Saudi Journal of Biological Sciences (2014) 21, 109–117

King Saud University

Saudi Journal of Biological Sciences

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REVIEW

Role and implications of nanodiagnostics in the changing trends of clinical diagnosis

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Received 16 September 2013; revised 3 November 2013; accepted 3 November 2013
Available online 19 November 2013

KEYWORDS
Nanodiagnostics;
PCR;
DNA sequencing

Abstract Nanodiagnostics is the term used for the application of nanobiotechnology in molecular diagnosis, which is important for developing personalized cancer therapy. It is usually based on pharmacogenetics, pharmacogenomics, and pharmacoproteomic information but also takes into consideration environmental factors that influence response to therapy. Nanotechnology in medicine involves applications of nanoparticles currently under development, as well as longer range research that involves the use of manufactured nano-robots to make repairs at the cellular level. Nanodiagnostic technologies are also being used to refine the discovery of biomarkers, as nanoparticles offer advantages of high volume/surface ratio and multifunctionality. Biomarkers are important basic components of personalized medicine and are applicable to the management of cancer as well. The field of nano diagnostics raises certain ethical concerns related with the testing of blood. With advances in diagnostic technologies, doctors will be able to give patients complete health checks quickly and routinely. If any medication is required this will be tailored specifically to the individual based on their genetic makeup, thus preventing unwanted side-effects.

Contents

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Peer review under responsibility of King Saud University.

ELSEVIER Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.sjbs.2013.11.001

1. Introduction

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 (Buxton et al., 2003). Its applications are seen in surgery, cancer diagnosis and therapy, bio-detection of molecular disease markers, molecular imaging, implant technology, tissue engineering, and devices for drug, protein, and gene and radionuclide delivery. Nano-materials are structurally and functionally prevalent in the organic, inorganic, and biological fields. Their unique size-dependent properties make these materials superior and indispensable in many areas of human activity. Nanotechnology is of great use for medical diagnosis and various nanoparticles have exhibited tremendous potential for detecting disease markers, pre-cancerous cells, fragment of viruses, specific proteins, antibodies and other indicators of disease. Many of the developments have already started and within a couple of years will show a definite impact in the field of diagnostics (Yezhelyev et al., 2006).

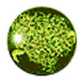
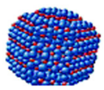
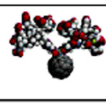
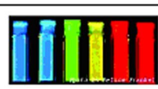
Nanotechnology will have an almost endless string of applications in biotechnology, biology, and biomedicine. New concepts for regenerative medicine give hope to many patients with organ failure or severe injuries. Nano-particle reinforced polymers, orally applicable insulin, and artificial joints made from nano-particulate materials, and low-calorie foods with nano-particulate taste enhancers (Barnes et al., 2007; Radwant and Aboul-Enein, 2002). Some products are already commercially available, such as surgical blades and suture needles, contrast-enhancing agents for magnetic resonance imaging, bone replacement materials, wound dressings, anti-microbial textiles, chips for in vitro molecular diagnostics, micro-cantilevers, and micro-needles. Nanotechnology and biology can address several biomedical problems, and can revolutionize the field of health and medicine (Curtis and Wilkinson, 2001). Nanotechnology is currently employed as a tool to explore the darkest avenues of medical sciences in several ways like imaging (Waren and Nie, 1998), sensing (Vaseashta and Dimova-Malinovska, 2005), targeted drug delivery (Langer R 2001) and gene delivery systems (Roy et al., 1999) and artificial implants (Sachlos et al., 2006). The new age drugs are nanoparticles of polymers, metals or ceramics, which can combat conditions like cancer (Farokhzad et al., 2006) and fight human pathogens like bacteria (Panacek et al., 2006; baker et al., 2005). Novel therapeutic strategies include the development of targeted transport vehicles allowing drug delivery to specific cells or cell structures. Of particular interest are bioengineered nano-particles, which can be utilized as transport vehicles of diagnostic or therapeutic agents.

1.1. Nanoparticles

Nanotechnologies represent an unprecedented recent advance that may revolutionize many areas of medicine and biology, including cancer diagnostics and treatment. In recent years there has been a rapid increase in nanotechnology applications in medicine in order to prevent and treat diseases in the human body (Angeli et al., 2008). Nano-medicine (the application nanotechnology to health) raises high expectations for millions of patients for better, more efficient and affordable healthcare and has the potential of delivering promising solutions to many illnesses. Nano-medicine, an offshoot of nanotechnology, refers to highly specific medical intervention at the molecular scale for curing disease or repairing damaged tissues (Campbell et al., 2008), such as bone (Zou et al., 2008; Mahony and Jones, 2008), muscle, nerve chronic pulmonary diseases and coronary artery disease (Schoenhagen and Conyers, 2008). Nanoparticle-based technologies have demonstrated especially high potential for medical purposes, ranging from diagnosing diseases to providing novel therapies. However, to be clinically relevant, the existing nanoparticle-based technologies must overcome several challenges, including selective nanoparticle delivery, potential cytotoxicity, imaging of nanoparticles, and real-time assessment of their therapeutic efficacy.

The size of nanomaterials is similar to that of most biological molecules and structures; therefore, nanomaterials can be useful for both in vivo and in vitro biomedical research and applications (Curtis and Wilkinson, 2001). The integration of nanomaterials with biology has led to the development of diagnostic devices, contrast agents, analytical tools, physical therapy applications, and drug delivery vehicles. Nanoparticles possess certain size-dependent properties, particularly with respect to optical and magnetic parameters, that can be manipulated to achieve a detectable signal. The primary event in most nanoparticle-based assays is the binding of a nanoparticle label or probe to the target biomolecule that will produce a measurable signal characteristic of the target biomolecules. A variety of probes have been used for this purpose, including QDs, nanoshells, and metal nanoparticles, quantum dots QDs are the most used and promising nanostructures for diagnostic applications (West and Halas, 2003; Jain, 1998). QDs are semiconductor nanocrystals, characterized by strong light absorbance that can be used as fluorescent labels for biomolecules. New nanodiagnostic tools include carbon nanotubes, nanoshells, gold nanoparticles, cantilevers and quantum dots (QDs). QDs, which are the most promising nanostructures for diagnostic applications, are semiconductor nanocrystals characterized by high photostability, single-wavelength

Table 1 Types of nanodevices are used in clinical Application.

Modality		Potential Applications
Cantilevers		• High-throughput screening • Disease protein biomarker detection • DNA mutation detection (SNPs) • Gene expression detection
Carbon Nanotubes		• DNA mutation detection • Disease protein biomarker detection
Dendrimers		• Image contrast agents
Nanocrystals		• Improved formulation for poorly soluble drugs
Nanoparticles		• Targeted drug delivery, permeation enhancers • MRI and ultrasound image contrast agents • Reporters of apoptosis, angiogenesis, etc.
Nanoshells		• Tumor-specific imaging
Nanowires		• High-throughput screening • Disease protein biomarker detection • DNA mutation detection (SNPs) • Gene expression detection
Quantum Dots		• Optical detection of genes and proteins in animal models and cell assays • Tumor and lymph node visualization

excitation, and size-tunable emission (Hassan et al., 2006). Different types of nanodevices are used for specific purposes (Table 1).

Ranging from the nano- to femtoliter range; droplet-based systems have also been used to directly synthesize particles and encapsulate many biological entities for biomedicine and biotechnology applications. This technology has the potential to provide novel solutions for today's biomedical engineering challenges and for advanced diagnostics and therapeutics.

Nanowires could lead to the fabrication of more sophisticated and high multispecificity biosensors via selective functionalization of individual segments for medical diagnostic applications. The surface of gold nanoparticles can be tailored by ligand functionalization to selectively bind biomarkers (Chandra et al., 2010). Thiol-linking of DNA and chemical functionalization of gold nanoparticles for specific protein/antibody binding are the most common approaches. Simple and inexpensive methods based on these bio-nanoprobes were initially applied for the detection of specific DNA sequences and are presently being expanded to perform a number of clinical diagnostic tests.

Nanoengineering of the coated colloids and microcapsules allows precision control over optical, mechanical, and catalytic properties to achieve sensitive response using a combination of polymers, fluorescent indicators, and glucose-specific proteins. Many of these nanodevices are available commercially and need to be adapted for the purpose required.

2. Nanotechnology in diagnosis of a number of diseases

Most of the current work was being done for diagnosing cancer but now it is being extended to other diseases. Nanodiagnostics, defined as the use of nanotechnology for clinical diagnostic purposes (Jain, 1998), was developed to meet the demands of clinical diagnostics for increased sensitivity and earlier detection of disease. The increased demand for sensitivity requires that a diagnostically significant interaction occurs between analyzed molecules and signal-generating particles, thus enabling the detection of a single analyzed molecule. Nanodevices can be used for in vitro or in vivo diagnosis where it is coupled with existing imaging technology.

Nanoparticles are considered to have the potential as novel intravascular or cellular probes for both diagnostic (imaging) and therapeutic purposes (drug/gene delivery), which is expected to generate innovations and play a critical role in medicine (Singh et al., 2007). Target-specific drug/gene delivery and early diagnosis in cancer treatment is one of the priority research areas in which nanomedicine will play a vital role. Some recent breakthroughs in this field recently also proved this trend. It is of great importance in the treatment and diagnosis of cancer. Some recent breakthroughs in the form of drug delivery are effective target therapies used in pre-sympathomimetic and diagnosis techniques (Torchilin, 2007). Biomolecule coated ultra small super paramagnetic iron oxide particles are used for detecting cancer cells. The particles can be safely injected into the blood stream of the patients and the presence of specific antigens/target molecular markers on the particles bind to the positive cells which induce a specific signal that can be detected by MRI (magnetic resonance imaging). Nanomaterials with a variety of unique intrinsic physical properties have attracted growing interest for use in biomedical imaging applications (Cho et al., 2010; Lee et al., 2011). Among the imaging nanoprobes, magnetic iron oxide nanoparticles have been widely used as MRI contrast agents for cancer imaging, helping to provide anatomical details and furthermore real-time monitoring of the therapeutic response (Kievit and Zhang, 2011; Varadan et al., 2008). This technology will help in detecting individual cancer cells months or years earlier than the conventional diagnostic tools. This will also be used for detecting residual or metastatic cancer cells after therapy.

Multilayer films and capsules synthesized from DNA have great potential as they are biodegradable, biocompatible, and the structure of the films can be finely controlled by base pairing of the nucleotides (Angus et al., 2008). These DNA capsules are envisaged to find applications in diagnosis of not only cancer but various other diseases which have specific known biomarkers.

Optical tweezers is one of the nano manipulation techniques, which can investigate pico-newton to femto-newton forces exerted on microscopic objects. With the Cell palpation system by use of optical tweezers an operator manipulates a probe particle that touches a certain location of a cell and feels the strength of the cell through a haptic device, which displays the force calculated and generated in a computer. This technique can be used in the differential diagnosis of tissue growths, calculi, cysts, abscess, as well as in hepato and spleno – megaly.

The newest technologies within nanodiagnostics involve microfluidic or ''lab on a chip'' systems, in which the DNA sample is completely unknown. The idea behind this kind of chip is impel: the combination of numerous processes of DNA analysis are combined on a single chip composed of a single glass and silicon substrate (Burns, 1998). The device itself is composed of micro fabricated luidic channels, heaters, temperature sensors, electrophoretic chambers, and fluorescence detectors to analyze nanoliter-sized DNA samples (O'Connor, 2005). Droplet-based microfluidic systems have been shown to be compatible with many chemical and biological reagents and are capable of performing a variety of ''digital fluidic'' operations that can be rendered programmable and reconfigurable. This platform has dimensional scaling benefits that have enabled controlled and rapid mixing of fluids in the droplet reactors, resulting in decreased reaction times. Coupled with the precise generation and repeatability of droplet operations, has made the droplet-based microfluidic system a potent high throughput platform for in vitro diagnostics. Microfluidic technologies illustrate a possible route for the

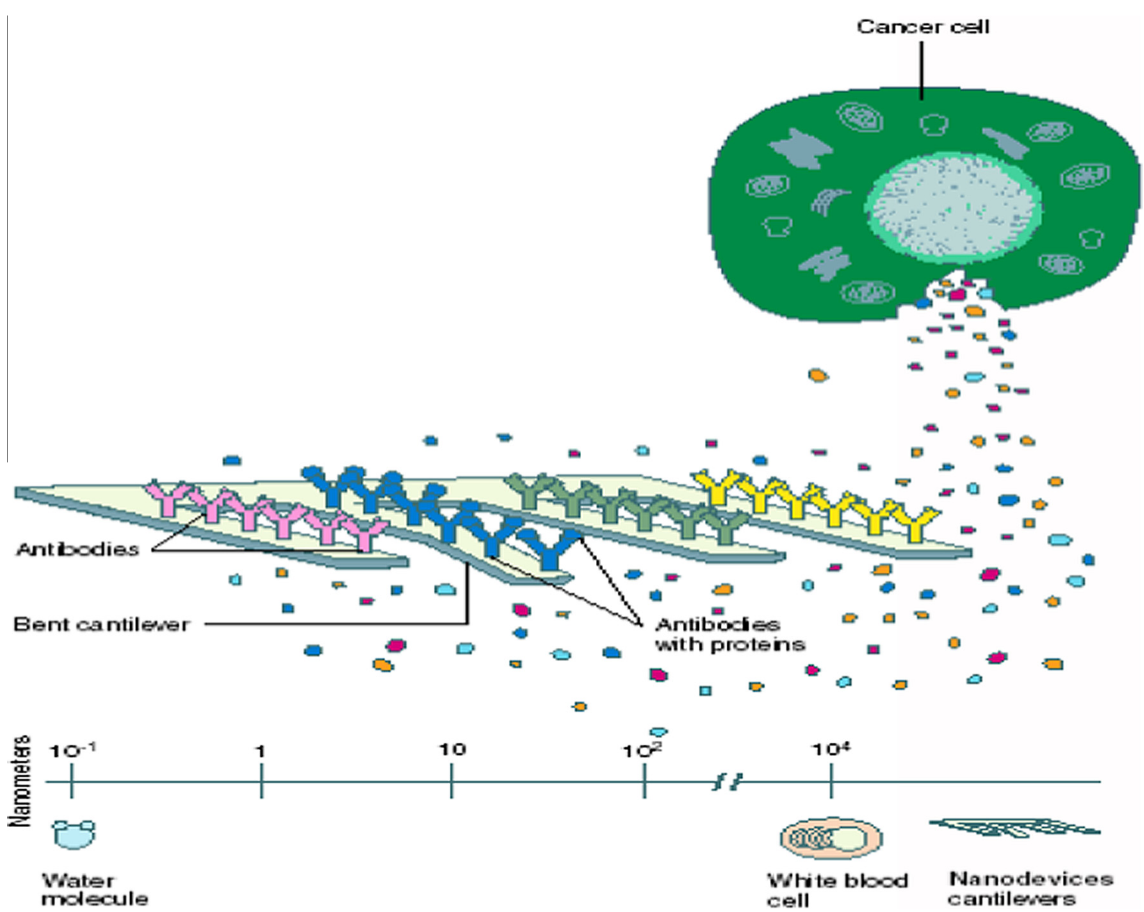


Figure 1 Nanoscale cantilevers concept explained by cancer markers.

development of tests which could fulfill the needs of clinicians for disease staging and monitoring (Walt, 2005).

Nanoscale cantilevers, microscopic, excitable beams resembling a row of diving boards, are built using semiconductor lithographic techniques. These can be coated with molecules capable of binding specific substrates—DNA complementary to a specific gene sequence. Such micron-sized devices, comprising many nanometer-sized cantilevers, can detect single molecules of DNA or protein. Nanoscale cantilevers, constructed as part of a larger diagnostic device, can provide rapid and sensitive detection of a number of disease specific molecules (Raiteri et al., 2001; Carrascosa et al., 2006).

In the Fig. 1, below the concept is explained by taking the example of cancer markers.

Similarly DNA-coated gold nanoparticles (NPs) can be used for developing tests more sensitive than ELISA. When the antigen is present in a complex protein mix, such as in serum-containing culture medium or in crude body fluids, and analyzed in a sandwich assay, Nano-i PCR and i PCR usually detect the antigen with 1–3 orders higher sensitivity than ELISA (Adler and Wacker, 2003; Chen et al., 2009; Perez et al., 2011) The basis of this system which uses larger magnetic micro particles (MMPs) to detect attomolar (10–18) concentrations of serum proteins is represented in Fig. 2. In this case, a monoclonal antibody to prostate specific antigen (PSA) is attached to the MMP, creating a reagent to capture free PSA. A second antibody to PSA, attached to the NPs, is then added, creating a "sandwich" of the captured protein and two particles that are easily separated using a magnetic field.

DNA-labeled magnetic nanobeads have the potential to serve as a versatile foundation for detecting virtually any protein or nucleic acid with far more sensitivity than is possible with conventional methods now in use. If this proves to be a general property of such systems, nanoparticle-based diagnostics could provide the means of turning even the rarest biomarkers into useful diagnostic or prognostic indicators.

"Smart" dynamic nanoplatforms have the potential to change the way diseases are diagnosed, treated, and prevented. The outside of such "nanoclinics" could have marker specific monoclonal antibodies coated with polyethylene glycol (PEG) to shield the device from immune system detection. The polymer matrix of such particles could be loaded with contrast agents, which would provide enhanced sensitivity for pinpointing the location of the problem within the body, and various types of therapeutic agents, such as reactive oxygen-generating photodynamic sensitizers that would be activated once the particle detects a malignant or abnormal cell could be developed

Dendrimers, 1- to 10-nm spherical polymers of uniform molecular weight made from branched monomers, are proving particularly adept at providing multifunctional modularity. Dendrimers can serve as versatile nanoscale platforms for creating multifunctional devices capable of detecting a number of proteins which are currently detected by individual ELISA test example Thyroid profile, Panel of Steroid hormones, Panel of inflammatory Cytokines (Chen and Kendler, 2008).

Circulating peptides and protein fragments are shed from all cell types in the tissue microenvironment, these vary in health and disease, as well as at different phases of our physiology. Proteolytic cascades within the tissue generate fragments that diffuse into the circulatory system. The identity and cleavage pattern of the peptides provide diagnostic information and these nanosensors can be used to understand the biological alterations in complex diseases like diabetes, asthma, cardiovascular diseases and cancer.

Diagnostics based on altered DNA sequence detection uses single-stranded oligonucleotide tagged magnetic nanobeads (de la Torre et al., 2011). The target DNA is recognized and volume-amplified to large coils by circularization of linear padlock probes through probe hybridization and ligation, followed by rolling circle amplification (RCA) (Strömberg et al., 2008). Upon hybridization of the nanobeads in the RCA coils, the complex magnetization spectrum of the beads changes dramatically, induced by the attached volume-amplified target molecules. This magnetization spectrum of the nanobeads can be used for concentration determination of RCA coils down to the pM range, thus creating the opportunity for nonfluorescence-based cost-efficient high-sensitivity diagnostics tool, more sensitive than PCR. This is useful for all genetic disorders like thalassemia, cystic fibrosis, muscular

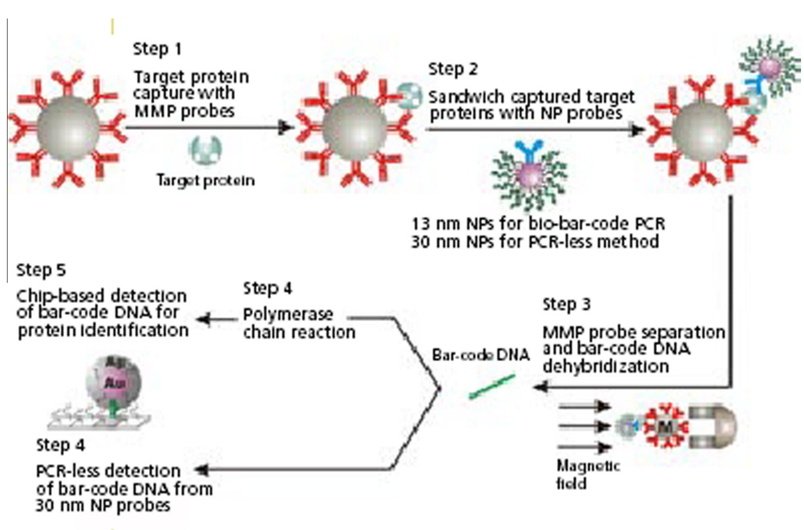


Figure 2 DNA labeled nano beads.

diseases, neurological and mitochondrial diseases. Apart from these SNP analysis gives an indication of susceptibility to complex diseases especially CVD, diabetes mellitus, HTN, etc. and is used for the prevention and management of these conditions.

2.1. Detection of disease genes

DNA-based electrochemical sensors offer sensitivity, selectivity and low cost for the detection of mutated genes associated with human disease (Drummond et al., 2003). All such sensors exploit nanoscale interactions between the target in solution, the recognition layer and a solid electrode surface. One of the innovations is in electrochemical amplifications with nanoparticles. Proof of principle, reproducibility, and the robust, simple and rapid characteristics of Clear Read nanoparticle technology have been used with unamplified DNA samples to demonstrate all possible forms of three genes implicated in hyper coagulation disorders.

2.2. Detection of single nucleotide polymorphisms

Nanosphere's Clear Read nanoparticle technology enables a microarray-based method for multiplex single nucleotide polymorphisms (SNP) genotyping in total human genomic DNA without the need for target amplification by PCR. This direct SNP genotyping method requires no enzymes and relies on the high sensitivity of the gold nanoparticle probes. Clear Read technology sandwiches a target DNA SNP segment between two oligonucleotide sequences to greatly increase detection specificity and sensitivity. One segment identifies any mutations in the DNA and the probe, a highly sensitive gold nanoparticle, creates a strong signal accurately indicating the presence of a specific target SNP (Bao et al., 2005). The assay format is simple, rapid and robust pointing to its suitability for multiplex SNP profiling at the point of care. Recently 20 Nan cantilevers labeled with 20 fluorescence dyes were able to detect multiple SNP's at the same time.

2.2.1. Glucose monitoring in vivo by nanosensors

It is possible to engineer fluorescent micro/nanoscale devices for glucose sensing. Deployment of micro/nanoparticles in the dermis may allow transdermal monitoring of glucose changes in the interstitial fluid.

Non-invasive glucose sensing will maximize acceptance by patients and overcome biocompatibility problems of implants. A new type of optical nanosensor, based on single walled carbon nanotubes that modulate their emission in response to the adsorption of specific biomolecules, can be used as a glucose sensor (Mano and Heller, 2005). Carbon nanotubes are coated with glucose oxidase, an enzyme that breaks down glucose molecules. Then ferricyanide, an electron-hungry molecule, is sprinkled, onto the nanotubes' surfaces (Zhang et al., 2005). Ferricyanide draws electrons from the nanotubes, quenching their capacity to glow when excited by infrared light. When glucose is present, it reacts with the oxidase, producing hydrogen peroxide. In turn, hydrogen peroxide reacts with ferricyanide in a way that reduces that molecule's hunger for electrons. The higher the glucose level, the greater is the nanotube's infrared fluorescence. This will monitor glucose continuously without multiple sample collection.

2.2.2. Nanotechnology and Diagnosis of infectious disease

Nanotechnology is research at the atomic, molecular, or macromolecular scale using nanomaterials that have a length scale of 1–100 nanometers (nm) (McNeil, 2005), Nanomaterials have some unique physical, chemical, and biological properties which are fundamentally different from those of the corresponding bulk material and could be widely used in medical testing (Rosi and Mirkin, 2005). One of the possible applications of bio nanotechnology is timely diagnosis of various diseases. Many infectious agents both bacterial and viral which cause SARS, dengue, Asian flu etc. spread out very fast and cause epidemic outbreaks with a very high morbidity and mortality. Therefore there is a great potential to use nanotechnology for diagnosing infectious agents.

Magnetic nanobeads as a nanosensor for clinically relevant viruses and bacteria can be developed. The nanobeads have a supramagnetic iron oxide core coated with dextran. Anti-viral antibodies are conjugated directly to nanobeads. By using a magnetic field, as few as five viral particles can be detected in a 10 mL serum sample. This system is more sensitive than ELISA-based methods and is an improvement over PCR-based detection because it is cheaper, faster and has fewer artifacts resulting in false positives. ELISA can decrease the lower limit of detection (LOD) of antigen detection to ~1 pg/mL. Another sensitive method called real-time immuno-polymerase chain reaction (IPCR) assay can detect 1000 HIV-1 RNA copies or 40 attograms of HIV-1 antigen per reaction (Barletta et al., 2004).

Direct, real-time electrical detection of single virus particles can be achieved with high selectivity by using nanowire field effect transistors. Measurements made with nanowire arrays modified with antibodies for influenza A showed discrete conductance changes characteristic of binding and unbinding in the presence of influenza A but not paramyxovirus or adenovirus, indicating how specific these tests are.

The rapid and sensitive detection of pathogenic bacteria is extremely important in medical diagnosis. Limitations of most of the conventional diagnostic methods are a lack of ultra sensitivity or delay in getting results because of the culturing involved. Several nanotechnology based methods have already been described including Ferro fluid magnetic nanoparticles and ceramic nanospheres. A bio conjugated nanoparticle based bioassay for in situ pathogen quantification can detect a single bacterium within 20 min.

Using nanolithography, antibodies could be localized in an exact place or spot, however, nonspecific background binding of the proteins and detection sensitivity is the major problem associated with this technique. Nanotechnology therefore helps in providing the technique that is capable of identifying and detecting a single viral particle. High sensitivity for detection is needed for diseases like AIDS for as few as 5–10 HIV viral particles can infect the other person and the current methods can assess either antibodies or a high load of viral antigens.

It is also necessary to identify the antibodies quickly as some of these infectious agents are quite stable, aggressive and these could be easily transmitted in trace amounts. Some of the infectious agents such as prions and infectious amyloidoses could be transmitted on fento molar concentration level. Therefore, diagnostics which are capable of identifying these infectious agents in extremely low concentration are important and bio-nanotechnology helps us in the early diagnosis of these infectious agents.

2.2.3. Diagnosis of tuberculosis based on BioMEMS

In India, the country with the highest estimated number of TB cases, nanotechnology can play a major role in addressing this issue. The diagnostic principle of nanomechanical deflection of the microcantilever due to adsorption of the TB specific antigens on its upper surface is employed for the diagnosis of TB. The deflection of the microcantilever would be measured in terms of piezoresistive changes by implanting boron at the anchor point. Such a biomicroelectromechanical system (BioMEMS) based microdiagnostic kit is highly specific as complementary biochemical interactions take place between TB antigens and the antibodies against them immobilized on the upper surface of the microcantilever. Measurement of antigens up to picogram levels can be done using this technique (Nelson et al., 2001).

NanoLogix's "Identikit" has shown great promise in detecting the presence of Mycobacterium avium complex (MAC) infection. The methodology provides for early detection of these pathogens in sputum and stool samples; thus allowing for treatment before the infection spreads throughout the body. Early detection of Mycobacterium avium complex (MAC) infection is rapidly becoming important, especially for those with AIDS who are living longer due to new methods of treatment.

A nanotechnology-based TB diagnostic kit, designed by the Central Scientific Instruments Organization of India and currently in clinical trials, does not require skilled technicians for use and offers efficiency, portability, user-friendliness and availability for as little as Rs.30 per test. Polylactide co-glycolide nanoparticles are being investigated by groups at the Harvard University (U.S.), the Postgraduate Institute of Medical Education and Research (India) and the Council for Scientific and Industrial Research (South Africa), as drug carriers for treating TB.

2.2.4. Nanotechnology and cardiovascular diseases

The rapid progress of nanoscience and the application of nanotechnology are changing the foundations of diagnosis, treatment, and prevention of cardiovascular diseases (Wickline et al., 2006; Lipinski et al., 2004). As the core of nanotechnology, nano- and microparticles offer "three-in-one" functions as imaging agents, target probes, and therapeutic carriers. While nano- and microparticle-based imaging of cardiovascular interventions is still in its developing phase, it has already presented exciting potential to monitor primary interventional procedures for precise therapeutic delivery, enhance the effectiveness of delivered therapeutics, and monitor therapeutic efficiency after interventions performed to treat cardiovascular diseases (Xiaoming, 2007).

3. Nanotechnology for clinical laboratory diagnostics – salient points

With advances in diagnostic technologies, doctors will be able to give patients complete health checks quickly and routinely. If any medication is required this will be tailored specifically to the individual based on their genetic makeup, thus preventing unwanted side-effects. As a result, the health system will become preventative rather than curative

- Nanoscale particles, used as tags or labels, increase the sensitivity, speed and flexibility of biological tests measuring the presence or activity of selected substances.
- Nanotechnology enables testing of relatively small sample volumes.
- Many of the technologies described are applicable both to DNA and proteins although some are better suited for proteins in body fluids.
- Nanotechnology enables the detection of a few microorganisms or molecules that would not be possible with conventional techniques.
- Early detection of disease such as CVD, PIH, and cancer improves the chances of cure.
- Nanotechnology, by reducing the time required for the test, has a positive impact on both clinical decision-making and treatment costs.
- Nanoparticles for targeted drug delivery in cancer enable combination of diagnostics and therapeutics and act as adjuncts to hyperthermia and photodynamic therapy.
- Nanobiotechnology will facilitate the development of personalized medicine, i.e. prescription of specific therapeutics best suited for an individual. The use of nanotechnologies for diagnostic applications shows great promise to meet the rigorous demands of the clinical laboratory for sensitivity and cost-effectiveness.

4. Insight of nano diagnostics

The biological application of nano-particles is a rapidly developing area of nanotechnology that raises new possibilities in the diagnosis and treatment of various diseases .The application of nanotechnology in the medical field must conform to requirements for public health, safety and environmental protection An important point is that, basically, what safeguards are needed with regard to a new technology depends mainly on the way it will be used.

- Nano particles can be much more reactive than larger volumes of the same substance. They are relatively cheap and can be manufactured in large quantities. They are already used in consumer products and can be highly reactive. Such particles often have unknown toxicity which can be difficult to quantify.
- Nanoparticles can cause chronic health effects. Carbon nanotubes are strong and can have a similar shape to asbestos fibers; several reviews conclude that carbon nanotubes are potentially toxic to humans. Given that nano-sized objects tend to be more toxic than their large scale form it would be unwise to allow the unnecessary build up of nanoparticles within the body until the toxicological effects of that nanoparticle are known.
- There is still too little research into the potential negative impacts of this technology on the environment Removing nanoparticles from the environment may also present a significant problem due to their small size. If absorbed, the particles may travel up the food chain to larger animals and have been shown to be harmful to Humans.

- There is some concern about the safety of nanoparticles introduced in the human body and released into the environment. Research is underway to address these issues. As yet there are no FDA directives to regulate nanobiotechnology, there are some legal issues of immediate and practical concern, related to the existing regulations in the field of nanodiagnostics.

The field of nano diagnostics raises certain ethical concerns related with the testing of blood.

- For example, if a nanochip were to be able to analyze our entire DNA sequence from a drop of blood, would it be morally correct for hospitals to know an individual's entire genetic makeup? Should not individuals have some say in whether or not hospitals have access to these records? Another area of concern is with the use of MEMS devices within the body. If the capsule device breaks down in one's stomach, harmful metal oxide particles could cause the introduction of free radicals that are harmful to cells. Researchers still do not know if the introduction of these devices within one's body would leave residual nanoparticles that would be harmful to the digestive system.

Acknowledgment

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding of this research through the Research Group Project no RGP-VPP-244. We are thankful to Dr. Rabbani Syed & Dr. Imran Ali Khan for helping in the manuscript preparation.

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