Toxicity of Engineered Metal Oxide Nanomaterials Mediated by Nano-Bio-Eco-Interactions: A Review and Perspective

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Nano impact statement

The behavior and fate of metal oxide nanomaterials (MONMs) are influenced by the dynamic interactions among different compartments in the natural environments. Thus, understanding the interactions at the nano-bio-eco interface is necessary for selecting and designing MONMs with minimum adverse impacts. This paper provides a comprehensive review of the recent experimental and theoretical studies on the toxicity of MONMs mediated by two-way or three-way interactions. In the Perspectives, we also call for more open collaborations between industry, academia, and research labs to facilitate nanotoxicological studies focused specifically on interactions at the nano-bio-eco interface, leading to safe and effective nanotechnology for commercial, environmental, and medicinal use.

Toxicity of Engineered Metal Oxide Nanomaterials Mediated by Nano-Bio-Eco-Interactions: A Review and Perspective

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Abstract

Along with expanding use of engineered metal oxide nanomaterials (MONMs), there is growing concern over their unintentional adverse toxicological effects on human health and the environment upon release and exposure. It is inevitable that biota will be exposed to nanomaterials, through intentional administration or inadvertent contact under such circumstances. Therefore, a thorough investigation of the potential nanotoxicity of MONMs at the nano-bio-eco interface is urgently needed. In general, nanomaterials interact with their surrounding environments, biotic and abiotic, immediately upon introduction into the environment. The behavior and fate of MONMs are influenced by the dynamics of the environment. Thus, understanding the interactions at the nano-bio-eco interface is necessary for selecting and designing MONMs with minimum adverse impacts. Despite the limitations of currently available techniques, careful characterization of nanomaterials and the choosing of methodologies that promote further risk assessment promise more reliable and accurate data output. Conventional toxicological analysis techniques lack the power to handle the large datasets generated from *in vitro*/*in vivo* observations. This paper provides a comprehensive review of the recent experimental and theoretical studies on the toxicity of MONMs mediated by two-way or three-way interactions. In the Perspectives, we also call for more open collaborations between industry, academia, and research labs to facilitate nanotoxicological studies focused specifically on interactions at the nano-bio-eco interface, leading to safe and effective nanotechnology for commercial, environmental, and medicinal use.

Keywords: Metal oxide nanomaterials, Nano-bio-eco interface, Nanotoxicology

1. Introduction

Nanotechnology is one of the rapidly developing and important research fields in the 21st century. The commercial use of nanomaterials for various novel applications is increasing drastically. Increasing use of manufactured nanomaterials in commercial products and environmental applications has substantially advanced our tactics against barriers in environmental and biomedical practices.[1-3] Manufactured metal oxide nanomaterials (MONMs), the theme of this review, are among the most widely used types of engineered nanomaterials. The metal elements on the periodic chart are capable of forming a large diversity of oxide compounds. They can adopt a vast number of structural geometries with electronic structures that exhibit metallic, semiconductor or insulator characteristics.[4] Conservative market estimates for the production of MONMs in 2020 are 1,663,168 tons, rising from 270,041 tons in 2012.[5] In the long-run, it is anticipated that improvements in thermal, mechanical, and other physiochemical properties of engineered nanomaterials, including MONMs, will fundamentally change the way they are used and associated risk assessments must keep pace. Ensuring the safe use of engineered nanomaterials, requires conscious and continuing efforts to establish and adhere to protocols in production, application, and disposal of these synthetic chemicals. Precautions and early actions have to be taken by researchers and scientists, as well as regulatory authorities, in order to minimize the potential hazards and maximize the benefits to humans.

It is inevitable that, during their manufacture, use, and disposal, engineered MONMs will be released into natural environments. Their appearance in soils, water and air could harm both environmental biota and humans upon exposure. Considerably unknown risks associated with MONMs have raised concerns from both public and authorities. Although some currently reported data suggest that very low concentrations of MONMs present in natural environments does no significant harm to biota, there still exists a huge knowledge gap with regard to the physicochemical properties of MONMs and their impact on environmental and human health. As reviewed and suggested in the existing literature,[6-13] the overall life-cycle-associated environmental impacts of those synthetic chemicals have to be cautiously addressed. Much of the published literature suggests that upon interaction with surrounding environments (chemicals, bacteria, biological contaminants…etc) present in the environment physically and chemically, their behavior and fate can be drastically altered, leading to unpredictable outcomes; therefore, one should consider the dynamics of particular environments when making nanotoxicological

assessments. Indeed, increased care has been taken in assessing their physical and chemical alteration in various environments for comparison with their intrinsic properties. For these and other reasons, thorough characterization of MONMs, taking into account the conditions of the particular environment under study is essentially indispensable.

Safe handling and disposal of nanomaterials nowadays receives increasing attention from both public and governmental authorities.[14, 15] It has been recognized that, among the 30 industrialized countries of the Organization for Economic Co-operation and Development (OECD), the United States, England, Germany, European commission and Australia have developed good practices documents for the safety of manufactured nanomaterials.[16, 17] Additionally, under the regulation of Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)[18-20] and International Organization for Standardization (ISO)[21, 22], generic recommendations for the exposure assessment and risk characterization of nanomaterials were addressed.[23] However, there are still many organizations working with nanomaterials currently using conventional chemical safety methods through the life-cycle of nanomaterials, especially in the process of disposal. Disposal is closely related to environmental health once MONMs are released or discharged, and it eventually affects human health.

Notably, issues gaining increased attention are the establishment of toxicological profiles of engineered nanomaterials with regard to the nano-bio-eco interface, which entails the cataloging of interactions among nanomaterials, biotic, and abiotic environments. Physicochemical properties at the nano-scale afford those artificial nanomaterials to be high reactivity compared to conventional counterparts. Thus the bioavailability and toxicity of nanomaterials can be altered at the nano-bio-eco interface. Control over physicochemical properties of nanomaterials makes possible the design and application of novel nanomaterials in a green and sustainable way. We have long been interested in studying the nanotoxicity of nanomaterials, (metal oxide nanomaterials in particular) to the human and ecological environment, with the research involving biological and computational studies.[6, 8, 24-37] In this review article, we summarize the findings of studies that have shown MONMs to interact with their immediate environments at nano-bio-eco interface. The mechanisms of their toxicity are briefly discussed. In addition this review highlights currently advanced toxicological analysis techniques in quantitative or qualitative approaches. The correlation between nano-bio-eco interactions and nanotoxicology is then further discussed with an emphasis on quantitative structure-activity relationship (QSAR).

2. Nano-bio-eco interactions

2.1. Nano-eco interactions

The term nano-eco interactions here refers to the interactions at the environment–nanomaterial interface, particularly in aquatic ecosystems[8] and terrestrial environments[38]. Nano-eco interactions generally involve physicochemical interactions with abiotic environments (e.g., surfactants, dissolved organic matter or DOM[39]). It becomes clear that physicochemical properties of nanomaterials are very likely to be altered once they are released or discharged. First and foremost, the aggregation/agglomeration status of MONMs can be substantially affected by various factors, including pH, ionic strength, organic matter (DOM in aqueous columns), surfactants, temperature, and even clay content in soil matrices. This leads to the alteration of their behavior and fate in environments. The interactions between MONMs and colloids can be electrostatic/steric[40] or related to collision efficiency[41]. At any given pH, an increase in ionic strength can increase aggregation profoundly.[42] It has been suggested that the aggregation rate of TiO_2 nanoparticles within porous media can be quite comparable to that of deposition, at ratios of porous media surfaces (collectors) to nanoparticle surface areas as high as 40.[43] In addition, one report indicates that the transition from reaction to diffusion limited aggregation occurs at an electrophoretic mobility from around -2 to -0.8 $\mu m\ s^{-1}\ V^{-1}cm$.[41] Numerous researches have demonstrated that MONMs exist as aggregates/agglomerates in water [41, 44] and soil matrices.[45, 46] Moreover, aggregation/agglomeration status could not only determine the mobility of nanomaterials, but also largely influence their bioavailability.

Secondly, surface chemistry of MONMs can be changed upon release or discharge. High specific surface area of MONMs may result in strong adhesion to colloids, e.g., minerals and organic matter, in water and soil columns,[47] leading to the alteration of surface properties. For instance, surface adsorption of phosphate to CeO_2 nanoparticles leads to a significant reduction of nonequilibrium retention (Kr) values upon addition of phosphate to soils.[48] Similarly, Xu et al (2012) also observed that ZnO and CuO nanoparticles can bind various constituents, such as Na, Ca, P, and Cl, from biological environments to form ion corona, as shown in **Fig. 1**, with or without addition of biological environment.[49] Surface charge and charge density may also be altered by the addition of organic matter and by ionic strength. Generally, an increase in organic matter may result in a domination of the charge of organic matter at the surface of MONMs; and the increase of ionic strength can neutralize surface charge of MONMs, for instance, TiO_2, ZnO

and CeO_2.[41] The alteration of surface chemistry could in turn change the aggregation/agglomeration status of MONMs. More importantly, modifying a surface with organic matter may also affect the potential nanotoxicity by altering reactive oxygen species (ROS) production. It was suggested that humic acid (HA) accounts for the prevention of adhesion and inhibition of ROS generation, thus leading to reduced nanotoxicity.[50]

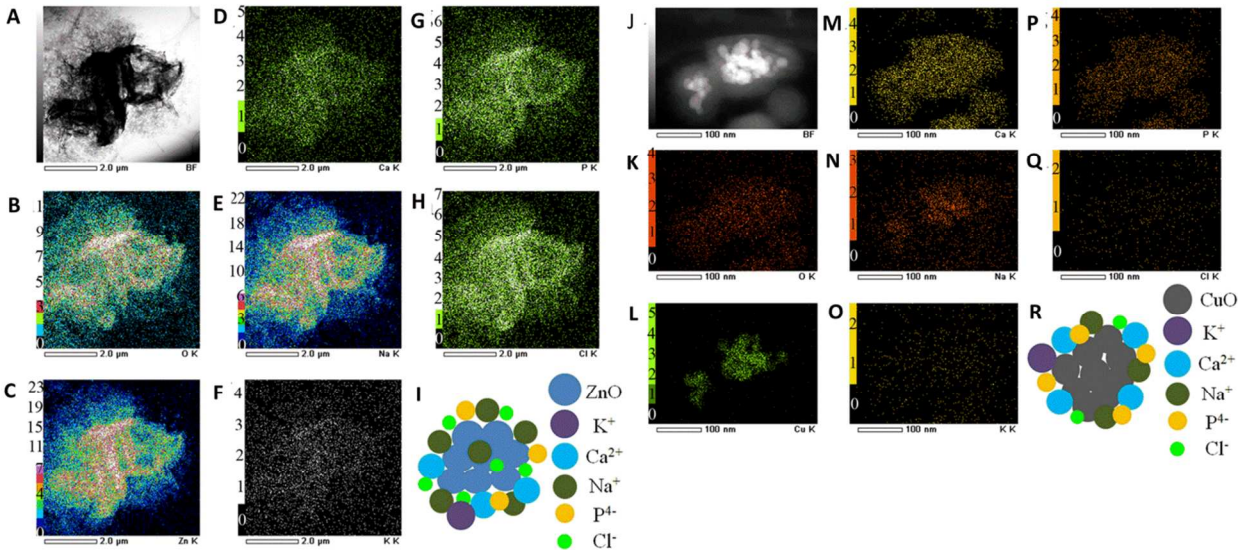

Fig. 1 Formation of ZnO nanoparticle complexes without fetal bovine serum (FBS) (A-I) and CuO nanoparticle complexes with FBS (J-R) in high-glucose Dulbecco's modified Eagle's medium (DMEM). TEM image (A) and dark-field TEM image (J) where the elemental maps were obtained; (B,K) TEM/EDS O-K map; (C,L) TEM/EDS Zn-K map; (D,M) TEM/EDS Ca-K map; (E,N) TEM/EDS Na-K map; (F,O) TEM/EDS K-K map; (G,P) TEM/EDS P-K map; (H,Q) TEM/EDS Cl-K map; (I,R) A simple model of ZnO bio-complexes. Permission obtained from *Sci. Rep.*, **2012**, *2,* 406.[49] Copyright Nature Publishing Group.

Additionally, metal ion dissolution can also be greatly affected by pH, ionic strength, and organic matter. It has been shown that the dissolution of ZnO nanoparticles is enhanced at both low and high pH,[51, 52] as well as high ionic strength[52]. However, natural organic matter either enhances or reduces ZnO dissolution, depending on their chemical composition and concentration.[51, 52] For instance, the presence of citric acid significantly enhanced the extent of Zn^{2+} release,[53] similar to the case that elevated Cu^{2+} release from CuO nanoparticles in the presence of Suwannee river fulvic acid.[54] Moreover, Zn^{2+} release can also be affected via ion trapping by organic matter complex, thereby resulting in decreased toxicity.[55]

Notably, in addition to chemical factors, physical factors such as sunlight, may trigger photocatalytic activity.[30, 56-58] Light irradiation may substantially affect the physicochemical properties of MONMs in various ways. Firstly, light irradiation may accelerate metal ion dissolution of MONMs. For instance, it is quite well known that the dissolution of ZnO NPs can be enhanced by UV or solar irradiation, which in turn leads to alteration of nanotoxicity.[59, 60] Secondly, crystallinity of MONMs can also be altered. It was reported that photo-induced phase transition (from anatase to rutile) of TiO_2 nanoparticles is initiated by intragap irradiation.[61] In addition, energy transition occurs within MONMs or their complexes upon the absorption of radiant energy. Photoinduced electron transfer in quantum dot-metal oxide nanoparticle junctions was also reported.[62, 63] It is also well known that light irradiation can initiate and enhance ROS formation in MONMs.[64]

2.2. Nano-bio interactions

In addition to nano-eco interactions, understanding the interaction of MONMs with macromolecules, tissues, and organs in biological systems *in vitro* and *in vivo* (**Fig. 2**) will permit us to design a safe nanomaterial for different application scenarios. Intuitively, nano-bio interactions often refer to biotic biomolecule–nanomaterial interaction.[8, 65] Nano-bio interactions are rather much more complex than those at the nano-eco interface. One should note that protein coronas play an important role in determining the behavior and fate of nanomaterials in biological environments. This particular phenomenon may result in denaturation of proteins, particle wrapping, and biocatalytic processes.[66-68] It should also be noted that nano-bio interactions may lead to phase transformations, free energy releases, particle aggregation, restructuring and dissolution at the nanomaterial surface.[69] Highly abundant proteins such as immunoglobulin G (IgG), fibrinogen, apolipoproteins, serum albumin, serotransferrin, prothrombin, alpha-fetoprotein, and kininogen-1 were commonly found on MONMs.[70, 71] The formation of nanomaterial-protein coronas is hydrodynamically associated with physicochemical properties of nanomaterials, involving different adsorption mechanisms, such as entropy-driven binding.[72] The thickness of protein coronas is related to various factors such as protein concentration, particle size, and surface properties of the particle.[73] It was reported that all tested MONMs, including Fe_3O_4, CoO, and CeO_2, form a hard protein corona through a dynamic process with different temporal patterns of the protein corona formation, suggesting a possible

fingerprint for nanoparticle identification.[74] Surface properties of MONMs profoundly affect the formation of protein coronas. It was suggested that surface coatings with negative and neutral surface charges adsorb more serum proteins than the positively charged ones, leading to a higher blood circulation time.[75] Similar results were also reported in a metallic nanoparticle-protein complex.[76] Thus the colloidal stability of MONMs can also be possibly altered along with the evolution of the protein corona.[70]

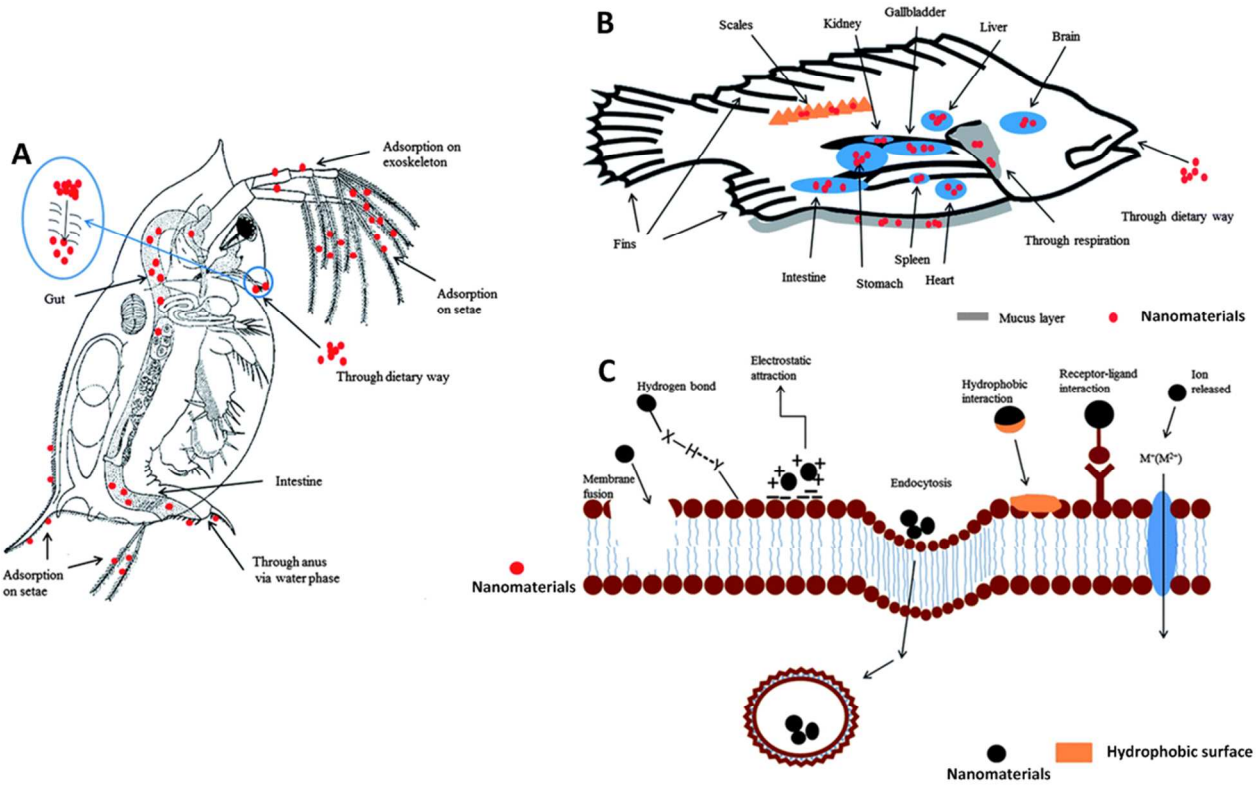

Fig. 2 Example of how nanomaterials interact with living organisms at nano–bio interface. Uptake and distributions of nanomaterials are illustrated in daphnia (A) and fish (B). The interactions between nanomaterials and cell membrane are illustrated in (C). Receptor–ligand interactions, hydrophobic interactions, electrostatic attractions and hydrogen bonds are often involved in the adsorption of nanomaterials onto cell membrane. Membrane fusion and endocytosis may occur during the internalization of nanomaterials. Metal ions released from dissolvable metal oxide nanomaterials can be transported into the cells via certain membrane channels. Permission obtained from *Environ. Sci.: Processes Impacts*, **2013**, *15*, 145-160.[77] Copyright Royal Society of Chemistry.

In addition, the nature and complexity of the protein coronas formed on the surface of MONMs can impact the distribution of nanomaterials in a biological system. Generally, there are two

types of protein coronas: soft and hard coronas. Nanomaterials that adsorb proteins with low affinity form soft protein coronas, and nanomaterials with tightly bound proteins form hard protein coronas. Thus, it is expected that hard coronas can directly interact with nanomaterials, whereas soft coronas interact with nanomaterials through protein-protein interactions with hard coronas. As we discussed above, surface coating normally allows the formation of soft coronas with weak coronal covering.[78] It was also observed that the tightly bound proteins occur only on MONMs with negatively charged surfaces after the strong protein elution.[75]

The interaction between nanomaterials and lipids is also critical in determining their behavior and fate in biological systems. It was found that the entrapment of superparamagnetic maghemite nanoparticles (γ-Fe_2O_3) in lipid bilayers reduces the lipid transition temperature and increases the membrane fluidity of all three types of lipid vesicles, including 1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine (SOPC), 1-stearoyl-2-oleoyl-snglycero-3-phosphocholine and 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine (SOPC-POPS).[79] They suggested that the negatively charged SOPC-POPS mixture is more predominant in this process due to high density encapsulation of nanoparticles via electrostatic interaction with positively charged γ-Fe_2O_3 nanoparticles.[79] Besides, the interaction of nanomaterials with nucleic acid has also been studied. Recently, Magro et al (2015) reported that γ-Fe_2O_3 nanoparticles interact chemically and electrically with DNA by direct covalent binding.[80] Reversible electron transfer at the interface betweenγ-Fe_2O_3 nanoparticles and DNA, as well as the generation of holes on the DNA bases, were observed. The interaction may be affected by nucleic acid length, presence of terminal phosphates, and types of DNA (dsDNA and ssDNA).[81]

2.3. Nano-bio-eco interactions

Studies on nano-bio interactions or nano-eco interactions have been relatively well covered. However, by comparison nano-bio-eco interactions remain as a critical topic worthy of additional research in the area of nanotoxicology.[31, 82-85] One should not ignore the fact that nano-bio and nano-eco interactions are often intertwined. For instance, ionic strength can affect protein corona formation on SPIONs.[75] Characterizing the nano-bio-eco interactions requires understanding of biotic and abiotic dynamics of the surrounding environments and their interaction with nanomaterials (see **Fig. 3**). The dynamic microenvironments could result in uncertainty to the fate and behavior of nanomaterials at the cellular level. For example, the

simultaneous interactions among a nanomaterial, ingredients of the abiotic environment (e.g. DOM, solar irradiation[8]), and a component of the target biota in a suspension medium can greatly modify the environmental fate of the nanomaterial and nanotoxicological response of the cellular system as the results of alteration of physicochemical properties of the biological system and the nanomaterial at the boundaries of the suspension. In addition, the quantitative characterization of such nano-bio-eco interactions could foster the development of better MONMs with low hazardous risk,[8, 29, 69, 86] and ultimately, enable their wider applications in medicine, commercial products, and environmental protection. It is noteworthy that while statistical analysis of studies at nano-bio-eco interface can mathematically indicate interactions between or among factorially-arranged biological and abiotic variables in an ecosystem experiment, interpretation of those interactions in an ecological sense is not an easy task.[31] Series of more carefully controlled experiments will be needed to reveal the separate nano-bio-eco interactions.

Besides the above mentioned issues, nanotoxicologists also face technical challenges while conducting ecotoxicity tests of engineered nanomaterials. The challenges include the transformations of studied engineered materials in environmental test media (eg., aggregation, dissolution, and other interaction of small molecules) and modes of nanomaterial interference (eg., adsorption to the test assay components and generation ROS).[83, 85] Therefore, combined knowledge and skills in the areas of physics, chemistry, and biology of nanomaterials are needed for improving the accuracy of the future toxicological assessments.

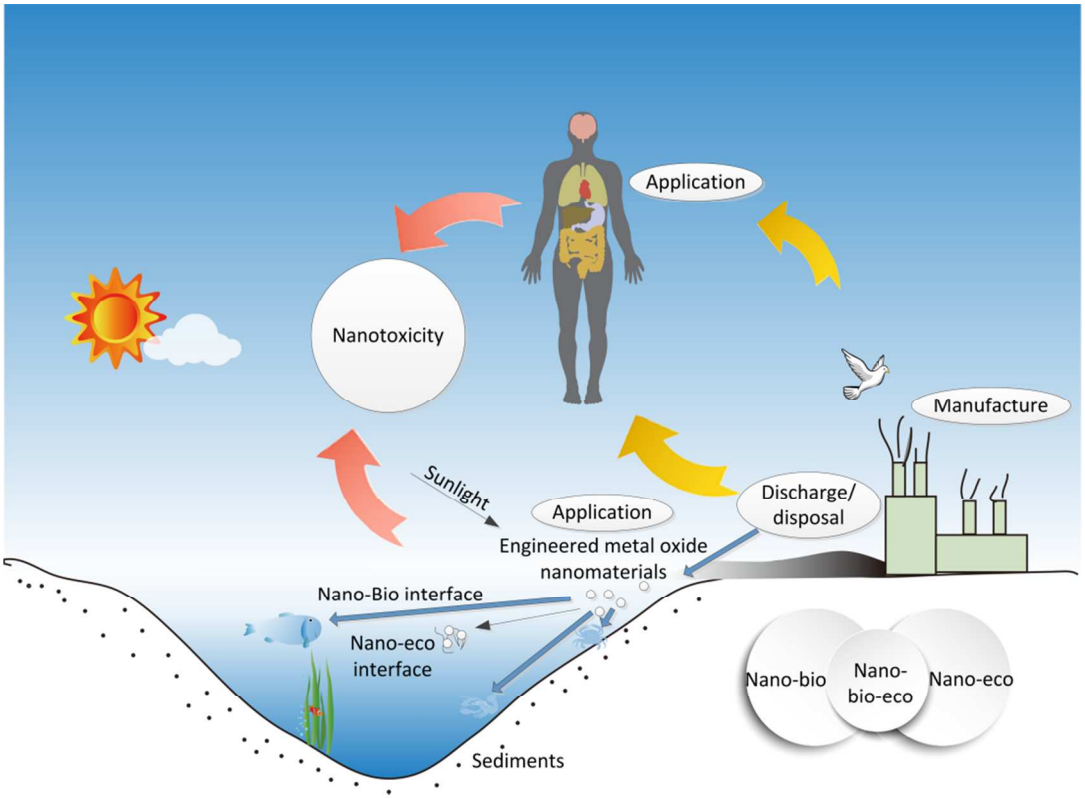

Fig. 3 Illustration for three-way nano-bio-eco interactions during the manufacture, use, and disposal of metal oxide nanomaterials.

3. Toxicity of engineered metal oxide nanomaterials mediated by nano-bio-eco-interactions

3.1. Mechanisms of nanotoxicity

Overproduction of ROS and the consequent production of oxidative stress induced by nanomaterials is a predominant mechanism leading to nanotoxicity.[87] It has been reported that oxidative DNA damage is associated with mutagenesis, carcinogenesis, and aging-related diseases in humans.[88, 89]

The level of ROS generation induced by nanoparticles is dependent on the physical and chemical nature of the nanoparticles and the surrounding environment and may proceed through different mechanisms.[87] The critical chemical and physical properties of engineered nanomaterials, including MONMS, that lead to the generation of ROS and nanotoxicity include molecular size, shape, oxidation status, surface area, bonded surface species, surface coating, solubility, and degree of aggregation and agglomeration.[88, 89]

It has been demonstrated that nanomaterials induce toxicity mediated by ROS in many biological systems, such as human erythrocytes and skin fibroblasts etc.[87] Apparently, nano-eco interactions occur. As aforementioned in the Section "Nano-eco interactions", engineered nanomaterials, including MONMS, involve physicochemical interactions with abiotic environments. All the above-described changes are the critical factors that lead to the generation of ROS, and thus afford nanotoxicity through nano-eco interactions. It becomes clear that nano-bio-eco interactions can easily mediate nanotoxicity.

Although there are reports claiming that there is no clear evidence of harm with regard to the current low discharge/release levels of nanomaterials (measured or measure-based predicted), it is well recognized that there is a knowledge gap in the behavior and fate of nanomaterials in dynamic environments that they may encounter.[90] Thus, their potential hazard to biological systems needs urgently to be understood and projected. In this context, nanotoxicology has been widely studied in recent years. Various mechanisms of nanotoxicity have been proposed and published. Oxidative stress via overproduction of ROS is regarded as one of the major underlying causes of cellular damage and death.[87] Other possible mechanisms include dissolution of metal ions,[91] physical damage via direct contact, etc. Internalization of nanomaterials in an organism may also lead to intracellular responses/alterations. Generally, all those factors do not act individually; instead, a combination of multiple factors may be involved in any process. For instance, increasing solution pH, HPO_4^{2-}, and DOM can reduce the availability and/or dissolution of Zn^{2+} from ZnO nanoparticles, thus reducing the nanotoxicity.[92] Below, we briefly illustrate principles that apply to studying nanotoxicology.

3.2. Approaches to studying nanotoxicology

3.2.1. Quantitative approaches

The continually expanding nanotechnology demands research methods with accuracy and reliability in a number of respects. The unique inherent characteristics of nanomaterials require a certain novelty of methodologies that may apply at the nano-scale, particularly in investigating nano-bio-eco interactions, such as biodistribution and internalization. In such scenario, paying prospectively rather than retrospectively will create superior incentives to develop novel approaches with high resolution, which in turn drives choice of methods powerfully. Firstly, there has been strong push for developing reliable methodologies in studying nanotoxicology at

the molecular level quantitatively for years. Traditionally, there are a large number of methods available that can be readily adapted to studying nanotoxicology. For example, assessing ROS formation by nanomaterials through various spectroscopic techniques, e.g. florescent and colorimetric-based methods, Raman spectroscopy,[93] and electron spin resonance (ESR) spectroscopy,[24, 94] has been successfully developed. The contents of MONMs in the whole organism or particular regions can also be quantitatively analyzed using inductively coupled plasma mass spectrometry (ICP-MS) , for instance, the content of TiO_2 in zebrafish (*Danio rerio*) embryos[28] and brain of CD-1(ICR) female mice.[95]

Recently, an integrative approach involving a microbeam mapping technique of Synchrotron Radiation X-Ray Fluorescence Analysis (SRXRF) was developed for studying the micro-distribution of TiO_2 nanoparticles.[95] Wang and co-workers highlighted this approach with an absolute detection limit of 10^{-12} to 10^{-15} g *in vivo*,[95] as shown in **Fig. 4**. Similarly, with a view to redesign safe nanoparticles, Vrainc et al (2013) reported an alternative method using innovative imaging flow cytometry in conjunction with confocal microscopy to identify the physico-chemical characteristics of SiO_2 nanoparticles involved in their uptake,[96] as shown in **Fig. 5**. However, quantitative approaches in evaluating the biodistribution of nanomaterials are still largely limited at the nano-scale.

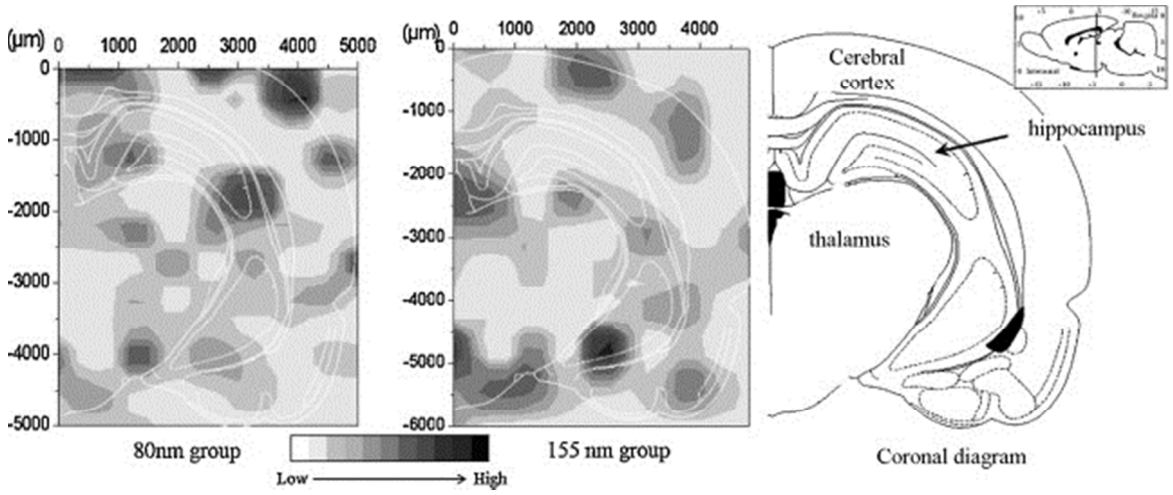

Fig. 4 SRXRF mapping of Ti-element distribution in the brain sections at 30 days after intranasal instillation of the different-sized TiO_2 particles[95]. Permission obtained from *Toxicol. Lett.*, **2008,** *183*(1-3), 72-80.[95] Copyright Elsevier B.V.

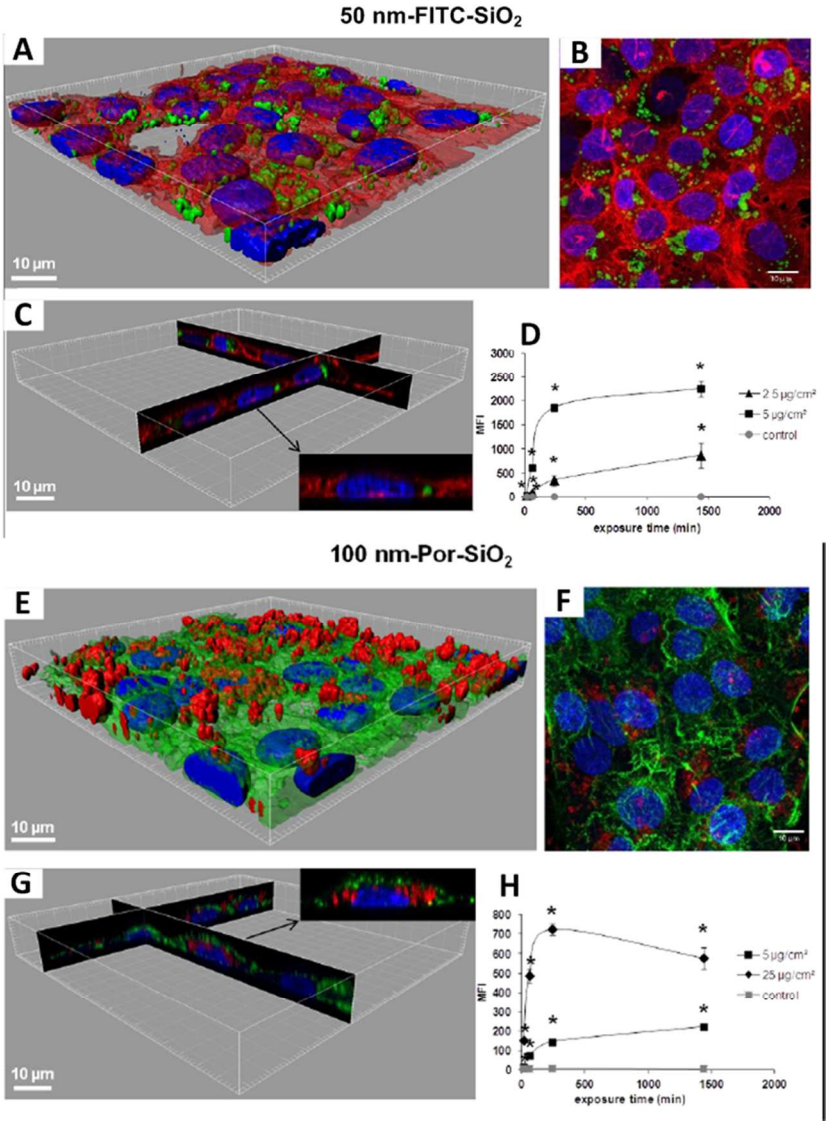

Fig. 5 Interaction of 50 nm-FITC-SiO_2 (A-D) and 100 nm-Por-SiO_2 (E-H) nanoparticles with Human lung adenocarcinoma (NCI-H292) cells. (A,E) 3D reconstruction of a confocal analysis of the cells exposed to SiO_2 NPs. Blue: DAPI-stained nuclei, Red: TRITC-phalloidin-stained actin filaments, Green: FITC/Por-labelled SiO_2 nanoparticles. (B,F). A projection of all images acquired in the stack of (A,E). (C,G). 3D reconstruction of x,z and y,z-slices of the corresponding regions on (A,E). The insert shows one selected representative cell. (D,H). Cells were exposed to nanoparticles, followed by flow cytometry (FCM) analysis of median fluorescence intensity (MFI). $*p < 0.05$, significantly different from previous time point. Permission obtained from *Part. Fibre Toxicol.*, **2013,** *10*(1), 2.[96] Copyright BioMed Central Ltd.

A single-nanoparticle detection method involving single-nanoparticle plasmonic microscopy and spectroscopy (dark-field optical microscopy and spectroscopy, DFOMS) and ultrasensitive *in*

vivo assay (cleavage-stage zebrafish embryos, critical aquatic species) has been developed to study transport and toxicity of single silver[97] and single gold[98] nanoparticles on embryonic developments. This technique may also be used for MONMs. Li et al (2012) successfully developed metal oxide nanoparticle-enhanced Raman scattering (MONERS) that can be applied for direct tracking and understanding of nano-bio-eco interactions at the single nanoparticle level.[99] They monitored the photocatalytic decomposition of methylene blue (MB) by TiO_2 NPs (P25, Degussa) using MONERS, suggesting its capability of direct molecular observation and understanding of chemical processes at a metal oxide interface. Notably, recent advances in hyperspectral microscopy with enhanced dark-field optical microscopy and hyperspectral imaging (HSI) enable the rapid identification of materials at the micro- and nanoscales with a detection limit of 10-15 nm for MONMs.[100-103] For instance, **Fig. 6** shows the identification of TiO_2 naoparticles in lung tissues. The emerging hyperspectral microscopy exhibits great potential for assessing spatial distribution and spectral characteristics of MONMs in biological and environmental systems, facilitating studies on the fate and transformation of these particles in various environments.[101]

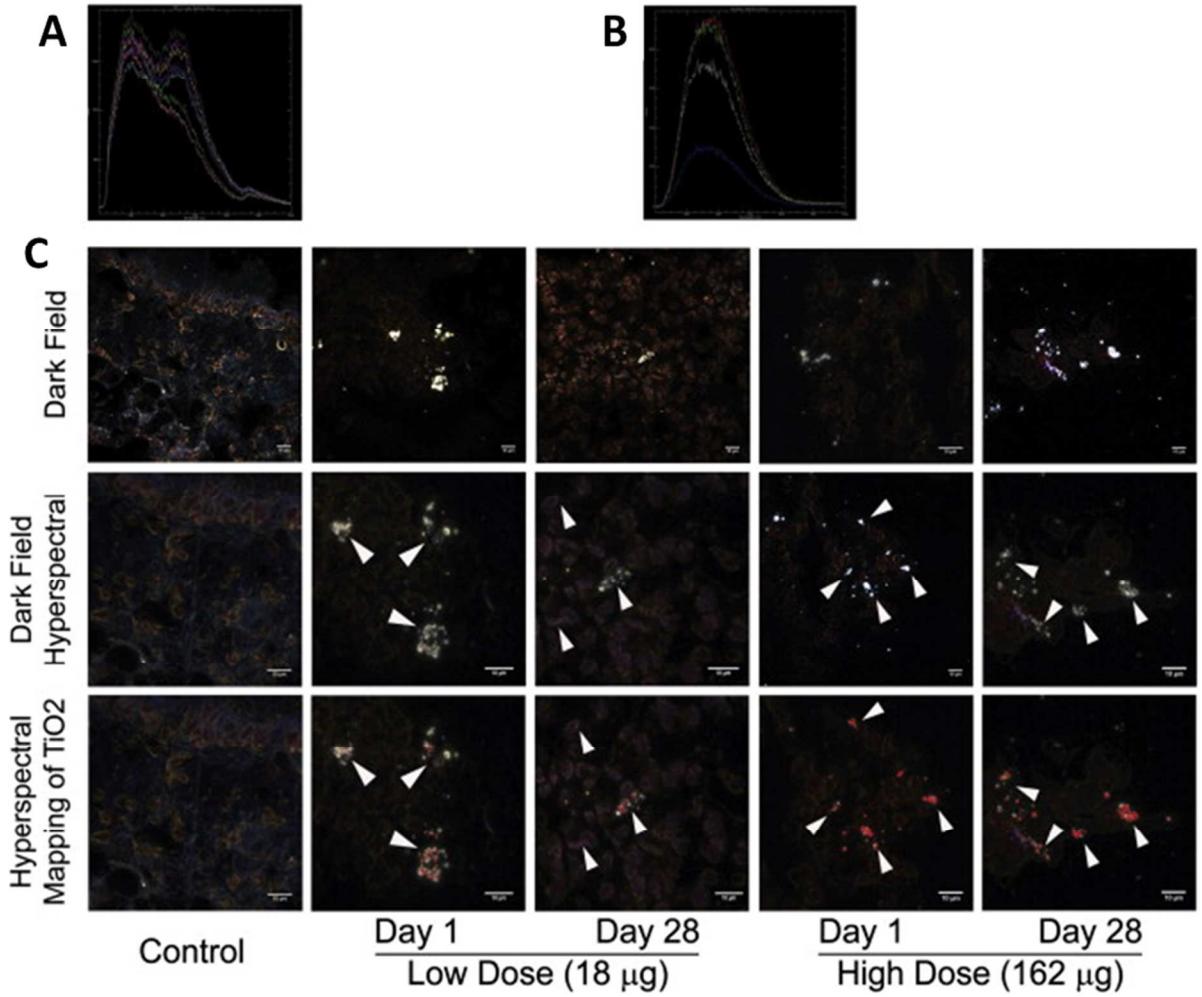

Fig. 6 Identification of TiO_2 naoparticles in lung tissues. (A) Reference spectral library from TiO_2 exposed tissue; (B) Reference spectral library from control tissue; and (C) Dark field images from nano-TiO_2 exposed tissues (upper panel). Dark field hyperspectral images from TiO_2 exposed tissues identifying these nanoparticles, which appeared as aggregates of white inclusions (middle panel). Hyperspectral mapping of TiO_2 nanoparticles in these tissues appeared as red dots or aggregates (bottom panel). Permission obtained from *Toxicol. Appl. Pharmacol.*, **2013,** *269*(3), 250-262.[103] Copyright Elsevier B.V.

In addition, studying nanotoxicity at the single cell level is also critical in establishing toxicological profiles quantitatively. Some cell populations, not all of them, may be vulnerable to the exposure to nanomaterials. The cell cycle is also one of the crucial factors underlying nanotoxicity and hence nanotoxicology. In light of this knowledge, nanotoxicity assaying at the single-cell level has been proposed based on flow and scanning image cytometry.[104] Furthermore,

magnetophoresis, combining fluorescence based cytotoxicity assaying, is also in practice for assessing the viability and uptake of the single-cellular magnetic nanoparticles (MNPs) simultaneously.[105] Notably, the integration of a cell-on-a-chip (CoC) with a microfluidic system has also been proposed for nanotoxicity assessments at the single cell level.[106]

3.2.2. Qualitative approaches

Although quantitative approaches have much to offer for studying nanotoxicology at the nano-bio-eco interface, yet qualitative analysis is always regarded as a valuable complement for quantitative research. Recently, attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy is used to probe surface adsorption of ligands on nanomaterials at the liquid–solid interface as it relates to biological and environmental systems.[107] ATR-FTIR gives a great insight into recognition and speciation of ligands adsorbed on the surface of nanomaterials, providing a comprehensive view on their surface chemistry. In addition, it could also aid in identifying the points of interactions and modes of adsorption through interpreting FTIR spectra. In the case of citric acid adsorption on TiO_2 nanoparticles in aqueous suspension, ATR-FTIR was employed to analyze surface speciation at different pH values, revealing that the nature of adsorption sites and coordination mode is critical in surface speciation.[108] In their study, for instance, protonated organic acids with carboxylic acid groups shows a peak at 1721 cm^{-1} in solution at pH =4 due to the υ[C=O] mode. However, peaks are shifted to 1571 and 1391 cm^{-1} upon deprotonation owing to $\upsilon_{as}[COO^-]$) and $\upsilon_s[COO^-]$ modes respectively. Moreover, success with ATF-FTIR has also been made to study the photocatalytic peroxidation of *E. coli*, lipo-polysaccharide (LPS), phosphatidyl-ethanolcholine (PE), and peptidoglycan (PGN) of the *E. coli* membrane wall on TiO_2 porous films,[109] and the settlement of *Undaria pinnatifida* kelp spores on anatase TiO_2 film[110]. Overall, ATR-FTIR can provide usable information, in a qualitative way, on surface functionality, which may further imply surface charge, the aggregation and sedimentation behavior, cellular uptake, and ultimately toxicity.

Other instrumentation techniques such as fluorescence microscopy and TEM are also frequently used in fulfilling such needs. Fluorescence microscopy is highly sensitive to specific fluorescent dyes at certain excitation and emission wavelength. Also, TEM is one of the most efficient way to identify the internalization of nanomaterials *in vivo*/*in vitro*. For example, as shown in **Fig. 7**, ROS production and biodistribution of TiO_2 nanoparticles is revealed in zebrafish larva on a

daily basis.[28] Both techniques are highly visualizable, and can be further modified and improved for multiple purposes, particularly enabling semi-quantitative or quantitative analysis. Tai et al. (2012) reported that a microchip nanopipet with a narrow chamber width could be applied to TEM image-based quantitative characterization.[111] They successfully developed a nanopipet with a narrow chamber width for sorting nanoparticles from blood and preventing the aggregation of the particles during the preparation process, thus enabling quantitative analysis of their aggregation/agglomeration states and the particle concentration in aqueous solutions. Techniques such as confocal microscopy and flow cytometry can also be used to study particle uptake and subcellular localization in a semi-quantitative approach.[112]

Notably, the integration of the microscope and the Raman spectrometer now allows rapid and easy sample collection, preparation, and analysis in a qualitative way.[113] In addition, developing a reliable qualitative method may provide a prototype that can be advanced for quantitative use. For instance, both DFOMS and Hyperspectral Imaging System are developed on the basis of optical microscopy.

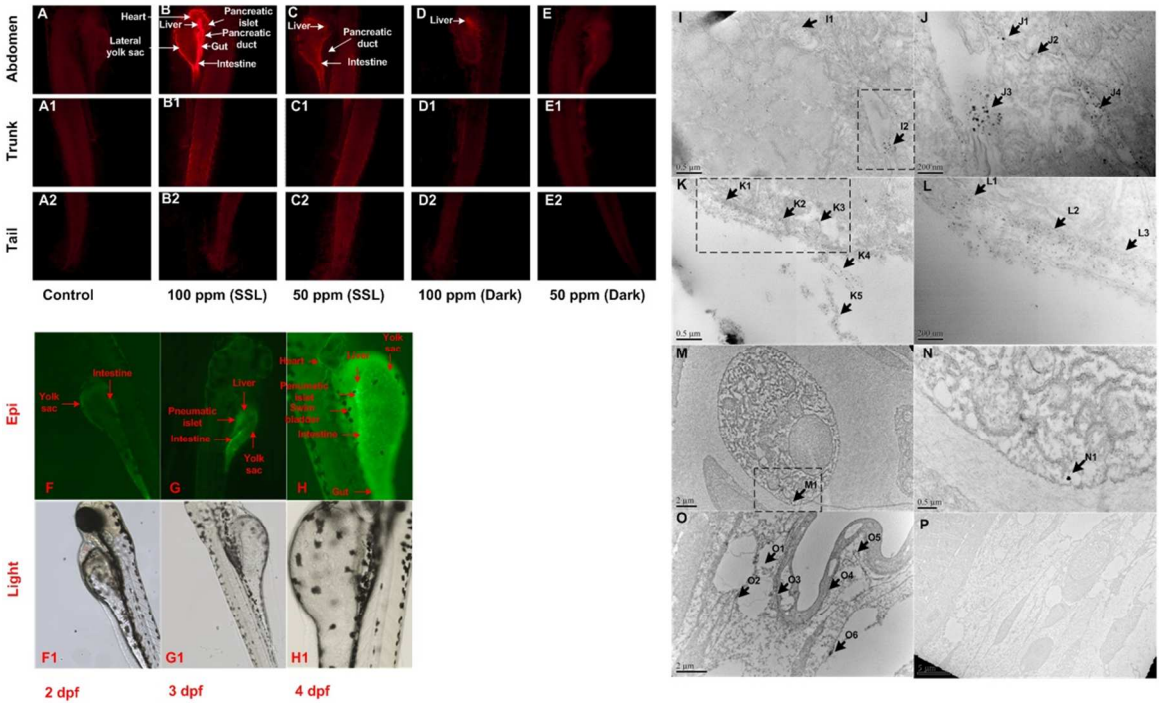

Fig. 7. Time-dependent biodistribution of TiO_2 nanoparticles and its ROS production in zebrafish larva (*Danio rerio*). (A-E): Dihydroethidium (DHE) detection of superoxide yield at 96 hpf. Epi-fluorescence (F-H) and light microscopy (F1-H1) images of FITC-S-TiO_2 treated zebrafish larvae at 2, 3, and 4 dpf. (I-P): TEM of S-TiO_2 NPs (100 ppm) treated embryos (120

hpf). Image (J) (L) and (N) are higher magnification images of NPs in the rectangular region of images (I) (K) and (M), respectively. Magnification for images: (I) 40 000 ×, (J) 100 000 ×, (K) 40 000 ×, (L) 100 000 ×, (M) 15 000 ×, (N) 30 000 ×, (O) 12 000 × and (P) 5000 ×. *hpf: hours post fertilization. FTIC: fluorescein isothiocyanate. Permission obtained from *Nanotoxicology*, 2014, 8(S1), 185-195.[28] Copyright Informa Plc.

3.3. Correlation between nano-bio-eco interactions and nanotoxicology

3.3.1. Experimental research

The understanding of nanomaterials and their interactions with surrounding environments, either biological or physical environments, and the subsequent toxicities largely relies on experimental researches. Data collected through *in vitro* and *in vivo* systems are most meaningful in unveiling possible pathways. They can also be used for validating the models built on theoretical/computational studies. Currently, the main emphases of experimental research are:

-to study the translocation/distribution of nanomaterials in biotic/abiotic systems;

-to study the exposure route to biota and humans;

-to identify and monitor the quantity of released nanomaterials in environments;

-and to study the relationship between physicochemical properties of nanomaterials and nanotoxicity with various biological endpoints.

It is prudent to characterize the physicochemical properties of nanomaterials thoroughly prior to any further studies.[114, 115] Indeed, measurements of particle characteristics in pure water may vary tremendously from that in cell culture media or water samples from lakes/rivers, etc. The alteration of their physicochemical properties tends to change their distribution and behavior in biotic/abiotic environments. Of course, the exposure route also matters most.[116, 117] The action mechanism and outcome may vary substantially with different routes. The exposure route often includes inhalation, direct contact (i.e. penetration through skin), and ingestion. Exposure may also occur in drug delivery and treatment. Meanwhile, the identification and monitoring of the release/discharge of nanomaterials is paramount in giving validation and credence to nanotoxicology in the long-run.[118, 119] Ultimately, our goal is to generate a group of computational models that can correlate and explain the relationship between physicochemical properties of nanomaterials and their toxicity, on the solid foundation of experimental researches.

3.3.2. Theoretical/computational study: QSAR

Theoretical/computational studies are increasingly involved in nanotoxicological researches, to circumvent the problems associated with field research, i.e., the limitations of resources, and reproducibility and reliability of first-hand data collected by experiments. Nowadays, a growing body of data shows the potential of QSAR as an alternative to interpret and model the physicochemical properties of nanomaterials and their toxicities.[120] Herein we mainly discuss the development of QSAR in nanotoxicology in this section. For other related computational modeling studies (e.g. relatively simple read-across, grouping and ranking), readers are encouraged to read [121], [122], [123], [124], [125], and [126]. QSAR modeling has shown its potential to relate the structural properties of nanomaterials with their experimentally measured biological endpoints quantitatively. As illustrated in Table 1, a large number of MONMs have been studied with various QSAR models involving a variety of biological systems,[36, 121, 127-133] including *E. coli*,[29, 36, 133-137] human keratinocyte cell line (HaCaT),[136, 137] human bronchial epithelial (BEAS-2B),[121, 128] murine myeloid (RAW 264.7) cells,[121] endothelial cells,[127, 131, 132, 138] smooth muscle cells,[127, 131, 132, 138] monocytes and hepatocytes,[127, 131, 132] human pancreatic cell lines (MIA PaCa-2),[127, 131] human lung fibroblasts,[139] human lung epithelial cells,[135, 140, 141] and L2 lung epithelial cells and lung alveolar macrophages[130]. In addition to cell lines, research has also been done on multiple bio-indicators with data extracted from published articles, including bacteria (*Vibrio fischeri*), crustaceans (*Daphnia magna* and *Thamnocephalus platyurus*), zebrafish *(Danio rerio)*, plant species (radish, rape, ryegrass, lettuce, corn, and cucumber), plant cucumber (*Cucumis sativus*), microalgae species (*Scenedesmus sp., Chlorella sp., Chlorella vulgaris,* and *Pseudokirchneriella subcapita*).[142]

Oftentimes, nanotoxicological profiles vary not only on biological models, but also on biological endpoints. Till now, across a wide range of biologic endpoints, a considerable number of studies have been conducted to evaluate the impact of MONM exposure with regard to QSAR modeling. The majority of laboratory studies on biological endpoints are mainly *in vitro* studies, including linear/log-linear regression models of EC_{50}/LC_{50} cytotoxicity,[29, 36, 133, 134, 136, 137] the concentration of nanoparticles leading to 50% reduction in cell viability (TC_{50}),[143] damage to cellular membranes (units L^{-1}) via lactate dehydrogenase (LDH) release,[130, 135, 140, 141] oxidative stress,[121] intracellular calcium flux,[121] mitochondrial membrane potential,[121, 132, 138] surface membrane permeability,[121] cytotoxic inhibition ratio with MTT assay,[139] cell apoptosis,[131, 132] ATP

content,[132, 138] apoptosis,[138] reducing equivalents,[132, 138] plasma membrane leakage,[128] and cell membrane damage via propidium iodide uptake[140, 144]. A single indicator may not be sufficient sometimes; therefore, multiple cell types, at multiple doses and with multiple endpoints may provide a more comprehensive view of the biological effects resulting from certain nanomaterials.[138] Although QSAR with *in vitro* studies can imply some correlations with *in vivo* observations, QSAR with a direct observation *in vivo* can further promote predicting nanotoxicity with high accuracy and reliability.

One should not ignore that sufficiently large nanotoxicity datasets can be rapidly acquired with the advance of High Throughput Screening (HTS) assay.[145] For instance, ten independent toxicity-related signaling pathways associated with murine macrophage cell line exposed to a library of MONMs can be readily obtained via HTS assay.[146] Later, those data can either be classified through the use of certain computational techniques, such as SOM,[147] or further analyzed via QSAR modeling[128]. Such hierarchical ranking and clustering of MONMs based on HTS basically provide an enormous *in vitro* profile network for further testing *in vivo*.[121, 138] In addition, HTS has shown promising potential for us to perform *in vivo* hazard risk assessment with high volume datasets.[148, 149] *In vivo* studies are generally considered to be more definitive regarding nanotoxicity assessment. This is typically valuable in facilitating the establishment and utilization of QSAR models in designing safe nanomaterials. Conventionally, obtaining valid scientific data is quite slow and somewhat objective in some cases. With the help of HTS assay, scientists and researchers can be relieved from intensive lab work and focus more on methods development and data analysis. There is a trend in academia for universities and institutions to apply this relatively novel technique in their researches.

Note that the rising popularity of QSAR modeling is essentially associated with a question over their reliable predictions. Thus, various modeling techniques have been acquired and applied in such context. Modeling techniques, such as decision tree forest (DTF) and decision treeboost (DTB),[133] multiple linear regression (MLR),[36, 131, 142, 150] naive Bayesian classifier (NBC) modeling,[132] self-organizing map (SOM),[132] Random Forest (RF) regression,[136] logistic regression (LR),[128] k-nearest neighbor (k-NN),[127] partial least square regression (PLSR),[150] support vector machines (SVM),[121, 127] ensemble learning (EL),[133] linear discriminant analyses (LDA),[130, 131, 142] sparse linear modeling and feature selection linear modeling,[131] and Bayesian regularized artificial neural network methods[131] have exhibited great potential in underlying the

quantitative relationships between the molecular structures and biological activities of MONMs. It is noteworthy that most of predictive outcomes generated by those modeling techniques are within acceptable range.

Not only a range of pristine MONMs have been involved in QSAR studies,[29, 36, 128, 133-137, 142, 150] but also surface functionalization of certain MONMs is also studied and reported[127, 131]. There are a large number of molecular, chemical and physical descriptors of those MONMs available in databases. Selection of proper descriptor is the most critical step in generating valid QSAR models with acceptable accuracy. Many descriptors can be obtained readily based on the molecular structure and atomic or group contributions, e.g., molecular weight, van der Waals, surface area, and size. Descriptors that relate to electronic structure, for instance, molar heat capacity, alpha and beta LUMO energies, and electronegativity, are available from quantum chemical calculations. Currently, simplified molecular input-line entry system (SMILES),[134, 135, 140, 150] density functional theory (DFT),[29, 36] "liquid drop" model (LDM) derived descriptors,[136] molecular operating environment (MOE),[127] and optimal descriptors[139] are the most frequently used databases for descriptor selection.

Table 1. Summary of Recent Progress in the Development of QSAR modeling for MONMs*

Tested MONMs	Modeling techniques	Descriptors	Description system	Correlation efficient (represented by (R^2, RMSE), if applicable)		Biological model	Reference
				Correlation (training set)	Correlation (validation set)		
17 metal oxide NPs	Monte Carlo	SMILES-based optimal descriptors	SMILES	R^2=0.90–0.94	R^2=0.73–0.98	*E. coli*	[135]
17 metal oxide NPs	DTB	oxygen percent, molar refractivity and polar surface area	Molecular descriptor	(0.955, 0.11)		*E. coli*	[133]
	DTF			(0.896, 0.19)			
17 metal oxide NPs	MLR	enthalpy of formation (ΔH_{Men+})	DFT	(0.85, 0.20)	(0.83, 0.19)	*E. coli*	[36]
17 metal oxide NPs	Monte Carlo	SMILES-based optimal descriptors	SMILES	R^2=0.83–0.96		*E. coli*	[134]
17 metal oxide NPs		absolute electronegativity	DFT	F=33.83, R^2=0.87 (dark exposure)		*E. coli*	[29]
		molar heat capacity and average of the alpha and beta LUMO energies		F=20.51, R^2=0.804 (photo exposure)			
18 metal oxides	RF regression	van der Waals interactions, electronegativity and metal–ligand binding characteristics	LDM-based descriptors	(0.96, 0.10)	(0.93,0.13)	HaCaT cells	[136]
				(0.92,0.12)	(0.78,0.32)	*E. coli*	
18 metal oxides	GA-MLR	ΔH_f^c: enthalpy of formation of metal oxide nanocluster representing a fragment of the surface and χ^c the Mulliken's electronegativity of the cluster	27 nano-descriptors including 16 quantum-mechanical descriptors and 11 image descriptors	R^2=0.93, RMSE=0.12		*E. coli* and HaCaT cells	[137]
TiO_2	MLR and LDA	engineered size, size in ultrapure water, size in PBS, and	General descriptor	With R^2 up to 0.77		rat L2 lung epithelial cells and rat lung alveolar	[130]

Tested MONMs	Modeling techniques	Descriptors	Description system	Correlation efficient (represented by (R^2, RMSE), if applicable)		Biological model	Reference
				Correlation (training set)	Correlation (validation set)		
		concentration in ultrapure water				macrophages	
ZnO		Engineered size, size in ultrapure water, size in PBS, and size in CCM		R^2= 0.94–0.99			
ZnO and TiO_2	Monte Carlo	optimal descriptor	squasi-SMILES	R^2=0.78-0.92	R^2=0.67-0.83	human lung epithelial cells	[135]
TiO_2	Monte Carlo	optimal descriptor	SMILES-based optimal descriptors	(0.9639, 0.049); (0.9893, 0.025); (0.9792, 0.049)	(0.9263, 0.123); (0.8959, 0.118); (0.9647, 0.066)	human lung epithelial cells	[141]
17 metal oxide NPs	MLR	metal electronegativity (χ), the charge of the metal cation corresponding to a given oxide (χ_{ox}), atomic number and valence electron number of the metal	SMILES	(0.81–0.90, 0.16–0.22)	(0.73–0.96, 0.15–0.26)	*E. coli*	[150]
	PLSR			(0.73–0.87, 0.19–0.27)	(0.70–0.96, 0.17–0.29)		
15 metal oxide nanoparticles	PM6 method	spherical size of nanoparticles and the weighted energy of the highest occupied molecular orbital	microscopic-image-based and theory-based (calculated) descriptors	R^2=0.82-0.94		NA	[151]
9 metal oxide nanoparticles	logistic regression	atomisation energy, period of the nanoparticle metal, primary size, and volume fraction	molecular, chemical and physical information and different concentrations	accuracy >95%		bronchial epithelial (BEAS-2B) cells	[128]
24 metal oxide nanoparticles	SVM	conduction band energy and ionic	an initial pool of 30 NP	Accuracy ~94% and confidence level of 80%		Human bronchial	[121]

Tested MONMs	Modeling techniques	Descriptors	Description system	Correlation efficient (represented by (R^2, RMSE), if applicable)		Biological model	Reference
				Correlation (training set)	Correlation (validation set)		
		index	descriptors			epithelial (BEAS-2B) and murine myeloid (RAW 264.7) cells	
24 metal oxide nanoparticles	Monte Carlo	optimal descriptors	SMILES	Best model with R^2= 0.8824, RMSE 0.214 for calibration set; and R^2= 0.7809, RMSE = 0.348 for validation set		human bronchial epithelial cells (BEAS-2B)	[140]
24 metal oxide nanoparticles	Markov Chain Monte Carlo (MCMC)	Conduction band energies, dissolution	General descriptor	NA		human bronchial epithelial cells (BEAS-2B)	[144]
SiO_2	Monte Carlo	mathematical functions of size and concentration	optimal descriptors	R^2= 0.9837, s = 2.53 %, F = 483	R^2= 0.9269, s = 7.94 %	human lung fibroblasts	[139]
70 metal oxide nanoparticles	NA	band energy	reactivity descriptors	accuracies of ca. 99% in both training and prediction sets		NA	[129]
41 nanoparticles with 6 metal oxide nanoparticles	Perturbation approach	molar volume, polarizability, size	molar volume (V), electronegativity (E), polarizability (P), and nanoparticle size (L)	accuracy > 93% for both training and prediction sets		15 mammalian cell lines, including A549 human cells	[143]
11 metal oxide and 7 metallic nanoparticles	MLR and LDA	Four types of 9-variable descriptors	molar volume (V), electronegativity (E), polarizability (P), and nanoparticle size (L)	accuracies of ca. 99% in both training and prediction sets		Multiple bio-indicators, including *D. magna*, *P. subcapitata*, *D. rerio*, etc	[142]
48 Fe_2O_3 and Fe_3O_4 metal	MLR, and sparse	relaxivities (R1 and R2) and the	a set of 691 molecular	training set R^2 = 0.81; test set regression coefficient R^2= 0.86;		endothelial and smooth	[131]

Tested MONMs	Modeling techniques	Descriptors	Description system	Correlation efficient (represented by (R^2, RMSE), if applicable)		Biological model	Reference
				Correlation (training set)	Correlation (validation set)		
oxide nanoparticles and 3 CdSe quantum dots) core with various coating combinations	linear modeling and feature selection, MLR-EM	zeta potential	descriptors	SEE = 3.6; and SEP = 3.3		muscle cells, monocytes, and hepatocytes	
	nonlinear Bayesian regularized artificial neural network methods			training set R^2 = 0.80; test set R^2 = 0.90; SEE = 2.8; and SEP = 2.9			
109 nanoparticles sharing a superparamagnetic core and dextran coating	linear	11 descriptors	derived from a set of 124 chemically interpretable descriptors	training set R^2 = 0.74; test set R^2 = 0.63; SEE = 0.34; and SEP = 0.36		significant variation in HUVEC	
	Non-linear			training set R^2 = 0.70; test set R^2= 0.66; SEE = 0.30; and SEP = 0.33			
	linear	19 descriptors		training set R^2 = 0.76; test set R^2 = 0.79; SEE = 0.19; and SEP = 0.24		significant variation in PaCa2 cells	
	Non-linear			training set R^2= 0.77; test set R^2= 0.54; SEE = 0.15; and SEP = 0.28			
109 nanoparticles sharing a superparamagnetic core and dextran coating	kNN QSAR models	Lipophilicity, van der Waals surface area, molecular refractivity, electrostatic descriptors	2-D MOE descriptors	coefficients of correlation R_{abs}^2 ranged from 0.65 to 0.80 for external sets		significant variation in PaCa2 cell line	[127]
48 Fe_2O_3 and Fe3O4 metal oxide nanoparticles and 3 CdSe quantum dots) core with various coating combinations	SVM	size, relaxivities, and zeta potential	molecular descriptors	external prediction accuracies of 56−88% for the five independent external validation sets, with the mean external accuracy of 73%		endothelial and smooth muscle cells, monocytes, and hepatocytes	
44 iron oxide core nanoparticles	H4 class definition and naive Bayesian classifier (NBC)	spin-lattice relaxivity and zeta potential	molecular descriptors	classification accuracy > 78%		aorta endothelial, vascular smooth muscle, hepatocyte,	[132]

Tested MONMs	Modeling techniques	Descriptors	Description system	Correlation efficient (represented by (R^2, RMSE), if applicable)		Biological model	Reference
				Correlation (training set)	Correlation (validation set)		
	model					and monocyte/macrophage	
	'hit' (i.e., significant bioactivity) identification analysis and SOM based consensus clustering	primary size, spin-lattice and spin-spin relaxivities, and zeta potentials					

*: Density functional theory (DFT), decision treeboost (DTB), decision tree forest (DTF), genetic algorithm- multiple linear regression (GA-MLR), Human Umbilical Vein Endothelial Cells (HUVEC), "liquid drop" model (LDM), multiple linear regression (MLR), Molecular Operating Environment (MOE), spin-lattice relaxivity (R1) and spin-spin relaxivity (R2), Random Forest (RF), root mean square error (RMSE), standard error of estimation (SEE), standard error of external prediction (SEP), simplified molecular input-line entry system (SMILES), self-organizing map (SOM), support vector machine (SVM).

Ultimately, the aim of the QSAR approach is to predict the toxicological behavior of nanomaterials at the nano-bio-eco interface. With the advance of computational technology, QSAR studies are likely to play a vital role in the design of novel nanomaterials on the basis of acceptable reliability and accuracy. Prediction performances of QSAR models have shown great value in fulfilling such needs. Successful implementation of QSAR can certainly facilitate current progress on nanotoxicology *in vitro* and *in vivo* with reasonable cost. Computational scientists in related disciplines can directly retrieve data from published literature, without being troubled by intensive lab work. Such "collaboration" could eventually not only benefit the research groups mutually, but also move the scientific community forward. One can envision that QSAR as a computational strategy can be a powerful tool in the future.

4. Perspective

It is clear that MONM behavior and fate at the nano-bio-eco interface is much more complicated than pristine ones regarding the way in which nanomaterials interact with biotic (cells, tissues and organisms) and abiotic (pH, ionic strength, organic matter, etc.) environments. Acquiring key data at the nano-bio-eco interface is crucial in understanding the relationship between physicochemical properties of nanomaterials and their related toxicity. Although a holistic approach is suggested and demanded in order to fully understand how nanomaterials behave in a connected ecosystem,[8] conclusive answers to the question over the threats posed by engineered nanomaterials are perhaps unlikely to be revealed, and will probably need to be assessed on a case-by-case basis within any given context. In addition, numerous data published in literature sometimes may seem controversial. Not only should we continue building database profiles for existing MONMs, but also start establishing a standard system as a reference. Building up routine methods for nanotoxicity evaluation warrants higher reproducibility and reliability of data.

One should recognize that quantitative analysis is always necessary in the quest to understand nanotoxicity at nano-bio-eco interface. With the help of qualitative approaches, one can observe in a wide view, not limited to some definitive figures. We should also be cautious when it comes to the comparison between *in vitro* and *in vivo* observations because failure may often exist. Despite the different levels of complexity between the *in vitro* and *in vivo* studies at the nano-bio-eco interface, observations *in vitro* are less labor-intensive and more cost-efficient in most cases. Such merits also allow assessing nanotoxicity rapidly with sensitive and reliable HTS assay. Toxicological profiles can be readily generated with multiple biological models, multiple biological endpoints, and multiple types of nanomaterials. Collectively, the sum of the responses across *in vitro* and *in vivo* experiments can be retrieved and analyzed with QSAR repeatedly over time. Unlike laboratory work, the computational approach has its merits of being reliable and reproducible. One fundamental thing that needs to be handled properly is to provide a toxicological profile based on reliable experimental studies with as much accuracy as possible. Due to their capability of forming a vast number of structural geometries with an electronic structure, MONMs play an important role in nanotechnology and possess advantages for propelling QSAR model development.[90] Nowadays, most QSAR studies still mainly focus on the risk assessment of pristine MONMs, namely, with no consideration of doping or surface

modification. Increasing demand on novel MONMs with intentional tailoring forces us to pay more attention to newly developed nanomaterials.

Till now, many challenges remain before the above mentioned approaches in the study of nanotoxicology can be put into practice, though the rate of progress has been laudable. Further studies are still required, further advances are still occurring, and more remain to be revealed. Greater knowledge of how MONMs interact at the nano-bio-eco interface is also needed. Ultimately, more open collaboration between industry, academia, and research labs needs to be formed. Moreover, there is a need to take a broader look at facilitating nanotoxicological studies, to focus specifically on the interactions at the nano-bio-eco interface, leading to safe and effective nanotechnology driven MONMs for commercial, environmental and medicinal use.

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