International Journal of Engineering Research and General Science Volume 3, Issue 2, Part 2, March-April, 2015
ISSN 2091-2730

Nanomolecular diagnostics: Recent trends and future perspectives

Kaliyaperumal Rani* and Barindra Sana

Division of Bioengineering, School of Chemical & Biomedical Engineering, Nanyang Technological University, 70 Nanyang Drive, Singapore 639457. E-mail: kranimani@gmail.com

Abstract— Nanobiotechnology refers to the unique fusion of two emerging technologies, biotechnology and nanotechnology with the tremendous potential of characterizing biological processes at the nanoscale levels. Nanomolecular diagnostics encompasses various molecular biological techniques that integrate nanotechnological concepts to make the diagnosis accurate and expedited. Unlike conventional molecular diagnostics, which require bulky instrumentations, high sample volume and extended analysis, nanodiagnostics enable simultaneous real time evaluation of various disease markers with exalted accuracy and sensitivity. Eventhough some of the nanodiagnostics are currently at the initial stage of evolution, while others are at testing phases, the current diverse applications of nanodiagnostics apparently evince its prodigious potential in molecular diagnosis. Hence this review presents an insight into the recent progresses in the field of nanomolecular diagnosis. The applications described here strongly suggest that nanodiagnostics continue to make tremendous strides and wide ranging impact in the scope of molecular diagnosis and lay the foundation for the establishment of novel therapeutic strategies for deadly diseases.

Keywords— Nanobiotechnology, Nanomolecular diagnostics, Molecular imaging, Nanoparticles, Nanosensors, Nanoarrays, Cancer research, Nanopores, Personalized medicine

INTRODUCTION

Nanotechnology, at its simplest refers to engineering functional systems at the nanoscale, which is about 1 to 100 nanometers. It pertains to the projected ability to fabricate items from the bottom up, using contemporary techniques at the nanoscale level to hatch complete, high performance articles. On the other hand, biotechnology refers to technology based on biology, which harnesses cellular and biomolecular processes to develop services and products that help to enrich our lives and the health of our planet. Nanobiotechnology or bionanotechnology refers to the unique fusion of two major fields; biotechnology and nanotechnology. The nanotechnical approach to biology is being considered pivotal as it offers unprecedented possibilities in studying and modulating biological processes on a molecular and atomic scale [1]. Nanotechnology, may, thus open up innovative ideas in virtually all sorts of disciplines spanning from medicine, food and agriculture to environmental, cosmetics and chemical industry. Nano-optics, novel nanoscale materials, nanosensors, nanocosmetics, nanocomposites, nanofoods and nanomedicines are just a few examples from this revolutionary field.

Nanotechnology enacts an imperative role in clinical medicine and its potential applications include disease diagnosis, molecular imaging, 3D nanostructured scaffolds and target-specific drug delivery [2]. In medicine, nanotechnology revolutionizes current treatments and facilitates target-specific drug delivery. Further, it introduces innovative concepts including novel tools into regenerative medicine to rejuvenate impaired tissues. In disease diagnosis, this technology helps to revolutionize many of the existing diagnostic tools and procedures to be more personalized, efficient, faster and cheaper. Taken together, the present scenario of nanotechnology apparently evinces its immense potential across a wide range of disciplines. Therefore, the current review explores the distinct nanotechnological applications in the realm of molecular diagnosis.

MOLECULAR DIAGNOSTICS AT A GLANCE

Molecular diagnosis, the emerging segment of clinical testing today, refers to the identification of abnormal mutations in DNA and RNA samples in order to diagnose and monitor diseases. It comprised of various molecular biological techniques (Molecular diagnostics) used to spot the defective genes and characterize the molecular bases of diseases. It also bolsters to interpret the genetic interactions including protein-protein, protein-DNA and their contribution in genetic abnormalities. Further, It also aids to understand expression patterns of protein-coding genes in different types of cells. Hence, molecular diagnostics play an instrumental role in disease prevention and treatment. Molecular diagnostics effectively translate novel findings and innovative technologies into pragmatic clinical assessments and propound the potential for advancing from diagnostics to prognostics. For instance, cancers, the leading causes of morbidity and mortality worldwide, a significant increment in recovery rate would be possible only if the novel molecular mechanisms that drive the pathophysiology of cancer could be explored which will establish the fundament for cutting-edge cancer therapeutics [3].

Molecular diagnostics are currently used in the following areas, which entail infectious disease molecular testing, molecular oncology testing, inherited diseases molecular testing, identity testing (DNA fingerprinting), histocompatibility testing and pharmacogenetics testing.

NANOTECHNOLOGY IN MOLECULAR DIAGNOSTICS: NANOMOLECULAR DIAGNOSTICS

Nanomolecular diagnostics, also termed as "Nanodiagnostics" refers to the inclusion of nanotechnological concepts in molecular diagnostics. Owing to the nanoscale nature of surface receptors, membrane pores and other vital constituents of cells, the anatomy and functions of these constituents could be explored with the aid of nanoscale probes [4]. The ultimate mission of any diagnostic regimen involves non-invasiveness, primitive and precise diagnosis with greater sensitiveness and cost effectiveness. Nanotechnology protracts the frontiers of molecular diagnostics to the nanoscale, which enable simultaneous real-time detection of a wide spectrum of diseases with exalted accuracy and greater cost-effectiveness [5]. For instance, nanoparticles immobilized on amorphous or nanocrystalline materials provoke greater functionality and bioavailability [6]. Molecular imaging strategies such as Infrared (IR) or Magnetic Resonance Imaging (MRI) are the promising approaches for early and explicit prediction of diseases. The resolution and sensitivity of imaging techniques are greatly enhanced with the aid of nanoparticles, quantum dot and nanocrystals. For instance, superparamagnetic iron oxide nanoparticles (SPION) with phosphorothioate-modified oligo DNA sequences (PS-ODNs) complementary to c-fos mRNA (SPION-cfos) was employed in magnetic resonance imaging (MRI) to diagnose neurodegenerative diseases [6-8]. Further, functionalized nanoparticles have gained much attention recently owing to their potential in detecting complementary functional groups present on cell outer layers, which are characteristics of deadly diseases such as cancer [6]. Taken together, nanodiagnostics act as vital means for establishing new therapeutic approaches for various deleterious diseases.

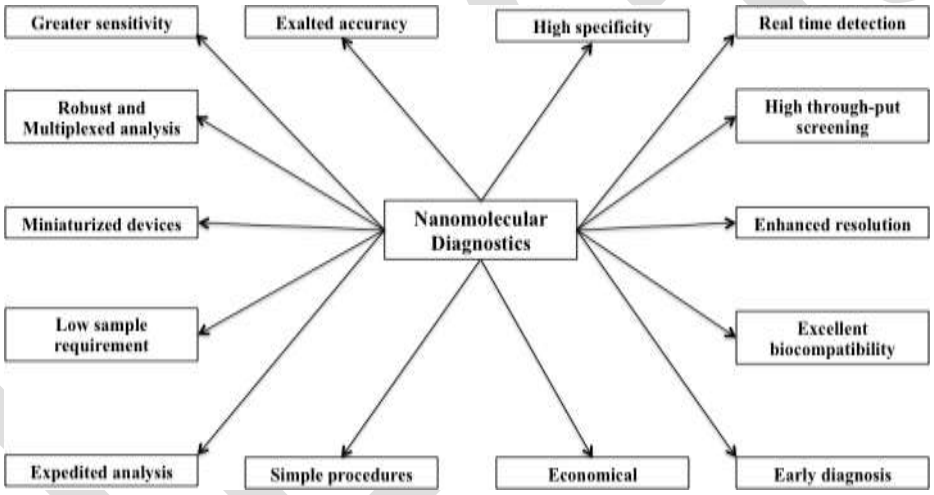

Fig. 1. Salient features of nanomolecular diagnostics, which make them superior to conventional molecular diagnostics.

NANOPARTICLES FOR MOLECULAR DIAGNOSTICS

The unique nanoscale characteristics of nanoparticles make them a promising nanoplatforms for molecular diagnostics. They are used as molecular imaging probes where the nanoparticles are functionalized with a typical targeting agent that could recognize cell surface biomarkers. Nanoparticles based probes bind with the healthy tissues and thus lifting the contrast between malignant and healthy tissues. This feature is of great value for diagnosing malignancies via magnetic resonance imaging. Nanoparticles that are currently used for molecular diagnosis include gold nanoparticles, magnetic nanoparticles, and quantum dots (QD).

GOLD NANOPARTICLES

Gold nanoparticles typically comprised of a metal core with quasi-continuous electronic conduction bands [9]. Gold nanoparticles are facile to make and are non-cytotoxic, surpassingly biocompatible with lesser nonspecific binding. These salient characteristics offer the possibility to use them as attractive nanomaterial for molecular diagnosis purpose. Electrons present in the conduction band bum around the metal core and are excited by light results in plasmonic responses [9]. The surface plasmon resonance of gold nanoparticles has diverse application and has drawn tremendous attention in recent years.

Gold nanoparticles are employed for sample labeling in transmission electron microscopy. Owing to their enduring electron absorbing properties, gold nanoparticles provide enhanced contrast as compared to conventional contrast agents and thus, act as a stain for samples with low contrast, such as tissue specimens. For instance conjugation of gold nanoparticles with antibodies provoke enhanced spatial resolution and specificity and are considered crucial in the labeling procedure [10]. Meanwhile, the exceptional optical features of the gold nanoparticles including robust absorption, scattering and in particular plasmon resonance make them amicable to use in a broad-spectrum of imaging techniques including Fluorescence Reflectance Imaging (FRI) systems and Optical Coherence Tomography (OCT). Also, in Photoacoustic imaging (PAI), an emergent hybrid biomedical imaging modality, gold nanoparticles are employed as contrast agents for functional, structural and molecular imaging [11-13].

A central theme of any molecular diagnostic procedure especially for molecular imaging, involves the use of radiolabelled tracers. The added value of gold nanoparticles is that they can be radiolabelled by neutron activation, thus enabling greater sensitivity in detection process, and are well accepted as x-ray contrast agents [14]. The optical properties of gold nanoparticles could alter upon conjugation with certain compounds, facilitating the accurate sensing and quantification of wide-spectrum of analytes. Upon aggregation, the absorption spectra of gold nanoparticles alter remarkably and this salient feature is of most beneficial in detecting DNA strand with single-base mismatch [10]. In Surface-Enhanced Raman Spectroscopy (SERS), gold nanoparticles are conjugated with specific-antibodies to detect pathogenic microbes [15]. This approach potentially obviates PCR and fluorescent tags used in the detection procedures.

Fluorophores tagged to nucleic acid–gold nanoparticle conjugates hold pledge in the area of biological sensing. This novel approach is considered quiet significant as the inclusion of fluorophores provides the complementary effect. One of the greatest advancements in this realm was the introduction of nanoflare constructs, which enable specific and real time detection of intracellular molecules such as mRNAs, microRNAs and other tumor markers present in blood [16, 17].

QUANTUM DOTS

Quantum Dots (QDs) are nanocrystals of semiconducting materials with peculiar optical and electrical characteristics. The unique advantages of QDs include tunable sizes and composition, broad adsorption and emission profile across a wide spectral range and greater sensitivity with strong photostability as compared to the classical organic dyes [18]. QDs have found applications in in vitro real time imaging of single cell migration, labeling of biomolecules in fixed cells and tissue parts, biosensors and in vivo imaging of cells and organs [19]. Enhanced photostability and exalted brightness of QDs are other striking features, which are of highly beneficial for live animal targeting and imaging. Further, recent researches have proven that QDs exhibit extremely higher two-photon cross sections of up to 50,000 GM and hence, considered as potential contrast agents in imaging applications. [20].

Fluorescent dyes are virtually applied in a wide spectrum of imaging applications in order to visualize cells, cell organelles and molecules. However, the photobleaching natures of those dyes limit their applications. QDs are applied as fluorescent tags in various immunoassays in place of fluorescent dyes [21, 22]. For instance in immunoassays for detection of Salmonella Typhi, a potent foodborne pathogen, quantum dots act as fluorescent tags [23]. Moreover, the ability of QDs to detect viruses even at trace amounts makes them an excellent nanomaterial in the detection of infectious microbes [24].

Another category of QD known as Bioconjugated QDs also attracted much attention in the recent years. Conjugations of QDs with biomolecules for instance, antibodies or peptides maximize their specificity and sensitivity and hence bioconjugated QDs are of great use in targeting cancer biomarkers. In QD-based multiplexed molecular imaging, several tumor biomarkers are simultaneously stained which reveals the tempo-spatial pattern among molecules. This feature is rather significant as it could help to delineate the molecular

mechanism behind cancer invasion and analyze tumor microenvironment [25, 26]. This holds the pledge in understanding the tumor invasion mechanism and paves the way for improved personalized treatments.

MAGNETIC NANOPARTICLES

Magnetic nanoparticles are spherical nanocrystals of 10-20 nm of size, which are lured to a high magnetic flux density. In general, these particles comprised of magnetic components such as iron, nickel, cobalt and their chemical mixture. As for magnetic nanoparticles, it's unique magnetic resonance behaviors is a big advantage to use as contrast agents in Magnetic Resonance Angiogrpahy (MRA) and Molecular Resonance Imaging (MRI) [27]. For instance, superparamagnetic iron oxide particles (SPIO) act as potential MR contrasts in diagnosing hepatic metastases owing to its rapid hepatic uptake [28, 29]. Also, in Diffusion Weighted MRI (DW-MRI), SPIO has tremendous potential in diagnosing nodal metastases and prostate cancer [30]. Iron-oxide or iron-cored nanoshells have been employed to diagnose colorectal cancers [31]. Macrophages play crucial roles in atherosclerotic plaque developments and SPIOs of great use in tracking macrophage activities and therefore lay foundation for establishing novel therapeutic strategies against atherosclerosis. In addition, SPIOs role in demonstrating stem cell activities in host organs such as brain yield novel opportunities in the field of regenerative medicine [32].

Diagnostic magnetic resonance (DMR) technology that uses magnetic nanoparticles as sensors holds considerable promise to identify targets such as DNA/mRNA, enzymes, proteins and peptides, drugs and microbes [33-35]. This technology is more robust and highly sensitive enabling multiplexed analysis using microliter samples.

NANOARRAYS

Microarrays remain as novel platform for high-throughput diagnosis of biomolecules such as multiplexed DNA and proteins. However, microarrays require relatively large sample volumes, prolonged incubation time and bulky instrumentation. Also, microarray analysis typically requires robust amplification and labeling, which make the analysis quite laborious and expensive. Nanoarrays are the miniaturization of microarrays and comprised of an array of molecules scattered in micron or sub-micron spatial range. Biological samples such as protein, DNA, RNA and whole viruses as well as nonbiological samples such as solutions, colloids, and particle suspension could serve as spots of nanoarray. The advantages of Nanoarrays over classic microarrays are manifold. Nanoarrays take only 1/10000 of the surface area utilized by a classic microarray system and over 1500 nanoarray spots could be embedded in the space required for a single microarray spot [36]. For instance, one nanoarray system imprints biological and nonbiological sensors onto silicon chips with ultra-micro spot sizes ranging from 1 to 20 μm and in the nanometer range to 250 nm. This distinctive advantage translate to economized reagent costs, expedited analysis and potentially greater specificity and sensitivity [37].

Nanoarrays are employed for biomolecular analysis in personal healthcare. These arrays exhibit higher sensitivity and selectivity and hence are potent in detecting pathogens even at trace quantities [38]. They are applied in bioaffinity tests for identifying DNA/RNA targets, proteins and receptor-ligand bindings. They are of great use in attaching diverse chemical and biological moieties, biomolecular materials and specific molecular segments such as single-stranded DNA for hybdridization [39-44]. IL-6 is considered as a crucial bioprognostic marker in prostate cancer and nanoarrays constructed on amine-reactive surface using antibodies against IL-6 and PSA were potentially beneficial in capturing their cognate antigen with greater specificity and sensitivity (10 pg/ml). In addition to serum PSA, nanoarrays are found equally efficient in detecting cellular PSA [45].

Biochips such as protein or antibody arrays seem to be a reliable approach for detecting disease markers at smaller quantities, which is of most helpful for early and accurate diagnosis of diseases, examining disease progression and scanning drug responses. Thus, this approach fosters to understand the functional and molecular characterization of diseases [46, 47]. Further, the excellence of nanoarrays in depicting tumor subtypes help to establish new and effective treatment strategies to treat tumors of different stage and nature [48-56].

The ultimate aim of molecular diagnostics involves single cell analysis. Nanoarrays are potentially useful to differentiate healthy cells from diseased cells at a single-cell resolution scale. Such ultra-high sensitivity aids to detect minor differences among cell types or debilitated effects of therapeutics, which might not be feasible with classical biochemical techniques. Thus, nanoarrays are potentially

beneficial in analyzing cell mixtures. Another great advantage of nanoarrays lies in its ability to detect targeted species by measuring atomic vibrational frequency, without labeling. This sort of label-free detection is considered highly beneficial so that biological activity of the target molecule remains intact for subsequent analysis.

NANOCHIPS : NANOBIOTECHNOLOGY ON A CHIP

The classical analysis of DNA sequencing is performed by hybridization or by probing with the complementary strands of known sequence. However, the major demerits of these techniques are non-specific hybridization of DNA. Thus, there is a need to construct a system that can eliminate non-specific DNA bindings. Nanogen's nanotechnology-based chips promise here superior alternatives to conventional DNA sequencing strategies. The chips are integrated with electronic current and DNA probes are separated on the basis of charge and size, to appropriate locations on the chip. The DNA probes hybridize with the cognate DNA present in the sample and the fluorescence emitted by hybridized DNA is detected and transmitted to an onboard computer by means of platinum wiring, installed in the chip. The salient feature of this device is that the test sites are electronically governable through an onboard computer. Also, the chip facilitates the simultaneous placement of multiple probes at distinct locations according to the sequence of interest, which makes the diagnosis process expedited and easier.

MICRO-ELECTROMECHANICAL SYSTEMS (MEMS)

Microfluidic and microcantilever devices are micro-electromechanical (MEM) systems fabricated using nanotechnology for in vitro diagnostics.

MICROFLUIDICS (LAB-ON-A CHIP)

Microfluidics represent an another contemporary technologies involve manipulating and controlling fluids and particles such as protein, DNA, cells, viruses, etc. at micrometer and sub micrometer dimensions based on the particle's electro kinetic properties including size, density, charge, light scattering and antigenic properties. These chips are easy to use, as the entire procedure of DNA analysis is integrated into a single chip comprised of a glass and silicon substrate. The chip contains micro fabricated fluidic chambers, heating systems, temperature sensors and fluorescence monitors to examine DNA specimens of nanolitre size [57]. The potential applications of microfluidics involve monitoring allergic response. To monitor this, cells are cultivated in the chamber of a chip and the fluorescence tagged dyes released upon allergic stimulation were detected by virtue of photomultiplier tube (PMT) connected with microscope [58].

Recently it was demonstrated that microfluidic systems could be fabricated to analyze the composition of cells, however, the samples analyzed need to have fluidity [59]. One of the most promising applications of microfluidic devices is in point-of-care diagnosis, for instance, in detecting bacterial infections in the mouth [60]. Microfluidic based techniques are cost efficient, fast, highly sensitive and require minimal sample volume. Inclusion of materials such as poly dimethylsiloxane in microfluidic techniques implies additional advantages, as these materials are of biocompatible and provoke less endotoxin contamination and complement activation [61-63]. These devices were shown to have potential in capturing substantial amount of cancer cells from cell mixture. Early and accurate detection of PSA (Prostate Specific Antigen) is of great significance to distinguish prostate cancer from other benign prostatic states. The lack of specificity of the serum marker PSA is a solemn concern since its elevated level could be linked to several abnormalities. Microfluidic chips offer possible solution to overcome the drawbacks. Further, they are also playing vital part in monitoring allergic responses [63, 64].

MICROCANTILEVERS

Micro cantilevers sensors function through measuring variations in cantilever bending or vibration spectrum. These sensors are known to have numerous merits such as minimal sample requirement, immense sensitivity, low cost, precise testing procedure and rapid response. In this sense, they turned out to be great use to detect disease markers, blood glucose level and chemical and biological warfare agents. Their potential use as biological sensor has been recently demonstrated by Arun Majumdar and co-workers in the diagnosis of prostate cancer [65]. In this approach, PSA specific anitbodies were coated on microcantilever's surface. The cantilever's

bending due to antigen-antibody interaction was detected optically using a photo detector. The microcantilever's extra sensitiveness in detecting PSA even at lower concentrations holds the key to future diagnostic applications.

In an other approach, blood glucose concentration was measured through immobilizing the enzyme so called glucose oxidase on microcantilever's surface [66]. Due to microcantilever's efficiency in surface stress measurements, they are useful in detecting low-density lipoprotein (LDL) and oxidized LDL (oxLDL). This is of great significance as oxLDL is correlated to cholesterol deposition in aorta, a pivotal step in coronary heart disease.

Single nucleotide polymorphisms (SNPs) are key areas of interest in genomics research. Several genetic abnormalities such as Tay Sachs syndrome, β- Thalassemia and Alzheimer's disease are evoked by single base mutation. Hence, detecting SNPs is of most helpful for early diagnosis of such abnormalities and Microcantilevers are of great benefit to capture DNA targets and detects point mutations.

Nanocantilevers: Nanocantilevers are the next generation tools in the evolution of cantilevers. Harold Craighead and co-workers demonstrated the practicability of employing 90 nm thick, silicon nitride nanocantilevers to distinguish DNA strand of 1578 base pairs. These nanocantilevers potentially eliminate the amplification step as it could accurately detect 0.23 attograms (1 attogram = 10^{-18} gram) of molecules [67]. Thus, these nanodevices have great impact to make molecular diagnosis procedures simplified.

NANOPORES

A nanopore is a nano-scale aperture found in membranes, which permits DNA molecules to penetrate through. This nanoaperture could be of biological (protein pores in lipid bilayers), solid state (fabricated in artificial membranes, such as graphene) or hybrid (pore-forming protein fabricated in artificial membranes) type. Nanopores serve as single-molecule analytical devices and the operating principle of this nanopore technique corresponds to that of a Coulter counter [68]. The nanopore is made in the membrane bifurcating the electrochemical chambers containing conductive electrolyte. Under the influence of an applied electric field, the charged molecules are galvanized through the aperture. The resultant changes in ionic current provoke details on the structural features and dynamic movements of the molecules. Nanopore analysis is of great use as it could help to analyze charged polymers such as single and double stranded DNA and RNA at sub nanometer resolution and eliminating the necessity of amplification, chemical modification, surface adsorption and probe hybridization Church et al., first demonstrated the real potential of nanopore analysis in the process of DNA sequencing [69].

Biological nanopores, as naturally occurring nanopores, are of great use in single-molecule DNA analysis due to their abundant nature with remarkable heterogeneity in terms of size and composition. For instance, α-haemolysin, a heptameric transmembrane nanopore has been extensively employed in DNA sequencing. This nanopore comprises 3.6 nm diameter vestibule coupled to transmembrane β-barrel of ~5 nm long and ~2.6 nm wide. Owing to the nature of the pore size, α-haemolysin only permits the translocation of single-stranded DNA (ssDNA). Translocation of ssDNA and ssRNA molecules through α-haemolysin was first reported by Kasianowicz *et al* [70]. Further native α-haemolysin has been employed to differentiate between RNA homopolymers made of adenylic and cytidylic acid [71] and ssDNA of deoxyadenylic acid and deoxycytidylic acid [72].

MspA (*Mycobacterium smegmatis* porin A) is another well-studied octameric channel pore which contains a single constriction of ~ 0.6 nm long and ~1.2 nm wide. It can distinguish trinucleotide sets of AAA, GGG, TTT and CCC at greater efficiency [73]. Similarly, the bacteriophage Phi29 DNA-packaging nanomotor (dodecamer) serves as a channel for the transit of DNA double-strands. The channel size of phi29 connector not only permits the translocation of ssDNA and dsDNA but it also allows small peptides and proteins. Owing to its bigger size, channel modifications such as insertion of chemical groups could be easily achieved which in turn enhances the efficiency of sensing and diagnosis process.

Solid-state nanopores possess outstanding characteristics such as well-confined geometries, sound mechanical stability, perfect sensitivity and exceptional compatibility with other analytical techniques and serve as a versatile alternative to biological nanopores [74-76]. They hold great promise for DNA sequencing and bar coding, characterizing protein interactions and detecting molecular transport such as individual DNA/RNA molecules and ions [77-80]. Recently, Graphene, a two-dimensional layer of carbon atoms, becomes an exceptional choice for single molecule detection and DNA sequencing processes by virtue of its remarkable mechanical, optical, electrical, magnetic and thermal characteristics [81]. Of all features, its sub-nm thickness is quite attractive as the thickness

corresponds to spaces among nucleotides of single-stranded DNA. This standout characteristic makes graphene nanopore an excellent tool for nucleic acid analysis and DNA sequencing.

DNA hypermethylation serves as a valuable biomarker in diseases such as cancer, lupus and muscular dystrophy. Synthetic nanopores have tremendous potential in detecting abnormal DNA methylation [82]. Given the crucial role of single nucleotide polymorphisms (SNPs) in causing phenotypic differences among individuals, it seems apparent that SNPs play significant part in tumor progression. Owing to its DNA sensing ability, nanopores might be able to distinguish the SNPs and thereby facilitate early diagnosis of malignancies. Asides, nanopore analysis has also been used to analyze RNA/Antibiotic complexes at single molecular level suggesting its potential in diagnosing RNA-mediated diseases [78].

CONCLUSION AND FUTURE PERSPECTIVES

Nanomolecular diagnostics have the potential to conquer many of the challenges associated with conventional molecular diagnostics, in spite of its intrinsic features such as facile procedures, miniaturized devices, expedited and high-throughput screening processes. Since the field of nanomolecular diagnosis is presently burgeoning expediously, this review presented an overview of some enticing progresses. Nanomolecular diagnostics have incredible potential to analyze single cells with high sensitivity. This notable proficiency of nanomolecular diagnostics opens completely new perspectives for cancer detection. Nanoparticles, quantum dot and nanoclusters enhance the resolution and sensitivity of imaging techniques by acting as contrast agents. Q-dots offer the possibility to detect low abundance antigens. Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) is a crucial mediator of cystic fibrosiss (CF) and quantum dots labeled anti CFTR antibodies are employed in AFM to detect and quantify CFTR.

Other promising candidates in this field are microfluidic chips, which are of immense use in isolating and manipulating living cells and diagnose inflammatory processes at early stage. Furthermore, biochip with an anisotropic nanofluidic sieving structure serves as an appropriate tool to isolate and categorize biological molecules such as DNAs or peptides. Modification of biochip surfaces using nanotechnological methods makes them more effective in RNA analysis at the single-cell level. Antibody arrays offer an attractive tool for characterizing cancer subtypes and thus, establishing molecular basis of diseases.

Nanosensors have emerged as a versatile alternative to conventional biosensors. Cantilever based sensors can be employed for the effective measurement of various disease markers such as myoglobin, glucose and lipoproteins. Silicone nanowire sensors show potential in the rapid and effective detection of influenza A virus. The use of nanopores in the detection of cancer biomarkers is of paramount significance as it facilitates early diagnosis, staging, advancement and most significantly chemotherapeutic drug response.

The applications described here suggest that nanodiagnostics have been stepping closer to the goal of accurate diagnosis of various life-threatening diseases in the early stages. Without doubt nanodiagnostics will make tremendous strides and wide ranging impact in the field of molecular diagnosis and lay the groundwork for the establishment of novel therapeutics and personalized medicine (PM) for various diseases.

REFERENCES:

[1] Fakruddin, M., Hossain, Z. & Afroz, H., "Prospects and applications of nanobiotechnology: a medical perspective", J Nanobiotechnology, 10, 31, 2012.
[2] Moghimi, S.M., Hunter, A.C. & Murray, J.C., "Nanomedicine: current status and future prospects", FASEB J, 19, 311-330, 2005.
[3] Ries, L.H., D.; Krapcho, M.; Mariotto, A.; Miller, BA.; Feuer, EJ.; Clegg, L.; Eisner, & MP.; Horner, M.H., N.; Hayat, M.; Hankey, BF.; Edwards, BK., editors. "SEER Cancer Statistics Review", National Cancer Institute; Bethesda, MD, 1975–2003, 2004.
[4] Jain, K.K., "Nanodiagnostics: application of nanotechnology in molecular diagnostics", Expert Rev Mol Diagn, 3, 153-161, 2003.
[5] Azzazy, H.M., Mansour, M.M. & Kazmierczak, S.C., "Nanodiagnostics: a new frontier for clinical laboratory medicine", Clin Chem, 52, 1238-1246, 2006.
[6] Riehemann, K. et al., "Nanomedicine--challenge and perspectives", Angew Chem Int Ed Engl, 48, 872-897, 2009.
[7] Liu, C.H. et al., "Noninvasive delivery of gene targeting probes to live brains for transcription MRI", FASEB J, 22, 1193-1203, 2008.
[8] Liu, C.H. et al., "MR contrast probes that trace gene transcripts for cerebral ischemia in live animals", FASEB J, 21, 3004-3015,

2007.
[9] Qian, H., Zhu, M., Wu, Z. & Jin, R., "Quantum sized gold nanoclusters with atomic precision", Acc Chem Res, 45, 1470-1479, 2012.
[10] Sperling, R.A., Rivera Gil, P., Zhang, F., Zanella, M. & Parak, W.J., "Biological applications of gold nanoparticles", Chem Soc Rev, 37, 1896-1908, 2008.
[11] Yang, X., Stein, E.W., Ashkenazi, S. & Wang, L.V., "Nanoparticles for photoacoustic imaging", Wiley Interdiscip Rev Nanomed Nanobiotechnol, 1, 360-368, 2009.
[12] Feis, A., Gellini, C., Salvi, P.R. & Becucci, M., "Photoacoustic excitation profiles of gold nanoparticles", Photoacoustics, 2, 47-53, 2014.
[13] Schultz, D.A., "Plasmon resonant particles for biological detection", Curr Opin Biotechnol, 14, 13-22, 2003.
[14] Hainfeld, J.F., Slatkin, D.N., Focella, T.M. & Smilowitz, H.M., "Gold nanoparticles: a new X-ray contrast agent", Br J Radiol, 79, 248-253, 2006.
[15] Cheng, H.W., Huan, S.Y., Wu, H.L., Shen, G.L. & Yu, R.Q., "Surface-enhanced Raman spectroscopic detection of a bacteria biomarker using gold nanoparticle immobilized substrates", Anal Chem, 81, 9902-9912, 2009.
[16] Heuer-Jungemann, A., Harimech, P.K., Brown, T. & Kanaras, A.G., "Gold nanoparticles and fluorescently-labelled DNA as a platform for biological sensing", Nanoscale, 5, 9503-9510, 2013.
[17] Halo, T.L. et al., "NanoFlares for the detection, isolation, and culture of live tumor cells from human blood", Proceedings of the National Academy of Sciences, 111, 17104-17109, 2014.
[18] Chan, W.C. et al., "Luminescent quantum dots for multiplexed biological detection and imaging", Curr Opin Biotechnol, 13, 40-46, 2002.
[19] Michalet, X. et al., "Quantum dots for live cells, in vivo imaging, and diagnostics", Science, 307, 538-544, 2005.
[20] Larson, D.R. et al., "Water-soluble quantum dots for multiphoton fluorescence imaging in vivo", Science, 300, 1434-1436, 2003.
[21] Ness, J.M., Akhtar, R.S., Latham, C.B. & Roth, K.A., "Combined tyramide signal amplification and quantum dots for sensitive and photostable immunofluorescence detection", J Histochem Cytochem, 51, 981-987, 2003.
[22] Wegner, K.D. et al., "Nanobodies and nanocrystals: highly sensitive quantum dot-based homogeneous FRET immunoassay for serum-based EGFR detection", Small, 10, 734-740, 2014.
[23] Yang, L. & Li, Y., "Quantum dots as fluorescent labels for quantitative detection of Salmonella typhimurium in chicken carcass wash water", J Food Prot, 68, 1241-1245, 2005.
[24] Tully, E., Hearty, S., Leonard, P. & O'Kennedy, R., "The development of rapid fluorescence-based immunoassays, using quantum dot-labelled antibodies for the detection of Listeria monocytogenes cell surface proteins", Int J Biol Macromol, 39, 127-134, 2006.
[25] Xing, Y. et al., "Bioconjugated quantum dots for multiplexed and quantitative immunohistochemistry", Nat Protoc, 2, 1152-1165, 2007.
[26] Tholouli, E. et al., "Quantum dots light up pathology", J Pathol, 216, 275-285, 2008.
[27] Howles, G.P., Ghaghada, K.B., Qi, Y., Mukundan, S., Jr. & Johnson, G.A., "High-resolution magnetic resonance angiography in the mouse using a nanoparticle blood-pool contrast agent", Magn Reson Med, 62, 1447-1456, 2009.
[28] Thorek, D.L., Chen, A.K., Czupryna, J. & Tsourkas, A., "Superparamagnetic iron oxide nanoparticle probes for molecular imaging", Ann Biomed Eng, 34, 23-38, 2006.
[29] Corot, C., Robert, P., Idee, J.M. & Port, M., "Recent advances in iron oxide nanocrystal technology for medical imaging", Adv Drug Deliv Rev, 58, 1471-1504, 2006.
[30] Triantafyllou, M. et al., "Diffusion-weighted MRI to detect pelvic lymph node metastases in patients with bladder or prostate cancer: comparison with histopathology as gold standard", Proceedings of the ISMRM, Hawaii, 2009.
[31] Fortina, P. et al., "Applications of nanoparticles to diagnostics and therapeutics in colorectal cancer", Trends Biotechnol, 25, 145-152, 2007.
[32] Hill, J.M. et al., "Serial cardiac magnetic resonance imaging of injected mesenchymal stem cells", Circulation, 108, 1009-1014, 2003.
[33] Perez, J.M., Josephson, L., O'Loughlin, T., Hogemann, D. & Weissleder, R., "Magnetic relaxation switches capable of sensing molecular interactions", Nat Biotechnol, 20, 816-820, 2002.
[34] Lee, H., Sun, E., Ham, D. & Weissleder, R., "Chip-NMR biosensor for detection and molecular analysis of cells", Nat Med, 14, 869-874, 2008.
[35] Lee, J.H. et al., "Artificially engineered magnetic nanoparticles for ultra-sensitive molecular imaging", Nat Med, 13, 95-99, 2007.
[36] Lynch, M. et al., "Functional protein nanoarrays for biomarker profiling", Proteomics, 4, 1695-1702, 2004.
[37] Silzel, J.W., Cercek, B., Dodson, C., Tsay, T. & Obremski, R.J., "Mass-sensing, multianalyte microarray immunoassay with imaging detection", Clin Chem, 44, 2036-2043, 1998.
[38] Chen, H. & Li, J., "Nanotechnology: moving from microarrays toward nanoarrays", Methods Mol Biol, 381, 411-436, 2007.
[39] Maurya, D.K., Ng, W.Y., Mahabadi, K.A., Liang, Y.N. & Rodriguez, I., "Fabrication of lab-on chip platforms by hot embossing and photo patterning", Biotechnol J, 2, 1381-1388, 2007.

[40] Truskett, V.N. & Watts, M.P., "Trends in imprint lithography for biological applications", Trends Biotechnol, 24, 312-317, 2006.
[41] Lee, K.B., Lim, J.H. & Mirkin, C.A., "Protein nanostructures formed via direct-write dip-pen nanolithography", J Am Chem Soc, 125, 5588-5589, 2003.
[42] Bruckbauer, A. et al., "Multicomponent submicron features of biomolecules created by voltage controlled deposition from a nanopipet", J Am Chem Soc, 125, 9834-9839, 2003.
[43] Zimmermann, J., Rabe, M., Verdes, D. & Seeger, S., "Functionalized silicone nanofilaments: a novel material for selective protein enrichment", Langmuir, 24, 1053-1057, 2008.
[44] Geho, D. et al., "Fractionation of serum components using nanoporous substrates", Bioconjug Chem, 17, 654-661, 2006.
[45] Nettikadan, S. et al., "Detection and quantification of protein biomarkers from fewer than 10 cells", Mol Cell Proteomics, 5, 895-901, 2006.
[46] Lin, Y. et al., "Profiling of human cytokines in healthy individuals with vitamin E supplementation by antibody array", Cancer Lett, 187, 17-24, 2002.
[47] Zhu, X., Gerstein, M. & Snyder, M., "ProCAT: a data analysis approach for protein microarrays", Genome Biol, 7, R110, 2006.
[48] Usui-Aoki, K., Shimada, K. & Koga, H., "A novel antibody microarray format using non-covalent antibody immobilization with chemiluminescent detection", Mol Biosyst, 3, 36-42, 2007.
[49] Kato, K., Toda, M. & Iwata, H., "Antibody arrays for quantitative immunophenotyping", Biomaterials, 28, 1289-1297, 2007.
[50] Nedelkov, D., Tubbs, K.A. & Nelson, R.W., "Surface plasmon resonance-enabled mass spectrometry arrays", Electrophoresis, 27, 3671-3675, 2006.
[51] Koga, H., Kyo, M., Usui-Aoki, K. & Inamori, K., "A chip-based miniaturized format for protein-expression profiling: the exploitation of comprehensively produced antibodies", Electrophoresis, 27, 3676-3683, 2006.
[52] Sanchez-Carbayo, M., "Antibody arrays: technical considerations and clinical applications in cancer", Clin Chem, 52, 1651-1659, 2006.
[53] Watanabe, M. et al., "Antibody array analysis of peripheral and blood cytokine levels in rats after masseter inflammation", Neurosci Lett, 382, 128-133, 2005.
[54] Haab, B.B., "Antibody arrays in cancer research", Mol Cell Proteomics, 4, 377-383, 2005.
[55] Ivanov, S.S. et al., "Antibodies immobilized as arrays to profile protein post-translational modifications in mammalian cells", Mol Cell Proteomics, 3, 788-795, 2004.
[56] Lal, S.P., Christopherson, R.I. & dos Remedios, C.G., "Antibody arrays: an embryonic but rapidly growing technology", Drug Discov Today, 7, S143-149, 2002.
[57] Mueller, O. et al., "A microfluidic system for high-speed reproducible DNA sizing and quantitation", Electrophoresis, 21, 128-134, 2000.
[58] Matsubara, Y., Murakami, Y., Kobayashi, M., Morita, Y. & Tamiya, E., "Application of on-chip cell cultures for the detection of allergic response", Biosens Bioelectron, 19, 741-747, 2004.
[59] Walt, D.R., "Chemistry. Miniature analytical methods for medical diagnostics", Science, 308, 217-219, 2005.
[60] Chen, Z. et al., "A microfluidic system for saliva-based detection of infectious diseases", Ann N Y Acad Sci, 1098, 429-436, 2007.
[61] Vickers, J.A., Caulum, M.M. & Henry, C.S., "Generation of hydrophilic poly(dimethylsiloxane) for high-performance microchip electrophoresis", Anal Chem, 78, 7446-7452, 2006.
[62] Takayama, S. et al., "Subcellular positioning of small molecules", Nature, 411, 1016, 2001.
[63] Du, Z., Colls, N., Cheng, K.H., Vaughn, M.W. & Gollahon, L., "Microfluidic-based diagnostics for cervical cancer cells", Biosens Bioelectron, 21, 1991-1995, 2006.
[64] Majumdar, A., "Bioassays based on molecular nanomechanics", Dis Markers, 18, 167-174, 2002.
[65] Wu, G. et al., "Bioassay of prostate-specific antigen (PSA) using microcantilevers", Nat Biotechnol, 19, 856-860, 2001.
[66] Chen, G.Y., Thundat, T., Wachter, E.A. & Warmack, R.J., "Adsorption-induced surface stress and its effects on resonance frequency of microcantilevers", Journal of Applied Physics, 77, 3618-3622, 1995.
[67] Ilic, B. et al., "Enumeration of DNA molecules bound to a nanomechanical oscillator", Nano Lett, 5, 925-929, 2005.
[68] Hu, J. & Zhe, J. (Google Patents, 2008).
[69] Church, G., Deamer, D. W., Branton, D., Baldarelli, R. & Kasianowicz, J., "Characterization of individual polymer molecules based on monomer-interface interactions.", US patent 5,795,782, 1995.
[70] Kasianowicz, J.J., Brandin, E., Branton, D. & Deamer, D. W., "Characterization of individual polynucleotide molecules using a membrane channel", Proc. Natl Acad. Sci. USA, 93, 13770–13773, 1996.
[71] Akeson, M., Branton, D., Kasianowicz, J.J., Brandin, E. & Deamer, D.W., "Microsecond time-scale discrimination among polycytidylic acid, polyadenylic acid, and polyuridylic acid as homopolymers or as segments within single RNA molecules", Biophys J, 77, 3227-3233, 1999.
[72] Meller, A. & Branton, D., "Single molecule measurements of DNA transport through a nanopore", Electrophoresis, 23, 2583-2591, 2002.
[73] Derrington, I.M. et al., "Nanopore DNA sequencing with MspA", Proc Natl Acad Sci U S A, 107, 16060-16065, 2010.

[74] Kim, M.J., Wanunu, M., Bell, D. C. & Meller, A., "Rapid fabrication of uniformly sized nanopores and nanopore arrays for parallel DNA analysis", Adv. Mater., 18, 3149–3153 2006.
[75] Nam, S.W., Rooks, M.J., Kim, K.B. & Rossnagel, S.M., "Ionic field effect transistors with sub-10 nm multiple nanopores", Nano Lett, 9, 2044-2048, 2009.
[76] McNally, B. et al., "Optical recognition of converted DNA nucleotides for single-molecule DNA sequencing using nanopore arrays", Nano Lett, 10, 2237-2244, 2010.
[77] Schneider, G.F. et al., "DNA translocation through graphene nanopores", Nano Lett, 10, 3163-3167, 2010.
[78] Wanunu, M. et al., "Nanopore analysis of individual RNA/antibiotic complexes", ACS Nano, 5, 9345-9353, 2011.
[79] Haque, F., Li, J., Wu, H.-C., Liang, X.-J. & Guo, P., "Solid-state and biological nanopore for real-time sensing of single chemical and sequencing of DNA", Nano Today, 8, 56-74, 2013.
[80] Atas, E., Singer, A. & Meller, A., "DNA sequencing and bar-coding using solid-state nanopores", Electrophoresis, 33, 3437-3447, 2012.
[81] Geim, A.K., "Graphene: status and prospects", Science, 324, 1530-1534, 2009.
[82] Mirsaidov, U. et al., "Nanoelectromechanics of methylated DNA in a synthetic nanopore", Biophys J, 96, L32-34, 2009.