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Understanding Nanomaterial Biotransformation: An Unmet Challenge to Achieving Predictive Nanotoxicology

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More than a decade has passed since the first concepts of predictive nanotoxicology were formulated. During this time, many advancements have been achieved in multiple disciplines, including the success stories of the fiber paradigm and the oxidative stress paradigm. However, important knowledge gaps are slowing down the development of predictive nanotoxicology and require a mutidisciplinary effort to be overcome. Among these gaps, understanding, reproducing, and modeling of nanomaterial biotransformation in biological environments is a central challenge, both in vitro and in silico. This dynamic and complex process is still a challenge for today's bioanalytics. This work explores and discusses selected approaches of the multidisciplinary efforts taken in the last decade and the challenges that remain unmet, in particular concerning nanomaterial biotransformation. It highlights some future advancements that, together, can help to understand such complex processes and accelerate the development of predictive nanotoxicology.

1. Strategy Development toward Predictive Nanotoxicology

Nanomaterials (NM) have shown great promise ever since their first use 12 centuries ago for decoration and steel enhancement[1], and even more so with the development of scanning tunneling microscopy in the 1980s, which enabled NM to be visualized and better understood.[2] This improved understanding of NM led to their refinement and ultimately attracted scientific as well as industrial interest across all disciplines, a trend that is expected to increase in the coming years. The use of NM in the industrial area and their availability in consumer products results in human exposure, which raises the question of their safety.[3] As the number of newly developed NM increased over the years, it became ever more evident that relying on each NM to be tested individually would push nanotoxicology into a frustratingly inadequate position.

Ten years ago, Meng et al. were among the first to propose a clear strategy for developing a predictive nanotoxicology.[4] In their paper, they highlighted the need for a platform that could deal with the immense number of biophysical interactions that occur once NM are introduced into a biological environment. In the development of the platform, pitfalls should be avoided, such as choosing end-points, model systems, and techniques that, although successfully used in classical toxicological assessment, are not applicable to NM. Their general concept is to consider the mechanisms of injury linked to disease pathogenesis, or in vivo toxicological outcomes, while taking into account the physicochemical properties of NM.[4]

For example, the generation of reactive oxygen species (ROS) can occur in the presence of certain NM and induce (pro)inflammatory effects in cells. Through cytokine production and the stimulation of inflammatory pathways, this can further lead to oxidant injury and disease development. As a strategy, it was suggested that, for NM observed to cause inflammation on an organ level in vivo, the presence of oxidative stress and inflammation at the cellular level should be tested as well and linked to the physicochemical properties of the material. To assess the link between ROS production and disease outcome, the authors propose a three-tier approach where antioxidant defense, proinflammatory effects, and cytotoxicity are assessed via cellular assays.[4]

Correlating the physicochemical properties of NM with biological outcomes is the ultimate goal of predictive nanotoxicology.[5] The potent way to enable predictive nanotoxicological assessment is to develop in vitro and in vivo quantitative structure–activity relationships (QSARs) models to correlate, through their mechanisms of injury, adverse health effects with NM physicochemical properties: thus ultimately limiting the need for in vivo testing.[6] To further increase the efficiency of screening, we should aim for high-content and high-throughput testing strategies, while standard reference nanomaterial libraries would elucidate the material properties that are most likely to lead to biological injury.[7]

The pathway to achieving predictive nanotoxicology is still paved with hindrances, especially when it comes to the knowledge gaps on the biotransformation of NM in biological environments. However, there are success stories where predictive nanotoxicology has been achieved: the fiber paradigm, for long, stiff, and biopersistent fibers,[8,9] and the bandgap paradigm,[10,11] for NM with electronically active surfaces, containing transition metals or redox-cycling organic chemical impurities.

The fiber toxicology structure–activity paradigm is related to biopersistence, fiber diameter, and length. The fiber diameter

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influences pulmonary deposition, while the fiber length is thought to be the most important factor in fiber pathogenicity by contributing to inflammation, tumor and fibrosis response, and formation of granuloma. Furthermore, biopersistent long fibers remain in the respiratory system, as their clearance by macrophages is hindered due to their dimensions: thus leading to frustrated phagocytosis, which can ultimately lead to chronic mesothelioma inflammation.[8,9]

While for the fiber paradigm geometry is the most important toxicological characteristic, the bandgap paradigm is based on the conduction band energy. Past research showed that the conduction band energy levels of different NM can be used to predict in vivo toxicological scenarios based on induced oxidative stress in vitro.[10,11] Analysis of 24 metal oxide particles showed that, when their conduction band energy levels overlapped the cellular redox potential, NM induced oxygen radicals, oxidative stress, and inflammation, both in vivo and in vitro.[10] In the past decade, a strong emphasis was put on establishing a connection between NM physicochemical properties and observed toxicity effects, which resulted in i) a striking correlation between the metal oxide nanoparticle bandgap and biological stress outcome,[10] ii) the fiber paradigm[8] as well as shedding light on iii) the increased toxicity of highly positively charged particles, compared to neutral or negatively charged ones.[12] All three examples show a high predictive power within a subclass of NM. An alternative research direction focused instead on the correlation between initial biological responses and overall toxicity to verify the predictivity of early events that can be tested in vitro, as opposed to costly in vivo studies. In this research direction, Meng et al.[4] suggested that both reactive oxygen species production and protein unfolding could represent initial indicators of a toxic response.

While the need for a predictive nanotoxicology was highlighted a decade ago,[4] it is still regarded as a goal for the future.[5] In this essay, we explore and discuss a qualitative balance of the past 10 years of multidisciplinary nanosafety research, highlighting the role of nanomaterial biotransformation as the common challenge for predictive nanotoxicology.

2. Ten Years Later: Achievements and Challenges

"When things are large, they are what they are. When they are small, it's a different game: they are what our measurements make them." – George M. Whitesides, No Small Matter. Science on the Nanoscale. [2009]

Since the development of the first paradigm, the nanofield has shown promising achievements both in terms of knowledge gains and development and refinement of tools, all of which assist the advancement toward predictive nanotoxicology (**Table 1**).

However, one of the great remaining challenges is the limited availability of complete data sets, which hinders not only the development of nontesting strategies but also the mechanistic interpretation of experimental data.

Often, available data fail to represent the complexity inherent to NM and their interactions with biological systems and therefore have limited effectiveness when looking for correlations between NM properties and toxic effects. This is the case for the characterization of NM physicochemical properties, which are often measured in dry-state conditions. Correlating such "powder-form" properties (intrinsic properties) to toxicity can be difficult, since, once in contact with a biological environment, NM and their properties will be modified by a system of competing and/or synergic processes (**Figure 1**).[13]

Colloidal stability, dissolution, and reprecipitation of NM affect both the particles themselves (e.g., size and surface area), and their behavior (e.g., sedimentation and diffusion), which ultimately influence the cellular response in vitro, and the biodistribution, pharmacokinetics, and systemic toxicity in vivo.[14]

Peter Wick has been the head of the research laboratory for Particles-Biology Interactions at the Federal Laboratories on Materials Science and Technologies Empa in St. Gallen since 2010. His general research interest is to study the interactions of nanomaterials with human tissues, including barrier tissue in vitro and ex vivo to obtain a detailed mechanistic understanding of their uptake, accumulation, transport, and effect on different types of cells or entire tissue.

Table 1. Some of the achievements that have marked the progress toward predictive nanotoxicology and some remaining challenges to address in the next 10 years.

	Achievements	Remaining challenges
Predictive	Bandgap paradigm for metal oxides	Correlating the intrinsic nanomaterial properties to observed effects
	Fiber paradigm	Characterizing NM after biotransformation
Knowledge gains	Protein corona	Predicting and controlling protein corona evolution and interaction
	NM charge-toxicity correlation	Clear predictive paradigm—lack of values
	Organ-level biodistribution	Data for biodistribution modeling
Tools	Advanced characterization techniques including adv. human in vitro models	Real-time, label-free, and nondestructive characterization techniques
	QSAR models	Complete data sets
	A standardized system for data storage and reuse	Wide acceptance and compliance by the community

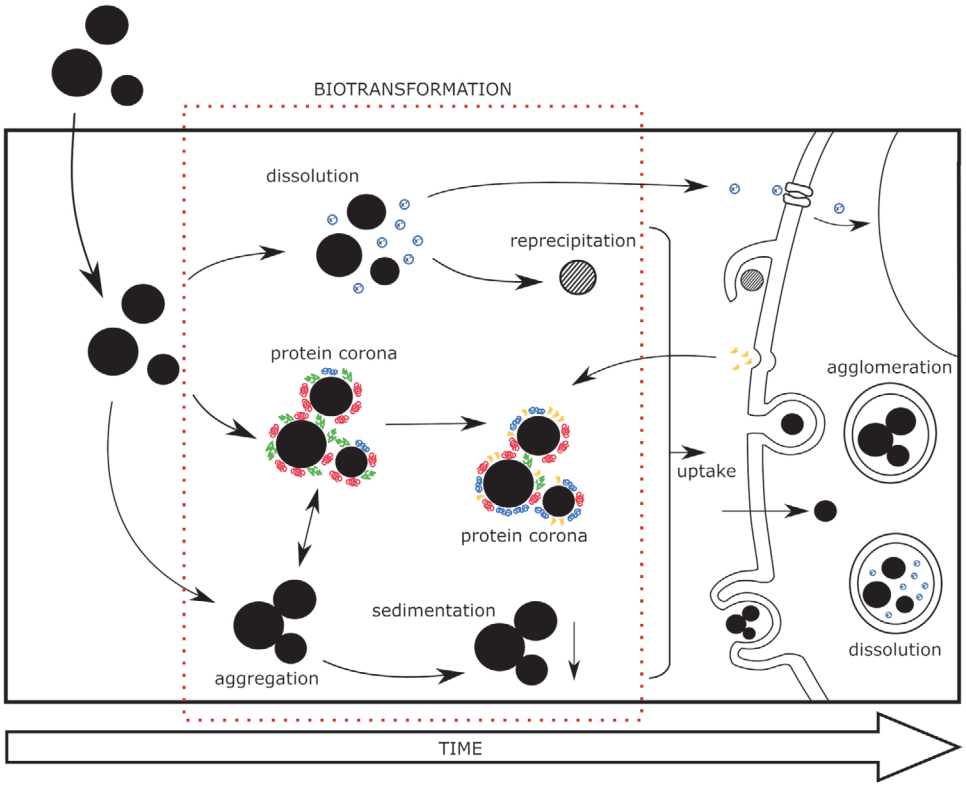

Figure 1. Nano–bio dynamic interactions: as soon as the particles get into the contact with a biological environment, the particles undergo partially dramatic changes from dissolution and reprecipitation as well as biomolecule (protein) coatings and agglomeration summarized as biotransformation. These transformations lead to the generation of a set of particles with different properties compared to the original particles.

At the same time, proteins interact with the surface of NM, creating a dynamic protein corona that changes with time and the surrounding environment (extrinsic properties).[15] While the adsorption of proteins on biomimetic surfaces has been known for a long time, a clearer understanding of the protein corona formation and its behavior upon interaction with NM was reached only by the research done in the last 12 years.[16] For example, while protein coronas in vitro form over seconds to hours, in vivo the corona may evolve along with the NM transport in the body, carrying a "fingerprint" of the prior biological fluid.[16] All these physicochemical and biological processes, summarized as NM biotransformation, transform a single type of NM in a population of particles/molecules with heterogeneous properties, each one interacting differently with living systems. At the nano–bio interface, such heterogeneous properties, together with cell characteristics, affect NM cellular contact, determining whether particles will be taken up, through which uptake pathway, and at which rate. Inside the cells, changes in the environmental conditions, such as pH change in lysosomes, further trigger NM modification and toxicity.[17,18]

The need for understanding and taking into account these dynamic and complex NM transformations has been known for more than a decade.[19] The advancements in characterization techniques and the development of experimental endpoints looking at the nanocellular interface have made it possible to measure NM properties and study the interactions of NM with biological entities.[20] However, despite multiple requests for standardized reporting,[13,21] critical information is still often not disclosed, demonstrating a lack of consensus in the scientific community. More efforts are needed in this direction, both in adopting a common characterization reporting standard and also in mechanistically and dynamically describing the transformations of NM in biological media (see the work by Faria et al.[22] and the responses generated by their proposal[23]).

Assessing the biotransformation processes of NM in biological systems requires appropriate models, which should be cost-effective, allow high-throughput screening, and have the potential for standardization.[24] Over the past decade, the success of advanced in vitro models was evident as the research moved from the classical 2D cancer cell monocultures toward 3D organoid-like primary-cell cocultures, which, when exposed to NM, better represent the intricate cell-to-cell signaling typical of in vivo situations.[24,25] Besides the presence of associated cells, advanced in vitro models, e.g., organ-on-a-chip and microfluidic technologies, can further model physiological stimuli found in specific organs, such as shear stress in vessels, bringing the model system even closer to realistic conditions. Advanced in vitro models have been particularly useful for the assessment of interactions and toxicological outcomes at the barriers, such as the air-lung barrier, the blood-brain barrier, and the gastrointestinal-tract barrier.[26] A successful in vitro model for predictive nanotoxicology is able to properly replicate all critical events that occur in vivo.

The knowledge gained about NM biodistribution in vivo shows that a complex system of interactions determines the fate of NM in the body and that NM properties play a major

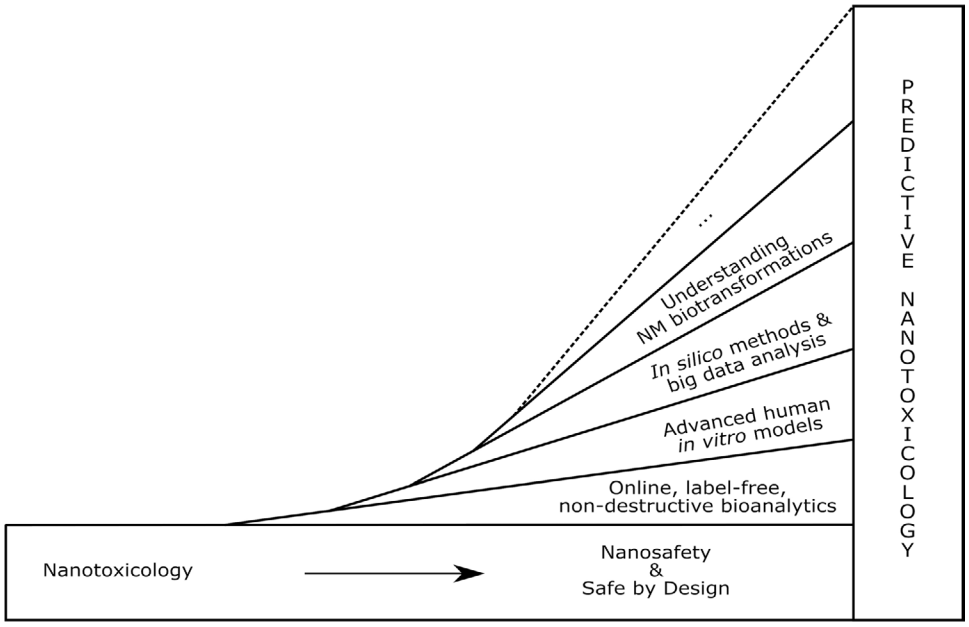

Figure 2. Graphical representation of the most promising developments needed to bring the current nanosafety research to the next level.

role in it, as observed in case-by-case studies.[27] As NM biodistribution in the body over time cannot (yet) be assessed in vitro, complementary methodologies, such as in silico physiologically based pharmacokinetic modeling, will be necessary to support the further growth and development of in vitro systems.[26]

Predictive in silico modeling is a nontesting data-generating strategy that can accelerate the assessment of NM toxicity.[28] Its foundation lies in the hypothesis that structurally similar NM should have similar biological activities, making it possible to infer toxicological information for untested NM.[29] Successful models have been developed in the last years, most of them addressing the in vitro effects of metal oxide NM,[30] and guidelines have been published to support the use of in silico modeling for regulatory purposes.[29] However, despite the fast development in computational nanosafety, the number of predictive models is still limited.[31,32] One of the main reasons is the scarcity of high-quality data and standardized or at least verified experimental methods with appropriate controls, which results in incomplete and eventually unreliable datasets.[7] Data generation, which is an interdisciplinary effort, should go hand in hand with a standardized system for data storage and reuse. Notably, such a system is being developed in the nanoinformatics field, in the shape of a framework for harmonization and interconnectivity of databases,[33] but it will need the full support of the nanotoxicology community to be operational and efficient.

3. Directing Future Multidisciplinary Effort toward Predictive Nanotoxicology

Nanotoxicology has without a doubt progressed in the mechanistic understanding and prediction of toxicity, taking into account multiple challenges and taking action on different fronts, including advanced methodologies, increased computing power, standardization, and data availability.

The complexity of nano–bio interactions calls for a multidisciplinary effort and new solutions to address all the relevant aspects needed to describe and model such processes (**Figure 2**). First, we face a turning point in the analytical field where many classical biochemical analyses have reached their limits since they are unable to monitor dynamic interactions. Instead, to detect such complex kinetics we need advanced methods that can be conducted in real-time and in situ without interfering with the biotransformation processes (label-free methods).[5]

Second, the development of advanced in vitro models should continue, to reach a sufficient complexity to represent in vivo conditions, but also of obtaining a level of standardization that assures reliable results.

Currently, we are convinced that the combination of advanced in vitro models, relevant endpoints, and adequate analytical tools will generate a considerable flow of data for in silico modeling,[34] which will provide precious insights into early events and kinetics of NM biotransformation. Moreover, supported by the exponential increase in computational power, big data analysis will extract patterns of toxicity from standardized, high-quality data, unlocking the development of new toxicity paradigms.

Whereas the road to predictive nanotoxicology is still long, the last years have proven to be fruitful. Thanks to the dedication and collaboration of scientists from different disciplines, which built the knowledge basis and shaped the tools needed to investigate the effects of nanomaterials, we are now equipped to face the next challenges with optimistic determination.

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Conflict of Interest

The authors declare no conflict of interest.

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