## Title

Facilitating Translational Nanomedicine via Predictive Safety Assessment



## Journal

Molecular Therapy, 25(7)

## ISSN

1525-0016

## Authors

Mirshafiee, Vahid
Jiang, Wen
Sun, Bingbing
et al.

## Publication Date

2017-07-01

## DOI

10.1016/j.ymthe.2017.03.011

Molecular Therapy
Review

# Facilitating Translational Nanomedicine via Predictive Safety Assessment

Vahid Mirshafiee,[1,5] Wen Jiang,[1,5] Bingbing Sun,[2,3] Xiang Wang,[1] and Tian Xia[1,2,4]

[1]Center for Environmental Implications of Nanotechnology (CEIN), California NanoSystems Institute (CNSI), University of California Los Angeles, 570 Westwood Plaza, Los Angeles, CA 90095, USA; [2]Division of NanoMedicine, Department of Medicine, University of California Los Angeles, 10833 Le Conte Ave., Los Angeles, CA 90095, USA; [3]State Key Laboratory of Fine Chemicals, Department of Chemical Engineering, Dalian University of Technology, 2 Linggong Rd., Dalian 116024, China; [4]State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

**Extensive research on engineered nanomaterials (ENMs) has led to the development of numerous nano-based formulations for theranostic purposes. Although some nano-based drug delivery systems already exist on the market, growing numbers of newly designed ENMs exhibit improved physicochemical properties and are being assessed in preclinical stages. While these ENMs are designed to improve the efficacy of current nano-based therapeutic or imaging systems, it is necessary to thoroughly determine their safety profiles for successful clinical applications. As such, our aim in this mini-review is to discuss the current knowledge on predictive safety and structure-activity relationship (SAR) analysis of major ENMs at the developing stage, as well as the necessity of additional long-term toxicological analysis that would help to facilitate their transition into clinical practices. We focus on how the interaction of these nanomaterials with cells would trigger signaling pathways as molecular initiating events that lead to adverse outcomes. These mechanistic understandings would help to design safer ENMs with improved therapeutic efficacy in clinical settings.**

During the past decades, engineered nanomaterials (ENMs) have been widely used in biomedical applications, such as nanomedicine, bioimaging, and tissue engineering, because of their unique biophysicochemical properties.[1,2] With regard to nanomedicine applications, ENMs could be formulated as drug carriers that deliver the therapeutic reagents within the human body and increase their bioavailability and blood circulation half-life.[2] Moreover, these nanocarriers could be functionalized to deliver the drug specifically to the desired target tissues (e.g., tumors) and release the payload sustainably over long periods, thereby eliminating the necessity of multiple drug administration and reducing its cytotoxic side effects toward healthy cells.[2] In addition to their implementation in drug delivery, ENMs could be used for diagnostic and imaging purposes that would help to monitor the disease and administer the treatment more accurately.[2]

Past research in nanomedicine has led to the design of various nanomaterial-based therapeutic and diagnostic systems that are being investigated in experimental stages (e.g., in vitro and in vivo) and clinical trials or are commercially available to the corresponding patients (Table 1). Most commercialized nanomaterial-based therapeutic reagents use lipids, proteins, and polymers that could self-assemble and biodegrade with few side effects.[3] Because of high biocompatibility of lipids, several commercial liposome-drug formulations have been developed, mostly relying on polyethylene glycol (PEG)-conjugated lipids, such as DOXIL, AmBisome, Epaxal, and Inflexal V, and are under clinical trials focusing on various types of disease treatments.[4,5] Polymer-based nanoparticles (NPs) also present low toxicity, but their surface functionalization may affect their safety. The most commonly used materials include PEG, ploy(lactic-co-glycolic) acid (PLGA), polylactic acid (PLA), and polycaprolactone (PCL).[6,7] Some polymer-based therapeutics, such as Zinostatin Stimalmer, Oncaspar PEG-L-asparaginase, and Neulasta PEG-GCSF, have been clinically approved for cancer treatment, while more formulations are undergoing clinical investigations.[7] Although these clinically approved nano-based treatments exhibit promising results with reduced hazard potential, they have some drawbacks. For example, liposome-based therapeutic NPs lack sufficient mechanical stability, and their undesirable disruption within the in vivo environment could lead to the burst release of their therapeutic cargo.[8] In addition, liposome- and polymer-based nanotherapeutics that use PEG-lipid conjugates in their structure could induce complement activation-related pseudo-allergic reactions.[9,10] Thus, the necessity to address the challenges associated with these nanomaterial-based drug delivery and diagnostic systems has promoted the development of newly designed ENMs with unique characteristics that could enhance the efficacy and accuracy of medical treatments. For example, multifunctional nanocarriers with cationic surface functionalization are developed for in vitro and in vivo delivery of nucleic acids, including DNA and RNA.[11,12] In addition, a new class of luminescent nanoprobes termed as upconversion nanoparticles (UCNPs) has been developed that presents higher photostability compared to traditional fluorophores and has growing applications in bioimaging and photodynamic therapy. However, it is important to thoroughly

http://dx.doi.org/10.1016/j.ymthe.2017.03.011.

[5]These authors contributed equally to this work.

**Correspondence:** Tian Xia, Ph.D., Division of NanoMedicine, Department of Medicine, University of California Los Angeles, 10833 Le Conte Ave., Los Angeles, CA 90095, USA.
**E-mail:** txia@ucla.edu

 

**Table 1. Biomedical Applications, Development Stages, and Biological Effects of ENM Libraries**

| NPs | Materials | Applications | Development Stages | Toxicity | References |
|---|---|---|---|---|---|
| Polymer-based NPs | polymer NPs, micelles, dendrimers made by PEG, PLGA, PLA, PCL, or polypeptides | cancer therapy<br>gene delivery<br>drug delivery<br>biomedical diagnosis<br>bioimaging | commercialized, clinical trials | low with possible toxicity induced by linkers for surface functionalization | 7,9 |
| Liposomes | lipids, cross-linked lipids, PEG-lipid conjugates | cancer therapy<br>gene delivery<br>drug delivery<br>biomedical diagnosis | commercialized, clinical trials | low but with potential toxicity induced by surface modification and degradation | 4,5,8 |
| Metal-based NPs | gold | biomedical diagnosis<br>cancer therapy<br>drug delivery | experimental, clinical trials | low with potential subchronic effects | 36–38 |
| | silver | antibacterial treatment | commercialized | pro-inflammatory effects | 40,41,45–47 |
| | QDs: II–IV, IV–VI, or III–V (CdSe, CdTe, InAs) | biomedical diagnosis<br>bioimaging | experimental | pro-inflammatory effects induced by released heavy metals (Cd, As) | 58,60,63 |
| Metal oxides | rare earth | biomedical diagnosis<br>bioimaging<br>cancer therapy | experimental | pro-inflammatory and profibrogenic effects | 31,32,34 |
| | SPION | biomedical diagnosis<br>bioimaging | commercialized | toxicity induced by iron core after bioaccumulation | 54 |
| carbon nanotubes | single-walled and multi-walled carbon nanotubes | tissue engineering<br>biomedical diagnosis<br>cancer therapy | experimental | pro-inflammatory and profibrogenic effects | 69,72 |
| Graphene | graphene and graphene oxide | biomedical diagnosis<br>tissue engineering<br>regenerative medicine<br>stem cell differentiation<br>antibacterial treatment | experimental | pro-inflammatory and profibrogenic effects | 66,81,85 |

investigate the interaction of these ENMs with biological systems and assess their toxicity before making judgements on their translational value in nanomedicine. Even though there are many types of nanomaterials with novel designs, such as biomimetic NPs and nanolipoproteins that were shown to be applicable for therapeutic purposes,[13] this mini-review specifically focuses on some of the most common inorganic ENMs, including colloidal solid silica NPs (Si-NPs), rare-earth based nanomaterials, gold NPs (Au-NPs) and silver NPs (Ag-NPs), superparamagnetic iron oxide nanoparticles (SPIONs), and quantum dots (QDs), as well as carbonaceous nanomaterials that are being assessed in numerous preclinical studies. We aim to present how recent mechanistic studies based on structure-activity relationship (SAR) analysis could help to design safer ENMs for therapeutic and diagnostic applications.

## Safety of Therapeutic and Diagnostic ENMs

Nanomaterials exhibit significantly varied properties (e.g., high surface area-to-volume ratio) compared to the same material at the larger scales, which could subsequently affect their interaction with cells and other biomolecules, as well as biodistribution within the biological systems, and thereby their safety profiles. The physicochemical properties of ENMs, including composition and crystal structure, size, shape, dissolution, surface charge, and functionalization, could significantly affect their cytotoxicity (Figure 1).[14,15] Furthermore, adverse outcomes induced by ENMs are dependent on the route of administration, which has been thoroughly reviewed in previous studies.[1] Here, we discuss the recent findings about how the variation of the aforementioned physicochemical properties would determine the safety of ENMs, including both acute response and chronic effects in preclinical experiments, in addition to some major long-term safety assessments that need to be explored further for expediting their translation into clinics.

### *Colloidal Solid Si-NPs*

Synthetic Si-NPs exist in different forms, including crystalline (e.g., quartz) and amorphous (e.g., fumed, mesoporous, and colloidal) particles, depending on their synthesis process, thus displaying different physicochemical properties (e.g., crystallinity, porosity, and surface reactivity).[16,17] Within the recent decades, colloidal solid Si-NPs have been applied in numerous therapeutic and diagnostic applications,[18] and our aim here is to focus on the nanotoxicity of this specific form of Si-NPs. These NPs could serve as a promising delivery vehicle for small molecules, such as therapeutic drugs or imaging probes. As an example, previous studies have demonstrated that the cargo of interest could be chemically conjugated to a silica precursor and incorporated into the structure of solid Si-NPs during the synthesis procedure.[18,19] The resulting Si-NPs display high loading efficiency and are capable of being functionalized with targeting moieties

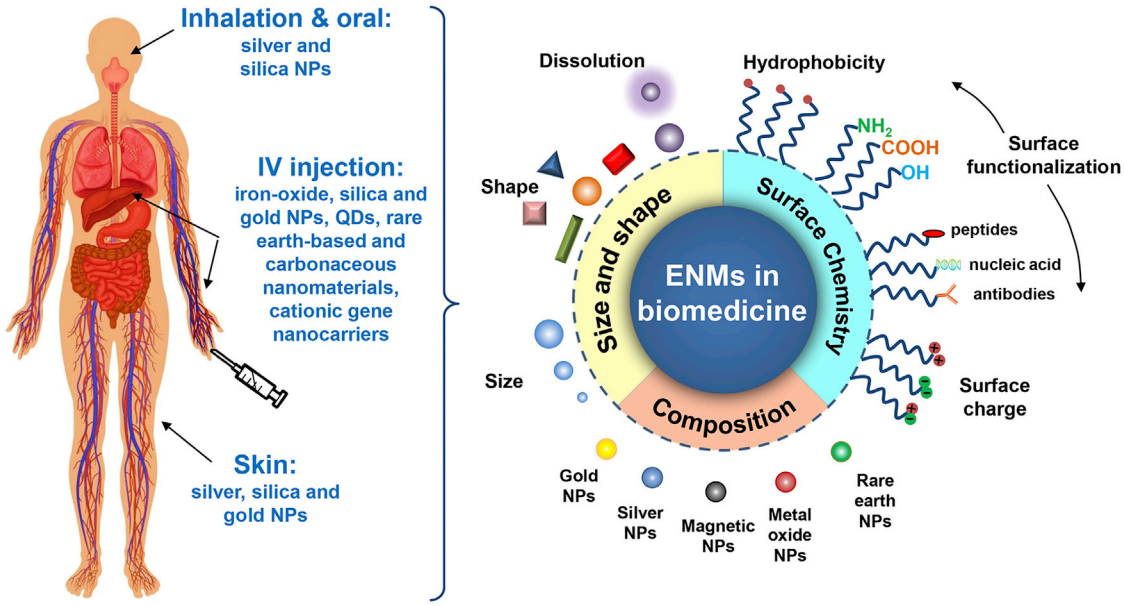


**Figure 1. Major Exposure Routes of ENMs with Distinct Physicochemical Properties for Medicinal Applications**

Physicochemical properties of ENMs and their route of administration determine their interaction at the nano-bio interface and cytotoxicity.

for targeted theranostic purposes.[20] Considering these particles possess a high potential to be explored in clinical investigations, it is necessary to study their interaction with biological systems in depth and evaluate their cytotoxicity and safety profiles comprehensively.

Most toxicity studies have shown that colloidal Si-NPs are less toxic than other forms, such as fumed Si-NPs.[21] The cytotoxic effect of colloidal Si-NPs is mainly attributed to the high levels of oxidative stress and generation of reactive oxygen species (ROS), mitochondrial damage, and autophagy.[22–24] Based on hierarchical oxidative stress hypothesis, high levels of ROS production could cause damages to DNA, proteins, lipids, and cell organelles and eventually lead to the cell death.[25] These particles show toxicity at higher doses, which is both size and surface chemistry dependent. For example, surface modification of bare Si-NPs with amine groups improves the biocompatibility of Si-NPs both in vitro and in vivo.[24,26] Other findings with regard to the size effect show that smaller particles induce higher cytotoxicity and inflammation in epithelial cells and macrophages, respectively.[27,28] This could possibly be attributed to the higher density of silanol groups on the surface of smaller Si-NPs compared to the large ones. Although these findings have improved our understanding with respect to the correlation between Si-NPs physicochemical characteristics and their toxicity, further in vivo analyses are needed to facilitate their application in translational nanomedicine. A finding by Sun et al.[29] indicated that although a single high-dose exposure of fumed Si-NPs to pulmonary system did not induce a profibrogenic effect, repetitive exposure at a lower dose led to a significant subchronic inflammatory response in the murine lungs. Though this study was focused on pulmonary toxicity of fumed silica, repetitive administration of colloidal Si-NPs might show similar pro-inflammatory effects in the long-term. Colloidal Si-NPs exhibit high biopersistence and retention in the body tissues (e.g., liver and spleen) that could further affect their toxicity.[30] Thus, even if colloidal Si-NPs may not induce acute toxicity in vivo, it is important to determine their long-term toxicity profile, as well as the consequences of repetitive administration over extended periods.

### *Rare Earth-Based ENMs*

Rare earth (RE)-based ENMs are increasingly used in various biological applications, including cell biology and nanomedicine, as imaging and sensing probes.[31] For example, numerous studies explored the application of these nanomaterials in MRI and showed that they could significantly improve the image contrast. RE-based nanomaterials such as UCNPs often outperform conventional imaging probes in terms of photostability and detection efficiency.[31] In addition to imaging applications, UCNPs could be applied for photodynamic therapy and cancer treatment.[32] However, the growing application of RE-based nanomaterials could increase the possibility of human exposure and adverse health outcomes. For instance, an increasing number of studies reported a direct correlation between administration of gadolinium (Gd)-based contrast agents and induction of nephrogenic systemic fibrosis (NSF) in patients with renal deficiency.[33] Thus, it is necessary to examine the toxicity of these materials in various biological environments as a function of their physicochemical properties, which could help to obtain a better understanding of their adverse health effects. In one study, Li et al.[34] performed a mechanistic study to understand how RE oxide (REO)-NPs induce cellular and profibrogenic damages to the lung and pulmonary system. The study demonstrated that enhanced dissolution of these NPs and shedding of REO ions within the acidic

environment of the lysosome leads to the deposition of $REPO_4$ complexes on the NP's surface and particle transformation into urchin-shaped structures. Following the depletion of free phosphate groups within the lysosome, the REO ions could strip the phosphates from the lysosome's lipid membrane and induce lysosomal damage. This phenomenon is followed by the release of lysosomal contents including cathepsin B, leading to activation of nonobese diabetic (NOD)-like receptor protein 3 (NLRP3) inflammasome and release of interleukin-1β (IL-1β), which eventually triggers a series of events, causing lung fibrosis.[34] To reduce this adverse effect and provide a safer design for REO-NPs, these researchers showed that precoating of REO-NPs with phosphate groups at a neutral pH could prevent the particle biotransformation and reduce their potential hazard. Furthermore, they found that ethylenediamine tetra(methylenephosphonic acid) (EDTMP) coating not only protects the surface of RE-based UCNPs from $REPO_4$ deposition and transformation, but also improves UCNPs stability and reduces their profibrogenic effects while maintaining particles' upconversion fluorescence efficiency.[35] Although these examples were focused on the pulmonary system, RE-based ENMs may present similar toxicological impacts on other organs, depending on their route of administration and biodistribution. For example, intravenous injection of UCNPs may result in their prolonged accumulation in the liver and spleen as part of the reticuloendothelial system (RES). Therefore, it is necessary to perform further mechanistic studies that examine chronic toxicological effects of RE-based ENMs in vivo. Such studies would provide a better knowledge to design safer NPs with minimized hazard potential that could be applied for translational nanomedicine.

### *Gold and Silver NPs*

Au-NPs are one of the ENMs that have been extensively studied in nanomedicine for various applications, such as cellular and diagnostic imaging, photothermal therapy, and drug delivery.[36] Au-NPs could be easily synthesized within a range of sizes and shapes and functionalized with a variety of biomolecules through simple bio-conjugation techniques. This implies that Au-NPs represent varied physicochemical characteristics in terms of size, shape, and surface functionality, which could considerably affect their cytotoxicity. For example, Au-NPs that are smaller than 2 nm could induce mitochondrial damage and oxidative stress in cultured cells due to their high surface reactivity, whereas bigger Au-NPs (above 4 nm) are considered mostly nontoxic to the cells.[36] Because Au-NPs that are used in nanomedicine are typically bigger than 5 nm, it is generally assumed that these particles are safe to be used in photothermal therapy and drug delivery applications.[36] However, one of the major concerns that has not been fully investigated consists of the long-term toxicological effects of these particles after in vivo administrations. Au-NPs are known to primarily accumulate in the organs of the RES (e.g., liver) and show slow clearance over several months.[37,38] Therefore, this persistent retention could result in chronic toxicity within these tissues. In addition, degradation of Au-NPs could result in formation of smaller particles that are known to be toxic, as previously mentioned. Falagan-Lotsch et al.[39] explored the long-term effect of exposing well-characterized Au-NPs to human dermal fibroblasts in vitro and found that even a single exposure of a subcytotoxic dose of Au-NPs could change the expression of genes that are associated with oxidative stress and inflammation. Moreover, these changes surprisingly lasted over 20 weeks.[39] Therefore, we predict the persistent accumulation of Au-NPs in the body organs could present similar toxicological responses. We emphasize that exploring the cytotoxicity of Au-NPs in extended long-term studies rather than solely investigating their acute short-term responses will not only elucidate more information about their safety profile, but also help to design safer Au-NPs for nanomedicine.

Ag-NPs have been widely used in antimicrobial applications including wound care, as well as coating of medical devices and implants. Despite their practical anti-infective characteristics and broad implementation in nanoproducts, major health and environmental concerns have been raised as a result of their toxicological impacts on various organs. For example, Ag-NPs have a high potential to detach from colloidal silver wound-dressing products and possibly invade the bloodstream from the injured tissues, which is followed by their migration to major organs, including liver, spleen, kidneys, lungs, and brain.[40,41] Furthermore, use of silver sprays might lead to pulmonary exposure that could also potentially induce extrapulmonary translocation to secondary organs such as liver. In contrast to the gold particles, Ag-NPs show significant cytotoxicity that mainly arises from the particle dissolution and release of toxic silver ions.[42–44] The mechanism of their toxicity is known to be via ROS generation that induces DNA damage, mitochondrial dysfunction, and lipid peroxidation in the membrane, which could eventually lead to cell death.[45–47] Similar to other nanomaterials, the toxicity of Ag-NPs significantly depends on their size and surface chemistry. For example, Wang et al.[43] have demonstrated that small polyvinylpyrrolidone and citrate-coated Ag-NPs (20 nm) cause more cellular toxicity and oxidative stress than large particles (110 nm) because of a higher rate of dissolution and silver bioavailability. For in vivo studies, dissolution rate of Ag-NPs determines the biopersistence and lung inflammation. In vivo toxicity analysis show that faster dissolution rate of smaller silver particles (20 nm) induces higher acute lung inflammation.[43] However, large Ag-NPs (110 nm) have a slower dissolution rate that could lead to their longer biopersistence in the lung and more significant subchronic lung injury at a longer time point (21 days).[43] There are several proposed methods to reduce the cytotoxicity of Ag-NPs, such as surface coating by using polymers.[48–50] Although these methods might improve the safety of Ag-NPs and resolve their acute toxicity, the subchronic effect of Ag-NPs even at low-dose exposure is a key issue that needs further in vivo assessment.

### *SPIONs*

SPIONs have been shown to be an ideal contrast agent for MRI and present great potential to be applied in cancer therapy.[51,52] SPIONs display lower toxicity compared to the other contrast agents, such as gadolinium-based compounds, and are mostly considered safe.[51] Examples of their application in MRI are clinically approved SPIONs, Feridex IV and Resovist, which could be used for liver imaging. The

rationale behind their design is that once SPIONs are administered into human body, the particles are expected to accumulate within the liver and phagocytosed by Kupffer cells, the residing macrophages in the liver.[53] Because the Kupffer cells in the diseased region of the tissue have lower particle uptake, SPIONs can provide an enhanced signal that helps to identify the lesion regions of liver more precisely.[54] After the intracellular uptake, it is anticipated that SPIONs dissolve into a nonsuperparamagnetic form of iron ions in the acidic lysosomes, which is metabolized further in the liver and subsequently used in the formation of red blood cells or excreted via kidneys.[53] However, this could result in high accumulation of iron in the liver for an extended time. Lunov et al.[55] reported that SPIONs triggered increased ROS production, followed by sustained activation of c-Jun N-terminal kinase (JNK) and production of pro-inflammatory cytokine tumor necrosis factor alpha (TNF-α) that led to the apoptosis of Kupffer cells. This was partially due to the Fenton reaction, in which the released ferrous ions ($Fe^{2+}$) have a high potential for reacting with hydrogen peroxide and oxygen produced by the mitochondria to form highly reactive hydroxyl radicals that could induce oxidative stress, leading to DNA, protein, and lipid damages.[56] Thus, overload or recurrent administration of SPIONs in prolonged treatments can result in high accumulation of SPIONs in the RES, elevate lipid metabolism, disrupt iron homeostasis, and affect liver functions.[57] This adverse effect could be more severe in patients with liver chronic diseases such as cirrhosis.[57] Thus, despite applying surface coatings (e.g., silicon and dextran) could improve the biocompatibility of SPIONs, this would not address the issue of iron overload in the body tissues. As such, new designs that could facilitate the rapid body clearance of iron are imperative.

### QDs

QDs have been used in bioimaging and cell labeling because of their promising optical properties. These nanomaterials could be stimulated by a single light source and emit multiple colors in a range of varied wavelengths by changing their size, shape, and composition.[58,59] QDs are frequently compromised of II–IV, IV–VI, or III–V materials, as well as transition metal doping. To date, the most commonly used QDs in biomedical research are cadmium (Cd)-based QDs.[58,60] However, their toxicological impact is a major concern that has hindered further clinical applications. The dissolution and release of heavy-metal Cd ions play a major role on their cytotoxicity.[61] With regard to the impact of QDs' physicochemical properties on their toxicity, Oh et al.[62] demonstrated that surface ligands and modifications, as well as size, are key parameters that contribute to the QD-induced toxicity by performing a comprehensive computational analysis across 307 previously published articles. To improve the biocompatibility and aqueous dispersibility, cadmium-based QDs are commonly coated with ZnS, ZnSe, and CdS, which form a core-shell structure.[63,64] This has been proved to effectively reduce the release of Cd from the core and improve biosafety even at a high concentration (16 μM).[64] However, the core-shell surface modification might only delay the cytotoxic effect of QDs and turn it into a subchronic effect. Furthermore, as the shells degrade gradually in the lysosome after the cellular uptake, the release of Cd ions could be inevitable during the long incubation. Because QDs could remain in the liver and spleen for more than 90 days[65] and their clearance is slow, this could result in severe subchronic cytotoxicity to the RES system, such as oxidative stress, genotoxicity, and neurotoxicity.[60,64] Therefore, novel designs that could more effectively inhibit their dissolution, as well as expedite their body clearance, could help to transit their application into clinic.

### Carbonaceous Nanomaterials

Engineered carbonaceous nanomaterials (ECNs) include carbon-based materials such as carbon nanotubes (CNTs), graphene and graphene oxides (GOs), and emerging graphene QDs and carbon dots.[66–68] Intrinsic electrical and optical characteristics, as well as the possibility to finely tune physicochemical properties such as size, hydrophobicity, durability, and surface functionalization (chemistry), render them as great candidates for use in biomedical settings.[69–71] Therefore, numerous carbonaceous nanomaterial-based scaffolds have been fabricated to be used in tissue or organ constructs and drug delivery systems.[69,71] With regard to the drug delivery purposes, the ultrahigh surface area of ECNs can be highly decorated, through both covalent and noncovalent methods, with pharmaceutical reagents such as anticancer drugs (e.g., doxorubicin, cisplatin, and methotrexate) and anti-inflammation therapeutics (e.g., dexamethasone).[70,72] Despite ECNs presenting great potential to be applied in nanomedicine, their translation into clinical investigations has been considerably slow because of safety concerns.

Extensive previous studies regarding the safety of ECNs indicate that ECNs have the potential to induce both acute or chronic inflammation and profibrogenic effects upon contact with cells and tissues.[14,73–76] ECNs' toxicity is known to be derived by their interactions with cells or organelles that generate pathogenic signals and adverse outcomes, including ROS, lysosomal damage, NLRP3 inflammasome activation, and cytokine release, leading to inflammation and fibrosis.[14,67,77–80] A number of ECN characteristics determine the induction of such adverse outcomes, including size, aspect ratio, hydrophobicity, state of agglomeration, impurities, durability, and surface chemistry.[14,81–83] For example, it has been found that the bare (nonfunctionalized), hydrophobic, and positively charged (e.g., polyetherimide [PEI]-modified) CNTs generate pro-fibrogenic effects in vitro and in vivo, whereas the hydrophilic or negatively charged (e.g., COOH- or PEG-modified) CNTs show less or no toxic effects.[84] Graphene and GO show a similar SAR.[85] In addition, they could attach to cell membrane as a 2D planar material and induce membrane damage and cytotoxicity.[86] One possible approach to reduce ECN cytotoxicity is surface functionalization. Wang et al.[83] demonstrated that coating multiwall CNTs with a tri-block copolymer (Pluronic F108) mitigates their toxicity by preventing the lysosomal membrane damage and subsequent inflammatory pathways. Therefore, surface coating of ECNs could be a safer design approach to improve their stability and biocompatibility. The other major toxicity issue for ECNs is their prolonged retention in the organs such as liver because of their size, which slows particle clearance. Thus, it is necessary to address this challenge with or without surface

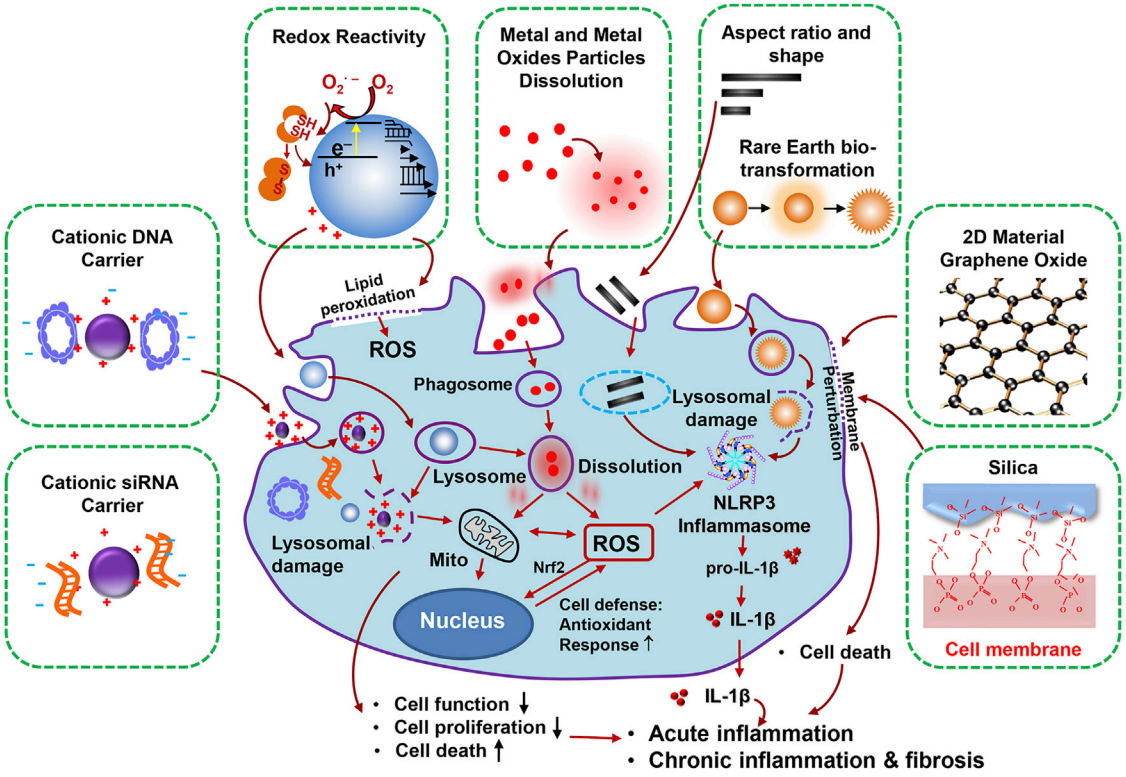


**Figure 2. SARs Linking ENM Physicochemical Properties to Adverse Outcome Pathways**

Examples of ENM-induced toxicity mechanisms include lysosomal damage by cationic nanocarriers, NLRP3 inflammasome activation for REO NPs and high aspect ratio nanomaterials, membrane perturbation by 2D nanomaterials and Si-NPs, and lipid peroxidation and ROS generation by silver and metal oxide NPs.

functionalization. With respect to the other ECNs, such as graphene QDs and carbon dots, even though their small size could improve body clearance, their long-term toxicity profile is still unknown and needs to be investigated in depth.

## Conclusions and Perspectives

The physicochemical characteristics of ENMs such as their composition, size and surface chemistry are the key factors that determine their toxicity and interactions with biological systems. These interactions at the nano-bio interface can initiate plasma membrane perturbation, lysosomal damage, ROS generation, and a series of subsequent signaling transductions that lead to cytokine production and pro-inflammatory responses or eventually cell death (Figure 2). Nanomaterials will first interact with biomolecules in the physiological environment (e.g., plasma proteins), which leads to protein adsorption onto their surface and corona formation. Although this matter was not discussed in this mini-review, formation of protein corona could change nanomaterial stability, bio-identity, targeting capability, cellular uptake, dissolution characteristics, and thereby their biodistribution and toxicity in vivo.[87–90] Thus, presence of protein corona is an important factor, in addition to the nanomaterials' physicochemical properties, that needs to be considered for safety assessment of ENMs in biomedicine and has been comprehensively discussed before.[88,89]

For safety analysis of ENMs, it is essential to first identify their in vivo biodistribution and target organs after administration. As mentioned earlier, RES, including liver and the immune system, is frequently targeted by various ENMs after administration, especially intravenous injection. Therefore, exploring adverse acute and chronic effects of these materials on the liver and immune system (e.g., hepatocytes, Kupffer cells, macrophages, and dendritic cells) is important. The preferred way to study these effects is by using an in vitro-to-in vivo predictive approach, which is recommended by a 2007 National Academy of Sciences report, *Toxicity Testing in the 21st Century: A Vision and a Strategy*.[91] For liver toxicity, future studies that focus on predictive in vitro analysis of toxicity pathways and mechanisms such as oxidative stress, NLRP3 inflammasome activation, and pro-inflammatory cytokine production, along with validating in vivo screening for acute and chronic liver inflammation or fibrosis, would provide important insights. In addition to 2D cell culture, engineered 3D coculture systems, including hepatocytes and Kupffer cells or other liver cell types, could maintain hepatocyte function for a longer period and would better resemble liver responses in vivo to ENMs. With regard to the immune system, the effect of ENM exposure on spleen and innate or adaptive immune responses will also need to be studied by using splenocytes and in vitro predictive toxicological approaches, as reviewed in detail previously.[92,93] These in vitro results need to be

validated by limited but focused in vivo experiments to demonstrate the value of predictive methods.

Predictive in vitro studies have to consider sedimentation, diffusion, and dosimetry models for relevant exposure doses and aim to construct intellectual frameworks such as adverse outcome pathways (AOPs) and SAR analysis, rather than solely reporting descriptive toxicological impacts of ENMs, which would provide invaluable knowledge for regulatory purposes. In addition, these mechanistic studies could help with devising ingenious approaches to mitigate the toxicity induced by ENMs, such as signaling pathway inhibition, and safer design by modification of major toxic properties of ENMs. Surface coatings or optimization of size and charge of the nanomaterials are among potential methods that could help with designing safer ENMs. For instance, nanocarriers for nucleic acid delivery typically have positive charge, and these exhibit stronger plasma membrane binding due to electrostatic effects and higher cellular uptake than negatively charged and neutral NPs. However, once inside the lysosomes after endocytosis, a high cationic charge could induce proton sponge effects, which leads to lysosomal swelling, membrane damage, and release of cathepsin B that results in NLRP3 inflammasome activation and pro-inflammatory IL-1β production (Figure 2).[84] Extensive lysosomal damage could also lead to cell death and inflammation.[12,94] Therefore, designing nanocarriers with the optimal cationic charge for gene delivery could facilitate endosomal escape of nucleic acids without triggering extensive lysosomal damage and subsequent pro-inflammatory effects and cell death.[12] In addition, it is essential to assess the safety profiles of ENMs at low, nonlethal dosages and extended repetitive exposures, which could help us to understand long-term consequences of ENMs' interactions with biological environments and further expedite their translation into clinical applications.

## AUTHOR CONTRIBUTIONS

V.M., W.J., B.S., X.W., and T.X. wrote the manuscript.

## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

## ACKNOWLEDGMENTS

This work was primarily supported by the National Institute of Environmental Health Sciences (NIEHS) R01 ES016746 and ES022698, as well as U01 ES027237, with leveraged support from the National Science Foundation (NSF) and the Environmental Protection Agency (EPA) under Cooperative Agreement No. DBI 0830117 and 1266377. Additional support is from Hundred Talents Program and National Natural Science Foundation of China (31570899). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH, NSF, or EPA.

## REFERENCES

1. Yildirimer, L., Thanh, N.T.K., Loizidou, M., and Seifalian, A.M. (2011). Toxicology and clinical potential of nanoparticles. Nano Today *6*, 585–607.
2. Zhang, L., Gu, F.X., Chan, J.M., Wang, A.Z., Langer, R.S., and Farokhzad, O.C. (2008). Nanoparticles in medicine: therapeutic applications and developments. Clin. Pharmacol. Ther. *83*, 761–769.
3. Bozzuto, G., and Molinari, A. (2015). Liposomes as nanomedical devices. Int. J. Nanomedicine *10*, 975–999.
4. Sercombe, L., Veerati, T., Moheimani, F., Wu, S.Y., Sood, A.K., and Hua, S. (2015). Advances and challenges of liposome assisted drug delivery. Front. Pharmacol. *6*, 286.
5. Chang, H.I., and Yeh, M.K. (2012). Clinical development of liposome-based drugs: formulation, characterization, and therapeutic efficacy. Int. J. Nanomedicine *7*, 49–60.
6. Li, C., and Wallace, S. (2008). Polymer-drug conjugates: recent development in clinical oncology. Adv. Drug Deliv. Rev. *60*, 886–898.
7. Prabhu, R.H., Patravale, V.B., and Joshi, M.D. (2015). Polymeric nanoparticles for targeted treatment in oncology: current insights. Int. J. Nanomedicine *10*, 1001–1018.
8. Liu, X., Situ, A., Kang, Y., Villabroza, K.R., Liao, Y., Chang, C.H., Donahue, T., Nel, A.E., and Meng, H. (2016). Irinotecan delivery by lipid-coated mesoporous silica nanoparticles shows improved efficacy and safety over liposomes for pancreatic cancer. ACS Nano *10*, 2702–2715.
9. Crist, R.M., Grossman, J.H., Patri, A.K., Stern, S.T., Dobrovolskaia, M.A., Adiseshaiah, P.P., Clogston, J.D., and McNeil, S.E. (2013). Common pitfalls in nanotechnology: lessons learned from NCI's Nanotechnology Characterization Laboratory. Integr. Biol. *5*, 66–73.
10. Moghimi, S.M., Andersen, A.J., Hashemi, S.H., Lettiero, B., Ahmadvand, D., Hunter, A.C., Andresen, T.L., Hamad, I., and Szebeni, J. (2010). Complement activation cascade triggered by PEG-PL engineered nanomedicines and carbon nanotubes: the challenges ahead. J. Control. Release *146*, 175–181.
11. Meng, H., Liong, M., Xia, T., Li, Z., Ji, Z., Zink, J.I., and Nel, A.E. (2010). Engineered design of mesoporous silica nanoparticles to deliver doxorubicin and P-glycoprotein siRNA to overcome drug resistance in a cancer cell line. ACS Nano *4*, 4539–4550.
12. Xia, T., Kovochich, M., Liong, M., Meng, H., Kabehie, S., George, S., Zink, J.I., and Nel, A.E. (2009). Polyethyleneimine coating enhances the cellular uptake of mesoporous silica nanoparticles and allows safe delivery of siRNA and DNA constructs. ACS Nano *3*, 3273–3286.
13. Fischer, N.O., Weilhammer, D.R., Dunkle, A., Thomas, C., Hwang, M., Corzett, M., Lychak, C., Mayer, W., Urbin, S., Collette, N., et al. (2014). Evaluation of nanolipoprotein particles (NLPs) as an in vivo delivery platform. PLoS ONE *9*, e93342.
14. Nel, A., Xia, T., Meng, H., Wang, X., Lin, S., Ji, Z., and Zhang, H. (2013). Nanomaterial toxicity testing in the 21st century: use of a predictive toxicological approach and high-throughput screening. Acc. Chem. Res. *46*, 607–621.
15. Sharifi, S., Behzadi, S., Laurent, S., Forrest, M.L., Stroeve, P., and Mahmoudi, M. (2012). Toxicity of nanomaterials. Chem. Soc. Rev. *41*, 2323–2343.
16. Napierska, D., Thomassen, L.C.J., Lison, D., Martens, J.A., and Hoet, P.H. (2010). The nanosilica hazard: another variable entity. Part. Fibre Toxicol. *7*, 39.
17. Sun, B., Pokhrel, S., Dunphy, D.R., Zhang, H., Ji, Z., Wang, X., Wang, M., Liao, Y.P., Chang, C.H., Dong, J., et al. (2015). Reduction of acute inflammatory effects of fumed silica nanoparticles in the lung by adjusting silanol display through calcination and metal doping. ACS Nano *9*, 9357–9372.
18. Tang, L., and Cheng, J. (2013). Nonporous silica nanoparticles for nanomedicine application. Nano Today *8*, 290–312.
19. Tang, L., Fan, T.M., Borst, L.B., and Cheng, J. (2012). Synthesis and biological response of size-specific, monodisperse drug-silica nanoconjugates. ACS Nano *6*, 3954–3966.
20. Vivero-Escoto, J.L., Huxford-Phillips, R.C., and Lin, W. (2012). Silica-based nanoprobes for biomedical imaging and theranostic applications. Chem. Soc. Rev. *41*, 2673–2685.
21. Zhang, H., Dunphy, D.R., Jiang, X., Meng, H., Sun, B., Tarn, D., Xue, M., Wang, X., Lin, S., Ji, Z., et al. (2012). Processing pathway dependence of amorphous silica nanoparticle toxicity: colloidal vs pyrolytic. J. Am. Chem. Soc. *134*, 15790–15804.
22. Herd, H.L., Malugin, A., and Ghandehari, H. (2011). Silica nanoconstruct cellular toleration threshold in vitro. J. Control. Release *153*, 40–48.

23. Duan, J., Yu, Y., Li, Y., Yu, Y., Li, Y., Zhou, X., Huang, P., and Sun, Z. (2013). Toxic effect of silica nanoparticles on endothelial cells through DNA damage response via Chk1-dependent G2/M checkpoint. PLoS ONE *8*, e62087.

24. Lehman, S.E., Morris, A.S., Mueller, P.S., Salem, A.K., Grassian, V.H., and Larsen, S.C. (2016). Silica nanoparticle-generated ROS as a predictor of cellular toxicity: mechanistic insights and safety by design. Environ. Sci. Nano *3*, 56–66.

25. Nel, A., Xia, T., Mädler, L., and Li, N. (2006). Toxic potential of materials at the nanolevel. Science *311*, 622–627.

26. Morris, A.S., Adamcakova-Dodd, A., Lehman, S.E., Wongrakpanich, A., Thorne, P.S., Larsen, S.C., and Salem, A.K. (2016). Amine modification of nonporous silica nanoparticles reduces inflammatory response following intratracheal instillation in murine lungs. Toxicol. Lett. *241*, 207–215.

27. Li, Q.L., Hu, H.J., Jiang, L.Z., Zou, Y., Duan, J.C., and Sun, Z.W. (2016). Cytotoxicity and autophagy dysfunction induced by different sizes of silica particles in human bronchial epithelial BEAS-2B cells. Toxicol. Res. (Camb.) *5*, 1216–1228.

28. Kusaka, T., Nakayama, M., Nakamura, K., Ishimiya, M., Furusawa, E., and Ogasawara, K. (2014). Effect of silica particle size on macrophage inflammatory responses. PLoS ONE *9*, e92634.

29. Sun, B., Wang, X., Liao, Y.P., Ji, Z., Chang, C.H., Pokhrel, S., Ku, J., Liu, X., Wang, M., Dunphy, D.R., et al. (2016). Repetitive dosing of fumed silica leads to profibrogenic effects through unique structure-activity relationships and biopersistence in the lung. ACS Nano *10*, 8054–8066.

30. Cho, M., Cho, W.S., Choi, M., Kim, S.J., Han, B.S., Kim, S.H., Kim, H.O., Sheen, Y.Y., and Jeong, J. (2009). The impact of size on tissue distribution and elimination by single intravenous injection of silica nanoparticles. Toxicol. Lett. *189*, 177–183.

31. Bouzigues, C., Gacoin, T., and Alexandrou, A. (2011). Biological applications of rare-earth based nanoparticles. ACS Nano *5*, 8488–8505.

32. Ai, X., Ho, C.J.H., Aw, J., Attia, A.B.E., Mu, J., Wang, Y., Wang, X., Wang, Y., Liu, X., Chen, H., et al. (2016). In vivo covalent cross-linking of photon-converted rare-earth nanostructures for tumour localization and theranostics. Nat. Commun. *7*, 10432.

33. Marckmann, P., Skov, L., Rossen, K., Dupont, A., Damholt, M.B., Heaf, J.G., and Thomsen, H.S. (2006). Nephrogenic systemic fibrosis: suspected causative role of gadodiamide used for contrast-enhanced magnetic resonance imaging. J. Am. Soc. Nephrol. *17*, 2359–2362.

34. Li, R., Ji, Z., Chang, C.H., Dunphy, D.R., Cai, X., Meng, H., Zhang, H., Sun, B., Wang, X., Dong, J., et al. (2014). Surface interactions with compartmentalized cellular phosphates explain rare earth oxide nanoparticle hazard and provide opportunities for safer design. ACS Nano *8*, 1771–1783.

35. Li, R., Ji, Z., Dong, J., Chang, C.H., Wang, X., Sun, B., Wang, M., Liao, Y.P., Zink, J.I., Nel, A.E., and Xia, T. (2015). Enhancing the imaging and biosafety of upconversion nanoparticles through phosphonate coating. ACS Nano *9*, 3293–3306.

36. Dreaden, E.C., Alkilany, A.M., Huang, X., Murphy, C.J., and El-Sayed, M.A. (2012). The golden age: gold nanoparticles for biomedicine. Chem. Soc. Rev. *41*, 2740–2779.

37. Kolosnjaj-Tabi, J., Javed, Y., Lartigue, L., Volatron, J., Elgrabli, D., Marangon, I., Pugliese, G., Caron, B., Figuerola, A., Luciani, N., et al. (2015). The one year fate of iron oxide coated gold nanoparticles in mice. ACS Nano *9*, 7925–7939.

38. Khlebtsov, N., and Dykman, L. (2011). Biodistribution and toxicity of engineered gold nanoparticles: a review of in vitro and in vivo studies. Chem. Soc. Rev. *40*, 1647–1671.

39. Falagan-Lotsch, P., Grzincic, E.M., and Murphy, C.J. (2016). One low-dose exposure of gold nanoparticles induces long-term changes in human cells. Proc. Natl. Acad. Sci. USA *113*, 13318–13323.

40. Dziendzikowska, K., Gromadzka-Ostrowska, J., Lankoff, A., Oczkowski, M., Krawczyńska, A., Chwastowska, J., Sadowska-Bratek, M., Chajduk, E., Wojewódzka, M., Dušinská, M., and Kruszewski, M. (2012). Time-dependent biodistribution and excretion of silver nanoparticles in male Wistar rats. J. Appl. Toxicol. *32*, 920–928.

41. Ahamed, M., Alsalhi, M.S., and Siddiqui, M.K.J. (2010). Silver nanoparticle applications and human health. Clin. Chim. Acta *411*, 1841–1848.

42. Osborne, O.J., Lin, S., Chang, C.H., Ji, Z., Yu, X., Wang, X., Lin, S., Xia, T., and Nel, A.E. (2015). Organ-specific and size-dependent Ag nanoparticle toxicity in gills and intestines of adult zebrafish. ACS Nano *9*, 9573–9584.

43. Wang, X., Ji, Z., Chang, C.H., Zhang, H., Wang, M., Liao, Y.P., Lin, S., Meng, H., Li, R., Sun, B., et al. (2014). Use of coated silver nanoparticles to understand the relationship of particle dissolution and bioavailability to cell and lung toxicological potential. Small *10*, 385–398.

44. Zhang, H., Wang, X., Wang, M., Li, L., Chang, C.H., Ji, Z., Xia, T., and Nel, A.E. (2015). Mammalian cells exhibit a range of sensitivities to silver nanoparticles that are partially explicable by variations in antioxidant defense and metallothionein expression. Small *11*, 3797–3805.

45. Hackenberg, S., Scherzed, A., Kessler, M., Hummel, S., Technau, A., Froelich, K., Ginzkey, C., Koehler, C., Hagen, R., and Kleinsasser, N. (2011). Silver nanoparticles: evaluation of DNA damage, toxicity and functional impairment in human mesenchymal stem cells. Toxicol. Lett. *201*, 27–33.

46. Avalos, A., Haza, A.I., Mateo, D., and Morales, P. (2014). Cytotoxicity and ROS production of manufactured silver nanoparticles of different sizes in hepatoma and leukemia cells. J. Appl. Toxicol. *34*, 413–423.

47. Zhornik, E.V., Baranova, L.A., Drozd, E.S., Sudas, M.S., Chau, N.H., Buu, N.Q., Dung, T.T., Chizhik, S.A., and Volotovskiĭ, I.D. (2014). Silver nanoparticles induce lipid peroxidation and morphological changes in human lymphocytes surface. Biophysics *59*, 380–386.

48. George, S., Lin, S., Ji, Z., Thomas, C.R., Li, L., Mecklenburg, M., Meng, H., Wang, X., Zhang, H., Xia, T., et al. (2012). Surface defects on plate-shaped silver nanoparticles contribute to its hazard potential in a fish gill cell line and zebrafish embryos. ACS Nano *6*, 3745–3759.

49. Gorka, D.E., Osterberg, J.S., Gwin, C.A., Colman, B.P., Meyer, J.N., Bernhardt, E.S., Gunsch, C.K., DiGulio, R.T., and Liu, J. (2015). Reducing environmental toxicity of silver nanoparticles through shape control. Environ. Sci. Technol. *49*, 10093–10098.

50. Niskanen, J., Shan, J., Tenhu, H., Jiang, H., Kauppinen, E., Barranco, V., Picó, F., Yliniemi, K., Kontturi, and Kyösti. (2010). Synthesis of copolymer-stabilized silver nanoparticles for coating materials. Colloid Polym. Sci. *288*, 543–553.

51. Li, L., Jiang, W., Luo, K., Song, H., Lan, F., Wu, Y., and Gu, Z. (2013). Superparamagnetic iron oxide nanoparticles as MRI contrast agents for non-invasive stem cell labeling and tracking. Theranostics *3*, 595–615.

52. Mahmoudi, M., Sant, S., Wang, B., Laurent, S., and Sen, T. (2011). Superparamagnetic iron oxide nanoparticles (SPIONs): development, surface modification and applications in chemotherapy. Adv. Drug Deliv. Rev. *63*, 24–46.

53. Weissleder, R., Stark, D.D., Engelstad, B.L., Bacon, B.R., Compton, C.C., White, D.L., Jacobs, P., and Lewis, J. (1989). Superparamagnetic iron oxide: pharmacokinetics and toxicity. AJR Am. J. Roentgenol. *152*, 167–173.

54. Wang, Y.X. (2011). Superparamagnetic iron oxide based MRI contrast agents: current status of clinical application. Quant. Imaging Med. Surg. *1*, 35–40.

55. Lunov, O., Syrovets, T., Büchele, B., Jiang, X., Röcker, C., Tron, K., Nienhaus, G.U., Walther, P., Mailänder, V., Landfester, K., and Simmet, T. (2010). The effect of carboxydextran-coated superparamagnetic iron oxide nanoparticles on c-Jun N-terminal kinase-mediated apoptosis in human macrophages. Biomaterials *31*, 5063–5071.

56. Singh, N., Jenkins, G.J., Asadi, R., and Doak, S.H. (2010). Potential toxicity of superparamagnetic iron oxide nanoparticles (SPION). Nano Rev. *1*, 5358.

57. Wei, Y., Zhao, M., Yang, F., Mao, Y., Xie, H., and Zhou, Q. (2016). Iron overload by superparamagnetic iron oxide nanoparticles is a high risk factor in cirrhosis by a systems toxicology assessment. Sci. Rep. *6*, 29110.

58. Wang, F., Shu, L., Wang, J., Pan, X., Huang, R., Lin, Y., and Cai, X. (2013). Perspectives on the toxicology of cadmium-based quantum dots. Curr. Drug Metab. *14*, 847–856.

59. Zhang, F., Yi, D., Sun, H., and Zhang, H. (2014). Cadmium-based quantum dots: preparation, surface modification, and applications. J. Nanosci. Nanotechnol. *14*, 1409–1424.

60. Yong, K.-T., Law, W.-C., Hu, R., Ye, L., Liu, L., Swihart, M.T., and Prasad, P.N. (2013). Nanotoxicity assessment of quantum dots: from cellular to primate studies. Chem. Soc. Rev. *42*, 1236–1250.

61. Wang, Y., Hu, R., Lin, G., Roy, I., and Yong, K.-T. (2013). Functionalized quantum dots for biosensing and bioimaging and concerns on toxicity. ACS Appl. Mater. Interfaces *5*, 2786–2799.

62. Oh, E., Liu, R., Nel, A., Gemill, K.B., Bilal, M., Cohen, Y., and Medintz, I.L. (2016). Meta-analysis of cellular toxicity for cadmium-containing quantum dots. Nat. Nanotechnol. *11*, 479–486.

63. Chen, N., He, Y., Su, Y., Li, X., Huang, Q., Wang, H., Zhang, X., Tai, R., and Fan, C. (2012). The cytotoxicity of cadmium-based quantum dots. Biomaterials *33*, 1238–1244.

64. Su, Y., He, Y., Lu, H., Sai, L., Li, Q., Li, W., Wang, L., Shen, P., Huang, Q., and Fan, C. (2009). The cytotoxicity of cadmium based, aqueous phase—synthesized, quantum dots and its modulation by surface coating. Biomaterials *30*, 19–25.

65. Ye, L., Yong, K.T., Liu, L., Roy, I., Hu, R., Zhu, J., Cai, H., Law, W.C., Liu, J., Wang, K., et al. (2012). A pilot study in non-human primates shows no adverse response to intravenous injection of quantum dots. Nat. Nanotechnol. *7*, 453–458.

66. Jin, Z.H., Owour, P., Lei, S.D., and Ge, L.H. (2015). Graphene, graphene quantum dots and their applications in optoelectronics. Curr. Opin. Colloid Interface Sci. *20*, 439–453.

67. Eatemadi, A., Daraee, H., Karimkhanloo, H., Kouhi, M., Zarghami, N., Akbarzadeh, A., Abasi, M., Hanifehpour, Y., and Joo, S.W. (2014). Carbon nanotubes: properties, synthesis, purification, and medical applications. Nanoscale Res. Lett. *9*, 393.

68. Lim, S.Y., Shen, W., and Gao, Z. (2015). Carbon quantum dots and their applications. Chem. Soc. Rev. *44*, 362–381.

69. Hong, G., Diao, S., Antaris, A.L., and Dai, H. (2015). Carbon nanomaterials for biological imaging and nanomedicinal therapy. Chem. Rev. *115*, 10816–10906.

70. Liu, Z., Robinson, J.T., Tabakman, S.M., Yang, K., and Dai, H.J. (2011). Carbon materials for drug delivery & cancer therapy. Mater. Today *14*, 316–323.

71. De Volder, M.F.L., Tawfick, S.H., Baughman, R.H., and Hart, A.J. (2013). Carbon nanotubes: present and future commercial applications. Science *339*, 535–539.

72. Ajima, K., Yudasaka, M., Murakami, T., Maigné, A., Shiba, K., and Iijima, S. (2005). Carbon nanohorns as anticancer drug carriers. Mol. Pharm. *2*, 475–480.

73. Mutlu, G.M., Budinger, G.R.S., Green, A.A., Urich, D., Soberanes, S., Chiarella, S.E., Alheid, G.F., McCrimmon, D.R., Szleifer, I., and Hersam, M.C. (2010). Biocompatible nanoscale dispersion of single-walled carbon nanotubes minimizes in vivo pulmonary toxicity. Nano Lett. *10*, 1664–1670.

74. Wang, X., Zang, J.J., Wang, H., Nie, H., Wang, T.C., Deng, X.Y., Gu, Y.Q., Liu, Z.H., and Jia, G. (2010). Pulmonary toxicity in mice exposed to low and medium doses of water-soluble multi-walled carbon nanotubes. J. Nanosci. Nanotechnol. *10*, 8516–8526.

75. Valle, R.P., Wu, T., and Zuo, Y.Y. (2015). Biophysical influence of airborne carbon nanomaterials on natural pulmonary surfactant. ACS Nano *9*, 5413–5421.

76. Bonner, J.C., Silva, R.M., Taylor, A.J., Brown, J.M., Hilderbrand, S.C., Castranova, V., Porter, D., Elder, A., Oberdörster, G., Harkema, J.R., et al. (2013). Interlaboratory evaluation of rodent pulmonary responses to engineered nanomaterials: the NIEHS Nano GO Consortium. Environ. Health Perspect. *121*, 676–682.

77. Barreto, J.A., O'Malley, W., Kubeil, M., Graham, B., Stephan, H., and Spiccia, L. (2011). Nanomaterials: applications in cancer imaging and therapy. Adv. Mater. *23*, H18–H40.

78. Shvedova, A.A., Kisin, E., Murray, A.R., Johnson, V.J., Gorelik, O., Arepalli, S., Hubbs, A.F., Mercer, R.R., Keohavong, P., Sussman, N., et al. (2008). Inhalation vs. aspiration of single-walled carbon nanotubes in C57BL/6 mice: inflammation, fibrosis, oxidative stress, and mutagenesis. Am. J. Physiol. Lung Cell. Mol. Physiol. *295*, L552–L565.

79. Sun, B., Wang, X., Ji, Z., Li, R., and Xia, T. (2013). NLRP3 inflammasome activation induced by engineered nanomaterials. Small *9*, 1595–1607.

80. Sun, B., Wang, X., Ji, Z., Wang, M., Liao, Y.-P., Chang, C.H., Li, R., Zhang, H., Nel, A.E., and Xia, T. (2015). NADPH oxidase-dependent NLRP3 inflammasome activation and its important role in lung fibrosis by multiwalled carbon nanotubes. Small *11*, 2087–2097.

81. Hu, W., Peng, C., Lv, M., Li, X., Zhang, Y., Chen, N., Fan, C., and Huang, Q. (2011). Protein corona-mediated mitigation of cytotoxicity of graphene oxide. ACS Nano *5*, 3693–3700.

82. Wang, X., Mansukhani, N.D., Guiney, L.M., Lee, J.H., Li, R., Sun, B., Liao, Y.P., Chang, C.H., Ji, Z., Xia, T., et al. (2016). Toxicological profiling of highly purified metallic and semiconducting single-walled carbon nanotubes in the rodent lung and *E. coli*. ACS Nano *10*, 6008–6019.

83. Wang, X., Duch, M.C., Mansukhani, N., Ji, Z., Liao, Y.P., Wang, M., Zhang, H., Sun, B., Chang, C.H., Li, R., et al. (2015). Use of a pro-fibrogenic mechanism-based predictive toxicological approach for tiered testing and decision analysis of carbonaceous nanomaterials. ACS Nano *9*, 3032–3043.

84. Li, R., Wang, X., Ji, Z., Sun, B., Zhang, H., Chang, C.H., Lin, S., Meng, H., Liao, Y.P., Wang, M., et al. (2013). Surface charge and cellular processing of covalently functionalized multiwall carbon nanotubes determine pulmonary toxicity. ACS Nano *7*, 2352–2368.

85. Duch, M.C., Budinger, G.R.S., Liang, Y.T., Soberanes, S., Urich, D., Chiarella, S.E., Campochiaro, L.A., Gonzalez, A., Chandel, N.S., Hersam, M.C., and Mutlu, G.M. (2011). Minimizing oxidation and stable nanoscale dispersion improves the biocompatibility of graphene in the lung. Nano Lett. *11*, 5201–5207.

86. Li, R., Mansukhani, N.D., Guiney, L.M., Ji, Z., Zhao, Y., Chang, C.H., French, C.T., Miller, J.F., Hersam, M.C., Nel, A.E., and Xia, T. (2016). Identification and optimization of carbon radicals on hydrated graphene oxide for ubiquitous antibacterial coatings. ACS Nano *10*, 10966–10980.

87. Mahmoudi, M., Bertrand, N., Zope, H., and Farokhzad, O.C. (2016). Emerging understanding of the protein corona at the nano-bio interfaces. Nano Today *11*, 817–832.

88. Corbo, C., Molinaro, R., Parodi, A., Toledano Furman, N.E., Salvatore, F., and Tasciotti, E. (2016). The impact of nanoparticle protein corona on cytotoxicity, immunotoxicity and target drug delivery. Nanomedicine (Lond.) *11*, 81–100.

89. Lee, Y.K., Choi, E.-J., Webster, T.J., Kim, S.-H., and Khang, D. (2014). Effect of the protein corona on nanoparticles for modulating cytotoxicity and immunotoxicity. Int. J. Nanomedicine *10*, 97–113.

90. Mirshafiee, V., Kim, R., Park, S., Mahmoudi, M., and Kraft, M.L. (2016). Impact of protein pre-coating on the protein corona composition and nanoparticle cellular uptake. Biomaterials *75*, 295–304.

91. National Research Council (NRC) (2007). Toxicity Testing in the 21st Century: A Vision and A Strategy (National Academy Press).

92. Dobrovolskaia, M.A., Aggarwal, P., Hall, J.B., and McNeil, S.E. (2008). Preclinical studies to understand nanoparticle interaction with the immune system and its potential effects on nanoparticle biodistribution. Mol. Pharm. *5*, 487–495.

93. Dobrovolskaia, M.A., and McNeil, S.E. (2013). Understanding the correlation between in vitro and in vivo immunotoxicity tests for nanomedicines. J. Control. Release *172*, 456–466.

94. Xia, T., Kovochich, M., Liong, M., Zink, J.I., and Nel, A.E. (2008). Cationic polystyrene nanosphere toxicity depends on cell-specific endocytic and mitochondrial injury pathways. ACS Nano *2*, 85–96.