Promise and peril in nanomedicine: the challenges and needs for integrated systems biology approaches to define health risk

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In the 1966s visionary film 'Fantastic Voyage' a submarine crew was shrunk to 100 nm in size and injected into the body of an injured scientist to repair his damaged brain. The movie (written by Harry Kleiner; directed by Richard Fleischer; novel by Isaac Asimov) drew attention to the potential power of engineered nanoscale structures and devices to construct, monitor, control, treat, and repair individual cells. Even more interesting was the fact that the film elegantly noted that the structure had to be miniaturized to a size that is not detected by the body's immune surveillance system, and highlighted the many physiological barriers that are encountered on the submarine's long journey to the target. Although the concept of miniaturizing humans remains an element of science fiction, targeted drug delivery through nanobots to treat diseases such as cancer is now a reality. The ability of nanobots to evade immune surveillance is one of the most attractive features of nanoscale materials that are exploited in the field of medicine for molecular diagnostics, targeted drug delivery, and therapy of diseases. This article will provide a concise opinion on the state-of-the-art, the challenges, and the use of systems biology—another equally revolutionary field of science—to assess the unique health hazards of nanomaterial exposures. © 2017 Her Majesty the Queen in Right of Canada. *WIREs Nanomedicine and Nanobiotechnology* published by Wiley Periodicals, Inc.

NANOMATERIALS AND NANOMEDICINE

Nanomaterials are engineered at an unprecedentedly small scale (nano is a prefix meaning one billionth) and exhibit novel chemical, physical and biological properties that are distinct from bulk materials (larger than 100 nm). As a result of these distinct features, applications of nanomaterials are versatile and extend into all aspects of modern life style including the energy, biomedical, consumer, and industrial sectors. With the immense growth of nanotechnology (the field of science that exploits the distinct physicochemical properties of nanomaterials) in the last decade, nano-enabled products (products containing nanomaterials) have become increasingly common. More than 1,600 products containing nanomaterials are currently on the market,[1] and

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there is an increasing reliance on these novel materials. One of the most exciting applications of nanotechnology is in field of medicine.

Nanomedicine is a new field of molecular medicine that exploits the ability to control individual atoms and molecules, and associated properties, to generate complex functional drug delivery vehicles, diagnostic and analytical tools for application in medicine.[2] Nanomaterials are not drugs themselves, but can be loaded with drugs, genes, antibodies, or radioactive materials and their surface can be functionalised with antibodies, peptides, or small molecules.[2] Several types of nanomaterials including liposomes, polymers, metals and metal oxides, and composites are used in nanodrug delivery systems. Once in the host, the activity of these drug-carrying vehicles called nanovectors is controlled in such a way that the content of the vector is unloaded only under specific conditions such as appropriate pH, temperature, or light (reviewed in Ref 3). While controlled delivery helps to protect the host from unintended exposure to active drug and protect the drug from being detected by the host's surveillance system, surface functionalization helps achieve delivery of the vector contents with great specificity. Other factors, such as enhanced cellular penetration due to their nanosize and their ability to load mixtures of active ingredients onto the nanomaterial to enable *in vivo* tracking of drug delivery, makes them attractive options for diagnostics and treatments.[3]

A recent detailed literature review[4] identified 247 nano-enabled applications and products that were either approved for use, under clinical study, or in the process of going into clinical trials. The details of nano-enabled therapeutics in phases I, II, and III clinical trials are summarized along with the type of the nanomaterial used, the names of the drugs, and the company synthesizing them.[5] The types of uses for the various products identified in these studies varied from treatment of cancers to antibacterial gels. Some of the nano-enabled medicines, such as liposomes, have been used for treating Kaposi's sarcoma, and recurring breast and ovarian cancer.[6] Albumin-coated paclitaxel particles have also been used to treat metastatic breast cancer.[6] Overall, there are many opportunities for use of engineered nanomaterials in therapeutics as reviewed in Refs 7,8 including:

1. *ex vivo* diagnostics—nanomaterial-based bio-barcode assays are being validated for early detection of cancer or neurological diseases, which involve surface functionalization of nanomaterials with DNA sequences or antibodies to detect expression of a gene or protein

biomarker of a disease in a specific cell type or biological fluid;

2. *in vivo* imaging—superparamagnetic nanomaterials are being validated for their application in imaging of tumor lesions, to track *in vivo* cellular movement following transplantation;

3. targeted delivery—ligand-attached nanomaterials are being developed for noninvasive detection and monitoring of in situ changes in the expression of biomarkers in tumor sites;

4. theranostics—integrated strategies to deliver the imaging and therapeutic components via a single nanovector are emerging and will permit accurate diagnosis of disease stage and real time monitoring of drug delivery and distribution, and effective assessment of post-therapy outcomes[9];

5. co-delivery of multiple therapeutic drugs against different targets, increasing the efficacy of anticancer treatments.

Thus, it is widely touted that nanomedicine will considerably improve the way many complex diseases are treated.

The other exciting nano-enabled innovation is in personalized medicine, a health care strategy where patient-specific treatments are prescribed on the basis of genetic, phenotypic, and environmental factors that are known to influence the outcome of a therapy.[10] It is well-known that drug efficacy and safety is individual-specific[11]; the precise control over particle size, shape, surface modifications, and other characteristics enables design of patient-specific therapeutics.

While nanomedicines and nano-enabled diagnostic tools continue to gain wide acceptance and applications in disease diagnosis and treatment, a clear and precise understanding of the interactions between nanomaterials and the surrounding biological milieu is integral to the success and sustainability of such applications. This understanding is critical particularly with respect to adverse toxicological properties that nanomaterials might possess.

CURRENT ISSUES WITH TOXICITY TESTING OF NANOMATERIALS

It was established early on that nanomaterials are not benign and that they can affect biological systems at the molecular and cellular levels resulting in detrimental effects. Observed biological effects induced by nanomaterials were also clearly identified as uniquely influenced by their varying physicochemical properties.[12,13] Over the last decade, considerable efforts

have been made to develop suitable *in vivo* and *in vitro* toxicity testing assays, or to adapt methods developed originally for bulky materials, to assess nanomaterial-induced toxicity. However, most nanomaterials are insoluble and have a tendency to aggregate, influencing exposure doses in a variety of ways including interference with optical measurements, and inducing nonlinear dose–response relationships. Moreover, the currently applied test strategies lack sensitivity to detect effects at low doses.[14,15] Thus, validation of the test systems for nanomaterials assessment has been challenging.

Issues for nanomaterial toxicity testing go beyond the lack of validated methods. Given the size (1–100 nm), shape (sheet, sphere, cube, fiber, tube), and surface (crystalline structure, impurities, charge, presence of coatings, defects) permutations, which may be the fundamental drivers of nanomaterial functionality (e.g., optical, electrical, mechanical, and chemical behavior), a clear picture of the scale of the library of nanomaterials combining these variables becomes apparent. For example, in nanomedicine, a simple form of a nanovector may consist of entry categories such as a shape, biological targeting agent, and specific type of payload for treatment. Considering that each of these entry categories can be replaced by 100 different choices, combinations of the categories will result in 10 possible vector candidates.[16] Thus, the high degree of nanomaterial variables in the context of toxicity testing is a challenge because it is simply impossible to test each nanomaterial variant for all of its toxicological properties. Moreover, it is known that nanomaterials can impact multiple biological functions and pathways that are yet to be elucidated. In addition, hazard characterization of this diversity of forms using traditional animal toxicity studies would be prohibitively costly, time consuming and present ethical problems. Indeed, if one were to use the conventional strategy for targeted testing of chemical-induced toxicity, it is estimated that it would require over a billion dollars and about 50 years to effectively assess the nanomaterials that are currently on the market that require immediate assessment.[17] As a result, while the widespread synthesis and uses of nanomaterials continue to grow in general, an accepted strategy for safety testing of nanomaterials/nano-enabled products and human health risk assessment is yet to be established.

Thus, new tools that are more comprehensive and high-throughput that (1) adequately address the unique challenges of nanomaterial research, and (2) can clearly link the toxicity to their specific physicochemical properties, is essential. It is envisioned that comprehensive information derived from high-throughput, high-content toxicological tests can help build knowledge-based property-response matrices to identify nanodescriptors that can eventually be incorporated into drug discovery, drug development, and predictive toxicity assessment models.[18,19]

POSSIBLE ALTERNATIVES TO CONVENTIONAL TOXICITY TESTING

The issues associated with the use of targeted, endpoint-based conventional toxicity assays are problematic across various areas of regulatory toxicology; for example, the challenge of conducting risk assessments of the ~80,000 industrial chemicals estimated to be on the global market. It is clear that the existing toxicity testing framework is ineffective for assessing the large volume of chemicals. To deal with this challenge, a call for transforming toxicology was made in a paradigm shifting report 'Toxicity Testing in the 21st Century: A Vision and A Strategy' by the National Research Council.[20] In this report, a transition from the conventional toxicity testing approach that is reliant on whole animal testing to one that applies mechanism-based measures in human cell culture models to develop predictive toxicological tools was proposed.[20] This and other complementary initiatives and reports have led to widespread increase in the development of new molecular biology, computational, and statistical tools. However, basic, but critical, elements for the test strategies, such as selection of cell systems (with intact metabolic systems) that adequately reflect complex *in vivo* biology, the scope of the biological space covered and the specific endpoints measured, and, most importantly, the empirical relationships between substance-induced molecular perturbations in a cell line and response (or adverse effects) in complex multicellular organisms, remain to be clearly defined.

SYSTEMS BIOLOGY APPROACHES FOR NANOTOXICOLOGY AND NANOMEDICINE

Inherent to the challenges outlined above is the reality that cellular function is controlled by sophisticated communication between cells, facilitated by the complex networks of genes/proteins/metabolites that interact with each other serving as sensors, regulators, and messengers of internal and external signals. Comprehensive understanding of this elegant complexity (i.e., systems biology) is a prerequisite to establishing sensitive mechanism-based alternative toxicity testing approaches, and drug safety

evaluation strategies.[21] Systems biology technologies are commonly employed by the pharmaceutical companies during the drug development and drug safety assessment stages.[22] However, a paradigm change toward systems biology approaches in toxicology also requires a shift in the regulatory toxicology community to more broadly embrace nonhypothesis-driven and mechanism-based strategies. While some typecast nonhypothesis driven approaches as 'fishing expeditions,' unbiased profiling tools that are not endpoint-specific and that do not require prior knowledge of toxicant mode-of-action offer the opportunity to comprehensively understand the various biological processes, functions and pathways perturbed in a cell type or a tissue, and to then build tailored assays that are pertinent and appropriate to assessing specific effects of toxicants or drugs. Indeed, the examples of tiered testing strategies, in which the first tiers include high-throughput and high-content mechanism-based screening assays that are used as triggers for subsequent tiers applying more conventional tests at higher levels of biological organization, are available (e.g., Ref 23).

Systems Biology[24,25] is a holistic approach to studying the complex interactions occurring within living cells and organisms. It combines computational modeling, bioinformatics tools, and quantitative molecular biology techniques to reveal the dynamic interactions between the different components of a biological system providing a deeper understanding of the system's behavior as a whole. This type of approach will prove especially important in the context of nanomedicine, where nanodrugs with improved half-lives administered intravenously remain in circulation for long periods of time before they find their target, during which they interact with various cell types and physiological barriers, and are retained in the target cells or tissues long after (days and potentially for years) offloading their payload.[3] In addition to benefitting toxicological understanding, the molecular documentation of the long journey of nanodrugs and their interactions with other biomolecules at different levels of molecular organization will improve their application.

Although, systems biology has a long history, owing to lack of appropriate modeling tools, there have been few success stories. The virtual human heart built over half a century, which is used in the clinical settings is one of the true examples of the benefits of understanding the system as a whole.[26] In the last decade or two, sophisticated tools have been developed to assemble biological knowledge, enabling the creation of comprehensive inventories of molecular entities such as genes, proteins, small biomolecules, and pathways. Recent advances in next generation research

tools such as genome-scale sequencing models have brought a renaissance of big data generation, enabling visualization of the genetic landscape of an organism under normal and stress conditions. This, in turn, has helped to develop mathematical models to understand how genes and proteins function at the molecular level, and effectively design the biological systems. The molecular and pathway perturbations, and their relationships with toxicological effects, are beginning to be assembled and cataloged (e.g., the Adverse Outcome Pathway of the Organisation for Economic Cooperation and Development).[27,28] Parallel advances in our ability to manipulate DNA and gene/pathway engineering have enabled construction of synthetic tissues, immune cells and viruses for targeting specific disease cells, synthetic drug delivery vectors, and tumor-targeting bacteria, a few recent successes of systems biology approaches (reviewed in Refs 29,30). However, it is clear that there is no immediate or simple solution to the complex challenge of how best to integrate and apply novel data streams in toxicological evaluations and risk assessments of toxicants or newly developed drugs to understand systems-level responses. Nevertheless, in the context of nanotoxicology, application of these systems biology tools enables the generation of hypotheses to further inform experimental designs for follow-up testing. In the context of nanomedicine, they allow investigation of the global interactions between drugs and the biological and macromolecular environment, thereby supporting targeted drug discovery, drug toxicity and safety assessment, and the development of other molecular diagnostic approaches.

The other important area of application for systems biology is in the field of personalized nanomedicine, which is an offshoot of personalized medicine (defined as a predictive and preventive approach with an individual patient at its core) that combines nanotechnology, bioinformatics, and patient/individual-specific molecular and physiological information to achieve the best medical outcome for a specific individual. Because of their noninvasiveness, improved half-lives, retention time, and higher specificity or targeting efficiency causing fewer side-effects, nano-enabled biomaterials, drug carriers, and sensor devices are rapidly being implemented in diagnosis and personalized treatments.[31] However, successful implementation of personalized medicine requires a thorough understanding of the interactions between the nanosurfaces and the biological surrounding at both individual and broader population levels. Moreover, as stated earlier, fundamental knowledge of what is normal, how deviations from normal at molecular levels (i.e., changes

in DNA, RNA, proteins, metabolites, and their interactions at different levels of organizations) lead to a disease, and how these various processes are influenced by nanomaterials is important. This information can be derived using systems biology tools. For example, individual responses to cues from the environment are based on the digital codes of our genome. With the progression in sequencing methodologies, it is now possible to sequence genomes and transcriptomes of individuals and families,[32] specifically identifying the relevant genomic changes that render an individual susceptible to disease. Such information may now constitute an individual's medical record, leading to: (1) deeper understanding of the genetic makeup of the individual; (2) understanding of how an individual might respond to a specific stimulus or treatment with a drug; and (3) identification of the individual's susceptibility to drug sensitivity or diseases. Such comprehensive information linking genetics with genomic observations, together with the versatility of nanomaterial applications, can then be employed to inform individually tailored health care,[33] where nanotechnology and noninvasive nanomedicines can play a significant role.

THE NEXT CHALLENGES

However important and promising nanotechnology might be in treating complex diseases such as cancer, and revolutionizing the individualized health care system, the dichotomy of the nanotechnology and nanotoxicology paradigms must be given proper considerations. Whereas exposure of living organisms to specific concentrations of nanomaterials leads to adverse outcomes, targeted delivery of the same nanomaterials loaded with payload to cancerous cells can prolong life expectancy for cancer patients. Thus, there is continuing need for more research to understand the mechanisms of nanomaterial uptake, interaction with the biological moieties in the surrounding environment, and toxicity, which can then inform 'safe by design' leading to safer nanomaterials for applications in the medical field. Although the sheer number of nanomaterials may be a significant challenge, with systems biology approaches at its core, it is feasible to build smarter, more efficient molecular and computational screening tools to overcome some of the hurdles. However, systems biology itself must overcome its own challenges to realize these goals.

As described above, although systems level approaches are not fully developed at present, the scientific community can now assess individual parts of the system very precisely, accurately, rapidly, and comprehensively. Indeed, high-content omic technologies are now used to generate terabytes of data within a single experiment. Assessing and controlling the quality and reproducibility of the data, and organizing and interpreting big data generated by each of these systems, are the next challenges facing the systems biology community.[34] The scale of the data generated from each of the parts of the system is colossal and requires collaboration across disciplines for correct synthesis and interpretation (e.g., biology, toxicology, genomics, bioinformatics, computer science, and mathematics). The analytical scale of this task (analyzing and integrating big data from different components) to understand the biology perturbed following exposure to a stimulus or otherwise is intimidating to say the least. Intelligent infrastructures for data curation, data mining, visualization, interpretation, and presentation of big data in individual areas such as genomics, proteomics, and metabolomics have been established. However, the field presently lacks the infrastructure and tools that are capable of amalgamating the data across hierarchical levels such as cells, tissues, organs, and individuals that is essential to gain the holistic view required to develop predictive toxicology computational systems-level models (the ultimate goal in toxicology).

In conclusion, programmable nanomaterials offer great promise to the field of medicine. However, before nanomedicine is routinely integrated into mainstream therapeutics, a variety of key gaps must be addressed. Seven areas were identified as critical priorities in this field by attendees of a workshop organized by the US Food and Drug Administration and the Alliance for NanoHealth in 2008[35] that remain as priorities to date and include: (1,2) the development of imaging technologies and determination of the distribution of nano-vehicles in the body upon their systemic administration; (3) the biological affinity of nanodrugs, the pathways by which they are internalized, retention time, and their ability to translocate across barriers; (4, 5) the development of new computational models for predicting the human health risks of nanomaterial exposures; (6) the establishment of consensus toxicity testing protocols; and (7) understanding the unanticipated secondary effects. While some efforts have been made to address these priorities over the past decade, the potential of nanomaterials to cause harm to humans and the environment remain largely undetermined, undermining opportunities for regulatory approval and commercialisation of nanomedicines. As stated

by Sanhai et al.[35] for meaningful and effective translation into benefits for patients, innovation in this area must apply the pillars of evidence-based medicine in parallel with predictive molecular toxicology paradigms that can be built with the aid of systems biology thinking.

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