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Metal/metal oxide nanoparticles: Toxicity concerns associated with their physical state and remediation for biomedical applications

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ABSTRACT

Metal/metal oxide nanoparticles show promise for various applications, including diagnosis, treatment, theranostics, sensors, cosmetics, etc. Their altered chemical, optical, magnetic, and structural properties have differential toxicity profiles. Depending upon their physical state, these NPs can also change their properties due to alteration in pH, interaction with proteins, lipids, blood cells, and genetic material. Metallic nanomaterials (comprised of a single metal element) tend to be relatively stable and do not readily undergo dissolution. Contrarily, metal oxide and metal alloy-based nanomaterials tend to exhibit a lower degree of stability and are more susceptible to dissolution and ion release when introduced to a biological milieu, leading to reactive oxygen species production and oxidative stress to cells. Since NPs have considerable mobility in various biological tissues, the investigation related to their adverse effects is a critical issue and required to be appropriately addressed before their biomedical applications. Short and long-term toxicity assessment of metal/metal oxide nanoparticles or their nano-formulations is of paramount importance to ensure the global biome's safety; otherwise, to face a fiasco. This article provides a comprehensive introspection regarding the effects of metal/metal oxides' physical state, their surface properties, the possible mechanism of actions along with the potential future strategy for remediation of their toxic effects.

1. Introduction

Nano-intervention has turned out to be inherent to numerous state-of-the-art technological developments in diverse scientific avenues, including biomedical and pharmaceutical perspectives. Metals/metal oxide nanoparticles display their ability for various applications. Various researchers have used gold nanoparticles (NPs), iron/iron oxide NPs, zinc oxide NPs, silver NPs, copper oxide NPs, titanium oxide NPs, cobalt oxide NPs, aluminum oxide NPs for diagnostics, including imaging, drug delivery, therapy, and theranostics [1–12]. Factors that offer the highest promise of technological advancement also pose a threat to humans, animals, and the environment. Despite stringent regulations towards nano-intervention in biomedical applications, nano-science has achieved considerable improvement in 'bench to bedside' conversion in recent times, augmenting the probability of human exposure with myriads of metallic nano-formulations. The enhanced production of nanomaterials leads to an increased probability of environmental release, either deliberately in discharge or accidentally in spillages, and poses a higher risk of adverse effects. Understanding the nano-bio interactions, associations of physicochemical characteristics of nanoparticles or formulations with biological setup, and their kinetics are of keen interest in explicating the basic relationship of NPs with biological systems. Usually, the toxicity of NPs is related to their nano size and large surface area and is theoretically expected to be more toxic than their bulk counterparts. Many of these inherent characteristics of NPs, such as size, surface area, shape, charge, crystal structure, and solubility, have possible implications in their toxicity [13–16].

The toxicity of different NPs has been extensively explored [17–21]. Due to their nano size, the particles can access the circulatory/lymphatic systems and subsequently to the tissues and organs [22]. Metallic nanomaterials (comprised of a single metal element) tend to be relatively stable and do not readily undergo dissolution. Contrarily, metal

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oxide and metal alloy-based nanomaterials tend to exhibit a lower degree of stability and are more susceptible to dissolution and ion release when introduced to a biological milieu, leading to reactive oxygen species (ROS) production and oxidative stress to cells [23–28]. The potential toxic effects of NP on a host can occur due to accidental or intentional exposure via ingestion, dermal absorption, inhalation, or parenteral administration. The type of NPs, their route of entry, and quantum are essential factors that affect different organs and tissues with significant health issues. It is due to different pharmacological behavior, their inherent properties and interaction with different biological fluids or environments like plasmic proteins which create a "corona" upon entering the blood; "Corona" refers to a layer of organic/inorganic molecules (which can include biological macromolecules like proteins, lipids, and nucleic acids) that are adsorbed to the nanomaterial surface upon entry into biological systems. Hence NPs may or may not pursue the predictable absorption/distribution/metabolism/excretion i.e ADME model in the host. For example, nanomaterials generally have longer maximal half-lives than traditional counterparts extending to years in some tissues [29]. These NPs can change their properties due to alteration in pH, interaction with proteins, lipids, blood cells, and genetic material. Since NPs have considerable mobility in various biological tissues, the investigation related to their adverse effects is a significant issue and needs to be appropriately addressed before their specific applications. This article focuses on the physicochemical properties of metal/metal oxide nanoparticles, their toxic effects, and computational approaches to predict their toxicity, further strategies for remediation, and a framework to ameliorate the toxic effects of nanoparticles.

2. Physicochemical interactions and molecular mechanism of toxicity

Various distinct properties of NPs like size, shape, charge, crystal structure, surface area, dose, mass, susceptibility for particular cell type, determine not only the mechanism of action for suitable biological application but also determine their mechanism of toxicity. Numerous *in-vitro* and *in-vivo* introspection has also confirmed that certain categories of NPs are more frequently toxic at the molecular, cellular, or tissue level [30]. Brand et al. [31] identified a set of specific deleterious effects specific to NP-based drug formulations compared to products with similar active pharmaceutical ingredients (API). Various physicochemical characteristics impacting the toxicological profiles of the NPs are discussed below:

2.1. Size

The particle size can also influence the approach of internalization in the cells and particle processing competence in the endocytic pathway [32]. For example, the diameter of DNA is 2 nm whereas the general cell membrane thickness ranges in the order of 10 nm hence the size of the particle will help in the internalization potential of NP within a cell [32]. Huo and co-workers demonstrated the deeper internalization of 6 nm gold NPs into the cell nucleus whereas 10−16 nm NPs were more concentrated in the cytosol [33]. As the particle size decreases, it prompts an exponential increment in surface area relative to volume, making the nanomaterial surface more receptive to itself (aggregation) and its adjacent milieu (biological components) [34]. When the nanomaterial uptake is augmented into particular tissues, it may prompt aggregation, which may hamper the critical biological functions [32, 35].

As nanoparticles' size diminishes, the energy barrier related to the uptake also reduces, permitting better cellular penetration or transdermal migration. Nanoparticles can exploit the enhanced permeation and retention effect ascribed to extremely vascularized regions like a tumor [36]. A microscopic study revealed that after inhalation, the aggregated or agglomerated TiO_2 NPs (20 nm) penetrated rapidly into the lung epithelium and translocated into the interstitial areas and vascular endothelium [37–39]. The diminished energy necessity for endocytosis increases the likelihood of phagocytic utilization. The overall increment in molecule surface region connected to a decrease in volume likewise uplifts the molecule's responsiveness and empowers more take-up through receptor-interceded endocytosis and non-phagocytic components [40]. Furthermore, the size has an impact on the uptake mechanism and hence the ease of accumulation. NPs smaller than 5 nm enter via a non-specific translocation technique, whereas NPs larger than 25 nm enter via pinocytosis. Other significant routes of NP larger than 5 nm include phagocytosis, macropinocytosis, and nonspecific transport processes. The size does not only determine the mechanism of uptake and localization, but also the mechanism of action of toxicity. The NP with a size of 1.2 nm has a major pathway of apoptosis for toxic effect while for 1.4 nm AuNP major mechanism is cell necrosis [41].

Recently, the influence of particle dimension, surface modification, and surface charge of AuNPs on genotoxicity has been described [42]. Gold NPs of 40–50 nm are taken up by cells most easily [43]. In other studies, the cell uptake of smaller gold NPs proved to be higher with severe toxicity [43,44]. NPs uptake also relies upon the cell type and mechanism involved for absorption by the cells. Similar to the normal process, macrophages or the same cells distinguish and are likely to uptake larger NPs, identical to large proteins (> 100 nm), e.g., superparamagnetic iron oxide NPs (SPIONs) or cell debris [45]. Hence larger NPs are mostly taken up by mononuclear phagocytes hence are more concentrated in blood, liver, and spleen. This further masks another cell from potential toxicity of larger NP as their availability is hindered to another cell whereas small size NP has larger distribution. Receptor-mediated endocytosis is used to uptake 40–50 nm NPs by other cell types [46].

2.2. Shape

Nanoobjects/nanomaterials are categorized on basis of dimensions in the nanoscale (1−100 nm) (ISO/ TC 229). "Nanoparticles have all the three dimensions in the nanoscale." "Nanoplates possess one external dimension in nanoscale and the two other external dimensions are significantly larger" and "nanoobjects with two similar external dimensions in the nanoscale and the third dimension significantly larger" are termed as nanofibre (ISO/ TC 229). Nanomaterials are fabricated in various shapes like spheres, ellipsoids, cylinders, sheets, cubes, and rods which further can be of solid and hollow type. The shape of NPs plays an important role in the metabolism of their components in the body. The shapes can differ from homogeneous/heterogeneous solids to hollow micellular rods depending upon the content and synthesis methods used [47,48]. Spherical NPs are phagocytosed and released at a faster rate than their high-aspect-ratio counterparts i.e NPs having length many times that of width e.g nanotubes; nanorods etc. [49]. This practice is thought to be caused by the narrowing of the contact area across the cell membrane and the effect of the additional energy related to the internalization [50]. When the long axis of the cylindrical particle is aligned parallel to the surface of the membrane, distended and deformed deformities are required to cover the particle and are considered weak in strength as compared to similar segments. In other embodiments, when the surface of the tube begins to attach to the cell membrane, phagocytosis may begin outside the term of completion. This occurrence can lead to frustrated phagocytosis with concluding cell rupture and localized inflammation. The uptake by HeLa cells (human cancerous cells) of 14 and 74 nm nanospheres was greater than that of nanorods of size 74 × 14 nm [43]. Some researchers also reported a reduction in cellular exposure to NP-shaped cells by increasing their size [43,51] and, in one study, nanorod (15 × 50 nm) was significantly better than nanospheres (15 and 50 nm) [52]. In a contradictory study, however, Gratton and his associates observed that high-aspect-ratio NPs have four-times augmented uptake for HeLa cells compared to low aspect proportion

particles of similar size and chemistry [53]. Therefore, the shape of NPs pays significantly to their performance and represents an imperative feature in their internal penetration and clearance potential.

In another study, comparison of toxicity of the block and the sphere morphology of cobalt oxide (Co_3O_4) magnetic NPs revealed that spherical Co_3O_4 NPs were more effective in generating nitric oxide than that produced by block morphology of Co_3O_4 NPs, whereas, block Co_3O_4 NPs were more effective at inhibiting liver GSH and brain AChE activities [54]. It suggests that toxicity is influenced by nanoparticle morphology and surface area, which could have implications for their biological application. The effect of shape on the adverse biological outcomes (cytotoxicity) requires further precision and is expected to be cell type-dependent [55,56]. Additional forms such as nonspherical, homogeneous/heterogeneous agglomerates, circular or tube-like or micelle-like capsules, and dendritic forms influence various favored uptake options that obscure their ADME profiles [47,48].

2.3. Surface charge

The surface charge similarly underlies a deterministic part in the cell take-up of NPs. The net positive and negative charges are associated with increased toxicity, while neutral surfaces should have tremendous biosafety [47]. Zwitterionic particles (containing an equal number of positively and negatively charged ions) are usually considered harmless, because of the self-managed balance of their charge and have been thoroughly investigated as antibacterial agents [57]. Cationic charged gold NPs are more likely to be taken up by the biological milieu (cells/proteins) and subsequently lead to higher cytotoxicity than the anionic NPs [58]. It is due to the strong interaction with negatively charged lipid bilayers. Cationic NPs express affinity for the anionic phospholipid membranes and energize endocytosis. Once penetrated, the positive charge performs as a proton entity that interrupts usual lysosomes' function and commences cell death [47]. In contrast, anionic particles display higher potency in breaking the skin barrier through charge density and signal coagulation cascades. Using adequate doses, the negative charged NPs may cause thrombosis and eventually embolism. Similarly, the platelets around cationic particles aggregate and form coronas that camouflage their exposed chemical characteristics and offer another biological entity. Firmly bound protein aggregates initially make a "hard" corona, and then a "soft" corona outside that regularly exchanges proteins with the plasma [59,60].

The charge of particle surface also depends on pH and influences the speed and orientation pathways of their cells' uptake. The binding areas on the NPs' surface increase the possibility of interacting with biomolecules like nucleic acids (DNA/RNA), proteins, and lipids and influence the degree of cytotoxicity [16].

2.4. Solubilization and agglomeration

Solubilising media or solvent also influence the particle size,dispersion and, agglomeration of metal NPs, thus affecting the toxicity. It has been seen that particles of TiO_2, ZnO have a larger size in phosphate-buffered saline than in water. The NPs showed different diameters in the biological milieu [61,62]. Accordingly, the manifestation of toxic effects varied depending upon the solvent composition and state. Nanoparticles can be formed in single particles as well as agglomerates (adhesion of particles by weak forces) or aggregates (formation of metallic or covalent bonds). Regardless of the physicochemical properties of metal nanoparticles, aggregates/agglomeration could be an inducer of toxicity. The total surface area of agglomerates does not vary substantially from the computed surface area of individual particles. Agglomerates are not constant entities, however may alter their sizes/shapes. Varying temperature/pressure/viscosity/pH, or other conditions of the encompassing environment lead to various agglomerates [63]. The bigger agglomerates may also split into shorter agglomerates or, another way around. Whereas, aggregates are accumulattion of NPs that developed collectively, aligned/united, and having remarkably lesser surface area as compared to the total surface area of the primary NPs. The primary NPs may be inadequately soluble and may feature to a granular biopersistent dust. The extremely soluble primary NPs would provide local/systemic accessibility of metal ions, consequently produce a "particle effect" where ROS may intrude with the whole surface area, thus causing local effect or systemic effect by phagocytosis [63]. In another study, Singer and associates observed single and agglomerated NPs of silver, manganese, and aluminium within the cells whereas agglomerates were observed on the cell surfaces of rat liver and macrophages cells [64].

The dissolution of metals from oxides depends on pH. Among the different oxides NPs studied viz: TiO_2, Cr_2O_3, Mn_2O_3, Fe_2O_3, NiO, CuO, and ZnO, the release of Cu^{2+} and Zn^{2+} from their oxides may have an impact on toxicity [16]. It has been suggested that the cytotoxicity of fourth period metal oxide NPs upsurges with the atomic number of the transition metal oxide. The chemical composition, particle size, temperature, and many other properties influence the propensity for metal nanomaterials to disperse into ionic constituents. Furthermore, the copper oxide was more toxic in cultured human laryngeal epithelial cells than amorphous silicon dioxide and ferric oxide of the similar particle size: this contradicts the influence of particle amount and surface area, as well as a reduced antioxidative defense. The toxicity of the oxides was noticed as reduced cell-viability, the generation of ROS, and changes in antioxidant enzyme activity, and the intensity of oxidized glutathione [65]. Higher extracellular solubilization of copper oxide could explain its increased toxicity. Rather, copper's intracellular bioavailability may be critical. Nanoparticles and microparticles are most likely taken up by endocytosis and deposited in lysosomes. They are dissolved here due to the acidic pH, delivering large amounts of copper ions close to the nucleus [63,66,67].

2.5. Crystallinity

The various conditions including the nature of surfactant, used in the fabrication of NPs determine the size, structure, crystal formation, and to some extent their morphology. The surfactant increases the particle size and improves crystalline character [68]. The polymer concentration, nanoparticle size, and composition of mixtures containing amorphous polymers such as poly (vinyl formal) and polystyrene all influence the crystallinity of the NPs [69]. The surfactant improves the crystallinity of NPs while also marginally increasing the particle size. The processes chosen for synthesis also impact the physiochemical properties of metal NPs. For example, in a study, Vidyasagar and colleagues synthesized ZnO NPs utilizing PEG 400 as a surfactant, using a one-step solid-state reaction technique [68]. This method not only reduced the time to synthesize ZnO NPs but also improved their crystallinity to submicron order. The bandgap energy dropped as the lattice constants increased, which can be attributed to the samples' improved crystallinity. The bandgap of ZnO can be set between 3.37 and 3.33 eV, depending on the application. The rate of particle aggregation is a major determinant of the final product's shape and crystallinity. The size and shape of the product can be amended by modifying the amount of PEG. The crystallinity improved as the amount of surfactant was reduced. Consequently, adding PEG to the reaction system changed the kinetics of the growing process, which is attributable to the fact that adding PEG causes fast nucleation and nanoparticle aggregation. As a result, adding PEG to samples increased crystallinity and altered product morphology. With decreasing NPs diameter, crystallinity dropped considerably. In another study, Jiang et al. [70] explored the relationship between the physicochemical parameters of nanoparticles (e.g. size, surface area, and crystal phase) and their oxidant producing ability. TiO_2 NPs were fabricated using the gas phase synthesis method that allows for precise control of size and crystal phase. The oxidant capacity (ROS) of thirteen larger-sized TiO_2 samples with varied crystal structures was compared to get a comprehensive picture of the effect of crystal structure on TiO_2

hazardous potential [71]. The oxidant reactivity exhibited by TiO_2 particles with similar size but different crystal structures was the highest for amorphous samples, followed by pure anatase, and lower for anatase/rutile mixtures, and lowest for pure rutile. They also observed no variation with the change of size of NPs.

2.6. Surface reactivity

Molecular and classical thermodynamics and kinetics of the bulk are traditionally thought to govern environmental impact. The fate of nanoparticles, on the other hand, is largely determined by surface interactions in the nanoscale regime [72–74]. Several parameters to studying these surface interactions and consequently their environmental impact includes dissolution, morphology/structural changes, and aggregation or stabilization. It has been noticed that nanomaterial shape and, in some cases, aspect ratio influence nanoparticle cellular internalization pathways [53,75]. Furthermore, when the two nanomaterial morphologies are compared under identical mass concentrations and time points, the nanosheets release more metal cations than the nano blocks [74]. For example, the nanosheets released four times as many Ni cations as the nano blocks after 72 h in solution. The incongruent dissolution trend of NMC (Nickel Manganese, Cobalt) oxides has also been observed [76]. Notably, this research revealed how the chemical transformations of NMC materials are influenced by a variety of surface terminations and water pH exchange to produce hydroxylated basal surfaces. Maximum toxicological studies are based on mass, but surface processes are critical chemical phenomena for reactive NP types like NMC. It is necessary to understand the microscopic chemical processes that control biological impact. ZnO and MgO, have been used as the model systems for probing surface reactivity. Tuckett and Baer demonstrated that ZnO nano-powders behave as multi-facet single crystals with the polar orientations corresponding to 25 % of the total surface area [77].

The mechanism of toxicity of various metal NPs and their compounds particularly oxides differs due to their inherited chemical property and expresses varied toxic effects. A study of silicon dioxide (SiO_2) and ZnO NPs of similar shape and size had revealed that they act by a different mechanisms. Oxidative stress production is a major mechanism of toxicity by ZnO whereas SiO_2 shows toxicity effect by altering DNA [78]. Altered cell viability, mitochondrial function, and oxidative stress are major mechanisms of toxicity by aluminum oxide NPs [79].

Surface characteristics like surface charge and surface hydrophilicity/hydrophobicity play a big role in NP dispersion and biological destiny. Aside from NP size, the amount of adsorbed blood components, primarily proteins, is determined by their surface hydrophobicity (opsonin). As part of the body's defense system, phagocytes suck up the opsonized particles to remove foreign chemicals. To prevent this *in vivo* outcome, NPs' surfaces are frequently coated with a hydrophilic polymer that acts as a barrier between the NPs and the opsonin. The surface charge of the NPs is normally neutral or slightly negative when a neutral polymer is used, but the zeta potential of the NPs is positive when a cationic polymer is used.

Apart from these major properties various other properties like dose, side-chain (cationic), functionalization, and the use of stabilizer are other important factors that affect the toxicity potential of metals [80]. The surface area of NP is the outcome of charge, size, shape, hollow or solid nature, functionalization, and unit of repetition.

2.7. Mechanism of toxicity of nanoparticles after internalization into the cell

The usual mechanisms of nanotoxicity include but are not limited to cytotoxicity, genotoxicity, production of reactive oxygen species (ROS), oxidative stress and inflammation, modulation of cell signaling, apoptosis, and cancer, etc. [81–86]. Nanoparticles trigger the cellular mechanism via receptors present on the membrane during internalization, leading to ROS production, resulting in oxidative stress. This happens when it interacts with mitochondria physically or chemically leading to alteration of uncoupling oxidation of phosphorylation membrane system after the internalization. This activity depends upon the concentration of NP and its functionalization. For example, Citrate-AgNPs have negative charge hence at lower dose are repelled by cellular membranes containing similar charges but lead to rapid internalization and thus ROS effect when the dose is increased which help in neutralizing the electrostatic barrier [87]. The other method of apoptosis or necrosis can also include pathways of genotoxic effect, lipid peroxidation, and even down-regulation of antioxidant enzymes or genes related to such enzymes [88]. NPs might induce activation of epidermal growth factors (EGF) or integrin receptors that can directly lead to inflammation, proliferation, or apoptosis (Fig. 1). Either it produces ROS in the cellular environment directly or triggers the activation of transcription factors (AP-1, NF-kB, or Nrf2) and activation of redox-dependent signaling pathways such as mitogen-activated protein kinase (MAPK) within the cells. After activation, it enters into the nucleus and alters gene expression of phase 2 enzymes glutathione S transferase (GST) and quinine oxidoreductase 1 (NQO-1), cytokines, and heme oxygenase 1 (HO-1) antioxidant enzymes. In addition to this oxidative stress, it also damages various organelles such as the lysosomes, mitochondria, and nucleus, consequential to apoptosis. For example, activation of procaspases and triggering of the intrinsic mitochondrial pathway is a major cause of apoptosis of ZnO NPs [89]. Apoptosis is one of the common cellular responses in NP-based toxicity but other responses like necrosis, endoplasmic reticulum (ER) autophagy, mitotic catastrophe leading to cell cycle arrest in dividing cells can be also the cause of cell death [88].

3. Remediation to reduce the toxicity of metal nanoparticles

Many challenges are facing the harmful impacts of metal/metal oxides NPs, which can be ameliorated by proficient conceptualization and development of nano/microstructures.

3.1. Delivery through polymers

The delivery of metal/metal oxide NPs can be achieved by proper entrapment, attachment, or encapsulation of NPs into the matrix to avoid side effects. The incorporation or encapsulation, or capping of polymers during the synthesis of these polymeric metal nanoparticles could help overcome the limitations of toxicity, aggregation, and instability [12,90,91,92]. The best way to increase biocompatibility and mitigate particle aggregation is by coating nanoparticles with discrete-sized polymers that deliver it with lower toxicity but with augmented efficiency when given at reduced doses. For example, an investigation of the toxicity of two different types of cadmium oxide NPs prepared by calcination of $Cd(OH)_2$ with and without coordination polymer clearly depicted that surface coverage by carbon produced by conversion of organic unit (polymer) can remarkably reduce the toxicity of CdO NPs in zebrafish [93]. In comparison to non-covered CdO NPs, this carbon surface coverage can control the release of Cd^{2+} ions in polymeric CdO-NPs, mitigating the toxicity.

The researchers create desired forms of metal composites and maximize their performance by modifying the structures using various additives. Metal composites that take complete advantage of each constituent's properties can benefit from the manipulation of their nanostructures. In addition to progress in the synthesis of nanostructure methods, choosing the specific constituents has excellent potential for stabilizing metal/metal oxides. Incorporating metal NPs into the polymeric matrix reduces the toxic effects and improves the efficiency owing to the continuous and controlled release. Nanoformulation of metal/metal oxide using a biocompatible polymer can enhance the drug efficacy at lower doses with sustained release and minimum undesirable side effects. It is well recognized that the reticuloendothelial system,

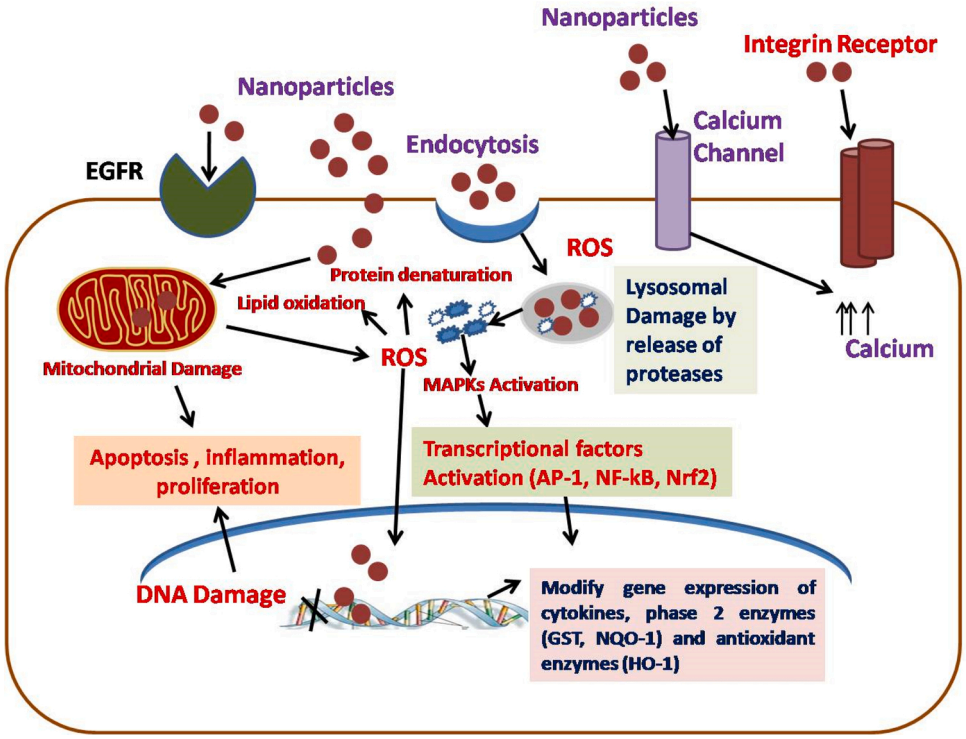

Fig. 1. Mechanism of toxicity of nanoparticles after internalization into the cell.
The figure shows the events leading to activation of growth factors, transcription factors, receptors, and inflammatory molecules leading to oxidative stress, inflammation, proliferation, or apoptosis after cellular internalization of nanoparticles.

primarily the liver and spleen, are the significant hurdle to active targeting, owing to their capability to recognize and eliminate NPs from the systemic circulation and, thus, avoid the effective delivery of the NPs to organs other than those of the reticuloendothelial system [22].

Polyethylene glycol (PEG) is usually employed as coating for remedial NPs due to its capability to save the particles from circulating proteins [94]. The inhibition of opsonization with a PEG steric hindrance enables the NPs with stealth characteristics that prolong their availability in the body [95,96]. The chance for NPs to impact both the target (for example tumor) and unintentional areas in the affected regions concurrently enhances [95]. Even though elevated delivery of the NPs to the affected region fundamentally improves its efficiency, otherwise the particles' entry to normal sites may result in adverse side effects that contradict the therapeutic beneficial effects. We may use other polymers like chitosan and polylactic acid along with PEG to present both "stealth" and therapeutic assets in parallel.

3.2. *Responsive surface alteration*

Surface alteration of these nanoparticulate systems with hydrophilic polymers is the most widely recognized approach to control the opsonization process and better surface properties [97]. The transformation of surface to contain environmentally friendly degradation mechanisms can motivate even toxic NP cells to perform as pseudotherapeutic agents. Protection of ROS production by metallic NPs using antioxidant-impregnated polymers such as polyTrolox ester with a meticulous reduction provides a way to reduce unwanted oxidative harm and local transport of hydrolyzed therapeutic agents by enzymes while storing the cytotoxic capability as a secondary treatment [98]. A cationic NP neutralized by an anionic shell may utilize prolonged blood flow from a neutral charge in an environment caused by leaking vessels to affect the tumor cells specifically [120]. The breakdown caused by the acidic tumor milieu permits the nanocarrier to escape the lysosomal destruction and expose the active substance. Alternatively, premature degeneration of the coating may transform the inert NP into a toxic agent by promoting immunogenicity or inflammation from its unprotected core before embracing its target [48,94]. Unexpected exposure causes agglomeration, corona formation, and uncontrolled chemistry, which may have detrimental physical effects.

Polymeric NPs also need assessment for their toxicity, degradation in the body, and biocompatibility of the metabolites. Administered metallic NPs exhibit the propensity to release toxic metal ions in variable pH segments in the host, and the circulating ions accumulate at the vital organs (liver and kidney), causing genotoxic and cytotoxic effects [48]. For example, gold, silver, ZnO, titanium oxide, aluminium oxide NPs were originally considered passive but gained attention as promoters of oxidative damage and inflammation [99,100]. Iron oxide or oxide NPs classify as Fenton or Fenton-like substances of the radical generation that pay to lipid peroxidation and DNA degradation. Therapeutic polymer-based metallic nanocomposites often have been designed to undermine continuous hydrolysis and breakdown in their monomers/analogs [121]. However, careful designing is needed to prevent the entrance of NPs into circulation and unleashing destruction downstream. The interactions of measuring the therapeutic potential and toxicity of NPs and their metabolites describe the underlying concerns of reducing ROS and inflammation and testing them using the *in vitro* assays.

Biocompatible colloidal suspensions were fabricated by coating the surface of magnetic iron oxide NPs formed during solution combustion synthesis, with a double layer of oleic acid, as a potential carrier for delivery in skin disorders [101]. Oleic acid is an FDA-approved agent for increasing skin permeation because it interacts with the stratum corneum's lipid content and facilitating the entry of different molecules into the deeper layers of the skin.

3.3. *Metal-organic frameworks*

Metal-organic frames (MOFs) are the latest categories of crystalline

hybrid materials made from seamless combinations of metal subunits and organic ligands (aromatic acids/foundations) by coordination bonding. These are also known as porous coordination polymers [102]. Because of their large surface area, contact, high pore volume, high density, non-toxicity, cohesiveness, and small size, they are considered potential nanocarriers in the biomedical field. MOFs are capable of providing more effective therapies and lowering adverse effects. Preliminary studies revealed that Zn, Zr, Mg, and Fe's toxicity is drastically reduced through MOFs [103]. The organic ligands, such as polycarboxylic acid, being highly polar, less harmful, and can be effortlessly removed [104,105].

Different anticancer therapies could improve the therapeutic efficacy of anticancer drugs using MOFs. For example, researchers wrapped Zr-MOFs in MnO_2 to combine photodynamic therapy with antiangiogenic drugs [106]. In order to use MOFs for immunotherapy applications, the immunogenic antigens or adjuvants can be incorporated into the system. For instance, aluminium has traditionally been used as an adjuvant in vaccines, aluminium based MOFs and aluminium incorporated MOFs have been reported for vaccine-related applications [107].

Functional modifications can also alter the physicochemical properties of engineered materials, thus before the active use of any new modification, a detailed investigation of their biocompatibility and compliance is necessary.

3.4. Artificial intelligence-based computational approaches

The computational approaches using artificial intelligence-based mathematical/ simulation models can be applied to develop predictive software to envisage their behavior in the biological system, thus allowing the high throughput screening before *in vitro* and *in vivo* studies [108–112]. Predicting the probable cytotoxicity of NPs based on geometric and their physicochemical properties can reduce the possible risks associated with the biological interactions [113,114]. The design of libraries of nanomaterials and high throughput screenings for toxicity is described [115]. Milli-fluidic benchtop equipment could fabricate a library of nanosized materials with desirable functionalities [111]. Liu et al. mentioned an adaptable and robust microfluidic platform for fabricating various uniform NPs with varying physical properties and drug-loadings [116]. The computational approach for designing and developing safe ZnO NPs is reported [117]. The researchers used experimental facts from a library of ZnO NPs or their modification to create quantitative structure-activity relationship models using biological endpoints for predicting the biocompatibility/toxicity of the NPs. Researchers reported that the concentration of NPs is the most crucial feature for cytotoxicity, whereas coated surface, doping, and aspect proportion also contributed significantly towards cytotoxicity. In another study, the gold NPs library has helped in the unearthing cell-specific and high-affinity binding NPs that can differentiate between associated cell types, therefore, suggesting the possible applications of safe NPs in diagnostics and therapeutics [118,119].

4. Conclusions

The common concerns that emerged underline the importance of handling the metallic NPs with caution since their effects are incredibly variable. Although studies disagree on the extent and mechanism of toxicity, it is obvious that some nanomaterials that have previously been considered compatible due to the safety of many substances may be toxic in their nanoforms. All things considered, the pharmaco-kinetic characteristic of different forms of NPs requires a thorough examination and a database of health hazards related to different NPs. Existing research on nanotoxicity has focussed on the empirical investigation of various NPs' harmfulness by creating libraries and databases and computational approaches to predict the toxicity of the metal-containing nanoformulations. This information can provide a way for further *in vitro* and *in vivo* evaluation of NPs. Studies should include research into the mechanisms of transmission of NPs, accumulation, long-term and long-term safety /toxicity, their interaction with cells, receptors, affective signaling pathways, and their global phagocytosis activity. Understanding the connection between these "new building materials" and biological systems is a way to protect these items in a variety of medical fields such as diagnosis and treatment.

Data availability

No data was used for the research described in the article.

Conflict of interest

The authors declare no conflict of interest.

Declaration of Competing Interest

The authors report no declarations of interest.

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