ANNUAL REVIEWS

Annual Review of Pharmacology and Toxicology

Nanoparticle Toxicology

Wen Yang,[1] Lin Wang,[1] Evan M. Mettenbrink,[1] Paul L. DeAngelis,[2] and Stefan Wilhelm[1,3,4]

[1]Stephenson School of Biomedical Engineering, University of Oklahoma, Norman, Oklahoma 73019, USA; email: stefan.wilhelm@ou.edu

[2]Department of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73104, USA

[3]Institute for Biomedical Engineering, Science, and Technology (IBEST), Norman, Oklahoma 73019, USA

[4]Stephenson Cancer Center, Oklahoma City, Oklahoma 73104, USA

Annu. Rev. Pharmacol. Toxicol. 2021. 61:269–89

First published as a Review in Advance on August 25, 2020

The *Annual Review of Pharmacology and Toxicology* is online at pharmtox.annualreviews.org

https://doi.org/10.1146/annurev-pharmtox-032320-110338

Keywords

nanoparticles, toxicology, physicochemical properties, anti-PEG antibody, protein corona, ROS

Abstract

Nanoparticles from natural and anthropogenic sources are abundant in the environment, thus human exposure to nanoparticles is inevitable. Due to this constant exposure, it is critically important to understand the potential acute and chronic adverse effects that nanoparticles may cause to humans. In this review, we explore and highlight the current state of nanotoxicology research with a focus on mechanistic understanding of nanoparticle toxicity at organ, tissue, cell, and biomolecular levels. We discuss nanotoxicity mechanisms, including generation of reactive oxygen species, nanoparticle disintegration, modulation of cell signaling pathways, protein corona formation, and poly(ethylene glycol)-mediated immunogenicity. We conclude with a perspective on potential approaches to advance current understanding of nanoparticle toxicity. Such improved understanding may lead to mitigation strategies that could enable safe application of nanoparticles in humans. Advances in nanotoxicity research will ultimately inform efforts to establish standardized regulatory frameworks with the goal of fully exploiting the potential of nanotechnology while minimizing harm to humans.

1. INTRODUCTION

Nanoparticles comprise a class of materials with dimensions in the 1–100-nm range that exhibit unique physical, chemical, and biological properties, making them distinct from their corresponding bulk materials or small molecules (1, 2). Owing to their unique material characteristics, nanoparticles are broadly used in a range of applications and products, including industrial catalytic processes, energy conversion and storage, and image display technologies as well as cosmetics, medical devices, and therapeutics and diagnostics (3–5). In addition to such rationally designed nanoparticles, we are constantly surrounded by substantial amounts of naturally and incidentally formed particles, such as corrosion- or erosion-derived nanoparticles in water and airborne nanoparticles from traffic and industrial combustion (6, 7). The abundance of nanoparticles in the environment and in everyday consumer products makes human exposure inevitable. However, the potential acute and chronic health risks that nanoparticles may pose to humans are poorly investigated and understood.

In this review, we introduce the major nanoparticle classes and explore how their corresponding physicochemical properties affect toxicity. Our discussion of the main nanotoxicity mechanisms provides an overview of how nanoparticles interact with the body at organ, tissue, cell, and biomolecular levels. Such mechanistic understanding is enabled by diverse experimental and theoretical methods that have been developed and applied for the assessment and evaluation of nanotoxicity. We conclude our discussion with a perspective on potential strategies to mitigate nanotoxicity, with the goal of exploiting the full potential of nanotechnology for safe applications in humans. We hope that our review serves as a valuable resource that covers the current landscape of nanotoxicity research and that it will inspire new studies focused on expanding our understanding of nanoparticle toxicity. Improved understanding of nanotoxicity may ultimately inform and guide the development of regulatory frameworks to minimize potential harm to humans.

2. NANOPARTICLE CLASSIFICATION AND PHYSICOCHEMICAL PROPERTIES

Nanoparticles can be grouped into three main classes: (*a*) natural, (*b*) incidental, or (*c*) engineered nanoparticles (**Figure 1*a–c***). The first class, natural nanoparticles, is ubiquitous in the environment and generated via normal physical, chemical, and biological processes. Examples of such natural nanoparticles include inorganic metal-based nanoparticles, e.g., naturally formed silver (Ag) nanoparticles, and organic nanoparticles, e.g., virus nanoparticles and exosomes (**Figure 1*a***). The second class, incidental nanoparticles, is generated unintentionally as byproducts of both industrial and nonindustrial processes such as corrosion, combustion, and cooking. Examples include inorganic and organic combustion products such as metal- and carbon-based nanoparticles, respectively (**Figure 1*b***). The third class, engineered nanoparticles, is intentionally designed and fabricated for specific industrial and/or medical applications. Examples include zinc oxide (ZnO) and titanium dioxide (TiO_2) nanoparticles in sunscreen and liposomes for drug delivery applications (8–10) (**Figure 1*c***). An alternative terminology for natural and incidental nanoparticles is ultrafine particles (UFPs). These UFPs are airborne particulates of less than 100 nm in aerodynamic diameter. While incidental and engineered nanoparticles are typically of anthropogenic origin, i.e., caused and/or prepared by human activity, natural nanoparticles are generated without human intervention (1, 6).

Beyond classification by origin, the various nanoparticle types can be further differentiated by their physicochemical properties (**Figure 1*d***). Physicochemical properties such as nanoparticle

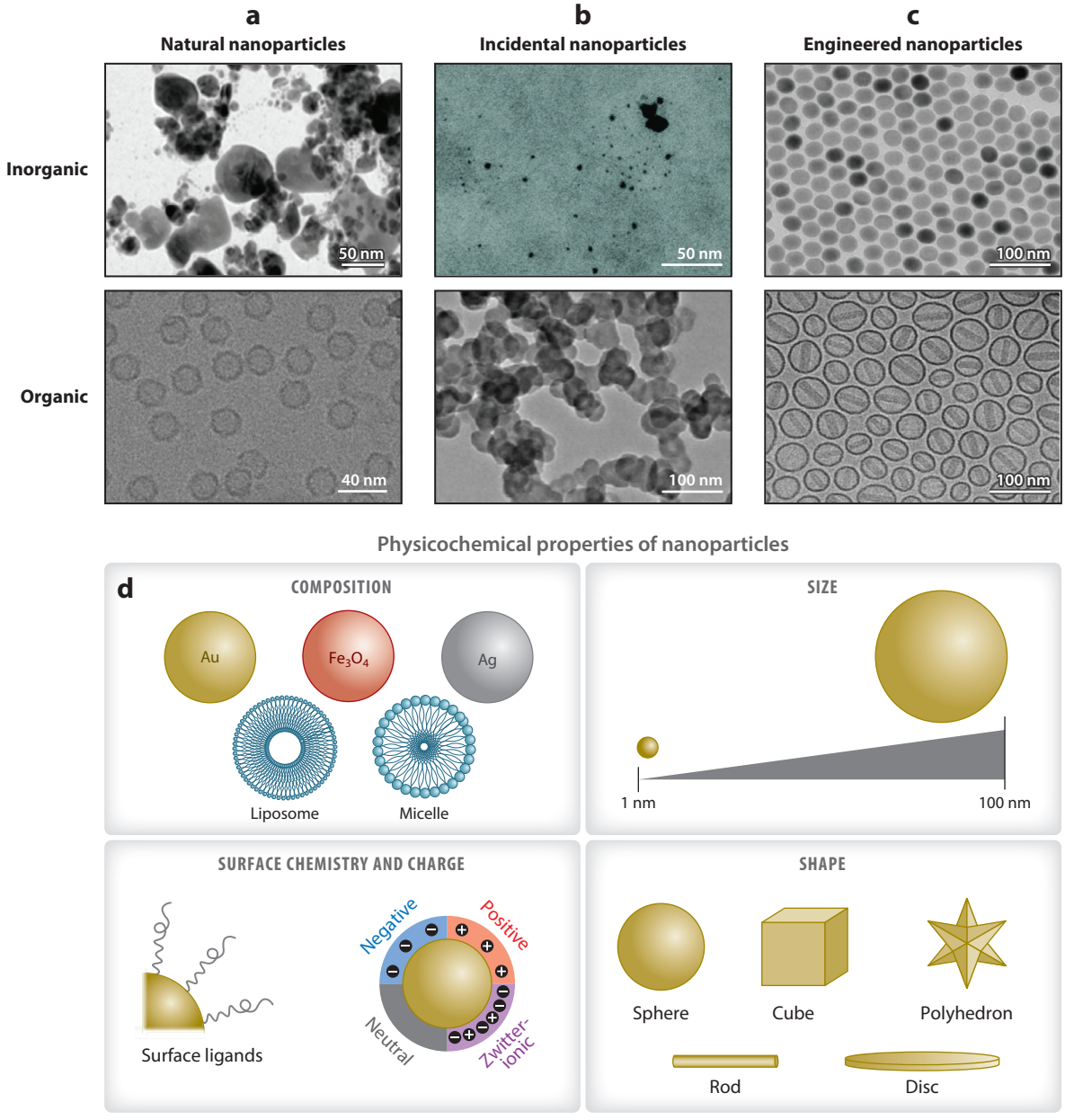

Figure 1

Nanoparticle classification and physicochemical properties. Nanoparticles can be broadly organized into three different classes: (*a*) natural, (*b*) incidental, or (*c*) engineered nanoparticles. Nanoparticles from all of these classes can be made from inorganic or organic materials. In contrast to the majority of natural and incidental nanoparticles, engineered nanoparticles typically exhibit narrow size distributions as well as defined shapes and surface properties. Panels *a–c* display transmission electron micrographs of different inorganic nanoparticles [(*a*,*b*, *top*) silver (Ag) nanoparticles, (*c*, *top*) upconversion ($NaYF_4$/Yb,Er) nanoparticles] and organic nanoparticles [(*a*, *bottom*) cowpea mosaic virus–like nanoparticles, (*b*, *bottom*) carbon black nanoparticles, (*c*, *bottom*) doxorubicin-loaded liposomes]. (*d*) Schematic of various nanoparticle physicochemical properties, including different nanoparticle compositions, sizes, surface chemistries, and shapes. Panel *a* adapted with permission from References 138 and 140, panel *b* adapted with permission from References 139 and 141, and panel *c* adapted with permission from References 110 and 142.

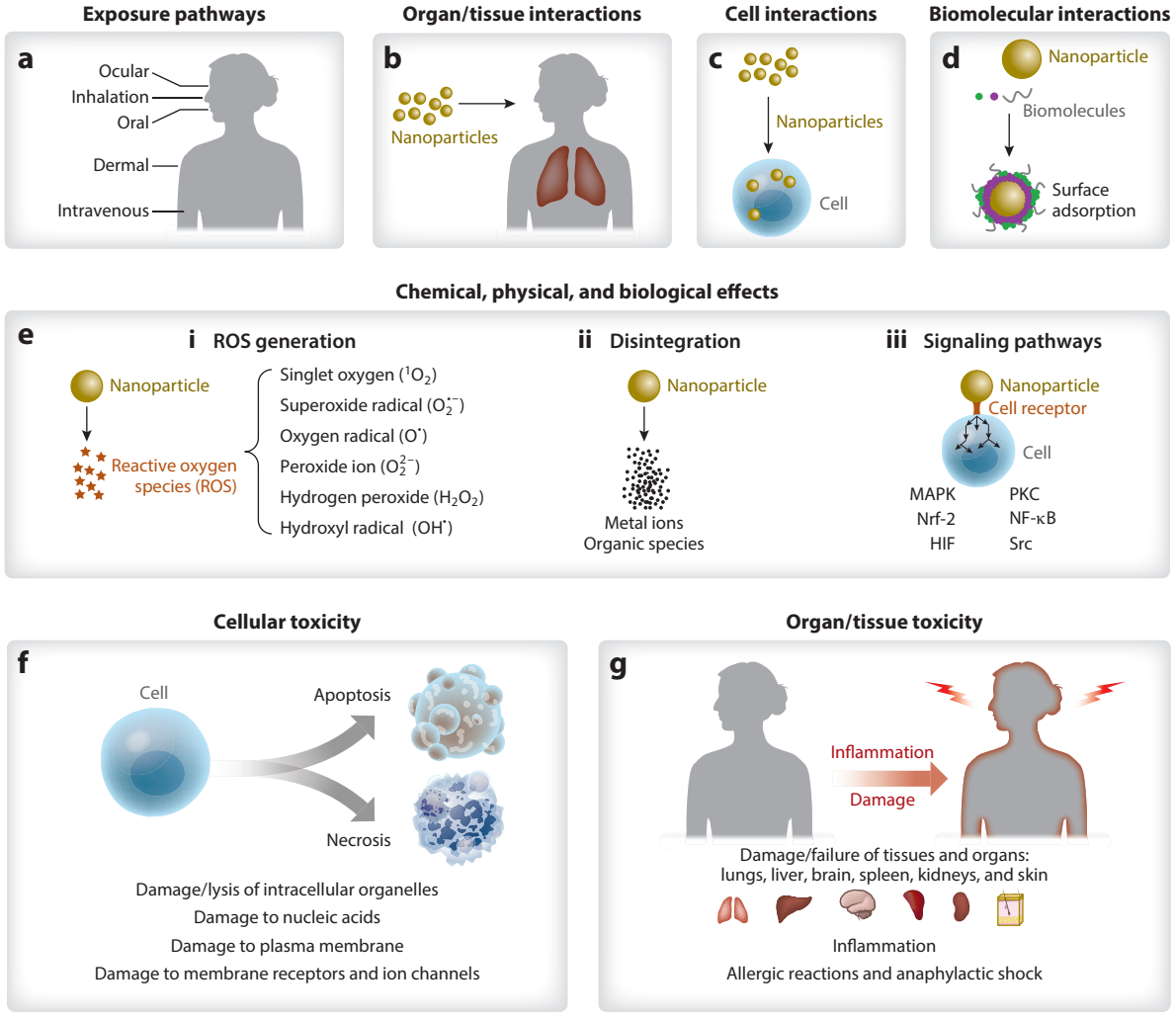

Figure 2

Schematic representation of nanoparticle adverse effects and nanotoxicity. (*a*) Nanoparticle exposure pathways. Upon exposure, nanoparticles can interact with (*b*) organs/tissues, (*c*) cells, and (*d*) biomolecules. Major nanoparticle toxicity mechanisms include (*e*, *i*) the generation of reactive oxygen species (ROS), (*e*, *ii*) nanoparticle disintegration and release of metal ions and organic species, and (*e*, *iii*) nanoparticle-mediated activation of cell signaling pathways. Nanoparticle adverse effects and toxicity can lead to (*f*) cell apoptosis and necrosis and (*g*) tissue/organ damage, inflammation, and anaphylactic shock.

composition, size, surface chemistry, and shape are key factors that govern nanoparticle interactions with biological systems and biomolecules. These interactions can affect biomolecular and cellular signaling, biological kinetics and transport, nanoparticle biodistribution, immunogenicity, and toxicity (11) (**Figure 2**). Compared to samples of engineered nanoparticles, natural and incidental nanoparticles tend to exhibit more heterogeneous physicochemical properties, with substantial variations in nanoparticle composition, size, surface chemistry, and shape (**Figure 1*a–c***). This heterogeneity complicates the assessment and understanding of nanoparticle biological interactions, adverse effects, and toxicity.

3. NANOTOXICOLOGY

The study of nanoparticle adverse effects and toxicity is commonly referred to as nanotoxicology (12). Upon exposure, all three classes of nanoparticles, i.e., natural, incidental, and engineered nanoparticles, may interact with organs, tissues, cells, and biomolecules (**Figure 2**). Consequently, nanoparticle exposure may induce undesirable and harmful nano-bio interactions and other downstream mechanisms that can potentially result in adverse effects and nanotoxicity.

Nanoparticle toxicity may occur as a function of exposure route, dose, concentration, time, and/or frequency. Traditionally, these fundamental toxicity factors are relevant for the assessment of small-molecule drugs and other compounds. In the evaluation of nanotoxicology, these parameters are also widely used. However, beyond these traditional toxicology parameters, other important factors that may affect nanoparticle toxicity need to be considered, including nanoparticle physicochemical properties such as material composition, size, surface chemistry, and shape (**Figure 1*d***). Compared to small molecules, these additional physicochemical variables make nanotoxicity assessment complex, and evaluation of nanoparticle toxicity on a case-by-case basis may be required. For example, slight variations in nanoparticle surface chemistry can result in significantly different toxicity, biodistribution, and elimination profiles, even if the nanomaterial core is the same (13–15).

To fully evaluate nanoparticle toxicity, nanoparticle structure and corresponding physicochemical properties need to be completely characterized and understood. In this way, observed toxic effects can be better attributed to certain nanoparticle properties for establishing specific nanoparticle structure-activity/toxicity functional relationships. As nanoparticle structural properties significantly affect toxicity, it is even more challenging to evaluate the safety of nanoparticles that exhibit large variations in physicochemical properties, as is often seen in natural and incidental nanoparticles (**Figure 1*a*,*b***). Therefore, it is challenging to draw general conclusions about nanoparticle toxicity, as nanotoxicity is dependent on complex interactions between different physicochemical properties and the corresponding biological environment. Based on this complexity, it is important to establish well-defined, standardized methodologies for the systematic evaluation of nanotoxicity under relevant conditions to achieve comparable toxicological data sets. However, this level of standardization has not yet been achieved, which makes it difficult to provide general trends of nanotoxicity for acute (<14 days) and chronic (>4 months) exposure regimens (16).

To provide examples of the broad range of potential nanoparticle adverse effects and toxicity, including neurotoxicity, pulmonary toxicity, vascular dysfunction, genotoxicity, and immunotoxicity, we have summarized studies that assessed the nanotoxicity of different nanoparticle classes and types in human subjects (**Table 1**). **Table 1** also includes studies that evaluated the toxicity of relevant engineered nanoparticles, such as Ag and ZnO nanoparticles, on human subjects but without any reported clinical or pathological findings, implying that the tested nanoparticles were safe and without noticeable adverse effects under the specific testing conditions. For context, Ag and ZnO nanoparticles are used in over-the-counter consumer products such as antiviral, antibacterial, and anti-inflammatory products and compounds as well as sunscreen (17–19). We want to emphasize that detailed reports and systematic clinical studies of nanoparticle toxicity in humans for various nanoparticle types are limited. Most published reports focus on the assessment of nanoparticle toxicity in cell culture and animal models. However, these models do not fully recapitulate nanoparticle toxicity responses in humans and are therefore limited in their predictive power of possible hazards to humans (20).

To emphasize the importance of composition and other physicochemical properties on nanoparticle adverse effects and toxicity, we highlight a study by Mills et al. (7) that assessed adverse

Table 1 Examples of nanoparticle toxicity in human subjects

Adverse effect	Nanoparticle class and type	Exposure route, dose, duration, number of human subjects	Toxicity mechanism	Toxicity assessment	Reference
Neurotoxicity	Natural Fe_3O_4 (<20 nm)	NA NA Chronic (many years) 22 human subjects	Abnormal, age-associated biomineralization of Fe_3O_4 in the brain	Quantitative magnetometry; correlation between Fe_3O_4 nanoparticle concentration in the human brain and Alzheimer's disease	143
	Incidental Fe_3O_4 and Fe_2O_3 (up to 150 nm)	Inhalation NA Chronic (many years) 37 human subjects	Inhalation of airborne pollutant nanoparticles; potentially enhanced ROS generation leading to neurodegenerative diseases	High-resolution TEM, EELS, and EDX analyses of human brain samples	43
Pulmonary toxicity	Incidental Chemically complex mixtures (10–80 nm)	Inhalation 30,000 NPs/cm^3 (>10 times background levels) Acute (6 h/day for 3 days) 17 human subjects	Upper airway inflammation and systemic oxidative stress with generation of proinflammatory cytokines	Analysis of 14 cytokines in nasal lavage samples and analysis of 8-OH-dG and creatinine in human urine samples	144
Vascular dysfunction	Incidental Diesel exhaust nanoparticles (<100 nm)	Inhalation 1.2×10^6 NPs/cm^3 Acute (up to 14 days) 16 human subjects	Increased systolic blood pressure and attenuated vasodilation due to nanoparticle-induced vascular oxidative stress	Measurement of forearm blood flow and blood pressure and biomarker analysis of human blood samples	7
Genotoxicity	Incidental Ag	Inhalation NA Chronic 76 human subjects	DNA damage in mononuclear leukocytes due to oxidative stress induced by Ag nanoparticles	Blood analysis for DNA damage using alkaline comet assay and analysis of total antioxidant status, total oxidant status, total thiol, and ceruloplasmin in human blood plasma samples	145
Immunotoxicity	Engineered PEGylated liposomes (Doxil, ~100 nm)	IV 40–306 mg Acute (infusion for 1 h) 29 human subjects	Hypersensitivity reaction and anaphylatoxin release due to complement activation of PEGylated liposomes	Analysis of human blood samples for complement terminal complex (SC5b-9) to correlate complement activation with hypersensitivity reaction	83
None reported	Engineered Ag (A: 5–10 nm; B: 25–40 nm)	Ingestion A: 100 μg/day; B: 480 μg/day Acute (up to 14 days) 60 human subjects	No clinically important changes in weight, BMI, blood pressure, heart rate, or laboratory findings in blood and urine samples	Analysis of human blood and urine samples, including hematology; ELISA for ROS and proinflammatory cytokines; and MRI	146
	Engineered ZnO with and without silane coating (up to 74 nm)	Topical dermal application Up to 100 mg/mL daily Acute (up to 5 days) 5 human subjects	No nanoparticle penetration through stratum corneum; no morphological or redox changes	Analysis of nanoparticle skin penetration using multiphoton tomography and fluorescence lifetime imaging microscopy	10

Abbreviations: Ag, silver; BMI, body mass index; EDX, energy dispersive X-ray spectroscopy; EELS, electron energy loss spectroscopy; ELISA, enzyme-linked immunosorbent assay; IV, intravenous administration; MRI, magnetic resonance imaging; NA, not available; 8-OH-dG, 8-hydroxydeoxyguanosine; PEG, poly(ethylene glycol); ROS, reactive oxygen species; TEM, transmission electron microscopy.

vascular side effects in 16 healthy human subjects exposed to combustion-derived nanoparticles from diesel exhaust over an acute exposure duration of 14 days. Impaired vascular function in study subjects was observed due to oxidative stress caused by inhalation of diesel exhaust nanoparticles. In contrast, when study subjects were exposed to filtered exhaust, i.e., exhaust without nanoparticles, or air containing pure carbon nanoparticles, vascular impairment was not observed. These findings indicate that nanoparticle composition and other physicochemical properties play key roles in nanotoxicity.

Other important nanoparticle physicochemical properties that affect nanotoxicity include size, surface chemistry, and shape (**Figure 1*d***). For more detailed information on how nanoparticle physicochemical properties affect nano-bio interactions, adverse effects, and toxicity, we refer interested readers to excellent review articles by the Chan (11) and Howard groups (12).

Nanoparticle size is a physicochemical parameter that has been reported to affect cellular uptake efficiency and cytotoxicity (21). A study by Pan et al. (22) reported size-dependent cytotoxicity of gold nanoparticles with identical surface chemistry in fibroblasts, epithelial cells, macrophages, and melanoma cells in cell culture. The researchers reported that nanoparticles with a diameter of 1.4 nm exhibited the highest cytotoxicity, while nanoparticles with a diameter of 15 nm had no reported toxicity. As potential reasons for the observed differences in cytotoxicity, the researchers listed size-dependent nanoparticle cell uptake kinetics and interactions with the cell plasma membrane promoting cell apoptosis and necrosis.

In addition to nanoparticle size, surface chemistry is another important parameter that directly affects nanotoxicity. For example, a study by Bozich et al. (23) concluded that gold nanoparticles with an overall positive surface charge exhibited greater toxicity on *Daphnia magna* model organisms compared to negatively charged gold nanoparticles of the same core size. Similarly, Lee et al. (24) reported that positively charged gold nanoparticles caused an ~50% reduction in cell viability compared to identical neutrally charged particles in cultured mouse breast cancer 4T1 cells. In comparison to neutral nanoparticles, there was a substantial reduction in cell viability of ~50% for positively charged nanoparticles. Potential reasons for the increased toxicity of positively charged nanoparticles include a higher electrostatic attraction of nanoparticles to negatively charged cell surfaces and overall increased nanoparticle cellular uptake, potentially leading to increases in oxidative stress and reactive oxygen species (ROS) (25, 26). As the nanoparticle surface interacts directly with biomolecules and biological systems, it is a driver of cellular uptake and intracellular transport kinetics (21). In addition, surface chemistry and surface charge are key factors of nanoparticle agglomeration and aggregation, which are additional variables that need to be considered in the assessment of nanotoxicity (27, 28).

Besides size and surface chemistry, nanoparticle shape may significantly affect nanotoxicity (26). For example, a study by Zhao et al. (29) reported increased cytotoxicity of needle- and plate-shaped nanosized hydroxyapatite compared to sphere- and rod-shaped nanoparticles in human lung BEAS-2B epithelial cells. A potential reason for the increased cytotoxicity may be that needle- and spike-like nanoparticle shapes potentially puncture cellular membranes, leading to compromised cellular integrity and cell death. The shape properties of micro- and nanoparticles can also induce physical activation of innate immunity. As reported by Wang et al. (30), TiO_2 microparticles exhibiting nanospikes can exert mechanical stress on cells, which can lead to potassium efflux and inflammasome activation in macrophages and dendritic cells. These findings highlight the potential of nanoparticle shape as a means to tune nanoparticle immunogenicity by physical cues, which could potentially be attractive for more efficient and effective vaccination and immunotherapy approaches.

Nanoparticle physicochemical properties not only affect cellular interactions but may also determine biodistribution, clearance, and elimination (31–33). For example, nanoparticles with sizes

smaller than 5.5 nm will be eliminated rapidly into urine via the kidneys (34). Nanoparticles larger than the renal cutoff size are often efficiently sequestered by cells in the liver and spleen, including Kupffer cells, B cells, T cells, and endothelial cells (33), and may be eliminated to varying extents via the hepatobiliary pathway (15). Understanding how nanoparticle physicochemical properties affect nano-bio interactions will provide an opportunity to control nanoparticle fate and toxicity inside the body. Such control may ultimately lead to more potent nanoparticle-based medical treatments and diagnostics with reduced side effects and toxicity for patients. An example of this control is the application of liposomes to encapsulate the small-molecule cancer drug doxorubicin. Compared to treatment with the free (i.e., unencapsulated) drug, US Food and Drug Administration–approved doxorubicin liposomes (i.e., Doxil) can reduce cardiotoxicity and other adverse effects to improve the quality of life for cancer patients (35).

4. NANOTOXICITY MECHANISMS

There are a number of different pathways by which nanoparticles can enter the body. These pathways include inhalation, oral ingestion, ocular exposure, application and deposition on skin, and intravenous administration (10, 36–39) (**Figure 2*a***). The inhalation of airborne nanoparticles is a major exposure pathway that allows nanoparticles to enter and deposit in lung tissues and the alveolar region (40) (**Figure 2*b***). Accumulation of nanoparticles in the lung can lead to oxidative stress–mediated lung inflammation at both acute and chronic stages (41, 42). Inhalation may also lead to the accumulation of nanoparticles in the brain. Maher et al. (43) reported that airborne magnetite nanoparticles can enter the brain via the olfactory bulb. Accumulation of magnetite nanoparticles in the brain that are abundant in airborne particulate matter pollution can lead to enhanced production of ROS, which is causally linked to neurodegenerative diseases such as Alzheimer's disease (43, 44).

After entering the body, nanoparticles may interact with the initially encountered organ or tissue. Nanoparticles may also subsequently translocate and enter the bloodstream (for example, from lungs to the capillary network to bigger vessels) to access distant organs/tissues via systemic transport (45, 46) (**Figure 2*b***). Within organs, tissues, and blood, nanoparticles can interact with cells and intracellular organelles to potentially cause toxicity at cellular and subcellular levels (**Figure 2*c***). It is important to point out that, upon entry into the body, nanoparticles interact with a variety of different biomolecules, including proteins, carbohydrates, lipids, and nucleic acids (**Figure 2*d***). These interactions result in the formation of a biomolecular nanoparticle surface corona, often referred to as the protein corona. Protein corona (or biomolecular corona) formation may change nanoparticle surface chemistry or stimulate the complement system substantially and ultimately affect nanotoxicity or the efficacy of nanomedicine (47–49). The damage of proteins to nanoparticle surfaces may also lead to protein unfolding (50, 51). This process may induce the loss of protein function and may cause immunotoxicity (52, 53). In addition, the protein configuration change can lead to adverse effects and toxicity via cell signaling pathway activation (51), enzyme function loss (54), nanoparticle aggregation (50), new antigenic site formation (55), and protein fibrillation (56).

At the cellular level, direct interaction between nanoparticles and cells may result in physical damage of cell membrane structures (57, 58). For example, graphene nanoparticles have been reported to cause physical damage, cytoskeletal dysfunction, and abnormal morphological stretching in different cell types as a result of the blade-like shape of these materials (57, 58). In addition, nanoparticles may be able to block cell membrane receptors and membrane ion channels, which may interrupt normal cellular biofunctions and homeostasis (59). Leifert et al. (59) reported that 1.4-nm gold nanoparticles were able to block voltage-gated potassium channels in vitro, which may lead to unwanted cardiac malformation in mice.

A major nanotoxicity mechanism is the generation of ROS such as singlet oxygen, superoxide anion radicals, oxygen radicals, peroxide ions, hydrogen peroxide, and hydroxyl radicals (**Figure 2*e***). ROS generation can occur in different ways. One route is through one-electron oxidative reactions with transition metals or nanoparticle surface groups (60, 61). It is important to note that a nanoparticle exhibits a relatively large surface area compared to the particle volume. An increase in surface area is typically accompanied by an increase in chemical reactivity potentially leading to increased ROS production. Another ROS generation mechanism is via mitochondrial respiration and subsequent ROS release into the cytoplasm through pores in mitochondrial membranes created by nanoparticles (60). In healthy cells, an equilibrium is maintained between intracellular antioxidants and ROS. However, intracellular nanoparticles can directly damage mitochondria, causing an increase in intracellular ROS and oxidative stress (62). Enhanced intracellular ROS levels may stimulate further ROS release from mitochondria through a process called ROS-induced ROS release. This process can substantially increase intracellular ROS levels and amplify the oxidative imbalance (63). High levels of ROS can cause oxidative stress and damage to cellular organelles, DNA, cell membranes, ion channels, and cell surface receptors, leading to adverse effects and toxicity.

Metal or metal oxide nanoparticles are used in preclinical and clinical applications such as imaging, photothermal therapy, and biosensors (64). However, corrosive tissue microenvironments and lysosomal degradation may disintegrate nanoparticles to release potentially harmful metal ions (**Figure 2*e***). For many nanoparticles, including Ag, cadmium selenide (CdSe), ZnO, and ferrosoferric oxide nanoparticles, released metal ions may generate high levels of oxidative stress and are primary sources of nanotoxicity. For example, Ag(I) ions released from Ag nanoparticles can cause DNA damage, ROS generation, and cell membrane destruction as reported from cell culture studies (65). We want to emphasize that nanotoxicity results obtained in cell culture studies do not necessarily recapitulate the nanoparticle toxicity potential in animal models or human subjects. For example, CdSe quantum dots were found to be toxic in cell culture; however, no toxicity was observed in animal models under the specific reported testing conditions (66–69).

The generation of high levels of ROS and the release of harmful metal ions from nanoparticles have been reported to affect a variety of cell signaling pathways such as nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB), mitogen-activated protein kinase (MAPK), Akt, and Src (70–73) (**Figure 2*e***). Activation and modulation of these signaling pathways can affect cell proliferation, differentiation, and cell survival. Nyga et al. (74) reported that cobalt nanoparticles can stabilize hypoxia-inducible factor (HIF) protein and upregulate *HIF* gene expression in human macrophages. HIF pathway activation can affect cell growth, cell survival, apoptosis, and metabolic adaptation (75). Importantly, there can be interplay and synergistic effects between ROS generation, cell signaling modulation, and nanoparticle disintegration. For example, nanoparticle disintegration may lead to modulation of signaling pathways and/or induce ROS generation (76), which can activate numerous signaling pathways or cause nanoparticle disintegration; in turn, different cell signaling pathways can subsequently induce ROS generation (77). These mechanisms may cause damage to cell membranes, intracellular organelles, and nucleic acids and eventually lead to cell apoptosis or necrosis (**Figure 2*f***). Loss of functional cells may compromise organ function and result in organ damage or inflammatory responses (**Figure 2*g***). Moreover, cell apoptosis, necrosis, and pyroptosis may lead to the release of large amounts of intracellular content to potentially cause local inflammation or systemic immune responses (78–80) (**Figure 2*g***).

In addition to the nanoparticle core material, surface components may also contribute significantly to nanoparticle adverse effects and toxicity. For example, researchers coat nanoparticle surfaces with polymers such as dextran or poly(ethylene glycol) (PEG) to reduce adsorption of proteins and other biomolecules and to prolong nanoparticle blood circulation times

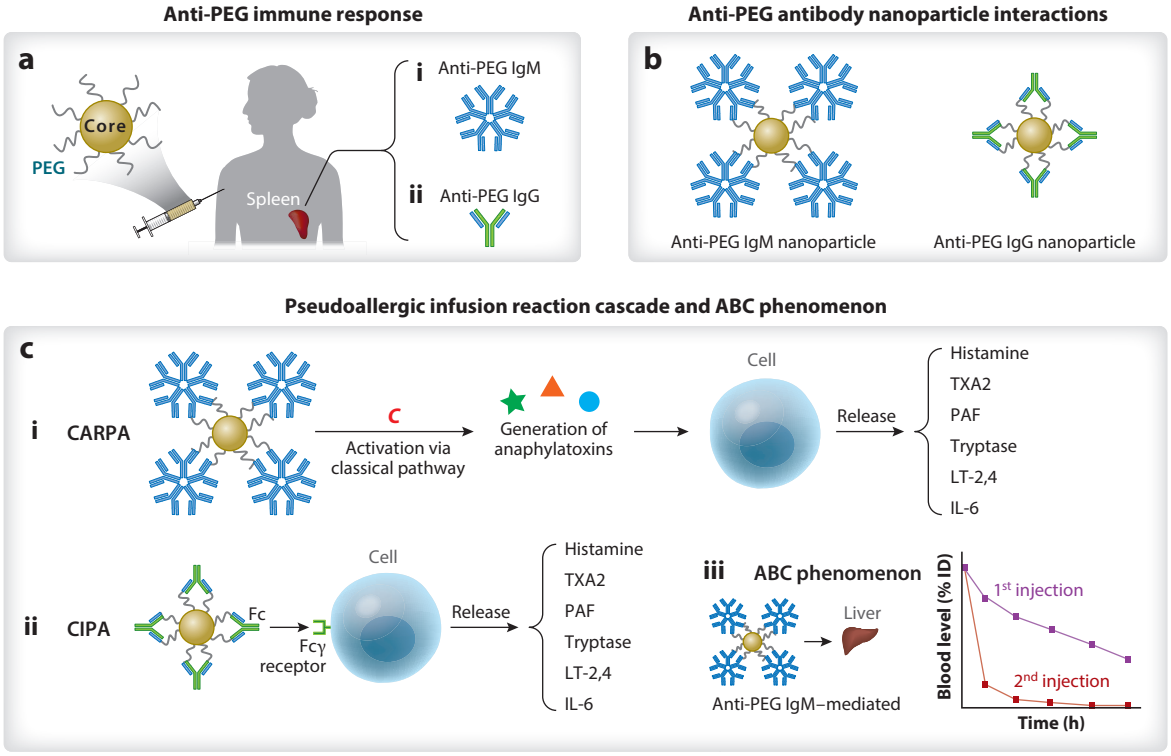

Figure 3

Anti-poly(ethylene glycol) (PEG) immunogenicity–induced mechanisms of nanoparticle pseudoallergic infusion reaction cascade and accelerated blood clearance (ABC) phenomenon. (*a*) Anti-PEG immune responses can be stimulated by intravenous administration of PEGylated nanoparticles. These nanoparticles can stimulate spleen marginal zone B cells and plasma B cells to produce anti-PEG immunoglobulin (Ig)M and IgG, respectively. (*b*) Anti-PEG IgM and IgG can bind efficiently to PEGylated nanoparticles. (*c*, *i*) The complement (*C*) activation–related pseudoallergy (CARPA) mechanism is initiated by anti-PEG IgM binding to the nanoparticle surface. In a subsequent step, the complement system is activated via the classical pathway. This activation leads to the generation of anaphylatoxins that stimulate different types of innate immune and blood cells, including macrophages, mast cells, basophils, and granulocytes, to release secondary mediators of pseudoallergy, such as histamine, TXA2, PAF, tryptase, LT-2 and 4, and IL-6. (*c*, *ii*) The complement-independent pseudoallergy (CIPA) is characterized by anti-PEG IgG binding to nanoparticles. The fragment crystallizable (Fc) portion of the anti-PEG IgG can bind to Fcγ receptors on macrophages, mast cells, and basophils to release secondary mediators of pseudoallergy. (*c*, *iii*) The rapid blood clearance of PEGylated nanoparticles upon repeated administration is referred to as the ABC phenomenon and is mediated in part by anti-PEG IgM opsonization, leading to efficient nanoparticle phagocytosis and accumulation in cells and organs of the mononuclear phagocyte system, including the liver. The second dose of PEGylated medicine (*red*) has a shorter half-life than the initial dose (*purple*) due to the patient's immune reaction.

(81, 82). Therefore, PEG is widely used in preclinical and clinical studies for surface modification of nanomedicines. However, PEG may induce hypersensitivity reactions and anaphylaxis mediated by anti-PEG antibodies in humans (83–85).

After an initial systemic administration of PEGylated nanoparticles, anti-PEG immunoglobulins (Igs; IgM initially, then IgG) may be generated by marginal zone spleen B cells (**Figure 3*a*,*b***). The anti-PEG IgM then targets PEGylated nanoparticles during subsequent administrations, causing complement activation via the classical pathway (48, 86–88) (**Figure 3*c***). Upon activation of the complement system, anaphylatoxins will be released, including platelet-activating factor, histamine, or cytokines, resulting in hypersensitivity reactions (89). Kozma et al. (90) documented the causal relationship between complement activation by anti-PEG IgM and hypersensitivity

reactions in pig models. Although the study was conducted in pigs, it provides valuable insights into potential PEG-related toxicity mechanisms in humans. Other reported studies indicate that hypersensitivity to nanoparticle surface components may be induced by complement activation via the alternative pathway (47, 91). This pathway does not depend on anti-PEG antibodies and is not limited to PEGylated nanoparticles (47, 91). More in-depth studies are needed to fully elucidate the underlying mechanisms of hypersensitivity reactions. In addition to complement activation–related pseudoallergy reactions, complement-independent pseudoallergy is caused by anti-PEG IgG (**Figure 3*c***). Mechanistically, a PEGylated nanoparticle is bound by anti-PEG IgG forming the nanoparticle-IgG complex, which subsequently can interact with the Fcγ receptors on mast cells, basophils, and neutrophils, resulting in the release of platelet-activating factor, histamine, or cytokines, to induce hypersensitivity reactions (92–94).

Hypersensitivity reactions often occur during second- and later-stage administration of PEGylated nanoparticles. However, hypersensitivity reactions have also been observed during the first dosage in human subjects. A potential rationale for this side effect is the abundance of preexisting anti-PEG antibodies in some of the patient population (93). Many humans who had never received PEGylated materials or drugs still possess preexisting anti-PEG IgM and IgG in various amounts, which is likely due to exposure to PEG-containing over-the-counter medication (e.g., daily multigram doses in some laxatives), cosmetics, and other everyday consumer products (89, 95–97). According to a study by Yang et al. (96), anti-PEG antibodies were detected in 72% of human samples collected after 1999, while 56% of historical samples from the previous 30 years (1970–1999) exhibited anti-PEG antibodies. In other words, a large number of humans exhibit detectable levels of anti-PEG IgG and IgM, and these numbers are expected to increase in humans in the future as a result of wider exposure to products that contain PEG.

Besides hypersensitivity reactions, complement activation can also lead to a direct attack of the lipid membrane of drug-carrying nanoparticles, such as doxorubicin liposomes, to prematurely release encapsulated chemotherapy drugs. Such premature drug release can affect the therapeutic effect of nanomedicines and could potentially contribute to additional nanotoxicity concerns (98). Anti-PEG immunity may also contribute to the so-called accelerated blood clearance (ABC) phenomenon (49, 99) (**Figure 3*c***). Upon repeated administration of PEGylated nanoparticles, anti-PEG IgM opsonization may trigger efficient nanoparticle phagocytosis. As a result, nanoparticles may accumulate to a large extent in cells and organs of the mononuclear phagocyte system, including the liver, after their first administration, and thus not arrive at the intended target location such as a tumor. Besides the observed decrease in nanoparticle therapeutic efficacy upon ABC, acute and chronic nanotoxicity of sequestered nanoparticles are substantial concerns.

5. NANOTOXICITY ASSESSMENT

Toxicity assessments are used to evaluate the safety of nanoparticles. In **Table 2**, we have summarized examples of commonly used cell culture and animal toxicity tests used for nanotoxicity assessment. Cell culture studies enable nanotoxicity evaluation of various model animal and human cell lines and are beneficial due to their simplicity, scalability, low cost, and throughput. However, cell culture studies, in contrast to animal models, lack complex physiology and are limited in their predictive power of nanotoxicity for other species and humans. Animal model testing can account for complex physiological environments during nanotoxicity assessment but may be limited in predicting toxic responses and adverse effects in humans. Computational nanotoxicity methods can assist with bridging the gaps between cell culture, animal models, and human subjects. If the underlying assumptions and models are not flawed, then these methods should substantially assist nanotoxicity modelling and prediction in the future for broad and routine

Table 2 Examples of nanoparticle toxicity assessment tools

Toxicity tests	Assessment tool(s)	Reference(s)
Cell culture level		
Cell membrane integrity	LDH assay	147
Cell morphology	Microscopy	148
Cell necrosis and apoptosis	Flow cytometry	149
Cell viability and cell death	MTT assay, live/dead assay, flow cytometry, trypan blue, WST	150
DNA damage and gene expression	Comet assay with Fpg treatment Gene expression levels monitored by qPCR	151
Hemoglobin release	Hemolysis assay	152
Inflammation and immune responses	ELISA	153
Ion channel disruption	Patch-clamp experiment	154
Mitochondrial damage	Mitochondrial membrane potential measurements	155
Protein structure	CD, DSC, FTIR, cryo-EM	156
ROS generation	DCFH assay, fluorescence lifetime imaging microscopy	157, 158
Animal and human level		
Biochemistry	Tissue-damaging enzymes (ALP, LDH, ALAT), cytokine analysis	120
Hematology	Hemoglobin content, total protein, total erythrocyte and leukocyte counts	159
Histopathology	Tissue sections (hematoxylin/eosin, immunohistochemistry)	120, 160
Pharmacokinetics and pharmacodynamics	MRI, PET, SPECT, CT, ICP-MS, fluorescence, biodistribution, clearance, and elimination	39, 161
Skin test	Skin penetration and skin allergic reactions	10
Survival studies	Kaplan-Meier analysis, survival curves, median survival, LC_{50}, LD_{50}	159
Clinical trials (phase I–IV)	Safety and toxicity data on human subjects	162

Abbreviations: ALAT, alanine aminotransferase; ALP, alkaline phosphatase; CD, circular dichroism; cryo-EM, cryogenic electron microscopy; CT, X-ray computed tomography; DCFH, 2′7-dichlorodihydrofluorescein; DSC, differential scanning calorimetry; ELISA, enzyme-linked immunosorbent assay; Fpg, formamidopyrimidine-DNA glycosylase; FTIR, Fourier transform infrared spectroscopy; ICP-MS, inductively coupled plasma mass spectrometry; LC_{50}, lethal concentration 50%; LD_{50}, lethal dose 50%; LDH, lactate dehydrogenase; MRI, magnetic resonance imaging; MTT, methyl tetrazolium; PET, positron emission tomography; qPCR, quantitative polymerase chain reaction; SPECT, single-photon emission computed tomography; WST, water-soluble tetrazolium salt.

applications (100). Computational studies can reduce the need, cost, and time required for animal and cell nanotoxicity testing (101–103). However, due to the lack of standardized protocols for nanotoxicity testing, published studies exhibit substantial heterogeneities in terms of nanoparticle characterization, dose metrics, experimental methods, and data completeness, reducing the overall statistical power and accuracy of computational models for nanotoxicity predictions (104, 105).

6. STRATEGIES TO MITIGATE NANOTOXICITY

By understanding the underlying toxicity mechanisms, researchers can start to devise strategies for mitigating nanoparticle adverse effects and nanotoxicity. While few studies focus on manipulating the nanoparticle core composition, most reported approaches center around the modification of nanoparticle surface chemistry and surface properties. For example, silica coating and polymer encapsulation strategies can be used to control nanoparticle disintegration and ion release kinetics of metal and metal oxide nanoparticles to mitigate metal ion–induced toxicities and ROS

production (106–110). Another popular approach is to passivate the nanoparticle surface with PEG for reducing biomolecular corona formation and for camouflaging nanoparticles. However, due to the immunogenic potential of PEG, other nanoparticle surface–coating technologies are urgently needed that provide similar camouflaging properties as PEG but without side effects for patients, including hypersensitivity, allergic reactions, and anaphylactic shock. A creative approach to address this challenge is to wrap nanoparticles in cell plasma membranes such as membranes derived from erythrocytes (111). Red blood cell membrane–coated nanoparticles exhibit minimal protein corona formation, toxicity, and immunogenicity. This camouflage strategy can be effective in mitigating the potential nanotoxicity of engineered nanoparticles to provide safer and more potent nanomedicines in the future and to use nanoparticles for safe applications in consumer products and industrial processes. However, it is difficult to apply such a strategy to natural and incidental nanoparticles as well as mitigate concerns for contaminating adventitious agents. The nanotoxicity of these natural and incidental nanoparticles may be mitigated by antioxidant therapy or by reducing human exposure to these nanoparticles via respiratory protection, including masks and other protective equipment (106, 112).

7. PERSPECTIVES AND CONCLUSION

One of the most pressing questions in conversations about nanoparticles is whether nanoparticles are toxic. The question has no direct answer due to the multiparameter nature of nanoparticle toxicity. Great caution is required to not generalize nanoparticle safety and toxicity concerns. In its current state, the evaluation of nanoparticle toxicity requires careful case-by-case assessment, because biological and pathological effects are determined by a number of variables, including nanoparticle physiochemical properties, exposure route, dose, and duration, to name a few. This opinion is in line with recent thoughtful editorials, viewpoints, and correspondences on nanoparticle risk assessment and nanosafety (113–118). Nanotoxicity is a highly important and timely research area, as human exposure to different nanoparticle classes and types will continue to increase in the future.

One of the main barriers to advancing progress in nanotoxicology is the lack of unified and standardized procedures for nanoparticle characterization, risk assessment methods, and reporting (105, 113, 119). For example, nanoparticle dose metrics are used and reported differently in different research studies, including mass-, surface area–, and nanoparticle number–based metrics (12). Standardization of nanoparticle dose metrics in experimental design and data reporting will be critical in the facilitation of data mining and development of computational approaches, such as the NanoSolveIT project, that use multiscale physics-based and data-driven models, including toxicogenomics and biokinetics, for integrated in silico nanoparticle risk assessment (100). In addition to dose metrics, standardized experimental design and data reporting will be required in all aspects of nanoparticle toxicity testing in order to train predictive computational models.

These data sets are typically obtained from conventional nanotoxicity studies that are based on in vitro cell culture and/or in vivo animal model experiments. There are ongoing debates within the research community about the potential and power of these models for predicting nanotoxicity in humans (101, 103, 104, 120). A limitation of nanoparticle risk assessment in those simplified models is that it is difficult to model chronic long-term exposure in laboratory animals that exhibit significantly shorter life spans than humans. However, data on chronic, low-dose nanoparticle exposure might provide valuable new insights on the long-term toxic effects of nanoparticles. Great attention and care should be placed on evaluating and understanding the mechanisms of nanotoxicity in biologically and physiologically relevant models. This may require new models of toxicity evaluation that exploit high-throughput screening methods (121, 122), machine learning

approaches (101), and the development of new three-dimensional microfluidic-based tissue chips and organoids that are aimed at better recapitulating human physiology (123–125). Such advanced tissue models combined with advanced optical imaging and single-cell analytical methods, such as single-cell RNA sequencing and single-cell elemental quantification, could provide powerful tools to assess nanotoxicity at the individual cell level (126–131).

Since nanoparticles may trigger immunogenicity and immunotoxicity, as observed in some cases with PEGylated nanomedicines, the formulation of nonimmunogenic nanoparticles is required. This goal may be achieved by coating nanoparticles with PEG alternatives such as zwitterionic polymers, cell-derived plasma membranes, or self (i.e., identical to human) carbohydrate polymers such as hyaluronan, polysialic acid, or heparosan (132–136). Recently, Lazarovits et al. (137) reported a method to create a new class of size- and shape-tunable nanoparticles that are made entirely from (human) patient-derived proteins. These nanoparticles are biodegradable and have not been observed to activate innate or adaptive immunity following systemic administration in animal models.

Nanotoxicology research will greatly benefit from the convergence of disciplines such as materials science, chemistry, engineering, biology, data science, medicine, and toxicology to answer pressing questions regarding the conditions required for the safe application and exposure of nanoparticles in humans. Ultimately, such concerted research will inform regulatory agencies and catalyze the generation of frameworks to exploit the full potential of safe nanoparticle exposure and application in humans.

DISCLOSURE STATEMENT

P.L.D. has a financial stake in Caisson Biotech, LLC, which is commercializing heparosan for drug delivery.

ACKNOWLEDGMENTS

The authors thank N. Donahue, V. Sheth, and S. Quine for fruitful discussions and assistance with illustrations. S.W. and P.L.D. acknowledge funding support from the University of Oklahoma IBEST-OUHSC Seed Grant for Interdisciplinary Research and OCAST (HR20-106).

LITERATURE CITED

1. Br. Stand. Inst. 2007. *Terminology for nanomaterials*. Publicly Availab. Specif., Br. Stand. Inst., London
2. Li N, Georas S, Alexis N, Fritz P, Xia T, et al. 2016. A work group report on ultrafine particles (American Academy of Allergy, Asthma & Immunology): why ambient ultrafine and engineered nanoparticles should receive special attention for possible adverse health outcomes in human subjects. *J. Allergy Clin. Immunol.* 138(2):386–96
3. Santos AC, Morais F, Simões A, Pereira I, Sequeira JAD, et al. 2019. Nanotechnology for the development of new cosmetic formulations. *Expert Opin. Drug Deliv.* 16(4):313–30
4. Hajba L, Guttman A. 2016. The use of magnetic nanoparticles in cancer theranostics: toward handheld diagnostic devices. *Biotechnol. Adv.* 34(4):354–61
5. Liu Y, Bhattarai P, Dai Z, Chen X. 2019. Photothermal therapy and photoacoustic imaging via nanotheranostics in fighting cancer. *Chem. Soc. Rev.* 48(7):2053–108
6. Hochella MF, Mogk DW, Ranville J, Allen IC, Luther GW, et al. 2019. Natural, incidental, and engineered nanomaterials and their impacts on the Earth system. *Science* 363(6434):eaau8299
7. Mills NL, Miller MR, Lucking AJ, Beveridge J, Flint L, et al. 2011. Combustion-derived nanoparticulate induces the adverse vascular effects of diesel exhaust inhalation. *Eur. Heart J.* 32(21):2660–71

8. Narum SM, Le T, Le DP, Lee JC, Donahue ND, et al. 2020. Passive targeting in nanomedicine: fundamental concepts, body interactions, and clinical potential. In *Nanoparticles for Biomedical Applications*, ed. EJ Chung, L Leon, C Rinaldi, pp. 37–53. Amsterdam: Elsevier
9. Sindhwani S, Syed AM, Ngai J, Kingston BR, Maiorino L, et al. 2020. The entry of nanoparticles into solid tumours. *Nat. Mater.* 19:566–75
10. Mohammed YH, Holmes A, Haridass IN, Sanchez WY, Studier H, et al. 2019. Support for the safe use of zinc oxide nanoparticle sunscreens: lack of skin penetration or cellular toxicity after repeated application in volunteers. *J. Investig. Dermatol.* 139(2):308–15
11. Albanese A, Tang PS, Chan WCW. 2012. The effect of nanoparticle size, shape, and surface chemistry on biological systems. *Annu. Rev. Biomed. Eng.* 14:1–16
12. Elsaesser A, Howard CV. 2012. Toxicology of nanoparticles. *Adv. Drug Deliv. Rev.* 64(2):129–37
13. Muhr V, Wilhelm S, Hirsch T, Wolfbeis OS. 2014. Upconversion nanoparticles: from hydrophobic to hydrophilic surfaces. *Acc. Chem. Res.* 47(12):3481–93
14. Elci SG, Jiang Y, Yan B, Kim ST, Saha K, et al. 2016. Surface charge controls the suborgan biodistributions of gold nanoparticles. *ACS Nano* 10(5):5536–42
15. Poon W, Zhang Y-N, Ouyang B, Kingston BR, Wu JLY, et al. 2019. Elimination pathways of nanoparticles. *ACS Nano* 13(5):5785–98
16. Liu J, Feng X, Wei L, Chen L, Song B, Shao L. 2016. The toxicology of ion-shedding zinc oxide nanoparticles. *Crit. Rev. Toxicol.* 46(4):348–84
17. Sun D, Zhang W, Mou Z, Chen Y, Guo F, et al. 2017. Transcriptome analysis reveals silver nanoparticle-decorated quercetin antibacterial molecular mechanism. *ACS Appl. Mater Interfaces* 9(11):10047–60
18. El-Rafie HM, Hamed MA-A. 2014. Antioxidant and anti-inflammatory activities of silver nanoparticles biosynthesized from aqueous leaves extracts of four *Terminalia* species. *Adv. Nat. Sci. Nanosci. Nanotechnol.* 5(3):035008
19. Holmes AM, Song Z, Moghimi HR, Roberts MS. 2016. Relative penetration of zinc oxide and zinc ions into human skin after application of different zinc oxide formulations. *ACS Nano* 10(2):1810–19
20. Rivera Gil P, Oberdörster G, Elder A, Puntes V, Parak WJ. 2010. Correlating physico-chemical with toxicological properties of nanoparticles: the present and the future. *ACS Nano* 4(10):5527–31
21. Donahue ND, Acar H, Wilhelm S. 2019. Concepts of nanoparticle cellular uptake, intracellular trafficking, and kinetics in nanomedicine. *Adv. Drug Deliv. Rev.* 143:68–96
22. Pan Y, Neuss S, Leifert A, Fischler M, Wen F, et al. 2007. Size-dependent cytotoxicity of gold nanoparticles. *Small* 3(11):1941–49
23. Bozich JS, Lohse SE, Torelli MD, Murphy CJ, Hamers RJ, Klaper RD. 2014. Surface chemistry, charge and ligand type impact the toxicity of gold nanoparticles to *Daphnia magna*. *Environ. Sci.: Nano* 1(3):260–70
24. Lee JC, Donahue ND, Mao AS, Karim A, Komarneni M, et al. 2020. Exploring maleimide-based nanoparticle surface engineering to control cellular interactions. *ACS Appl. Nano Mater.* 3:2421–29
25. Fröhlich E. 2012. The role of surface charge in cellular uptake and cytotoxicity of medical nanoparticles. *Int. J. Nanomed.* 7:5577–91
26. Sukhanova A, Bozrova S, Sokolov P, Berestovoy M, Karaulov A, Nabiev I. 2018. Dependence of nanoparticle toxicity on their physical and chemical properties. *Nanoscale Res. Lett.* 13(1):44
27. Tripathy N, Hong T-K, Ha K-T, Jeong H-S, Hahn Y-B. 2014. Effect of ZnO nanoparticles aggregation on the toxicity in RAW 264.7 murine macrophage. *J. Hazard. Mater.* 270:110–17
28. Albanese A, Chan WCW. 2011. Effect of gold nanoparticle aggregation on cell uptake and toxicity. *ACS Nano* 5(7):5478–89
29. Zhao X, Ng S, Heng BC, Guo J, Ma L, et al. 2013. Cytotoxicity of hydroxyapatite nanoparticles is shape and cell dependent. *Arch. Toxicol.* 87(6):1037–52
30. Wang J, Chen H-J, Hang T, Yu Y, Liu G, et al. 2018. Physical activation of innate immunity by spiky particles. *Nat. Nanotechnol.* 13(11):1078–86
31. Dai Q, Wilhelm S, Ding D, Syed AM, Sindhwani S, et al. 2018. Quantifying the ligand-coated nanoparticle delivery to cancer cells in solid tumors. *ACS Nano* 12(8):8423–35
32. Wilhelm S, Tavares AJ, Dai Q, Ohta S, Audet J, et al. 2016. Analysis of nanoparticle delivery to tumours. *Nat. Rev. Mater.* 1(5):16014

33. Tsoi KM, MacParland SA, Ma X-Z, Spetzler VN, Echeverri J, et al. 2016. Mechanism of hard-nanomaterial clearance by the liver. *Nat. Mater.* 15(11):1212–21
34. Du B, Yu M, Zheng J. 2018. Transport and interactions of nanoparticles in the kidneys. *Nat. Rev. Mater.* 3(10):358–74
35. Tahover E, Patil YP, Gabizon AA. 2015. Emerging delivery systems to reduce doxorubicin cardiotoxicity and improve therapeutic index: focus on liposomes. *Anticancer Drugs* 26(3):241–58
36. Westerhoff P, Atkinson A, Fortner J, Wong MS, Zimmerman J, et al. 2018. Low risk posed by engineered and incidental nanoparticles in drinking water. *Nat. Nanotechnol.* 13(8):661–69
37. Zhu S, Gong L, Li Y, Xu H, Gu Z, Zhao Y. 2019. Safety assessment of nanomaterials to eyes: an important but neglected issue. *Adv. Sci.* 6(16):1802289
38. Liou S-H, Tsou T-C, Wang S-L, Li L-A, Chiang H-C, et al. 2012. Epidemiological study of health hazards among workers handling engineered nanomaterials. *J. Nanopart. Res.* 14(8):878
39. Ye L, Yong K-T, Liu L, Roy I, Hu R, et al. 2012. A pilot study in non-human primates shows no adverse response to intravenous injection of quantum dots. *Nat. Nanotechnol.* 7(7):453–58
40. Qiao H, Liu W, Gu H, Wang D, Wang Y. 2015. The transport and deposition of nanoparticles in respiratory system by inhalation. *J. Nanomater.* 2015:394507
41. Zhang H, Ji Z, Xia T, Meng H, Low-Kam C, et al. 2012. Use of metal oxide nanoparticle band gap to develop a predictive paradigm for oxidative stress and acute pulmonary inflammation. *ACS Nano* 6(5):4349–68
42. Adamcakova-Dodd A, Stebounova LV, Kim JS, Vorrink SU, Ault AP, et al. 2014. Toxicity assessment of zinc oxide nanoparticles using sub-acute and sub-chronic murine inhalation models. *Part. Fibre Toxicol.* 11:15
43. Maher BA, Ahmed IAM, Karloukovski V, MacLaren DA, Foulds PG, et al. 2016. Magnetite pollution nanoparticles in the human brain. *PNAS* 113(39):10797–801
44. Lin Y, Hu C, Chen A, Feng X, Liang H, et al. 2020. Neurotoxicity of nanoparticles entering the brain via sensory nerve-to-brain pathways: injuries and mechanisms. *Arch. Toxicol.* 94(5):1479–95
45. Choi HS, Ashitate Y, Lee JH, Kim SH, Matsui A, et al. 2010. Rapid translocation of nanoparticles from the lung airspaces to the body. *Nat. Biotechnol.* 28(12):1300–3
46. Raftis JB, Miller MR. 2019. Nanoparticle translocation and multi-organ toxicity: a particularly small problem. *Nano Today* 26:8–12
47. Moghimi SM, Simberg D. 2017. Complement activation turnover on surfaces of nanoparticles. *Nano Today* 15:8–10
48. Szebeni J. 2014. Complement activation-related pseudoallergy: a stress reaction in blood triggered by nanomedicines and biologicals. *Mol. Immunol.* 61(2):163–73
49. Abu Lila AS, Kiwada H, Ishida T. 2013. The accelerated blood clearance (ABC) phenomenon: clinical challenge and approaches to manage. *J. Control. Release* 172(1):38–47
50. Dominguez-Medina S, Kisley L, Tauzin LJ, Hoggard A, Shuang B, et al. 2016. Adsorption and unfolding of a single protein triggers nanoparticle aggregation. *ACS Nano* 10(2):2103–12
51. Deng ZJ, Liang M, Monteiro M, Toth I, Minchin RF. 2011. Nanoparticle-induced unfolding of fibrinogen promotes Mac-1 receptor activation and inflammation. *Nat. Nanotechnol.* 6(1):39–44
52. Saptarshi SR, Duschl A, Lopata AL. 2013. Interaction of nanoparticles with proteins: relation to bioreactivity of the nanoparticle. *J. Nanobiotechnol.* 11(1):26
53. Neagu M, Piperigkou Z, Karamanou K, Engin AB, Docea AO, et al. 2017. Protein bio-corona: critical issue in immune nanotoxicology. *Arch. Toxicol.* 91(3):1031–48
54. Nel A, Xia T, Mädler L, Li N. 2006. Toxic potential of materials at the nanolevel. *Science* 311(5761):622–27
55. Cedervall T, Lynch I, Lindman S, Berggård T, Thulin E, et al. 2007. Understanding the nanoparticle-protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles. *PNAS* 104(7):2050–55
56. Linse S, Cabaleiro-Lago C, Xue W-F, Lynch I, Lindman S, et al. 2007. Nucleation of protein fibrillation by nanoparticles. *PNAS* 104(21):8691–96
57. Akhavan O, Ghaderi E. 2010. Toxicity of graphene and graphene oxide nanowalls against bacteria. *ACS Nano* 4(10):5731–36

58. Chen KL, Bothun GD. 2014. Nanoparticles meet cell membranes: probing nonspecific interactions using model membranes. *Environ. Sci. Technol.* 48(2):873–80
59. Leifert A, Pan Y, Kinkeldey A, Schiefer F, Setzler J, et al. 2013. Differential hERG ion channel activity of ultrasmall gold nanoparticles. *PNAS* 110(20):8004–9
60. Fu PP, Xia Q, Hwang H-M, Ray PC, Yu H. 2014. Mechanisms of nanotoxicity: generation of reactive oxygen species. *J. Food Drug Anal.* 22(1):64–75
61. Abdal Dayem A, Hossain MK, Lee SB, Kim K, Saha SK, et al. 2017. The role of reactive oxygen species (ROS) in the biological activities of metallic nanoparticles. *Int. J. Mol. Sci.* 18(1):120
62. Xia T, Kovochich M, Brant J, Hotze M, Sempf J, et al. 2006. Comparison of the abilities of ambient and manufactured nanoparticles to induce cellular toxicity according to an oxidative stress paradigm. *Nano Lett.* 6(8):1794–807
63. Zorov DB, Juhaszova M, Sollott SJ. 2014. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiol. Rev.* 94(3):909–50
64. Pelaz B, Alexiou C, Alvarez-Puebla RA, Alves F, Andrews AM, et al. 2017. Diverse applications of nanomedicine. *ACS Nano* 11(3):2313–81
65. McShan D, Ray PC, Yu H. 2014. Molecular toxicity mechanism of nanosilver. *J. Food Drug Anal.* 22(1):116–27
66. Tsoi KM, Dai Q, Alman BA, Chan WCW. 2013. Are quantum dots toxic? Exploring the discrepancy between cell culture and animal studies. *Acc. Chem. Res.* 46(3):662–71
67. Chen N, He Y, Su Y, Li X, Huang Q, et al. 2012. The cytotoxicity of cadmium-based quantum dots. *Biomaterials* 33(5):1238–44
68. Derfus AM, Chan WCW, Bhatia SN. 2004. Probing the cytotoxicity of semiconductor quantum dots. *Nano Lett.* 4(1):11–18
69. Hauck TS, Anderson RE, Fischer HC, Newbigging S, Chan WCW. 2010. In vivo quantum-dot toxicity assessment. *Small* 6(1):138–44
70. Nishanth RP, Jyotsna RG, Schlager JJ, Hussain SM, Reddanna P. 2011. Inflammatory responses of RAW 264.7 macrophages upon exposure to nanoparticles: role of ROS-NFκB signaling pathway. *Nanotoxicology* 5(4):502–16
71. Son Y, Cheong Y-K, Kim N-H, Chung H-T, Kang DG, Pae H-O. 2011. Mitogen-activated protein kinases and reactive oxygen species: How can ROS activate MAPK pathways? *J. Signal Transduct.* 2011:792639
72. Walter PL, Kampkötter A, Eckers A, Barthel A, Schmoll D, et al. 2006. Modulation of FoxO signaling in human hepatoma cells by exposure to copper or zinc ions. *Arch. Biochem. Biophys.* 454(2):107–13
73. Frame MC. 2002. Src in cancer: deregulation and consequences for cell behaviour. *Biochim. Biophys. Acta* 1602(2):114–30
74. Nyga A, Hart A, Tetley TD. 2015. Importance of the HIF pathway in cobalt nanoparticle-induced cytotoxicity and inflammation in human macrophages. *Nanotoxicology* 9(7):905–17
75. Weidemann A, Johnson RS. 2008. Biology of HIF-1α. *Cell Death Differ.* 15(4):621–27
76. Angelé-Martínez C, Nguyen KVT, Ameer FS, Anker JN, Brumaghim JL. 2017. Reactive oxygen species generation by copper(II) oxide nanoparticles determined by DNA damage assays and EPR spectroscopy. *Nanotoxicology* 11(2):278–88
77. Manke A, Wang L, Rojanasakul Y. 2013. Mechanisms of nanoparticle-induced oxidative stress and toxicity. *Biomed. Res. Int.* 2013:942916
78. Campisi L, Cummings RJ, Blander JM. 2014. Death-defining immune responses after apoptosis. *Am. J. Transplant.* 14(7):1488–98
79. Frank D, Vince JE. 2019. Pyroptosis versus necroptosis: similarities, differences, and crosstalk. *Cell Death Differ.* 26(1):99–114
80. Wang Q, Wang Y, Ding J, Wang C, Zhou X, et al. 2020. A bioorthogonal system reveals antitumour immune function of pyroptosis. *Nature* 579(7799):421–26
81. Bergström K, Holmberg K, Safranj A, Hoffman AS, Edgell MJ, et al. 1992. Reduction of fibrinogen adsorption on PEG-coated polystyrene surfaces. *J. Biomed. Mater. Res.* 26(6):779–90
82. Banerjee I, Pangule RC, Kane RS. 2011. Antifouling coatings: recent developments in the design of surfaces that prevent fouling by proteins, bacteria, and marine organisms. *Adv. Mater.* 23(6):690–718

83. Chanan-Khan A, Szebeni J, Savay S, Liebes L, Rafique NM, et al. 2003. Complement activation following first exposure to pegylated liposomal doxorubicin (Doxil): possible role in hypersensitivity reactions. *Ann. Oncol.* 14(9):1430–37
84. Ingen-Housz-Oro S, Pham-Ledard A, Brice P, Lebrun-Vignes B, Zehou O, et al. 2017. Immediate hypersensitivity reaction to pegylated liposomal doxorubicin: management and outcome in four patients. *Eur. J. Dermatol.* 27(3):271–74
85. Szebeni J, Simberg D, González-Fernández Á, Barenholz Y, Dobrovolskaia MA. 2018. Roadmap and strategy for overcoming infusion reactions to nanomedicines. *Nat. Nanotechnol.* 13(12):1100–8
86. Ishida T, Wang X, Shimizu T, Nawata K, Kiwada H. 2007. PEGylated liposomes elicit an anti-PEG IgM response in a T cell-independent manner. *J. Control. Release* 122(3):349–55
87. Wang X, Ishida T, Kiwada H. 2007. Anti-PEG IgM elicited by injection of liposomes is involved in the enhanced blood clearance of a subsequent dose of PEGylated liposomes. *J. Control. Release* 119(2):236–44
88. Shimizu T, Mima Y, Hashimoto Y, Ukawa M, Ando H, et al. 2015. Anti-PEG IgM and complement system are required for the association of second doses of PEGylated liposomes with splenic marginal zone B cells. *Immunobiology* 220(10):1151–60
89. Mohamed M, Abu Lila AS, Shimizu T, Alaaeldin E, Hussein A, et al. 2019. PEGylated liposomes: immunological responses. *Sci. Technol. Adv. Mater.* 20(1):710–24
90. Kozma GT, Mészáros T, Vashegyi I, Fülöp T, Örfi E, et al. 2019. Pseudo-anaphylaxis to polyethylene glycol (PEG)-coated liposomes: roles of anti-PEG IgM and complement activation in a porcine model of human infusion reactions. *ACS Nano* 13(8):9315–24
91. Chen F, Wang G, Griffin JI, Brenneman B, Banda NK, et al. 2017. Complement proteins bind to nanoparticle protein corona and undergo dynamic exchange in vivo. *Nat. Nanotechnol.* 12(4):387–93
92. Povsic TJ, Lawrence MG, Lincoff AM, Mehran R, Rusconi CP, et al. 2016. Pre-existing anti-PEG antibodies are associated with severe immediate allergic reactions to pegnivacogin, a PEGylated aptamer. *J. Allergy Clin. Immunol.* 138(6):1712–15
93. Ganson NJ, Povsic TJ, Sullenger BA, Alexander JH, Zelenkofske SL, et al. 2016. Pre-existing anti-polyethylene glycol antibody linked to first-exposure allergic reactions to pegnivacogin, a PEGylated RNA aptamer. *J. Allergy Clin. Immunol.* 137(5):1610–13.e7
94. Finkelman FD. 2007. Anaphylaxis: lessons from mouse models. *J. Allergy Clin. Immunol.* 120(3):506–15
95. Zhang P, Sun F, Liu S, Jiang S. 2016. Anti-PEG antibodies in the clinic: current issues and beyond PEGylation. *J. Control. Release* 244:184–93
96. Yang Q, Jacobs TM, McCallen JD, Moore DT, Huckaby JT, et al. 2016. Analysis of pre-existing IgG and IgM antibodies against polyethylene glycol (PEG) in the general population. *Anal. Chem.* 88(23):11804–12
97. DeAngelis PL. 2015. Heparosan, a promising 'naturally good' polymeric conjugating vehicle for delivery of injectable therapeutics. *Expert Opin. Drug Deliv.* 12(3):349–52
98. Chen E, Chen B-M, Su Y-C, Chang Y-C, Cheng T-L, et al. 2020. Premature drug release from polyethylene glycol (PEG)-coated liposomal doxorubicin *via* formation of the membrane attack complex. *ACS Nano* 14(7):7808–22
99. Yang Q, Lai SK. 2015. Anti-PEG immunity: emergence, characteristics, and unaddressed questions. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* 7(5):655–77
100. Afantitis A, Melagraki G, Isigonis P, Tsoumanis A, Varsou DD, et al. 2020. NanoSolveIT Project: driving nanoinformatics research to develop innovative and integrated tools for in silico nanosafety assessment. *Comput. Struct. Biotechnol. J.* 18:583–602
101. Furxhi I, Murphy F, Mullins M, Poland CA. 2019. Machine learning prediction of nanoparticle in vitro toxicity: a comparative study of classifiers and ensemble-classifiers using the Copeland Index. *Toxicol. Lett.* 312:157–66
102. Oh E, Liu R, Nel A, Gemill KB, Bilal M, et al. 2016. Meta-analysis of cellular toxicity for cadmium-containing quantum dots. *Nat. Nanotechnol.* 11(5):479–86
103. Kolanjiyil AV, Kleinstreuer C, Kleinstreuer NC, Pham W, Sadikot RT. 2019. Mice-to-men comparison of inhaled drug-aerosol deposition and clearance. *Respir. Physiol. Neurobiol.* 260:82–94
104. Ha MK, Trinh TX, Choi JS, Maulina D, Byun HG, Yoon TH. 2018. Toxicity classification of oxide nanomaterials: effects of data gap filling and PChem score-based screening approaches. *Sci. Rep.* 8(1):3141

105. Leong HS, Butler KS, Brinker CJ, Azzawi M, Conlan S, et al. 2019. On the issue of transparency and reproducibility in nanomedicine. *Nat. Nanotechnol.* 14(7):629–35
106. Eftekhari A, Dizaj SM, Chodari L, Sunar S, Hasanzadeh A, et al. 2018. The promising future of nano-antioxidant therapy against environmental pollutants induced-toxicities. *Biomed. Pharmacother.* 103:1018–27
107. Buchman JT, Hudson-Smith NV, Landy KM, Haynes CL. 2019. Understanding nanoparticle toxicity mechanisms to inform redesign strategies to reduce environmental impact. *Acc. Chem. Res.* 52(6):1632–42
108. Wilhelm S, Kaiser M, Würth C, Heiland J, Carrillo-Carrion C, et al. 2015. Water dispersible upconverting nanoparticles: effects of surface modification on their luminescence and colloidal stability. *Nanoscale* 7(4):1403–10
109. Wilhelm S. 2017. Perspectives for upconverting nanoparticles. *ACS Nano* 11(11):10644–53
110. Wilhelm S, Hirsch T, Patterson WM, Scheucher E, Mayr T, Wolfbeis OS. 2013. Multicolor upconversion nanoparticles for protein conjugation. *Theranostics* 3(4):239–48
111. Rao L, Meng Q-F, Bu L-L, Cai B, Huang Q, et al. 2017. Erythrocyte membrane-coated upconversion nanoparticles with minimal protein adsorption for enhanced tumor imaging. *ACS Appl. Mater. Interfaces* 9(3):2159–68
112. Dolez PI, Bodila N, Lara J, Truchon G. 2009. Personal protective equipment against nanoparticles. *Int. J. Nanotechnol.* 7(1):99–117
113. Nat. Nanotechnol. Ed. Board. 2020. The risks of nanomaterial risk assessment. *Nat. Nanotechnol.* 15:163
114. Hansen SF, Lennquist A. 2020. Carbon nanotubes added to the SIN List as a nanomaterial of Very High Concern. *Nat. Nanotechnol.* 15(1):3–4
115. Fadeel B, Kostarelos K. 2020. Grouping all carbon nanotubes into a single substance category is scientifically unjustified. *Nat. Nanotechnol.* 15(3):164
116. Heller DA, Jena PV, Pasquali M, Kostarelos K, Delogu LG, et al. 2020. Banning carbon nanotubes would be scientifically unjustified and damaging to innovation. *Nat. Nanotechnol.* 15:164–66
117. Shatkin JA. 2020. The future in nanosafety. *Nano Lett.* 20(3):1479–80
118. Fadeel B. 2019. The right stuff: on the future of nanotoxicology. *Front. Toxicol.* 1:1
119. Faria M, Björnmalm M, Thurecht KJ, Kent SJ, Parton RG, et al. 2018. Minimum information reporting in bio-nano experimental literature. *Nat. Nanotechnol.* 13(9):777–85
120. Schrand AM, Rahman MF, Hussain SM, Schlager JJ, Smith DA, Syed AF. 2010. Metal-based nanoparticles and their toxicity assessment. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* 2(5):544–68
121. Nel A, Xia T, Meng H, Wang X, Lin S, et al. 2013. Nanomaterial toxicity testing in the 21st century: use of a predictive toxicological approach and high-throughput screening. *Acc. Chem. Res.* 46(3):607–21
122. Watson C, Ge J, Cohen J, Pyrgiotakis G, Engelward BP, Demokritou P. 2014. High-throughput screening platform for engineered nanoparticle-mediated genotoxicity using CometChip technology. *ACS Nano* 8(3):2118–33
123. De Simone U, Roccio M, Gribaldo L, Spinillo A, Caloni F, Coccini T. 2018. Human 3D cultures as models for evaluating magnetic nanoparticle CNS cytotoxicity after short- and repeated long-term exposure. *Int. J. Mol. Sci.* 19(7):1993
124. Steger-Hartmann T, Raschke M. 2020. Translating in vitro to in vivo and animal to human. *Curr. Opin. Toxicol.* 23–24:6–10
125. Zink D, Chuah JKC, Ying JY. 2020. Assessing toxicity with human cell-based in vitro methods. *Trends Mol. Med.* 26(6):570–82
126. Sindhwani S, Syed AM, Wilhelm S, Glancy DR, Chen YY, et al. 2016. Three-dimensional optical mapping of nanoparticle distribution in intact tissues. *ACS Nano* 10(5):5468–78
127. Sindhwani S, Syed AM, Wilhelm S, Chan WCW. 2017. Exploring passive clearing for 3D optical imaging of nanoparticles in intact tissues. *Bioconjug. Chem.* 28(1):253–59
128. Syed AM, Sindhwani S, Wilhelm S, Kingston BR, Lee DSW, et al. 2017. Three-dimensional imaging of transparent tissues via metal nanoparticle labeling. *J. Am. Chem. Soc.* 139(29):9961–71
129. Syed AM, MacMillan P, Ngai J, Wilhelm S, Sindhwani S, et al. 2020. Liposome imaging in optically cleared tissues. *Nano Lett.* 20(2):1362–69
130. Merrifield RC, Stephan C, Lead JR. 2018. Quantification of Au nanoparticle biouptake and distribution to freshwater algae using single cell–ICP-MS. *Environ. Sci. Technol.* 52(4):2271–77

131. Wilhelm S, Bensen RC, Kothapalli NR, Burgett AWG, Merrifield R, Stephan C. 2018. *Quantification of gold nanoparticle uptake into cancer cells using single cell ICP-MS*. Appl. Note, PerkinElmer, Waltham, MA
132. Fang RH, Kroll AV, Gao W, Zhang L. 2018. Cell membrane coating nanotechnology. *Adv. Mater.* 30(23):e1706759
133. Golabchi A, Wu B, Cao B, Bettinger CJ, Cui XT. 2019. Zwitterionic polymer/polydopamine coating reduce acute inflammatory tissue responses to neural implants. *Biomaterials* 225:119519
134. Kim K, Choi H, Choi ES, Park M-H, Ryu J-H. 2019. Hyaluronic acid-coated nanomedicine for targeted cancer therapy. *Pharmaceutics* 11(7):301
135. Zhang T, Zhou S, Hu L, Peng B, Liu Y, et al. 2016. Polysialic acid-modifying liposomes for efficient delivery of epirubicin, in-vitro characterization and in-vivo evaluation. *Int. J. Pharm.* 515(1):449–59
136. Lane RS, Haller FM, Chavaroche AAE, Almond A, DeAngelis PL. 2017. Heparosan-coated liposomes for drug delivery. *Glycobiology* 27(11):1062–74
137. Lazarovits J, Chen YY, Song F, Ngo W, Tavares AJ, et al. 2019. Synthesis of patient-specific nanomaterials. *Nano Lett.* 19(1):116–23
138. Akaighe N, Maccuspie RI, Navarro DA, Aga DS, Banerjee S, et al. 2011. Humic acid-induced silver nanoparticle formation under environmentally relevant conditions. *Environ. Sci. Technol.* 45(9):3895–901
139. Glover RD, Miller JM, Hutchison JE. 2011. Generation of metal nanoparticles from silver and copper objects: nanoparticle dynamics on surfaces and potential sources of nanoparticles in the environment. *ACS Nano* 5(11):8950–57
140. Zheng Y, Lee PW, Wang C, Thomas LD, Stewart PL, et al. 2019. Freeze-drying to produce efficacious CPMV virus-like particles. *Nano Lett.* 19(3):2099–105
141. Guo B, Zebda R, Drake SJ, Sayes CM. 2009. Synergistic effect of co-exposure to carbon black and Fe2O3 nanoparticles on oxidative stress in cultured lung epithelial cells. *Part. Fibre Toxicol.* 6:4
142. Andriyanov AV, Koren E, Barenholz Y, Goldberg SN. 2014. Therapeutic efficacy of combining pegylated liposomal doxorubicin and radiofrequency (RF) ablation: comparison between slow-drug-releasing, non-thermosensitive and fast-drug-releasing, thermosensitive nano-liposomes. *PLOS ONE* 9(5):e92555
143. Pankhurst Q, Hautot D, Khan N, Dobson J. 2008. Increased levels of magnetic iron compounds in Alzheimer's disease. *J. Alzheimer's Dis.* 13(1):49–52
144. Khatri M, Bello D, Gaines P, Martin J, Pal AK, et al. 2013. Nanoparticles from photocopiers induce oxidative stress and upper respiratory tract inflammation in healthy volunteers. *Nanotoxicology* 7(5):1014–27
145. Aktepe N, Kocyigit A, Yukselten Y, Taskin A, Keskin C, Celik H. 2015. Increased DNA damage and oxidative stress among silver jewelry workers. *Biol. Trace Elem. Res.* 164(2):185–91
146. Munger MA, Radwanski P, Hadlock GC, Stoddard G, Shaaban A, et al. 2014. In vivo human time-exposure study of orally dosed commercial silver nanoparticles. *Nanomedicine* 10(1):1–9
147. Hussain SM, Hess KL, Gearhart JM, Geiss KT, Schlager JJ. 2005. In vitro toxicity of nanoparticles in BRL 3A rat liver cells. *Toxicol. In Vitro* 19(7):975–83
148. Lee J, Lilly GD, Doty RC, Podsiadlo P, Kotov NA. 2009. In vitro toxicity testing of nanoparticles in 3D cell culture. *Small* 5(10):1213–21
149. Kumar G, Degheidy H, Casey BJ, Goering PL. 2015. Flow cytometry evaluation of in vitro cellular necrosis and apoptosis induced by silver nanoparticles. *Food Chem. Toxicol.* 85:45–51
150. Lewinski N, Colvin V, Drezek R. 2008. Cytotoxicity of nanoparticles. *Small* 4(1):26–49
151. Trouiller B, Reliene R, Westbrook A, Solaimani P, Schiestl RH. 2009. Titanium dioxide nanoparticles induce DNA damage and genetic instability in vivo in mice. *Cancer Res.* 69(22):8784–89
152. Evans BC, Nelson CE, Yu SS, Beavers KR, Kim AJ, et al. 2013. Ex vivo red blood cell hemolysis assay for the evaluation of pH-responsive endosomolytic agents for cytosolic delivery of biomacromolecular drugs. *J. Vis. Exp.* 73:50166
153. Elsabahy M, Wooley KL. 2013. Cytokines as biomarkers of nanoparticle immunotoxicity. *Chem. Soc. Rev.* 42(12):5552–76
154. Kirchner C, Liedl T, Kudera S, Pellegrino T, Muñoz Javier A, et al. 2005. Cytotoxicity of colloidal CdSe and CdSe/ZnS nanoparticles. *Nano Lett.* 5(2):331–38

155. Wörle-Knirsch JM, Pulskamp K, Krug HF. 2006. Oops they did it again! Carbon nanotubes hoax scientists in viability assays. *Nano Lett*. 6(6):1261–68
156. Lynch I, Dawson KA, Linse S. 2006. Detecting cryptic epitopes created by nanoparticles. *Sci. STKE* 2006(327):pe14
157. Zhao J, Riediker M. 2014. Detecting the oxidative reactivity of nanoparticles: a new protocol for reducing artifacts. *J. Nanopart. Res.* 16(7):2493
158. Balke J, Volz P, Neumann F, Brodwolf R, Wolf A, et al. 2018. Visualizing oxidative cellular stress induced by nanoparticles in the subcytotoxic range using fluorescence lifetime imaging. *Small* 14(23):e1800310
159. Rajkumar KS, Kanipandian N, Thirumurugan R. 2016. Toxicity assessment on haemotology, biochemical and histopathological alterations of silver nanoparticles-exposed freshwater fish *Labeo rohita*. *Appl. Nanosci*. 6(1):19–29
160. Yang Y, Qin Z, Zeng W, Yang T, Cao Y, et al. 2017. Toxicity assessment of nanoparticles in various systems and organs. *Nanotechnol. Rev.* 6(3):279–89
161. Hoshyar N, Gray S, Han H, Bao G. 2016. The effect of nanoparticle size on in vivo pharmacokinetics and cellular interaction. *Nanomedicine* 11(6):673–92
162. Bobo D, Robinson KJ, Islam J, Thurecht KJ, Corrie SR. 2016. Nanoparticle-based medicines: a review of FDA-approved materials and clinical trials to date. *Pharm. Res.* 33(10):2373–87