Trends in Biotechnology

CellPress
REVIEWS

Opinion

Debugging Nano–Bio Interfaces: Systematic Strategies to Accelerate Clinical Translation of Nanotechnologies

Morteza Mahmoudi[1,*,@]

Despite considerable efforts in the field of nanomedicine that have been made by researchers, funding agencies, entrepreneurs, and the media, fewer nanoparticle (NP) technologies than expected have made it to clinical trials. The wide gap between the efforts and effective clinical translation is, at least in part, due to multiple overlooked factors in both *in vitro* and *in vivo* environments, a poor understanding of the nano–bio interface, and misinterpretation of the data collected *in vitro*, all of which reduce the accuracy of predictions regarding the NPs' fate and safety in humans. To minimize this bench-to-clinic gap, which may accelerate successful clinical translation of NPs, this opinion paper aims to introduce strategies for systematic debugging of nano–bio interfaces in the current literature.

Highlights

Critical information on nano–bio interfaces (e.g., biomolecular corona) and cells, including their sex, type, size, and passage number, should be considered.

Standardization communities should propose standard units for nanoparticle dosage.

Mathematical and computational approaches should be developed to define underlying mechanisms at the nano–bio interfaces.

Interlaboratory comparison of characterization of nanoparticles, nano–bio interfaces, nanotoxicities, therapeutic efficacies, and others should be conducted to prevent conflicts produced by different instruments.

Researchers, various well-established laboratories, funding agencies, entrepreneurs, and the media should work closely to prepare reliable and precise data sets, not only to prevent further clutter in the nanomedicine literature but also to accelerate successful clinical translation of nanomedicine.

Why Has Therapeutic Nanotechnology Failed Clinically?

Nanotechnology is now being used in many non-biomedical applications like displays, automobile parts, clothing, and kitchen appliances. However, the progress we have made in understanding and integrating nanotechnology has not extended to nanomedicine because the very qualities of nanoparticles (NPs) that make them so well suited to industry also happen to make them toxic and harmful in biological and medical contexts. Consequently, the use of nanomaterials in nanomedicine products (e.g., therapeutics; please note that medical devices are not considered in this category) has been fairly limited [1]. In cancer therapies, for example, there are fewer than expected FDA-approved products on the market; notable exceptions include ABRAXANE, which is an albumin-stabilized paclitaxel NP; DOXIL, a liposomal formulation of doxorubicin; and ONIVYDE, a liposomal formulation of irinotecan[i].

The reasons for nanotechnologies' lack of success in medical applications, and specifically therapeutic NPs as distinct from medical devices, are diverse, but include (i) considerable inconsistency in the biological impact of nanotechnologies (ranging from toxicity to therapeutic efficacy); (ii) misinterpretations of the current literature (basically due to our poor understanding of nano–bio interfaces); (iii) interference of nanotechnologies with available assays/kits, which has been widely ignored in the literature; (iv) a failure to determine essential parameters and controls in *in vitro* settings; (v) missing data/information in the current literature on the nano–bio interface, nanotechnology aspect, and biological system parts of *in vitro* and *in vivo* assays/models; and (vi) overlooking essential/critical phenomenon of disease occurrence and progress; for example, epithelial–mesenchymal transition (EMT) in cancer [2]. These serious challenges in nanomedicine hinder accurate prediction of biological readouts (e.g., therapeutic efficacy) of nanotechnologies based on available *in vitro* knowledge. An informative example is the therapeutic efficacy of nanotechnologies in cancer treatment. A very recent in-depth

[1]Department of Anesthesiology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA
[@]Twitter: @MoriMahmoudi

*Correspondence:
mmahmoudi@bwh.harvard.edu
(M. Mahmoudi).

analysis of the delivery of a wide range of NPs to different tumors reported over the past 10 years revealed that only 0.7% (median) of the NPs administered were actually delivered to solid tumors [3]. Therefore, it is unsurprising that many nanomedicines fail clinical trials [4], and even among the few successful products that do pass clinical trials, there is not yet any FDA-approved nanomedical product that markedly improves patient survival or quality of life. If these serious challenges are not addressed in future publications, I predict that many nanomedicine products currently in clinical trials will also fail. A prime example: most nanomedicines currently in clinical trials include targeted therapies using ligands to selectively identify tumor cells and deliver drugs (either single or multiple drugs delivered simultaneously). These new modalities may be subject to several crucial but unpredicted drawbacks based on factors including the effect of the biomolecular corona [5] (the layer of specific proteins rapidly but reproducibly adsorbed onto the surface of NPs) and its shielding role on both targeting species [6] and drug release [7].

Systematic debugging of the nano–bio interfaces is one of the approaches I offer in this opinion paper as a potential means to overcome these serious challenges. However, systematic debugging requires tight controls and a deep understanding of three elements: the nano parameters (e.g., physicochemical properties of NPs), nano–bio parameters (e.g., important factors affecting the composition and evolution of the biomolecular corona), and the biosystem (e.g., cell type). Although the nano element (reproducibility of NP preparations with distinct physicochemical properties) has been extensively investigated [8–12], neither the nano–bio parameters nor the biosystem element has received proper study. Therefore, most of the inconsistencies in nanomedicine data spring from poor control of the conditions related to the nano–bio parameters and the biosystem element [13,14]. Herein, I will focus on concerns related to the nano–bio parameters and suggest that the biosystem element should be carefully addressed in future publications through the creation of reproducible and robust data sets that will ultimately culminate in the successful clinical translation of nanotechnologies.

Modifications of Conventional Toxicity Assays

Most toxicological assays were created to monitor the toxicity of soluble chemicals, long before they were applied to toxicity evaluations of NPs. For example, the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay and comet assay were developed in 1983 [15] and 1984 [16], respectively; however, they were not originally designed for nanotoxicity evaluation, to which they were applied a decade later [17,18]. Using long-standing assays for nanotoxicological purposes and seeking robust and accurate results requires essential modifications to the assay and consideration given to all possible degrees of interference in the assays by the NPs themselves, as outlined in the following section.

Interference of Nanoparticles with Toxicity Assays

The interaction between the NPs themselves and toxicity assays is one of the primary sources of errors in toxicological readouts. For example, several NPs (e.g., carbon nanomaterials, nanoporous silicon, and silver NPs) can interfere with cell viability and proliferation assays (e.g., MTT, WST-1, XTT, and alamarBlue) to produce false-positive/false-negative results [19,20]. In this regard, even a cursory review of the literature turns up a few reports probing the toxicity of NPs via proliferation assays where there are clearly interactions with the assays. This strongly suggests that the vast majority of the toxicity data in the literature are not accurate, explaining at least in part why the current literature reflects a wide range of toxicity for the exact same type of NPs. More specifically, one recent report revealed that about 95% of the publications using different assays to probe the toxicity of NPs did not mention the potential of interference from

the NPs [21]. This strongly suggests that the field of nanomedicine still suffers from a lack of robust and precise toxicological data.

Issues with Static *In Vitro* Conditions

Because of their high surface-to-volume ratio, NPs are likely to interact with the biomolecules, amino acids, and nutrients in cell-culture media. As NPs can easily change the nutritional balance of the cell-culture media in terms of biomolecules and pH, the media could exert their own toxic effect on cells, and create false results. Several strategies to minimize such false readouts have been considered, including creating a dynamic environment using microfluidics approaches and modifying the assay protocols [22–24]. As a very simple example, one suggestion to diminish the likelihood of errors in the MTT assay was to introduce NPs to the cell-culture medium prior to incubating them with cells; this would saturate the surface of the NPs with the preferred biomolecules. Thereafter one would collect the biomolecule-coated particles, re-disperse them into fresh media, and then apply them to the cells [24]. Aside from these efforts, which are reflected in only a very small portion of the current literature, there are essentially no other reports that consider this important issue; thus, many published *in vitro* outcomes may contain errors.

Issues with Nanoparticles' Biological Characteristics and Dosage

In cell-culture media, the synthetic identity of NPs becomes their biological identity [13]. The surface of NPs becomes coated with a wide range of proteins and nutrients, which leads to significant changes in their physicochemical characteristics, including size and surface properties. Such changes may cause aggregation/agglomeration and sedimentation of NPs in cell-culture media, which alter toxicology responses, cellular uptake of NPs, and intracellular trafficking outcomes [25]. To minimize these errors in assessing the biological responses to NPs, detailed examinations of the physicochemical properties of NPs and their biological identities should be performed and published. In addition, there should be a concerted effort to create a unifying literature on the biological effects of nanotechnologies and use it to identify particles with negligible aggregation/agglomeration and sedimentation for biological readouts. In addition, NP dosage should be carefully analyzed and reported in a standard and/or similar way, which should be introduced by standardization communities; unfortunately, due to the lack of standard unit, the current reports on the NPs' dosage contain different units (e.g., mg/mL, mole, mg/kg body weight, and number of NPs in cells) [26], which prevent precise comparison between the outcomes of various reports. Aside from the reported dosage, almost none of the nanotoxicology reports consider or report the actual dose of NPs in biological systems; instead, the incubated dosage of NPs with cell media and biological systems are reported, which could be substantially misleading. To define the actual dose of NPs in cells, the supernatant solution should be considered as an essential negative control [27]. More specifically, the cell-culture supernatant should be collected and analyzed to accurately assess the number or amount of NPs remaining in the media. More important information that can be derived from the supernatant control is the contribution to toxicology outcomes of leftover compounds and ions still present in the solution. As an example, a gold nanorod solution and its corresponding supernatant had very similar toxicity on cultured cell lines, indicating that the toxicity of the NP solution originated from chemicals used in processing (e.g., free cationic capping agent) rather than the NPs themselves [28].

To summarize, I ask authors of new manuscripts to comprehensively consider the modification of conventional toxicity assays to build a robust and accurate new source of NP toxicity data. As suggested by Krug and Wick [20], I also call on editors and reviewers to avoid publishing

manuscripts that do not include in-depth information on (or experiments to assess) NPs' interference with the toxicity assays employed, the role of static substrates, full characterization of the biological identity of NPs, and essential supernatant controls for defining the actual dosage of NPs and the possible interference of leftover chemicals/ions in the assays' readouts. Last but not least, I ask science media to cover reports on robust modifications of the conventional toxicity assays for nanotoxicity purposes; in addition, the steering committees and clinicians that approve particular NP usage in clinical trials are encouraged to look into the toxicity and safety data sets of that distinct NP to make sure that robust and precise outcomes, without misinterpretation, are provided.

Missing Critical Information about Cells and Their Set Ups

Cell Shape

Cells in their native tissues have different morphologies compared with the same cells grown on a culture plate. One simple reason is that because 2D cell-culture plates somewhat flatten cells, they never attain their normal 3D shape. As cell shape can affect surface area, which in part determines cells' interactions will the environment, one expects cells with greater exposed surface area to have much higher possibility of interaction (see Figure 1 for details). To that end, and because the adsorption and internalization of NPs by cells are heterogeneous [29], cells cultured on flat substrates would be expected to demonstrate substantially different uptake of NPs and intracellular trafficking compared with the same cells in their native tissues. In addition to heterogeneous adsorption and internalization of NPs by cells (as shown by an overdispersed Poisson probability distribution [29]), another mechanism affecting cell–NP interactions is the number of cell membrane receptors exposed to the NPs. Therefore, if cells cannot assume their *in vivo* shapes in *in vitro* settings, the outcomes of NP and drug-screening readouts may contain considerable errors. Despite the importance of shape in cell function and behavior, the effect of variation in shape among the same type of cells on cellular uptake of NPs and intracellular trafficking and toxicology data has so far been very poorly investigated.

Another reason for cell shape variation between *in vitro* and *in vivo* settings is the significant difference in stiffness between native tissue and cell-culture plates, a factor practically ignored in the current literature. *In vivo*, different cells are subject to a wide range of stiffness depending on their native tissue (i.e., there is a ~50 000-fold difference in stiffness from very soft to hard tissues [30]). It is known that substrate stiffness has crucial effects on cell shape and function. For example, the morphology and cytoskeletal structure of three different cells (fibroblasts, endothelial cells, and neutrophils) were probed after being cultured on surfaces with stiffness ranging from 2 to 55 000 Pa. Outcomes revealed substantial effects of substrate stiffness on cell shape, morphology, and cytoskeleton (including variation of actin stress fibers) in all cell types [31]. A good example of the effect of substrate stiffness on cell function is its effective use in directing the fate of mesenchymal stem cells toward neurogenic, myogenic, or osteogenic phenotypes [32].

The shape-related cellular effects associated with conventional culture plates may cause substantial misinterpretation of the safety and therapeutic efficacy of NPs. To overcome this major issue, new cell-culture methodologies should be developed that mimic the native tissue stiffness of the cells being cultured and also provide surface features that encourage cultured cells to recapitulate their 3D structures. Culturing of cells on such substrates should provide readouts with superior predictive accuracy compared with conventional cell-culture plates.

Several attempts have been made to optimize culture conditions (e.g., microfluidics and 3D culture plates) to maximally encourage cells to assume the morphology and shape found in

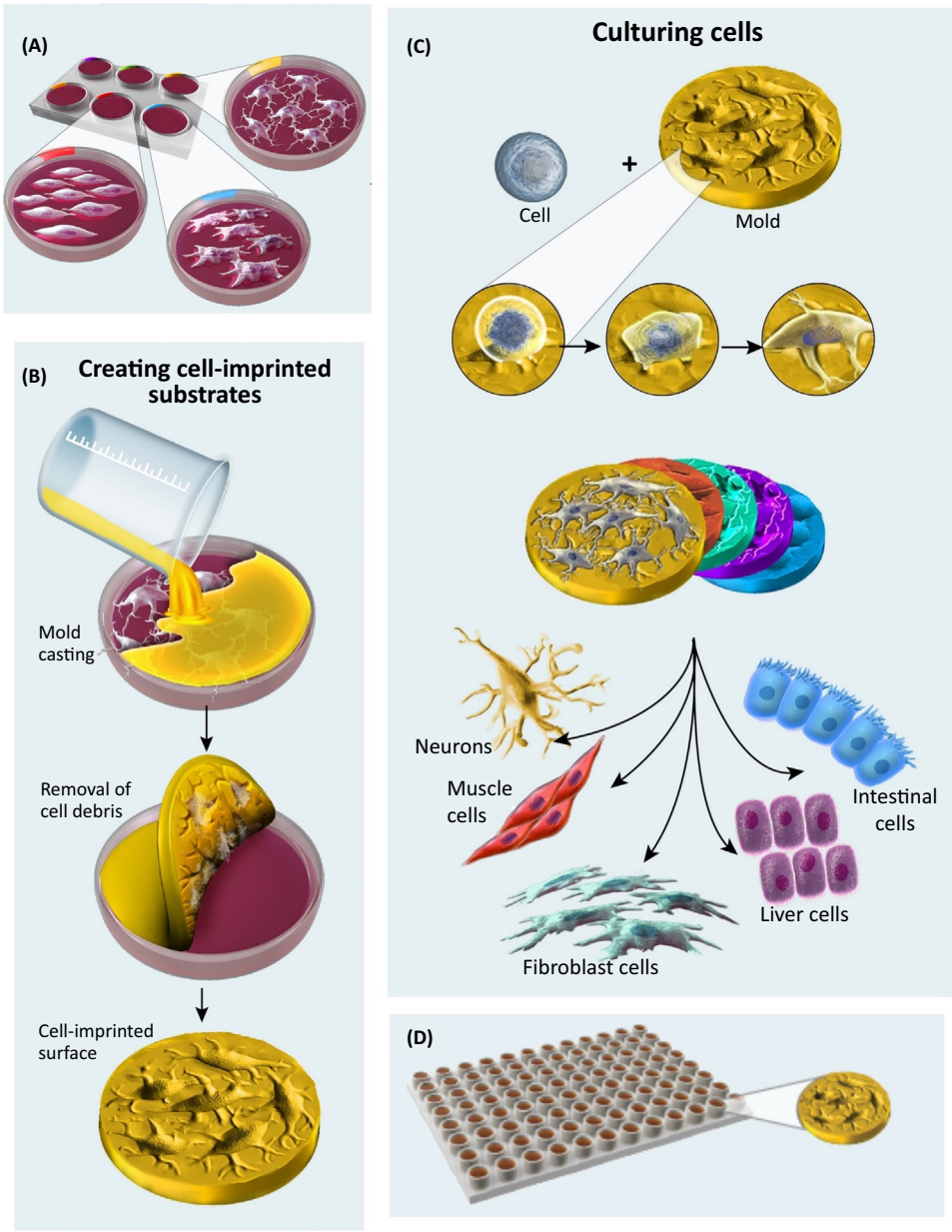

Figure 1. Schematic Showing a Representative Cell-Imprinting Method for Production of Substrates to Achieve More Robust and Reliable Nanoparticle–Cell Interaction Readouts. (1) Desired cells are cultured on 2D substrates approximating the stiffness of the corresponding cells' native tissue; (2) cell shape and morphologies are transferred by mold casting using a material such as polydimethylsiloxane. After a curing step, the cell debris are removed and the cast serves as a negative replica with an imprinted pattern of the cell surfaces. (The casting materials and curing steps should be selected and designed in a way to provide the required stiffness for Stage 3.) (3) Cells are cultured on substrates having the shape, morphologies, and stiffness of their corresponding tissues, which may help cells to drive their 3D native tissue shapes. To achieve accurate and reproducible toxicity outcomes, nanoparticle uptake, and intracellular trafficking, each cell type should be cultured on molds with the appropriate shape and tissue stiffness. (4) Example of future mass-produced, cell-specific well plates having desired cell shape and stiffness.

native tissues [22,23,33]. However, to the best of our knowledge, no 2D approach has provided both the surface features of the desired cells and the stiffness of their corresponding tissues (e.g., ~50, ~12 000, and ~3 000 000 Pa for neurons, skeletal muscle, and bone cells, respectively). To overcome this issue, I propose a straightforward approach to preparing a substrate with stiffness similar to the actual cells' native tissue that also encourages cells to assume their pseudo-3D shapes in 2D culture plates (see Figure 1 for details). The first step is to fabricate a smooth substrate recapitulating the stiffness of the desired cells' native tissue, drawing upon a wide range of biocompatible materials such as polymers and polydimethylsiloxane (PDMS). The cells themselves would be used as templates, transferring their shapes and surface morphologies to the casting materials (e.g., polymers and PDMS), a technique known as cell imprinting [34]. The resulting substrates will provide the necessary combination of the surface features of the desired cells and the stiffness of their corresponding tissues.

For mass production of such a substrate (with the imprinted shape of the desired cells and proper stiffness to mimic the mechanical properties of those cell's native tissue), it is necessary to create a master mold from which replicas would be produced. One approach likely suitable for mass production of cell-imprinted surfaces with proper stiffness is electroforming [35,36] a nickel mold, followed by replication via injection molding, casting, hot embossing processes, or photo-polymerization. Electroforming is a metal-forming process that uses electrodeposition or electroplating onto a master, and its tolerances in replicating surface features usually lie in the range of 1.5–3 nm [35,36]. This process can be used to deposit nickel or other electroformable metals onto gold-coated cell-replicate masters. The metallic molds can then be used for direct hot embossing of the surface of any desired substrate (with stiffness adjusted according to the native stiffness of the cell to be cultured) to impart cell-surface topography.

This proposed approach is only one example of many possible strategies to produce substrates toward the goal of reliable and efficient culturing of a wide range of cells of varying origins (e.g., heart, liver, skin, or brain), effectively mimicking the *in vivo* shape and function of the cells for assessment of NP uptake and intracellular trafficking as well as drug-screening applications (see Figure 1 for details). These new substrates may yield important insights into NP–cell interactions and reduce the current gap between *in vitro* and *in vivo* outcomes. In other words, using substrates that mimic both the shape and stiffness of cells in their native tissues could go a long toward resolving many of the existing misunderstandings regarding the cellular uptake of NPs and their intracellular trafficking and toxicology. Last but not least, cell confluency should be carefully considered in *in vitro* settings, as it critically changes mechanical structure of cells [37], and thus their shapes, which may affect cellular responses to NPs.

Cell Type/Origin/Size

Just as human beings have different ways to solve a problem, various cells can also have different responses to a foreign material such as NPs. This is mainly thought to be due to the differences in the surface properties of the cell membrane, as well as different types and numbers of receptors on the surface of each cell, given its distinct size. Based on these hypotheses, it was shown that the impact of the exact same NPs on different cell types varies significantly [38]. There are several mechanisms underlying these variations, including (i) numerous detoxification strategies that any particular cell (based on its function and membrane properties) can employ when reacting with NPs and (ii) different cell sizes, which significantly affect NP uptake, intracellular trafficking, and the corresponding nanotoxicity. For the exact same cell type, and mainly due to the heterogeneous adsorption and internalization of NPs by cells [29], one can expect that NP uptake would increase with cell size. For example, daughter cells produced by division of mother cells (approximately double the size of the daughter cells

[39]) may have lower uptake of NPs compared with the mother cells, as demonstrated by Panet and colleagues [40]. These results were first reported by another group [41] from a different view point, which was the fact that NP uptake depends on the cell cycle state; more specifically, mother cells can take up more NPs than daughter cells. Although the outcomes of these two studies were the same, their conclusions reflected two distinct interpretations of the underlying mechanism. Both interpretations seem reasonable and together help provide a mechanistic understanding of the effect of cell cycle on NP uptake, which will also help avoid misinterpretation of outcomes by relying on only one mechanism.

To obtain more robust and accurate information/libraries about cell uptake, intracellular trafficking profiles, and toxicity outcomes of NPs, authors should include in all reports the cell type, size, and proliferation stages (i.e., proportions of cells at different cycle stages: G_1, S, G_2, and M). That information will help the nanomedicine community design safer NPs with the greatest possible therapeutic efficacy. For example, cancer cells may be at various different cell cycle stages, making their NP uptake even more different from that of normal cells. This may also affect the side effects of nanodrugs. Since cancer cells grow and divide very quickly, drug uptake by newly born cells is much lower than that of normal surrounding cells, mainly mother cells. On top of these challenges, the random and asymmetric partitioning of NPs in cell division [29] also adds another layer of complexity to the therapeutic efficacy of NPs on cancer cells, which along with their toxic effects on normal cells should be considered in the design of future studies.

Cell Passage

Cell passage number is a type of data typically missing from the current literature on cell–NP interactions. It is known that as the number of passages increases, cells experience alterations in morphology and function (e.g., response to stimuli, growth rates, protein expression, and transfection efficiency) [42–45][ii]. For example, increasing the passage number of a prostate cancer cell line (LNCaP) from 25 to 60 can substantially change phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) pathway-regulated androgen receptor activity, which may have consequences during the various stages of prostate cancer, as these two oncogenic pathways cross-regulate each other by reciprocal feedback (inhibition of one activates the other, maintaining tumor cell survival) [46]. These variations in cellular physical characteristics and functions may substantially change nanotoxicology, NP uptake, and cellular trafficking outcomes. Thus, the nearly complete absence of cell passage numbers across publications in the field should produce considerable concern regarding the reliability and reproducibility of studies on all these aspects of NPs. Future publications should (i) use high-quality, low-passage-number cells from well-known biological resource centers and (ii) concisely and consistently report cell passage numbers.

Cell Sex

Variations in molecular function (e.g., protein and hormone secretion and mechanistic signaling pathways) and cytoskeletal differences between male and female cells are increasingly being investigated [47–50]. Sex-dependent differences in cellular function can substantially direct both the cause and treatment of disease (e.g., cardiovascular disease [51]). Sex-specific differences in protein and hormone secretion can change the biological identity and cellular fate of NPs (in terms of toxicity and trafficking pathways). For example, Hayashi and colleagues [52] demonstrated differences in how female and male zebrafish blood plasma affect the biological identity of silica NPs and their biological outcomes. Another recent study showed that cell sex can directly affect uptake of quantum dots in two different cell lines, that is, early human amniotic stem cells (extracted from placentas of male and female fetuses) and primary

fibroblasts (derived from the salivary glands of adult male and female donors of similar ages) [53]. The underlying mechanism of the different NP uptake by male and female cells is supposed to be dependent on, but not limited to, variation in secreted paracrine factors, cellular cytoskeleton, and mitochondrial and endoplasmic reticulum structures. These differences between male and female cells can change biological identity of NPs together with their mechanism of entrance. Interestingly, it was also found that the difference in NP uptake was strongly dependent on cell type. For example, uptake of quantum dots in female human amniotic stem cells was higher than the male ones, while the reverse trend was observed for fibroblasts.

Similar to cell passage number, important information on cell sex is nearly absent from most publications in the field. Researchers are encouraged to consider variation of biomolecular secretion, intracellular organelle shapes/functions, cell cytoskeleton, cell membrane receptors, and cell stiffness in male and female cells and report the sex data [53].

Cell Transition

Perhaps one of the central overlooked factors in nanotechnologies for cancer therapeutic applications is the EMT. The EMT is recognized as one of the key cellular plasticity characteristics during embryonic development [54]; however, it is now well established that the EMT has more critical effects than expected in tumorigenic process, specifically at the metastatic stages [2]. The EMT concept in therapeutic efficacy of nanotechnologies in both cell culture and *in vivo* is widely overlooked. As the EMT phenomena, which has crucial roles in a wide range of cancers and specifically in metastatic cancer cells [2], is almost ignored in the cancer nanotechnology research and clinical trials, there is no wonder that cancer nanoproducts have a higher chance of failure in successful translation to clinical practice.

Recent advances in the field demonstrated that matrix stiffness can substantially affect EMT and its resistance to chemotherapeutics [55]; therefore, in future *in vitro* use of EMT in cancer nanotechnology, the cell-culture stiffness should be carefully matched to the EMT's original tissue site, for any specific type of cancer, to achieve robust and precise outcomes.

Implications of Nanoparticles' Biological Identity

Once in the body, the surface of NPs is rapidly covered with a layer of biomolecules (i.e., 'biomolecular corona') whose composition strongly determines its biological identity [13], regulating interactions with biological entities including cells and the immune system [56]. The field of biomolecular corona work is fairly complex and requires precise protocols to make both *in vitro* and *in vivo* data more reliable and reproducible. The current literature reflects a few conflicts in results; in addition, there is poor correlation between *in vitro* corona data and *in vivo* outcomes.

Importance of Protein Source

Currently, one of the most important sources of conflict in biomolecular corona readouts is the use of different protein sources to form and characterize the biomolecular corona and connect these outcomes to justify and/or predict the *in vivo* fate of NPs. Just some of the protein sources being used are fetal bovine serum, animal serum (e.g., mouse, rat, and monkey), human serum, animal plasma, and human plasma. Several reports have revealed the significant role of protein source in the composition of the biomolecular corona and corresponding biological readouts (toxicity, cell response, cellular trafficking, and pharmacokinetics) [57–60]. In addition, even given a single plasma sample, the type of anticoagulant used can dramatically change the corona composition [59].

To more completely understand the biomolecular corona, the scientific community should focus on the use of plasmas, either animal or human, with distinct anticoagulant compounds. To probe possible connections between *in vitro* and *in vivo* outcomes, one should use the most relevant protein source that is compatible with the *in vivo* model. For example, to connect the role of the biomolecular corona to *in vivo* readouts in a mouse model, plasma from that particular mouse model should be used. To facilitate clinical translation, the composition of the biomolecular corona formed on NPs should be carefully probed in plasmas of different species (including animal models and humans) *in vitro* and compared with their biological readouts in animal models. If we are to use these data to define corona differences between animal models and humans *in vitro* and apply that information to predicting the biological fate of NPs in humans (according to the available *in vivo* readouts on animal models), future reports should employ advanced mathematical approaches such as multianalyte analysis, supervised and unsupervised clustering approaches, machine/deep learning algorithms, and quantitative structure–activity relationship models [61–63]. The combination of high-quality data and appropriate mathematical models greatly increases the likelihood of optimizing the biological identity of NPs for achieving the desired biological fates in clinical settings. Unfortunately, so far there are few reports that use such mathematical approaches to predict the cellular responses to corona-coated NPs [64–66]. This represents a huge opportunity for mathematical scientists (and more specifically scientists well versed in deep-learning strategies) to study the biological identity of NPs in a wide range of different *in vitro* and *in vivo* settings (as training and validation sets) with the ultimate aim of predicting the biological identity and readouts of those NPs in humans.

Importance of Protein Conformation and Spatial Location

For designing safe NPs with high targeting efficacies, it is essential to be able to define proteins' conformation and spatial location in the biomolecular corona. Conformation of important proteins (e.g., fibrinogen) is of critical importance as exposure of specific protein section can activate immune system [56] and thus, at least, can shorten NPs' circulation time. Therefore, monitoring and reporting important protein's conformational changes in the biomolecular corona are of crucial importance and can enable scientists to more accurately predict biological fate of NPs. For this purpose, new approaches should be developed as the available techniques (e.g., circular dichroism) do not have a proper capacity to monitor protein conformation in complex biomolecular layer. Recently, fluorescence resonance energy transfer was used to label proteins of interest and showed great capacity to probe their conformational changes in complex biomolecular corona [66]. Aside from protein conformational changes, it is now possible to also define the spatial location of specific proteins, their functional motifs, and their binding sites using a combination of various techniques including monoclonal antibody-targeted gold NPs, differential centrifugal sedimentation, and imaging techniques [67]. The protein conformation and spatial location should be considered in highly promising and well-characterized NPs before starting the clinical trials to achieve more in-depth details on possible side effects of biomolecular corona, which may affect safety and targeting/therapeutic efficacy of NPs.

Disease-Specific Biomolecular Corona

Besides the importance of protein source in directing corona composition, it was revealed in 2014 [5] that different conditions (e.g., health vs. disease) of the same protein source (i.e., human plasma) have significant effects on the composition/structure of the biomolecular corona (and thus the ultimate physicochemical properties of NPs). This concept is now being increasingly accepted, and several investigators have shown that the plasma composition evolves during the disease progress, substantially altering the biomolecular corona

composition and consequently nano–bio interactions such as efficacy, toxicity, and eventual biological fate [8,68–76]. In addition, recent findings suggest that age, gender, and ethnicity may also affect the plasma composition, with similar alterations to the biomolecular corona and biological readouts [52,70].

One issue in the current literature that produces severe conflicts in understanding the biomolecular corona is the absence of detailed information on plasma donors, including age, sex, medical history, and disease stage as well as time, date, and condition of blood extraction. For example, the majority of biomolecular corona studies to date were carried out using commercially available pooled plasma (i.e., combined from multiple sources) from donors reflecting a wide range of health, disease, age, gender, ethnicity, etc. To avoid these sources of errors, future reports should provide the important information on plasma donors to characterize variations in the biological identity of the exact same type of NP at different disease stages and how changes in biological identity may actually change the biological fates of NPs as the disease progresses. This information, along with mathematical approaches, can substantially improve the capacity of scientists to design safer NPs with more predictable therapeutic efficacy and biological fate across patients of different ages and genders and suffering from different diseases.

Implications in Correlating *In Vitro* and *In Vivo* Biomolecular Corona Information

Understanding the *in vivo* biological identity of NPs is of crucial importance for predicting the biological fate, possible toxicity, and therapeutic efficacy of NPs. Unlike the *in vitro* biomolecular corona, our knowledge about the *in vivo* biomolecular corona is very limited, mainly due to the difficulty of recapturing NPs after *in vivo* administration [58,77,78]. Therefore, careful consideration of all the important but overlooked factors relevant to the nano–bio and biosystem elements as well as others presented in this subsection will substantially enhance our capacity to use *in vitro* settings to predict the *in vivo* biological identity of NPs and their biological fates.

Another major factor that should be considered in the *in vitro* setting is mimicking the dynamic features of physiological fluids. Dynamic flow has a unique capacity to create shear stress on NPs and also provides a continual source of new biomolecules. This induces more complexity to the *in vivo* biomolecular corona in animal models of disease or humans, as the particles' environment is constantly changing. For example, in cancer models, NPs experience tumor accumulation, penetration, and distribution through several pathways including the enhanced permeability and retention effect once they leave the blood vessels. The tumor microenvironment provides significantly differing biological fluids compared with the circulatory system (in terms of pH and plasma compositions) [79] and can substantially change the biological identity of NPs, which may markedly alter their therapeutic efficacy. In addition, cancer models may have impaired lymphatic drainage and production of permeability-enhancing factors (including nitric oxide, vascular endothelial growth factors, and cytokines) [80], which affects the composition of plasma and thus the biomolecular corona composition. To capture this important information *in vitro*, plasmas used in the *in vitro* setting should be exposed to a dynamic environment, which is now possible using a wide range of microfluidic approaches [22,23]. As the current literature, for example in cancer nanotechnology, is mainly focused on the accumulation and intratumoral distribution of NPs based on their synthetic identity [8], our knowledge of how disease stage affects the biological identity of NPs and how the disease-specific biomolecular corona composition affects tumor accumulation and intratumoral distribution remains limited. The changes to *in vitro* settings described here may substantially expand our knowledge of the *in vivo* biological identity of NPs and their biological readouts in a

manner that depends on cancer stage, eventually enabling us to identify the optimal physicochemical properties of NPs for the desired tumor distribution and the highest possible therapeutic efficacy. It is noteworthy that the biomolecular corona may have substantial effects on the drug-release profiles of therapeutic NPs [7,81,82], though this important effect has been being widely ignored in the field of drug delivery. The literature on *in vivo* drug release should take into account this important effect to better predict the therapeutic efficacy of nanocarriers.

Another important issue is the difference in incubation time between the *in vitro* biomolecular corona setting and the *in vivo* blood availability of NPs. In most *in vitro* settings, NPs are incubated with proteins for 1 hour [13], while many particles have much shorter *in vivo* blood residency, and thus experience less incubation time. As the protein exchange to the surface of NPs is slow and the biomolecular corona changes over time [65,83–87], the incubation time for *in vitro* testing of a particular NP should match its *in vivo* blood circulation residency. In addition, if the particles leave the bloodstream and enter another tissue/environment (e.g., the tumor microenvironment), the *in vitro* setting should mimic the *in vivo* environment as closely as possible, including changing the incubating plasma at a rate similar to biological conditions to best define the *in vivo* biological identity of NPs. This is mainly because the composition of biomolecular corona changes during incubation; thus, if the incubation period is kept consistent, one gets reproducible and robust corona characterization, simplifying the correlation of *in vitro* outcomes with *in vivo* readouts.

Concluding Remarks and Future Perspectives

Given the points of incomplete understanding and missing information regarding the nano element, nano–bio parameters, and the biosystem element outlined above, it is not surprising that the current nanomedical literature suffers from many conflicts and misinterpretations. In the form of inaccuracies in biological readouts for nanotechnologies, these are unfortunately reflected in our inability to bring nanomedicine from the bench to the clinic, and the road has been paved with plenty of setbacks. To correct these misinterpretations and minimize conflicts, researchers, funding agencies, entrepreneurs, and the media should collaborate to ensure all required controls and experimental settings are carefully considered, and that all required information is comprehensively and consistently reported (Figure 2 and see Outstanding Questions). Aside from debugging nano–bio interfaces from a scientific viewpoint, there are many 'overlooked factors' in development and commercialization of bench-to-clinic discoveries. I personally believe the central overlooked factor is the necessity of building the entire foundation of nanomedicine companies from people (at all levels including venture capitalists, founders, entrepreneurs, scientific advisory boards, managing team, and staff members) who are wholeheartedly persuaded by changing the course of the world in terms of healing people suffering from catastrophic diseases (such as cancer, cardiac, and neurodegenerative disorders), and relieving the agony of their families. By having other motives as our highest priority (e.g., profits and fame), we should expect seeing the continued failure of nanomedicine products together with a substantial increase in prevalence and incidence of diseases. At least from what history has thought us, only a small fraction of people, like Louis Pasteur and Marie Curie, who strongly believe in changing the course of the world with great empathy and genuine care for all humans (and not for their own profit and fame) can change our lives. The second important and often overlooked factor is the gaping chasm between the support of funding agencies, entrepreneurs, and the media of therapeutic companies in comparison to companies that are focused on disease prevention. It is clear that our need for therapeutic measures will decrease if robust prevention strategies can reduce the devastating costs of therapeutic approaches together with the agony of disease for both patients and their families.

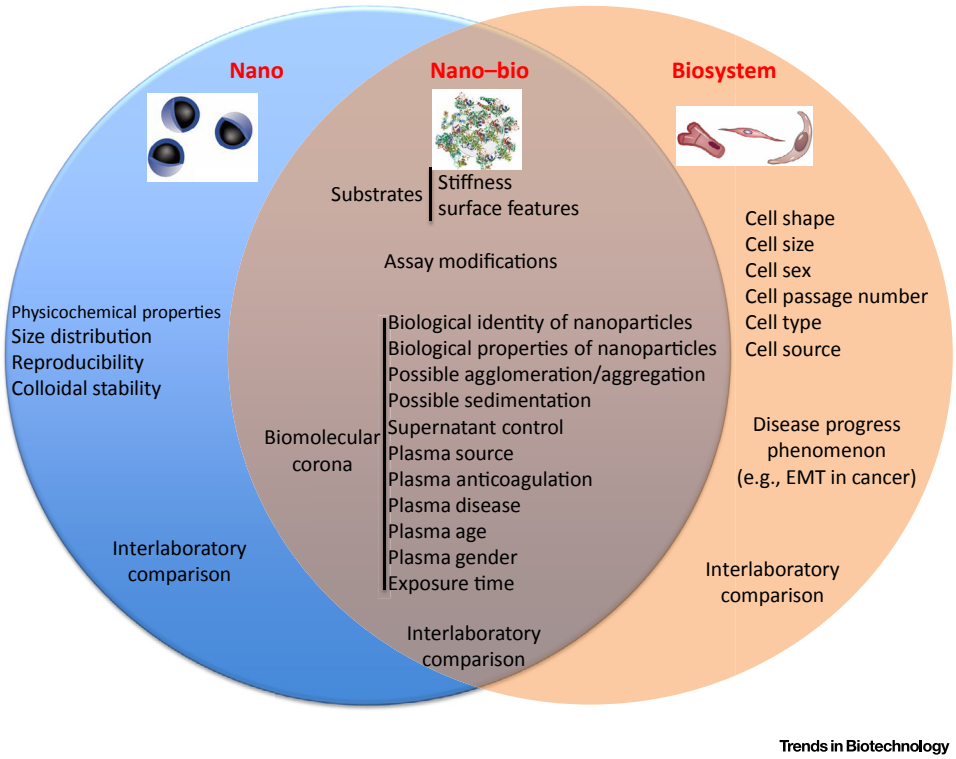

Figure 2. Schematic Showing the Minimum Set of Required Information and Experimental Setups from Nano-, Nano–Bio Parameters, and the Biosystem Elements for Careful Consideration by Authors, Reviewers, and Editors in Future Publications for Achieving Robust and Reliable Nanomedicine Readouts.

The decision of prioritizing one of these fields, prevention or therapeutic research, is a no-brainer. I call upon funding agencies, researchers, entrepreneurs, and the media to take a second glance at these overlooked factors, which might pave the way for a great cost reduction in the medical field, save families much emotional strain, and ultimately save potential patients from having to endure these catastrophic diseases.

In addition, computational scientists and software companies may play an important role in defining the actual mechanisms underlying some complex situations (e.g., learning the details of protein conformational changes after its interaction with the surface of NPs and their corresponding biological outcomes) – see Box 1 for details. Last, but not least, a very recent report probed outcomes of the exact same type of NPs through different laboratories and revealed significant variations in the results [88]. To avoid future source of conflicts through interlaboratory variations, robust and precise standard operating procedures should be developed not only for NP characterizations, but also for all characteristics of nano–bio interfaces (e.g., biomolecular corona) and the biological readouts including nanotoxicology, cellular uptake and intracellular trafficking of NPs, and targeting and therapeutic efficacies. Without resolving these important issues in the field and establishing constructive collaboration among researchers, various well-established laboratories, funding agencies, entrepreneurs, and media, future progress in the field of nanomedicine will only further clutter and complicate the literature. Instead, I believe that incorporating the strategies proposed above into future progresses will

Box 1. The Importance of Computer Simulations in Debugging Nano–Bio Interfaces

Over the past decade, combinations of computer simulation and experimental approaches have provided in-depth information about the mechanisms underlying important observations at nano–bio interfaces and, in particular, have shed light on the interactions between nanoparticles and the binding sites/functional units of proteins and resulting structural alteration [65,89]. The beauty of simulation approaches (if the system is small enough to run the simulation in a reasonable amount of time and the required force-field parameters are available) is that it can substantially help scientists identify the mechanisms underlying nanoparticle–protein interactions and also provide information on the structural/conformational changes in proteins that guide the biological fate of nanoparticles. For example, using a molecular dynamics simulation, interactions of insulin and fibrinogen with gold nanoparticles were probed and the effects of the nanoparticles on the proteins' structural/conformational changes determined [90]. This kind of detailed proteomic information will provide a unique opportunity to the scientific community to reduce misinterpretation of solely experimental outcomes and also can substantially increase the predictive accuracy of the biological outcomes of nanoparticles. Although concurrent simulation and experimental approaches are extremely useful for resolving existing conflicts in the literature on protein–nanoparticle interactions [91], at the moment such simulations can be applied only in very limited circumstances (mainly for single protein–nanoparticle studies) due to the technical shortcomings of computer simulations (the common simulation approaches together with their advantages and limitations have been discussed in [92]). Overall, the field of nanomedicine would benefit greatly from the contribution of experts in computer simulation and computational biology toward developing new methodologies to overcome the current limitations of these approaches for nanomedicine applications.

Aside from protein–nanoparticle interactions, the latest advances in the field of computer simulation provide a unique opportunity to quantitatively and qualitatively consider the effect of substrates' mechanical and morphological properties on cell shape, membrane exposure to cell-culture media, and cell nucleus characteristics, in terms of shape, direction, and even chromatin conformation. In a prime example, a unifying computational framework was employed to create a multicomponent 'virtual cell model' with promising capability to predict changes in cell and cell nucleus characteristics in a range of cell substrates with different physico-mechanical properties (see [92] for details). These computational approaches may represent an exciting opportunity to correct many misinterpretations in the current literature by providing insights into variations in cell morphologies, membrane exposure to cell-culture media, and nucleus characteristics of cells cultured on appropriate substrates (i.e., mimicking the shape of cells and their native tissue stiffness) compared with the same cells cultured on conventional plastic-well plates.

Another important field within computer science that may have a dramatic impact on nanomedicine is machine/deep learning, which is an artificial-intelligence approach that enables computers to reach a mode of self-learning without being explicitly programmed [93–95]. When exposed to a new set of data, these programs are capable of learning, growing, changing, and developing by themselves. Machine learning, though now only tangentially involved in nanomedicine, may revolutionize the field, and more specifically, our understanding of the complex nano–bio interface [62,96]. For example, machine learning can be trained using biomolecular corona data from different nanoparticles with distinct physicochemical properties (after incubation with plasma of different species, including animal models and humans, *in vitro*) and their biological readouts; these training sets could then be employed to predict either the biological identity of other nanoparticles in different plasmas or the fate of nanoparticles in human systems.

Outstanding Questions

Why there is a huge gap between bench discoveries and clinical translation of nanoparticles?

Why are critical factors in disease progress, such as the epithelial–mesenchymal transition in cancer, poorly considered in nanomedicine?

Why does the current literature contain significantly conflicting biological data on even the exact same types of nanoparticles?

How can debugging nano–bio interfaces and systematic strategies help to market nanomedicine technologies?

What critical pieces of information, which may be sources of conflicts in the literature, are yet overlooked?

Why does interlaboratory comparison of the exact same nanoparticles, using the same assays, etc., provide different outcomes?

generate comprehensive and unbiased data that, in combination with advanced mathematical approaches such as machine/deep learning, may significantly accelerate the discovery and successful clinical translation of nanomedicines for a wide range of diagnostic and therapeutic applications.

Resources

[i] www.cancer.gov/news-events/cancer-currents-blog/2016/grodzinski-nanotechnology

[ii] www.atcc.org/~/media/PDFs/Technical%20Bulletins/tb07.ashx

References

1. Etheridge, M.L. *et al.* (2013) The big picture on nanomedicine: the state of investigational and approved nanomedicine products. *Nanomedicine* 9, 1–14
2. Brabletz, T. *et al.* (2018) EMT in cancer. *Nat. Rev. Cancer* 18, 128–134
3. Wilhelm, S. *et al.* (2016) Analysis of nanoparticle delivery to tumours. *Nat. Rev. Mater.* 1, 16014
4. Ledford, H. (2016) Bankruptcy filing worries developers of nanoparticle cancer drugs. *Nature* 533, 304–305
5. Hajipour, M.J. *et al.* (2014) Personalized protein coronas: a "key" factor at the nanobiointerface. *Biomater. Sci.* 2, 1210–1221
6. Salvati, A. *et al.* (2013) Transferrin-functionalized nanoparticles lose their targeting capabilities when a biomolecule corona adsorbs on the surface. *Nat. Nanotechnol.* 8, 137–143

7. Cifuentes-Rius, A. *et al.* (2013) Optimizing the properties of the protein corona surrounding nanoparticles for tuning payload release. *ACS Nano* 7, 10066–10074
8. Shi, J. *et al.* (2017) Cancer nanomedicine: progress, challenges and opportunities. *Nat. Rev. Cancer* 17, 20–37
9. Xia, X.-R. *et al.* (2010) An index for characterization of nanomaterials in biological systems. *Nat. Nanotechnol.* 5, 671–675
10. Saei, A.A. *et al.* (2017) Nanoparticle surface functionality dictates cellular and systemic toxicity. *Chem. Mater.* 29, 6578–6595
11. Dawson, K.A. (2013) Leave the policing to others. *Nat. Nanotechnol.* 8, 73
12. Wills, J.W. *et al.* (2017) Characterizing nanoparticles in biological matrices: tipping points in agglomeration state and cellular delivery *in vitro*. *ACS Nano* 11, 11986–12000
13. Monopoli, M.P. *et al.* (2012) Biomolecular coronas provide the biological identity of nanosized materials. *Nat. Nanotechnol.* 7, 779–786
14. Mahmoudi, M. *et al.* (2017) Multiscale technologies for treatment of ischemic cardiomyopathy. *Nat. Nanotechnol.* 12, 845–855
15. Mosmann, T. (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* 65, 55–63
16. Ostling, O. and Johanson, K.J. (1984) Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells. *Biochem. Biophys. Res. Commun.* 123, 291–298
17. Hoffmann, F. *et al.* (1997) Preparation, characterization and cytotoxicity of methylmethacrylate copolymer nanoparticles with a permanent positive surface charge. *Int. J. Pharm.* 157, 189–198
18. Dhawan, A. *et al.* (2006) Stable colloidal dispersions of C60 fullerenes in water: evidence for genotoxicity. *Environ. Sci. Technol.* 40, 7394–7401
19. Azhdarzadeh, M. *et al.* (2015) Nanotoxicology: advances and pitfalls in research methodology. *Nanomedicine* 10, 2931–2952
20. Krug, H.F. and Wick, P. (2011) Nanotoxicology: an interdisciplinary challenge. *Angew. Chem. Int. Ed. Engl.* 50, 1260–1278
21. Ong, K.J. *et al.* (2014) Widespread nanoparticle-assay interference: implications for nanotoxicity testing. *PLoS One* 9, e90650
22. Huh, D. *et al.* (2011) From 3D cell culture to organs-on-chips. *Trends Cell Biol.* 21, 745–754
23. Griffith, L.G. and Swartz, M.A. (2006) Capturing complex 3D tissue physiology *in vitro*. *Nat. Rev. Mol. Cell Biol.* 7, 211–224
24. Mahmoudi, M. *et al.* (2010) A new approach for the *in vitro* identification of the cytotoxicity of superparamagnetic iron oxide nanoparticles. *Colloids Surf. B Biointerfaces* 75, 300–309
25. Cho, E.C. *et al.* (2011) The effect of sedimentation and diffusion on cellular uptake of gold nanoparticles. *Nat. Nanotechnol.* 6, 385–391
26. Hardman, R. (2006) A toxicologic review of quantum dots: toxicity depends on physicochemical and environmental factors. *Environ. Health Perspect.* 114, 165
27. Alkilany, A.M. *et al.* (2016) Misinterpretation in nanotoxicology: a personal perspective. *Chem. Res. Toxicol.* 29, 943–948
28. Alkilany, A.M. *et al.* (2009) Cellular uptake and cytotoxicity of gold nanorods: molecular origin of cytotoxicity and surface effects. *Small* 5, 701–708
29. Summers, H.D. *et al.* (2011) Statistical analysis of nanoparticle dosing in a dynamic cellular system. *Nat. Nanotechnol.* 6, 170–174
30. Butcher, D.T. *et al.* (2009) A tense situation: forcing tumour progression. *Nat. Rev. Cancer* 9, 108
31. Yeung, T. *et al.* (2005) Effects of substrate stiffness on cell morphology, cytoskeletal structure, and adhesion. *Cytoskeleton* 60, 24–34
32. Engler, A.J. *et al.* (2006) Matrix elasticity directs stem cell lineage specification. *Cell* 126, 677–689
33. Mashinchian, O. *et al.* (2015) Regulation of stem cell fate by nanomaterial substrates. *Nanomedicine* 10, 829–847
34. Ren, K. and Zare, R.N. (2012) Chemical recognition in cell-imprinted polymers. *ACS Nano* 6, 4314–4318
35. Schlesinger, M. and Paunovic, M. (2011) *Modern Electroplating*, John Wiley & Sons
36. Ansari, K. *et al.* (2006) Stamps for nanoimprint lithography fabricated by proton beam writing and nickel electroplating. *J. Micromech. Microeng.* 16, 1967
37. Pegoraro, A.F. *et al.* (2017) Mechanical properties of the cytoskeleton and cells. *Cold Spring Harb. Perspect. Biol.* 9, a022038
38. Mahmoudi, M. *et al.* (2012) Cell "vision": complementary factor of protein corona in nanotoxicology. *Nanoscale* 4, 5461–5468
39. Tzur, A. *et al.* (2009) Cell growth and size homeostasis in proliferating animal cells. *Science* 325, 167–171
40. Panet, E. *et al.* (2017) The interface of nanoparticles with proliferating mammalian cells. *Nat. Nanotechnol.* 12, 598–600
41. Kim, J.A. *et al.* (2012) Role of cell cycle on the cellular uptake and dilution of nanoparticles in a cell population. *Nat. Nanotechnol.* 7, 62–68
42. Esquenet, M. *et al.* (1997) LNCaP prostatic adenocarcinoma cells derived from low and high passage numbers display divergent responses not only to androgens but also to retinoids. *J. Steroid Biochem. Mol. Biol.* 62, 391–399
43. Chang-Liu, C.-M. and Woloschak, G.E. (1997) Effect of passage number on cellular response to DNA-damaging agents: cell survival and gene expression. *Cancer Lett.* 113, 77–86
44. Wenger, S.L. *et al.* (2004) Comparison of established cell lines at different passages by karyotype and comparative genomic hybridization. *Biosci. Rep.* 24, 631–639
45. Sambuy, Y. *et al.* (2005) The Caco-2 cell line as a model of the intestinal barrier: influence of cell and culture-related factors on Caco-2 cell functional characteristics. *Cell Biol. Toxicol.* 21, 1–26
46. Lin, H.-K. *et al.* (2003) Suppression versus induction of androgen receptor functions by the phosphatidylinositol 3-kinase/Akt pathway in prostate cancer LNCaP cells with different passage numbers. *J. Biol. Chem.* 278, 50902–50907
47. Magazines, T. and Savers, S. (2001) Exploring the biological contributions to human health: does sex matter? *J. Womens Health Gend. Based Med.* 10, 433–439
48. Shah, K. *et al.* (2014) Do you know the sex of your cells? *Am. J. Physiol. Cell Physiol.* 306, C3–C18
49. Taylor, K. *et al.* (2011) Reporting of sex as a variable in cardiovascular studies using cultured cells. *Biol. Sex Differ.* 2, 152
50. Park, H.-J. *et al.* (2016) Gender-specific differences in PPARγ regulation of follicular helper T cell responses with estrogen. *Sci. Rep.* 6, 28495
51. Straface, E. *et al.* (2013) Sex differences at cellular level: "cells have a sex". In *Sex and Gender Differences in Pharmacology*, pp. 49–65, Springer
52. Hayashi, Y. *et al.* (2017) Female versus male biological identities of nanoparticles determine the interaction with immune cells in fish. *Environ. Sci. Nano* 4, 895–906
53. Serpooshan, V. *et al.* (2018) The effect of cell sex on uptake of nanoparticles: the overlooked factor at the nanobio interface. *ACS Nano* Published online 14 March 2018. http://dx.doi.org/10.1021/acsnano.7b06212
54. De Craene, B. and Berx, G. (2013) Regulatory networks defining EMT during cancer initiation and progression. *Nat. Rev. Cancer* 13, 97
55. Rice, A. *et al.* (2017) Matrix stiffness induces epithelial–mesenchymal transition and promotes chemoresistance in pancreatic cancer cells. *Oncogenesis* 6, e352
56. Deng, Z.J. *et al.* (2011) Nanoparticle-induced unfolding of fibrinogen promotes Mac-1 receptor activation and inflammation. *Nat. Nanotechnol.* 6, 39–44
57. Schöttler, S. *et al.* (2016) Protein source and choice of anticoagulant decisively affect nanoparticle protein corona and cellular uptake. *Nanoscale* 8, 5526–5536
58. Bertrand, N. *et al.* (2017) Mechanistic understanding of *in vivo* protein corona formation on polymeric nanoparticles and impact on pharmacokinetics. *Nat. Commun.* 8, 777

59. Mirshafiee, V. *et al.* (2016) The importance of selecting a proper biological milieu for protein corona analysis *in vitro*: human plasma versus human serum. *Int. J. Biochem. Cell Biol.* 75, 188–195
60. Mueller, L.K. *et al.* (2017) The transferability from animal models to humans: challenges regarding aggregation and protein corona formation of nanoparticles. *Biomacromolecules* 19, 374–385
61. Cohen, J.D. *et al.* (2018) Detection and localization of surgically resectable cancers with a multi-analyte blood test. *Science* 359, 926–930
62. Papa, E. *et al.* (2016) Investigation of the influence of protein corona composition on gold nanoparticle bioactivity using machine learning approaches. *SAR QSAR Environ. Res.* 27, 521–538
63. Walkey, C.D. *et al.* (2014) Protein corona fingerprinting predicts the cellular interaction of gold and silver nanoparticles. *ACS Nano* 8, 2439–2455
64. Bigdeli, A. *et al.* (2016) Exploring cellular interactions of liposomes using protein corona fingerprints and physicochemical properties. *ACS Nano* 10, 3723–3737
65. Vilanova, O. *et al.* (2016) Understanding the kinetics of protein–nanoparticle corona formation. *ACS Nano* 10, 10842–10850
66. Raoufi, M. *et al.* (2018) Probing fibronectin conformation on a protein corona layer around nanoparticles. *Nanoscale* 10, 1228–1233
67. Kelly, P.M. *et al.* (2015) Mapping protein binding sites on the biomolecular corona of nanoparticles. *Nat. Nanotechnol.* 10, 472
68. Strojan, K. *et al.* (2017) Dispersion of nanoparticles in different media importantly determines the composition of their protein corona. *PLoS One* 12, e0169552
69. Hajipour, M.J. *et al.* (2015) Personalized disease-specific protein corona influences the therapeutic impact of graphene oxide. *Nanoscale* 7, 8978–8994
70. Corbo, C. *et al.* (2017) Personalized protein corona on nanoparticles and its clinical implications. *Biomater. Sci.* 5, 378–387
71. Caracciolo, G. *et al.* (2014) Size and charge of nanoparticles following incubation with human plasma of healthy and pancreatic cancer patients. *Colloids Surf. B Biointerfaces* 123, 673–678
72. Colapicchioni, V. *et al.* (2016) Personalized liposome–protein corona in the blood of breast, gastric and pancreatic cancer patients. *Int. J. Biochem. Cell Biol.* 75, 180–187
73. Kokkinopoulou, M. *et al.* (2017) Visualization of the protein corona: towards a biomolecular understanding of nanoparticle-cell-interactions. *Nanoscale* 9, 8858–8870
74. Caputo, D. *et al.* (2017) A protein corona-enabled blood test for early cancer detection. *Nanoscale* 9, 349–354
75. Vlasova, M. and Smirin, B.V. (2017) Personalized approach in nanomedicine: understanding adverse effects and their risk assessment. In *Recent Advances in Drug Delivery Technology*, pp. 1–21, IGI Global
76. Park, S.-M. *et al.* (2017) Towards clinically translatable *in vivo* nanodiagnostics. *Nat. Rev. Mater.* 2, 17014
77. Sakulkhu, U. *et al.* (2014) *Ex situ* evaluation of the composition of protein corona of intravenously injected superparamagnetic nanoparticles in rats. *Nanoscale* 6, 11439–11450
78. Amici, A. *et al.* (2017) *In vivo* protein corona patterns of lipid nanoparticles. *RSC Adv.* 7, 1137–1145
79. Miller, M.A. *et al.* (2015) Predicting therapeutic nanomedicine efficacy using a companion magnetic resonance imaging nanoparticle. *Sci. Transl. Med.* 7, 314ra183
80. Nel, A. *et al.* (2017) New insights into "permeability" as in the enhanced permeability and retention effect of cancer nanotherapeutics. *ACS Nano* 11, 9567–9569
81. Kah, J.C.Y. *et al.* (2012) Exploiting the protein corona around gold nanorods for loading and triggered release. *ACS Nano* 6, 6730–6740
82. Behzadi, S. *et al.* (2014) Protein corona change the drug release profile of nanocarriers: the "overlooked" factor at the nanobio interface. *Colloids Surf. B Biointerfaces* 123, 143–149
83. Tenzer, S. *et al.* (2013) Rapid formation of plasma protein corona critically affects nanoparticle pathophysiology. *Nat. Nanotechnol.* 8, 772–781
84. Walczyk, D. *et al.* (2010) What the cell "sees" in bionanoscience. *J. Am. Chem. Soc.* 132, 5761–5768
85. Casals, E. *et al.* (2010) Time evolution of the nanoparticle protein corona. *ACS Nano* 4, 3623–3632
86. Röcker, C. *et al.* (2009) A quantitative fluorescence study of protein monolayer formation on colloidal nanoparticles. *Nat. Nanotechnol.* 4, 577–580
87. Lundqvist, M. *et al.* (2011) The evolution of the protein corona around nanoparticles: a test study. *ACS Nano* 5, 7503–7509
88. Langevin, D. *et al.* (2018) Inter-laboratory comparison of nanoparticle size measurements using dynamic light scattering and differential centrifugal sedimentation. *NanoImpact* 10, 97–107
89. Wei, S. *et al.* (2017) Exploring protein–nanoparticle interactions with coarse-grained protein folding models. *Small* 13, 201603748
90. Tavanti, F. *et al.* (2015) Competitive binding of proteins to gold nanoparticles disclosed by molecular dynamics simulations. *J. Phys. Chem. C* 119, 22172–22180
91. Mahmoudi, M. *et al.* (2011) Protein–nanoparticle interactions: opportunities and challenges. *Chem. Rev.* 111, 5610–5637
92. Heydari, T. *et al.* (2017) Development of a virtual cell model to predict cell response to substrate topography. *ACS Nano* 11, 9084–9092
93. Goldberg, D.E. and Holland, J.H. (1988) Genetic algorithms and machine learning. *Mach. Learn.* 3, 95–99
94. Andrieu, C. *et al.* (2003) An introduction to MCMC for machine learning. *Mach. Learn.* 50, 5–43
95. Shipp, M.A. *et al.* (2002) Diffuse large B-cell lymphoma outcome prediction by gene-expression profiling and supervised machine learning. *Nat. Med.* 8, 68–74
96. Findlay, M.R. *et al.* (2018) Machine learning provides predictive analysis into silver nanoparticle protein corona formation from physicochemical properties. *Environ. Sci. Nano* 5, 64–71