



Internet Journal of Medical Update

Journal home page: <http://www.akspublication.com/ijmu>

Review

Nanoscience in diagnostics: A short review

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(Received 21 October 2010 and accepted 04 December 2010)

ABSTRACT: Nanoscience is at the leading edge of the rapidly developing field of nanotechnology. Nanosciences and nanotechnology are transforming a wide array of products and services that have the potential to enhance the practice of medicine and improve public health. Several areas of medical care are already benefiting from the advantages that nanotechnology can offer. Applications of nanoscience are in biotechnology, medicine, pharmaceuticals, physics, material science and also electronics. Nanotechnology extends the limits of molecular diagnostics to the nanoscale. Nanotechnology on a chip is one more dimension of microfluidic/lab on a chip technology. We still suffer serious and complex illnesses like cancer, cardiovascular diseases, multiple sclerosis, Alzheimer's and Parkinson's disease, and diabetes as well as different kinds of serious inflammatory or infectious diseases (e.g. HIV). It is of extreme importance to face these diseases with appropriate means. The interplay between nanoscience and biomedicine is the hallmark of current scientific research worldwide. The use of nanoscience may open new vistas of improving the effectiveness and efficiency of medical diagnosis and therapeutics, so called nanomedicine. An appealing example is the use of quantum dots as fluorescent labels. Despite recent progress in the treatment of cancer, the majority of cases are still diagnosed only after tumors metastasize, leaving the patient with a grim prognosis. Nanotechnology is in a unique position to transform cancer diagnostics and to produce a new generation of biosensors and medical imaging techniques with higher sensitivity and precision of recognition. Novel nanotechnologies can complement and augment existing genomic and proteomic techniques employed to analyze variations across different tumor types, thus offering the potential to distinguish between normal and malignant cells. This brief review tries to reiterate the most contemporary developments in the field of applied nanoscience, particularly in their relevance in diagnosis of various diseases and discuss their future prospects.

KEY WORDS: *Nanoscience; Nanomedicine; Nanoparticles; Labs-on-a-chip; SNPs; Cancer*

INTRODUCTION

Nanoscience is the study of phenomena and manipulation of materials at atomic, molecular and macromolecular scales, where properties differ significantly from those at a larger scale; and nanotechnology is the design, characterization, production and application of structures, devices

and systems by controlling shape and size at the nanometre scale.¹ The prefix 'nano' is derived from the Greek word for dwarf. One nanometre (nm) is equal to one-billionth of a metre. A human hair is approximately 80,000nm wide, and a red blood cell approximately 7000nm wide. Nanobiotechnology is another chief field of investigation. The thrust is to combine nanoscale engineering with biology to manipulate living systems directly or build biologically inspired materials and devices at the molecular level. The origin of nanoscience and nanotechnologies is often attributed to a concept

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advanced by Nobel Laureate Richard P. Feynman, who in a 1959 lecture at the California Institute of Technology, stated “There is plenty of room at the bottom. Many of the cells are very tiny, but they are active: they manufacture substance; they walk around; they wiggle: and they do all kinds of marvelous things all on a very small scale. Also they store information. Consider the possibility that we too can make things very small which does what we want when we want-and that we can manufacture an object that maneuvers at that level.”^{2,3} The term ‘nanotechnology’ was not used until 1974, when Norio Taniguchi, a researcher at the University of Tokyo, Japan used it to refer to the ability to engineer materials precisely at the nanometre level.⁴ The primary driving force for miniaturisation at that time came from the electronics industry, which aimed to develop tools to create smaller (and therefore faster and more complex) electronic devices on silicon chips. Because of the small dimensions, most of the applications of nanobiotechnology in molecular diagnostics fall under the broad category of biochips/microarrays but are more correctly termed nanochips and nanoarrays. Microarray/biochip methods employing the detection of specific biomolecular interactions are now an indispensable tool for molecular diagnostics, but there are some limitations. DNA microarrays and ELISA rely on the labeling of samples with a fluorescent or radioactive tag – a highly sensitive procedure that is time consuming and expensive. The chemical modification and global amplification of the nucleic acid samples are achieved by PCR which can introduce artefacts caused by the preferential amplification of certain sequences. Alternative label-free methods include surface plasmon resonance and quartz crystal microbalance. Nanotechnologies also provide label-free detection. Nanotechnology is thus being applied to overcome some of the limitations of biochip technology.⁵

Applications of nanotechnologies in medicine are especially promising, and areas such as disease diagnosis, drug delivery targeted at specific sites in the body and molecular imaging are being intensively investigated and some products are undergoing clinical trials. Nanocrystalline silver, which is known to have antimicrobial properties, is being used in wound dressings in the USA.⁶ Applications of nanoscience and nanotechnologies are also leading to the production of materials and devices such as scaffolds for cell and tissue engineering, and sensors that can be used for monitoring aspects of human health. The interplay between nanoscience and biomedicine is the hallmark of current scientific research worldwide. The use of nanoscience may open new vistas of improving the effectiveness and efficiency of medical diagnosis and therapeutics. Applications of nanotechnology for the treatment, diagnosis,

monitoring, and control of biological systems has recently been referred to as ‘Nanomedicine’ by the National Institutes of Health (NIH). An appealing example is the use of quantum dots as fluorescent labels. On the other hand, the language of biomedicine can be translated to allow formation of nanostructures with biomimetic structural and functional complexity. For instance, DNA is extensively used to direct the organization of nanoparticles. The gap between nanoscience and biomedicine has to be bridged to establish a common understanding of how nanoscience and biomedicine can augment each other and what impact can be envisioned from this cooperation.

Nanostructures display unique mechanical, electrical, chemical, and optical properties. Understanding and controlling such properties is challenging, but harnessing them will provide exciting new opportunities for research, diagnosis, and therapy.⁷ Sensitive biosensors constructed out of nanoscale components (e.g., nanocantilevers, nanowires and nanochannels) can recognize genetic and molecular events and have reporting capabilities, thereby offering the potential to detect rare molecular signals associated with malignancy. Such signals may then be collected for analysis by nanoscale harvesters that selectively isolate cancer-related molecules from tissues. Another area with near-term potential for the early detection of cancer is the identification of mutations and genomic instability.⁸

A large subject area, nanomedicine includes, for example, nanoparticles that act as biological mimetics, nanofibers and polymeric nanoconstructs as biomaterials, sensors and laboratory diagnostics.⁹ It has become increasingly clear that progress forward in nanoscience and nanotechnology requires a particularly wide spectrum of skills and knowledge. As such, a number of interdisciplinary research programs and other efforts have been established to promote collaboration among relevant fields. An example is the National Cancer Institute's Nanobiology Think Tank, an outreach program to promote collaborations, disseminate knowledge and enhance the development of novel creative ideas for research and biomedical application. With similar objectives, a number of academic institutions have established special institutes and centers dedicated to nanotechnology.³

Nanotechnology research has emerged a bench-top tool for rapid diagnosis of infectious disease and detection of tiny genetic mutations known as single-nucleotide polymorphisms (SNPs). This could well expedite a physician's determination of the status of an infectious patient and have broader benefits for public health as infectious diseases continue to be a growing threat to all nations. Other nanomedicine projects are aimed at rational delivery and targeting of pharmaceutical,

therapeutic and diagnostic agents via intravenous and interstitial routes of administration with nanosized particles. The promising application of nanoscience and nanotechnology may well contribute important tools for promoting health, preventing disease and otherwise improve the quality of life for all segments of the population.¹⁰

NANOSCIENCE IN HEALTHCARE

Healthcare has been evolving from reactive medicine (disease is detected and treated in late stages) to a personalized, proactive and preventative medicine (presymptomatic disease is detected and treated on an individual basis). At the heart of this transformation will be a host of new, miniaturized technologies that permit biological information to be acquired and analysed quickly and cheaply. As genomic sequencing becomes more affordable, patients will have at least some information relevant to their probabilistic future health, which can be used to schedule physician visits before the development of symptoms. Given advances in the knowledge of disease biomarkers such as proteins, circulating tumour cells¹¹ and microRNAs¹² it is likely that, for a given disease, a panel of blood-based biomarkers will be assessed to provide pre-symptomatic information relevant to the onset and progression of the disease.

Nanotechnology is already starting to have an impact on the diagnosis, treatment and prevention of disease, especially by enabling early disease detection and diagnosis, as well as precise and effective therapy. It approaches in surgery, cancer diagnosis & therapy, bio-detection of molecular disease markers, molecular imaging, implant technology, tissue engineering, and devices for drug, protein, gene and radionuclide delivery. While many of these medical nanotechnology applications are still in their infancy. Nanoparticles or nanostructures are utilized as novel drug delivery systems.^{13,14} Systemic administration of chemotherapeutic agents, in addition to its anti-tumor benefits, results in indiscriminate drug distribution and severe toxicity. This shortcoming may be overcome by targeted drug-carrying platforms that ferry the drug to the tumor site while limiting exposure to non-target tissues and organs.¹⁵

The rapid and sensitive detection of pathogenic bacteria is extremely important in medical diagnosis and measures against bioterrorism. Recent advances in the field of nanotechnology led several groups to recognize the promise of recruiting nanomaterials to the ongoing battle against pathogenic bacteria.¹⁶ Rapid, selective, and sensitive detection of viruses is crucial for implementing an effective response to viral infection, such as through medication or quarantine. Direct, real-time electrical detection of

single virus particles can be achieved with high selectivity by using nanowire field effect transistors.¹⁷

Sensor, able to detect the presence of a single molecule in samples like blood represents one of the greatest challenges for nanotechnologies. Not only does it require precision in manufacture, but it also requires a unique mixture of electronics, optics, chemistry, biochemistry and medicine to make devices that can be used routinely, cheaply and reliably to monitor the state of human health. An example of this is in point-of-care health screening where a single drop of blood placed on a sensor chip would be almost instantaneously analysed to provide data to aid a diagnosis. This will require the processing power of a silicon chip with biochemical sensitivity to identify many blood components. This type of monitoring could also begin to be incorporated within the body to provide constant monitoring of health, such as in the control of diabetes or in critical care. There are many other potential applications of such devices in medicine, making this an area of increasing investment.

NANOSCIENCE IN DIAGNOSTICS

The enormous increase in knowledge of the human genome (genomics) and of expression products, proteins (proteomics), makes it possible in an increasing number of cases to trace diseases to abnormalities at the molecular level. In theory, this gives rise to the possibility of making a diagnosis at a very early stage and of possibly starting treatment even before the initial symptoms of the disease appear.¹⁸ Attention in medicine is, therefore, increasingly focusing on prevention. Neonatal screening (by means of a heel prick) for metabolic diseases is a good example of this.¹⁹ The medical profession has an ever increasing number of technical tools at its disposal for detecting these molecular biomarkers. The diagnostic research can be conducted in the laboratory using samples taken from the human body (in vitro research) but it can also be carried out directly on the patient (in vivo). This distinction is important because, in the latter case, the tools/agents have to meet more stringent requirements.

Research into DNA can be conducted to measure gene expression in diseased tissue, or to ascertain which variant of a particular gene a person has. Many human genes exist in several forms, which only differ in a single base pair. These are known as SNPs. The corresponding protein variants may differ from each other in a single amino acid and then display a considerable difference in functionality. SNPs are the root of all kinds of genetic disorders and also affect a person's sensitivity to chemical substances, including medicines. This refers to their therapeutic effect as

well as their side effects.²⁰ Genetic research offers major possibilities for identifying gene types that predispose a person to certain diseases and for achieving better matches between individual patients and the medicines they are prescribed.^{21,22}

DNA chips used for analyzing DNA have been available for a few years now. They are currently widely used in scientific, biomedical research but they are rarely used in clinical practice. DNA chips and other biochips were originally an achievement of microtechnology but miniaturisation is advancing here too, as with computer chips. Nanotechnologies are also increasingly playing a role in producing the chips and in increasing their detection sensitivity and reliability.²³⁻²⁶ A new nanotechnological analytical method uses quantum dots.²⁷ DNA in a sample is identified on the basis of its bonding to DNA molecules of a known composition embedded in micrometre-sized polymer spheres containing various mixtures of quantum dots, each of which provides a unique spectral bar code (colour code).²⁸ American researchers have used this method to study SNPs in genes that code for enzymes of the cytochrome P450 family which are involved in the breakdown of substances (including medicines) in the body.²⁹ The method is very suitable for studying large quantities of samples on many SNPs simultaneously (multiplex analyses).

Antibodies placed on nanowires can be used in a similar way to detect viruses, for example in a blood sample.^{17, 30} The bonding of a single virus particle to an antibody results in a change in the nanowire's electric conductance. The method is extremely sensitive which means that an infection can be detected at a very early stage. It is also suitable for multiplex analyses. Work is also underway on sensors based on carbon nanotubes, for use in microarrays.³¹ Detection methods based on cantilevers, nanowires or nanotubes offer the added advantage that it is not necessary to label the sample. Labs-on-a-chip are pocket-sized laboratories. They can be used for analyzing biopolymers and also for research and for manipulating cells. They are expected to play an important role in the further development of biosensors for the detection of pathogenic bacteria.³² In due course, there will also be possibilities for point-of-care applications, in which simple analyses can be made in the general practitioner's clinics, in the patients' homes and carried out by the patients' themselves.³³

Multiple nanoparticle detection methods have been developed, including scanning and transmission electron microscopy³⁴ Raman spectroscopy³⁵ and the naked eye.³⁶ Detection by the naked eye may be preferable, as other techniques are expensive and require specialized equipment and additional preparations. Both a DNA microarray and a heterogeneous immunoassay have been developed

with 10-nm gold particles amplified with silver, which can be detected by the naked eye³⁶ Most recently, the light-scattering properties of submicroscopic metal particles, such as gold nanoparticles, have been investigated.³⁷ These particles scatter incident white light to generate monochromatic light and can be seen either by the naked eye or by dark-field microscopy. The intensity generated by a nanoparticle is 100,000 times that generated by a fluorescein-labeled molecule. The approach has been used with success in DNA hybridization arrays, immunohistochemistry, and immunoassays³⁸ The resonance light-scattering (RLS) properties of nanoparticles render them well suited for use in microchannel-based immunoassays.⁵

Long-term stability and biocompatibility with a variety of macromolecules, such as DNA, RNA, and proteins, indicate that the metal nanoparticles can be used successfully as a label for a variety of diagnostic approaches. The immunoassay proved to be capable of detecting *H. pylori* and *Escherichia coli* O157:H7 antigens at quantities as low as 10 ng. This detection limit is comparable to that of the conventional dot blot ELISA.³⁹ A monoclonal antibody raised against a specific bacterial antigenic epitope could be used in the future to potentially further improve the sensitivity of detection.⁴⁰

In the case of *in vivo* diagnostics, patients are given contrast agents or radiopharmaceuticals. Their specific properties mean that these agents are useful in imaging pathophysiological changes and functional changes such as changes in blood flow in cells, tissue, and organs. The term molecular imaging is often used, as today's imaging techniques are increasingly concerned with making molecular biomarkers of disease processes visible, for instance, a receptor protein on the surface of a cancer cell. Besides being given a contrast agent (the imaging component), a carrier molecule or particle is also given a molecule that specifically binds to the biomarker, such as an antibody (the targeting component). Imaging that focuses on molecular biomarkers makes early detection of diseases possible and provides information on appropriate therapies. Imaging is also very suitable for monitoring, evaluating and optimizing treatment, which is, being provided.¹⁸

NANOSCIENCE AND CANCER DIAGNOSIS

Cancer is a major public health problem in the world, and one in four deaths in the United States is due to cancer, with an estimated 1479350 new cancer cases and 562340 deaths from cancer expected in 2009.⁴¹ Nanotechnology is an emerging field that may have potentials to make paradigm changes in the detection, treatment, and prevention of cancer.⁴² Nanoscience raises new promises in the

diagnosis and treatment of various cancers. They can also facilitate important advances in detection, diagnosis, and treatment of human cancers and have led to a new discipline of nano-oncology.⁴³ Nanoparticles offer a new method of tumor targeting, already available in clinical practice, which can concomitantly improve the efficacy and decrease the toxicity of existing or novel anticancer agents. This makes them an ideal candidate for precisely targeting cancer cells. Molecular imaging is now considered as a high area in cancer diagnosis.⁴⁴

In the near future, the use of nanotechnology could revolutionize not only oncology, but also the entire discipline of medicine. The development of resistance to variety of chemotherapeutic agents is one of the major challenges in effective cancer treatment. Nanotechnology could enhance the precision of drugs that have one highly specialized mission, like finding and killing cancer cells or tumors. Additionally, multifunctional nanocarriers are developed to enhance drug delivery and overcome multidrug resistance (MDR) by either simultaneous or sequential delivery of resistance modulators (e.g., with P-glycoprotein substrates), agents that regulate intracellular pH, agents that lower the apoptotic threshold (e.g., with ceramide), or in combination with energy delivery (e.g., sound, heat, and light) to enhance the effectiveness of anticancer agents in refractory tumors.⁴⁵

Nanoparticle (NP) has large surface area which can be conjugated or coated with different molecular probes for diverse detection system (optical, electrical, magnetic etc.) as well as used as a vehicle to carry different biomolecules and anticancer drugs to tumor cells. The nanoparticles that can be used to replace conventional contrast agents include liposomes, dendrimers, and nanotubes. Surface or hollow cores of these nanoparticles can be loaded with imaging agents, such as paramagnetic or superparamagnetic metals or radionuclides. Semiconductor quantum dot (QD) with novel optical and electronic properties helped to devise a new class of NP probes for molecular, cellular, and in vivo imaging. A large variety of materials ranging from metal, ceramic, polymer, lipid, protein and nucleic acid are used for developing novel nanoparticles with multiple functions which can detect different aspects of cancer biology and progression. The major issue of concern is biocompatibility and safety of these materials and their fate after in-vivo use. However with collaborative interdisciplinary research it will be possible to develop safer nanomaterials in future. Optically efficient, cancer specific Quantum dots provide a new tool to enable noninvasive visualization of disease-specific molecular and tissue changes with subcellular spatial resolution.⁴⁶ Nanotechnology is in a unique position to transform cancer diagnostics and to produce a new

generation of fluorescent markers and medical imaging techniques with higher sensitivity and precision of recognition.⁴⁷

Nanotechnology offers a wide range of tools, from chip-based nanolabs, capable of monitoring and manipulating individual cells, to nanoscale probes that can track the movements of cells and even individual molecules as they move about in their environment.⁴⁸ As such, nanotechnology can complement other technology platforms, such as proteomics and bioinformatics. Another near-term application of nanotechnology to accelerate basic research is to use molecular-size nanoparticles with a wide range of optical properties (e.g., quantum dots) to track individual molecules or cells as they move through local environments, thereby monitoring multiplexed cellular and molecular events in real time.^{5,49}

In the USA the National Nanotechnology Initiative has claimed that nanotechnology has potential in the treatment of cancer. It has been stated that 'It is conceivable that by 2015, our ability to detect and treat tumors in their first year of occurrence might totally eliminate suffering and death from cancer'.⁵⁰ Although it is reasonable to hope that some measures based on nanotechnologies may make contributions to detection and treatment of some forms of cancer, other factors such as a greater understanding of environmental causes of cancer, public health measures, and advances in surgical, pharmacological and radiological management are important in the reduction of incidence of and death from cancer. In the more distant future we will likely see nanotherapeutics with release mechanisms that are customized for precisely controlled release rates, dependent upon the mechanism of the therapeutic to be delivered. It is also likely that nanotherapeutics will appear that have their size, shape and elastic modulus optimized to permit passage across the blood/brain barrier.⁵¹

BRIEF SUMMARY OF NANOTECHNOLOGY BASED DIAGNOSTICS IN USE

Various nanotechnologies with potential applications in molecular diagnostics are nanotechnology on a chip (nanoChipTM, nanoArraysTM), Nanoparticle technologies (Gold particles, Nanobarcodes, Quantum dot technology, Nanoparticle probes) and other technologies (Nanowires, Nanopore technology, Cantilever arrays, DNA nanomachines for molecular diagnostics, Nanosensors, Resonance light scattering (RLS) technology).⁵

Array technologies

The enormously powerful array technologies, which use relatively large biological samples at the

micrometer scale, are continuously being enhanced for sensitivity, size and data analysis. The original DNA chip approach, which carries an array of DNA molecules on an inert carrier, is now routinely used in gene and protein analysis. The push towards higher resolution and smaller sample volume makes this an emerging nanotechnology. Lab-on-a-chip technologies, which are used for sensing and supporting disease diagnosis, are also currently in the micrometre range, but progress in nanofluidic systems will potentially lead to integrated nanoscale systems becoming available. These could have a range of applications, for example in improved devices for detection of biological and chemical agents in the field.

Gold particles

Gold nanoparticles are effective labels for sensors since a variety of analytical techniques can be used to detect them, like optical absorption, fluorescence, Raman scattering, atomic and magnetic force, and electrical conductivity. This technique has been used to detect life threatening bacteria and viruses, such as anthrax and HIV, and could replace PCR and the fluorescent tags commonly used today. Labeling oligonucleotide targets with gold nanoparticles rather than fluorophore probes substantially alters the melting-profiles of the targets from an array substrate. A nanoparticle-based DNA detection system is ten-times more sensitive and 100,000-times more specific than current genomic detection systems. Recently nanoparticles, especially gold and silver nano particles, have been successfully applied for labeling because of their controlled size distribution, long-term stability, and compatibility with biological macromolecules, including proteins and nucleic acids. Most recently, the light-scattering properties of submicroscopic metal particles, such as gold nanoparticles, have been investigated. The resonance light-scattering (RLS) properties of nanoparticles render them well suited for use in microchannel-based immunoassays and nucleic acid studies.⁴⁰ With the advent of nanoscience and nanotechnology, rapid progress has been achieved for the effective, sensitive and selective detection of bio- molecules. For example the functionalized semiconductor nanoparticles (Quantum Dots) are applied to determine proteins and nucleic acids.

Nanoparticle probes

In nanoparticle probes, DNA is attached to the nanoparticles. The probes are then used to bind and signal the presence of a specific DNA sequence. The nanoparticle probes change color if the target is present in a sample. Products based on it are Nanosphere Spot Assay and Gold nanoparticle

probe assays. Nanosphere Spot Assay is colorimetric detection of amplified DNA sequences. Whereas, Gold nanoparticle probe assays are in microarray format for analysis of DNA targets and eliminates the need for target amplification processes, such as PCR, saving both time and cost. Applications of nanoparticle probes are antibiotic resistant bacterial infection diagnostics, infectious disease diagnostics, detection of SNPs, detection of biological markers for cancer and blood screening.

Quantum Dots

Applications of quantum dot technology are DNA mapping, genotyping, multiplexed diagnostics and ultrasensitive viral RNA detection. The use of quantum dots in cancer investigations has increased intensely due to their unique size-dependent optical properties. Bioconjugated near-infrared quantum dots probes are highly sensitive molecular imaging tools for in vivo study. Further development of quantum dots might enable their application in detecting and localizing metastasis, quantitative measurement of molecular targets to facilitate targeted therapy, tracking drug delivery, and monitoring the efficacy of therapeutics noninvasively in real time.⁵²

Nanowires

Potential applications of nanowires are nucleic acid detection without amplification, quantitative PCR, tissue analysis, characterization of protein-protein interactions and integrated drug delivery & detection.

CONCLUSION

Nanodiagnostics will improve the sensitivity and integration of analytical methods to yield a more coherent evaluation of life processes. An important benefit would be extension of the present limits of molecular diagnostics. Nanoparticles are the most versatile material for developing diagnostics. This will have applications in genomic analysis, proteomics and molecular diagnostics. Nanotechnology has potential advantages in applications in point-of-care diagnosis, like on patients' bedside, self-diagnostics for use in the home and for field use of sensors for lab-on-a-chip. Novel methods to manage the symptoms of cancer that adversely impact quality of life and research tools that will enable rapid identification, as well as validation of new targets for clinical development and prediction of drug resistance. Developing nanotechnologies are being pursued for general cellular processes such as ubiquitous signaling pathways that may benefit numerous physiological systems, as well as being targeted toward the

particular challenges of specific disorders such as diabetes mellitus, cancer, Alzheimer's, arteriosclerosis, and AIDS. Ethical implications in the use of this technology and its impact on medicine should be considered, as it is in only in an emerging stage today. Current promises offered by the use of this technology will touch all spheres of our life. We need to have a balanced view about the prospects of this technology in the future, so that it does not offer us unsubstantiated hope.

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