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Perspective

Nanotoxicology and Nanomedicine:

The Yin and Yang of Nano-Bio Interactions for the New Decade

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ABSTRACT

Nanotoxicology and nanomedicine are two sub-disciplines of nanotechnology focusing on the phenomena, mechanism, and engineering at the nano-bio interface. For the better part of the past three decades, these two disciplines have been largely developing independently of each other. Yet recent breakthroughs in microbiome research and the current COVID-19 pandemic demonstrate that holistic approaches are crucial for solving grand challenges in global health. Here we show the Yin and Yang relationship between the two fields by highlighting their shared goals of making safer nanomaterials, improved cellular and organism models, as well as advanced methodologies. We focus on the transferable knowledge between the two fields as nanotoxicological research is moving from pristine to functional nanomaterials, while inorganic nanomaterials – the main subjects of nanotoxicology – have become an emerging source for the development of nanomedicines. We call for a close partnership between the two fields in the new decade, to harness the full potential of nanotechnology for benefiting human health and environmental safety.

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Nanotoxicology and Nanomedicine are two sub-disciplines of nanotechnology concerning the adverse effects and health application of nanomaterials, respectively. This *Perspective* draws parallels and contrast between the two fields and calls for their close collaboration to address emerging challenges in global health.

1. Introduction

Nanomaterials (NMs) and their functional derivatives have enabled a wide range of breakthroughs in technology, engineering and medicine, and the advent of nanotechnology is considered as important as the Industrial Revolution [1]. However, as with other innovative substances (e.g., pesticides or antibiotics), the commercialization of NMs preceded their extensive safety evaluation in relation to human and environmental health. Overlooking existing toxicological knowledge in the development of nanotechnologies, especially in biomedical applications, can be costly. Thus, the effective and safe use of nano-biomedical applications necessitates the development and partnership of the disciplines that are historically referred to as "nanotoxicology" and "nanomedicine".

Nanotoxicology emerged when toxicologists in the 1990s extended their research from the pulmonary effects of airborne particles to these of engineered NMs such as metal oxides and carbon nanotubes (CNTs) [2, 3]. Nano(eco)toxicology was later supported by European legislation mandating systematic studies on the toxicity of NMs [4]. To date, nanotoxicology has developed into a relatively mature discipline, generating systematic knowledge for risk assessment of NMs and for the development of safer-by-design nano-enabled products, which are also an integral part of the processes needed for successful clinical translation of nanomedicines.

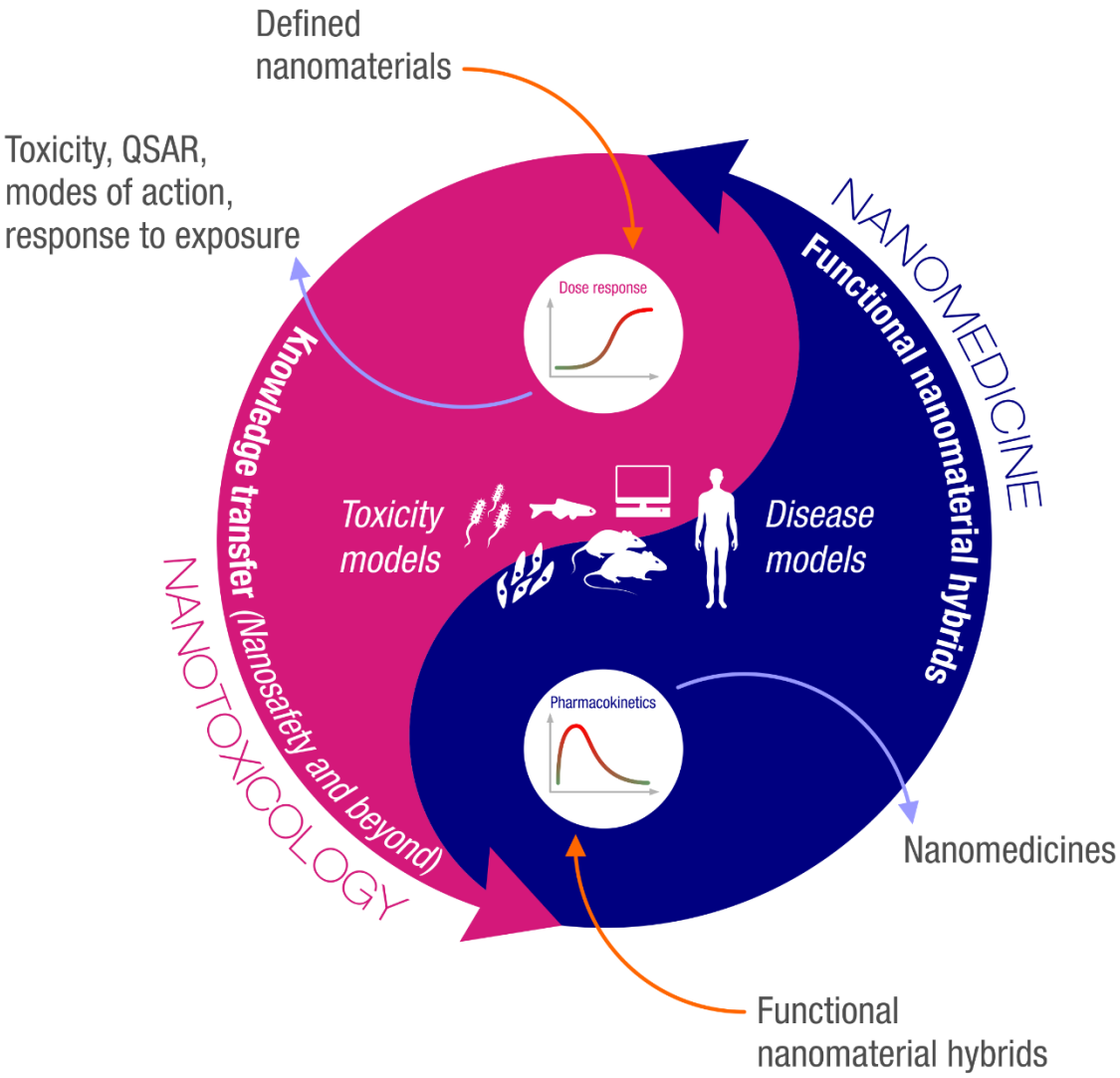
The development of nanomedicine was driven by the progress in the pharmaceutical industry in the 1960s that resulted in the development of NM-based systems for controlled drug release [5]. Despite the relatively long history of nanomedicine, there were only approximately 50 US Food and Drug Administration (FDA)-approved nanomedicines on the market and 77 in clinical trials in 2016 [6], most based on liposomes and protein complexes. Nanomedicine as a field has faced challenges in clinical translation, with the major obstacle being low efficacy due to

limited understanding of nano-bio interactions, NM biocompatibility, NM-specific toxicity, targeted delivery and NM fate and degradation [7].

Despite apparent differences in definition, nanotoxicology and nanomedicine are both fundamentally focused on the dose-response relationship of NMs. Nanotoxicology is concerned with determining the NM concentrations that cause unintended effects, i.e., toxicity, or cause toxicity to non-target cells, organs, or organisms. On the other hand, nanomedicine aims to increase the specificity and efficacy of drugs, bioimaging or diagnostic agents at the lowest possible doses. The favorable efficacy/toxicity ratio, tumor targeting and release tunability are major benefits of nanomedicines over conventional drugs and are thus important driving forces for the development of nanomedicine. In addition, from the perspective of nanomedicine, nanotoxicology has often been viewed as a discipline that provides toxicology information to guide the design of safer NMs and therefore has been suggested to be renamed as "nanosafety" [8]. However, within the context of this *Perspective*, we consider "nanosafety" as being a constituent of the broader definition of nanotoxicology that encompasses both unintended (for safety to human health and the environment) and intended (for nanodrug efficacy) toxicity of NMs, and use the term "nanotoxicology" here to signify primarily the investigation of the unintended effects of NMs.

The goal of this *Perspective* is to demonstrate that nanotoxicology and nanomedicine share many overlapping interests and challenges (**Scheme 1**), and bridging these two disciplines, accordingly, would be mutually beneficial. From the viewpoint of nanotoxicology, nanomedicine is a major outlet for applying existing knowledge and elevating the translational value of nanotoxicology through advocating safer and more efficacious NMs for medicine. Conversely, nanomedicine can benefit from existing knowledge and methodologies of nanotoxicology, from NM-cell interactions, complex mixture characterization techniques and cellular and organism models to the chemical and biological effects arising from the interplay

of physicochemical properties of NMs on their exposure, biodistribution, biotransformation, accumulation, and toxicity. Moreover, considering the importance of the microbiome in human diseases, as recently revealed, the expertise of nano(eco)toxicology has become precipitously relevant to the development of nanomedicine targeting cancer, neurological disorders as well as metabolic diseases. In addition, we elaborate on the idea that engineered nanoparticles can be exploited in different ways to fight biological nanoparticles such as coronaviruses, using the complementary know-how of nanotoxicology and the pragmatic approaches of nanomedicine. Thus, both disciplines would gain from the transfer of knowledge and sharing of cellular and organism models as well as analytical techniques as delineated in this article.



Scheme 1. Nanotoxicology and nanomedicine, *at a glance*. Both fields have been developed largely independently over the past decades, yet both fields are concerned with the fate and behaviour of engineered as well biological nanoparticles in biological systems and, as a result, share much in common in terms of their goals, methodologies, and biological model systems. Knowledge transfer from nanotoxicology to nanomedicine, beyond the framework of nanosafety and in the forms of toxicity data, quantitative structure–activity relationship (QSAR) and other nanoinformatics models, modes of action, and responses to NM exposure, would greatly benefit nanomedicine. Conversely, research in nanotoxicology, currently focused on defined NMs, could gain an enhanced purpose and applicability by investigating functional NM hybrids designed for nanomedicine (their safety, interactions with the microbiome and endogenous proteins, as well as the fate and behaviour of wear-off NMs from medical devices for example). Here the bold arrows indicate current deficiencies where improvements may be made to benefit the development of both fields. Thin orange arrows denote inputs and thin blue arrows outputs from each field.

2. Nanotoxicology and nanomedicine: different vocabularies, common key problems

Currently, the fields of nanotoxicology and nanomedicine are developing mostly in parallel, as illustrated by the verbiage of these two disciplines (**Figure 1**). While nanotoxicology focuses on NM toxicity and the corresponding mechanisms, nanomedicine in the context of delivery aims to develop new drugs or drug carriers and translate them into clinical applications. These different aims bred largely diverse vocabularies in the scientific publications of the two fields. For example, when comparing the frequency of words in the category of "nano-bio interface, distribution" (**Figure 1**), it appears that nanotoxicology-related publications more frequently report on pulmonary toxicity of NMs than nanomedicine articles (keyword "lung"), likely because of inhalation being one of the most relevant environmental and occupational exposure routes to NMs. In contrast, the keyword "brain" is the most frequently mentioned organ in nanomedicine articles, followed by "lung" and "liver". This can be explained by the focus on NM-mediated delivery of drugs through the blood-brain barrier (BBB) and NM-enabled targeting of tumors in various organs, as revealed by the high word counts for "drug", "cancer" and "tumor".

As indicated by the high frequency use of “size” and “material” in both nanotoxicology and nanomedicine articles, these are two areas of interest that the disciplines share. The attention on “size” could be extended to other physicochemical characteristics (such as surface modification or surface chemistry) of NMs that determine their application and toxicity potential. “Material” is another common word, but often entails different meanings to the two disciplines. For nanotoxicology, the material of interest typically includes defined pristine materials, *i.e.*, metals, metal oxides, CNTs, graphene, graphene oxide, fullerene and 2D transition metal dichalcogenides. For nanomedicine, nanoscale materials are often decorated by FDA approved polymers, lipids, and proteins. For example, nanomedicines are often PEGylated to improve the stability and reduce uptake by the reticuloendothelial system for prolonged drug circulation; here function and efficacy are the foci while toxicity is generally an unwanted side effect.

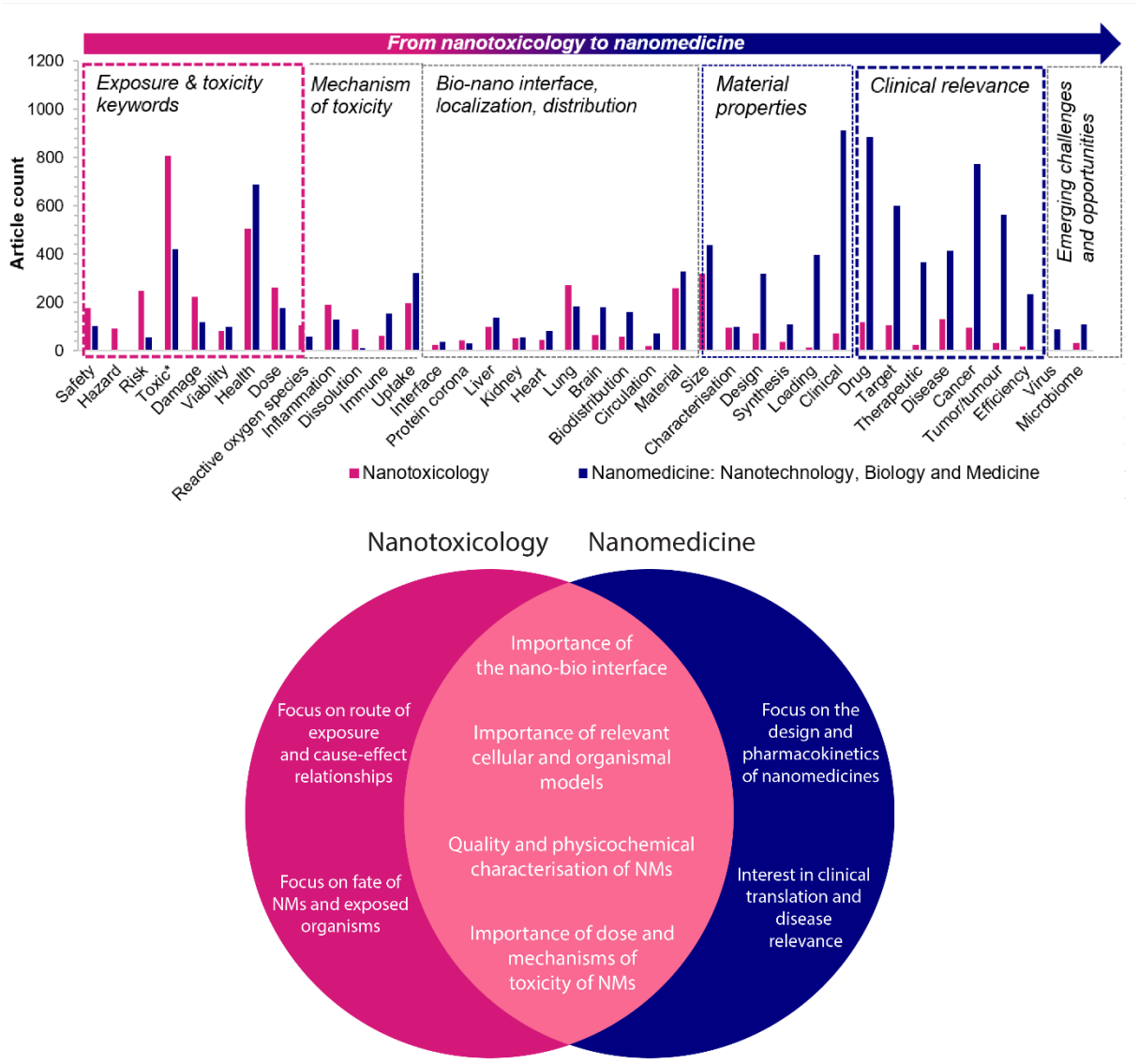


Figure 1. A meta-analysis of research emphases in nanotoxicology *versus* nanomedicine. (Top) Lexicons of nanotoxicology (magenta) and nanomedicine (navy blue), extracted on the 31st of July, 2020 from two representative journals: *Nanotoxicology* (Taylor and Francis) and *Nanomedicine: Nanotechnology, Biology and Medicine* (Elsevier) using Web of Science (Clarivate Analytics). The keywords used for the search (except for "virus" and "microbiome", which were added as emerging research directions) were selected from the most frequently used words from the Perspectives by Fadeel et al. (2018) [9], Faria et al. (2018) [10] and Pelaz et al. (2017) [11], identified by WordCounter. (Bottom) Similarities and differences between nanotoxicology and nanomedicine research foci, extracted from the top panel.

3. Transferable knowledge

In the following subsections we discuss the main areas where the interests of nanomedicine and nanotoxicology overlap, and where nanomedicine can benefit from the knowledge generated by the nanotoxicology community beyond the scope of nanosafety.

3.1 Linking NM physicochemical properties with bioactivity

The physicochemical properties of NMs are transitional between molecular and bulk systems [11]. The bioactivity of NMs is dictated by both the intrinsic (e.g., chemical composition, size and shape) and extrinsic (e.g., protein corona, surface speciation) properties that are material and biological system dependent [12]. Linking the physicochemical properties of NMs with their bioactivity necessitates comprehensive characterization of the NMs and reporting of their properties in both simple suspensions and complex biological environments. Detailed description of the experimental conditions, techniques and procedures for the synthesis and characterization of NMs has been proposed to be made mandatory for enhanced repeatability in nanomedicine reporting [13].

Several recent key articles pointed out the lack of a universal guideline for the physicochemical characterisation of NMs and biological test systems for their safety evaluation [9, 10]. While the nanomedicine community highlighted the need for minimum information on materials and test system characterisations that should be reported in the nano-bio literature [10], the nanotoxicology community proposed to use novel omics-based high-throughput bioassays to link the physicochemical properties of NMs to their hazards and define the minimal set of NM-related features that predict toxicity [9]. Clearly, insufficient quality of NMs (especially, if produced on large scale) and inadequate reporting of physicochemical properties (including acquired corona composition) hamper both nanosafety studies and nanomedicinal applications [14, 15]. Precision NM synthesis is of crucial importance to ensure desired and specific function and properties of the NM (size, targeting, loading, ligand density and orientation, etc.) [16].

3.2 The nano-bio interface shared by nanotoxicology and nanomedicine

The current FDA-approved anti-cancer nanomedicines rely on passive targeting of tumors, i.e., enhanced permeability and retention (EPR), and only a small percentage (0.7% median) of the administered NM dose is able to reach the tumor [17] (though this has recently been refuted by meta-analyses using the standard AUC_{tumor}/AUC_{blood} ratio) [18]. A main cause of these poor statistics is our limited understanding of the nano-bio interface, which is central to the interests of nanotoxicology and nanomedicine and is where the boundaries between the two disciplines blur.

The protein "corona" [19] refers to a biological-synthetic hybrid arising from surface energy minimisation of a NM by its biological host – a targeted cell, a tissue, an organ, the gut immune barrier, the bloodstream, or the BBB. As a result, the NM acquires a dynamic camouflage mostly of proteins and lipids within an intra- or extracellular matrix. The protein corona impacts the cellular uptake, translocation, biodistribution, toxicity and non-specific clearance of the NM by immune cells and is considered a major hindrance to the targeting capacity of nanomedicines [20, 21]. Accordingly, a great effort in nanomedicine involves the development of stealth polymers to fend off opsonization and elimination of the NMs by the immune system, as well as mitigating, reinventing or baiting the protein corona for smart NM design and drug delivery [22, 23]. In addition, the protein corona may elicit conformational changes and toxicity to NM-bound amyloid proteins that are associated with neurological disorders and type 2 diabetes, an emerging frontier of nanomedicine [24, 25]. On the other hand, the field of nanotoxicology has developed a range of analytical methods to characterize the bio-corona [26], as well as nanoinformatics models for prediction of protein corona from NM physicochemical descriptors and for prediction of cellular uptake of NMs [27, 28]. Continued effort in this area should take into consideration of functional NMs and nanocomposites pertinent to nanomedicine.

3.3 The microbiome, a kingmaker of the nanotoxicology-nanomedicine entanglement

The role of the microbiome in human disease has been suggested for some time. However, evidence of definitive links and underlying mechanisms has just started to emerge [29-31]. Host-gut microbiota metabolic interactions have been linked to colorectal cancer [31], depression [32], neurological disorders [29], obesity [32], type 2 diabetes [30], dysfunction of the immune system [33, 34] and social behaviour [35]. The human microbiome is also linked to the development of the central nervous system and maturation of the immune system [36, 37]. The pathological and physiological relationships between the microbiota and the host have positioned the microbiota as a key element in determining the pharmacology and toxicology outcome of nanomedicines (**Figure 2**). For example, TiO_2 nanoparticles have found a range of applications in cosmetics, food packaging and cancer nanomedicine. However, when administered orally, TiO_2 nanoparticles can influence the metabolic profile of the gut microbiota, inducing an over-production and systemic translocation of bacterial lipopolysaccharides (LPS) [38] that have a toxicological role in carcinogenesis, metastasis and pathogenesis of neurodegenerative diseases [39-46]. In contrast, while CNTs and C_{60} have been proven ecotoxic, hydroxylated C_{60} promoted the gut microbiota to metabolically produce short-chain fatty acids (SCFAs) [47] that were protective against carcinogenesis [48]. Antibiotics, antidiabetic and antacid medications have been shown to significantly influence the gut microbial community composition [52]. This implies that the future design, efficacy, and toxicity testing of nanomedicines should focus on their toxicities to both eukaryotic and prokaryotic cells, ensuing that the developed drugs would not destroy beneficial microbiome but support the function, survival, and metabolism of beneficial microbiome in the host. Indeed, functionalized gold NMs have been shown to target bacterial infection induced by *Escherichia coli* (*E. coli*) in murine gut without harming intestinal microflora, demonstrating the feasibility of NM-based antibacterial treatments that have both higher efficacy and specificity than conventional antibiotics [58]. Knowledge on the molecular mechanisms of NMs interacting

with microorganisms, gained from nano(eco)toxicology studies, would be useful for developing targeted nanomedicines with reduced side effects. Future research in this area, pertinent to both nanotoxicology and nanomedicine, should focus on elucidating how NMs, including potential nanomedicines, affect microbial metabolic pathways that have been connected to human diseases, i.e., targeted metabolomics, combined with transcriptomics approaches, integrated into beneficial off-target effects of nanomedicines.

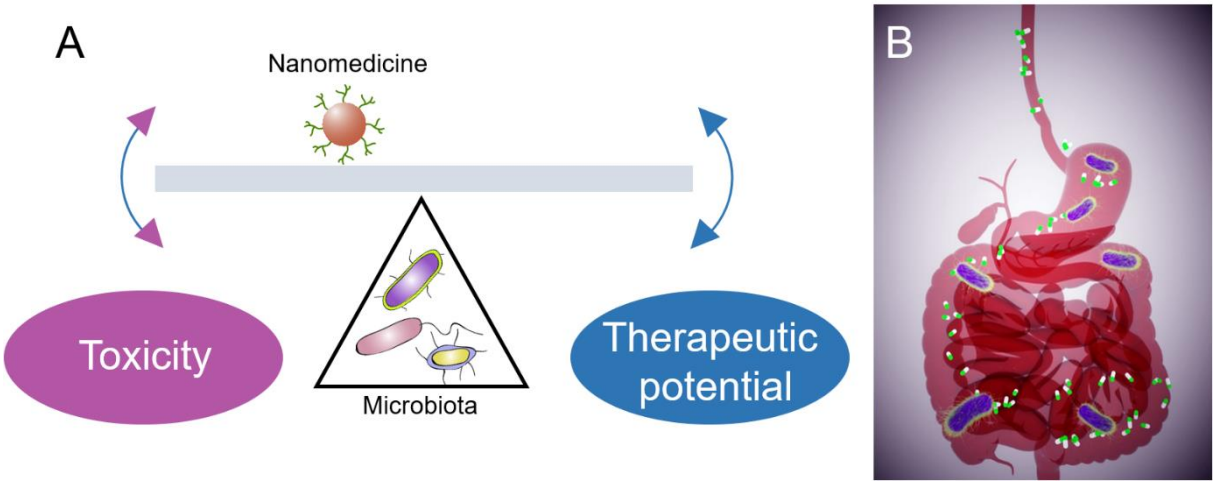


Figure 2. The microbiota profoundly influences the toxicity-therapeutic potential balance of nanomedicines (A). Such complex involvement of the gut microbiota in modulating the efficacy and toxicity of oral nanomedicines remains to be understood (B).

3.4 Transferable knowledge of intrinsic NM bioactivities

The definition of nanomedicines for treatment has expanded over the past decade from "carriers of conventional drugs" to NMs that have intrinsic therapeutic properties. To date, the intrinsic bioactive properties of NMs (such as antibacterial or anticancer properties) are by far more exploited on the market than the NM potential as drug carriers. Currently, silver NMs are the most commercialised NMs being used in over 350 consumer products. In nanomedicine, one of the major tasks is to develop NMs with high efficacy but low toxicity, two stringent criteria which often hinder the translation of nanomedicines from bench to bedside. At the same time,

the nanotoxicology community has, over the years, acquired in-depth knowledge on the intrinsic bioactive properties of NMs [1] that is highly transferable to nanomedicine.

Nanomedicines may exert biological effects directly affecting the cellular viability or indirectly via the bio-corona, including complement activation and interactions with the immune cells (relevant for most NMs), or affecting the organism's microflora [60]. The direct cytotoxic mechanisms of NMs are relatively well-described: dissolving metal-based NMs mostly exert toxicity *via* dissolution and/or oxidative stress [61, 62], CNTs via shape and agglomeration-dependent immunotoxicity [63], and positively charged NMs via enhanced interactions with outer cell membranes and intracellular organelles [64]. The indirect effects of NMs, however, have been significantly less addressed, and we suggest that these aspects may be the focus of future nanotoxicology studies. These include, e.g., the potential effects of NMs on the immune system (such as immunoglobulin deposition on NMs, complement activation and inflammatory responses) [65] and on the composition and function of the microbiota. In addition, nanomedicines will be transformed in the body and, eventually, discharged into the environment. The biotransformation of nanomedicines and their discharge into the environment is an aspect often overlooked by the nanotoxicology community [66, 67].

It has been well established that the toxicities of certain NMs, such as Ag, ZnO NMs, and partially CuO NMs, largely correlate with the dissolution of these NMs, regardless of the organism type [68]. However, nanotoxicology has also revealed that NM dissolution in test tubes or growth medium may not accurately predict the rate of metal ion release from NMs, when the NMs are in close vicinity or adsorbed to cell membranes or internalised by cells. Most importantly, toxicity also depends upon where the ion release takes place, extracellularly or intracellularly [12]. Hence, translating this knowledge to nanomedicine requires careful characterisation of the dissolution of metal NMs in physiologically relevant matrices. The ecotoxicity data also suggests that, when developing NM-enabled antimicrobials, it should be

taken into consideration that NMs commonly employed for their antimicrobial properties (e.g., Ag, ZnO and CuO) are not specific to prokaryotic organisms and thus, could induce side effects when used as antimicrobials. Modification of NM surface properties and shapes may provide opportunities for fine-tuning the biodistribution and efficacy of antimicrobial NMs to targeted cell types [69]. Another issue regarding the use of metal-based NMs as antimicrobials that should be considered is the co-regulation of metal and antimicrobial resistance (AMR) in bacteria [70]. NMs that do not release metal ions may prove promising for preventing AMR. While CNTs have been shown to act as efficient antibacterial agents [60], CNTs at non-growth inhibitory concentrations can increase bacterial susceptibility to conventional antibiotics, rendering the latter more efficient [71].

3.5 Transferable knowledge of cellular and organism models

One common research strategy in nanotoxicology and nanomedicine is the use of cellular and animal models. *In vitro*, the assays and biological endpoints are often similar, *e.g.*, lethality, metabolic disruption or membrane damage of cells, uptake, localisation and mechanisms of action of NMs, usually assessed as a function of NM dose. Hence, the methodologies applied, and knowledge generated *in vitro* are mostly transferable between the two fields. Eukaryotic unicellular organisms, for example, provide a model system that entails the simplicity, cost-efficiency and fast generation time of the cellular assays and yet is composed of independently functioning organisms [68]. Such organisms include ciliated protozoa (e.g., *Tetrahymena thermophila*) and yeast (e.g., *Saccharomyces cerevisiae*) that have been successfully incorporated into nano(eco)toxicology research but could prove useful for nanomedicine, considering these organisms have been employed as models for studying the fundamental aspects of eukaryotic cell biology and human diseases for decades. The ease of genetic manipulation of fully sequenced genomes, well-established biochemistries, and naturally high

exposure to NMs in the case of the filter-feeding protozoa, make these model organisms well suited for high-throughput toxicity screening and mechanistic studies of nanomedicines.

While the above-mentioned cost- and labour-efficient toxicological tests allow *in vitro* comparisons between a wide variety of NM physicochemical characteristics, experiments with conventional *in vivo* models can only include a limited number of replicates and exposure conditions but are often unavoidable. Wild-type rodents are simple, multipurpose and, therefore, widely used animal models in nanotoxicology and nanomedicine [72, 73]. In comparison, transgenic models can provide much insight into specific pathological, toxicological, or pharmacological pathways [74, 75]. Despite such specificity, transgenic rodent models can still be used interchangeably in nanotoxicology and nanomedicine. For example, monocyte chemoattractant protein-1 (MCP-1) transgenic models display an overexpression of MCP protein and an enhanced recruitment of anti-inflammatory cells [76]. These models have been used to study the toxicity of fullerene [77] as well as the therapeutic potentials of liposomes and ceria nanoparticles [78, 79]. Similarly, APP/PS1 transgenic mice overexpressing human amyloid beta have been employed for testing nanomedicines against Alzheimer's disease [80] as well as the toxicity of environmental aluminium [81]. In contrast to rodents, the most widely used test organism in nanomedicine, zebrafish (*Danio rerio*), including transgenic zebrafish, is a less expensive and advantageous *in vivo* vertebrate model not only for nanoecotoxicology (for chemical hazard evaluation) and pharmacology (drug screening), but also for nanotoxicology and nanomedicine [82, 83]. Given the unique features of large fecundity, fast and synchronised development, as well as embryonic translucency, zebrafish is highly suitable for screening the *in vivo* toxicity of NMs of large quantities. Additionally, the availability of full genome sequence, feasibility of live staining and *in situ* hybridization allow in-depth mechanistic investigations of NM effects with the zebrafish model. Moreover, the optical transparency of zebrafish larvae is suited for performing real-time visualization of NM circulation through

cardiac or tail vein microinjection. Furthermore, the increasing number of human disease models, such as skin cancer, hematopoietic disorders, and neurological disorders, also continues to elevate the clinical importance of the zebrafish model [84]. Nanotoxicology research is often interested in wild-type animals to represent a general population, while nanomedicine is interested in the use of "disease models" representing human diseases. Additionally, the exposure routes and organs of interest for the two fields differ. The NMs of interest to nanotoxicology are administered through inhalation, aspiration, dermal exposure, and ingestion to simulate natural exposure conditions. Upon NM exposure, nanotoxicology mostly examines the biological consequences of exposure to the animal lung, skin, gastrointestinal tract, and liver. In nanomedicine, the administration route largely relies on direct injection of NMs into blood circulation or specific tissues. As a result, the NM biodistribution is mostly governed by circulation time and organs of interest, which typically include major internal organs such as liver, spleen, brain, and gastrointestinal tract. Due to these differences, direct transfer of nanotoxicology data to nanomedicine, or *vice versa*, remains a challenge. However, as nanotoxicological research is moving from pristine to advanced functional materials for biomedical applications while nanomedicine is expanding to include extremely complex composite materials [85] and inorganic NMs for imaging and therapeutics, knowledge transfer between the two fields has become necessary.

While knowledge of animal testing data from nanotoxicology has been transferred to nanomedicine to some extent, better collaboration between the two fields is essential for cost-effective research and development, harnessing the medicinal potential of NMs and nanocomposites. For instance, the nanotoxicology community developed numerous experimental and theoretical approaches (cell co-cultures, artificial *in vitro* models, test standards and systems biology approaches) to improve testing throughput and *in vitro-in vivo* correlation and translation, and adopted protocols and documents (such as Guidance Document

on Good *In Vitro* Method Practices, GIVIMP) to ensure that data obtained in *in vitro* tests are rigorous and reproducible [86]. These elements are, clearly, beyond the scope of nanosafety and are highly pertinent to the interest of nanomedicine as improved and increasingly realistic tissue models for targeting and diagnosis.

3.6 Novel coronaviruses, a common challenge for nanotoxicology and nanomedicine

Recent coronaviral epidemics and pandemics, especially the ongoing COVID-19 pandemic, have drastically changed social life on the global scale and presented grand challenges to the scientific community. The culprit of the current COVID-19 pandemic, the novel coronavirus SARS-CoV-2, is a biological particle (60-140 nm in size) in essence that can be studied both as a nano-toxin for nanotoxicology and a disease model for nanomedicine (**Figure 3**). Similarly to toxic engineered nanoparticles, SARS-CoV-2 has been found to compromise the lungs, the liver, the brain, the pancreas and other organs eliciting both short- and long-term impacts on infected subjects [87, 88]. Mechanistically, SARS-CoV-2 enters the cell via the angiotensin-converting enzyme 2 (ACE2) receptor [89] that has been known in the field of nanotoxicology as a target of cationic polyamidoamine (PAMAM) dendrimer to induce acute lung injury in animal models [90]. Thus, we advise to use nanotoxicological cellular and organism exposure methodologies and knowledge on nano-bio interactions to examine the behaviour, toxicity and pathogenesis of SARS-CoV-2 and other emerging viruses. In addition, existing nanotoxicology data on antibacterial and virucidal agents may be repurposed for testing coronaviral mitigation strategies. For example, silver nanocluster/silica composite can inactivate SARS-CoV-2, while exposure to metal catalysts Ag/Al_2O_3 and Cu/Al_2O_3 can destroy the replication and propagation abilities of SARS-CoV, baculovirus and *E. coli* [91, 92]. Emerging coronaviruses may also be studied as a class of disease models for nanomedicine, like cancer or neurodegeneration, where existing tools may be exploited for developing novel vaccine adjuvants, diagnostic tests [93, 94] and, possibly, therapeutic solutions targeting the structure, function, replication and

signalling pathways of the viruses. This holistic approach may transform the potential of nanotechnology against COVID-19 and future viral epidemics and pandemics.

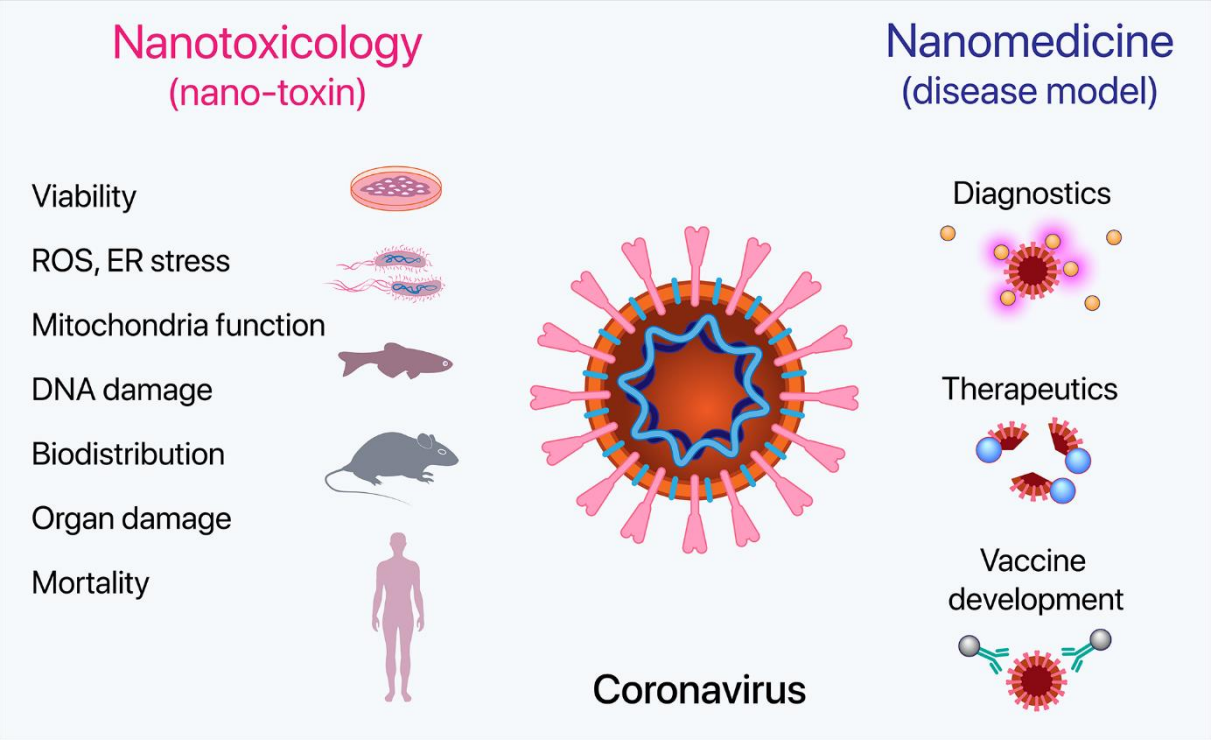


Figure 3. Nanotoxicology and nanomedicine may offer mechanistic insights and theranostic solutions for combating emerging novel coronaviruses such as SARS-CoV-2.

3.7 NM-induced endothelial leakiness: from nanosafety to therapeutic strategies

NM-induced endothelial leakiness (NanoEL) is a nano-bio interfacial phenomenon juxtaposing both nanosafety concerns and nanomedicine opportunities [95, 96]. Microscale endothelial leakiness, induced through exposure to high density, negatively charged and ultrasmall NMs (e.g., TiO_2, SiO_2 and Au) lowered the biological barrier for intravasation and extravasation of metastatic and circulating tumor cells, respectively [97, 98]. This raised nanosafety concerns for the prevalent use of these NMs and their derivatives in nanomedicine. However, the same induced leakiness in the endothelium may increase critical drug access with favourable pharmacodynamics in the pathological context with little or no EPR effect [99], thus opening new opportunities for improved efficacy and targeting of nanomedicines against vascular, metabolic and brain diseases [100] (**Figure 4**).

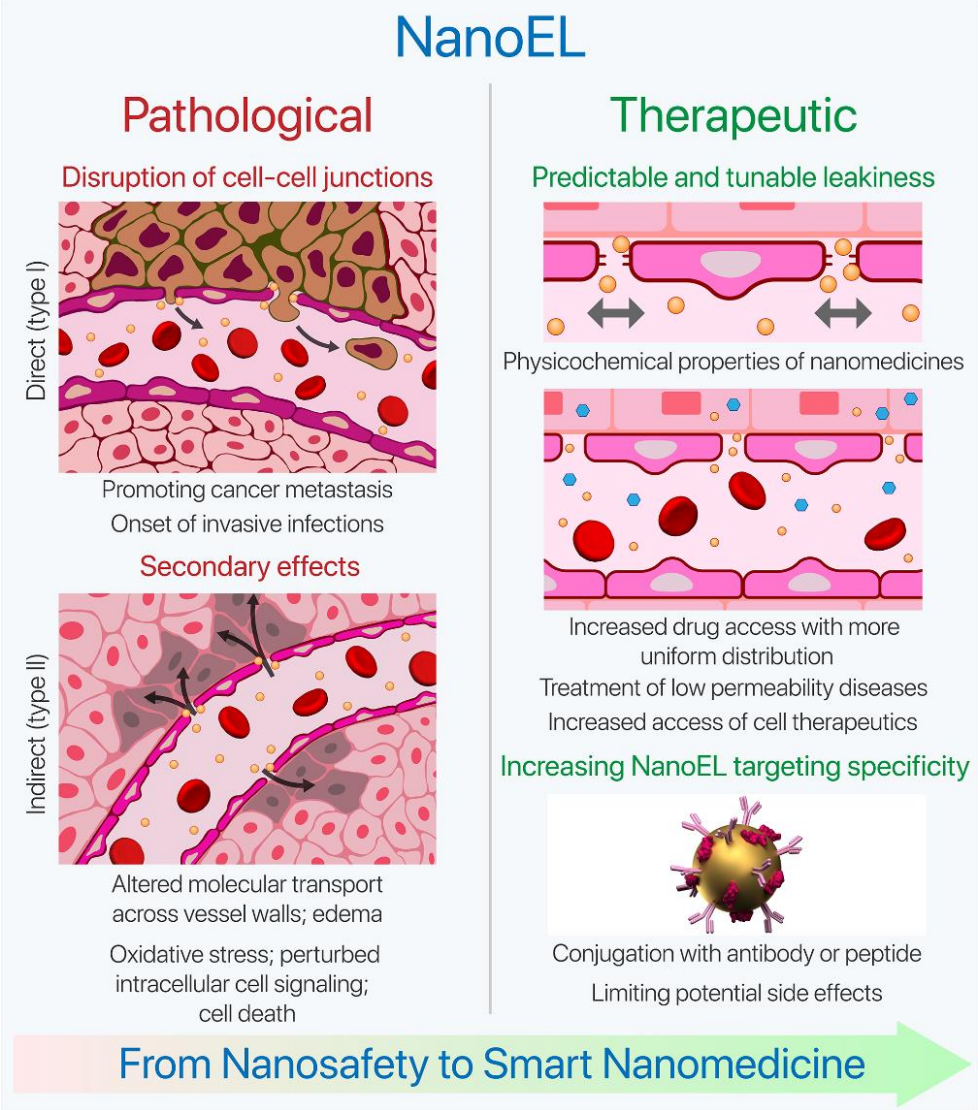


Figure 4. NM-induced endothelial leakiness (NanoEL) is a tissue level phenomenon originated from nanoscale interactions between NMs and endothelial adherens junctions. Originally established as a nanosafety concept, NanoEL may have huge therapeutic implications for guiding vascular transport and tumor targeting of nanomedicines.

3.8 Transferable knowledge of computational and statistical modelling

Over the past two decades, multiscale computer modelling has demonstrated increased value for delineating and predicting the atomistic and molecular details and dynamics of nano-bio interactions that are nontrivial to obtain from experimental studies in nanotoxicology and nanomedicine. These efforts range from simulations of NM membrane translocation to the acquisition of the protein corona, protein fouling, and protein aggregation mitigation by a NM

inhibitor, taking into consideration the NM size, shape, surface charge, surface chemistry and functionalisation. The established QSAR profiles of NMs, a statistical modelling approach in the field of nanotoxicology [101], can be utilised to select prototype nanomedicines and scaffolds. Together, we envision that computer simulations, statistical modelling and machine learning can significantly contribute to improved prediction of the *in vivo* behaviour, efficacy and fate of nanomedicines as well as the biological and toxicological responses of nanomedicines and will provide a route towards personalised nanomedicine [102].

3.9 Transferable knowledge of nanotoxicology findings: additional examples

NM-induced activation of the NLRP3 inflammasome is a well-acknowledged phenomenon in nanotoxicology that can be used to facilitate the development of new vaccine adjuvants for nanomedicine purposes [103]. For example, it was found that the shape, crystallinity, and hydroxyl content of aluminium oxyhydroxide NMs and nanocellulose play an important role in NLRP3 inflammasome activation and are therefore useful parameters to tune for enhancing antigen-specific immune responses [104, 105].

The liver is a major accumulation site for incidental NMs entering the systemic circulation after extrapulmonary translocation, so liver toxicity is a major concern for nanotoxicology. Similarly, for nanomedicine, liver accumulation of most injected NMs reduces the efficacy of nanodrug delivery to disease sites. However, this phenomenon of liver accumulation offers opportunities for nanomedicine. For example, NMs have been developed to target liver sinusoidal endothelial cells, which serve as a major antigen-presenting cell type in the liver capable of generating regulatory T-cells (Tregs) to induce immune tolerance in autoimmune disease and allergy models [106, 107].

Finally, basic understanding of the mechanisms of toxicity of NMs (nanotoxicology) facilitates the development of new NM-based drugs (nanomedicines) exemplified by the cases of graphene oxide, where understanding of lipid membrane peroxidation-induced toxicity of

graphene enabled the creation of more efficient antimicrobials thereby overcoming antibiotic resistance [108, 109].

4. Conclusions

For nearly three decades, the field of nanotoxicology has accumulated a wealth of data and knowledge concerning the biological responses of living cells and animals to exposure of NMs. Much of this information has been reviewed elsewhere, serving as reflections on the fate of NMs from cradle to grave and offering guidance for safe nanotechnology for human health and the environment. In this *Perspective*, we contended that the two predicaments, i.e., real-world relevance of nanotoxicology and efficacy of nanomedicines, may be mitigated by improved collaboration and knowledge-sharing between the mechanism-focused field of nanotoxicology and the application-oriented field of nanomedicine. The field of nanosafety intends to bridge that gap, but the principles, methodologies, and observations of nanosafety originate from nanotoxicology. We exemplified routes of knowledge transfer from nanotoxicology to nanomedicine concerning the nano-bio interface, the physicochemical, intrinsic bioactive and antibacterial properties of NMs, the microbiome, novel coronaviruses, the pathogenic-therapeutic duality of the NanoEL effect, statistical modelling, as well as cellular and organism models, summarized in the recommendations for two-way knowledge transfer in Box 1. We highlighted a crucial need for the field of nanotoxicology to investigate the toxicity, modes of action and responses to exposure for functional hybrids over defined NMs for improved translation of the nanotoxicity database to safe nanomedicines, and for nanomedicine to leverage the extensive modelling and machine learning approaches emerging in nanotoxicology. Together, this *Perspective* intended to illustrate the Yin and Yang relationship between nanotoxicology and nanomedicine, for their shared interest and utility in the nano-bio interface, and for their contrasting approaches and goals. Regardless, from a more philosophical

point of view, nanotoxicology and nanomedicine are inherently two complementary elements of one discipline in nanotechnology striving for the sustainability and betterment of life.

Box 1. Key recommendations for bidirectional knowledge transfer between nanotoxicology and nanomedicine.

For nanotoxicology:

- ✓ Adaptation of new endpoints to address the indirect effects of NMs, such as the potential effects of NMs on the immune system (e.g., immunoglobulin deposition on NMs, complement activation and inflammatory responses) and the effects of the NMs on the composition and function of the microbiome.
- ✓ Testing of nanomedicine-relevant NMs and conditions to address the toxicity of functional NM hybrids in parallel to defined NMs and the impact of discharged nanomedicines on the environment.
- ✓ Consideration of the potentially added value of nanotoxicology to nanomedicine by converting undesired toxic effects into desired therapeutic outcomes (e.g., killing of cancer and bacterial cells by intrinsically toxic NMs, and development of new vaccine adjuvants based on inflammasome-activating NMs).
- ✓ Understanding the toxicology relevance of the EPR and NanoEL effects for facilitating the development of anti-tumor and the blood-brain barrier penetrating nanomedicines.
- ✓ Elucidating the toxicology and pathology of novel viruses as nanoparticulate toxins.

For nanomedicine:

- ✓ Adaptation of the know-how developed by the nanotoxicology community for the physicochemical characterization of NMs in their pristine form as well as after bio-transformations (including evolution of the bio-corona).
- ✓ Incorporation of microbiological knowledge gained from nano(eco)toxicology studies, including the potential of nanomedicines to affect microbial metabolic pathways that have been connected to human diseases, i.e., targeted metabolomics, combined with transcriptomics approaches.
- ✓ Incorporation of nanotoxicology knowledge into the design of nanomedicines, e.g., factors related to the intrinsic toxicities of NMs to both eukaryotic and prokaryotic cells (i.e., microbiome); avoiding cytotoxicity issues by fine-tuning the biodistribution, surface properties, shape and composition of NMs.
- ✓ Translating the knowledge on endothelial leakiness to guide the design and data interpretation of nanomedicines against cancer, diabetes as well as neurological disorders associated with the body vasculature as well as the BBB.
- ✓ Developing nanomedicines targeting the structure, toxicity and signaling pathways of novel viruses.
- ✓ Adaptation of experimental and theoretical approaches developed by nanotoxicology (e.g., eukaryotic unicellular organisms such as protozoa or yeast as model systems, cell co-cultures, artificial *in vitro* models, zebrafish as an *in vivo* model, test standards, systems biology approaches, QSARs and nanoinformatics approaches).

Declaration of Competing Interest

None.

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