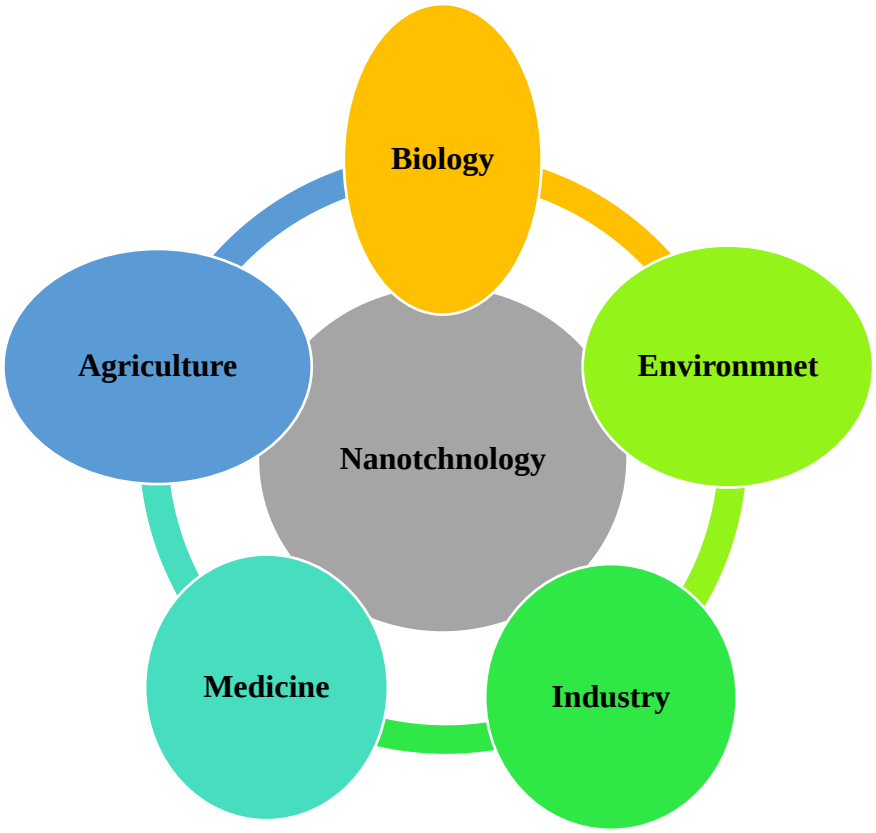


Figure 1: Nanotechnology and their application in various areas for combating issues

Nanoparticles Applications of Medical Sciences

Nanoparticles (NPs) are extensively used in medicine for both diagnostic and therapeutic purposes. In diagnostics, NPs serve as fluorescent labels for detecting biomolecules and pathogens, and as contrast agents in magnetic resonance imaging (MRI) and other imaging techniques (11). Additionally, NPs are employed for targeted drug delivery, including the delivery of protein and polynucleotide substances, photodynamic therapy, thermal ablation of tumors, and prosthetic repair (12). Various types of NPs, particularly gold and silver nanometals, have been widely used in drug delivery, disease diagnosis, and as biological sensors (13). These particles can be synthesized in different sizes and shapes with a narrow size distribution. A unique feature of NPs is their optical behavior, which changes with particle size, allowing NPs of different sizes to exhibit different colors at visible wavelengths (14). This property is useful for disease diagnosis and drug delivery.

The surface of NPs can be easily modified to bind various ligands such as sugars, peptides, proteins, and DNA (15). Iron oxide superparamagnetic NPs are a significant category of

inorganic materials used in drug delivery, prepared through chemical methods like co-precipitation or biological means using bacteria (16). These NPs can be surface-modified for direct ligand bonding, and their superparamagnetic properties enable targeted drug delivery via an external magnetic field (17). For instance, Fe3O4 (magnetite), γ-Fe2O3 (maghemite), and superparamagnetic iron oxide NPs (SPIONs) are commonly used in drug delivery, often coated with polymers such as dextran or chitosan to enhance biocompatibility (18).

Recent advancements emphasize the use of carbon nanotubes and fullerenes (Buckyballs) in drug delivery due to their size, shape, and surface properties (19). Single-wall carbon nanotubes and C60 fullerenes, with diameters around 1 nanometer, can easily pass through biological membranes and barriers, penetrating cells. These structures allow for surface engineering, enhancing solubility and biocompatibility, and facilitating the delivery of biological molecules such as proteins, DNA, and drugs. Pharmaceutical compounds can be loaded onto or inside these structures, enabling targeted and simultaneous delivery of multiple compounds.

The term "liposome" was coined in 1961 by Alec D. Bangham. These double-layer vesicles consist of an aqueous core enclosed within a lipid bilayer, typically composed of natural or synthetic phospholipids (20). The amphiphilic nature, biocompatibility, and ease of surface modification make liposomes a valuable option for drug delivery (21). Another example of lipid nanostructures is solid lipid nanoparticles (SLNs), which form a solid lipid matrix composed of triglycerides, lipids, fatty acids, steroids, and waxes, with sizes typically less than 1 μm (22). To enhance the stability of these particles, surfactants are often included in their formulation (23). SLNs can encapsulate drugs with low aqueous solubility, allowing for controlled release and targeted delivery via oral or injectable routes (24).

Polymer nanoparticles (PNPs), both natural and synthetic, are also widely used in drug delivery. These materials must be biocompatible, non-toxic, and free from leachable impurities, with appropriate physical structures and desired half-lives (25). Biodegradable polymers are often preferred due to their high stability and scalability (26). PNPs can form vesicular systems (nanocapsules) or matrix systems (nanospheres), where the drug is either encapsulated within a polymeric cavity or dispersed in a polymer matrix (27). Polymer micelles, self-assemblies of block copolymers, have a core-shell structure and exhibit properties such as low critical micellization concentration (CMC), which enhances drug solubility and stability (28). These structures offer greater mechanical and biological stability compared to liposomes, reducing macrophage interaction and providing better drug protection (29).

Hydrogel nanoparticles are three-dimensional polymer networks used to encapsulate and deliver drugs. These hydrogels swell in water or biological environments, carrying large amounts of fluids (30). Stimulus-responsive hydrogels can release drugs in response to specific environmental changes, such as temperature and pH (31). These systems have applications in delivering DNA and proteins, wound healing, biosensing, and tissue engineering.

Nanoparticle Toxicity and their Mechanisms

The surface properties of nanoparticles (NPs), such as hydrophobicity and hydrophilicity, significantly influence their biological interactions, including plasma protein binding, cellular uptake, phagocytosis, immune system stimulation, and particle removal (32). These properties result in varied cellular responses, such as adhesion, growth, and differentiation (33). NPs induce oxidative stress through physicochemical interactions with the cell membrane, generating ions that cause toxicity, which can be exploited to target cancer cells (34). Larger NPs have increased interactions with the cell membrane, leading to higher cellular toxicity (35). The cell membrane, composed of proteins and extracellular polymeric materials, is complex and dynamic (36). NPs penetrate cells through diffusion, endocytosis, and interactions with membrane proteins such as the phospholipid layer (37). Once inside, NPs are localized in endosomes and the nucleus, degraded in lysosomes, or recycled back to the plasma membrane (38). The toxicity of gold nanoparticles (Au NPs) with diameters under 100 nm has been studied, revealing that both the smallest and largest sizes (3, 5, 50, and 100 nm) induce toxicity, including apoptosis, oxidative stress, organelle and DNA damage, and mutagenesis (39). NPs primarily enter cells through endocytosis, with their toxicity largely attributed to the increase in reactive oxygen species (ROS) they generate (40).

Reactive Oxygen Species Levels in the Cell

Nanoparticles (NPs) can increase inflammatory factors such as TNF-α, IL-8, IL-6, and IL-1, ultimately causing mitochondrial damage (41). The interaction of NPs with cell surface ligands and membrane receptors is a primary route for drug delivery, typically implemented through endocytosis (42). Recently, amphipathic gold nanoparticles (Au NPs) have been used to reduce toxicity in drug delivery. Their hydrophobic nature protects them against microbial attacks, swelling, and pH-induced pore changes, allowing them to pass through membranes without damage (43). This behavior is reminiscent of cyclic citrullinated peptide (CCP) used in rheumatoid arthritis therapy (44). The α-helix protein, with its hydrophilic and hydrophobic parts, binds to cationic groups, enters the cell, and interacts with the negatively charged

membrane (45). Factors crucial for NP interaction with cell surface proteins include surface charge and hydrophobicity, with cationic interactions being stronger than anionic ones (46). The hydrophobic properties of NPs facilitate drug delivery for medications that are otherwise difficult to transfer (47). Coating NPs with ligands affects their size, ligand density, receptor emission, and free energy changes (48). Rod and cylindrical NPs require more time for cellular wrapping compared to spherical NPs due to the thermodynamic forces involved in engulfment (49).

The interaction of NPs with macromolecules such as proteins can lead to structural changes in the proteins, affecting their function (50). NPs like C60 fullerenes and single-walled carbon nanotubes (SWCNTs) are used to inhibit enzymes such as HIV-1 protease and S-DNA-glutathione for therapeutic purposes (51). However, these features can also contribute to NP toxicity, primarily through oxidative stress, which disrupts intracellular harmony and increases reactive oxygen species (ROS) (52). DNA strand damage, such as hydroxy deoxyguanosine formation, can occur if DNA repair mechanisms fail, leading to cancer progression (53). Oxidative stress activates various signaling pathways that may result in cell death (54).

The most common mechanisms of NP cytotoxicity entail the following :

1. NPs may cause oxidation via increase of reactive oxygen species (ROS)

2. NPs may damage cell membranes by perforating them

3. NPs damage components of the cytoskeleton, disturbing intracellular transport and cell division

4. NPs disturb transcription and damage DNA, thus accelerating mutagenesis

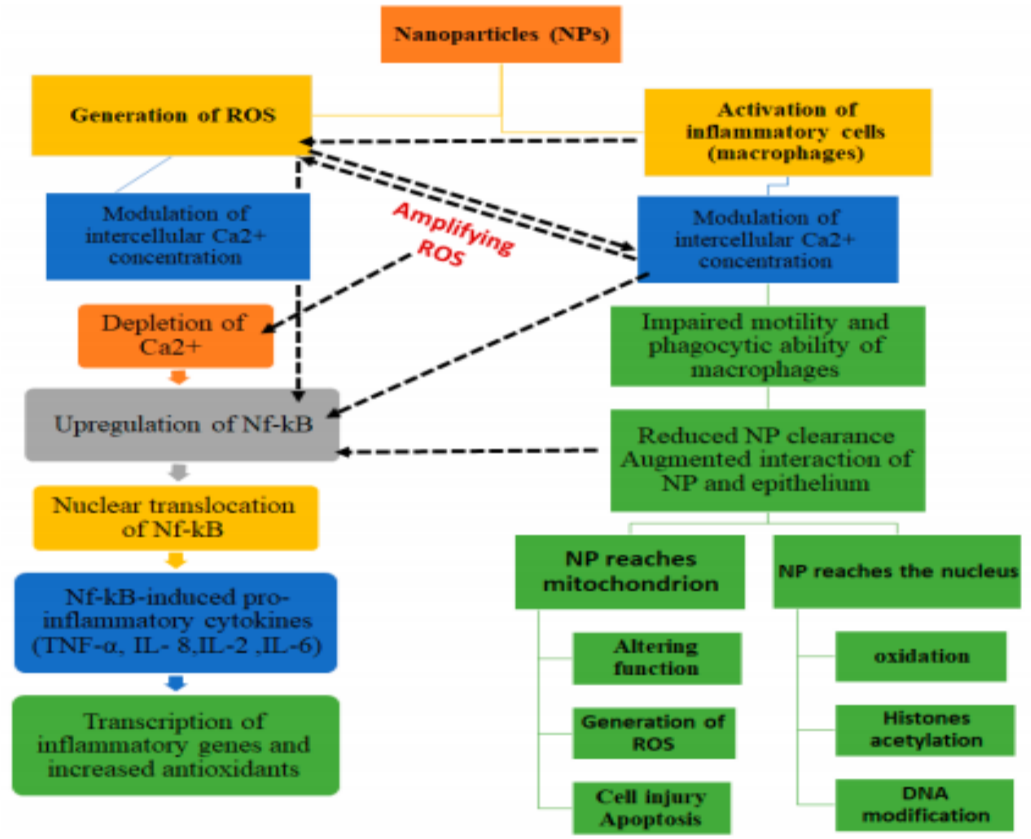
5. NPs damage mitochondria and disturb their metabolism, which leads to cell energy imbalance

6. NPs interfere with the formation of lysosomes, thereby hampering autophagy and degradation of macromolecules and triggering the apoptosis

7. NPs cause structural changes in membrane proteins and disturb the transport of substances into and out of cells, including intercellular transport

8. NPs activate the synthesis of inflammatory mediators by disturbing the normal mechanisms of cell metabolism, as well as tissue and organ metabolism The penetration of NPs can occur

through diffusion, endocytosis and membrane receptor proteins. NPs are then localized in late endosomes, mitochondria, endoplasmic reticulum (ER) or nucleus, then induce signaling pathways that are mostly depended on ROS. Mitochondrial ROS can lead to accumulation of more levels of ROS and resultant oxidative stress may disrupt protein folding process, causing ER stress and induce DNA damage, leading to activation of cell death pathways. Although



some NPs, such as Ag NPs, are used as an antimicrobial agent because of this mechanism, inappropriate use of these NPs can damage other cells instead of microbes. For example, Ag NPs can be used to disinfect wounds and prevent the growth of bacteria in that area. They can prevent bacterial growth and replication through the above mechanisms and heal the wound. But, it should be noted that the same NPs can also affect the cells of human body around the injury site and cause cell death.

Figure 2: Nanoparticles and their interaction with cellular functions to enhance the antioxidants to inhibit the ROS level.

The Effect of NP on the Protein Conformational Changes

A variety of techniques, including nuclear magnetic resonance (NMR) spectroscopy, X-ray crystallography, circular dichroism spectroscopy, isothermal titration calorimetry, differential scanning calorimetry, fluorescence spectroscopy, and UV-visible spectroscopy, have been widely used to analyze protein-nanoparticle (NP) interactions (55). NP-induced conformational changes and subsequent protein corona formation depend on several factors, such as protein type, NP type, NP size, shape, pH, and temperature (53).

Subtle changes in NP structure affect their surface properties and subsequent interactions with proteins (56). The interaction of single-wall carbon nanotubes (SWCNTs) and multi-wall carbon nanotubes (MWCNTs) of varying diameters with tau protein has been investigated using various methods (57). Circular dichroism spectroscopy revealed that increasing concentrations of SW63CNTs led to a significant increase in β-sheet content in tau protein, indicating that SWCNT binding causes tau protein to fold into a more compact structure (58). In contrast, MWCNT binding did not alter the secondary structure of tau protein but resulted in protein aggregation (59). Transmission electron microscopy (TEM) showed that tau protein can bind to the surface of SWCNTs, dispersing them, whereas tau protein cannot attach to the MWCNT surface, leading to MWCNT agglomeration (60).

Surface functionalization of NPs also influences protein adsorption and subsequent NP-induced conformational changes (61). Protein surface residues interact with energetically favorable counterparts on the NP surface based on charge, hydrophobicity, and hydrophilicity (62). Thermodynamic parameters, such as standard enthalpy change (ΔH°), standard entropy change (ΔS°), and standard Gibbs free energy change (ΔG°), can indicate the nature of NP-protein interactions (63). When ΔH° and ΔS° are negative, hydrogen bonds and van der Waals interactions are the main forces between NPs and proteins. If ΔH° is nearly zero and ΔS° is positive, electrostatic interactions are predominant (64).

Effect of Protein Corona on the Toxicity of NPs

Upon injection of nanoparticles (NPs) into the bloodstream, various biological molecules compete to interact with the NP surface, a phenomenon known as the Vroman effect. Initially, the smallest and most abundant proteins adsorb onto the NP surface. However, over time, these proteins are replaced by those with higher affinity (65). The structure and composition of the protein corona depend on the physicochemical properties of the NPs, the physiological environment, and the duration of exposure (66). The protein corona alters the size and surface composition of nanomaterials, providing them with a new biological identity. This new identity

influences physiological responses, including aggregation, cellular uptake, half-life in the bloodstream, signaling, synthesis, transfer, accumulation, and toxicity (67). The protein corona is complex, with no universal composition specific to all NPs. Common proteins found in the corona include albumin, immunoglobulin G (IgG), fibrinogen, and apolipoproteins. These proteins, prevalent in blood plasma, may be replaced over time by proteins with lower concentrations but higher affinities for the NP surface.

Proteins weakly attached to the NP form a "soft corona," while NPs with pre-formed agent groups, such as polyethylene glycolated (PEGylated) NPs, typically have only a weak covering corona and no "hard corona". The presence of a protein corona can reduce the toxicity of NPs by decreasing their cellular uptake. Consequently, NPs with a less developed protein corona exhibit higher cellular uptake and increased cytotoxicity. This phenomenon has been observed in various cell environments for carbon nanotubes (CNTs), graphene oxide nanosheets, and biopolymer NPs. For commonly toxic nanomaterials, such as positively charged polystyrene NPs, the protein corona plays a protective role against membrane damage.

Effect of Protein Corona on Non-specific Cellular Uptake

The specific entry of nanoparticles (NPs) into cells is facilitated by receptor-specific ligands. Non-specific cellular uptake, on the other hand, is a random process performed without biomolecular control. The extent of NP entry into cells is influenced by the protein corona. Studies have shown that the non-specific cellular uptake of oligonucleotide-mediated gold nanoparticles (AuNPs) significantly increases in environments devoid of serum proteins (68). Similarly, the cellular absorption of iron-platinum (FePt) NPs with quantum dots (QDs) is dramatically reduced in HeLa cells due to the formation of a protein corona (69).

Effect of Protein Corona on Bio-distribution of NPs

The nature of the nanoparticle (NP) core, whether non-polymeric or polymeric, significantly influences its persistence in the bloodstream and clearance rate. Pre-coating NPs with specific agents, such as bovine serum albumin (BSA), has been shown to increase their persistence in the blood and reduce the clearance rate. For instance, a study revealed that BSA-coated nanodrugs had a lifespan six times longer than their non-coated counterparts (70).

Effect of Surface Charge of NPs on their Toxicity

Nanoparticle (NP) hydrophobicity and surface charge significantly influence their biological distribution due to their interactions with the immune system, plasma proteins, extracellular

matrix, and non-target cells. Hydrophobic or charged NPs are less persistent in circulation because they are more readily opsonized by plasma proteins and subsequently cleared by the reticuloendothelial system (RES) (71). Positively charged NPs non-specifically attach to negatively charged non-target cells, while hydrophobic groups on the NP surface promote aggregation, accelerating identification and clearance by the RES (72).

To mitigate these interactions, NPs are often coated with hydrophilic polyethylene glycol (PEG). This coating reduces opsonization levels, thereby increasing the persistence of NPs in circulation (73). PEGylation creates a "stealth" effect, minimizing recognition and clearance by the immune system (74).

Effects of Physicochemical Properties of NPs on Cytotoxicity

A unique property of nanomaterials is their high surface-to-volume ratio, which endows them with useful characteristics. However, this trait is also associated with unique mechanisms of toxicity. Toxicity is generally thought to originate from nanomaterials' size, surface area, composition, shape, and other factors (75). The surface charge of nanoparticles (NPs) affects biological aspects such as absorption, colloidal behavior, plasma protein binding, and passage through the blood-brain barrier (76). Negatively charged NPs exhibit higher cellular absorption than positive and neutral NPs due to resistance by plasma proteins, which causes hemolysis, platelet aggregation, and eventually toxicity (77).

The surface of NPs influences the absorption levels of ions and biomolecules, potentially altering cellular responses (78). Additionally, surface charge determines colloidal behavior, which affects how organisms respond to changes in NP shape and size, leading to cellular accumulation (79). The effect of NP surface chemistry on human immune cells and red blood cells (RBCs) has been investigated in both in vivo and in vitro models (80). For instance, the effect of silicon surface charge on cell lines showed reduced ATP levels and genotoxicity for negatively charged hydrophilic and hydrophobic surfaces compared to positively charged amine-modified surfaces (81).

The interaction between NPs and cells initially depends on the nature of the NP surface. Incubation of NPs with cells may interfere with cell adhesion, affecting cellular properties such as morphology, cytoskeleton, proliferation, and survival (82). It is worth noting that the surface of NPs and the groups on their surface significantly impact adhesion. For example, bare iron oxide NPs with an approximate diameter of 50 nm exhibit 64% less cell adhesion compared to

polyethylene glycol (PEG)-coated ones (83). This difference can be attributed to the varying interactions between NPs and cells with different charges in the presence or absence of surface-coating agents (84).

In Vivo Study of Nanoparticle Toxicity

In addition to numerous studies on the behavior of nanoparticles (NPs) in in vivo models, their biomedical applications and toxicity for living organisms remain important topics. Although NPs are highly promising for various medical applications, they can potentially cause side effects. These side effects cannot be precisely estimated in vitro, as comparisons between in vivo and in vitro effects of NPs often show differences.

Metal oxide NPs, such as titanium dioxide (TiO2), are among the most widely used, particularly in environmental protection measures. Therefore, it is important to evaluate their toxicity and bioavailability through experiments involving their injection into experimental animals. In a study by Kiss et al. (2016), experimental animals (rats) were injected with a suspension of TiO2 NPs at a dose of 15 µg/cm². The biodistribution and general condition of the animals were monitored. The results showed inflammation or other manifestations of toxic effects within 24 hours, suggesting that TiO2 NPs are relatively hazardous (85).

Silver NPs are another example of NPs potentially useful in medicine due to their antimicrobial activity. Their toxicity and biodistribution were analyzed by Mitra Korani in an experiment where guinea pigs were dermally exposed to 100, 1,000, and 10,000 ppm of silver NPs of different sizes (less than 100 nm). The results showed a close correlation between dermal exposure and tissue levels of Ag NPs, with the following ranking: kidney–muscle–bone–skin–liver–heart–spleen (86).

Histopathological studies revealed severe proximal convoluted tubule degeneration and distal convoluted tubule damage in the kidneys of middle and high-dose animals. Narrowed marrow space and separated lines were identified as major signs of bone toxicity observed at all three dose levels of Ag NPs. Increased dermal doses of Ag NPs caused cardiocyte deformity, congestion, and inflammation. The three different AgNP concentrations produced comparable results for several endpoints measured in the heart, bone, and kidney, but differed in tissue concentrations and the extent of histopathological changes. Ag ions were detected in different organs after dermal exposure, indicating potential target organ toxicities in a time- and dose-dependent manner (87).

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Gold NPs have been shown to be toxic for mice, causing weight loss and a decrease in hematocrit and red blood cell count (92). In drug delivery using gold NPs, it is important to understand their toxic properties, as the positive effects of their use should outweigh the negative ones. One study found that gelatin NPs modified with polyethylene glycol, designed for the delivery of ibuprofen sodium salt, were nontoxic at the necessary dose for effective drug delivery (1 mg/kg), as confirmed by the estimation of inflammatory cytokine levels in an in vivo model and histological analysis of organs (93).

Carbon Nanotubes (CNTs) in Medicine

Drug Delivery and Cancer Treatment: CNTs are highly effective in drug delivery due to their ability to penetrate cell membranes and deliver drugs directly to target cells. Functionalized CNTs can increase the lifespan of drugs in the human body and facilitate targeted delivery, which is crucial for cancer treatment (94).

Biosensors and Bioimaging: CNTs exhibit intrinsic fluorescence properties, making them suitable for biosensing and bioimaging applications. They can detect specific targets in human tissues, such as cancer tumors, by binding to DNA molecules and proteins on their surfaces (94).

Tissue Engineering: CNTs have shown significant potential in tissue engineering. They play a key role in the culture of tissue cells, such as fibroblasts, and have been used to create scaffolds that support cell growth and tissue regeneration (94).

Toxicity Concerns: Despite their promising applications, the cytotoxicity of CNTs remains a concern. Studies have shown that CNTs can pass through membrane barriers and enter organs, potentially causing inflammatory and fibrotic responses (95).

Quantum Dots (QDs) in Medicine

Biomedical Applications: QDs are nanoparticles with exceptional photobleaching-resistant fluorescence, making them ideal for various optical-based biomedical applications. They are used in bioimaging, drug delivery, and as biosensors (96).

Cancer Detection and Treatment: QDs are extensively used in the detection of cancerous tumors. They can pass through the blood-brain barrier (BBB) and target brain tumors. CdSe/Zn QDs with a diameter of 13 nm have been shown to reach tumor tissue in laboratory mice without causing astrocyte damage or nerve inflammation (98).

Toxicity Concerns: The toxicity of QDs is size-dependent, with smaller QDs (below 20 nm) accumulating in the brain parenchyma. Studies have shown that CdTe QDs predominantly accumulate in the liver, decreasing antioxidant levels and inducing oxidative stress in liver cells (97, 98). The degradation of QDs can release toxic cadmium and tellurium ions, which accumulate in various organs and tissues (99).

Study of Toxicity in Cell Cultures

1. Oral Uptake: Intestinal epithelium cells are commonly used in experimental models to study the toxicity of ingested NPs. These studies focus on the kinetics of NP uptake by cells and the viability of cells upon NP uptake. Key markers such as reactive oxygen species (ROS), glutathione (GSH), and inflammatory cytokines (IL-1β, IL-6, IL-8, TNF-α) are often measured (100).

2. Transdermal Uptake: The toxicity of NPs that enter the body through the skin is usually studied in keratinocytes, fibroblasts, and, more rarely, sebocytes. These studies help understand the potential effects of NPs used in topical applications and transdermal drug delivery systems (101).

3. Inhalation Uptake: The toxicity of inhaled NPs is studied using primary cell lines and different tissues of the respiratory system. Common cell models include:

- Primary rat brain microvessel endothelial cells (rBMEC)
- Murine neural stem cells (NSCs)
- Human pulmonary cell line (A549)
- Human epithelial cells and fibroblasts (102)

4. Injection (Drug Delivery and Imaging): NPs used in drug delivery or imaging are administered by injection, and their toxicity is studied in primary epithelial cell cultures. Various tumor cells are used to evaluate the toxic effects of NPs in cancer chemotherapy, including:

- Gastrointestinal cells
- Human colon cells
- Skin cells
- Pancreatic PANC-1 cells
- Human lung adenocarcinoma cells
- Human hepatocellular carcinoma HepG2 cells
- Human skin carcinoma A431 cells (103).

Recent Advancements

1. Cancer Chemotherapy: Recent studies have focused on the use of NPs in cancer chemotherapy, evaluating their toxic effects on different cancer cell lines. These studies aim to optimize NP formulations to minimize toxicity while maximizing therapeutic efficacy (104).

2. Respiratory System: Research has shown that inhaled NPs can penetrate deep into the lung space, potentially causing respiratory conditions such as asthma and chronic obstructive

pulmonary disease (COPD). The extent of toxicity depends on NP characteristics, dose, and exposure duration (105).

3. Transdermal Applications: Advancements in transdermal NP delivery systems have led to the development of more effective and less toxic formulations. Studies continue to explore the mechanisms of NP penetration through the skin and their interactions with skin cells (106).

Conclusions

Nanoparticles have many biomedical applications due to their unique characteristics such as size, shape, chemistry, and charge. However, the signaling pathways through which NPs produce toxic effects need to be better understood. Recent studies have shown that inflammation, necrosis, ROS, and apoptosis are key factors mediating the mechanism of NP toxicity. These results may create barriers to the use of NPs in diagnosis and treatment of diseases for which they are ideally suited.

It is important to identify the dose, shape, and properties of NPs responsible for their toxicity to reduce side effects by appropriately modifying the formulation or using NPs with lower toxicity. The dose of NPs is a crucial factor in their toxicological profile, along with their accumulation, distribution, metabolism, and disposal. Intravenously injected NPs have higher toxicity than those administered to the skin.

According to various studies, protocols should be established to determine which doses and structures of NPs are more toxic. The evaluation of NP toxicity is complicated by the disparity between different toxicological studies performed on NPs of diverse origins and compositions. Therefore, studying NP toxicity in various applications, especially in biomedicine such as drug delivery and biosecurity, is crucial.

There is a need for the development of accepted and specific protocols to identify the actual particles, their surface surroundings, and the composition of NPs that render them toxic. It is

hoped that increased knowledge of NPs will lead to their safer design with reduced toxicity, enabling their use in the treatment of various diseases and drug delivery.

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