Predictive toxicological paradigm and high throughput approach for toxicity screening of engineered nanomaterials

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Abstract: Nanotechnology is developing rapidly and numerous engineered nanomaterials (ENMs) with various design strategies have been produced and used in commercial products that affect many aspects of life. This increases the potential of human and environmental exposure to nanomaterials and there is an urgent need to have a platform to investigate the potential adverse effects of these materials. We propose a predictive toxicological paradigm that utilises high throughput mechanism-based *in vitro* screening to make predictions about the physicochemical properties of ENMs that may lead to generation of pathology or disease outcomes *in vivo*. In this review, we describe the tools required for establishing predictive toxicology paradigms together with examples of successful approaches in the UC Centre of Environmental Implications of Nanotechnology (UC CEIN) and the UCLA Centre for Nanobiology and Predictive Toxicology (CNPT).

Keywords: engineered nanomaterials; ENMS; high throughput screening; HTS; predictive toxicological paradigm; nano-structure-activity relationships; nano-SARs; compositional and combinatorial libraries.

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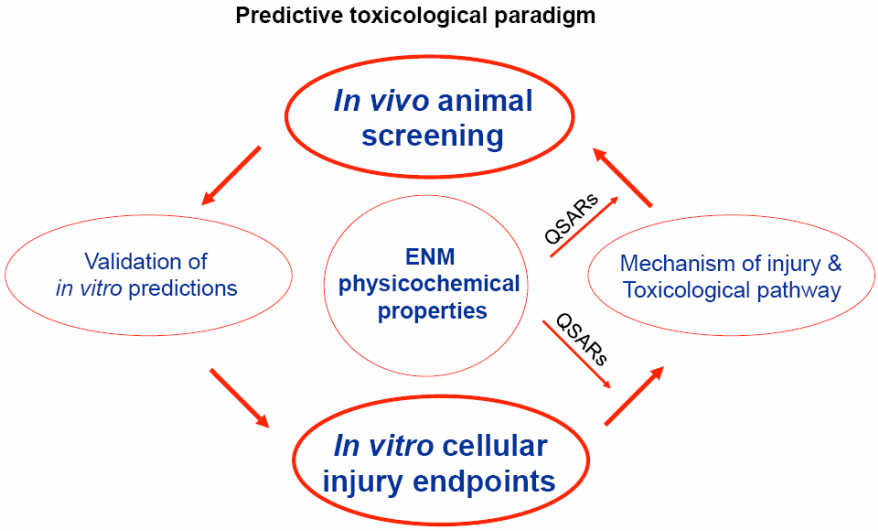
1 Introduction

The production of engineered nanomaterials (ENMs) represents a significant scientific advancement in material design and their uses in new consumer products are expected to impact every aspect of life. Currently, ENMs are being widely used in sunscreens, cosmetics, electronics, drug delivery systems, medical devices and vaccines, etc. (Chen and Mao, 2007; Jain et al., 2008; Nel et al., 2006). The rapid commercialisation of nanotechnology increases the opportunity of nanomaterial exposure to humans and environments. This has raised concern in the public, academia, and industry that the novel physicochemical properties of ENMs could generate hazardous biological outcomes (Colvin, 2003; Donaldson et al., 2004; Oberdorster et al., 2005). To investigate the potential adverse effects of ENMs, a key challenge is the ability to evaluate large numbers of new ENMs and develop reliable and cost-effective methods that can be used for hazard screening (Nel et al., 2006). Here, we propose a predictive toxicological paradigm, which can be defined as the use of high throughput mechanism-based *in vitro* screening to make predictions about the physicochemical properties of ENMs that may lead to the generation of pathology or disease outcomes *in vivo* (Meng et al., 2009; Nel et al., 2012) (Figure 1). Our opinion is that safety screening could commence at biomolecular and cellular level, where high throughput platforms can be used to generate the knowledge that is needed for our advertising and conducting animal studies, which can then be used more sparingly but essential to validate the relevance of the *in vitro* studies (Meng et al., 2009). Both the National Toxicology Program as well as the National Research Council (NRC) in the US National Academy of Sciences (NAS) have recommended that toxicological testing in the 21st-century evolve from a predominantly descriptive science in animal models to a predictive scientific model that is premised on target-specific, mechanism-based biological screening (NRC, 2007; NTP, 2004). It was further recommended that the biological testing should be based on robust scientific paradigms that can be used to screen multiple toxicants concurrently instead of costly animal experiments examining one toxicant at a time (Meng et al., 2009; Walker and Bucher, 2009).

Figure 1 The elements of predictive toxicological paradigm for ENM hazard testing (see online version for colours)



Notes: We define a predictive toxicological approach as establishing and using *in vitro* mechanisms and pathways of injury that are directly related to the physiochemical properties of nanomaterials as well as to disease mechanisms *in vivo*. The *in vivo* output is used to validate the *in vitro* screening method as being 'predictive' and therefore valid for high volume screening of large batches of materials to obtain quantitative structure-activity relationships.

2 Infrastructure required for establishing a predictive toxicological paradigm

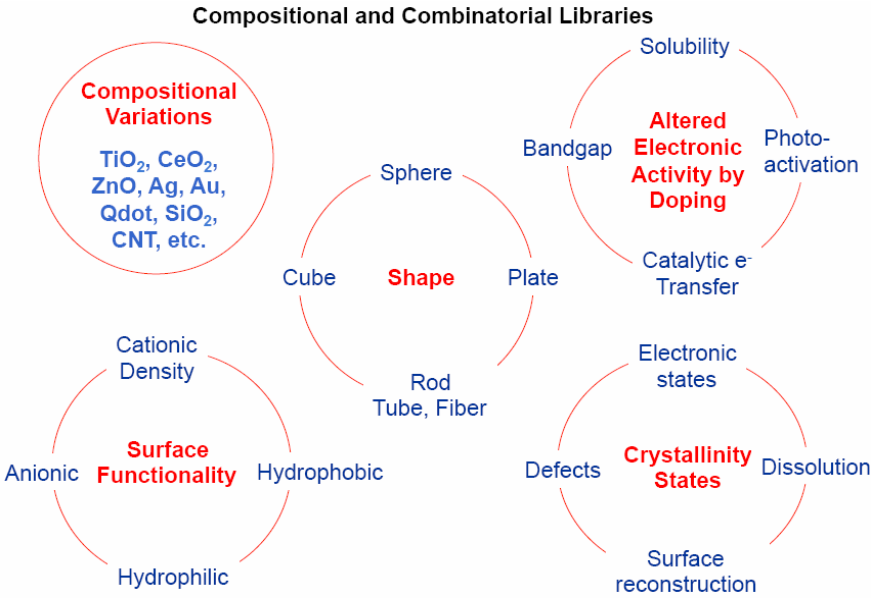
To establish the predictive toxicological paradigm, there are four major considerations. The first is to acquire or synthesise compositional and combinatorial ENM libraries that can be used for knowledge generation of material properties that may lead to biological injury. The second requirement is to develop *in vitro* cellular screening assays that elucidate mechanisms and pathways of injury. Third, it is important to develop high content or rapid throughput screening platforms to assess large number of material compositions and properties, dosage and time points that are likely to lead to biological injury. Finally, the *in vitro* data could be used for *in silico* modeling to establish hazard ranking and structural-activity relationships (SARs) that can be used to predict ENM toxicity.

2.1 Establishment of compositional and combinatorial ENM libraries

Acquisition and characterisation of ENM libraries are essential for toxicity screening and elucidating the material properties that are most likely to lead to biological injury (Nel et al., 2009; Oberdorster et al., 2005). The selection of compositional library materials should consider the commercial production volume so that the major classes of ENMs are

incorporated, including metals, metal oxides, silica, carbon-based nanomaterials, etc. The choice of materials should also consider the exposure potential, route of exposure and portal-of-entry. For example, free nanoparticles or powders are more likely to become airborne with the potential to generate pulmonary toxicity after inhalation. Thus, it is appropriate to investigate this exposure scenario using lung cells (*in vitro*) and pulmonary exposures (*in vivo*) that should ideally be linked to the mechanisms of injury. Ideal ENM libraries should also include positive and negative control ENMs to provide a reference point for the evaluation of material toxicity. To establish the link between specific physicochemical properties of ENMs to toxicity, it is also important to establish combinatorial ENM libraries, which are synthesised to vary or alter major physicochemical properties that may be involved in toxicity. Property variations include size, surface area, shape, crystallinity, bandgap, porosity, solubility, charge, and surface functionalisation (George et al., 2011a; Ji et al., 2012; Meng et al., 2011; Wang et al., 2012; Xia et al., 2008b; Zhang et al., 2011) (Figure 2).

Figure 2 Building compositional and combinatorial ENM libraries (see online version for colours)



Notes: Combinatorial libraries are built by using one of the compositional materials synthesised to vary or alter one of the major physicochemical properties that may be involved in toxicity. Property variations include size, shape, porosity, hydrophilicity/hydrophobicity, crystallinity, band gap, photoactivation, solubility, charge, and surface area.

2.2 Developing mechanism-based in vitro high throughput screening assays that link to ENM physicochemical properties

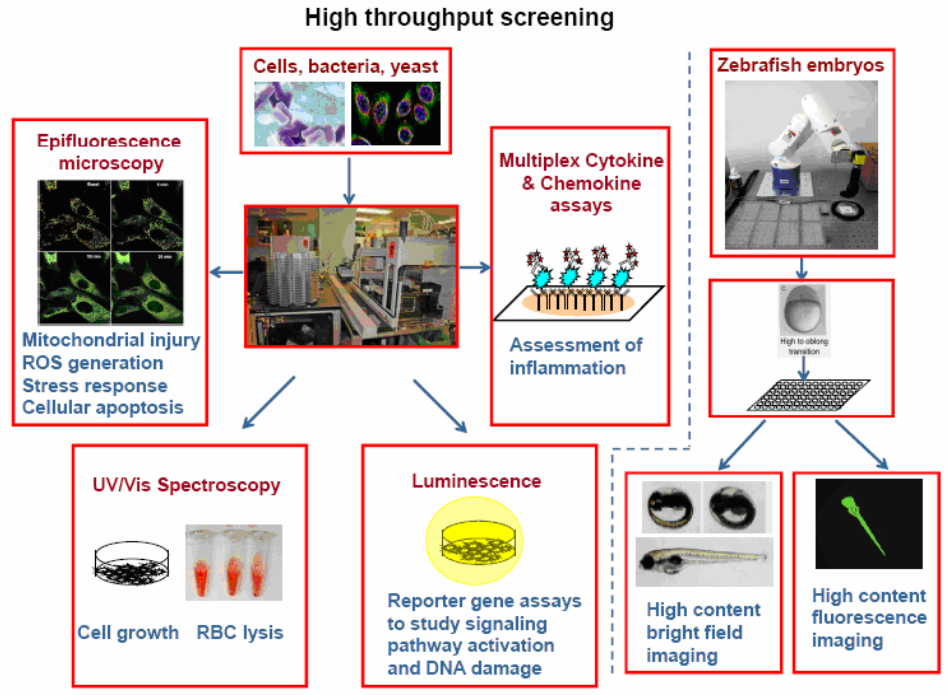
While much of the knowledge about ENM cellular toxicity has been generated by fairly straightforward cellular viability assays such as lactate dehydrogenase (LDH) assay,

MTT/MTS assay and propidium iodide (PI) staining, the major drawback of these endpoint assays is that they are often not informative of specific toxicological pathway because multiple stimuli can result in the same outcome and there is little connectivity between the biological outcome and specific ENM properties. Moreover, cellular viability assays do not reflect sub-lethal effects. For these reasons we advocate developing mechanism-based *in vitro* assays because it is conceptually the most appropriate way to link *in vitro* toxicity with pathological effects *in vivo*. Currently, there are approximately ten major mechanistic pathways of toxicity that have been linked to ENMs. These include injury paradigms such as the generation of reactive oxygen species (ROS) and oxidative stress, frustrated phagocytosis for long aspect ratio materials, changes in protein structure and function (e.g., loss of enzymatic activity), protein unfolding response, immune activation (e.g., through exposure of cryptic epitopes or immunostimulatory effects), fibrogenesis and tissue remodelling, hemolysis, vascular injury, neurotoxicity (e.g., oxidative stress, protein fibrillation) and genotoxicity (Table 1). It is important to note that additional mechanisms of toxicity are possible given the wide range of novel ENM physicochemical properties (Meng et al., 2009; Nel et al., 2012). For the purpose of this article, we will focus on the generation of oxidative stress.

Table 1 Experimental examples of major toxicological pathways that could lead to nanomaterial toxicity

Toxicological pathway	*Example nanomaterials*
Membrane damage/leakage/thinning	Cationic NPs (Xia et al., 2008b), crystalline silica (Lin et al., 2006)
Protein binding/unfolding responses/loss of function/fibrillation	Cationic NPs (Xia et al., 2008b), metal oxide NPs, Dendrimer, CNTs (Linse et al., 2007)
DNA cleavage/mutation	UFP (Kumagai et al., 1997), metal/metal oxide NPs (AshaRani et al., 2009),
Mitochondrial damage: e^- transfer/ATP/PTP opening/apoptosis	UFP (Li et al., 2003), cationic NPs (Xia et al., 2008b)
Lysosomal damage: proton pump activity/lysis/frustrated phagocytosis	UFP (Li et al., 2003), cationic NPs (Xia et al., 2008b), CNTs (Poland et al., 2008), long aspect ratio materials (Ji et al., 2012)
Inflammation: signalling cascades/ cytokines/ chemokines/adhesion	Metal oxide NPs (Xia et al., 2008a), CNTs (Poland et al., 2008), UFP (Pietropaoli et al., 2004), long aspect ratio materials (Ji et al., 2012)
Fibrogenesis and tissue remodelling injury	CNTs (Poland et al., 2008), metal oxides (Churg and Wright, 2002), long aspect ratio materials (Wang et al., 2012)
Blood platelet, vascular endothelial and clotting abnormalities	SiO_2 (Chen et al., 2008)
Oxidative stress injury, radical production, GSH depletion, lipid peroxidation, membrane oxidation, protein oxidation	UFP (Li et al., 2003), CNTs (Poland et al., 2008), metal/metal oxide NPs (Xia et al., 2008a), cationic NPs (Xia et al., 2008b)

Figure 3 *In vitro* and *in vivo* HTS assays and response readouts in cells and zebrafish embryos (see online version for colours)



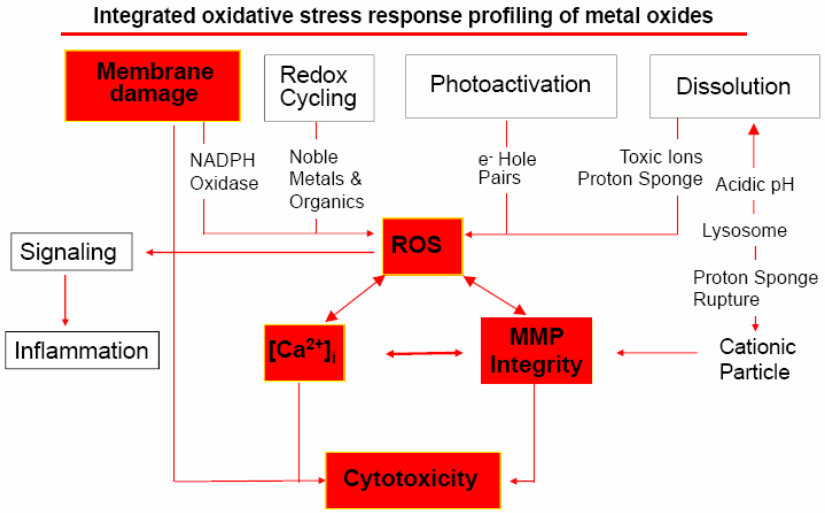
Notes: The high throughput screening was used to study biological pathways of injury to facilitate *in vivo* toxicological assay procedures that are cost-effective for rapid screening. The cell seeding, liquid handling, imaging, image analysis, etc., were automated; and multi-parametric assays could be completed in a single day compared to two to three weeks that are required to complete the full array of classical assays (Epifluorescence microscopy, UV/V is spectroscopy, cytokine and chemokine assays and luminescence). The high content screening platform with robotic arm system was used for automatic embryo picking and plating, and the high content imaging was used for bright field and fluorescence analysis.

2.3 High throughput screening system for ENM toxicity assessment

Particle-induced oxidative stress invokes three tiers of cellular responses including cellular antioxidant defence, activation of pro-inflammatory signalling pathways leading to the production of cytokines/chemokines, and mitochondria-mediated cell death (George et al., 2010; Li et al., 2008; Xia et al., 2006, 2009). However, in order to perform the entire panel of tests or three tiers of oxidative stress, it requires at least two to three weeks of labour-intensive effort. A high throughput screening approach could offer several advantages over conventional assays (Abraham et al., 2004, 2008) (Figure 3). First, this approach speeds up the pace of knowledge generation that is possible with compositional and combinatorial ENM libraries. High throughput screening (HTS) provides a rapid readout because of the standardisation of the procedure, automation (e.g., cell seeding, liquid handling, imaging, image analysis), and miniaturisation (requires less amount of reagents, lowers the cost per assay). Not only is HTS capable of

screening large libraries, but it can also accommodate multiple cell lines, time points and doses in the same experiment (Figure 3). Coupled with computer-assisted tools, this approach can significantly improve the reliability of toxicological screening as well as establishment of property-activity relationships. To develop rapid throughput platforms based on mechanisms of toxicity, it is advantageous to combine different steps or nodal points in the injury pathway. Such multi-parametric screening efforts enhance the utility of the procedure, cover lethal and sublethal cellular responses and improve the predictive value of the assay. As an example of such an assay, we recently developed a multi-parametric screening procedure that incorporates several cellular oxidative stress responses involved in the advanced level (Tier 3) of oxidative stress (George et al., 2010) (Figure 4). Also, we recently have developed an *in vivo* high content screening system using zebrafish embryos, which uses robotic arms to automatically pick-and-plate the embryos into 96-well plates and high content bright-field and fluorescence-based imaging to compare the toxicological effect of ENMs in zebrafish embryos and larvae (Lin et al., 2011) (Figure 3). More specifically, ENMs-induced hatching interference in the embryos and the expression of the stress marker (including oxidative stress) heat shock protein 70: enhanced green fluorescence protein (*hsp70*: eGFP) in transgenic zebrafish larvae were examined using the high content screening system. These high throughput screening approaches accelerate the hazard assessment of ENMs and allow the development of nano-structure-activity relationships (nano-SARs) and hazard ranking of ENMs.

Figure 4 Schematic flow diagram to illustrate the interrelatedness of cellular responses induced by metal oxides (see online version for colours)



Notes: We established multi-parametric HTS assays that are based on nanoparticle-induced ROS production and oxidative stress. Nanomaterials induce ROS production as a direct consequence of specific material properties or as a consequence of triggering cellular injury responses leading to oxidant radical generation. ROS production could trigger a range of oxidative stress effects. The induction of cellular toxicity at the highest level of oxidative stress involves a number of interrelated cellular responses that include intracellular Ca^{2+} release and mitochondrial perturbation leading to cell death with accompanying changes in cell membrane integrity and nuclear PI uptake. The parameters chosen in multi-parametric HTS assays are highlighted in red.

2.4 Develop hazard ranking and nano-SARs to prioritise in vivo animal assays

Although *in vivo* animal models are considered the 'gold standard' for toxicological research, *in vivo* screening is time-consuming and expensive. A complete set of toxicological assays for a single chemical, including assessment of carcinogenicity, chronic, reproduction and developmental effects could involve hundreds of animals and costs of $1–3 million. As a result, less than 2% of industrial chemicals have undergone toxicity testing using rodents. We believe that by using a predictive toxicology approach it is possible to avoid a similar conundrum in nano safety testing. Through mechanism-based *in vitro* HTS screening, we should be able to identify the major mechanisms of toxicity and perform hazard ranking according to which we can begin to prioritise the *in vivo* testing of ENMs. This approach also allows generation of data on dose, kinetics, nanomaterial physicochemical properties, and quantifiable biological response outcomes. However, the *in vivo* results are important to validate the *in vitro* screening as 'predictive', thereby allowing the *in vitro* platform to be used as the primary screening procedure. *In vitro* HTS data will also be used to establish *in vitro* nano-SARs by *in silico* modeling that will use statistics, mathematics and machine learning to perform hazard ranking as a prelude to future risk prediction studies. As an example of the nano-SAR model, we recently developed a classification nano-SAR for the cytotoxicity of a small set of metal oxide nanoparticles as quantified by high throughput screening of the PI uptake by transformed bronchial epithelial (BEAS-2B) cells (Liu et al., 2011). This model demonstrates remarkable prediction accuracy and low false-negative classification rates as confirmed in both the internal and external validation. Another example is the development of self organising maps (SOM), which are presented as data clusters to provide both quantitative and visual representation of pathway similarities and possible relationships between the biological effects induced by different nanomaterials (Rallo et al., 2011). In addition to identifying and visualising clusters and quantifying similarity measures, the SOM approach will help in developing predictive quantitative structure-activity relationships.

3 Demonstration of the use of a predictive toxicological paradigm in the UC Centre for the Environmental Implications of Nanotechnology (UC CEIN)

Based on our proposed predictive paradigm for nanomaterial hazard assessment, the NSF- and EPA-funded UC CEIN have developed a multidisciplinary and broad-based model of predictive toxicology premised on QSARs and nanomaterial injury mechanisms at the molecular, cellular, organismal, and ecosystems levels (Damoiseaux et al., 2011; Thomas et al., 2011). Similar approaches have also been applied to predict the toxicity of organic compounds, carbonaceous nanomaterials, and various metal oxides by other research groups (Burello and Worth, 2011; Puzyn et al., 2011; Sayes and Ivanov, 2010; Toropova et al., 2011). Since the inception of UC CEIN, we have established compositional and combinatorial libraries representing materials that are being produced in the largest volumes and therefore more likely to come into contact with the environment (Damoiseaux et al., 2011; Thomas et al., 2011). We consider the physicochemical properties that allow these materials spread to the environment, become bio-available through cellular/organismal uptake, and perform biocatalytic activities that

could lead to toxicity in bacteria, yeasts, algae, protozoa, mammalian cells and a series of trophic life forms that can be studied in terrestrial, fresh water and marine mesocosms (Damoiseaux et al., 2011; Thomas et al., 2011). We have also developed a high content screening platform using zebrafish embryos including robotic arm for automatic embryo picking and plating as well as high content imaging for bright field and fluorescence analysis. We used mechanisms and biological pathways of injury that can be used to perform HTS with a view to facilitate *in vivo* toxicological procedures that are cost-effective and useful for rapid screening (Damoiseaux et al., 2011; Thomas et al., 2011). All of the above nanomaterial physicochemical properties and biological and toxicological data are being fed into a computerised data collection and *in silico* decision-making tools to establish QSARs that help with risk predictions (Liu et al., 2011; Rallo et al., 2011). This model building has been carried out in collaboration with multiple partners nationally and internationally.

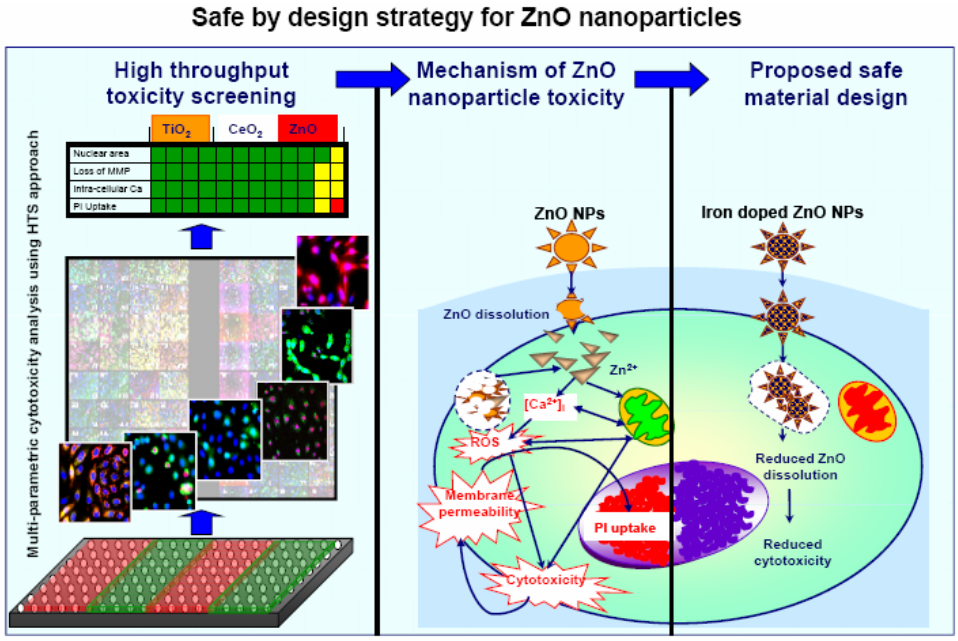
3.1 Implementation of a safe design strategy that lessens the toxicity of ZnO nanoparticles

Once toxicity of nanoparticles has been assessed, it is important to use the knowledge to develop methods to engineer safer nanomaterials (George et al., 2011b; Xia et al., 2011). Based on detailed physicochemical characterisation of the nanoparticles, the UC CEIN and the NIEHS-funded UCLA Centre for NanoBiology and Predictive Toxicology identified the importance of particle dissolution and shedding of toxic Zn-ions in ZnO-induced toxicity in mammalian cells (Xia et al., 2008a). To study the dissolution property of ZnO, we performed detailed analysis of ZnO nanoparticles in water, PBS, and cell culture media using ICP-MS (Xia et al., 2008a, 2011). The result showed ZnO nanoparticles were highly soluble in these media (Xia et al., 2008a, 2011). To study ZnO dissolution in cellular studies, we used fluorescently labelled ZnO to treat the cells and performed confocal microscopy (Xia et al., 2008a). We found that no labeled ZnO particles could be seen in the acidic lysosomal compartments of RAW 264.7 cells, while fluorescent particles could be observed in a less acidic caveolar compartment in BEAS-2B cells (Xia et al., 2008a). These data showed that dissolution of ZnO nanoparticles inside cells could play an important role in cellular toxicity (Gilbert et al., 2012; Xia et al., 2008a). Based on the importance of dissolution to ZnO nanoparticle toxicity, we hypothesised that lowering the rate of dissolution could decrease its toxicity (George et al., 2010; Xia et al., 2011). Since mixed zinc-iron oxides are significantly more resistant to proton-assisted dissolution than pure zincite, we investigated the possibility of deliberate doping of ZnO with iron to reduce dissolution (George et al., 2010; Xia et al., 2011).

Dr. Mädler (IWT, Germany) generated a panel of ZnO nanomaterials with an increasing atomic percentage of iron in the crystal matrix using flame spray pyrolysis. Iron doping enhanced the aqueous stability of ZnO through strengthening of iron versus zinc binding to oxygen (George et al., 2010; Xia et al., 2011). Iron is typically found to be present at high spin Fe^{2+} ions substituted for Zn^{2+} at lattice sites and based on the crystal field splitting of the Fe *3d* states, we infer Fe^{2+} to be more strongly bound than Zn^{2+} (George et al., 2010; Xia et al., 2011). Consistent with this mechanism, we observed reduced dissolution in buffered solution for iron-doped nanomaterials (George et al., 2010; Xia et al., 2011).

The panel of iron-doped ZnO was assessed using the multi-parametric HTS platform that was described earlier (George et al., 2010; Xia et al., 2011). Consistent with the slow dissolving iron-doped ZnO, we found that reduction in the cytotoxicity of ZnO follows with an increasing atomic percentage of iron (George et al., 2010; Xia et al., 2011). We tested the toxicity of these nanoparticles *in vitro* using a multi-parametric rapid throughput screening assay and found that the cytotoxicity decreased as the percentage of Fe doping increases (Figure 5) (George et al., 2010; Xia et al., 2011). Furthermore, we performed *in vivo* tests using the Fe-doped ZnO library in zebrafish (Xia et al., 2011). The zebrafish studies examined embryo hatching and mortality rates as well as the generation of morphological defects. We found that Fe-doped ZnO could decrease the hatching interference of zebrafish embryos without causing changes in viability or morphology compared to undoped ZnO nanoparticles (Xia et al., 2011). We also found that iron doping, similar to the effect of a metal chelator, DTPA, interfered in the inhibitory effects of ZnO on zebrafish hatching (Xia et al., 2011). These data showed that dissolution plays an important role under both *in vitro* and *in vivo* conditions (Xia et al., 2011). The importance of ZnO dissolution in the excitation of acute pulmonary inflammation in rodents was also demonstrated through the use of iron-doped ZnO as will be discussed in the next section.

Figure 5 Use of Fe-doping as a safe-by-design strategy for ZnO nanoparticles (see online version for colours)



Notes: The toxicity of ZnO nanoparticles was tested *in vitro* using a multi-parametric rapid throughput screening assay, and the dissolution of ZnO nanoparticles inside cells was found to play an important role in cellular toxicity. Iron-doped ZnO nanoparticles were synthesised and shown to exhibit the lower rate of dissolution in biological and environmental media. Cellular HTS and *in vivo* studies in zebrafish embryos and the rodent lung confirmed a reduction in toxicity by Fe-doping.

4 Predictive pulmonary toxicity studies in the UCLA Centre for Nanobiology and Predictive Toxicology

The Centre for Nanobiology and Predictive Toxicology aims to obtain a fundamental understanding of how the physical and chemical properties of carefully selected compositional and combinatorial ENM libraries through their effect on cellular injury responses to relate to disease pathogenesis in the lungs of exposed rodents (Xia et al., 2011). These studies are being executed through the acquisition, synthesis, and characterisation of compositional and combinatorial ENM libraries that focus on the major physicochemical properties of nominated metal, metal oxide, and silica nanoparticles hypothesised to play a role in pulmonary toxicity through the generation of oxidative stress, inflammation, signal pathway activation and membrane lysis. These efforts are being assisted by *in silico* modelling that use heatmaps, mathematical models and machine learning to perform hazard ranking and risk prediction (Liu et al., 2011; Rallo et al., 2011).

In the exploration of the acute pro-inflammatory effects of ZnO nanoparticles in the rodent lung, we hypothesised that iron doping on this material will decrease pulmonary inflammation, in the same way as Fe-doping leading to a decrease in acute cytotoxicity and oxidative stress parameters in the high throughput screening of bronchial epithelial and macrophages cell lines (George et al., 2010; Xia et al., 2011). We confirmed that similar to the effects of ZnO doping in mammalian cells that the decreased rate of dissolution and shedding of toxic Zn^{2+} lead to a reduction in bronchoalveolar PMN counts, protein and cytokine levels compared to non-doped particles in the lungs of rats and mice (George et al., 2010; Xia et al., 2011). This suggests that Fe-doping is a possible safe design strategy for preventing ZnO toxicity (George et al., 2010; Xia et al., 2011). We recently have compared an extensive list of 24 metal oxide nanoparticles that were chosen based on variation in bandgap energy and dissolution properties to delineate their toxicological potential at cellular and whole animal levels (Zhang et al., 2012b). We demonstrate that the overlap of conduction band energy levels with the cellular redox potential is strongly correlated to the ability of Co_3O_4, Cr_2O_3, Ni_2O_3, Mn_2O_3, and CoO nanoparticles to induce oxygen radicals, oxidative stress, and lung toxicity. Importantly, we show the possibility to predict the toxicity of metal oxide nanoparticles in the lung based on their semiconductor properties and an integrated *in vitro*/*in vivo* hazard ranking model based on oxidative stress (Zhang et al., 2012b). We are also using a silica nanomaterial library to demonstrate whether the pulmonary injury potential can be predicted by surface silanol groups that vary according to the silica composition, which are being studied in a library of silica nanoparticles that include amorphous, mesoporous, fumed and a series of different crystalline polymorphs (Zhang et al., 2012a).

5 Limitations of the predictive toxicological approach

Although we showed some successful examples of predictive toxicological approach on ENM safety assessment we know that *in vitro* cellular assays have limitations in predicting the *in vivo* outcomes because of the complex nature of *in vivo* systems that involves the interaction of multiple cell types, tissues, and organs. Even though in some cases the *in vitro* and *in vivo* toxicological outcomes could be bridged through the use of assays developed based on toxicological mechanisms, the generation of disease outcomes

in human or environmental systems is highly dependent on real-life exposures of ENMs (Meng et al., 2009; Nel et al., 2006). So fate, transport and life cycle analysis need to be considered for ENM risk assessment (Thomas et al., 2011). Compared to *in vitro* false-positive results that can be corrected by *in vivo* testing, we are confronted with the possible *in vitro* false-negative data generation. If a disease mechanism is involved that does not show up in the *in vitro* assays, we could be faced with unanticipated disease outcomes (Donaldson et al., 2009). In this case, further development of complementary *in vitro* assays, machine learning, and establishment of QSAR could be helpful to limit the number of false negatives in toxicity screening (Liu et al., 2011; Rallo et al., 2011). Currently, there are only a limited number of mechanism-based *in vitro* assays available, further development of *in vitro* assays based on toxicity mechanisms, especially in HTS format, is needed for toxicity screening of ENMs. In spite of these shortcomings, we think the predictive toxicological approach and HTS are the appropriate safety testing strategies for ENMs to ensure the sustainable development of nanotechnology.

6 Conclusions

We advocate the implementation of predictive toxicology for toxicity screening of ENMs. It is based on the establishment of compositional and combinatorial libraries, the development of mechanism-based *in vitro* toxicity screening assays, the development of high throughput screening assays, the building of computerised nano-SAR models, and the prioritisation of *in vivo* assays to validate the predictability of *in vitro* assays. This predictive toxicological platform has been successfully implemented in the UC Centre of Environmental Implications of Nanotechnology (UC CEIN) and the UCLA Centre for Nanobiology and Predictive Toxicology (CNPT) for nanomaterial hazard assessment. We believe this is an appropriate approach to build a knowledge-base that can meet the challenges of an expanding nanotechnology enterprise.

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