Chapter 6

Nanotechnology-Based Rapid Diagnostic Tests

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Additional information is available at the end of the chapter

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Abstract

Recently, various nanomaterials are used in order to develop nanotechnology-based rapid diagnostic tests, such as metallic nanoparticles, quantum dots (QDs), silica nanospheres, magnetic nanoparticles, carbon nanotubes (CNTs), silicon nanowires (SiNWs), nanopores, graphene, nanostructured surfaces, and metal films. This novel nanodiagnostic approach will further develop point-of-care (POC) diagnostics and monitoring technologies. Nanobiosensors and microarrays of biosensors can create biochip systems and microfluidic platforms that are the most used nanofabrications for rapid diagnostic tests. These nanoplatforms are constructed for the rapid detection of various diseases or pathogen-specific biomolecules/markers, such as DNA, proteins, whole cells (e.g., circulating tumor cells), and others. The fabrication of small-scale portable devices with the incorporation of nanostructures will offer many advantages in the early detection of various diseases and health-threatening infections by pathogens and in the treatment selection and treatment monitoring. The use of nanostructures in *in vitro* diagnostics gives the opportunity to augment the sensitivity and specificity required in clinical practice, lowers the cost and test time of the assays, and enables portable microfluidic platforms suitable for resource-constrained settings. In this chapter, all the state-of-the-art advantages in this field are discussed, starting with the nanostructures used for the fabrication of nanobiosensors, nanobiosensors arrays, and nanofluidic platforms and the nanodiagnostic use of rapid tests in the detection of pathogens, in cancer management, and glucose monitoring for the management of diabetes disease.

Keywords: Nanotechnology, Nanostructures, point-of-care devices, nanodiagnostics, low-cost and rapid diagnosis

1. Introduction

Nanostructures are used in order to create specific nanodevices for the manipulation of biological systems at the molecular level, and this is what currently defines *nanomedicine*. So far, the

integration of nanoparticles with biology has led to the development of diagnostic devices, contrast agents, advanced therapy applications, drug delivery therapy, and imaging approaches. Nanomedicine offers many advantages in everyday clinical practice, taking into consideration the non-invasive approach of the samples used, fast reaction times, specificity, and sensitivity that nanoparticles can offer. Therein, these advantages will lead us closer to the construction of point-of-care mobile nanodevices. In the context of *in vitro* nanodiagnostics, nanotechnology allows the construction of novel sensors and *in vitro* tests, in order to improve the sensitivity of existing tests, to develop new diagnostic test platforms, and to allow point-of-care applications. Thus, *Nanodiagnostics* is defined as the application of nanotechnology for the diagnosis of a dysfunction and/or disease in human, at the earliest stage possible, ideally at the level of a single cell. To achieve this goal, various types of nanotechnologies are currently being explored for use in nanodiagnostic applications. Furthermore, an increase in knowledge in basic research will be seen through the development of microscopic and spectroscopic techniques towards ultrahigh spatial resolution, molecular resolution, and ultrahigh sensitivity that will also help in the creation of advanced *in situ* diagnostics tools. More specifically, nanotechnology-based diagnosis techniques offer great opportunities such as

1. Rapid diagnostic test, potentially in the doctor's office or bedside tests, for the initial diagnosis and treatment selection and treatment monitoring to the doctor/hospital or even at home.

2. The early detection of several diseases in comparison with the efficiency of current techniques. The early detection is very important as it offers the opportunity for earlier diagnosis and thus more therapeutic opportunities.

This chapter summarizes the nanostructures used in nanodiagnostic tests and their applications for the development of rapid diagnostic tests for point-of-care disease management and public health.

2. Nanostructures used in nanodiagnostics

The term *nanostructures* includes materials of <100 nm in size in at least one dimension. There are nanostructures in zero-dimensional (0D), one-dimensional, (1D), and two-dimensional (2D) systems. The dimensions of nanostructures are advantageous for use in diagnostics because they are in the range of the size of various biomolecules such as nucleic acids, small proteins, and viruses. An *in vitro* diagnostic tool is always comprised of an element that is able to identify a biochemical change, activity or concentration of a specific molecule of biological importance in the solution of interest. In a nanobiosensor, a transducer is used to convert this biochemical signal into a quantifiable signal.

The use of nanomaterials in the design of *in vitro* diagnostic systems is offering types of sensors that are characterized by specificity, sensitivity, and robustness. Several kinds of nanomaterials have found attractive applications in *in vitro* diagnostic tests, such as metallic nanoparticles, quantum dots (QDs), silica nanospheres, magnetic nanoparticles, which belong to the

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zero-dimensional (0D) systems, carbon nanotubes (CNTs), silicon nanowires (SiNWs), nanopores, which belong to the one-dimensional (1D) systems, and graphene, nanostructured surfaces, and metal films, which belong to the two-dimensional (2D) systems.

2.1. Metallic NPs

Metals, especially gold and silver, have the advantageous ability to interact with external fields such as light, radiofrequency, and X-rays. Under a specific wavelength, metals exhibit surface plasmon resonance (SPR), the oscillation of free electrons in a particle's surface; thus, they can successfully be combined with conventional methods such as colorimetry or absorption spectroscopy. A typical example of SPR biosensing consists of the liquid sample part and an immobilized ligand (e.g., an antibody) on an SPR-active gold-coated glass slide. This system can create a thin flow cell in which the sample will be able to flow in the aqueous solution, and when light (visible or near infrared) is projected through the glass slide and onto the gold surface at angles and wavelengths near the SPR condition, the optical reflectivity of the gold changes in a specific way when an actual interaction occurs between the sample and the ligand of the solid phase. The most frequent medical use of these NPs is the rapid tests, for example, pregnancy test kits, where gold nanoparticles are used as a color marker [1]. Moreover, metallic NPs are suitable for surface-enhanced Raman spectroscopy (SERS), since they produce Raman signal. So, when molecules are in close proximity to a metal surface, they exhibit a dramatic augmentation in the electromagnetic field, yielding high Raman intensity. Thus, SERS surface biosensors are usually performed on Ag, Au, or Cu surfaces. SERS is an excellent assay for the sensitive and specific detection of low-concentration molecules, for example, the detection of biomarkers for bacillus spores or the measurement of glucose after the appropriate chemical modifications of the SERS surfaces [2]. Another example is the molecular sentinels, which are comprised of metal NPs decorated with a Raman label-conjugated stem-loop DNA. Thus, when the DNA molecule is in close proximity to the metal surface, the Raman intensity is maintained high. In contrast, in the bound state, there is a disruption of the stem-loop and the Raman label is no longer in close proximity to the metal. This approach was used to detect the *gag* gene of the HIV-1 in PCR amplicons [3] and several single-nucleotide polymorphisms (SNPs) such as *BRCA1* gene of breast cancer, using plasmonic nanoprobes that detach from the Raman tag when the conjugated oligonucleotide is hybridized, thus decreasing the plasmonic effect and change the SERS [4].

2.2. Quantum dots (QDs)

In the field of *in vitro* applications, nanoparticles are mainly used as markers for biomolecules, since they have many optical advantages that make them suitable for several diagnostic assays such as PCR, for the construction of biochips or suitable for multiplexing, conversely to the traditional dyes used in every day clinical practice. To this end, inorganic fluorescent nanoparticles are being investigated such as semiconductor nanoparticles (quantum dots) or nanoparticles-like nanophopsphors, resulting in an increase in sensitivity and specificity and the possible analysis of multiple analytes that offer opportunities of mass production [5].

Quantum dots, semiconductor nanocrystals coated with inorganic materials, are also currently used in the field of basic research of cell biology, and their use in clinical diagnostic tests is already under investigation with great progress as markers, especially in image-guided techniques, and at the same time, some nanobiochip platforms are already in the market. More importantly, QDs are very efficient donors of energy compared to traditional organic dyes, especially dye acceptors in FRET-based assays (fluorescence resonance energy transfer) [6].

2.3. Silica nanospheres

Just like QDs, inorganic dye-loaded silica particles are characterized by good photostability, sharp emission peaks, and long-lasting fluorescence lifetimes. They are appropriate for dispersion aqueous solutions, due to their hydrophilic surface. They are usually used to conjugate optical labels in order to increase the detection signal, such as organic or inorganic dye molecules (lanthanide-based and ruthenium-based) [7].

2.4. Magnetic NPs

Last but not least is the use of *supermagnetic iron oxide nanoparticles (SPIOs)*, which are used for magnetic separation in several immunomagnetic applications such as cell sorting, nucleic acid extraction, purification, and detection of pathogens, cancer cells, and generally rare populations in a solution/sample [8].

2.5. Carbon nanotubes or nanopores (CNTs–CNPs)

There are a variety of challenges associated with the flow of liquids through carbon *nanotubes* and *nanopores*. These are small electrically insulated tubes or pores which can detect a single molecule when this passes through the tube or pore. The molecule's detection is based on the change of the ionic current of the electrolyte solution containing the molecules of interest, which results in a change of the electrical current (translocation event signal) [7]. The incorporation of biochips and nanofluidics with nanopores or nanotubes will be able to replace the existing sequencing approaches of DNA in the clinical practice, as each DNA base has unique molecular structure and thus a unique translocation event signal. To this aim, nanofluidic devices are developed that employ multiple measurements on single molecules to enhance the ability to size DNA molecules. Techniques to integrate membranes contain nanopores into microfluidic devices, which decrease noise and enable the design of networks containing nanopores.

2.6. Silicon nanowires (SiNWs)

Nanowires are currently believed to be unique and advantageous for the construction of a nanobiosensing device. Nanowires are nanoscale channels through which current is passed and can be constructed from carbon nanotubes, metal oxides or silicon, and they require high temperatures to be synthesized and are usually prepared on silicon wafers. Antibodies are

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usually used as detectors in the surface of the nanowire. Antibodies interact with the biological target of interest, and the conformational change results in a change in the current that passes through the nanowire, allowing a sensitive and specific detection. The use of nanowires in an array mode, where different antibodies are conjugated to each nanowire, allows the mass detection of different types of disease or the creation of a personalized molecular profile in one type of disease [7].

2.7. Graphene, metal films and nanostructured surfaces

Graphene, metal films, and nanostructured surfaces are all in the class of 2D nanostructures which are structures with one dimension of ~100 nm in size. Their incorporation in nanodiagnostic is their use as racks in order to conjugate and immobilize ligands for targeted binding when the sample comes across them. They usually are sheets of a certain nanomaterial, which have special properties different from that of the corresponding bulk material. For example, metal films exhibit the same advantages with metal NPs, for example, the SPR effect, and thus, they are used in the construction of label-free SPR biosensors. As for nanostructured surfaces, they are in reality electrodes with their surface linked with nanotubes or nanoparticles. Finally, graphene will offer great sensitivity in rapid diagnostic tests, since it is an incredibly stable one-layer 2D surface of carbon atoms with unique optical and conductive properties [7].

3. Nanotechnology-on-a-chip or nanofluidics

A nanobiochip is comprised of integrated biomolecules or biologically active artificial structures which are usually smaller than that of cell's. The chip contains microarrays, which are minitest sites, on a solid surface, and this allows for multiple tests to be carried out simultaneously. Therefore, identification of a specific molecular signature, which will be unique to the diagnosis, can be done through thousands of biochemical reactions being performed on the nanobiochip [9, 10].

The identification and quantification of a variety of molecules will be permitted through the combination of nanotechnologies, such as nanofluids and nanobiosensors, with biochips, and this will lead to the generation of future *in vitro* diagnostic chip-based devices. Nanofluidic structures have small fluidic conducts which means they are automatically applied in situations where you have extremely small quantities of the sample. This includes Coulter counting, analytical separations and determinations of biomolecules, such as proteins and DNA and facile handling of mass-limited samples. Lab-on-a-chip structures comprise one of the more promising tools of nanofluidics. The advance and production of lab-on-chip devices for PCR and other related techniques mean that nanofluidics have had a substantial impact in biotechnology, medicine, and clinical diagnostics. In nanofluidics, a chamber of up to a few hundred nanometers in size contains a liquid sample which is then manipulated and analyzed. As the volume of the samples are so small, this allows for a substantial reduction in the amount of sample needed for the analysis as well as allowing for the advantages of laminar

flow conditions, high surface to volume rations, low concentrations, molecular confinement, and low heat capacity to be used. On the other hand, nanofluidics also generates new challenges in the device design and manufacture, the accurate control of flow and mixing, and the sensitivity of molecular detection, and they are starting to be used in many diagnostic and analytical devices [10, 11].

An *in vitro* simple detection system can therefore be established, which allows hundreds of cantilever biosensors to be used simultaneously on the same array. A lab-on-a-chip device could be produced through additional advances of this technique which include the complete integration with fluidic handling system, other analytical techniques and signal extraction electronic. The initial proposition, by Berger et al., was for a "laboratory-on-a-tip" which described the potential combination of cantilever sensors with atomic force microscopy (AFM). In this proposed technique, a cantilever with a tip could examine and detect with nanometer resolution where the biochemical analysis is executed. Thus, in conclusion, an alternative PCR which has the potential to replace microarray detection techniques is offered through cantilever nanobiosensing for the identification of SNPs, oncogenes, viruses, bacteria, and a variety of other pathogens. Additionally, the most common *in vitro* application of SPR biosensor chip devices is defining the affinity parameters of biomolecular interactions, where a sensorgram is used to report the association and dissociation of the ligand and its binding partner, which is added once the ligand has been immobilized on the sensor chip. There are numerous advantages of using this technique in comparison with conventional methods for affinity measurement, and these include little material being required, it is very quick, and finally, no tracer is needed for labeling. The expanding field of proteomics has seen the most recent developments in SPR where it is combined with matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF), and this permits the study of biomolecular interactions between molecules of which at least one is known. The interacting molecule cannot always be identified when a variety of proteins is used within the interaction study. However, using the MALDI-TOF technique allows you to determine the molecule that interacts with the sensor from the mixture [10, 11].

4. Applications of nanotechnology in *in vitro* nanodiagnostics

Up to now, nanostructures are successfully incorporated to *in vitro* diagnostics for the construction of nanobiosensors and *in vitro* rapid diagnostic tests mainly in order to improve existing tests and make them more effective or create innovative diagnostic test approaches that will be able to be incorporated in point-of-care applications of every day clinical practice. Nanodiagnostic applications are currently focused on two main approaches, such as the use of nanoparticles as biomarkers and the development of novel nanosensors that incorporate various nanostructures, such as carbon nanotubes, lateral nanostructures or nanothin surface layers via labeled nanosensors or label-free nanosensors. With the use of *in vitro* diagnostic tests based on nanotechnology, various clinical and research fields will be improved such as genotyping techniques, immunohistochemistry assays, detection of

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biomarkers, early cancer detection, and others. Below, we are going to describe some of these applications.

4.1. Nanosensors for glucose monitoring

The management of diabetes disease, besides the great progress in the maintenance of insulin, is still intriguing. In the current clinical practice, diabetes patients need to tolerate tandem blood samples in order to monitor blood glucose and therefore minimize the possibility of hyper- or hypoglycemia, together with the aftermaths. Thus, it is still now an essential task to design a management approach for the monitoring of blood glucose that is non-invasive, fast, and sensitive. The use of nanotechnology for the fabrication of a rapid and portable diagnostic test offers the aforementioned advantages. However, it is difficult task to design the ideal test, since the ideal biosensor has to be small, low-cost, with simple function, accurate in the measurement and of course portable. Furthermore, this future test will have to minimize the blood volume needed for the test and the possible contaminations and to assure the accuracy of the measurement. Even further, an implantable microfluidics biosensor could provide a more accurate management of *in vivo* glucose monitoring and insulin administration. Barone et al. [12] used solution-phase optical single-walled CNTs (SWCNTs) sensors which have a tunable near-infrared (NIR) emission that responds to changes in the local dielectric function, but remains stable to permanent photobleaching. SWCNTs are integrated with beta-D-glucose sensing as a model system; for example, they are conjugated with glucose oxidase, an enzyme that degrades glucose molecules and ferricyanide a molecule that takes electrons from the NTs, quenching their capacity to glow under NIR. In order to test the *in vivo* capacity of the nanosensors, they implanted them in situ in a human skin tissue sample and excited with NIR. The results demonstrated that the higher the glucose concentration, the greater the fluorescence. Thus, the nanosensors were found able to modulate their emission in response to the adsorption of specific biomolecules, suggesting that nanoparticle optical sensors are an attractive solution for glucose monitoring [12]. However, such techniques that use glucose consumption turned out to have many disadvantages regarding the continuous glucose monitoring. Therefore, technique based on competitive affinity binding of glucose and subcutaneously implanted enzymatic electrochemical detection is being recently a very attractive approach, since they can be highly stable and low drift. For example, the use of a polysaccharide solution such as dextran conjugated to a glucose-binding protein such as concanavalin A (Con A), which competitively binds glucose leading to reversible de-cross linking of the dextran–Con A complex. In the unbound state, the changes in the fluorescence or viscosity of the solution can be detected. Alternatively, synthetic glucose-responsive polymers have been recently fabricated from various research groups in order to substitute Con A, which is found to be cytotoxic and fast degradable. Huang et al. [13] fabricated a biocompatible, glucose-specific polymer (PAA-ran-PAAPBA) in order to create a sensor based on the sensing principle of the viscosity detection changes due to affinity binding between glucose and (PAA-ran-PAAPBA). Microelectromechanical system (MEMS) technology was incorporated for the fabrication of the sensor device that allowed long-term continuous glucose monitoring [14]. The device uses a magnetically responsive vibrating Parylene polymer

microcantilever as sensing element, situated in a microchamber, which was filled by PAA-ran-PAAPBA. Glucose passes through the membrane and binds reversibly to (PAA-ran-PAAPBA) polymer, causing a viscosity alteration in the solution. The MEMS device was suggested as a subcutaneously implanted sensor for stable and reliable continuous monitoring of glucose in practical diabetes management [13].

Recently, a type of miniaturized sensors called optodes has attracted the scientists' attention. Nano-optodes consist of a chemical that responds to an analyte, a polymer to immobilize the chemical transducer and instrumentation (optical fiber, light source, detector, and other electronics) [15]. They can be integrated with several optical measurement schemes such as reflection, absorption, evanescent wave, luminescence (fluorescence and phosphorescences) that is the most popular methodology, chemiluminescence and surface plasmon resonance. Balaconis et al. [16] used nanofiber fluorescent nano-optodes in order to measure the dynamic changes of glucose concentrations based on the competitive binding between a hydrophobic boronic acid recognition molecule, a chromophore and glucose. The concentration change of glucose in the membrane was monitored by measuring the change of the optical signal. Nano-optodes are proven to be functional both *in vitro* and *in vivo* and to be very sensitive, since they can detect even small molecules [15, 16].

4.2. Detection of bacteria and viruses

Nowadays, pathogen detection is performed using very sensitive techniques such as ELISA, PCR, and sequencing techniques. However, the aforementioned techniques are considered very expensive; they require excessive sample preparation and have long validation times with no early response; and they need expertise personnel to perform the test. Therefore, the advantageous optical, magnetic, electrical, and catalytic characteristics of nanomaterials can offer faster, more sensitive, specific, and cheaper diagnostic assays that no experts will be needed for their use, in order to detect microbial pathogenesis. Pathogens express on their membranes various molecules such as glyco-, lipoproteins, glycopeptides, carbohydrates, and lipids. Thus, nanotechnology usually uses antibodies as targeting ligands for the development of various immunoassays. For example, gold and silver NPs have been broadly used for conjugation with affinity ligands, finding attractive applications as chemical sensors or even further for the generation of nanoscale arrays of pathogen-targeting ligands. Moreover, NPs can also be conjugated with specific oligonucleotides sequences that bind pathogen nucleic acid sequences to generate colorimetric changes. Other nanoparticles including fluorescent QDs and CNTs have been used in various applications including DNA detection and the development of immunoassays for the detection of bacteria and viruses. Besides NPs based-assays, miniaturized microfluidic system or lab-on-a-chip (LOC) is a futuristic and attractive field of research for accurate and point of care management of microbial infections.

Very recently, Wu et al. [17] fabricated a Microbead Quantum-dots Detection System (MQDS) in order to identify and measure target DNAs of pathogenic microorganisms and substitute PCR amplifications. All reporter probes and internal control probes were conjugated

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with quantum dots that fluoresce at different emission wavelengths using the click reaction, in order to monitor the binding process by flow cytometry [17]. Zhang et al. [18] created an innovative microfluidic microbead array with QDs tags for HBV genotyping. This method detected *in vitro*-transcribed RNA in serum samples with increased sensitivity of 1000 copies/mL of HBV virus. Thus, they were able to create a QDs-based biochip of high specificity and sensitivity for virus genotyping based on DNA hybridization. This microfluidic device managed to incorporate the microarray technology with the advantages of QDs when used as fluorescent agents and thus suggested a microfluidic approach for the highly sensitive detection of virus DNA of analysis with the use of small sample amount and fast detection time [18]. Moreover, Fu et al. [19] used Raman reporter-labeled AuNPs as SERS nanotags which target the HIV-1 DNA marker. The oligonucleotide-conjugated AuNPs were anchored in user-friendly lateral flow (LF) strips that have been extensively used for point-of-care (POC) self-diagnostics. They managed to analyze HIV-1 DNA with high sensitivity by monitoring the characteristic of Raman peak intensity of the DNA-conjugated AuNPs. The detection limit of these SERS-based lateral flows was observed to be at least 1000 times more sensitive compared to colorimetric or fluorescent detection methods. These results demonstrate the potential feasibility of the proposed SERS-based lateral flow assay to quantitatively detect a wide range of genetic diseases with high sensitivity [19]. Tsang et al. [20] used upconversion nanoparticles (UNPs), based on the upconversion phenomenon where the absorption of photons results in a shorter wavelength emission of light compared to the excitation wavelength. So far, the most common UNPs are the lanthanide Yb^{3+} to Er^{3+} ions as used in this study, where Yb^{3+} ions have the ability to convert to Er^{3+} ions under NIR light and emit at green, visible wavelengths. These UNPs are linked with AuNPs which are conjugated with oligonucleotide probes (AuNPs) targeting Ebola virus oligonucleotide, and this nanocomposite is anchored on a nanoporous alumina (NAAO) membrane. Taking into consideration that Ebola outbreaks are currently of great concern, it is essential to investigate the feasibility of detection nanodevices of into low-cost, rapid, and with ultrasensitive detection of various pathogens, especially epidemic viruses [20].

Besides DNA, antimicrobial peptides (AMPs) are promising affinity agents for the development of biosensors due to the possibility of recognizing a various pathogenic biomarkers (bacteria, fungi, toxins, viruses), in order to design biosensors that exhibit more specificity and sensitivity regarding the detection limits. In the bound state, the biosensor can be evaluated via electrochemical impedance and fluorescence spectroscopies. Mannoor et al. [21] fabricated an array electrobiosensor functionalized with the AMP magainin I on the surface of AuNPs, in order to detect pathogenic bacteria. When the specific reaction occurs between magainin I and the sample, dielectric alterations of the surface's properties are detected, a change that allows the selective detection of pathogenic Gram-negative bacteria *E. coli* and *Salmonella typhimurium* related to the non-pathogenic *E. coli* and the Gram-positive species *Listeria monocytogenes* [21]. It is also possible to use synthetic peptides in order to maximize the selectivity like Lillehoj et al. [22] who designed a microelectromechanical sensor using two synthetic peptides (C16G2cys and G10KHc) for the detection of *Streptococcus mutans* and *Pseudomonas aeruginosa.* On the other hand, Cho and Irudayaraj [23], proposed an *in situ* immuno-AuNP network-based ELISA biosensor to detect pathogens with high sensitivity. The

in situ ELISA biosensor was able to detect *E. coli* and *S. typhimurium* in real sample conditions within 2 h of inoculation pathogens at extremely low concentrations.

As already mentioned, 2D nanostructures can offer great sensitivity in rapid diagnostic tests. Mevold et al. [24] used graphene–PDDA nanosheets absorbed with AuNPs. PDDA is a homopolymer for the dispersibility of graphene, since it charges positively the graphene and protects the solution from the aggregation of graphene. The positive charge of AuNPs/graphene–PDDA nanosheets serves to easily capture the negative charge of *Staphylococcus aureus* and small molecules (adenine) for SERS rapid detection [24]. Moreover, Li et al. [25] aimed to detect foodborne pathogens, such as *Escherichia coli* O157:H7, *Salmonella enterica*, *Vibrio cholera*, and *Campylobacter jejuni*, all at the same time using multiplex PCR and magnetic nanoparticle probes (MNPs). The MNPs were conjugated with streptavidin, immobilized in an oligonucleotide array and used in order to visualize the hybridization between the oligonucleotide array and the 5′ biotinylated single-strand PCR products. Interestingly, the signal could be easily detected by naked eye or a microscope. The streptavidin–MNPs microarray detected 316 foodborne pathogens/mL and thus suggested as a sensitive, specific, and easy-to-use tool for the fast detection of foodborne pathogens in a modestly equipped laboratory, being an attractive approach for future rapid diagnostic tests [25].

4.3. Nanotechnology in cancer diagnosis

The incorporation of nanotechnology in cancer diagnosis is essential, since early detection of the disease can improve the chances of treatment. In addition, the reduction of the needed time for the nanotest will lead in more precise decision-making in every day clinical practice and treatment costs.

Up to now, several nanomaterials, such as AuNPs, semiconductor II–VI QDs, silicon nanowires (SNWs), carbon CNTs, and graphene, have been used in order to detect various cancer markers (proteins/peptides or DNA/RNA) in a sensitive and specific manner, especially when used for the construction of high-performance nanobiosensors. For instance, FET-SNWs have been used for the detection of several prostate cancer biomarkers, such as prostate-specific antigen (PSA) at the level of fg/ml of PSA for monitoring prostate cancer and predicting the risk of early biochemical relapse and the prostate biomarker 8-hydroxydeoxyguanosine (8-OHdG) by using a SNWs functionalized with antibodies against 8-OHdG [26]. PSA, prostate-specific membrane antigen, platelet factor-4, and interleukin-6 prostate cancer biomarkers have also been detected by electrochemical NTs [27, 28].

Furthermore, using a nanowire technology (nCounter Analysis System), ribonucleic acid (RNA) expression levels of cancer-testis antigens (CTAs) have been measured, as biomarkers for aggressive prostate cancer. This nanowire technology offers the possibility of a sensor chip, is able to simultaneously detect more than one of cancer marker, and measures a panel of biomarkers related to a specific cancer type and/or individual, thus contributing to the personalization of cancer diagnosis [29]. Lee et al. [30] developed a nanowire substrate-enabled laser-scanning imaging combined with flow cytometry for the isolation and quantitation of

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circulating tumor cells from a human lung carcinoma sample mixture of tumor cells and leukocytes.

Interestingly, CNTs and SNWs have been utilized for detection of various volatile organic compounds (VOCs) in breath samples of lung and gastric cancer patients, respectively [31]. Thus, Tran et al. [32] constructed a portable read-out NWs on-a-chip device, by the addition of a complementary metal-oxide semiconductor (CMOS) on FET-SiNWs, creating a nanoplatform that could detect ALCAM in serum at a detection limit of 15.5 pg/ml, in <30 min. Besides cancer biomarker detection, FET-SiNWs and zinc oxide nanowires (ZnONWs) have been used to detect ssDNA and mi-RNAs related to the initiation and progression of various cancer types [33].

Just like other nanobiosensors, nanocantilevers were demonstrated to be able to detect PSA at low levels (0.2 ng/ml–60 μg/ml) for the detection of prostate cancer. Huber et al. [34] used microcantilever arrays to detect BRAFV600E mutation nanomechanically without amplification, from total RNA samples isolated from malignant melanoma cells. Wang et al. [35] fabricated a new cantilever array-based biosensor based on MEMS for the detection of alpha-fetoprotein (AFP), a liver cancer biomarker, with high accuracy, while Liu et al. [36] detected the same biomarker for hepatocellular carcinoma at the level of ng/ml, using a resonant microcantilever electromagnetic resonance-exciting and piezoresistive read-out elements on-chip integrated, in order to measure frequency-shift versus specific-adsorbed mass.

So far, nanostructures such as QDs, AuNPs, and superparamagnetic NPs have been the most successfully incorporated in *in vitro* diagnostic applications, due to their potential to be functionalized by several biomolecules (antibodies, oligonucleotides) against the target biomolecules of interest. In the field of *in vitro* diagnostics, nanoparticles are mainly used as markers for biomolecules. On the other hand, conventional fluorescent dyes already used in medical laboratory tests, in PCR assays, and in biochips are not photostable or suitable for multiplexing. Jokerst et al. [37] used semiconductor nanoparticle QDs combined with a microfluidic biosensor for the multiplex quantitation of cancer biomarkers such as carcinoembryonic antigen (CEA), cancer antigen 125 (CA125), and Her-2/Neu (C-erbB-2) in serum and whole saliva specimens. This QD nanobiochip assay system resulted in a 30 times signal amplification, compared with standard molecular fluorophores [37]. Moreover, supermagnetic iron oxide nanoparticles are used, integrated with applications such as cell sorting, nucleic acid extraction and purification for the detection and isolation of circulating tumor cells of several types of cancer such as colorectal, lung, and breast cancer [38–40]. Besides circulating tumor cells, recently circulating extracellular vesicles and exosomes are demonstrated to serve as cancer biomarkers. Kanwar et al. [41] fabricated a microfluidic device (ExoChip) in polydimethylsiloxane (PDMS) and conjugated with antibodies against CD63, overexpressed mainly in exosomes. The ExoChip was able to measure fluorescent-carbocyanine-dyed exosomes from pancreatic cancer patients, compared to those from healthy subjects. Overall, the aim of this study was to suggest a novel approach for cancer molecular profiling that can be applied in various cancer types. Specifically, they managed to create an

exosomal-microRNA profiling that could enable the future molecular screening and diagnosis of human cancers [41].

5. Discussion and future perspectives

Up to now, the incorporation of nanostructures in medicine is offering the development of diagnostic tools of high sensitivity, advantageous contrast agents compared to traditional dyes already in use, novel personalized treatment approaches, and drug delivery vehicles. Taking into consideration small-sized sample volumes, fast reaction times, specificity and sensitivity of nanosystems, in the near future they will be able to bring mobile testing devices into every day clinical practice. Regarding *in vitro* nanodiagnostics, nanotechnology allows the construction of high sensitive nanosensors and *in vitro* tests to develop new diagnostic nanoplatforms and to allow point of care applications. Many of the technologies described in this chapter are demonstrated to be versatile; for instance, they are suitable for DNA and protein detection, and they detect very few pathogens, such as bacteria and viruses, or molecules such as low concentration of glucose that would not be detected with conventional techniques. The incorporation of nanotechnology in medicine will lead to the development of rapid diagnostic tests, which will result in the improvement of clinical decision-making and treatment costs. For example, rapid diagnostic nanotests offer early detection of disease such as cancer improving this way the possibilities of treatment.

NPs are the most versatile material for developing diagnostics, since they can be conjugated with various agents and serve as tags or labels. Thus far, there are several efforts in the way in order to develop nanoparticle-based systems for disease detection. Nanosphere, Inc. has launched the Verigene system which uses AuNPs. Verigene system is a molecular diagnostic system for rapid diagnostic evaluation that enables rapid treatment decisions regarding targeted therapy for various infections in bloodstream, respiratory tract, and gastrointestinal tract. The technology can also be applied in the future for other life-threatening diseases such as cardiovascular, autoimmune diseases, and cancer. T2 Biosystems developed T2MR, a diagnostic detection method that uses miniaturized magnetic resonance technology in order to measure how water molecules react under magnetic fields. The T2MR technology platform offers a fast, simple, and sensitive alternative to existing diagnostic methodologies and uses magnetic nanoparticles to identify proteins, nucleic acids, and other materials. T2MR technology enables low limit of detection, as low as 1 cell/mL, compared to the 100–1000 cell/mL required by PCR-based *in vitro* diagnostics. Up to now, T2Candida is in clinical trials and can identify the five clinically relevant species of Candida with 99.4% specificity and 91.1% sensitivity, directly from whole blood which enables physicians to initiate appropriate therapy on the same day. All other FDA-cleared Candida diagnostics require a blood culture to determine the Candida species, which takes up to 6 days for species identification or negative result. On the other hand, the conjugation of virus-specific antibodies in AuNPs can enable the rapid diagnosis of flu virus. The AuNPs–antibodies complex targets the virus in a way that larger AuNPs-virus aggregates are formed. Subsequently, in the presence of light, the sample leads to an increase of the reflected light due to these aggregates, allowing a much faster

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validation and virus detection than with the tests currently used. The same notion of the formation of aggregates around the biomolecule of interest is used for the fast and specific detection of various diseases.

Currently, QD technology is the most broadly employed nanotechnology for diagnostic developments, especially for cancer management. The only concern about QDs is the *in vivo* toxicity. However, researchers suggest the use of QDs composed of silicon, which is believed to be less toxic than the cadmium contained in many QDs.

Regarding circulating cancer cell detection, researches have recently published the NanoFlare genetic-based technology that enables the detection of living circulating tumor cells in bloodstream. A NanoFlare is designed to enter cells and to hybridize with cancer-specific oligonucleotide sequences. NanoFlare has a great advantage, such as all nanoparticles due to their size: the fact that they can enter inside the cell gives the opportunity of the use of various biomolecules that are present inside the cell and not only markers anchored on the cell's surface. So when NanoFlare attaches to the cancer-specific target into the cell, a reporter "flare" is released that produces a detectable fluorescent signal.

Nanosensors and blood sensors capable of detecting multiple pathogens or chemical compounds are one such example. Point-of-care diagnostics are possible with nanosensors and also an attractive technology towards point-of-care diagnosis that will be easy for the patient to use at home and will enable the integration of diagnostics with therapeutics and the development of personalized treatment approaches. Blood sensors, especially cantilevers arrays, are characterized by important advantages since the technology of nanomechanical detection requires no labels and/or external probes, and optical excitation and is rapid, highly specific, sensitive, and portable. The above give the opportunity to detect pathogens or molecules in blood samples and are a great example of future point-of-care diagnostic tools. Furthermore, the upper goal regarding the construction of diagnostic biochips will be the miniaturization of the biosensor chips to range of "nano"-dimensions. Thus, the use of nanotechnology in rapid diagnostic tests will lead to devices with nanodimensions, sensitive, easy to use, and non-expensive in order to allow direct signal observation, manipulation, analysis, and result validation of a single biological molecule from a single cell. This offers new opportunities and provides powerful tools in the fields of genomics, proteomics, molecular diagnostics, and high-throughput screening.

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