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Nanotechnology-Based Biosensors and Diagnostics: Technology Push versus Industrial/Healthcare Requirements

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Abstract There have been considerable advances in the field of nanotechnology-based biosensors and diagnostics (NBBD) during the last two decades. These include the production of nanomaterials (NMs), employing them for new biosensing and diagnostic applications, their extensive characterization for in vitro and in vivo applications, and toxicity analysis. All these developments have led to tremendous technology push and successful demonstrations of several promising NBBD. However, there has been a significant lag in their commercialization, especially due to the lack of international regulatory guidelines for evaluating the safety of NMs and the growing public concerns about their toxicity. Despite these numerous advances and the recent regulatory approval of several NMs, it still remains to be seen if NBBD are superior to conventional ones (not based on NMs), reliable, reproducible, cost effective, and robust enough to meet the requirements of industries and healthcare. This manuscript provides a critical review of NBBD, the technology push, and the industrial/healthcare requirements.

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1 Introduction

Nanotechnology is not a single technology or discipline, but it encompasses various technologies that cross sectors, such as nanomaterials (NMs), medicine, devices, fabrication, electronics, communications, and energy. It is the ability to measure and to control matter at the nanometer scale. The prefix "nano" was derived from the Greek word "dwarf", while the term "nanotechnology" was used by the Japanese researcher Norio Taguchi in 1974. However, the concept of nanotechnology was realized by the famous physicist Richard Feynman in 1959 in his landmark lecture "There's plenty of room at the bottom" at an American Physical Society meeting at Caltech, where he mentioned the possibility of manipulating material at the level of individual atoms and molecules. The major push for nanotechnology came from the electronics industry for the development of miniaturized electronic devices on silicon chips. There has been a phenomenal development in nanotechnology during the last two decades [1, 2], which resulted in highly diversified applications of nanotechnology in biosensors [3, 4], diagnostics [5–12], environmental monitoring [13], drug delivery [14–17], therapeutics [18–23], healthcare [24–30], medicine [31], textiles [32], food packaging and food safety [33], and information and communication technologies. Nanotechnology is also seen as the strongest candidate for personalized medicine that will enable individualized therapy [34]. All these developments have led to transformative changes in the scientific landscape [35], as revealed during a recent assessment of the global impact of the past decade of nanotechnology by Dr. Mihail Roco (Senior Advisor of Nanotechnology, National Science Federation) in his

extensive 500-page report known as Nano2 [36]. The major advances are the development of new fields of plasmonics, nanotoxicology, and environmental health and safety; use of graphene for carbon-based systems; devising the gene-sequencing solutions based on the combinations of near-field optical physics and biochemistry; development of hybrid materials/structures; and use of local probes of atomic- and molecular-scale structure for imaging complexity and function at atomic levels. These developments will have substantial impact in all fields in the next decade [35].

The first major initiative for pushing nanotechnology was taken by President Clinton in 2000 by establishing the National Nanotechnology Initiative (NNI), which was a multi-agency program comprised of National Science Foundation, Department of Defense, Department of Energy, National Institutes of Health, and National Cancer Institute. The main focus of the program was to build, characterize, and understand nanoscale devices. However, the realized economic impact was estimated to be greater than a trillion US dollars in the next two decades, which led almost all countries to start intensive and dedicated research efforts in nanotechnology [37–40]. Several tens of billions of dollars have already been invested worldwide in nanotechnology, thereby resulting in exponentially increased number of publications (Fig. 1) and patent applications.

The first decade of nanotechnology from 2000 onwards is regarded as the "hype cycle" (as described by Gartner Inc.) [41] (Fig. 2; Table 1). It consists of five phases: (1) technology trigger, (2) peak of inflated expectations, (3) trough of disillusionment, (4) slope of enlightenment, and (5) plateau of productivity. After the announcement of US NNI, the peak of inflated expectations quickly followed, as evident from President Clinton's State of the Union address. The Science magazine further led to inflated expectations about molecular computing by proclaiming

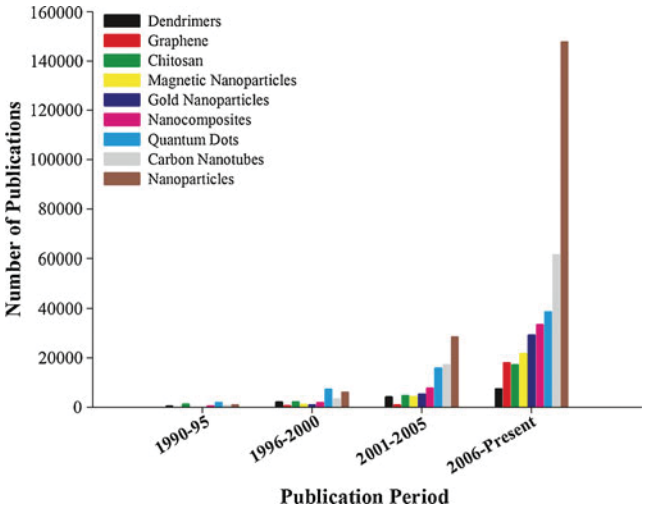

Fig. 1 Number of peer-reviewed articles published on nanomaterials during the past two decades. The data is taken from ISI Web of Knowledge on June 19, 2012

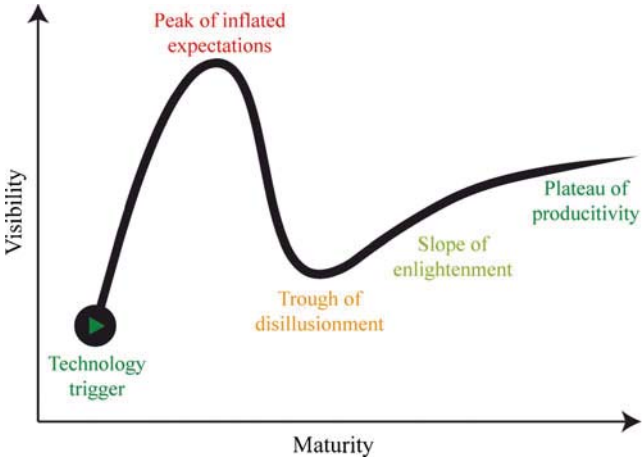

Fig. 2 The Gartner Hype cycle [41]

molecular electronics as the breakthrough of the year [42]. The initial era of nanotechnology saw important pioneering studies that were highly useful for the advancement of this new scientific discipline. However, during the subsequent peak of inflated expectations, it was observed that unsubstantiated and even fabricated results were published in highly reputed journals. The trough of disillusionment quickly followed, as evident from the most infamous data falsification case of Jan Hendick Schön at Bell Laboratories, who used fabricated data several times for many publications in Science and Nature [43, 44]. However, the quick investigation and resolution of this scandal was highly instrumental in putting nanotechnology again on the slope of enlightenment for the remaining decade. The multi-billion dollar microelectronics industry, based on the devised 32-nm silicon transistor technology, clearly demonstrates the case of productive nanotechnology. However, the applications of nanotechnology in biosensors, diagnostics, imaging, and therapeutics still need to be critically investigated and realized. There is an exclusive need of continued fundamental research in nanotechnology in addition to the critical evaluation of environmental health and safety of NMs.

The nanotechnology products can be classified into three categories based on the number of dimensions "pushed" to the nanometer scale: (1) thin films, such as coatings of implants for biocompatible purposes, anticoagulant coatings of stents, and coatings of pills and other therapeutic agents, have only one dimension pushed to the scale of few tens or hundreds of nanometers, while the other two dimensions can still extend up to millimeters; (2) NMs, such as carbon nanotubes (CNTs), silicon nanowires, nanorods, and fibers, have two dimensions pushed to the nanometer scale; and (3) NMs, such as quantum dots, gold, magnetic and polymeric nanoparticles, and liposomes, have all the three dimensions pushed to the nanometer scale. Both the top-to-bottom and bottom-up approaches have been used for the production of NMs. The top-to-bottom

Table 1 Description of the phases in the Gartner Hype cycle [41]

Phases	Description
Phase 1	Technology trigger: A potential technology breakthrough triggers significant publicity due to tremendous media interest. But there are usually no commercially viable products at this stage.
Phase 2	Peak of inflated expectations: The overenthusiasm and unrealistic expectations are generated due to the significant publicity in the previous phase. There are few successful applications of the developed technology but typically more failures.
Phase 3	Trough of disillusionment: The interest in the technologies fades as they do not meet the expectations due to the failures in experiments and implementations. There is usually no media interest and most of the producers generally abandon their developed technologies. However, some producers still manage to secure the investments to improve their products up to the expected standards.
Phase 4	Slope of enlightenment: During this phase, the businesses understand the benefits and applications of the developed technology, which leads to the development of highly refined products. There is increased funding from the investors and usually no media interest.
Phase 5	Plateau of productivity: The benefits of the technology are widely demonstrated, which increases its acceptability. There is continuous refinement of technology leading to the second and third generations. However, the final height of the plateau varies based on whether it has broad market applicability or a niche market.

approach involves micro-/nano-machining of macroscopic materials down to the desired nanometer scale using physical (anisotropic) or chemical (isotropic) processes. This process includes combination of techniques such as lithography, laser ablation, ion milling, and chemical etching. On the other hand, in the bottom-up approach, the material is "built" by the formation of an initial critical mass followed by the subsequent accumulation of material. Most commonly used techniques for bottom-up nanofabrication are molecular beam epitaxy, physical or chemical vapor deposition and evaporation, and the (bio)chemical processes for the production of (supra)molecular complexes, self-assembled monolayers, and protein–polymer nanocomposites.

Several promising NMs, such as carbon nanotubes (CNTs), graphene, quantum dots (QDs), nanoparticles (NPs), and nanocomposites, have been used for diagnostics and biosensors in the last decade. The first major application has almost always been the glucose sensing mainly due to the multi-billion dollar glucose monitoring market. The field of nanotechnology has grown by leaps and bounds in the last two decades. However, the post-hype era of nanotechnology [45] has posed serious challenges in the commercialization of nanotechnology-based products. The growing public concerns about the safety of NMs, the regulatory concerns in the absence of international guidelines for assessing the safety of NMs, and the industrial/healthcare (I/H) requirements are the most critical issues to be addressed before these products become commercially viable. This report provides the critical review of NBBD by evaluating the technology push versus the I/H requirements.

2 Developments in Nanotechnology-Based Biosensors and Diagnostics

The changing landscape of biomedical diagnosis [46, 47] is providing a continuous stimulus to the evolution of NBBD. The progress in miniaturization, microfluidics, and integration of all assay steps and/or reagents onto a miniaturized device has led to lab-on-a-chip. Nanotechnology will enable the further miniaturization of bioanalytical systems by integrating sensors, fluidics, and signal-processing circuits, which will provide the large-scale integration of different biochemical reactions on a smaller footprint. The on-going development of integrated lab-on-a-chip devices will employ various elements of nanotechnology.

During the last decade, NMs have been widely used in the fields of in vitro diagnostics, imaging, and therapeutics. They have enabled the simultaneous multiplex detection of many disease biomarkers [48, 49] and the diagnosis of diseases at a very early stage [8, 19, 50]. They have also opened the possibility to explore the detection of ultra-trace concentrations of target analytes and have led to ultra-sensitive, rapid, and cost-effective assays requiring minimum sample volume. The NMs are being seen as the most promising candidates for the development of high-throughput protein arrays [51]. The size, shape, composition, structure, and other physical/chemical properties of NMs can be tailored in order to produce the desired materials with specific absorptive, emissive, and light-scattering properties. The bioconjugated NPs have also been employed for signal amplification in assays and other biomolecular recognition events [49]. However, the most promising application of nanotechnology will be in the field of point-of-care diagnostics, which will enable the primary-care physician and patients to perform assays at their respective settings. The long-term stability of NPs in addition to their brightness and sharp bandwidth will be of tremendous significance to devise new methods for ultra-sensitive biomarker discovery, validation, and clinical use. The gold NPs (GNPs) tagged with short segments of DNA can be employed to detect the genetic sequence in a sample, while the use of nanostructures (nanopores, nanowires, nanopillars, and nanogaps)-based devices can further provide the single-molecule detection capability. The identification and

characterization of single-stranded genomic DNA or RNA without amplification has already been shown.

NMs such as QDs and NPs are good imaging agents due to their enhanced performance and functionality [52]. They can be targeted to the specific disease sites in the body by conjugating them to biomarker-specific vectors. The NMs-based imaging agents provide additional information pertaining to the physiology and function apart from the anatomical information, which enables more accurate and early disease diagnosis, such as the highly sensitive detection of early stage cancer, thereby leading to better therapy. Similarly, the effectiveness of treatments can be monitored more rapidly and accurately. The plasmonic NPs and drug delivery will be employed for targeted therapeutics, where the first impacts will certainly be in treating cancer. The use of NPs improves the bioavailability and pharmacokinetics of therapeutics. They take the drugs directly to the target sites of disease in the body by avoiding exposure of healthy tissues, which increases the availability of a drug at the target site and reduces the treatment dose. These developments in nanotechnology will be highly beneficial in shifting the late-stage diagnosis (involving expensive and socially burdensome treatment) to early-stage diagnosis (relatively less expensive and less invasive). The most widely used NMs in NBBD are described below.

2.1 CNTs

During the past decade, CNTs have been one of the most extensively used NMs in biosensors, diagnostics, tissue engineering, cell tracking and labeling, and delivery of drugs and biomolecules [15, 16, 53]. They are hollow cylindrical tubes composed of one, two, or several concentric graphite layers capped by fullerenic hemispheres, which are referred to as single-, double-, and multi-walled CNTs, respectively. They have unique structures, excellent electrical and mechanical properties, high thermal conductivity, high chemical stability, remarkable electrocatalytic activity, minimal surface fouling, low overvoltage, and high aspect ratio (surface to volume). CNTs-based biosensors and diagnostics have been employed for the highly sensitive detection of analytes in healthcare, industries, environmental monitoring, and food quality analysis. They have been predominantly used in electrochemical sensing, mainly for glucose monitoring but also for the detection of fructose, galactose, neurotransmitters, neurochemicals, amino acids, immunoglobulin, albumin, streptavidin, insulin, human chorionic gonadotropin, C-reactive protein, cancer biomarkers, cells, microorganisms, DNA, and other biomolecules [54].

2.2 Graphene

Graphene, an atomically thin layer of sp^2-hybridized carbon, is another most extensively used NM for diagnostics and biosensors in the last few years due to its interesting and exciting properties, such as high mechanical strength, high thermal conductivity, high elasticity, tunable optical properties, tunable band gap, very high room temperature electron mobility, and demonstration of the room temperature quantum Hall effect. It is a transparent material with a very low production cost and low environmental impact. It has been extensively employed in electrochemical, impedance, fluorescence, and electrochemiluminescence biosensors for the detection of a wide range of analytes such as glucose, cytochrome *c*, NADH, hemoglobin, cholesterol, ascorbic acid, dopamine, uric acid, hydrogen peroxide, horseradish peroxidase, catechol, DNA, heavy metal ions, and gases [55, 56].

2.3 QDs

QDs are inorganic nanocrystals, approximately 1–10 nm in size, with unique optical properties of broad excitation, narrow size-tunable emission spectra, high photochemical stability, and negligible photobleaching. They have been widely used [57], mainly as alternatives to fluorophores, for the development of optical biosensors to detect ions, organic compounds, pharmaceutical analytes, and biomolecules such as nucleic acids, proteins, amino acids, enzymes, carbohydrates, and neurotransmitters. They have also been employed for the in vivo detection of target sites in cancer. In fact, they are the ideal candidates for multiplexed optical bioanalysis due to their ultra-high sensitivity, high specificity, cost effectiveness, miniaturized size, size-dependent emission wavelength, and rapid analyte detection [48].

2.4 NPs

NPs have also been extensively used in various bioanalytical applications [58, 59], especially for the development of biosensors, diagnostics, imaging, drug delivery, and therapy, due to their unique optical and other properties. They change color in response to the binding of molecules to their surface. The change in the properties of nanoparticles by varying their size or shape has been exploited for various bioanalytical applications.

The most widely used NPs are GNPs, which have a nontoxic, biocompatible, and inert core. The prominent plasmon absorption and scattering properties of GNPs are highly useful for the early stage detection and photothermal therapy of cancer and other diseases. They have been used for the development of immunoassays, diagnostics, and biosensors for various analytes [60–66]. Based on their preferential accumulation at the tumor sites, they have been used for the therapy of cancer and other diseases by acting as nanocarriers for the delivery of drugs, DNA, and genes. The multivalent GNPs facilitate efficient drug delivery to the target sites by shielding the unstable drugs, while their

strongly enhanced surface plasmon resonance absorption enables the photothermal therapy of cancer. They have been extensively used in imaging due to their enhancement of the Raman and Rayleigh signals that provide greater chemical information. Therefore, it will be highly useful to combine all the benefits of GNPs, such as diagnostic, specific targeting, and therapeutic, into a single multifunctional GNPs-based platform, which can be chemically tailored for a particular disease.

Magnetic NPs are the second most widely used NPs, which have been extensively employed in biosensors and diagnostics for the detection of proteins, enzymes, DNA, mRNA, drugs, metabolites, pathogens, and tumor cells. Various types of magnetic sensors based on different signal transduction mechanisms, such as magnetic relaxation switch assay sensors, magnetic particle relaxation sensors, and magnetoresistive sensors, have been developed [67]. The diagnostic magnetic resonance (DMR) technology has also been employed extensively for magnetic biosensing [68]. The development of miniaturized chip-based nuclear magnetic resonance detector (μNMR) has further enhanced the capabilities of DMR for the highly sensitive analyte detection in microliter sample volumes, multiplex analysis, and development of cost-effective, portable, and high-throughput platforms for point-of-care diagnostics. The magnetic NPs are being extensively used by industries such as Phillips Research, Eindhoven, Netherlands for the development of immunoassays [69] and rapid integrated biosensor for multiplexed immunoassays [49].

2.5 Chitosan

Chitosan is one of the most promising NMs [70] for the integration of biological components in medical devices [71] due to its excellent biocompatibility, complete biodegradability, and non-toxic nature [72]. The degradation products of chitosan are harmless natural metabolites. It is obtained by the deacetylation of chitin, the second most abundant natural polymer after cellulose, which is found in the shells of crustaceans (crabs and shrimp), the cuticles of insects, and the cell walls of fungi. It is suitable for optical sensors due to its transparent nature. It is also appropriate for electrochemical sensors as the chitosan films are porous and highly permeable to ions. The pH-dependent solubility of chitosan enables the formation of stable films under neutral and basic pH conditions, whereas its amine groups aid in the covalent binding of biomolecules and the formation of nanocomposites with polymers or NPs. But it requires chemical modification such as carboxymethylation to increase its solubility in water and other common solvents. It has been extensively used in biosensors, diagnostics, lab-on-a-chip devices, and other biomedical or bioanalytical applications [70–72].

2.6 Dendrimers

Dendrimers are hyperbranched, monodispersed, star-shaped, and nanometer-scale three-dimensional macromolecules with a very high density of surface functional groups. They are composed of three distinct components, i.e., the core, the interior dendron, and the exterior surface with terminal functional groups. They have been used extensively in various biosensors and diagnostics [73], such as those based on electrochemistry, fluorescence, surface enhanced Raman scattering, impedimetry, and surface plasmon resonance, mainly as they increase the analytical sensitivity, stability, and reproducibility but reduce the non-specific interactions. They have also been used for other bioanalytical applications [50, 74] such as drug delivery, gene transfection, and catalysis.

2.7 Biological and Other NMs

Lipid vesicles, thin lipid films, and liposomes are biological NMs formed via the bottom-up nanotechnology approach. They have very similar composition to the cell membrane, being composed of phospholipids or other amphiphiles. The bilayer lipid membrane structure provides a biomimetic environment for embedding the biocomponents, such as receptors and proteins, under non-denaturing conditions. Due to their inherent biocompatibility, effective encapsulation of hydrophilic or hydrophobic drugs, and sensitivity to pH and temperature, they have been used as drug-delivery carriers for controlled drug release [75, 76] and for the development of biosensors and diagnostics [77]. The amphiphilic nature allows them to spontaneously form organized structures. They have been used for the amplification of optical, electrochemical, and acoustic signals. Hybrid nanoparticles composed of lipids and polydiacetylene (PDA) have been employed for the development of smart colorimetric biosensors, where the externally induced conformation change of PDA due to specific biomolecular interactions results in remarkable blue-to-red chromatic transition [78]. This approach has been employed for the rapid diagnosis of diseases, study of peptide–membrane interactions, and the colorimetric screening of enzyme catalysts, antibacterial peptides, and physiological ions.

Besides these, other NMs (such as cellulose nanocrystals [79, 80]), biomolecules [81–83], and a wide range of nanocomposites [84–86] with unique properties have also been used. The nanoscale features of the bioanalytical platforms have also been modified for signal enhancement and better assay sensitivity [87, 88]. Moreover, the tools and instruments being employed for nanoscale probing and manipulation have also evolved. The Scanning Probe Microscope [89] that was previously used only for the topographical mapping/imaging of surfaces can now be employed to probe nanometer-localized electrical, optical, and nanomechanical properties

[90], and to monitor interactions in real time. It has evolved from a tool to a nanotechnology instrument for bottom-up nanofabrication [91] and for imaging biomolecule assemblies, surfaces, and cells, both in ambient and liquid environment, with special modifications for the sensitive biological surfaces [92]. Therefore, the last decade has seen significant developments in nanotechnology and the continuously increased use of NMs in diagnostics and biosensors.

3 Technology Push versus Industrial/Healthcare Requirements

The numerous advances in NBBD have generated tremendous technology push, as evident from the exponentially increased number of publications (Fig. 1), patent applications, projects, and focused nanotechnology initiatives/themes. However, it is essential for the developed technologies to comply with I/H requirements in order to facilitate their market entry by generating the desired market pull. Most of the nanotechnology-based products have only been demonstrated in the research settings and are devoid of extensive validation and trials in industries and healthcare. There has been continuous decrease in the venture capital investment during the last few years mainly due to the relatively stagnant commercialization of nanotechnology-based products and the growing public concerns about the safety of NMs. The critical I/H requirements for nanotechnology-based products are discussed here.

3.1 Reproducible and Cost-Effective Production

The reproducible and cost-effective production of NMs is the most critical and in fact the preliminary requirement as it will directly influence the reproducibility in biosensing and diagnosis. The current state-of-the-art procedures have considerable variability in the production of NMs. However, there are substantial ongoing research efforts by researchers and industries in order to improve the production procedures so that they can offer significant cost savings for some of the NMs. On the other hand, it is quite clear that the use of NMs in biosensors and diagnostics will certainly incur additional costs in comparison to the conventional procedures. Therefore, the cost-effective production of NMs and their analytical benefits will be the determining factors in taking strategic decisions pertaining to whether nanotechnology-based products can substantially score over the conventional products in order to gain successful market entry.

3.2 Characterization

The NMs need to be more intensively characterized by developing the right tools and techniques. The commercially available NMs such as CNTs, graphene, GNPs, and QDs are characterized by routinely used analytical techniques such as scanning electron microscopy, Raman spectroscopy, Fourier transform infrared spectroscopy, etc. But there is a need for more stringent investigation of the properties of NMs, which will provide highly useful information pertaining to the storage, functionalization, modification, and use of NMs under optimum conditions. Apart from the material characterization, the nature of the metallic impurities, such as those in the CNTs, also needs to be determined as it can substantially affect the properties as well as the toxicity of NMs.

3.3 Material Safety

The material safety need to be evaluated individually for each NM as they are unique [93]. When the NM is intended to be used for in vivo applications, the critical physiological parameters such as absorption, distribution, metabolism, excretion, and toxicity should be determined. It has been demonstrated that some NMs have prolonged tissue retention and may also contain heavy metals, which increases the risk of cytotoxicity. The toxicological studies of NMs also need to be done according to the established regulatory guidelines along with the determination of their efficacy so that the risk-to-benefit assessments could be done. However, in the absence of such guidelines at the moment together with the lack of measurement tools and standard materials, the study of NMs' toxicity and their environmental impact is quite challenging. The well-drafted guidelines by the National Institute for Occupational Safety and Health for handling NPs should be followed for the development of new manufacturing processes to minimize the workplace exposure risks. The risk assessment and risk management paradigm for NPs, as described in Fig. 3, should also be considered.

3.4 Compliance with Regulatory Guidelines

The NBBD needs to adhere to strict health and safety protocols, and regulatory guidelines so that the potential hazards of nanotechnology [94] can be effectively addressed. The field of nanotechnology has raised potential scientific and policy issues pertaining to the risk assessment and standard setting, which has challenged the risk governance and decision-making processes [95, 96]. Presently, there are intensive efforts in drafting the claims for the Nanotechnology Regulation [97], where the main objective is to make claims leading to the development of nanotechnology but at the same time, evaluating critically the safety of nanotechnology in terms of its effects on the public health and the environment. There have been many instances such as tetraethyl lead and methyl *tert*-butyl ether, where the potentially hazardous technologies/products adversely affecting the health and the environment were commercialized. The

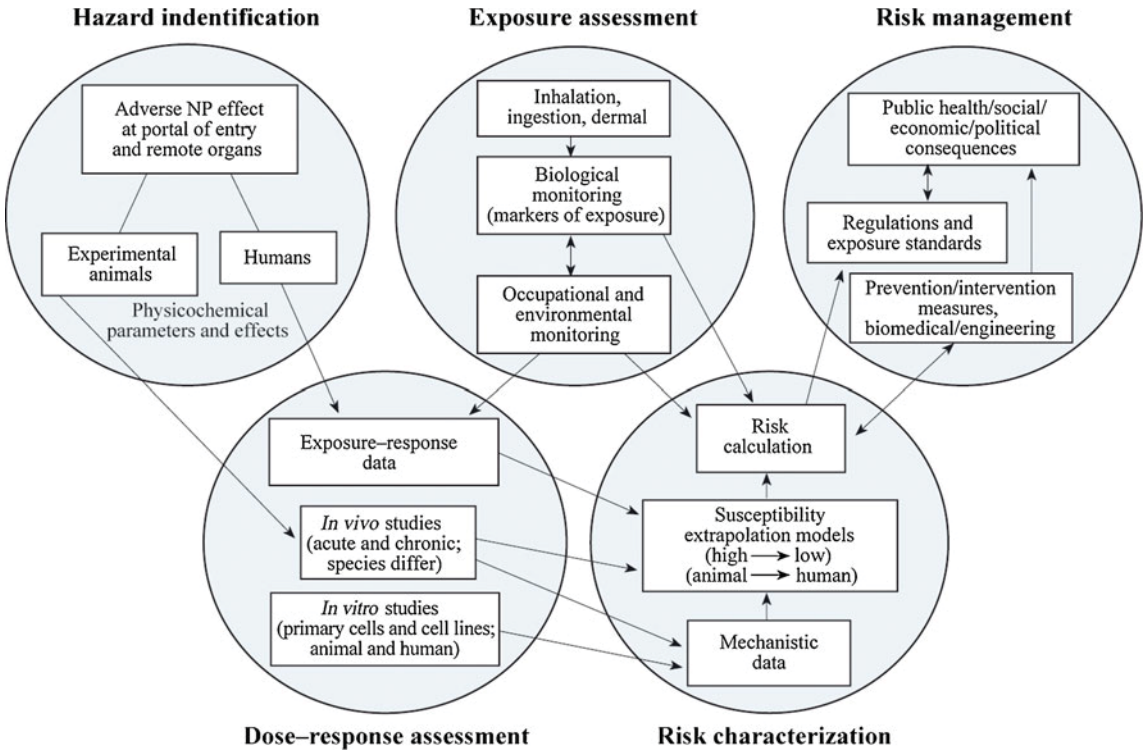

Fig. 3 Risk assessment and risk management paradigm for nanoparticles [99]. Reprinted with permission from Environmental Health Perspectives

conventional direct regulation and self-regulation approaches, often referred to as hard and soft law, respectively, are being explored for making the claims of the Nanotechnology Regulation. The conventional direct regulation is the "command and control" regulation that involves making prescriptive rules to directly control the private sectors. On the other hand, self-regulation measures include the industrial codes of conduct, voluntary guidelines, or decision frameworks. The Environmental Defense–DuPont Nano Partnership Nano Risk Framework is an example of the self-regulation measure to evaluate and address the potential risk of NMs.

3.5 Correlation with Established Technology

The efficacy of the developed NBBD needs to be demonstrated by correlating it with the established technologies, e.g., their use as biosensor with the performance of state-of-the-art enzyme-linked assays, scanning probe microscopy to standard imaging techniques such as electron microscopy (when it comes to imaging NPs). This will enable the determination of benefits (or drawbacks) of the developed nanotechnology-based products over the commercially available products, which will lend considerable support to their commercialization. The requirement of technology correlation is becoming more apparent to the researchers in nanotechnology as it has been accepted as a norm by almost all scientific journals and investment/certification/regulatory agencies for evaluating the developed technology. Thus, the researchers have already started to address this concern and adopted it as a standard practice in the development of NBBD.

3.6 Potential End-User Trials

Most of the NBBD are developed and evaluated in standard laboratory settings using commercially available analyte samples. However, based on their intended applications, there is an absolute requirement for their validation and testing in the actual end-user's settings in industries and healthcare using the "real world" samples. These end-user trials will enhance the credibility and commercial appeal of the developed nanotechnology-based products by demonstrating their robustness under the actual conditions prevalent at the end-user's settings, where they will be finally employed. Presently, this is a serious limitation for most of the nanotechnology researchers as it requires significant funding and efforts.

3.7 Toxicity Analysis

There are growing public concerns about the potential toxicity of NMs, especially for in vivo biomedical applications, due to their ultra-small size and unique properties. Most of these concerns are fueled by fundamental misconceptions about NMs and nanotechnology, where the risk of nanotechnology has been exaggerated. But these can be effectively addressed with information outreach if the scientific

community can clearly demonstrate the safety of NMs for diagnostics and biosensors before exploiting them commercially. This will abridge the information gap between the scientific community and public, which will lend considerable support to the acceptance of developed technology and its commercialization. There are ongoing research efforts to determine the toxicological profiles and potential adverse effects of NMs [98–102], understand their biological interaction mechanisms [103], develop robust and widely acceptable analytical tools and tests for characterizing NMs in various environments [104], and to determine the safety of NM throughout its life cycle, i.e., research and development, production, use, disposal, and/or recycle. The perceived and real adverse impacts of NMs need to be effectively addressed lest they become barriers for the future technology development.

The toxicity of NMs depends on numerous factors. The toxicity of CNTs depends on their dimensions, impurities, surface chemistry, dispersion, type, dose, and the interaction between various factors [105]. Similarly, the inherent toxicity of QDs that is made up of toxic materials combined with their clearance problems are the major factors why QDs have also not been approved for in vivo applications. Although the biocompatible QDs have also been demonstrated to prevent toxicity, they are very expensive. Despite the tremendous research efforts devoted to the use of chitosan during the last two decades, it is still not approved by the Food and Drug Administration (FDA) for drug delivery, which has led to diminishing interest of biotech companies. The side effects resulting from the toxicity of GNPs have also been demonstrated. But these can be diminished by devising new procedures of functionalizing GNPs with compounds that enhance their biocompatibility and clearance. The known and expected NPs exposure and clearance routes are shown in Fig. 4. A tiered testing system to assess NP toxicity was suggested [106], where the physico-chemical characterization needs to be done prior to and during subsequent testing in cell-free, cellular, and in vivo assays. However, it is difficult to predict the in vivo toxicity from the in vitro assays.

3.8 Evaluating the Need of NMs

A wide range of NMs have been employed in biosensors and diagnostics mainly due to their demonstrated benefits such as increased signal, higher analytical sensitivity, lower limit of detection, and better analytical characteristics. As an example, most of the nanotechnology-based concepts have been initially applied to blood glucose monitoring due to its enormous market potential. However, it is well known to the industries and experts in the field nowadays that the use of NMs does not provide any analytical advantage or cost effectiveness in comparison to the commercially available blood glucose monitoring devices. It only leads to increased signal and, in some cases, greater detection range and/or no requirement of external mediator. The increased detection range beyond the pathophysiological glucose range in diabetics is of no real use. On the contrary, the use of NMs increases the manufacturing cost, complexity, and hands-on time, while reducing the production and functional reproducibility. This is the main reason that despite the numerous publications and patents pertaining to the use of NMs for blood glucose monitoring [54, 56, 107], none of the nanotechnology-based concepts has actually been taken up by the industries for commercialization. On the other hand, the simplified technologies that do

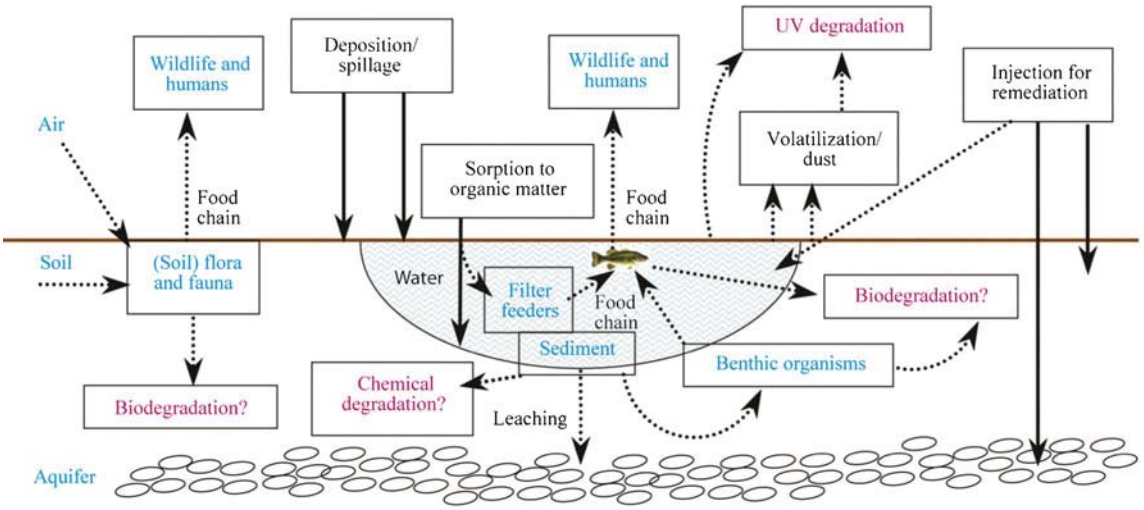

Fig. 4 Expected and known nanoparticle exposure and clearance routes [99]. Reprinted with permission from Environmental Health Perspectives

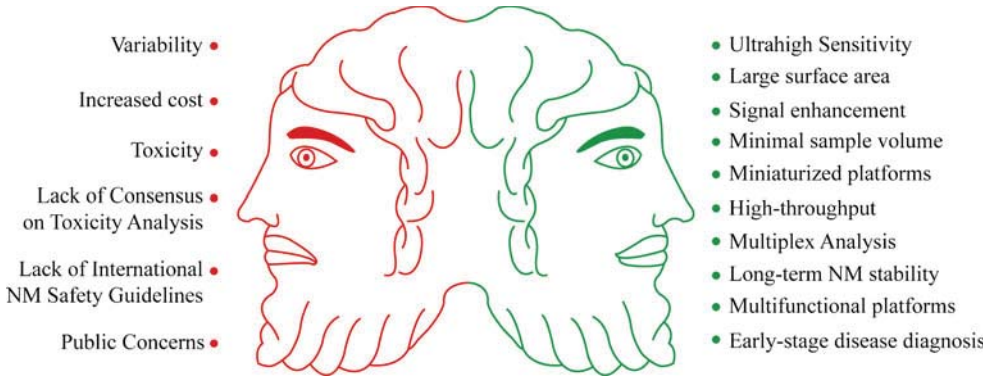

Fig. 5 Nanotechnology-based diagnostics and biosensors: the two sides of the Janus

not use NMs but increase the analytical performance and cost effectiveness are more readily adopted for glucose monitoring and other bioanalytical applications [108–110]. There has been lot of hype around the use of NMs in the last decade that resulted in unrealistic hopes [111]. The academic researchers have been forced to include elements of nanotechnology for the sole reason of securing research funding [112]. However, the recent years have seen a change in this trend as more focus is now provided to the improvement of bioanalytical applications. Therefore, the need of using NMs for a particular application should be critically assessed by the technology and business experts.

4 Conclusions and Future Trends

The significant advances in field of NBBD in the last two decades have generated tremendous technology push. Initially, most of these developments were motivated by hype, which led to inflated expectations and the inclined trend to employ nanotechnology-based concepts and devices. However, the field of nanotechnology has now progressed past the peak of hype, where increased attention is being paid to the toxicological and environmental effects of NMs. The post-hype era is mainly focused on determining the safety of NMs, arriving at the international regulatory guidelines for assessing the safety of NMs, and determining the robustness of NBBD in accordance with I/H requirements. The extensive benefits of employing NMs for biosensors and diagnostics have been widely demonstrated and are well known to the scientific community worldwide. The field of NBBD has progressed to the "slope of enlightenment" phase of the Gartner Hype cycle. However, extensive research efforts are still required to critically investigate the production reproducibility, analytical parameters, and the safety of NMs. The finalization of international regulatory guidelines for assessing the safety of NMs, which is the topmost priority and in full swing at the moment, will provide the much-needed momentum to this field. There is a critical need for the developed technologies to meet I/H requirements in order to become commercially viable. However, the commercial success of the developed NBBD will be determined by the key technology differentiators, cost effectiveness, reliability, and the generated market pull.

The interdisciplinary nature of nanotechnology is a major challenge in itself as it is difficult to find the expertise in all the fields at a particular setup or group. Therefore, the technical data pertaining to the applications of nanotechnology-based products in a particular field need to be critically reviewed by the experts in that field. As an example, most of the published reports have shown the intracellular delivery of nanoparticles to cells that were dead and permeable, while these studies should have been conducted in healthy cells to obtain accurate results.

Presently, many companies are investing their time, money, and efforts on the development of procedures for the production of reproducible, stable, and biocompatible NMs. The researchers have also started the testing of NMs-based biosensors and diagnostics on "real world" samples, which provides much better understanding of sample matrix effects and the highly useful information about the effects of physiological interferences. Similarly, various modifications of NMs have been demonstrated to reduce the NM's toxicity and make them biocompatible.

Despite the tremendous technology push, the NBBD represents the two sides of Janus (Fig. 5). The advantages of using NMs have been clearly demonstrated, while extensive efforts are still required to remove the limitations and comply with regulatory guidelines. The nanotechnology-based products are still restricted to research and development settings as they are unable to fulfill the quality control standards posed by certification agencies such as FDA. There is an immense need to reduce the production cost of NMs by developing cost-effective and reproducible manufacturing techniques as the current market price of NMs is too high for any realistic commercial application. However, these challenges would be effectively tackled in the near future by continuous technology developments. The ongoing efforts to meet I/H requirements and to determine the safety of

NMs will generate the desired market pull, which will boost the commercialization of NBBD.

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