

## Perspective on Current Alternatives in Nanotoxicology Research

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**T**HE EVOLUTION OF NANOTECHNOLOGY proceeds at an unprecedented rate due to major investments in research and development from public and private sectors. Incorporation of engineered nanomaterials (ENMs) into novel or replacement technologies has impacted a diverse number of industrial, commercial, consumer, and health-care services and products. Use of ENMs in many industries has, however, raised concerns with all routes of exposure (dermal, oral, inhalation, and parenteral) in occupational, consumer, and environmental settings. Given the sheer number, diversity, and wide use of ENMs, toxicology studies are unable to keep pace. Risk-assessment frameworks specific to nanomaterials that incorporate alternative testing have been proposed. However, most of the past and current models depend heavily on mammalian animal model testing.<sup>1,2</sup> These time- and resource-intensive studies, although informative, are clearly unable to assess the avalanche of ENM-enabled technologies and the wide range of exposures that may result. This fact, coupled with general societal pressure to reduce animal use, has resulted in calls for tiered and integrative testing strategies using high-throughput *in silico*, *in vitro*, and other alternative models to screen and assess ENMs along their chemical life cycle.<sup>3–6</sup>

Major goals of the OECD,<sup>7</sup> ENPRA,<sup>8</sup> Nano GO,<sup>9</sup> and NNI NEHI<sup>10</sup> working groups for nanotoxicology testing are the development and use of predictive models. Research has shown that the inherent small size, large surface area, and other unique physical and chemical properties of ENMs result in adsorption, distribution, metabolism, and excretion (ADME) and biological responses not observed in their larger counterpart materials. Toxicity responses following exposure can occur earlier (or on longer time frames), at lower doses, and in other organs far removed from the original site of exposure that are not observed with their larger counterparts. Interactions with biomolecules and organism environment can drastically change an ENM's physical (size, shape) and chemical (solubility) properties, resulting in differences in particle fate, ADME, and potential biological response, including novel effects on immune, cardiovascular, development (e.g., stem cell), reproductive, and neurological systems.<sup>11–16</sup>

These difficulties represent a major challenge for 21st-century toxicology and safe-by-design material development. Critical considerations for toxicological assessments include robust material characterization, transformations along an ENM's life cycle, understanding of potential particle transfor-

mations in different biological mediums (e.g., biocorona, solubilization, etc.), modeling and use of appropriate deposited dose and dose metrics,<sup>17</sup> appropriate *in vitro* model for both exposure and response that aligns with *in vivo* adverse outcome, and increased development and use of standardized assays for read-across purposes.<sup>6–8</sup>

Current advances in machine learning, *in silico*, *in vitro*, and tissue model techniques have placed predictive modeling of the *in vivo* response with an integrative alternative tiered toxicity testing strategy a potential attainable long-term goal for nanotechnology risk assessment.<sup>13,18</sup> Acellular assays, *in vitro* assays, and advances in “omics” coupled with computational modeling may provide a suite of biomarkers for high-throughput screening assays predictive of *in vivo* toxicity.<sup>19</sup> Further model and method development in complex cell and tissue culture systems, including air–liquid interface culture,<sup>20</sup> cellular co- and tri-cultures, biocorona and ADME systemic toxicology, organ-on-a-chip, and other alternative animal models,<sup>21</sup> has placed *in vitro* screening techniques at the forefront of nanotoxicology testing for responsible nanotechnology development. This special issue highlights three research papers that tackle several of these issues utilizing alternative approaches.

Nanomaterials encounter a milieu of proteins and lipids upon *in vivo* exposure, resulting in a unique surface biocorona that can change the surface properties of the material and influence its fate and biological reactivity.<sup>22</sup> Numerous studies have investigated this phenomenon, with the majority of the work focused on protein binding and using controlled or healthy model systems. Little information exists, however, on how biocorona formation differs in unhealthy, altered, or potentially compromised biological systems, such as preexisting disease states or genetic differences. A study by Kobos et al. investigates this possibility by incubating two different types of gold nanoparticles (AuNPs) in human serum followed by (1) an integrated proteomic/lipidomic analysis of the biocorona and (2) screening of human macrophage response upon exposure. Serum from obese individuals resulted in low lipid content in AuNP biocorona and a differential macrophage response compared to serum from healthy individuals. Improved screening techniques on human sera collected from different human subpopulations with specific comorbidities could help improve understanding of innate immune response and improve prediction of clinical outcomes.

Next, Kraeling et al. investigated how differing spherical dendrimers, along with changes in terminal chemistry and surface charge, impact their delivery into skin using either viable pig skin or human cadaver skin in a diffusion apparatus. The harvest of several samples of viable pig skin following euthanasia both minimizes pain and distress of *in vivo* dermal penetration studies and reduces the number of animals needed for studies, while maintaining the benefit of a physiologically viable and intact organ system. Dendrimers with highly branched and stable polymeric structures provide numerous options for skin delivery of either drugs or cosmetics by altering their physicochemical characteristics. In screening four different generations of polyamidoamine dendrimers with different terminal group function and surface charges with confocal fluorescent microscopy and radioactive labeling, a generation four dendrimer conjugated to glycolic acid resulted in a sixfold increase in stratum corneum uptake with dermal penetration compared to other generations and glycidol functionalization. Conducting cross-species comparisons on single and repeated doses of dendrimers with different physicochemical properties holds promise as a model to assess safety of these NPs in future drug and cosmetic development.

Lastly, use of human-derived stem-cell models for *in vitro* screening and predictive studies has gained traction due to stem cells' key roles in differentiation, development, immune response, wound repair, and xenobiotic insult, with potentially large impacts.<sup>23–25</sup> Nguyen et al. investigated how sub-toxic silver NP (AgNP) exposure impacts osteogenic differentiation of bone-marrow stem cells. AgNPs are increasingly used in the clinic for antimicrobial coatings on orthopedic devices and treatments during wound healing. Using acute and sub-chronic AgNP exposures at sub-toxic doses, the authors report that bone-marrow mesenchymal stem cells failed to show osteogenic differentiation that was dose and osteogenic growth factor dependent. With the increase in novel materials for biomedical implants, similar studies could be employed to screen and possibly predict tissue-level effects to key sensitive cell types.

To date, much progress has been made in improving *in silico*, *in vitro*, and *ex vivo* model systems with increased complexity and with promising predictivity of tissue-level responses. Minimally invasive collection of complex animal tissues for screening approaches to understand NP fate and bio-reactivity can help bridge the large divide between simple *in vitro* screening systems and whole-tissue or organ-system responses. However, we are increasingly learning that biological systemic responses and toxicities (e.g., adaptive immune, cardiovascular, and neurological responses) can occur systemically to the original portal of entry.<sup>26</sup> As alternative models improve their predictivity of tissue-level responses, organ-level and systemic responses remain a long-term goal and will require novel integration of a number of innovative approaches to get there.

#### Disclaimer

The views expressed here are those of the authors and do not necessarily reflect those of the National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention.

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