

A fresh look at nanomaterials

Dr Robert Tanguay provides an insight into his work on identifying hazards and risks of nanomaterials; research that has the potential to improve and protect human and environmental health

What are the central questions your research seeks to address?

Because whole animal testing is slow, labour intensive and expensive, we have not yet assessed the potential hazard posed by most environmental chemicals or nanomaterials. The situation gets significantly more complex when you consider that real-world exposures are to mixtures of chemicals. It is important to be clear that the absence of toxicity data does not mean these materials necessarily pose a risk, but comprehensive scientifically valid biological response data is needed to begin to prioritise chemicals for more detailed testing. In order to identify whether individual or mixtures of existing chemicals or nanomaterials pose a risk, innovative strategies must be developed to screen the backlog and to keep pace with the newly emerging materials.

We have developed the embryonic zebrafish assay as a rapid sensitive platform to identify whether chemicals or nanomaterials have the potential to adversely interact with biological targets. It is our expectation that using this assay will allow us to rapidly identify the nanomaterial properties required to produce toxicity. The practical application of this approach is to use this information to precisely engineer nanomaterials that are inherently safer. This basic concept is central to the Safer Nanomaterials and Nanomanufacturing Initiative (SNNI) of the Oregon Nanoscience and Microtechnologies Institute.

How can the principles of green chemistry be implemented through research?

Green chemistry is the design of products and processes that reduces or completely eliminates the use or generation of hazardous substances and can be applied during the entire life cycle of a product including design, manufacturing (including energy consumption), and ultimate use. If a researcher was challenged with designing an exciting new nanomaterial for a commercial application and wished to apply green chemistry principles to material design, the researcher would select the least toxic chemical constituents, minimise the use of toxic reagents in the synthesis, reduce waste products from the synthesis, and generate a safe end of life product.

How important do you consider the dissemination of research to the scientific community?

Researchers within SNNI have developed a collaborative knowledgebase of Nanomaterial-Biological Interactions (NBI). This database is

used to organise and warehouse data produced from SNNI research. We collect data and information on the physical and chemical properties of nanomaterials (eg. purity, electronic and photonic properties, size, shape, charge, composition, functionalisation, agglomeration state, etc.), how they are synthesised, their fate and transport in the environment, how they interact with and potentially alter living systems (ie. beneficial, benign or deleterious) and at multiple levels of biological organisation (eg. molecular, cellular, or organismal). We have begun to curate data and information from published literature to enhance our information base. The ultimate goal for the NBI is to provide the scientific foundation to identify structure and design principles of high-performance, environmentally-benign nanomaterials that can be applied to the development of future nanotechnology products.

How will your research contribute to the design of new, innovative, safer and more useful nanomaterials?

Through collaborations with our chemists, we believe we have an excellent opportunity to merge the principles

of green chemistry and nanomaterial design with high throughput sensitive *in vivo* biological testing to identify basic nanomaterial properties that are required to produce given biological responses. For example, an engineer may produce 10 unique nanomaterials in small quantities and each of these materials have valuable inherent properties for a product. With very small quantities of nanomaterials we could assess all 10 of them in just a few days in our sensitive *in vivo* assay. From our data we could identify the least toxic of the 10 and provide information to the chemists, who could move forward in scaling up production of the selected material. The added benefit is that we would now have biological response data from all 10 materials and we could begin to identify relationships between the nanomaterial structures to the observed biologic responses. With sufficient nano-bio data it should be possible to define the basic design rules for inherently safer materials. With the design rules in place, it would be possible to intelligently design the next generation of nano-products to be inherently safer.



Defining nanomaterial-biological interactions

An Oregon-led research team is studying precision-engineered gold nanoparticles; investigations which will help define which structural features of nanomaterials are linked to biological effects

IN THE RAPIDLY changing world of technology, with consumers demanding ever more advanced products, nanotechnology is quickly progressing from the discovery to application stage. This has meant that nanomaterials are now being widely used in commercial products and applications.

One particular challenge for scientists arises in understanding the risks and hazards of different nanomaterials – an area that has not kept up with the rapid rate of change. However, general themes are now emerging as a number of groups are assessing individual nanomaterials in various biological systems. For example, regardless of the biological assay, many nanoparticles are not toxic, and the ones that produce toxicity do so only at very high concentrations. This observation allows more attention to be focused on understanding what makes a given particle toxic. An illustration of this, from the Safer Nanomaterials and Nanomanufacturing Initiative (SNNI) in Oregon, is that scientists have shown that particles which are positively charged are more likely to be toxic.

A collaborative research team within SNNI at the Oregon State University and the University of Oregon are developing and refining these axioms to fuel safer design of nanomaterials and the nanomanufacturing processes. The SNNI research team led by Tanguay and Hutchison are hoping their work will help save resources by confirming those particles which have undesirable qualities, so they can be discarded from the technology development pipeline as early as possible or re-tailored, for example for use in target drug delivery in cancer therapies. Tanguay says that the key is to identify the nanomaterial properties that are necessary to produce toxicity, and just as importantly to identify the biological targets that they act upon to produce toxic responses.

A MATRIX OF NANOPARTICLES

The challenge of optimising both performance and safety in nanomaterials lies in the ability to resolve which structural features lead to the desired properties. It has been difficult to draw meaningful conclusions about biological impacts from many studies of nanomaterials, due to the lack of nanomaterial characterisation,

unknown purity, and/or alteration of the nanomaterials by the biological environment. Tanguay outlines some of the ways they have been addressing this: "To investigate the relative influence of core size, surface chemistry, and charge on nanomaterial toxicity, we tested the biological response of whole animals exposed to a matrix of nine structurally-diverse, precision-engineered gold nanoparticles (AuNPs) of high purity and known composition prepared and characterised by the Hutchison lab. This library of nanoparticles contained three core sizes and four unique surface coatings and positively or negatively charged headgroups. According to Tanguay, the mortality, morbidity, uptake, and elimination of AuNPs were all dependent on these parameters, showing the need for tightly controlled experimental design and nanomaterial characterisation. He believes the results illustrate the value of an integrated approach to identifying design rules that minimise potential hazards.

This simple example demonstrates it will be possible to discriminate between toxic and nontoxic nanoparticles

There are currently many assays being developed to assess the potential hazard of nanomaterials, the most prevalent being the use of cultured cells. Cell culture assays can be performed rapidly and at a reasonable cost, but cultured cells have limited complexity to

respond to insults (injury or trauma). Tanguay explains: "Embryonic development is exquisitely sensitive to chemical stressors because this is the most dynamic period in an organism's life". Embryonic development requires countless interaction between individual macromolecules and cells to achieve normal development.

THE VALUE OF ZEBRAFISH

The real innovation in the team's research work has been assay refinement and rapid throughput assay development. Tanguay's team has chosen the zebrafish (*Danio rerio*) because it offers a number of practical advantages as a model organism. The zebrafish is remarkably similar to humans and other vertebrates in regards to cellular structure, signalling processes, anatomy and physiology, and as such is particularly favourable for high-throughput assays.

Tanguay explains that individual female zebrafish are able to produce hundreds of eggs

INTELLIGENCE

DEFINING NANOMATERIAL-BIOLOGICAL INTERACTIONS TO ENHANCE BIOCOMPATIBILITY

OBJECTIVES

The project aims to utilise a systematic, iterative process of nanomaterial design, synthesis, and toxicity testing to develop inherently safer nanomaterials; specifically, to couple the power of precision engineering of gold nanomaterials with the rapid throughput embryonic zebrafish assay. This will provide essential biological-based information to reveal how nanomaterials interact with and modulate biological activity.

KEY COLLABORATORS

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weekly, so large sample sizes are easily achieved, allowing for statistically powerful dose-response studies. "We can simultaneously assess the toxicity of a large number of substances in a short period," he says. Compared to other models, the development of zebrafish is rapid: the neuronal plate formation for zebrafish occurs at 10 hours post fertilisation (hpf), followed by organogenesis at 24 hpf, as compared to a rat which occurs at 9.5 days and 5-6 days respectively, and the first heartbeat occurs at 30 hpf for the zebrafish and 10.2 days for rats. In addition, zebrafish are very small and can be individually exposed in wells of a multi-well plate, so the required volume needed for the model is small; thus, only limited amounts of materials are needed for toxicity studies. As Tanguay describes: "Zebrafish embryos develop externally and are optically transparent, so it is possible to resolve individual cells throughout the duration of an exposure using simple microscopic techniques and numerous effects can be assessed non-invasively over the course of development".

A GOLDEN FUTURE

Safe and practical applications of gold nanomaterial-derived products are now rapidly being developed for a variety of fields including medicine; functionalised gold nanomaterials are being produced for chemotherapy and medical diagnostics, because gold as an element has been shown to be safe. Through collaboration with the Hutchison group, Tanguay's research team has demonstrated proof of the concept that they can take an integrated approach to design, test and redesign of nanomaterials that are inherently safer. This is particularly beneficial for the technology and health industries.

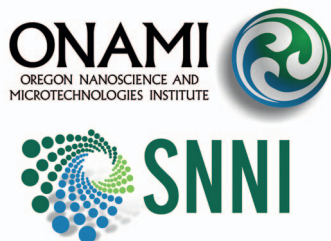
Hutchison's team synthesised three different sizes of gold core nanoparticles (0.8, 1.5 and 10 nm), each with a specific placement of positively, negatively or neutrally charged functionalised chemical groups. Under the electron microscope, particles with the same core size but with different functionalised groups look identical. Other characterisation methods are used to measure purity and important surface properties. Using these precision-engineered nanomaterials in their bioassays, Tanguay's group found that when the functional groups were positively charged, embryo lethality resulted. However, when the functional groups were negatively charged, fatality did not result but embryonic development was significantly altered by the exposure. Tanguay explains: "When the functional groups were uncharged, regardless of the concentration evaluated, the animals were completely unaffected. This simple example demonstrates it will be possible

to discriminate between toxic and nontoxic nanoparticles".

COLLABORATION LEADS TO SUCCESS

This innovative research demands input from a number of disciplines and the team has drawn on a range of technical skills, from chemical syntheses to toxicological assessment in whole animal bioassays. Embryonic zebrafish are used to assess the biological, physiological, behavioural and molecular responses to nanomaterial exposure, as well as behavioural assessment of adult zebrafish exposed to nanomaterials early in their development. The team is highly skilled in assessing nanomaterial purity and composition and biological impacts. In particular, Tanguay's team is exploring gene expression changes in response to nanomaterial exposure, microscopic analysis of biodistribution of nanomaterials and dynamic light scattering to assess nanomaterial size distribution. In collaboration with Hutchison's team, they are using techniques such as transmission electron microscopy and scanning electron microscopy to evaluate nanomaterial shape and size distribution, and techniques such as NMR, MALDI, XPS, UV/VIS and TGA to evaluate purity and composition. Informatics is integral to support development of structure-activity relationships for nanomaterials and mechanical automation of toxicological assessments, thus they are developing a comprehensive nanomaterials-biological interactions (NBI) knowledgebase in collaboration with Dr Stacey Harper at Oregon State University. The NBI serves as a repository for annotated data on nanomaterial characterisation, synthesis methods, and nanomaterial-biological interactions. Computational and data mining tools are also being developed to provide a logical framework to conduct species, route, dose, and scenario extrapolations and identify key data required to predict the biological interactions of nanomaterials.

Tanguay says the team takes an interdisciplinary approach daily to leverage data, information and knowledge from experts in diverse fields: "Combining the information from each research programme allows us to begin to describe how the physical and chemical features of a material may drive its fate in the environment and its impact on living systems". For example, a chemist who synthesises nanomaterials will have intimate knowledge of the chemistry and physical properties of those materials, while a toxicologist will have expert knowledge to interpret results of studies to determine the biological interactions of those materials. In working on assessing the effects of nanomaterials on biological systems, Tanguay believes collaboration to be imperative in this highly interdisciplinary work.



LARGE GROUP OF ZEBRAFISH EMBRYOS JUST 15 HOURS OF AGE