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Current Approaches for Safer Design of Engineered Nanomaterials

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Abstract

The surge of applications for engineered nanomaterials (ENMs) across multiple industries raises safety concerns regarding human health and environmental impacts. ENMs can be hazardous through various mechanisms, including, particle dissolution and shedding of toxic metal ions, surface reactivity and perturbation of cellular membranes, lysosomal membrane damage, activation of inflammation pathways (*e.g.*, NLRP3 inflammasome), *etc*. The aim of this review is therefore to discuss practical approaches for the safer design of ENMs through modification of their physicochemical properties that can lead to acute and/or chronic toxicity. This is premised on our understanding of how different ENMs induce toxicity within various biological systems. We will summarize studies that have investigated nanomaterial toxicity both *in vitro* and *in vivo* to understand the underlying mechanisms by which nanoparticles can cause inflammation, fibrosis, and cell death. With this knowledge, researchers have identified several design strategies to counter these mechanisms of toxicity. In particular, we will discuss how metal doping, surface coating and covalent functionalization, and adjustment of surface oxidation state and aspect ratio of ENMs could reduce their potential adverse effects. While these strategies might be effective under certain experimental and exposure scenarios, more research is required to fully apply this knowledge in real life applications of nanomaterials.

Graphical Abstract.

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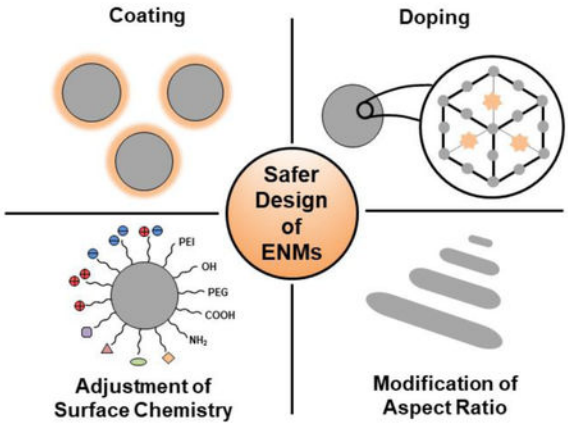

Keywords

Engineered nanomaterials; safer design; doping; coting; surface chemistry; aspect ratio

1. Introduction

Engineered nanomaterials (ENMs) are increasingly used in different scientific and industrial disciplines, including, but not limited to, medicine, transportation, electronics, agriculture, food, and cosmetics.1,2,3 For therapeutic and diagnostic purposes, ENMs have emerged as great candidates for targeted drug delivery and bioimaging.4,5 The electrical and waste water treatment industries utilize nanomaterials to improve sensing and conduction properties of materials and improve water quality, respectively.6,7,8 However, this growing application of nanomaterials in consumer products and various technologies could increase the possibility of ENMs entering into human bodies and the environment, and raise major safety concerns with regard to their potential adverse impacts. In fact, numerous studies have previously explored toxicological effects of ENMs on different biological species, and found some of these nanomaterials to be toxic towards mammalian cells, plants, and aquatic organisms.9,10,11,12,13,14,15,16,17,18,19 Moreover, some of these investigations were able to correlate the adverse biological outcomes of these cytotoxic nanomaterials to their physical or chemical characteristics (*e.g.*, size, surface charge, and aspect ratio) and identify the key factors that make them toxic to the biological organisms. These correlations between nanomaterial's physicochemical properties and its cytotoxicity (*i.e.*, structure-activity relationships) have been derived by preparing combinatorial libraries of nanomaterials with various, but well-defined, physicochemical properties such as size, surface chemistry, and shape, and their mechanistic toxicological profiling in tissue culture cell and animal models. 15,20,21 For example, comprehensive analysis of highly soluble metal oxides particles such as ZnO and CuO has shown that these nanoparticles induce significant cytotoxicity in mammalian cells and living animals because of their dissolution and release of toxic metal ions.22,23,24,25 Another example is silver nanoparticle that has been demonstrated to display a size-dependent cytotoxicity due to its dissolution and release of silver ions. 17,19,26 Comprehensive toxicological profiling of ENMs and development of structure-activity relationships not only helps to identify the key physical or chemical characteristics

of ENMs that render them toxic, but also helps to develop safer design strategies that minimize nanomaterials toxicity by optimizing their physicochemical properties.11,16,22,25 Thus, we aim to discuss exemplary studies in this review article that explored the underlying mechanisms linking nanomaterial toxicity to their physicochemical properties and proposed strategies to design safer ENMs premised on these correlations. While there might be various safer design techniques to develop less toxic nanomaterials, we will specifically summarize example approaches, including, doping, surface coating, adjustments of surface chemistry and charge, and modification of shape and aspect ratio.

2. Examples of Safer Design Approaches

2.1. Doping

Several studies have identified doping as an effective strategy to reduce the cytotoxicity of industrially important ENMs such as ZnO, CuO, and SiO_2 nanoparticles. Doping is a facile yet effective method that is used to modify a material's crystal structure by addition of impurities in order to achieve improved catalytic, electro-optical, magnetic, chemical, and physical properties.27 Dopants such as iron (Fe), titanium (Ti), and aluminum (Al) are typically evenly incorporated into the host lattice to change the binding energy of metal ions to oxygen, or reduce the density of reactive chemical groups on the particle surface.16,22 The working mechanisms of doping in reduction of ENM cytotoxicity is premised on changing nanoparticle's physicochemical properties, which involves either decrease in nanoparticle dissolution and release of toxic ions, modification of reactive surfaces to reduce generation of reactive oxygen species (ROS), or perturbation of the cellular membrane that leads to inflammation and cell death.16,22,24,25

Flame spray pyrolysis (FSP) is a well-established technique for the doping of nanomaterials, which employs rapid combustion during the synthesis procedure.28 Through a liquid precursor, a self-sustaining flame with high local temperature and large temperature gradient allows for the formation of homogenous crystalline nanoscale materials from droplet or gas to particle.28 This process is well-suited for industrial applications because of the facile one-step synthesis process and potential to scale-up the production of doped nanomaterials. Here we will give several examples of the FSP approach to reduce toxicity of nanomaterials.

One particular example is Fe-doped ZnO nanoparticles. ZnO is an important ENM that has wide industrial applications such as in cosmetics (*e.g.*, sunscreens) and electronics.3 However, there are clinical reports on ZnO-induced pulmonary inflammation in humans called metal fume fever that occurs when welders are exposed to metal fumes containing high concentrations of ZnO.29 This indicates that assessment of ZnO nanoparticle toxicity is highly relevant to human health. ZnO dissolution to Zn^{2+} ions has been known to play a major role in its induction of toxicity and inflammation, thus reduction of dissolution could potentially decrease its adverse effects.30 In order to explore the effect of doping on dissolution and toxicity of ZnO nanoparticles, George *et al.* prepared Fe-doped ZnO nanoparticles by FSP and assessed their cytotoxicity in RAW 264.7 and BEAS-2B mammalian cells.22 Characterization of particle dissolution indicated Fe-doped ZnO nanoparticles are less soluble than pure ZnO particles, which is attributed to the higher binding energy of iron to oxygen than zinc.22 The reduced dissolution further led to a

decrease in cytotoxicity, which was reflected by the decrease in number of cells positively stained by propidium iodide (PI), and preservation of mitochondrial membrane potential at even the lowest Fe-doping level of 1.02% (atomic percentage of Fe content).22 These *in vitro* data were also validated *in vivo* by examining the toxicity of Fe-doped ZnO nanoparticles in zebrafish embryos and rodent lungs.24 While ZnO nanoparticle exposure adversely interfered with embryo hatching, which ultimately caused starvation and death, hatching rate significantly improved upon exposure to Fe-doped ZnO nanoparticles. When rodents including mice and rats were exposed to Fe-doped ZnO nanoparticles, polymorphonuclear cell count, lactate dehydrogenase (LDH) release, and cytokine (IL-6) production in bronchoalveolar lavage (BAL) fluid were reduced compared to levels in rodents exposed to the un-doped ZnO controls. Hemeoxygenase-1 (HO-1) expression, an oxidative stress biomarker for inflammation, was also reduced in animals exposed to Fe-doped ZnO. These results demonstrate that doping partially inhibits cytokine production and can lessen the inflammatory response.24 While these two studies provide information about toxicity of Fe-doped and un-doped ZnO nanoparticles towards mammalian cells, another study assessed the viability of bacteria, including, *B. subtilis*, *P. putida*, and *E. coli*, in the presence of these nanoparticles.31 Interestingly, it was found that Fe-doping does not impact the IC_{50} values of these nanoparticles and bacteria viability.31 The difference behind these findings can be explained by variations with experimental cell type, exposure environment, and dose of ZnO nanoparticles. In fact, tannic acid that was added to the exposure media in order to mimic the natural aquatic environment increased the IC_{50} values of ZnO nanoparticles by uptake and chelating of Zn^{2+} ions. Therefore, ZnO nanoparticle toxicity was ameliorated by Zn^{2+} chelating action of tannic acid rather than Fe-doping.

Another example is CuO nanoparticles doped with iron via FSP. CuO nanoparticles are employed in large-scale production for semiconductors, antifouling paints, and sensors.25 Similar to ZnO nanoparticles, CuO nanoparticles are hazardous due to dissolution and shedding of toxic Cu^{2+} ions that generate ROS and induce inflammation.25 Copper can cause oxidative stress injury leading to inflammation,20 cellular DNA damage,32 and reduced reproductive capacity in aquatic organisms such as *Daphnia Magna*.33 The dissolution of un-doped and Fe-doped CuO nanoparticles in various aqueous solutions were compared by Naatz *et al.* and it was found that the dissolution rate of CuO decreased with increasing iron content in doped particles. Accordingly, cell viability was improved in THP-1 and BEAS-2B cells exposed to Fe-doped CuO, which resulted in a significant reduction of cell death.25 At the dose of 0.5 ppm in Holtfreter's medium, a balanced salt solution developed for maintaining zebrafish embryos, there was approximately 6% dissolution without Fe-doping and less than 1% dissolution with 10% Fe-doping after 24 hours.25 The oxidation reaction between sodium bicarbonate in Holtfreter's medium and CuO enhances dissolution which is reduced by Fe-doping. Decreasing dissolution was also observed at the dose of 50 ppm in cell culture media, including, RPMI 1640 and BEGM, as well as deionized (DI) water.25 The authors further assessed the toxicity of Fe-doped CuO nanoparticles in THP-1 and BEAS-2B cells as well as zebrafish embryos.25 The reduction in shedding of Cu^{2+} ions had a positive impact on viability of THP-1 and BEAS-2B cells as well as zebrafish embryo hatching rate.25 In another study, Adeleye *et al.* used similar FSP-fabricated Fe-doped CuO nanoparticles and showed Fe-doping could decrease colloidal

stability and increase the dissolution of CuO nanoparticles in natural waters.34 These contradictory results in terms of the effect of iron doping on CuO nanoparticle dissolution can be explained by enhanced reactivity at neutral pH and surface area after doping, which promotes nanoparticle dissolution.34 Therefore, these results further highlight the important role of exposure media in toxicological analysis of nanomaterials, as it was also significant in the previous section for Fe-doped ZnO nanoparticles.

Doping of amorphous fumed silica is another example for the safer design of ENMs by this technique. Fumed SiO_2 has increased applications in the food industry due to being generally recognized as safe for food additives and packaging.16 However, recent studies showed that fumed SiO_2 nanoparticles are hazardous due to their chain-like structure as well as high three membered siloxane rings and surface silanol density on the particle surface. The chain-like structure of fumed silica prevents cellular uptake and the particles are adsorbed on the cell membrane. The three membered siloxane rings on the surface are strained and they can be easily hydrolyzed to form hydrogen bonded silanol groups (*e.g.*, ≡Si-OH), which are highly active to generate ROS, leading to disruption of cell membranes. Membrane perturbation leads to potassium efflux, NLRP3 inflammasome activation and IL-1β production.16,35 To investigate the impact of doping on the toxicity of fumed SiO_2 nanoparticles, Sun *et al.* synthesized a library of Ti- and Al-doped fumed silica nanoparticles with varying concentrations of aforementioned dopants by FSP.16 Characterization of doped and un-doped SiO_2 nanoparticles showed that increasing the concentration of aluminum and titanium in doped fumed silica nanoparticles decreased the total surface silanol density.16 Additionally, authors observed a decline in abiotic ROS generation for doped silica nanoparticles, suggesting a direct relationship between density of surface silanol groups and ROS generation.16 The toxicological impact of pristine and doped fumed silica particles was assessed both *in vitro* and *in vivo*. While doped fumed silica was predominately taken up into the cytoplasm of THP-1 cells, un-doped particles adhered to and perturbed the cell membrane, indicating the key role of surface properties of fumed silica nanoparticle in determining its cellular uptake profile.16 Furthermore, cytotoxicity and pro-inflammatory cytokine (IL-1β) production were also decreased for doped fumed silica particles. Doped silica was unable to induce IL-1β production in THP-1 macrophages, indicating that doping inhibits NLRP3 inflammasome activation and cytokine production.16 Reduced acute pulmonary inflammation in mice additionally confirmed that doping was protective against fumed silica exposure.

These examples show that doping is an effective method to reduce the nanoparticle-induced toxicity and increase the safety profiles of ZnO, CuO, and SiO_2 ENMs that are widely used in industrial applications. However, it should be noted that many factors such as particle dose and exposure environment need to be considered when one is drawing conclusions regarding the effect of doping on particle toxicity. In addition, it is necessary to further evaluate the impact of doping on the applicability of the final product. If doping has no interference with the desirable properties of a nanomaterial, this technique can be considered as a safer design strategy.

2.2. Surface Coating

Coating is one of the major surface modification strategies to design safer ENMs. Unlike doping, which can be disadvantageous due to the irreversible chemical modifications that may alter the intrinsic properties of the ENMs, surface coating can be reversible by non-covalent modification. In addition, the dispersion state of nanomaterials is a key factor in determining their bioavailability, bioreactivity, and thus potential toxicological and pro-inflammatory responses. With that being said, different noncovalent coatings can alter the dispersion state of nanomaterials to influence their toxicity.37 In this section, we present examples of safer design by surface coating for carbon nanotubes (CNTs), rare earth oxide nanoparticles (REO NPs), and upconversion nanoparticles (UCNPs) that have wide biomedical applications. These examples will demonstrate how surface coating with pluronic F108 (PF108) or phosphate provides a layer of protection for particle-induced toxicity.

Due to unique mechanical strength and high electrical conductivity, the applications of single-walled and multi-walled CNTs have expanded to drug delivery (*e.g.*, drug nanocarriers), tissue engineering (*e.g.*, bone scaffolds), water purification, and sensors.38 CNTs induce inflammation and/or fibrosis as a result of their length, surface chemistry, and aggregation state.37,39 In order to design safer CNTs, one possible approach is to change the surface properties of these carbonaceous nanomaterials via coating. One example is surface coating of CNTs by a nonionic triblock copolymer, PF108. This copolymer contains a long hydrophilic segment that allows for better dispersion and reduced agglomeration, cellular uptake, and pro-fibrogenic effects of CNTS shown by Wang *et al.*40 These authors further demonstrated the protective effects of PF108 coating on toxicity of CNTs in BEAS-2B and THP-1 cells, as well as mice lungs.40 PF108 coating decreased cellular uptake of CNTs and nanotube-induced lysosomal membrane damage in phagocytic THP-1 cells.40 There was also a decrease in pro-inflammatory cytokine (IL-1β) production by THP-1 cells and pro-fibrogenic TGF-β1 production by BEAS-2B cells. In addition, *in vivo* experiments demonstrated that PF108-coated CNTs had reduced collagen production and deposition in the lung as compared to pristine CNTs, which further validates the protective effect of surface coating against pulmonary fibrosis.40 The mechanism of this protective effect is based on the stability of PF108 coating on CNTs that remained intact even under acidic lysosomal conditions. The relative available surface area on CNTs coated with PF108 decreased in acidic conditions, which reduces direct interaction with lyososomal membranes and consequential damage.40 These results were supported by Mutlu *et al.* that demonstrated CNTs dispersed in PF108 had no lung toxicity and were almost completely cleared from the lungs after 90 days.41 In contrast, the aggregated CNTs without PF108 coating induced granulomatous lung inflammation and fibrosis.41

Rare earth-based nanomaterials such UCNPs have been widely used in industrial and biomedical applications (*e.g.*, bioimaging) due to their unique anti-stoke effects and high photostability.42,43 Nonetheless, occupational exposures from respirable rare earth dust can result in pneumoconiosis,44 and clinical exposures to gadolinium contrast agents in magnetic resonance imaging (MRI) have been shown to cause nephrogenic systemic fibrosis in patients with renal impairment.45 The mechanism of REO's profibrogenic effects

involves nanoparticle-induced lysosomal damage in macrophages (*e.g.*, THP-1 and Kupffer cells) and NLRP3 inflammasome activation.11,21 REO NPs can quickly dissolve in the acidic environment of lysosomes after cellular uptake, and the released rare earth ions have high binding affinity to phosphate groups. These phosphates exist in the interior area of the lysosome as well as phospholipids of the lysosomal membrane. Lysosomal damage occurs when these rare earth ions strip away the phosphates in the phospholipid bilayer of this organelle. Next, lysosomal damage leads to the release of cathepsin B enzyme, which induces NLRP3 inflammasome activation and IL-1β production.11,21 A recent study by Mirshafiee *et al.* further demonstrated that the induction of lysosomal damage by REO nanoparticles and activation of NLRP3 inflammasome in macrophages could result in a highly inflammatory cell death pathway called pyroptosis.21 Release of IL-1β would then trigger a cascade of events leading to fibrosis.11 For example, pulmonary exposure of La_2O_3 nanoparticles resulted in increased neutrophil count and lung fibrosis with extensive collagen deposition.46 In order to design safer rare earth-based nanomaterials, Li *et al.* demonstrated that pre-treatment of REO NPs with phosphates at neutral pH could prevent their induced lysosomal damage and pro-fibrogenic effects.11 This effective method is also applicable to other rare earth-based nanomaterials such UCNPs that induce similar toxicological effects via lysosomal damage.47 UCNPs are generally prepared by dispersion of trivalent lanthanide ions (*e.g.*, Er and Yb) into a dielectric rare earth-based lattice.12 In one example, $NaYF_4$:Er/Yb UCNPs coated with ethylenediamine tetra(methylenephosphonic acid) (EDTMP), significantly reduced lysosomal damage, subsequent activation of NLRP3 inflammasome, and IL-1β production, as compared to pristine UCNPs.47 In addition, 92% of cells treated with coated UCNPs retained fluorescence after 24 hours, whereas only 18% of cells treated with pristine UCNPs displayed fluorescence intensity. This would indicate that surface coating not only reduces particle toxicity, but also helps to improve the photostability of UCNPs. In addition to rare earth-based nanoparticles, it was recently found that EDTMP coating could also passivate the surface of toxic metal oxide nanoparticles such as CoO, Ni_2O_3, Co_3O_4, and CuO and reduce their cytotoxicity and acute pulmonary toxicity effects.48 Therefore, this coating could be an effective safer design approach for a broad spectrum of toxic metal oxide nanoparticles to facilitate the safe use of various metal oxide nanoparticles in commercial nanoproducts.

2.3. Adjustment of surface chemistry and properties

Adjustments of charge density and hydrophobicity are other mechanisms by which surface chemistry modifications can ameliorate nanomaterial toxicity and improve functionality in biomedical applications such as for targeted drug delivery.49,50 These surface chemistry properties can be adjusted by covalent binding of functional groups onto the ENM's surface. 51 Functional groups include anionic, nonionic, and cationic groups that can impact both the surface charge density and hydrophobicity.52,53

Covalent functionalization is a facile and effective method to create functional and safer CNTs for commercial applications. In order to identify the effect of surface charge and different functional groups on the toxicity of CNTs, Li *et al.* prepared a library of CNTs functionalized with common anionic, nonionic, and cationic surface groups and assessed

their cytotoxicity in THP-1 and BEAS-2B cells and the pulmonary system.52 Of the three groups, the anionic group (carboxylate and polyethylene glycol), resulted in the lowest pro-fibrogenic effect and uptake in THP-1 and BEAS-2B cells, observed through decreased production of IL-1β, TGF-β1, and platelet derived growth factor (PDGF-AA). Neutral and weakly cationic functional groups, amine and sidewall amine, exhibited intermediary effects, while the strongly cationic group, polyetherimide (PEI), induced significant lysosomal damage followed by the release of cathepsin B enzyme and subsequent lung fibrosis.52 Evident from these results, pro-fibrogenic responses are highly dependent on the surface charge and functional groups of CNTs. Positively charged CNT surfaces interact with anionic groups on the cell surface, leading to enhanced cellular uptake.54 *In vivo* data from pulmonary macrophages extracted from mice BAL fluid confirmed these *in vitro* results.52 Another example of the differential toxicity of anionic versus cationic functional groups is on gold nanoparticles (GNPs). Goodman *et al.* compared positively charged cationic GNPs (ammonium-functionalized) to negatively charged anionic GNPs (carboxylate-functionalized) and found significantly higher LC_{50} values for the anionic particles in *E. coli*, Cos-1 cells, and red blood cells. These results indicate that negatively charged anionic particles are less likely to be drawn towards and damage negatively charged phospholipid bilayers which can be considered protective against cytotoxicity.55 The toxicity of GNPs is also correlated to charge density and hydrophobicity.56 In multiple human lung and kidney cell lines, greater positive charge density and hydrophobicity resulted in the release of heme oxygenase 1 (HO-1) and intracellular H_2O_2, indicators of oxidative stress.56 The study concluded that hydrophobic and positively charged GNPs induce oxidative stress through activation of NADPH oxidase and disruption of the mitochondria, respectively.56 Therefore, covalent functionalization of CNTs and gold nanoparticles with anionic and hydrophilic surface groups could potentially decrease their toxicity.

Another example where adjustment of surface properties could positively impact nanomaterial toxicity is graphene oxide (GO).57 Due to the large surface area, good dispersibility, and high stability, GO is rapidly being developed into biomedical applications (*e.g.*, biosensors and drug delivery nanocarriers).58 However, any biomedical use will be premised on the safety of GO. Since GO is well-known to kill bacteria through induction of oxidative stress,59 it is possible that surface oxidation state plays an important role in GO toxicity. To explore this role, Li *et al.* adjusted the surface oxidation state of GO by catalytic chemical reactions and assessed its toxicity.57 This included solvothermal reduction of GO by *N*-methyl-2-pyrrolidinone (NMP) to produce reduced GO and hydration in alkalized aqueous solvents to open the epoxy rings and increase the density of hydroxyl groups and carbon radicals to produce hydrated GO.57 Toxicological profiling of pristine, reduced, and hydrated GOs were performed in THP-1 cells, alveolar macrophages, and mice lung.57 Hydrated and pristine GOs were more prone to induce cell death and lung inflammation *in vitro* and *in vivo*, respectively, than reduced GOs.57 *In vitro* observations indicated pristine and hydrated GOs were attached to or inserted into the membrane of THP-1 cells, whereas reduced GOs were primarily internalized into the macrophages due to the increased hydrophobicity.57 Moreover, the hydration process opens the epoxy rings in pristine GO and increases the density of carbon radicals, which leads to extensive lipid peroxidation, followed by failure of membrane integrity, and ultimately cell death. Evidently, for human

and environmental exposures, reduced GO is a safer design option. In a specific clinical setting, however, hydrated GO with high radical carbon density is capable of killing drug resistant bacteria such as *E. coli* and it can be utilized as an antibacterial coating on catheters without direct contact to cells or tissues in the body.60 Application of iron oxide nanoparticles are increasing across multiple industries.61 Iron oxide nanoparticles readily produce hydroxyl radicals from catalytic reactions that occur at the particle surface that attribute to their cytotoxicity.62 Surface functionalization with organic compounds (*e.g.*, aldehyde, amino, and carboxyl groups) can stabilize the high chemical activity of iron oxide nanoparticles and improve biological compatibility.63 Thus, oxidation state modification could be useful in designing safer and effective ENMs for targeted applications.

2.4. Adjustment of aspect ratio

Optimizing the aspect ratio of a nanomaterial is another effective method to reduce its cytotoxicity. Preparation and synthesis of nanomaterials in wire or rod shapes could diversify their usage and develop new applications.64,65,66 Although both nanomaterials with high and low aspect ratios can be taken up into cells, studies have found fiber like particles with high aspect ratios can induce damage to the intracellular organelles (*e.g.*, lysosomes),1 and may fail to be fully engulfed, namely “frustrated phagocytosis”, by macrophages and result in inflammation and cytotoxicity.1,67,68 Therefore, size manipulation of nanorods and nanowires to produce materials with optimal aspect ratios that will not trigger inflammatory responses, will help to design safer nanomaterials.

In order to investigate how aspect ratio affects nanomaterial toxicity, Ji *et al.* synthesized seven different CeO_2 nanorods and nanowires with aspect ratios of 4, 8, 16, 22, 31, 52, and greater than 100 with respective lengths of 33.2, 50.8, 106.7, 197.2, 310.4, 495.7, and greater than1000 nm, and assessed their toxicity in THP-1 cells.1 The authors classified CeO_2 nanomaterials with aspect ratios of 30 or lower as nanorods and those with aspect ratios greater than 30 as nanowires. *In vitro* results in THP-1 cells indicated that nanorods with high aspect ratios greater than or equal to 22 were capable of inducing inflammation via lysosomal damage, cathepsin B release, and assembly of NLRP3 inflammasomes. Nanowires with aspect ratios of 52 and greater than 100 resulted in cell death, while medium ratios of 22 and 31 did not decrease cell viability but induced significant amounts of IL-1β production. Like nanowires with high aspect ratio, nanorods with low aspect ratio of 8 were readily taken up into cellular compartments but did not cause lysosomal damage or cytotoxicity.1 The mechanism by which high aspect ratio CeO_2 is hazardous can be attributed to strong van der Waals forces53 and dipole-dipole attractions, which cause long wires to form stacking bundles that can contact cellular surfaces and pierce membranes.1 When lysosomes are ruptured by nanowires, cathepsin B is released, signaling the assembly of NLRP3 inflammasome which triggers an inflammatory response.1 Damaged plasma membrane will lead to cell swelling and necrosis.69 Release of LDH, an enzyme found in all living cells, was a clear indicator of cell death as a result of membrane disruption.1 In order to determine whether a similar toxicological profile exists *in vivo* for these nanomaterials, Lin *et al.* further compared the toxicological impacts of CeO_2 nanospheres, nanorods, and nanowires in the gastrointestinal (GI) tract of zebrafish larvae and mice lung.67 Despite both CeO_2 nanosphere and nanorod induced acute lung inflammation in mice after a 40 hour

exposure by oropharyngeal aspiration, only the longest CeO_2 nanowires (>100) induced significant production of IL-1β and TGF-β1 in BAL fluid after 21 days, with no signs of pulmonary fibrosis. However, a sub-chronic exposure of 44 days to these longest nanowires resulted in more collagen deposition in the lungs, and pulmonary fibrosis. Furthermore, significant growth inhibition and delayed development of zebrafish larva was observed when they were exposed to CeO_2 nanorods with aspect ratios of 52 and greater than 100. This was suggested by the absence of dorsal and anal fin structures and fewer calcified vertebral segments. Assessment of CeO_2 nanomaterial uptake in the GI tract of zebrafish larvae revealed that nanowires were aggregated in the GI tract lumen and capable of piercing and disrupting the microvilli.

Park *et al.* compared the differential toxicity of high (6.2 ± 0.6) and low (2.1 ± 0.4) aspect ratio aluminum oxide nanorods to find that the nanorods with higher aspect ratio induced a greater inflammatory response in mice, observed through higher levels of inflammatory cytokines (*e,g.,* TNFα, MCP-1, and IL-6), histopathological lesions, and cell death. These results were further validated *in vitro* from cell lines of the kidney, skin, liver, lung, brain, and heart. Long aluminum oxide nanorods induced significant membrane damage in lung and kidney cells, whereas short nanorods did not. As expected, a significant decrease in cell viability for lung and kidney cell lines was confirmed with high amounts of LDH release, an indicator of membrane damage.70 Another study investigated short (1 μm) and long (10 μm) silver nanowires instilled into rat lungs. While both lengths were internalized into alveolar epithelial cells and phagocytized, silver nanowires with a higher aspect ratio caused more inflammation in the beginning and lasted longer with increased numbers of neutrophils and greater cytokine production.71 These results indicate that the shape and aspect ratio play a critical role in the cytotoxicity of rod and wire-shaped various nanomaterials, and they could also impact the safety of other industrial nanoparticles. Thus, the ability to synthesize nanomaterials with varying aspect ratios allows for the safer design of nanorods and nanowires.

3. Conclusions and Perspectives

Metal doping, coating, surface functionalization, oxidation state modification, and adjustment of aspect ratio are parts of various strategies for designing ENMs that are biologically and environmentally safer. Each one of these methods targets and prevents a mechanism of toxicity by modifying physiochemical properties of ENMs such as dissolution and release of toxic metal ions, agglomeration, damage to lysosomes, and perturbation of cellular membranes. These modifications ameliorate and in certain materials prevent toxic effects entirely. However, we also demonstrated that the safer designed nanomaterials were not always effective under certain exposure conditions and environments. Further testing of these materials will delineate the effective boundaries of this approach. With the increasing number of new ENMs and their biomedical applications, including, bioimaging, drug delivery, and cancer therapy, it is important to consider the safer design approaches for nanomaterials to make their use safe and sustainable.

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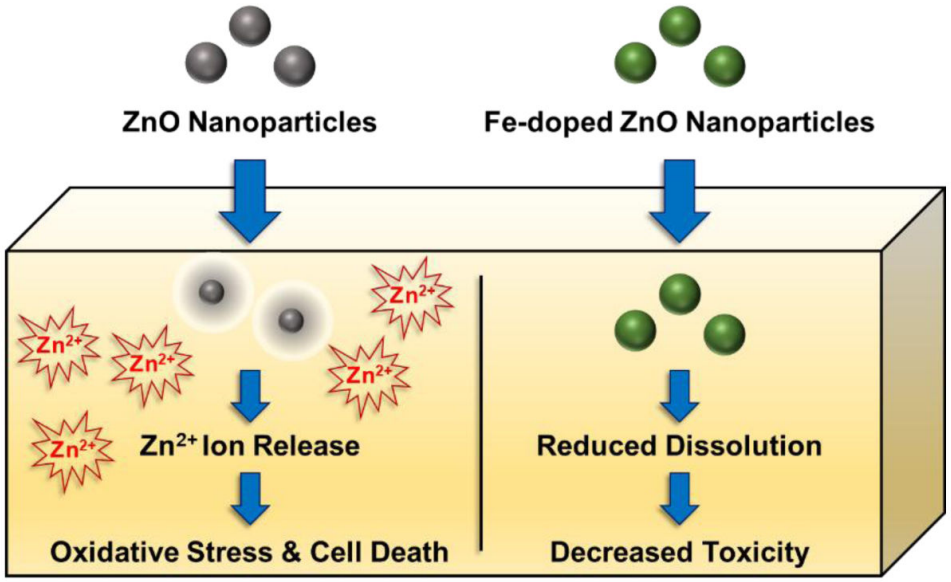

Figure 1.

Effect of Fe doping on cytotoxicity of ZnO nanoparticles. Doping ZnO particles with iron decreases their dissolution and shedding of toxic Zn^{2+} ions that further reduces their induced oxidative stress and cell death.

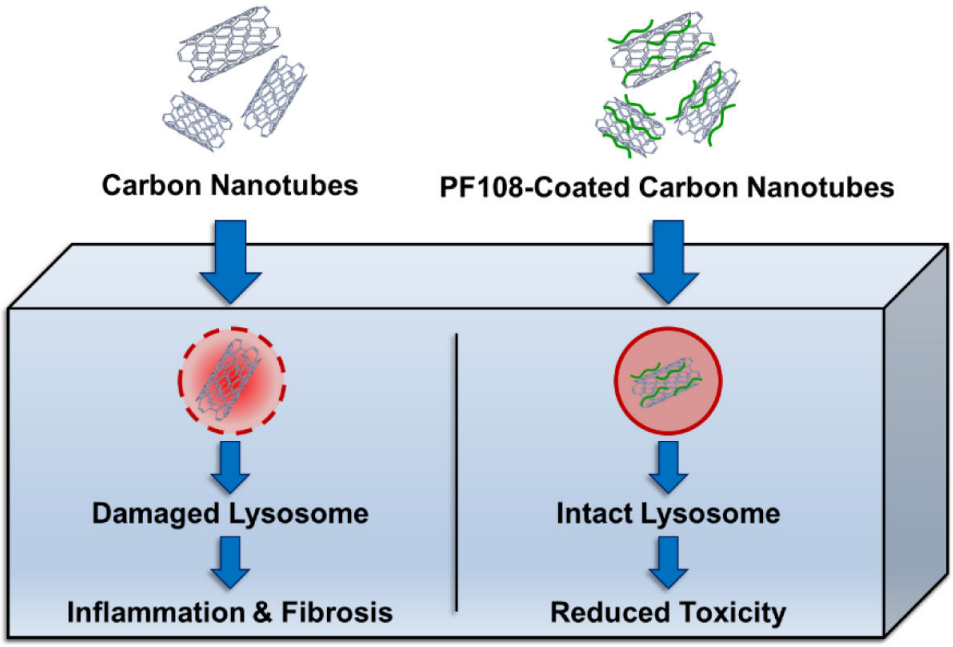

Figure 2.

Effect of surface coating on cytotoxicity of carbon nanotubes. Coating CNTs with Pluronic F108 prevents lysosomal damage and subsequent inflammation and fibrosis.

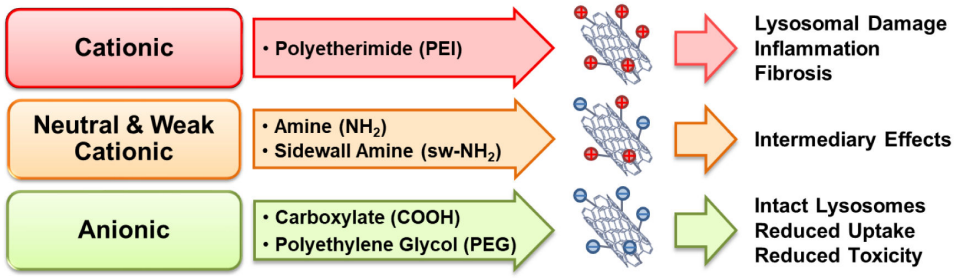

Figure 3.

Effect of surface chemistry on toxicity of CNTs. Cationic functionalization of CNTs promotes their toxicity by increasing cellular uptake, lysosomal damage, and inflammation, whereas anionic functionalization does not display these toxicological features.

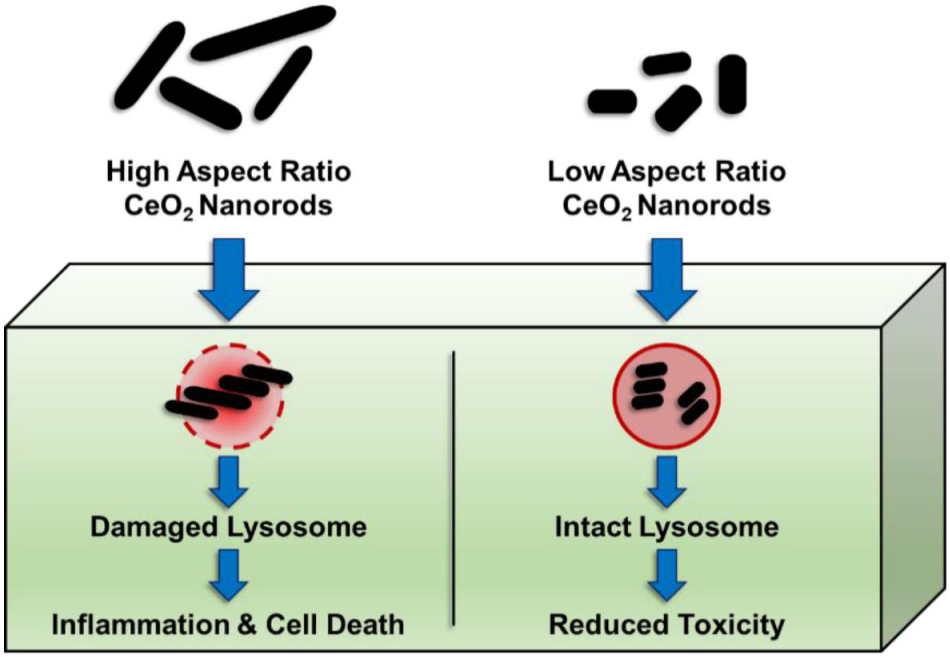

Figure 4.

Toxicity of CeO_2 nanowires and nanorods is dependent on aspect ratio. Long aspect ratio CeO_2 nanorods could trigger lysosomal damage after their cellular uptake, that could lead to inflammasome activation, inflammation, as well as cell death.