Chapter 4
Nanoparticles-Induced Oxidative Stress

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Abstract With the growing usage of nanoparticles (NPs) in industry, biomedicine, and daily life, an increasing chance for humans to be exposed to NPs has been issued. However, the basis of toxicity of most manufactured NPs is not fully understood. An important mechanism of nanotoxicity is reactive oxygen species (ROS) formation, which could cause oxidative stress, inflammation, and consequent cell death. NPs can interact with H_2O or O_2 in the physiological environment, resulting in the direct production of ROS, or affect the function of mitochondria and NADPH oxidase, resulting in the indirect production of ROS. ROS generation and oxidative stress were depicted by the hierarchical oxidative stress model. Critical determinants that can affect the generation of ROS, including NPs' composition, size, shape, and surface chemistry, are briefly discussed in this review.

Keywords Oxidative stress · Nanoparticles · Nanotoxicity · Reactive oxygen species · Physicochemical properties

4.1 Introduction

Nanotechnology is a rapid developing field that encompasses the production and usage of particles at the nanoscale (1–100 nm). Due to the excellent optical, electronic, and biological properties, nanoparticles (NPs) are widely used in industry, biomedicine, and over 1800 consumer products [1, 2]. For example,

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carbon nanotubes (CNTs) are used in energy field due to its electronic properties [3]. Various NPs have also been used in the area of biotechnology, biosensors, and nanomedicine [4–7].

The application of NPs enhances their contact probability with humans. Humans may be exposed to NPs through different pathways, such as inhalation, ingestion, skin contact, and injection. For example, NPs in plants, animal bodies, and microbes could transfer into human body through food chain [8, 9]. Airborne NPs could easily enter into respiratory tract by inhalation [10]. NPs in cosmetics and personal care products could enter into human body through skin penetration [11]. In addition, theronostic NPs could also be intravenously injected into human body for imaging and drug delivery [12]. The small size of NPs makes them easily to pass through cell membranes and penetrate into living organisms and consequently cause cellular dysfunction. In addition, the great surface area to volume ratio of NPs increases their chemical or catalytic activity, resulting in increased toxicity through different mechanisms. Thus, understanding and assessing of NPs' toxicity is necessary for the safety usage of NPs.

Reactive oxygen species (ROS) formation is one of the mechanisms of nanotoxicity, which could lead to cellular oxidative stress, inflammation, and cell death. NPs with specific properties could interact with H_2O or O_2 in the physiological environment, resulting in the direct production of ROS. Furthermore, NPs may affect the function of mitochondria and NADPH oxidase, resulting in the indirect production of ROS. The elevated ROS level leads to hierarchical oxidative stress [13]. The physicochemical properties of NPs, such as chemical composition, size, shape, and surface chemistry have been found to dictate oxidative stress level and their toxicity. In this review, we focus on introducing molecular mechanisms underlying NP-induced oxidative stress and hierarchical oxidative stress models used to study the oxidative stress-related mechanism. We also review the recent progress on regulation of oxidative stress and nanotoxicity through NPs' physicochemical properties.

4.2 Molecular Mechanisms Underlying Nanoparticle-Induced Oxidative Stress

The generation of ROS and related oxidative stress is considered as the main cause of nanotoxicity. The level of ROS generation depends on the physicochemical nature of NPs, including their composition, size, shape, and surface chemistry. Different NPs can induce the ROS generation through direct and indirect mechanisms.

4.2.1 Direct Mechanisms of ROS Generation

NPs could donate or receive electrons from intracellular and extracellular molecules, such as H_2O and O_2, resulting in the production of abiotic ROS. The composition, surface structure, and photosensitivity are key determinants to the direct production of abiotic ROS.

The composition of NPs affects the abiotic ROS level. Nel et al. demonstrated that when particles' concentration was 0.5 μg/mL, titanium dioxide (TiO_2) NPs induced the highest abiotic ROS level, followed by ambient ultrafine particles and fullerol, while carbon black (CB) and polystyrene (PS) NPs did not induce abiotic ROS [14]. In another paper, the induction ability of abiotic ROS was in the following order: ZnO > CeO_2 > TiO_2 > NH_2-PS (10 μg/mL) [15]. The abiotic ROS level was also screened in 24 metal oxide NPs. Co_3O_4, Mn_2O_3, CuO, Ni_2O_3, and CoO induced abiotic ROS in a dose dependent manner, while the rest NPs did not induce abiotic ROS [16]. PdO doping on Co_3O_4 NPs (200 μg/mL) dictates abiotic ROS. The abiotic ROS level is positively correlated to PdO content [17].

Specific surface structure could catalyze the production of abiotic ROS. For example, fumed silica NPs induced higher ROS level compared to colloidal silica due to the strained three-membered rings (3MRs) on the surface. 3MRs on fumed silica could be cleaved to release radicals, which further react with oxygen-containing molecules, such as water, to generate ROS [18].

NPs could induce abiotic ROS under photocatalysis. After irradiated by light with energy greater than band gap, NPs' electrons transform to the conduction band, leaving a hole in the valence band. Electrons in conduction band could react with O_2 to generate superoxide anion. Holes in the valence band could react with H_2O to produce hydroxyl radicals. For example, TiO_2 NPs (10, 20, 100 nm) induced abiotic ROS after photoactivation. Smaller TiO_2 NPs induced higher ROS level due to more photoactivated electrons and holes formed on NPs' surface and more H_2O and O_2 molecules were absorbed [19]. Under solar radiation, ZnO NPs induce the production of ROS in a dose-dependent manner [20]. Due to the local surface plasmon resonance (SPR), ROS generated on the surface of Ag NPs under UV-365 irradiation could be regulated by surface decoration of Ag NPs. Citrate-decorated Ag NPs could induce highest ROS level, followed by bare Ag NPs, while PVP-decorated Ag NPs did not elicit a detectable amount of ROS under UV-365 irradiation [21].

4.2.2 Indirect Mechanisms of ROS Generation

Besides ROS production and oxidative stress induced by direct reaction with NPs, ROS and oxidative stress could also be elicited through mitochondria and NADPH oxidase pathways, which are the main resources of ROS generation in cell. It was first reported in 1966 that the respiratory chain in mitochondria could produce ROS

[22]. The follow-up work demonstrated that the electron leakage in respiratory chain was captured by oxygen, resulting in ROS generation [23]. Previous papers demonstrated extracellular stimulus, such as hypoxia, cytokines, and grow factors, could stimulate the production of ROS through mitochondrial respiration [24]. NADPH oxidase is a plasma membrane-associated enzyme found in both phagocytic and non-phagocytic cells. Some of its subunits are located on the cell membrane and the rest parts are in cytoplasm in quiescent condition. When NADPH oxidase is activated, subunits in cytoplasm migrate to cell membrane and all the subunits are assembled [25]. Previous research confirmed NPs could induce ROS production by affecting the function of mitochondria and NADPH oxidase.

4.2.2.1 Mitochondrial Respiration

The toxicity of Ag NPs was investigated to find the possible molecular mechanisms associated with their toxic effects. When Ag NPs were exposed to NIH3T3 cells, ROS was produced through mitochondrial pathway and subsequent activation of the JNK and P53 pathway and apoptosis were found [26]. Plate-shaped Ag NPs induced high level of ROS than sphere Ag NPs [27]. TiO_2 NPs are widely used in human products such as sunscreen and paints. The impact of TiO_2 NPs on mitochondrial function isolated from lung tissue was investigated. TiO_2 NPs elevated ROS level in short exposure time, resulting in the decrease of the mitochondria membrane potential [28]. Besides TiO_2 NPs, hydroxyapatite (HA) NPs are also widely used in human life, such as the additives of oral hygiene products to resist dental decay. Both TiO_2 NPs and nano-HA were able to stimulate ROS production through mitochondrial pathway in TR146 epithelial cells and subsequently induced inflammation and apoptosis [29]. ZnO NPs could induce ROS in mitochondria, while CeO_2 NPs did not induce any ROS at the same dose [15].

Besides the daily use of NPs, they have also been used in biomedical field, The dendrimer phthalocyanine (DPc)-encapsulated polymeric micelle, which was designed for photodynamic therapy, could induce ROS in mitochondria and induce damages to the mitochondria [30].

4.2.2.2 NADPH Oxidase Pathway

Ambient ultrafine particles can pass from the lungs to the blood circulation after inhalation because of their small size, and subsequently induce lung oxidative stress. The ROS producing mechanism of ambient ultrafine particles exposed to mouse pulmonary microvascular endothelial cells was investigated. The result showed ROS induced by ambient ultrafine particles could be inhibited by DPI, a NADPH oxidase inhibitor, while the mitochondria respiratory chain inhibitor did not influence the ROS induced by ambient ultrafine particles [31]. The data confirmed that ambient ultrafine particles induced ROS through activation of NADPH oxidase. Carbon nanomaterials, which are widely used, arise concerns for their

possible harmful healthy effects. The inflammatory response induced by CNTs in human primary macrophages was conducted, with results showing CNTs induced ROS through NADPH oxidase and subsequently induced NLRP3 activation and IL-1β secretion [32, 33]. CeO_2 NPs are widely used in industry as fuel additives, catalysts, semiconductors, and oxygen sensors. The safety evaluation of CeO_2 NP showed that ROS induced by CeO_2 could be hampered by DPI, indicating CeO_2 NPs induced ROS through NADPH oxidase pathway [34].

4.3 The Hierarchical Oxidative Stress Model

ROS induced by NPs results in oxidative stress. To elucidate the oxidative stress-related mechanism, the hierarchical oxidative stress model was proposed, which contains three parts: antioxidant defense, inflammation, mitochondrial perturbation, and cell death (Fig. 4.1) [13].

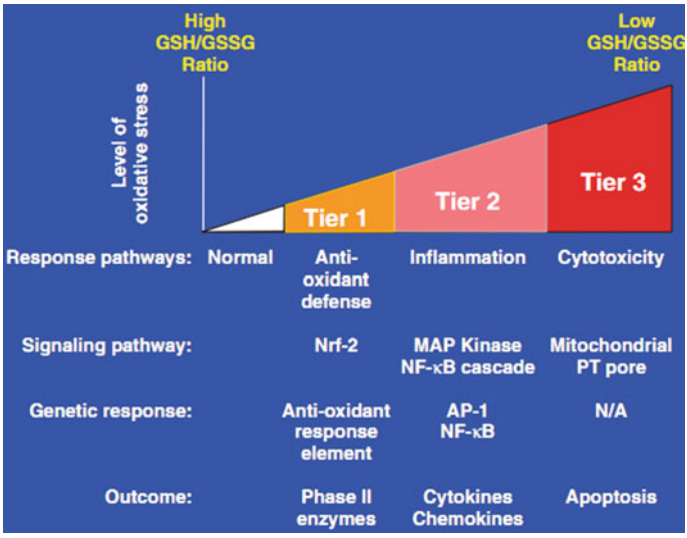

Fig. 4.1 The hierarchical oxidative stress model. At a lower amount of oxidative stress (tier 1), phase II antioxidant enzymes are induced via transcriptional activation of the antioxidant response element by Nrf-2 to restore cellular redox homeostasis. At an intermediate amount of oxidative stress (tier 2), activation of the MAPK and NF-kB cascades induces pro-inflammatory responses. At a high amount of oxidative stress (tier 3), perturbation of the mitochondrial PT pore and disruption of electron transfer results in cellular apoptosis or necrosis. From [13], Reprinted with permission from AAAS

4.3.1 Antioxidant Defense

Low levels of oxidative stress induce antioxidant defense, in which stage Keap1-Nrf2-ARE pathway plays an important role. The transcription factor Nrf2 is captured by the actin-anchored protein Keap1 in quiescent conditions, inducing the low expression of Nrf2-regulated genes. When cells are exposed to oxidative molecules, Nrf2 is detached from Keap1, translocates to nuclear and activates the ARE-responsive genes, which subsequently activates a lot of antioxidative enzyme, such as heme oxygenase 1 (HO-1), glutathione-*S*-transferase isoenzymes, NADPH quinone oxidoreductase, superoxide dismutase and glutathione peroxidase [35].

Among these enzymes, HO-1 is usually used as an antioxidant defense marker. ROS induced by NPs can promote the expression of HO-1. For example, when ambient ultrafine particles and NH_2-PS were exposed to RAW 264.7 cells, the intracellular ROS level increased as indicated by the enhancement of HO-1 expression, while other NPs, such as CB, TiO_2, and COOH-PS, did not affect the HO-1 expression [14]. The antioxidant response induced by TiO_2, ZnO, and CeO_2 were screened in RAW 264.7 and BEAS-2B cells. Among these NPs, ZnO NPs enhanced the intracellular HO-1 level, while TiO_2 NPs and CeO_2 NPs did not affect [15]. The HO-1 level in lung tissue of mice was enhanced by Co_3O_4, Cr_2O_3, and CuO NPs, while NiO, Fe_2O_3, and Fe_3O_4 NPs did not influence the HO-1 level [36].

4.3.2 Inflammation

Under moderate level of oxidative stress, the protective antioxidant defense is overtaken by inflammation. MAPK and NF-κB pathway, which is sensitive under redox condition, is activated at high level of oxidative stress condition, and eventually induces the release of cytokines and chemokines.

Several papers have discussed inflammation induced by oxidative stress. Although both ambient ultrafine particles and NH_2-PS could induce ROS and antioxidant defense, only ambient ultrafine particles can activate JNK pathway and induce the release of TNF-α [14]. ZnO NPs was also found to activate the JNK pathway and induce the release of TNF-a [15]. IL-6 secretion was investigated by a series of metal oxide NPs. Co_3O_4, Cr_2O_3, CuO, Mn_2O_3, CoO, and Ni_2O_3 NPs induced a higher level of IL-6 than NiO, Fe_2O_3, Fe_3O_4, Y_2O_3, ZrO_2, CeO_2, HfO_2, and In_2O_3 NPs [36]. Oxidative stress induced by nano-HA and TiO_2 NPs activated the NF-κB pathway and induced the release of TNF-α and IL-6 [29]. ROS induced by pristine graphene activated MAPK pathway, including JNK, ERK and p38 MAPK, promoted the release of TNF-α [37]. To evaluation the potential harmful effects of Ag NPs on immune system, oxidative stress-related toxicity assay was conducted, with results showing ROS induced by Ag NPs activated both MAPK and NF-κB pathways [38].

4.3.3 Mitochondrial Perturbation and Cell Death

The high level of oxidative stress also affects the mitochondrial membrane potential, inducing the release of pro-apoptotic factors and cell death.

An investigation was conducted to compare the effect of Fe_2O_3, Fe_3O_4, and CuO NPs on mitochondrial perturbation. Results showed Fe_2O_3 and CuO NPs induced mitochondrial depolarization in A549 cells while Fe_3O_4 did not affect the mitochondria membrane potential [39]. ROS induced by ambient ultrafine particles, NH_2-PS and ZnO NPs resulted in mitochondria membrane potential decrease and cell apoptosis [14, 15]. Nano-HA and TiO_2 NPs also induced the production of ROS and inflammation, and eventually cell apoptosis. It is interesting that TiO_2 NPs induced a higher percentage of early apoptosis than nano-HA, while nano-HA induced a higher percentage of late apoptosis [29]. ROS induced by Ag NPs on human Jurkat T cells resulted in a time-dependent apoptosis, in which both early and late apoptosis were observed [38]. PVP-decorated Ag NPs were observed to induce apoptosis and necrosis in THP-1 monocytes in a time-dependent manner [40]. ROS induced by pristine graphene in murine RAW 264.7 macrophages activated MAPK pathway, resulting in cell apoptosis. In addition, late apoptosis and necrosis increased in a dose-dependent manner while early stage apoptosis was not affected [37]. The impact of size on oxidative stress induced apoptosis was conducted, with results showing the percentage of early apoptosis was not affected by NPs' size. Silica NPs with a diameter of 19 nm induced higher percentage of late apoptosis than silica NPs with diameters of 43 and 68 nm [41].

4.4 Nanoparticles' Physicochemical Properties Regulate Oxidative Stress and Nanotoxicity

Although the induction of oxidative stress by NPs may proceed through a variety of mechanisms, the oxidative stress formation from a particular NP depends on the physicochemical properties of the NPs. The critical physicochemical properties that lead to the induction of oxidative stress and nanotoxicity include composition, size, shape, and surface chemistry.

4.4.1 Composition

The toxicity of spherical metal oxides NPs with different chemical composition [Fe_2O_3 (29 nm), Fe_3O_4 (20–30 nm), TiO_2 (63 nm) and CuO (42 nm)] has been compared [39]. CuO NPs were found to be the most toxic ones in A549 human lung cancer cells by inducing DNA damage and DNA lesions. A lot of polymer and inorganic nanomaterials can induce oxidative stress by generation of ROS.

For example, the widely used Ag NPs were reported to induce apoptosis by generation of ROS and inducing DNA damage in Jurkat T Cells [38]. Magnetite NPs, which can be used as contrast agents for MRI imaging, induced high level of ROS in A549 cells [42]. Previous results showed graphene oxide (GO) (thickness of about 1 nm and the size of 1–2 μm) induced ROS production in J774A.1 and RAW264.7 cells [43].

Some studies were conducted to investigate the impact of composition of NPs on ROS induction. Four types of nanomaterials with different compositions [CB (sphere, 12.3 ± 4.1 nm), single-walled carbon nanotubes (SWCNTs) (rope-shaped, diameters: 8 nm length: <5 μm), SiO_2 NPs (crystal structure, 20.2 ± 6.4 nm) and ZnO NPs (crystal structure, 19.6 ± 5.8 nm)] induced different levels of cytotoxicity on primary mouse embryo fibroblast cells [44]. ZnO NPs induced the highest cell death and oxidative stress among all NPs. However, SWCNTs exhibited highest genotoxicity, which might be attributed to particle shape. A high-throughput screening approach was used to analyze the toxicology of Au (sphere, 12 nm), Ag (sphere, 13 nm), Pt (sphere 13 nm), Al_2O_3 (sphere 12 nm), SiO_2 (sphere 19 nm), ZnO (sphere 10 nm), and CdSe/ZnS (QD) (dot, 6.5 nm) NPs in RAW 264.7 and BEAS-2B cells. The results showed QD and ZnO trigger the highest ROS level, while Au, Ag, Pt, Al_2O_3, and SiO_2 did not induce ROS [45]. The relation between intracellular ROS level and NPs' properties related to their composition, such as conduction band energy, was investigated among 24 MOx NPs (10–100 nm, except for Cr_2O_3 and Ni_2O_3 of 193 ± 90.0 and 140.6 ± 52.5 nm, respectively). NPs could induce high level of ROS when NPs' band energy was between cellular redox potential (−4.12 to −4.84 eV) (Fig. 4.2) [36]. Using this method, Nel et al. demonstrated that Co_3O_4 NPs induce high level of ROS and PdO doping could dictate the band energy of Co_3O_4 NP (11.1 ± 2.8–13.3 ± 3.7 nm), which could tune the oxidative stress level. The intracellular ROS level was positively correlated to percentage of PdO doping [17].

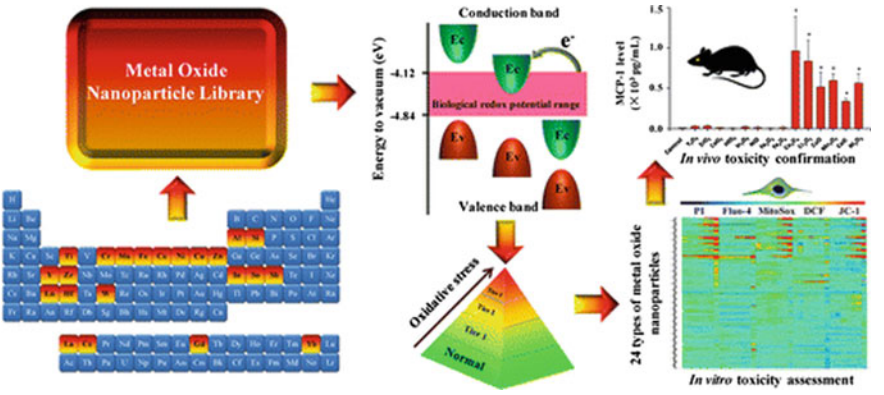

Fig. 4.2 Use of metal oxide nanoparticle band gap to develop a predictive paradigm for oxidative stress. Reprinted with the permission from [36]. Copyright 2012 American Chemical Society

4.4.2 Size

The toxicity is regularly negatively related to NPs' size. The cytotoxicity of Ag NPs with different sizes from 10 to 75 nm was evaluated in BEAS-2B cells [46]. Small NPs (10 nm) were found to be more toxic. In another paper, size-dependent toxicity of Ag NPs (15, 30, and 55 nm) has been reported. Smaller Ag NPs could induce higher apoptosis in macrophages than larger NPs at the same dose (10 μg/mL) [47]. Smaller ZnO NPs induced higher cell cytotoxicity in human $CD4^+$ T cells [48]. Silica NPs have been used in gene delivery and biomedical imaging. The hemolytic activity was investigated using silica NPs with different diameters. Smaller nano-silica induced higher percentage of hemolysis of red blood cells [49]. The interaction between NPs and biomolecules, such as protein and DNA, takes place on the nano-bio interface, and sequentially affects NPs' toxicity. NPs' curvature and surface area are crucial parameters for NP-biomolecule interactions and are determined by NPs' size. Therefore, small-sized NPs have great potential in inducing cell death. The toxicity of nano- and micrometer particles of CuO after exposure of A549 cells confirmed the above conclusion [39]. CuO NPs were more toxic in terms of cytotoxicity, mitochondrial damage, DNA damage and oxidative DNA lesions in A549 cells than micrometer particles.

The impacts of NPs' size on oxidative stress and ROS have also been investigated by several research groups. Because of the tiny size of NPs, they can easily penetrate cell membranes and other biological barriers into living organisms, so the intracellular ROS level is in general negatively correlated to NP's size. For example, Ag NPs was used to investigate the influence of size on intracellular ROS level in macrophage, indicating that Ag NPs with a diameter of 15 nm induced higher ROS level in alveolar macrophages than NPs with a diameter of 30–55 nm. The enhanced ROS was caused by the decrease of mitochondria membrane potential [47]. In another paper, the impact of Ag NPs' size on ROS in non-phagocyte was investigated, showing that small sized Ag NPs induced highest ROS level in hepG2 cells [50]. Silica NPs with a diameter of 19 nm induced higher ROS level than that of 43 and 68 nm in hepG2 cells. Cell apoptosis and necrosis were also found when incubated with silica NPs with a diameter of 19 nm [41].

4.4.3 Shape

Previous papers have demonstrated that the shape of NPs affects their nanotoxicity. Hemolytic activity of SiO_2 nanorods with aspect ratio of 2, 4, and 8 were investigated [51]. Spherical SiO_2 showed highest hemolysis activity, while mesoporous SiO_2 with high aspect ratio had lower hemolysis activity than mesoporous SiO_2 with low aspect ratio. TiO_2 nanomaterial with a fiber structure of longer than 15 nm showed higher toxicity when compared with TiO_2 nanospheres with a diameter of 5 nm [52] because that long TiO_2 nanobelts induced inflammasome activation and

release of inflammatory cytokines. The shape of Ni NPs also dramatically affected the toxicity upon exposure to zebrafish embryos. Dendritic clusters consisting of aggregated 60 nm particles resulted in higher toxicity than spherical Ni NPs [53].

The cytotoxicity, DNA oxidative damage, and apoptosis in HeLa cells were investigated when they were incubated with wire-shaped NPs and alpha-MnO_2 nanowires, respectively. Long nanowires in cultured fibroblasts cause failed cell division, DNA damage, and increased ROS, while vertical nanowire arrays induce cell motility and proliferation rate [54]. NPs' aspect ratio is negatively correlated to ROS level. For example, ROS in HCT116 cells was evaluated after exposed to CTAB-decorated gold nanorods (GNRs). GNRs with lowest aspect ratio induced the highest intracellular ROS [55]. In another paper, ROS induced by spherical gold nanoparticles (GNPs) and GNRs was investigated in MDCK II cells. Spherical GNPs with diameter of 43 nm induced higher ROS level than GNRs with a size of 38 nm $\times$ 17 nm [56]. The impact of mesoporous silica NPs' shape on intracellular ROS was investigated in A375 cells. Spherical NPs induced highest ROS level, while long rod-like NPs possess low intracellular ROS level [57]. Y_2O_3 NPs could be used in biological imaging and photodynamic therapy. Spherical Y_2O_3 NPs induced higher ROS level than rod-like NPs [58]. The aforementioned properties may be useful for design new NPs with expected biocompatibility and low toxicity.

Besides aspect ratio, other factors of NPs could also dictate ROS level. For example, our lab found that PS nano-disk did not induce ROS generation, while PS nanosphere elicited intracellular ROS production in BJ and jurkat cells. In addition, ROS level induced by PS NPs is correlated with cellular uptake [59]. Hexagonal plate-like ZnO nanocrystals were also reported to display significantly higher activity in ROS induction than rodshaped crystals [60].

4.4.4 Surface Chemistry

A wide variety of synthetic and natural ligands could be attached to NPs to tune the surface physicochemical properties of the NPs. Surface chemistry modification can be divided into non-covalent and covalent method. The non-covalent decoration is usually achieved by non-covalent interactions between NPs and ligands, including hydrophobic interactions [61, 62], ionic interactions [63, 64] and π–π interactions [65, 66]. Ligands can also be covalently linked to the surface of NPs through certain chemical reactions. Compared to non-covalent methods, covalent methods could afford more versatile decoration to NPs through stable chemical bonds. Different chemical reagents and linking methods can be used in chemical modifications of NPs. With strong oxidative acid treatment, abundant carboxylic groups can be introduced onto the surfaces of CNTs [67], thus providing the possibility of further immobilization of functional molecules on the nanotubes by reacting with carboxylic groups. Surface functionality of NPs with poly (ethylene glycol) (PEG) could effectively prevent NP agglomerations and protein adsorption, leading to the extended circulation time of NPs in biological systems. The chemical

couplings of PEG onto GNPs are often performed by Au-S bond or copolymerization of PEG with the bulk materials of NPs to form the PEGylated NP systems [68, 69].

The decoration on NPs may also alter NPs' surface charge density, resulting in the variation of nanotoxicity. NPs initially interact with plasma membranes when exposed to cells. The damage of model cell membranes was evaluated by GNPs and TiO_2 NPs with different surface charges [70]. Both kinds of NPs with positive charges on surface induced strong leakage (>80%), whereas only marginal leakage, was found for negatively charged NPs. When a human keratinocyte cell line (HaCaT) was exposed to three different GNPs (positively charged, neutral, and negatively charged), cell morphology was disrupted by all GNPs and charged GNPs displayed a lower LD_{50} than neutral GNPs. More oxidative stress was founded in cells treated with charged GNPs than neutral ones [71]. In addition, charged GNPs and neutral GNPs caused cell death through different mechanisms. Charged GNPs promoted the expression of p53 and caspase-3 in nuclear, while the neutral GNPs caused an increased expression of p53 in both nuclear and cytoplasm. Lin and Zhang et al. also investigated the effect of GNPs' surface charge on cytotoxicity [72]. They found that positively charged GNPs can be attracted by the cell membrane due to electrostatic interaction, while negatively charged GNPs were rejected. Increasing GNPs' surface charge density promoted cellular uptake and increased cytotoxicity. However, the level of penetration and membrane disruption were in different manner, with low charge densities inducing high penetration and high charge densities resulting in membrane disruption. Besides GNPs, it was also found that SWCNT coated with negatively charged ligands caused a minimal leakage of liposomes [73]. Meso-2,3-dimercaptosuccinic acid (DMSA) was used to modified Fe_2O_3 NPs for reducing cytotoxicity and genotoxicity [74]. Auffan et al. found that DMSA-Fe_2O_3 NPs caused lower toxicity in fibroblasts, which might be due to that the negative charge on the surface of DMSA-Fe_2O_3 NPs prevented cell contact and reduced the toxic effect. When superparamagnetic iron oxide NPs (SPIONs) were coated with different surface ligands [75], Mahmoudi et al. found that bare SPIONs were more toxic than charged SPIONs in three cell lines (HCM, BE-2-C, and 293T), while the SPIONs-COOH showed lower toxic than SPIONs-NH_2. The result was consistent with the alteration of genes expression levels. In conclusion, surface charge also plays an important role on intracellular ROS level, with positively charged NPs inducing higher intracellular ROS level than negatively charged and neutral NPs [14]. A similar phenomenon was found when positively and negatively charged silicon NPs were exposed to macrophage NR8383 cells [76]. The intracellular ROS level in HeLa cells was detected after exposure to a GNP library decorated with cationic ligands of different length. The results showed that intracellular ROS level was positively correlated with ligand length [77]. Poly(methacrylic acid) (PMAA) and oleic acid (OA) were used to decorate ZnO NP, resulting low level of ROS induction of PMAA and OA decorated ZnO NPs compared to undecorated ZnO NPs [78].

The cytotoxicity of NPs can also be modulated by altering the hydrophobicity. Our lab synthesized a GNP library with a continuous change in hydrophobicity. It was found that cell viability was negatively correlated with hydrophobicity [79].

The cytotoxicity of GNRs coated with CATB and PEG has been compared [80]. It showed that GNRs coated with CTAB resulted in more cell death than PEG-GNRs in Hela cell. The cytotoxicity of unmodified and PEG functionalized SWNTs in neuronal PC12 cells has been compared at cell and molecular level [81]. Unmodified SWCNTs showed higher cytotoxicity than PEG-SWCNTs because of the ROS level. Phospholipid-PEG (Pl-PEG)- coating CNTs was also found to enhance cell viability and proliferation [82].

Other polymers were also used for decorating NPs. Various layer-by-layer polyelectrolytes (PE) were used for surface decoration of GNRs. The cellular uptake, toxicity, and gene expression in HeLa cells could be regulated by PE density [83]. The cytotoxicity of uncoated and poly(vinyl alcohol) (PVA)-coated SPIONs in mouse fibroblasts has been compared in another report [84]. Cell viability of bare SPION was lower than PVA-coated SPIONs at the same concentration. Significant apoptosis could be found in cells treated with bare SPIONs at high concentration while PVA-coated SPIONs induced no apoptosis. In another paper, Poly(*N*,*N*-dimethylacrylamide) (DMAAm) was used for Fe_2O_3 NP functionality [85]. It was found that the cell viability of DMAAm-Fe_2O_3 NPs was higher than dextran-modified and unmodified Fe_2O_3 NPs.

4.5 Concluding Remarks

Production of manufactured NPs for commercial usage in various fields has been growing exponentially, therefore, the toxicity of NPs has become an urgent issue to the public. ROS generation and oxidative stress are usually considered to be the starting points of toxicology induced by NPs. Nanotoxicity such as inflammation and cell death has been recognized as the downstream effects to the ROS formation and oxidative stress. Oxidative stress can be directly induced by NPs or indirectly through mitochondrial respiration and activation of NADPH oxidase. NPs' physicochemical properties, such as composition, size, shape, and surface chemistry, have been found to affect the generation of ROS and oxidative stress.

Besides nanotoxicity, oxidative stress is also related to aging and diseases, such as cancer and neurodegeneration. For example, DNA damage induced by oxidative stress is responsible for cancer induction. Under high level of oxidative stress, cancer cells will be killed. Therefore, precise regulation of oxidative stress is important for both prevention of NP-induced toxicity and treating diseases. However, previous studies generally attempted to explore the correlations on a case-by-case basis, looking at one parameter while keeping others constant. In addition, the complex formation of NPs in biological systems (such as adsorption of serum proteins) further aggravates the difficulty in precise regulation of oxidative stress. Therefore, examining the collective impact of combined parameters of NPs on oxidative stress and nanotoxicity will be crucial for systemically understanding the interactions between NPs and biological systems and will also be helpful for design of new NPs with better safety profiles.

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