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Review

The rise of nanotoxicology: A successful collaboration between engineering and biology

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Abstract: The field of nanotechnology has grown exponentially in the last decade, due to increasing capabilities in material science which allows for the precise and reproducible synthesis of nanomaterials (NMs). However, the unique physicochemical properties of NMs that make them attractive for nanotechnological applications also introduce serious health and safety concerns; thus giving rise to the field of nanotoxicology. Initial efforts focused on evaluating the toxic potential of NMs, however, it became clear that due to their distinctive characteristics it was necessary to design and develop new assessment metrics. Through a prolific collaboration, engineering practices and principles were applied to nanotoxicology in order to accurately evaluate NM behavior, characterize the nano-cellular interface, and measure biological responses within a cellular environment. This review discusses three major areas in which the field of nanotoxicology progressed as a result of a strong engineering-biology partnership: 1) the establishment of standardized characterization tools and techniques, 2) the examination of NM dosimetry and the development of mathematical, predictive models, and 3) the generation of physiologically relevant exposure systems that incorporate fluid dynamics and high-throughput mechanisms. The goal of this review is to highlight the multidisciplinary efforts behind the successes of nanotoxicology and celebrate the partnerships that have emerged from this research field.

Keywords: nanotoxicology; dosimetry; characterization; in vitro exposure models

1. The Development of Nanotechnology and Nanotoxicology

In the last decade, the field of nanotechnology has risen to the forefront of research and

development, with applications extending into the medical, energy, consumer, and industrial sectors. Nanotechnology exploits the unique physicochemical properties of nanomaterials (NMs), which possess at least one dimension less than 100 nm in size. NMs are unique from their bulk counterparts due to distinctive physicochemical properties such as an enhanced surface area to volume ratio, tunable size and morphology, and the ability to be functionalized with numerous chemical moieties [1]. As a result of these properties, NMs display novel behaviors which make them advantageous for applications, such as increased intracellular transport and delivery, enhanced reactivity, augmented catalytic activities, and unique spectral and optical properties [2]. Currently, there are over 1,800 NM-containing products in production [3], highlighting the rapid development of nanotechnology and the fact that the NMs are becoming a permanent component in consumer goods and applications.

While nanotechnology has made significant progress and growth, one considerable drawback is that some NMs have been shown to induce negative cellular consequences following exposure [1]. When NMs are introduced into a biological system, the resulting response can range from no effect, to a minor stress induction, to significant cytotoxicity, based on their respective physiochemical properties [4,5]. The ability of NMs to induce a detrimental cellular consequence gave rise to the field of nanotoxicology, which evaluates the safety of and monitors health risks associated with NMs [6]. Nanotoxicology has grown and matured tremendously since its inception and has provided the scientific community a baseline of NM-induced responses. However, nanotoxicology suffered early difficulties due to challenges including but not limited to, an inability to accurately measure target physicochemical properties, non-standardized procedures, a lack of NM-specific controls, and inadequate characterization in peer-reviewed publications. In order to address the characterization concerns, an engineering-biology collaboration was formed. This partnership effectively merged key engineering aspects and design tools with intensive biological-based safety assessments, thereby transitioning the field of nanotoxicology into its next research era.

2. The Merging of Engineering and Nanotoxicology

2.1. Research challenges associated with NMs

From early nanotoxicological investigations it became apparent that due to their unique physicochemical properties, NMs behaved differently from traditional chemicals. As such, when examining the toxicity of NMs, additional experimentation metrics and controls were required to accurately assess their biological impacts. For example, most NMs are insoluble by nature, and while they can be dispersed and remain stable within a solution, some NM agglomerates can sediment out [7]. Therefore, NM transport is not solely dependent upon gradient-based diffusion, as for traditional chemicals [8]. Moreover, the majority of NMs have also been shown to agglomerate, due in part to their innate surface charge and inter-particle interactions [9]. The ability to form larger aggregates has presented a significant problem with regards to dosimetry, thereby complicating NM toxicity assessments [10]. Additionally, most metallic NMs undergo ionic dissolution, in which ions are generated from the surface of the individual particles due to an oxidation process [11]. Not only does this process produce a second toxicological influence, as both the NMs themselves and produced ions have been associated with stress and cytotoxicity, but introduces modifications to NM properties as a function of time [12]. When introduced into a biological system NMs bind local biomolecules and proteins, thereby forming a “protein corona” which has been shown to alter behavior and modify

toxicity [13]. The protein corona presents a novel challenge that needs to be addressed, as no two environments will possess the same biomolecular and protein profiles; meaning that the corona is highly variable and impossible to predict.

As NMs do not possess the same characteristics as traditional chemicals, challenges arise in executing standardized toxicological assessments and analyses. Given that NMs are smaller than the wavelength of visible light, their presence is able to interfere with absorbance- and fluorescent-based measurements. Additionally, many NMs possess an intrinsic fluorescence or are able to interact with surrounding molecules, including assay reagents, possibly introducing error to experimental analyses [14]. Taken together, these complications have produced significant inaccuracies and erroneous toxicological conclusions. For example, NMs are known to disrupt the standard, colorimetric [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) viability assessment by altering the solution color [15]. This NM-induced color shift, in particular with silver NMs, produced erroneous data and in some instances concealed toxicity [16]. In order to overcome these challenges, the current approach is to include multiple NM controls to account for any unintentional interactions or color change. Ideally, however, new assessments and strategies should be devised in order to standardize nanotoxicological endpoints and safety metrics.

2.2. Integrating engineering into nanotoxicology protocols

Early nanotoxicological investigations focused on exploring the ability of NMs to induce a toxic response within a standard in vitro model, which consists of a monoculture grown on a flat tissue culture dish in the presence of biological medium. However, as research into this area progressed it became apparent that experimental design needed to account for NM-introduced complications, such as their ability to agglomerate and sediment, and to correct characterization constraints plaguing the field. As NM synthesis was traditionally carried out in engineering or material science laboratories, their involvement in nanotoxicological investigations was not only convenient, but strategic. By integrating engineering fundamentals into experimental design and inquiry, this partnership launched the next, prolific phase of nanotoxicological research. This phase focused on improving characterization techniques, correlating physicochemical properties to observed bioresponses, devising deposition/dosimetry models, and generating physiologically-relevant exposure systems [6]. In this review, each of these developments will be discussed with a focus on how the successful merging of biology and engineering fundamentals supported nanotoxicological efforts.

3. Characterization of NM Properties and Behavior

3.1. The need for NM characterization

As previously discussed, NMs display differential behavior from traditional chemicals and toxins. To complicate matters, NM-induced responses are highly dependent upon target physicochemical properties, making NM characterization a necessity. Extensive work has been carried to correlate NM properties with observed toxicological effects, with some common themes emerging [17]. The key physical parameters that have been found to play a predominant role in determining the biocompatibility or toxicity of NMs include core composition [18], primary particle size [19], surface chemistry [20], porosity [21], surface charge [22], and morphology [23] (Figure 1).

For example, when comparing NMs with different primary sizes, smaller particles have been found to induce greater stress and toxicity than their larger counterparts, due to a higher degree of NM internalization [19]. Surface charge, which is determined by synthesis procedures, solution composition, and functionalization [24], is a key mediator of NM-cellular interactions and cytotoxicity, with positively charged NMs increasing toxicological effects [22]. Additionally, porous NMs have been linked with higher acute toxicity over their non-porous counterparts, due to greater available surface area [21]. Correlating physicochemical properties to observed biological effects has been a major nanotoxicological effort and the conclusions to date have been the focus of multiple review papers [17,25]. The undeniable conclusion is that the distinctive properties of each novel NM set will play a role in their induced-cellular responses, highlighting the importance of proper characterization.

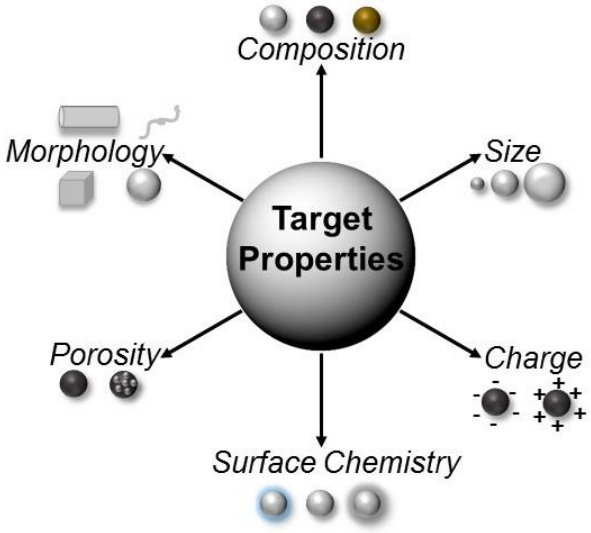

Figure 1. Identification of key NM physicochemical properties involved in nanotoxicity. Extensive efforts have been carried out that successfully identify the role of target physicochemical properties, such as core composition, primary particle size, surface charge and chemistry, porosity, and shape to NM-induced cellular consequences.

3.2. Characterization techniques

The development and employment of NM characterization techniques provided a major impact to the field of nanotoxicology as it allowed for the correlation between measured properties and observed bioresponses. The inclusion of NM characterization improved the scientific quality of individual investigations and allowed for a means of direct comparison between studies [26]. While it has become accepted that NM characterization is critical for nanotoxicology, the standardization of characterization efforts has yet to become a full reality.

A number of experimental tools and techniques have been employed to accurately assess the properties of NMs [27,28]. A succinct list of critical characterization techniques and their target endpoints are included in Table 1. Transmission electron microscopy (TEM) is a valuable asset to determine NM primary particle size and morphology following their drying on a copper grid [29].

Brunauer-Emmett-Teller (BET) analysis is a means to quantify NM surface area and can thereby be used to calculate the surface area to volume ratio [30]. Through the addition of an energy dispersive x-ray analysis (EDAX) attachment to TEM or scanning electron microscopy (SEM) machines, particle composition can be verified, either alone or within a cellular system [31]. Additionally, due to its high resolution, EDAX is able identify the composition of heterogeneous samples, making it an important tool for efforts into areas such as identification of NM air pollution [32]. Particle tracking through dynamic light scattering (DLS) and zeta potential analyses are used to determine the extent of agglomeration and surface charge in solution, respectively [33]. The agglomerate size of NMs has proven to be a critical physicochemical property as it influences particle stability in solution, mode of biotransport, and degree of NM-internalization [34]. Ultraviolet-visible (UV-VIS) identifies the NM's spectral signature, which varies as a function of composition, shape, and surface chemistry [35]. By application of the Beer-Lambert law, UV-VIS can be used to determine NM concentration within a solution. Additionally, by monitoring the number, wavelength, and width of spectral peaks over time it is possible to visualize if NMs are agglomerating or becoming unstable in solution [36]. Fourier transform infrared (FTIR) spectroscopy measures infrared intensity versus light wavelength. In addition to elucidating NM optical properties, FTIR is a means to determine and verify the functional groups attached to the NM surface [37].

Table 1. NM characterization techniques.

Characterization tool	Abbreviation	NM property
Transmission electron microscopy	TEM	Size and morphology [29]
Scanning electron microscopy	SEM	Size and morphology [31]
Brunauer-Emmett-Teller	BET	Surface area [30]
Energy dispersive x-ray	EDAX	Composition [31]
Atomic force microscopy	AFM	Size and morphology [38]
Dynamic light scattering	DLS	Agglomeration and uniformity [33]
Zeta potential	Z	Surface charge [33]
Ultraviolet-visible spectroscopy	UV-VIS	Spectral profile and purity [36]
Fourier transform infrared spectroscopy	FTIR	Surface functionalization [37]

3.3. Kinetic evaluation of NM properties

Recently, characterization efforts have emerged that are monitoring the time-dependent, or kinetic, properties of NMs, as physicochemical parameters can change over time. These preliminary efforts have identified 1) the degree of NM agglomeration and 2) the rate of ionic dissolution as the physicochemical properties which play a predominant role in nanotoxicological responses [17,25,39]. It has been shown that the extent of NM agglomeration can alter over time, potentially impacting biotransport rates, deposition, and the nano-cellular interface [40]. The production of metallic ions from NM surfaces, or ionic dissolution, has been acknowledged as a chief mechanism for NM-induced toxicity [12]. Studies have also identified that the kinetic rate of ion generation varies as a function of time and particle size, again highlighting the complexity of characterizing this phenomenon [41].

The evaluation of NM behavior becomes more complex when one considers that these traits are able to modify original physicochemical properties, such as primary size [9]. Therefore, it is

becoming increasingly important to characterize NMs periodically throughout a study, in order to identify if and to what extent properties are being modified [42]. To further complicate matters, reports have identified that NM behavior is strongly dependent upon environmental factors, such as fluid composition [9,34]. Following dispersion in numerous physiological fluids, such as lysosomal and interstitial, both final agglomerate sizes and the rate of ion production were significantly varied [9,13,34]. As these behavioral traits have been directly correlated to NM deposition and cytotoxicity, respectively, modification to agglomeration and dissolution will ultimately impact subsequent nanotoxicological profiles.

4. Understanding and Predicting NM Dosimetry

4.1. Understanding the complexities of NM deposition and dosimetry

What is true for all chemicals is that the dose makes the poison. Even water, which is required for life, can be toxic if too much is consumed [43]. Therefore, dosimetry is a crucial consideration and must be carefully designed and carried out to accurately interpret results. As NM deposition has been successfully correlated to cellular stress and toxicity [44], dosimetry selection and analysis has become increasingly important to current nanotoxicological efforts. Dosimetry analyses, which determine the amount or concentration of NM that interacts with the cellular system, involves complex analyses and the development of predictive modeling capabilities. Therefore, this is an emerging area that has thrived off the engineering – toxicology partnership.

In order to carry out a dosimetry analysis, it is necessary to quantify three different but interconnected dosages (Figure 2): administered, solution, and deposited [45]. The administered, or experimental, dose is the NM concentration that was added to the exposure system. The solution and deposited dosages are the NM concentrations within the fluid supernatant and deposited on the cell surface, respectively. The evaluation of these NM dosages can be carried out through inductively coupled plasma mass spectrometry (ICP-MS) analysis [46]. Dosimetry data is often presented as deposition, expressed as either a NM amount or as a percent of administered dose.

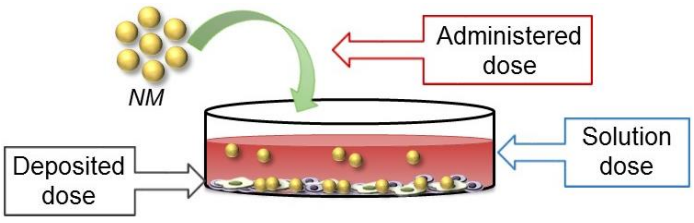

Figure 2. Breakdown of NM dosimetry. Three different dosimetric aspects are considered during analysis, the administered, solution, and deposited dosages. By determining these dosages and elucidating how they are dependent upon physiochemical and environmental properties, it will be possible to gain a better understanding of NM pharmacokinetic behaviors.

The deposited NM dosage can be further broken down into the internalized and extracellular dosages. Following binding to the cellular membrane, the NMs can either remain on the surface or

enter the cell through a number of endocytosis or direct diffusion mechanisms. While both surface bound and internalized NMs have the potential to induce cellular consequences, the accumulated, intracellular particles are believed to be the driving force for observed nanotoxicological outcomes [47]. These responses are due, in part, to the increased rate of ionic dissolution in lysosomes and a higher probability of NMs interacting with and disrupting essential cellular processes [47,48].

There are several techniques currently available to quantify the accumulated NM dosage for metal or metal oxide particles, including ICP-MS, TEM/SEM, and confocal imaging in conjunction with fluorescently tagged NMs [46,49,50]. Back-scattered electrons (BSE) imaging attached to an SEM has become the preferred imaging technique for intracellular NM identification due to the ability to increase the contrast between organic (cellular) and inorganic (metal-based NM) materials [51]. However, the evaluation of carbon NMs, such as carbon nanotubes (CNTs), poses a significant challenge as the cellular carbon content makes ICP-MS analysis unreliable. As CNTs are renowned for their mechanical and electrical properties, they are being employed in numerous applications including electrode design, composites, coatings, microelectronics, and energy storage devices [52]. However, methods are currently being explored to accurately determine the extent of carbon-NM accumulation within cellular systems, including multispectral imaging flow cytometry [53] and gel electrophoresis in conjunction with Raman spectroscopy [54]. These methods for carbon-based NMs, along with ICP-MS for metal-based NMs, allow for a direct correlation between intracellular NM accumulation and observed nanotoxicological effects: further strengthening the understanding of NM pharmacokinetics.

4.2. Development of predictive dosimetry mathematical models

As consensus is rising that nanotoxicology reports should be based upon deposited or accumulated dose, and not the administered concentration, an emerging priority for the field is the development of a mathematical model to accurately predict NM deposition [55]. The development of accurate pharmacokinetic models, based on NM physicochemical properties and environmental factors, would be greatly advantageous for the design of nano-based biomedical applications. To date, correct predictions of NM dosimetry has presented a major challenge, due to the complications that accompany working with NMs [56]. Of direct relevance to deposition rates is the ability of NMs to agglomerate and sediment out of solution. This gravitational force disrupts the normal gradient-based diffusion that most chemicals exhibit, making the accurate generation of pharmacokinetic profiles following NM exposure unlike any previously examined compound. To further complicate the issue, physicochemical properties and environmental variables are known to alter agglomeration and sedimentation [57–59]. Taken together, these challenges suggest that a complex, comprehensive model is needed, as a generic pharmacokinetic model will be unable to capture the true NM delivered dosage.

The arduous task of designing, constructing, and validating this dosimetry model has been tackled by a small number of researchers focused on developing an improved means for assessing NM deposition. Significant progress has been achieved in both understanding the required elements and the development of complex mathematical models. The theoretical in vitro sedimentation and diffusion deposition (ISDD) model was one of the first to successfully predict kinetic rates of NM deposition [10,60]. Building off this early platform, additional models were developed and implemented with great success. These subsequent models incorporated alternative media

environments [61], standardized the NM suspension process for density calculations [62], and constructed a data-driven transfer function via TEM analysis [63]. While these models slightly vary, they all incorporate similar experimental values that must be previously obtained, including particle size distribution, fractal dimension, NM permeability, agglomerate density, and exposure fluid characteristics. As NMs have the ability to sediment, which is a driving bio-transport mechanism, the evaluation of agglomerate density and sedimentation is a predominant influence in the mathematical prediction of deposition [64]. However, one significant drawback associated with these models is making these mathematically intensive methods more approachable and user friendly to a larger audience.

Beyond the development of pharmacokinetic models for NM dosimetry, extensive efforts have focused on predictive toxicity assessments. Quantitative structure-activity relationship (QSAR) methods have sought to correlate NM physicochemical properties to toxicity, in order to establish a computational framework for the prediction of NM toxicity [65]. These models are founded in the fact that NM structure and properties, or descriptors, directly influence the nano-cellular interface and biological responses [66]. To date, QSAR analyses have focused on the correlation between NM composition, aggregate size, and degree of toxicity [66,67]. Additionally, molecular dynamics (MD) have proven to be a powerful tool for the prediction and analysis of the nano-cellular interface and NM-internalization mechanisms [68]. For example, a recent MD study examined the mechanical responses at the interface of a cellular lipid membrane and graphene under different pressures and applied forces [69]. In addition to the discussed dosimetry models, QSAR and MD applications will be assets moving forward in the understanding of the complex nano-cellular interface and the development of NM safety guidelines.

5. The Development of Complex, Engineered NM Exposure Systems

5.1. The need for improved biological models for NM evaluation

Novel classes of NMs and nano-based products are being generated at a pace far surpassing current capabilities to screen for potential hazards and to evaluate application effectiveness [70]. While these NM assessments can be carried out in either in vitro or in vivo models, most evaluations are performed in vitro due to the innate advantages of experimental flexibility, rapid performance, and lower cost. Currently, one considerable drawback associated with traditional in vitro models is the lack of physiological relevance, as cell-based systems possess little to no resemblance of animal models. This lack of biological agreement between in vitro and in vivo models has resulted in poor correlations and conflicting results between cell-based and animal NM toxicity assessments [71,72]. For example, a recent study identified that polymeric NMs induced significant cytotoxicity within an in vitro model, but uncovered no discernable responses within rats [73].

These model-based discrepancies arise from a combination of particle and biological factors. Firstly, cell-based in vitro and animal-based in vivo models are vastly different: cell-based are static and utilize culture medium whereas animal-based are dynamic and encompass a variety of physiological fluids. Moreover, NM characteristics, including degree of agglomeration and rate of ionic dissolution, are a function of environment, and as such will exhibit altered characteristics between these two models [34,41,57,74]. Therefore, there is a critical need to develop enhanced cellular models that preserve in vitro advantages while incorporating in vivo influences to produce a

more realistic and relevant NM exposure scenario.

5.2. Incorporating physiological variations into in vitro exposure models

By transforming traditional in vitro systems to incorporate in vivo influences, a more accurate and relevant biological model can be generated. Nanotoxicology is currently in need of improved exposure systems that are capable of large-scale processing and analysis, to account for the large number of experimental NMs [70]. One current effort to improve cell systems is to generate an immune inclusive co-culture model, allowing for assessment of immune activation – a key biological endpoint frequently overlooked in in vitro investigations. Biologically-speaking, it is possible to elicit a strong inflammatory and immune response even in the absence of cell death, which could lead to long term health concerns, including neurological diseases and cancer [75]. Recent studies have uncovered an active immune/inflammatory response following NM exposure without any cytotoxicity, demonstrating their capability to induce cellular disruption and introduce potential long-term health concerns [76,77].

It is also possible to alter the immediate physical environment to reflect complex in vivo surroundings. For example, the inclusion of physiological fluids, either artificially synthesized or procured from an in vivo source, replicates an environment that NMs will likely encounter [9,34]. Studies have identified that when NMs are dispersed in biological fluids both their behavior and the resultant nano-cellular interface is modified. For example, following exposure to artificial biological fluids, gold nanorods underwent significant agglomeration, resulting in a loss of particle stability and photothermal efficiency [57].

Moreover, the cardiovascular system surrounds all tissue and produces either direct or indirect fluid movement, whereas current in vitro techniques are static; neglecting this significant physiological influence. Initial dynamic studies concluded that the presence of an additional transport force, lateral fluid flow, altered the balance between NM diffusion and sedimentation. This adjustment introduced significant variations to observed dosimetry, the surrounding protein corona, particle deposition, and resultant bioresponses [13,34,78,79] Of importance is the fact that dynamic flow within an in vitro environment was linked to a drop in rates of NM deposition. Taken together, inclusion of physiological enhancements, such as co-culture systems, biological fluids, and dynamic movement, allows for the identification of NM behaviors and consequences unobtainable in standard in vitro models.

5.3. Design of enhanced and high-throughput devices

Previously, in this review, the unique challenges associated with evaluating the behavior and safety of NMs were discussed. These distinctive physicochemical properties produced non-standard testing metrics, which are highly dependent upon local influences. Therefore, to accurately assess NM-induced cellular responses, we need to not only incorporate key physiological influences, but design controlled exposure systems. For example, inhalation is one of the predominant mechanisms of NM exposure in the real world. During exposure, NMs remain suspended in air within the lungs and first encounter a fluid when deposited onto the alveolus. When considering the external influences and exposure route involved in inhalation, a standard in vitro model is not relevant or appropriate. Instead, aerosol chambers have been developed in which NMs are dispersed in air and

are deposited onto cells at an air-liquid interface within a controlled environment. Through characterization of both NM properties and cellular outcomes, an engineered aerosol chamber was able to more accurately mimic lung exposure scenarios, while simultaneously allowing for controlled operation and dosimetry [80,81].

Another concern plaguing the field of nanotoxicology is that novel NMs are being synthesized at a pace that supersedes the ability to screen them for health concerns and occupational hazards. In order to keep up with the rapid development of new materials, an engineered high-throughput mechanism is required [82]. While numerous approaches are being currently explored, microfluidics have shown promising nanotoxicological results, including identification of cytotoxicity, stress, and genetic damage [83]. One benefit to microfluidics is that through proper fluid and cellular selection, the system can be individually designed to mimic a biological target. This would allow nanotoxicologists to focus on the key routes of NM exposure, including inhalation, ingestion, and dermal exposure [84]. Additionally, microfluidic platforms are able to incorporate engineering elements that are physiologically accurate such as dynamic flow, pressure gradients, and pulsatory behavior [85]. While these efforts show promise for future advancements, their designs still requires extensive work, optimization, and validation prior to their wide-spread utilization.

6. Conclusion

Due to the exponential growth of nanotechnology, which incorporates distinctive nano-sized materials, the field of nanotoxicology emerged. Since its inception, nanotoxicology has strived to elucidate cellular responses following NM exposure, evaluate the safety of NMs, and establish regulatory and occupational guidelines for their usage. However, these objectives proved to be a major challenge as NMs exhibited modified dosimetry and altered physicochemical characteristics within cellular systems that did not align with standardized assessments and procedures. Through building and sustaining an effective partnership, engineers and biologists seamlessly merged engineering approaches and toxicological practices, including enhanced NM characterization efforts, design of mathematical dosimetry models, and generation of enhanced in vitro exposure systems. As nanotoxicology is truly a multi-disciplinary field, this partnership proved to be prolific and helped shape the future course of successful research efforts and nanotechnology innovations.

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Conflict of Interest

The author declares no conflict of interest.

References

1. Oberdorster G, Oberdorster E, Oberdorster J (2005) Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. *Environ Health Perspect* 113: 823–839.
2. Kessler R (2011) Engineered nanoparticles in consumer products: understanding a new

ingredient. *Environ Health Perspect* 119: 120–125.
3. Vance ME, Kuiken T, Vejerano EP, et al. (2015) Nanotechnology in the real world: redeveloping the nanomaterial consumer products inventory. *Beilstein J Nanotechnol* 6: 1769–1780.
4. Comfort KK, Maurer EI, Braydich-Stolle LK, et al. (2011) Interference of silver, gold, and iron oxide nanoparticles on epidermal growth factor signal transduction in epithelial cells. *ACS Nano* 5: 10000–10008.
5. Dowding JM, Das S, Kumar A, et al. (2013) Cellular interaction and toxicity depend on physicochemical properties and surface modification of redox-active nanomaterials. *ACS Nano* 7: 4855–4868.
6. Hussain SM, Warheit DB, Ng SP, et al. (2015) At the crossroads of nanotoxicology in vitro: past achievements and current challenges. *Toxicol Sci* 147: 5–16.
7. Brunelli A, Pojana G, Callegaro S, et al. (2013) Agglomeration and sedimentation of titanium dioxide nanoparticles (n-TiO2) in synthetic and real waters. *J Nanopart Res* 15: 1684.
8. Cho EC, Zhang Q, Xia G (2011) The effect of sedimentation and diffusion on cellular uptake of gold nanoparticles. *Nat Nanotechnol* 6: 385–391.
9. Braydich-Stolle LK, Breitner EK, Comfort KK, et al. (2014) Dynamic characteristics of silver nanoparticles in physiological fluids: toxicological implications. *Langmuir* 30: 15309–15316.
10. Hinderliter PM, Minard KR, Orr G, et al. (2010) ISDD: a computational model of particle sedimentation, diffusion and target cell dosimetry for in vitro toxicity studies. *Part Fibre Toxicol* 7: 36.
11. Maurer-Jones MA, Mousavi MPS, Chen LD, et al. (2013) Characterization of silver ion dissolution from silver nanoparticles using fluorous-phase ion-selective electrodes and assessment of resultant toxicity to Shewanella oneidensis. *Chem Sci* 4: 2564–2572.
12. Bian SW, Mudunkotuwa IA, Rupasinghe T, et al. (2011) Aggregation and dissolution of 4 nm ZnO nanoparticles in aqueous environments: influence of pH, ionic strength, size, and adsorption of humic acid. *Langmuir* 27: 6059–6068.
13. Braun NJ, DeBrosse MC, Hussain SM, et al. (2016) Modification of the protein corona-nanoparticle complex by physiological factors. *Mat Sci Eng C* 64: 34–42.
14. Ong KJ, MacCormack TJ, Clark RJ, et al. (2014) Widespread nanoparticle-assay interference: implications for nanotoxicity testing. *PLoS One* 9: e90650.
15. Worle-Knirsch JM, Pulskamp K, Krug HF (2012) Oops they did it again! Carbon nanotubes hoax scientists in viability assays. *Nano Lett* 6: 1261–1268.
16. Liang L, Cui M, Zhang M, et al. (2015) Nanoparticles' interference in the evaluation of in vitro toxicity of silver nanoparticles. *RSC Adv* 5: 67327–67334.
17. Gatoo MA, Naseem S, Arfat MY, et al. (2014) Physiochemical properties of nanomaterials: implication in associated toxic manifestations. *Biomed Res Int* 2014: 498420.
18. Griffitt RJ, Luo J, Gao J, et al. (2008) Effects of particle composition and species on toxicity of metallic nanomaterials in aquatic organisms. *Environ Toxicol Chem* 27: 1972–1978.
19. Shang L, Nienhaus K, Nienhaus GU (2014) Engineered nanoparticles interacting with cells: size matters. *J Nanobiotechnol* 12: 5.
20. Hauck TS, Ghazani AA, Chan WCW (2008) Assessing the effect of surface chemistry on gold nanorod uptake, toxicity, and gene expression in mammalian cells. *Small* 4: 153–159.
21. Yu T, Greish K, McGill LD, et al. (2012) Influence of geometry, porosity, and surface characteristics of silica nanoparticles on acute toxicity: their vasculature effect and tolerance

threshold. *ACS Nano* 6: 2289–2301.
22. Zhu ZJ, Wang H, Yan B, et al. (2012) Effect of surface charge on uptake and distribution of gold nanoparticles in four plant species. *Environ Sci Technol* 46: 12391–12398.
23. Misra SK, Nuseibeh S, Dybowska A, et al. (2014) Comparative study using spheres, rods and spindle-shaped nanoplatelets on dispersion stability, dissolution, and toxicity of CuO nanomaterials. *Nanotoxicology* 8: 422–432.
24. Jana NR, Gearheart LA, Obare SO, et al. (2002) Liquid crystalline assemblies of ordered gold nanorods. *J Mat Chem* 12: 2909–2912.
25. Gil PR, Oberdorster G, Elder A, et al. (2010) Correlating physico-chemical and toxicological properties of nanoparticles: the present and the future. *ACS Nano* 5: 5527–5531.
26. Bouwmeester H, Lynch I, Marvin HJ, et al. (2011) Minimal analytical characterization of engineered nanomaterials needed for hazard assessment in biological matrices. *Nanotoxicology* 5: 1–11.
27. Baer D, Gaspar DJ, Nachimuthu P et al. (2010) Application of surface chemistry analysis tools for characterization of nanoparticles. *Anal Bioanal Chem* 396: 983–1002.
28. Lin PC, Lin S, Sridhar R (2013) Techniques for physicochemical characterization of nanomaterials. *Biotechnol Adv* 32: 711–726.
29. Braun NJ, Comfort KK, Schlager JJ, et al. (2013) Partial recovery of silver nanoparticle-induced neural cytotoxicity through the application of a static magnetic field. *Bionanoscience* 3: 367–377.
30. Baalousha M, Ju-Nam Y, Cole PA, et al. (2012) Characterization of cerium oxide nanoparticles-part 1: size measurements. *Environ Toxicol Chem* 31: 983–993.
31. Joshi M, Bhattacharyya A, Ali SW (2008) Characterization techniques for nanotechnology applications in textiles. *Ind J Fibre Text Res* 33: 304–317.
32. Pachauri T, Singla V, Satsangi A, et al. (2013) SEM-EDX characterization of individual coarse particles in Agra, India. *Aerosol Air Qual Res* 13: 523–536.
33. Murdock RC, Braydich-Stolle L, Schrand AM, et al. (2008) Characterization of nanomaterial dispersion in solution prior to in vitro exposure using dynamic light scattering technique. *Toxicol Sci* 10: 239–253.
34. Breitner EK, Hussain SM, Comfort KK (2015) The role of biological fluid and dynamic flow in the behavior and cellular interactions of gold nanoparticles. *J Nanobiotechnol* 13:56.
35. Untener EA, Comfort KK, Maurer EI, et al. (2013) Tannic acid coated gold nanorods demonstrate a distinctive form of endosomal uptake and unique distribution within cells. *ACS Appl Mat Interfaces* 5: 8366–8373.
36. Sethi M, Joung G, Knecht MR (2009) Stability and electrostatic assembly of Au nanorods for use in biological assays. *Langmuir* 25: 317–325.
37. Dablemont C, Lang P, Mangeney C, et al. (2008) FTIR and XPS study of Pt nanoparticle functionalization and interaction with alumina. *Langmuir* 24: 5832–5841.
38. Klapetek P, Valtr M, Necas D, et al. (2010) Atomic force microscopy analysis of nanoparticles in non-ideal conditions. *Nanoscale Res Lett* 6: 514.
39. Elzey S, Grassian VH. (2010) Agglomeration, isolation and dissolution of commercially manufactured silver nanoparticles in aqueous environments. *J Nanopart Res* 12: 1945–1958.
40. Woehl TJ, Park C, Evans JE, et al. (2014) Direct observation of aggregative nanoparticle growth: kinetic modeling of the size distribution and growth rate. *Nano Lett* 14: 373–378.

41. Comfort KK, Maurer EI, Hussain SM (2014) Slow release of ions from internalized silver nanoparticles modifies the epidermal growth factor signaling response. *Coll Surf B Biointerface* 123: 136–142.
42. Comfort KK, Braydich-Stolle LK, Maurer EI, et al. (2014) Less is more: in vitro chronic, low-level nanomaterial exposure provides a more meaningful toxicity assessment. *ACS Nano* 8: 3260–3271.
43. Gardner JW (2002) Death by water intoxication. *Mil Med* 167.5: 432–434.
44. DeBrosse MC, Comfort KK, Untener EA, et al. (2013) High aspect ratio gold nanorods displayed augmented cellular internalization and surface mediated cytotoxicity. *Mat Sci Eng C* 33: 4094–4100.
45. Luby AO, Breitner EK, Comfort KK (2015) Preliminary protein corona formation stabilizes gold nanoparticles and improves deposition efficiency. *Appl Nanosci* doi: 10.1007/s13204-015-0501-z.
46. Fabricius AL, Duester L, Meermann et al. (2014) ICP-MS based characterization of inorganic nanoparticles-sample preparation and off-line fractionation strategies. *Anal Bioanal Chem* 406: 467–479.
47. Xiao Y, Vijver MG, Chen G, et al. (2015) Toxicity and accumulation of Cu and ZnO nanoparticles in Daphnia magna. *Environ Sci Technol* 49: 4657–4664.
48. Sabella S, Carney RP, Brunetti V, et al. (2014) A general mechanism for intracellular toxicity of metal-containing nanoparticles. *Nanoscale* 6: 7052–7061.
49. Comfort KK, Maurer EI, Hussain SM (2013) The biological impact of concurrent exposure to metallic nanoparticles and a static magnetic field. *Bioelectromagnetics* 24: 500–511.
50. Maurer EI, Comfort KK, Hussain SM, et al. (2012) Novel platform development using an assembly of carbon nanotube, nanogold and immobilized RNA capture element towards rapid, selective sensing of bacteria. *Sensors* 12: 8135–8144.
51. Kempen PJ, Hitzman C, Sasportas LS, et al. (2013) Advanced characterization techniques for nanoparticles for cancer research: applications of SEM and NanoSIMS for locating Au nanoparticles in cells. *Mater Res Soc Symp Proc* 1569: 157–163.
52. DeVolder MF, Tawfick SH, Baughman RH, et al. (2013) Carbon nanotubes: present and future commercial applications. *Science* 339: 535–539.
53. Marangon I, Boggetto N, Menard-Moyon C, et al. (2013) Localization and relative quantification of carbon nanotubes in cells with multispectral imaging flow cytometry. *J Vis Exp* 82: e50566.
54. Chilek JL, Wang R, Draper RK, et al. (2014) Use of gel electrophoresis and Raman spectroscopy to characterize the effect of the electronic structure of single-wall carbon nanotubes on cellular uptake. *Anal Chem* 86: 2882–2887.
55. DeLoid G, Cohen JM, Dark R, et al. (2014) Estimating the effective density of engineered nanomaterials for in vitro dosimetry. *Nat Commun* 5: 3514.
56. Teeguarden JG, Hinderliter PM, Orr G, et al. (2007) Particokinetics in vitro: dosimetry considerations for in vitro nanoparticle toxicity assessments. *Toxicol Sci* 95: 300–312.
57. Comfort KK, Speltz J, Stacy BM, et al. (2013) Physiological fluid specific agglomeration patterns diminish gold nanorod photothermal characteristics. *Adv Nanopart* 2: 336–343.
58. Son J, Vavra J, Forbes VE (2015) Effects of water quality parameters on agglomeration and dissolution of copper oxide nanoparticles (CuO-NPs) using a central composite circumscribed

design. *Sci Total Environ* 521: 183–190.
59. Stacy BM, Comfort KK, Comfort DA, et al. (2013) In vitro identification of gold nanorods through hyperspectral imaging. *Plasmonics* 8: 1235–1240.
60. Khanbeig A, Kuman A, Sadouki F et al. (2012) The delivered dose: applying particokinetics to in vitro investigations of nanoparticle internalization by macrophages. *J Control Release* 162: 259–266.
61. Cohen J, DeLoid G, Pyrgiotakis G, et al. (2013) Interactions of engineered nanomaterials in physiological media and implications for in vitro dosimetry. *Nanotoxicology* 7: 417–431.
62. Cohen JM, Teeguarde JG, Demokritou P (2014) An integrated approach for the in vitro dosimetry of engineered nanomaterials. *Part Fibre Toxicol* 11:20.
63. Summers MRB, Brown MR, Hondow N, et al. (2015) Statistical prediction of nanoparticle delivery: from cell culture media to cell. *Nanotechnology* 26: 155101.
64. Liu R, Liu HH, Ji Z, et al. (2015) Evaluation of toxicity ranking for metal oxide nanoparticles via an in vitro dosimetry model. *ACS Nano* 9: 9303–9313.
65. Richarz AN, Madden JC, Robinson RLM, et al. (2015) Development of computational models for the prediction of the toxicity of nanomaterials. *Perspect Sci* 3: 27–29.
66. Burello E, Worth AP (2011) QSAR modeling of nanomaterials. *Wiley Interdiscip Rev Nanomed Nanobiotechnol* 3: 298–306.
67. Puzyn T, Rasulev B, Gajewicz A, et al. (2011) Using nano-QSAR to predict the cytotoxicity of metal oxide nanoparticles. *Nat Nanotechnol* 6: 175–178.
68. Desai T, Keblinski P, Kumar SK (2005) Molecular dynamics simulations of polymer transport in nanocomposites. *J Chem Phys* 122: 134910.
69. Song Z, Wang Y, Xu Z (2015) Mechanical responses of the bio-nano interface: a molecular dynamics study of graphene-coated lipid membrane. *Theor Appl Mech Lett* 5: 231–235.
70. Arora S, Rajwade JM, Paknikar KM (2012) Nanotoxicology and in vitro studies: the need of the hour. *Toxicol Appl Pharmacol* 258: 151–165.
71. Demokritou P, Gass S, Pyrgiotakis G, et al. (2013) An in vivo and in vitro toxicological characterization of realistic nanoscale CeO_2 inhalation exposures. *Nanotoxicology* 7: 1338–1350.
72. Frohlich E, Salar-Behzadi S (2014) Toxicological assessment of inhaled nanoparticles: role of in vivo, ex vivo, and in silico studies. *Int J Mol Sci* 15: 4795–4822.
73. Voight N, Henrich-Noach P, Kockentiedt S, et al. (2014) Toxicity of polymeric nanoparticles in vivo and in vitro. *J Nanopart Res* 6: 1–13.
74. Cho WS, Duffin R, Howie SEM, et al. (2011) Progressive severe lung injury by zinc oxide nanoparticles; the role of Zn^{2+} dissolution inside lysosomes. *Part Fibre Toxicol* 8: 27.
75. Shacter E, Weitzman SA (2002) Chronic inflammation and cancer. *Oncology* 16: 230–232.
76. Kusek ME, Pazos MA, Pirazi W, et al. (2014) In vitro coculture to assess pathogen induced neutrophil trans-epithelial migration. *J Vis Exp* 6: e50823
77. Walczak AP, Kramer E, Hendriksen PJ, et al. (2015) In vitro gastrointestinal digestion increases the translocation of polystyrene nanoparticles in an in vitro intestinal co-culture model. *Nanotoxicology* 9:886–894.
78. Fede C, Fortunati I, Weber V, et al. (2015) Evaluation of gold nanoparticles toxicity toward human endothelial cells under static and flow conditions. *Microvas Res* 97:147–155.
79. Ucciferri N, Collnot EM, Gaiser BK, et al. (2014) In vitro toxicology screening of nanoparticles on primary human endothelial cells and the role of flow in modulating cell response.

Nanotoxicology 8: 697–708.
80. Jeannnet N, Fierz M, Klaberer M, et al. (2015) Nano aerosol chamber for in vitro toxicity (NACIVT) studies. *Nanotoxicology* 9: 34–42.
81. Xie Y, Williams NG, Tolic A, et al. (2012) Aerosolized ZnO nanoparticles induce toxicity in alveolar type II epithelial cells at the air-liquid interface. *Toxicol Sci* 125: 450–461.
82. Macarron R, Banks MN, Bojanic D, et al. (2011) Impact of high-throughput screening in biomedical research. *Nat Rev Drug Discov* 10: 188–195.
83. Mahto SK, Charwat V, Rothen-Rutishauser B, et al. (2015) Microfluidic platforms for advanced risk assessments of nanomaterials. *Nanotoxicology* 9: 381–395.
84. Oberdörster G, Maynard A, Donaldson K, et al. (2005) Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. *Part Fibre Toxicol* 2: 8.
85. Esch E, Bahinksi A, Huh D (2014) Organs-on-chip at the frontiers of drug discovery. *Nat Rev Drug Discov* 14: 248–260.

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