Chapter 7
Medical Nanobiosensors

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Abbreviations

Abs	Antibodies
AD	Alzheimer's disease
AuNPs	Gold nanoparticles
CA125	Cancer antigen 125
CA15-3	Cancer antigen 15-3
CEA	Carcionoembryonic antigen
CJD	Creutzfeldt-Jakob disease
CNTs	Carbon nanotubes
cTnT	Cardiac troponin-T
EGFR	Epidermal growth factor receptor
ELISA	Enzyme linked immunosorbent assay
FRET	Fluorescence resonance energy transfer
HER2	Human epidermal growth factor receptor 2
HRP	Horseradish peroxidase
IUPAC	International Union of Pure and Applied Chemistry
MMP-9	Matrix metalloproteinase 9

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MNPs	Magnetic nanoparticles
MWCNTs	Multi-walled carbon nanotubes
oxLDL	Oxidized low density lipoprotein
PCR	Polymerase chain reaction
PD	Parkinson's disease
PrP	Prion proteins
PSA	Prostate specific antigen
QDs	Quantum dots
SWCNT	Single-walled carbon nanotubes

7.1 Introduction

…the preservation of health is … without doubt the first good and the foundation of all the others goods of this life…

(René Descartes and Discours de la method, 1637)

The concept of diagnosis based on biological samples dated back several thousand years ago documented from the ancient China, Egypt to the Middle Ages of Europe [1]. Nevertheless, it was not until the 1960s when Professor Leland C Clark Jnr., as the father of the biosensor concept, described how to perform reliable and robust measurements of analytes (molecules of interest) presents in the body [2]. Presently, cancer can be diagnosed by screening the levels of the appropriate analytes existing in blood and likewise diabetes is inspected by measuring glucose concentrations. Moreover, the most conventional techniques of diagnostic technologies are the enzyme-linked immunosorbent Assay (ELISA) and the polymerase chain reaction (PCR). Nevertheless, these techniques report different handicaps such as high cost and time required, significant sample preparation, intensive sample handling, and can become troublesome to patients. Accordingly, novel advances in diagnostic technology are highly desired.

Diagnostic technology is an important field for the progress of healthcare and medicine, specifically in early diagnosis and treatment of diseases (which can deeply reduce the expense of patient care related to advanced stages of several diseases). Since molecular diagnostics can profile the pathological state of the patient, nanobiosensors for molecular diagnostics represent a factual and interesting application of the nanotechnology in medicine.

A biosensor is defined by the International Union of Pure and Applied Chemistry (IUPAC) as a "device that uses specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles, or whole cells to detect chemical compounds usually by electrical, thermal, or optical signals". Generally, biosensors include biorecognition probes (responsible for the specific detection of the analytes) and a transducer element (that converts a biorecognition event into a suitable signal) [3, 4]. In the twenty-first century, nanotechnology has been revolutionizing many fields including medicine, biology, chemistry, physics, and electronics. In this way, biosensors have been also benefited by nanotechnology, which is an emerging

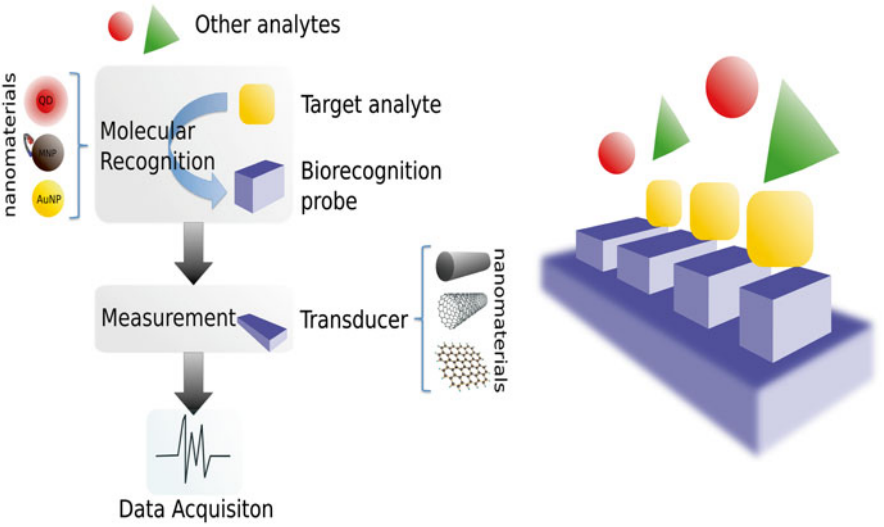

Fig. 7.1 Schematic representation of a nanobiosensor. Normally, a nanobiosensor relies on nanomaterials as transducer elements or reporters of biorecognition events

multidisciplinary field that entails the synthesis and use of materials or systems at the nanoscale (normally 1–100 nm). The rationale behind this technology is that nanomaterials possess optical, electronic, magnetic or structural properties that are unavailable for bulk materials. Since nanomaterials range in the same scale of the diagnostic molecules, when linked to biorecognition probes (such as antibodies, DNA and enzymes), nanostructures allow the control, manipulation and detection of molecules with diagnostic interest, even at the single molecule level. Normally, nanobiosensors are based on nanomaterials or nanostructures as transducer elements or reporters of biorecognition events [5, 6]. Figure 7.1 displays the schematic representation of a nanobiosensor.

This chapter aims at providing a description on the basic principles of the nanobiosensors and a brief survey on nanobiosensing strategies towards medical applications, specifically in the following disease categories: neurodegenerative diseases, cardiovascular diseases and cancer chosen as the most reported application fields.

7.2 Biorecognition Probes

Biorecognition probes, or molecular bioreceptors, are the key in the specificity of biosensors (a non-specific biorecognition event can yield a false result). Biomolecular recognition generally entails different interactions such as hydrogen bonding, metal coordination, hydrophobic forces, van der Waals forces, pi-pi interactions and electrostatic interactions. In this section the most common biorecognition probes in nanaobiosensors are briefly discussed (see Fig. 7.2a).

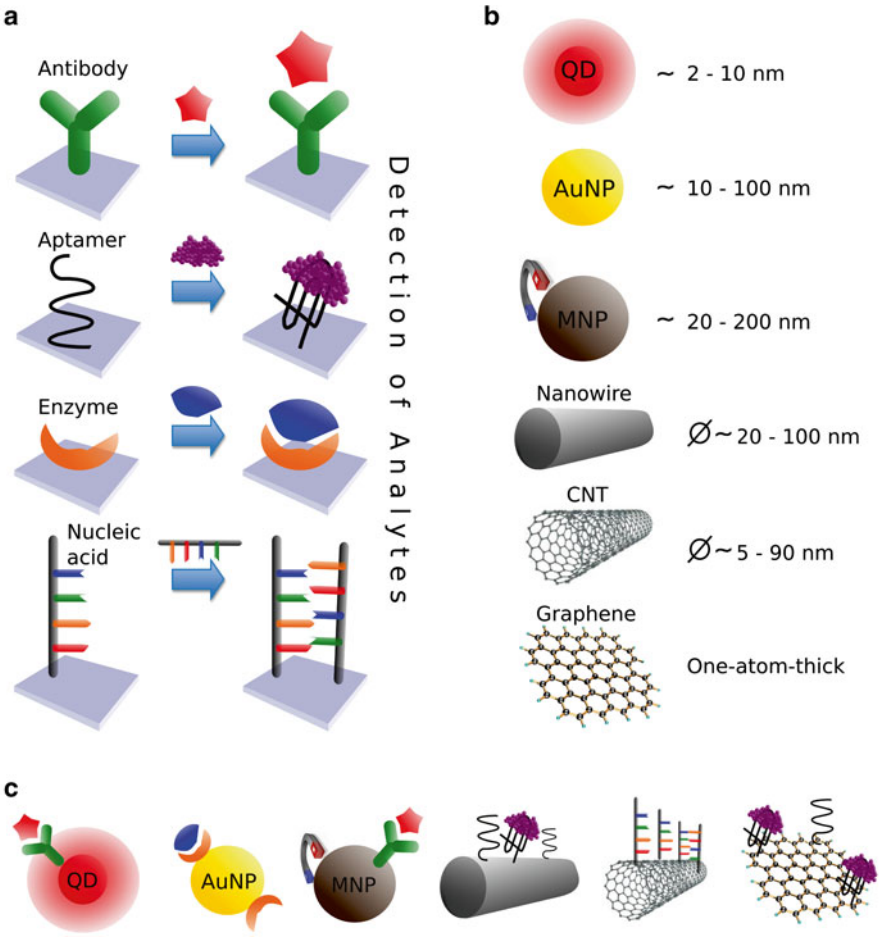

Fig. 7.2 Biorecognition probes and nanomaterials. (**a**) Biorecognition probes. (**b**) Nanomaterials. (**c**) Nanomaterials decorated with biorecognition probes. *QD* Quantum Dot, *AuNP* gold nanoparticle, *MNP* magnetic nanoparticle. Sketches are not at scale

7.2.1 Antibodies

Antibodies are soluble forms of immunoglobulin containing hundreds of individual amino acids arranged in a highly ordered sequence. These polypeptides are produced by immune system cells (B lymphocytes) when exposed to antigenic substances or molecules. Proteins with molecular weights greater than 5,000 Da are generally immunogenic. Antibodies contain in their structure recognition/binding sites for specific molecular structures of the antigen. Since an antibody interacts in a highly specific way with its unique antigen, antibodies are widely employed in biosensors.

7.2.2 Aptamers

Aptamers are novel artificial oligonucleic acid molecules that are selected (in vitro) for high affinity binding to several targets such as proteins, peptides, amino acids, drugs, metal ions and even whole cells [7–9].

7.2.3 Enzymes

Enzymes are protein catalysts of remarkable efficiency involved in chemical reactions fundamental to the life and proliferation of cells. Enzymes also possess specific binding capabilities and were the pioneer molecular recognition elements used in biosensors and continue still used in biosensing applications [10, 11, 126].

7.2.4 Nucleic Acids

Since the interaction between adenosine and thymine and cytosine and guanosine in DNA is complementary, specific probes of nucleic acids offer sensitive and selective detection of target genes in biosensors [12].

7.3 Transduction Modes

In order to detect biorecognition events, biosensors require a transduction mode. Transduction modes are generally classified according to the nature of their signal into the following types: (1) optical detection, (2) electrochemical detection, (3) electrical detection, (4) mass sensitive detection and (5) thermal detection.

7.3.1 Optical Detection

Optical biosensing is based on several types of spectroscopic measurements (such as absorption, dispersion spectrometry, fluorescence, phosphorescence, Raman, refraction, surface enhanced Raman spectroscopy, and surface plasmon resonance) with different spectrochemical parameters acquired (amplitude, energy, polarization, decay time and/or phase). Among these spectrochemical parameters, amplitude is the most commonly measured, as it can generally be correlated with the concentration of the target analyte [13].

7.3.2 Electrochemical Detection

Electrochemical detection entails the measurement of electrochemical parameters (such as current, potential difference or impedance) of either oxidation or reduction reactions. These electrochemical parameters can be correlated to either the concentration of the electroactive probe assayed or its rate of production/consumption [13].

7.3.3 Electrical Detection

Electrical detection is often based on semiconductor technology by replacing the gate of a metal oxide semiconductor field effect transistor with a nanostructure (usually nanowires or graphitic nanomaterials). This nanostructure is capped with biorecognition probes and a electrical signal is triggered by biorecognition events [14, 15].

7.3.4 Mass Sensitive Detection

Mass sensitive detection can be performed by either piezoelectric crystals or microcantilevers. The former relies on small alterations in mass of piezoelectric crystals due to biorecognition events. These events are correlated with the crystals oscillation frequency allowing the indirect measurement of the analyte binding [16]. Microcantilever biosensing principle is based on mechanical stresses produced in a sensor upon molecular binding. Such stress bends the sensor mechanically and can be easily detected [17].

7.3.5 Thermal Detection

Thermal biosensors are often based on exothermic reactions between an enzyme and the proper analyte. The heat released from the reaction can be correlated to the amount of reactants consumed or products formed [18].

7.4 Nanomaterials: The Nanobiosensors Toolbox

Recent advances of the nanotechnology focused on the synthesis of materials with innovative properties have led to the fabrication of several nanomaterials such as nanowires, quantum dots, magnetic nanoparticles, gold nanoparticles, carbon nanotubes and graphene. These nanomaterials linked to biorecognition probes are generally the basic components of nanobiosensors. In order to attach nanomaterials with biorecognition probes, nanomaterials are either electrostatically charged or functionalized with the suitable chemically active group [19–23] (see Fig. 7.2c).

In the following section the most widely used nanomaterials in biosensing are briefly described and they are sketched in Fig. 7.2b.

7.4.1 Zero-dimensional Nanomaterials

7.4.1.1 Quantum Dots (QDs)

QDs are semiconductors nanocrystals composed of periodic groups of II–VI (e.g., CdSe) or III–V (InP) materials. QDs range from 2 to 10 nm in diameter (10–50 atoms). They are robust fluorescence emitters with size-dependent emission wavelengths. For example, small nanocrystals (2 nm) made of CdSe emit in the range between 495 and 515 nm, whereas larger CdSe nanocrystals (5 nm) emit between 605 and 630 nm [24]. QDs are extremely bright (1 QD ≈ 10–20 organic fluorophores) [25]. They have high resistance to photobleaching, narrow spectral linewidths, large stokes shift and even different QDs emitters can be excited using a single wavelength, i.e. they have a wide excitation spectra [26, 27]. Because of their properties QDs are used in biosensing as either fluorescent probes [28, 29] or labels for electrochemical detection (Wang et al. 2011a).

7.4.1.1.1 Gold Nanoparticles (AuNPs)

Synthesis of AuNPs often entails the chemical reduction of gold salt in citrate solution. Their scale is less than about 100 nm. AuNPs have interesting electronic, optical, thermal and catalytic properties [30, 31]. AuNPs enable direct electron transfer between redox proteins and bulk electrode materials and are widely used in electrochemical biosensors, as well as biomolecular labels ([32]; Ambrosi et al. 2009b).

7.4.1.1.2 Magnetic Nanoparticles (MNPs)

MNP are often composed by iron oxide and due to their size (20–200 nm) they can possess superparamagnetic properties. MNP are used as contrast agents for magnetic resonance imaging and for molecular separation in biosensors devices [33–35].

7.4.2 One-Dimensional Nanomaterials

7.4.2.1 Carbon Nanotubes (CNTs)

CNTs consist of sheets (multi-walled carbon nanotubes, MWCNTs) or a single sheet (single-walled carbon nanotubes SWCNTs) of graphite rolled-up into a tube. Their diameters range about from 5 to 90 nm. The lengths of the graphitic tubes are normally in the micrometer scale. CNTs seem a remarkable scheme of excellent mechanical, electrical and electrochemical properties [36, 37] and even can display

metallic, semiconducting and superconducting electron transport [38]. The properties of carbon nanotubes are highly attractive for electrochemical biosensors and also has been used as transducer in bio-field-effect transistors [39, 40].

7.4.2.2 Nanowires

Nanowires are planar semiconductors with a diameter ranging from 20 to 100 nm and length from submicrometer to few micrometer dimensions. They are fabricated with materials including but not limited to silicon, gold, silver, lead, conducting polymer and oxide [41, 42]. They have tunable conducting properties and can be used as transducers of chemical and biological binding events in electrically based sensors such as bio-field-effect transistors [43–45] or even as nanomotors [46].

7.4.3 The Innovative Two-Dimensional Material: Graphene

Graphene is a recently discovered one-atom-thick planar sheet of sp^2 bonded carbon atoms ordered in a two-dimensional honeycomb lattice and is the basic building block for carbon allotropes (e.g., fullerens, CNTs and graphite). Graphene has displayed fascinating properties such as electronic flexibility, high planar surface, superlative mechanical strength, ultrahigh thermal conductivity and novel electronic properties [47]. Owing to its properties, graphene has been employed as transducer in bio-field-effect transistors, electrochemical biosensors, impedance biosensors, electrochemiluminescence, and fluorescence biosensors, as well as biomolecular label [48, 49, 133].

7.5 Nanobiosensing Strategies Toward Medical Applications in Health Priorities: Biomarkers Detection

Biomarkers can be altered genes, RNA products, proteins, or other metabolites that profile biological processes in normal, pathogenic or pathological states and even during pharmacologic or therapy responses of the patient [50–53]. Molecular diagnostics relies on the detection of these biomarkers sourced from biological samples such as serum, urine, saliva and cerebrospinal fluid.

Nanobiosensors can perform a key role in biomarker detection. As innovative devices, nanobiosensors often comprise the following requirements: a tiny amount of sample and assay reagents, fast response, highly sensitive, high accuracy and reproducibility, portability, multiplexing capabilities, user friendly and low cost. This section contains an overview of the powerful advantages of different nanobiosensing strategies for biomarker detection focused on the following disease categories: neurodegenerative diseases, cardiovascular diseases and cancer. Figures 7.3, 7.4 and 7.5 display different biosensors for neurodegenerative diseases, cardiovascular diseases and cancer (respectively). All the exposed applications are summarized in Table 7.1. Since this chapter cannot cover all applications and technical details, the interested reader is referred to some recent literature referenced across the content.

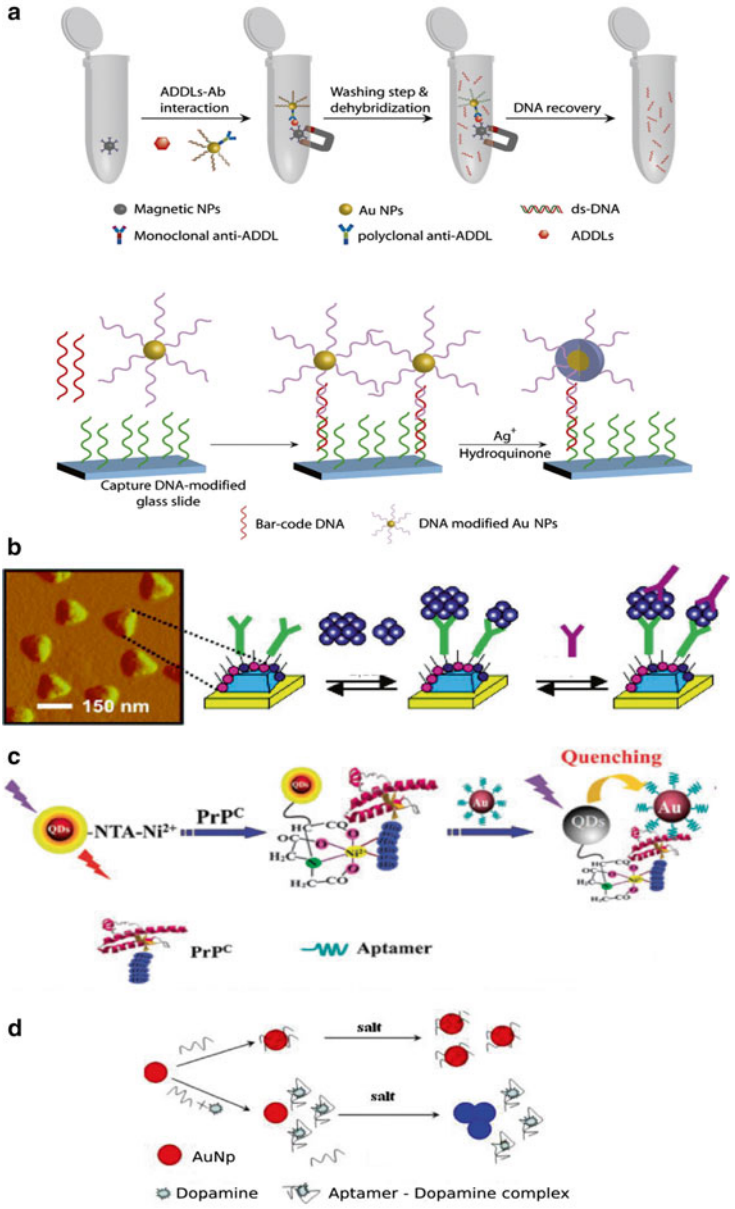

Fig. 7.3 Nanobiosensors for neurodegenerative diseases. (**a**) Bio-bar-code assay for amyloid β (ADDL) detection based on AuNP and MNP (Reprinted with permission from [121]. Copyright 2011, Elsevier). (**b**) Detection of amyloid β by using a nanopatterned optical biosensor based on silver nanoparticles and localized surface plasmon resonance (Modified with permission from Nam et al. 2005. Copyright 2005, American Chemical Society). (**c**) Detection of prion protein (PrP^C) with a long-range resonance energy transfer strategy based on quenching of the light of QDs by AuNPs (Modified with permission from [76]. Copyright 2010, Royal Society of Chemistry). (**d**) Aptamer-based colorimetric biosensing of dopamine using unmodified AuNPs (Modified with permission from [71]. Copyright 2011, Elsevier)

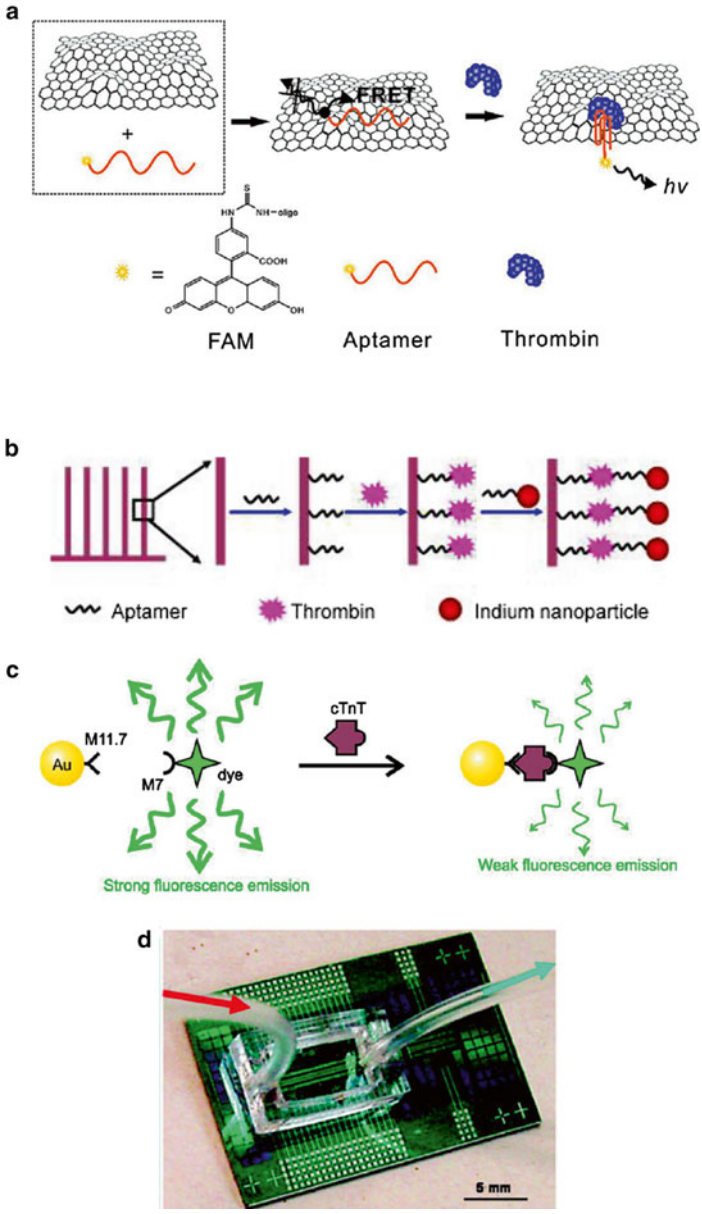

Fig. 7.4 Nanobiosensors for cardiovascular diseases. (**a**) Thrombin detection based on fluorescence resonance energy transfer through graphene as acceptor of organic dyes (FAM) donors connected with specific aptamers (Reprinted with permission from [88]. Copyright 2010, American Chemical Society). (**b**) Aptamer-functionalized indium nanoparticles as thermal probes for thrombin detection on silicon nanopillars using thermal detection (Reprinted with permission from [90]. Copyright 2010, Elsevier). (**c**) Long-range fluorescence quenching by gold nanoparticles in a sandwich immunoassay for cardiac troponin T (M11.7, specific antibody; M7, specific fragment antibody; cTnT, cardiac troponin T) (Reprinted with permission from [93]. Copyright 2009, American Chemical Society). (**d**) Setup for the detection of cardiac troponin T composed of arrays of highly ordered silicon nanowire clusters (Modified with permission from [92]. Copyright 2009, American Chemical Society)

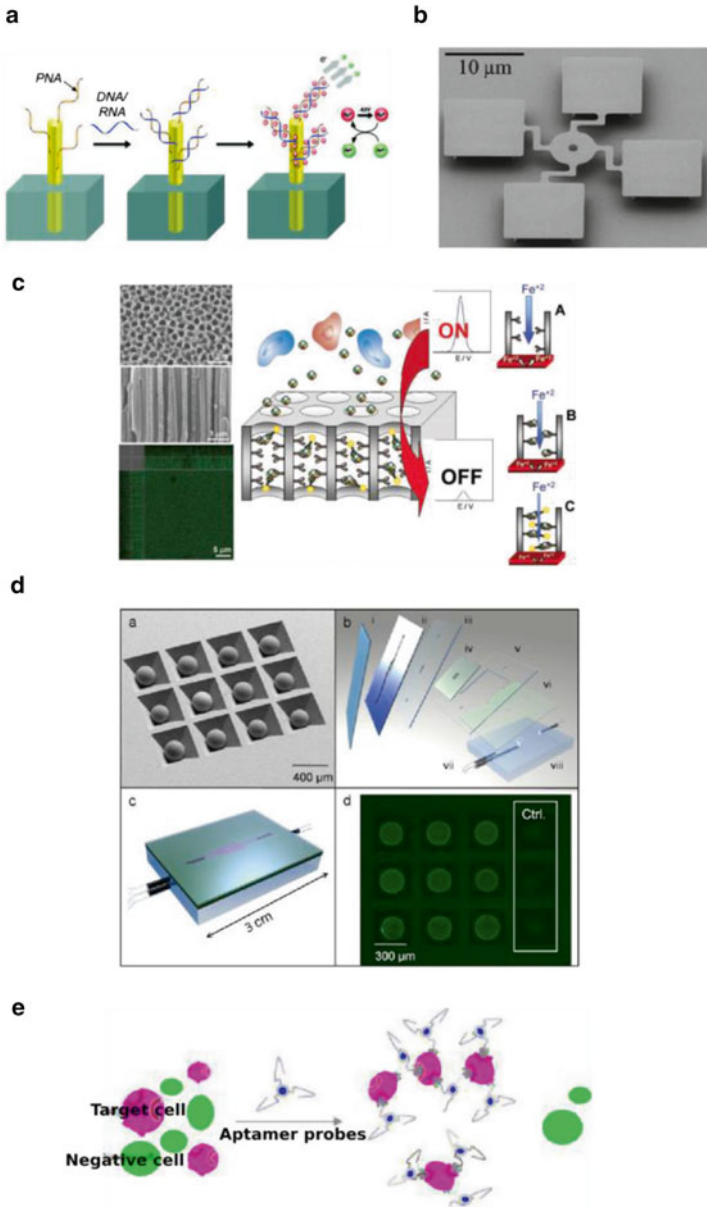

Fig. 7.5 Nanobiosensors for cancer. (**a**) Nanoscale electrode platform for the direct electrocatalytic mRNA (related with prostate cancer) detection using peptide nucleic acid-nanowire sensors (Reprinted with permission from [75]. Copyright 2009, American Chemical Society). (**b**) Trampoline shaped nanomechanical resonator made of silicon for the detection of prostate specific antigen (Reprinted with permission from [2]. Copyright 2009, Royal Society of Chemistry) (**c**) A nanochannel (porous alumina)/gold nanoparticle-based filtering and sensing platform for direct detection of cancer antigen 15-3 using Fe(2/3) as electrochemical signaling indicator (Reprinted with permission from [16]. Copyright 2011, John Wiley and Sons). (**d**) Silicon Nano-bio-chips for multiplexed protein detection: determinations of cancer biomarkers in serum and saliva using quantum dot bioconjugate labels (as fluorescent probes) (Reprinted with permission from [36]. Copyright 2009, Elsevier). (**e**) A sensitive fluorescence anisotropy method for the direct detection of cancer cells in whole blood based on aptamer-conjugated near-infrared fluorescent methylene blue nanoparticles (Modified with permission from [72]. Copyright 2010, Elsevier)

Table 7.1 Nanobiosensors for biomarker detection towards medical applications in different disease categories

Application/detection	Nanomaterials/platform	LOD	Characteristics	Reference
Neurodegenerative diseases				
Amyloid β	Magnetic beads and AuNPs, fluorescent detection (OD)	10 fM	Ultra-high sensitivity. A hopeful beginning towards a diagnosis tool	Khan et al. [57]
	Scanometric detection (OD)	10 aM		
	Nanoscale biosensor based on localized surface plasmon resonance (OD)	7.3 pM	Provides new information relevant to the understanding and possible diagnosis of Alzheimer	Haes et al. [59]
	AuNPs, surface plasmon resonance based sensor (OD)	1 fg/mL	Ultrasensitive detection, focused on early detection	Lee et al. [60]
Amyloid β aggregation inhibitor	Precipitation of AuNP. Optical density of supernatants (OD)	ND	Potential diagnostic tool for diseases involving abnormal protein aggregation as their key pathogenesis processes	Han et al. [62]
Apolipoprotein E	Quantum dots. Fluorescent microarray (OD)	62 pg/mL	High sensitivity. Can be extended to several AD biomarkers	Morales-Narváez et al. [122]
Dopamine	Electrodes modified with CNTs and other features (ECD)	1 nM	High sensitivity and selectivity	Ali et al. [69]
	SWCNTs (ECD)	15 nM	Selective, analyte is detected in the presence of ascorbic acid and uric acid at physiological pH	Alwarappan et al. [70]
	Graphene-modified electrodes (ECD)	ND	Selective detection of dopamine in a large excess of ascorbic acid	Wang et al. [47]
	AuNPs (OD)	0.36 μM	Simple, sensitive and selective aptamer based colorimetric detection	Zheng et al. [73]
	MWCNT as enhancer of electron transfer (ECD)	0.01 mM	Response enhanced by using CNT	Alarcón-Angeles et al. [71]
Prion proteins	Gold-coated magnetic nanoparticles (OD)	N/A	Can provide a useful insight into the affinity of PrP to nanoparticle-functionalized aptamers for diagnosis applications	Kouassi et al. [76]
	Nanomechanical resonator arrays and nanoparticles (MSD)	20 pg/mL	Can be suitably applied to develop ante mortem tests to directly detect prion proteins in body fluids	Varshney et al. [77]
	Long range resonance energy transfer from QDs to the surface of AuNPs (OD)	33 aM	Ultra-sensitive detection. Can be successfully applied in biological media	Hu et al. [78]
	QDs as a highly selective probe (OD)	3 nM	Sensitive, rapid and simple detection	Zhang et al. [79]

Cardiovascular diseases				
Matrix metalloproteinase 9	Peptide decorated MNP (MRI)	N/A	Good candidate for an enzyme reporter probe for in-vivo and for whole-body imaging of protease activity in humans	Schellenberger et al. [85]
Thrombin	Catalytic enlargement of aptamer-functionalized AuNPs (OD)	2 nM	Sensitive detection. One of the first nanobiosensors for thrombin detection	Pavlov et al. [89]
	FRET between graphene (acceptor) and organic dye (donor) (OD)	31 pM	High sensitivity in blood serum samples	Chang et al. [90]
	Aptamer-functionalized indium nanoparticles as thermal probes (TD)	22 nM	Sensitive, selective and low-cost method	Wang et al. [72]
	Label-free colorimetric detection using fribirnogen and AuNPs (OD)	0.04 pM	Highly sensitive, selective, rapid and simple method employed in blood plasma	Chen et al. [92]
Cardiac troponin T	Arrays of highly ordered silicon nanowire clusters (ED)	0.8 fM	Ultra-high sensitivity. Potential point-of-care application	Chua et al. [94]
	FRET between AuNP (acceptor) organic dye (donor) (OD)	0.02 nM	High sensitivity	Mayilo et al. [95]
Oxidized low density lipoprotein	Antibody decorated nanowires in field effect transistors (EC)	N/A	Selectivity	Rouhanizadeh et al. [97]
Myoglobin	Surface electrode modified with AuNPs (ECD)	0.56 nM	The whole procedure takes 30 min. Do not require plasma pretreatment. Small sample amount (1 μL).	Suprun et al. [99]
Cancer				
Probes made of peptide nucleic acid used to detect a gene fusion recently associated with prostate cancer	Nanowires, electrochemical detection	100 fM	One of the first electrochemical sensors to directly detect specific mRNAs in unamplified patient samples	Fang and Kelley. [105]
Prostate specific antigen	Arrays of nanomechanical resonators and nanoparticles (MSD)	1.5 fM	Ultra-high sensitivity in real samples	Waggoner et al. [106]
	Silver enhancement of AuNPs (OD)	15 fM	Diagnostic at earlier timepoint comparing with other procedures	Storhoff et al. [107]

(continued)

Table 7.1 (continued)

Application/detection	Nanomaterials/platform	LOD	Characteristics	Reference
Cancer antigen 15-3	Nanochannels and antibody decorated AuNPs (ECD)	52 U/mL	Simple procedure applied to whole blood that avoids tedious and time consuming labors	De la Escosura-Muñiz and Merkoçi. [108]
	Antibody decorated AuNPs (OD)	15 U/mL	High sensitivity	Ambrosi et al. 2009a
Carcinoembryonic antigen	Nanogold-enwrapped graphene nanocomposites (ECD)	10 pg/mL	Sensitive detection through novel nanocomposites that have a promising potential in clinical diagnosis	Zhong et al. [110]
	Quantum dots capped with silica onto gold substrates (ECD)	50 pg/mL	Satisfactory stability, acceptable veracity, proper for dual-tumor markers detection in clinic serum samples	Qian et al. [112]
Carcinoemrbionic antigen	Antibody decorated QDs and capture antibody capped agarose beads in microfluidic chip (OD)	20 pg/mL	Multiplexing capabilities. High sensitivity in saliva and serum samples	Jokerst et al. [113]
Cancer antigen 125		N/A		
Human epidermal growth factor receptor 2		0.27 ng/mL		
Human epidermal growth factor receptor 2	Antibody decorated graphene encapsulated nanoparticles are patterned as gate of a field effect transistor (ED)	1 pM	High specificity and sensitivity. Suitable for clinical applications	Myung et al. [111]
Epidermal growth factor receptor		100 pM		
Tumor cells	Antibody decorated AuNPs (ECD)	4×10^3 cells/ 0.7 mL	Rapid and simple assay	De la Escosura-Muñiz et al. [116]
Leukemia infected cells	Aptamer decorated near infrared fluorescence nanoparticles (OD)	4×10^3 cells/mL	Quick method, without the need of the complicated separation steps in whole blood samples	Deng et al. [117]
Tumor imaging	Antibody decorated MNP as contrast agent (MRI)	N/A	Could be considered for further research as an MRI contrast agent for the detection of tumors in human	Oghabian et al. [117], Rasaneh et al. [120]

LOD limit of detection, *AuNPs* gold nanoparticles, *QDs* quantum dots, *OD* optical detection, *CNT* carbon nanotube, *MWCNT* multi-walled carbon nanotube, *ECD* Electrochemical detection, *ED* electrical detection, *MSD* mass sensitive detection, *TD* thermal detection, *FRET* fluorescence resonance energy transfer, *MRI* magnetic resonance imaging, *N/A* not available

7.5.1 Neurodegenerative Diseases

Dementia is a meaningful health problem in developed countries with over 25 million people affected worldwide and probably over 75 million people at risk during the next 20 years [54]. Alzheimer's Disease (AD) is the most frequent cause of dementia and results in a progressive loss of cognitive function affecting one in eight people by the time they reach 65 years of age [55, 123]. Diverse sources of evidence suggest that amyloid-β (Aβ) have a causal role in its pathogenesis [56]. Therefore, Aβ is a potential AD biomarker. An overview on recent nanobiosensing approaches for Aβ detection is presented below.

Khan et al. [57] have employed the "bio-bar-code assay", previously developed by Mirkin and co-workers [58], to detect Aβ at femto molar level (10 fM by using fluorescent detection) and atomolar level (10 aM, by using scanometric detection). Plasma samples from control and Alzheimer's patients were assayed. This technic is based on oligonucleotide-modified gold nanoparticles. A complex: magnetic bead – capture antibody – Aβ – detection antibody – AuNP – DNA strands is performed. And finally, each analyte binding is reported by the presence of thousands of DNA strands; i.e. the more complexes are created, the more DNA released (see Fig. 7.3a). Despite the results were hard to reproduce, is a hopeful beginning to a clinical diagnostic tool. Haes and colleagues [59] have reported an optical nanobiosensing platform based on localized surface plasmon resonance spectroscopy, so as to monitor the interaction Aβ/specific antibodies. Clinical samples, extracted from cerebrospinal fluid, were assayed in this sensor based on nanopatterned surfaces achieving a picomolar sensitivity (7.3 pM) (see Fig. 7.3b). This technology provides new information relevant to the understanding and possible diagnosis of AD. Lee et al. [60] have developed a surface plasmon resonance based biosensor [61] for Aβ detection. The procedure enhances the surface plasmon resonance signal by using antibody decorated AuNP. This approach is focused on ultrasensitive detection towards early detection achieving a detection limit of 1 fg/mL. Han et al. [62] have proposed a screen for Aβ aggregation inhibitor by using Aβ-conjugated AuNPs. Aβ aggregation of AD patients serum was visualized through Aβ aggregation-induced AuNP precipitation. This approach is a potential diagnostic tool for diseases involving abnormal protein aggregation as their key pathogenesis processes. The authors of this chapter have recently studied a microarray for another potential Alzheimer Disease's biomarker screening; i.e., Apolipoprotein E. This microarray is reported by QDs nanocrystals, exploiting their advantageous photonics properties, a limit of detection up to 62 pg mL^{-1} can be achieved [63].

Parkinson's disease (PD) is a common chronic neurodegenerative disorder. The classical clinical features are progressive tremor, rigidity and bradykinesia [64] Dopamine (DA) is a neurotransmitter with a variety of functions in the central nervous system. It affects the brain's control of learning, feeding and neurocognition. Disorders in DA levels have been associated with Parkinson's disease, among others psychiatric disorders such as schizophrenia, and depression [65–66]. Thus, DA has an active research as biomarker and is a promising analyte toward molecular diagnosis of Parkinson's disease.

Since DA is electrochemically active, electrochemical detection of DA by oxidative methods is a preferred procedure. Nevertheless, DA is always subsisting with ascorbic acid in real samples and the products of DA oxidation can react with ascorbic and regenerate dopamine again impacting the accuracy of detection gravely. Different approaches have been reported to avoid this shortcoming. Ali et al. [67] demonstrated that DA can be electrochemically detected by altering the electrode surface with a thin layer of an in situ polymerized poly(anilineboronic acid)/CNT composite and a thin layer of the highly permselective Nafion film. The detection limit is of ~1 nM. Alwarappan and co-workers [68] explored the performance of electrochemically pretreated SWCNTs for the electrochemical detection of DA in the presence of ascorbic acid and uric acid at physiological pH. This approach showed a successful selective response with a detection limit of about 15 nM. A MWCNT as enhancer of electron transfer combined with β-Cyclodextrin (β-CD) as molecular receptor is also reported as a DA electrochemical sensor system. The proposed molecular host–guest recognition based sensor shows a electrochemical sensitivity for amperometric detection of DA over the range 0.01–0.08 mM [69].

Wang et al. [70] proposed a graphene-modified electrode that was applied in the selective detection of DA with a linear range from 5 to 200 **μ**M in a large excess of ascorbic acid. Zheng and co-workers [73] explored the interaction of DA-binding aptamer and DA by using unmodified citrate-coated AuNPs as colorimetric signal readout, where AuNP aggregation is induced in the presence of the analyte and the stability of the solution is preserved in the absence of the analyte (see Fig. 7.3d). A selective nanobiosensor was achieved with a detection limit of 0.36 **μ**M. The sensitivity and selectivity of these approaches enable their potential use in diagnosis of PD.

Creutzfeldt-Jakob disease (CJD) is a neurodegenerative disease characterized by rapidly progressive dementia, myoclonus, ataxia and visual disturbances, extrapyramidal and pyramidal involvement, as well as a kinetic mutism [74]. Prion proteins (PrP) once transformed from their normal cellular counterparts (PrP^{c}) into infectious form (PrP^{res}) are transmissible infectious particles, destitute of nucleic acid, that are believed to be responsible for causing the fatal CJD in humans [75]. Since disorders in PrP levels are expected to be present in samples derived from CJD patients, there is an enormous interest in PrP screening technologies; some nanobiosening approaches are presented below.

Kouassi et al. [76] have developed an assay for PrP assessment based on aptamer-mediated magnetic and gold-coated magnetic nanoparticles. Analyte detection was reported by using Fourier transform infrared spectroscopy. The proposed assay can provide a useful insight into the affinity of PrP to nanoparticle-functionalized aptamers for diagnosis applications. Varshney and co-workers [77] have reported PrP detection in serum by exploiting micromechanical resonator arrays. Secondary antibodies and nanoparticles were used as mass amplifiers to detect the presence of small amounts of PrP onto mechanical resonators. This device showed a limit of detection of about 20 pg/mL and the authors are currently working so as to enhance the detection limit. Hu et al. [78], have explored an ultra-sensitive detection strategy for PrP based on the long range resonance energy transfer from QDs to the surface of AuNPs. Energy transfer from QDs to the surface of AuNPs occurs with high

efficiency and the fluorescent signal of QDs was quenched as a consequence of the molecular recognition between PrP (bound with high specificity to QDs) and an aptamer specific for the PrP (conjugated to AuNPs) (see Fig. 7.3c). This procedure achieves a very low detection limit of 33 aM and might be successfully applied in biological media. Zhang and co-workers [79] have reported the use of QDs as a highly selective probe for PrP detection. When the suitable treated QDs were mixed with PrP, the concentration of QDs in supernatant decreased due to the precipitation resulting on a reduced fluorescence intensity of the supernatant. This phenomenon was used for quantitative detection with a detection limit of 3 nM. The reported method shows a sensitive, rapid and simple performance.

7.5.2 Cardiovascular Diseases

Cardiovascular diseases is the cause of nearly half of all deaths in the Western world [80] and is also a major cause of death, morbidity, and disability in Asia and Africa [81, 129–131]. Cardiovascular diseases include hypertension with or without renal disease, stroke, atherosclerosis, other diseases of arteries, arterioles, and capillaries, and diseases of veins and lymphatics. In addition, there are different forms of heart disease such as rheumatic fever/rheumatic heart disease, hypertensive heart disease, heart and renal disease, ischemic heart disease, diseases of pulmonary circulation and so on [82]

Matrix metalloproteinase 9 (MMP-9), also known as gelatinase B, is an enzyme important in inflammation, atherosclerosis and tumor progression processes; furthermore MMP-9 is a potential biomarker involved in cardiovascular diseases [83, 84]. Schellenberger and colleagues [85] have developed a technology for MMP-9 screening. They have reported a nanobiosensor based on peptide decorated MNPs that is proper for in vivo imaging of MMP-9 activity by magnetic resonance imaging. This system has a great potential as reporter probes for assessing enzyme activity of proteases by in vivo magnetic resonance imaging and can help towards diagnosis and monitoring applications [86].

Thrombin (also known as factor IIa) is the last enzyme protease involved in the coagulation cascade and it converts fibrinogen to insoluble fibrin, causing blood clotting [87]. Therefore, thrombin plays a central role in cardiovascular diseases [88]. Thrombin detection is under active research; for example, currently, an inquiry on the Web of Knowledge displays more than 50 approaches based on nanotechnology related with thrombin detection. About 30 of them exploit the use of AuNPs and aptamers are the most common biorecognition probes in these sensors.

As far as is known, one of the first nanobiosensors for thrombin detection was reported by Pavlov and colleagues [89]. They developed an amplified optical detection of thrombin onto solid phase by the catalytic enlargement of thrombin aptamer-functionalized AuNPs. This system exhibited a detection limit of ca. 2 nM. Chang and co-workers [90], have proposed a biosensor based on fluorescence (or Förster) resonance energy transfer (FRET) [91] between graphene sheets as acceptors and fluorescent dyes as donors. Fluorescence of dye labeled aptamer is quenched when aptamer attach to graphene due to energy transference between dyes and graphene.

Dyes emission is recovered by biorecognition events, i.e. when thrombin binds with aptamers to perform quadruplex-thrombin complexes (FRET is not present). Fluorescence emission is proportional to thrombin concentration (see Fig. 7.4a). The procedure was applied to blood serum samples achieving a high sensitivity with a detection limit of ca. 31 pM. Wang et al. [47] have reported an innovative thermal biosensor for thrombin detection in serum samples by using aptamer-functionalized indium nanoparticles as thermal probes onto silicon nanopillars (see Fig. 7.4b). This device is characterized by a sensitive (detection limit of ca. 22 nM), selective and low-cost method. Chen and co-workers [92], have published a label-free colorimetric thrombin detection by using fibrinogen decorated AuNPs. Mixing of thrombin into the fibrinogen decorated AuNPs solutions in the attendance of excess fibrinogen yields the agglutination of the capped AuNPs. The absorbance of the supernatant of the assayed solution is inversely proportional to the thrombin concentration. This procedure is highly sensitive, selective, rapid and simple and can be suitably employed in blood plasma with a detection limit of 0.04 pM.

Human cardiac troponin-T (cTnT) is a key protein biomarker related specifically to myocardial damage [93]. Since cTnT prevails in elevated concentrations in the bloodstream of a patient suffering from heart attack, this biomarker can be employed as a helpful linker between a patient suffering from unstable angina or a more dangerous case of myocardial infarction [94]. Mayilo and colleagues [95] have developed a biosensing platform based on FRET effect with AuNP as acceptors of organic dyes donors. The fluorescence of the antibody fragment – organic dye conjugated is quenched by performing an immunocomplex with an antibody decorated AuNP. The fluorescence quenching is proportional to the detected cTnT (see Fig. 7.4c). This platform accomplished a detection limit of 0.02 nM in serum. Chua and co-workers [94] have designed a label-free electrical detection of cTnT by using complementary metal-oxide semiconductor-compatible silicon nanowire sensor arrays. Nanowires are decorated with specific antibodies against cTnT and biorecognition events are reported by electrical signals. Setup is displayed in Fig. 7.4d. This system achieved a limit of detection as low as ca. 0.85 fM fg/mL in undiluted serum and it possesses portability characteristics for point-of-care application.

Oxidized low density lipoprotein (oxLDL) is recognized as a biomarker for acute myocardial infarction in patients with coronary artery disease. Specifically, oxLDL at elevated levels can forecast acute heart attack or coronary syndromes [96, 128]. Rouhanizadeh and colleagues [97] have reported a biosensor based on electrical detection by using antibody decorated nanowires in field effect transistors. This biosensor enables to differentiate the LDL cholesterol between the reduced (native LDL) and the oxidized state (oxLDL). Acute myocardial infarction can also be diagnosed preventively by measuring myoglobin protein levels [98]. Suprun et al. [99] have designed a electrochemical nanobiosensor for cardiac myoglobin screening based on direct electron transfer between Fe(III)-heme and electrode surface modified with antibody decorated AuNPs. Notably, 1 μL of undiluted plasma of healthy donors and patients with acute myocardial infarction was analyzed with a limit of detection as low as 0.56 nM. Since the procedure takes 30 min, it can be used for rapid diagnosis.

7.5.3 Cancer

Cancer is the predominant cause of death in economically developed countries and the second major cause of death in developing countries [100]. Cancer is a pathology mainly characterized by the chaotic growth of cells with an altered cell cycle control. Since the name of a specific cancer depends upon the tissue or body cells in which it originated, there are many different types of cancers and the most frequent are: breast, lung, prostate, skin, cervical, colon and ovarian cancer among others.

Cancer results in complex molecular alterations. These alterations can be unveiled by using technologies that assess changes in the content or sequence of DNA, its transcription into messenger RNA or microRNA, the production of proteins or the synthesis of several metabolic products [101]. Nevertheless, validation of accurate cancer biomarkers has been slow and is under active research [101, 102]. Table 7.2 shows a list of some potential cancer biomarkers. More details about cancer markers can be founded in the literature [103, 104,127, 129,134, 135].

Biosensors for cancer detection are highly attractive and they are under vigorous development. An overview of some recent nanobiosensing approaches is provided below (Table 7.2).

Fang et al. (2009) [105] have developed an electrochemical sensor to directly detect specific mRNAs in unamplified patient samples (without PCR amplification). The sensor relies on peptide nucleic acid decorated nanowires. Probes made of peptide nucleic acid have been used to detect a gene fusion recently associated with prostate cancer (see Fig. 7.5a). This system exhibits a sensitivity of 100 fM. Waggoner and colleagues [106] have designed a nanobiosensing platform based on arrays of nanomechanical resonators for PSA detection. The surfaces of the proposed trampoline-like devices (see Fig. 7.5b) are capped with specific antibodies and the mass of bound analyte is detected as a reduction in the resonant frequency. Antibody decorated nanoparticles are used in order to enhance sensitivity. Real samples were assayed with a detection limit of 1.5 fM. Storhoff et al. [107] have applied a procedure for the detection of prostate cancer recurrence by using AuNP in monitoring PSA levels of clinical samples. This strategy employs functionalized AuNPs as a probe of PSA captured onto antibody capped glass slides. PSA amount was quantified through silver enhancement of AuNPs [108]. Since this method show a detection limit of 15 fM, they achieved a diagnostic of prostate cancer recurrence in

Table 7.2 Potential cancer biomarkers and their applications

Biomarker	Abbreviation	Type of cancer	Application
Human epidermal growth factor receptor 2	HER2	Breast	Predictive
Epidermal growth factor receptor	EGFR	Breast	Predictive
Cancer antigen 15-3	CA15-3	Breast	Monitoring
Carcionoembryonic antigen	CEA	Colon	Monitoring
Cancer antigen 125	CA125	Ovarian	Monitoring
Prostate specific antigen	PSA	Prostate	Monitoring

clinical samples at earlier timepoint comparing with other procedures. In this regard, the device designed by Waggoner et al., probably might exhibit a similar clinical performance.

De la Escosura-Muñiz and Merkoçi [109] have designed a nanochannel/nanoparticle-based filtering and sensing platform for direct electrochemical detection of CA15-3 in blood. A membrane with nanochannels capped with specific antibodies enable both the filtering of the whole blood assayed without previous treatment and the specific detection of the target analyte. Captured analytes are reported by adding antibody decorated AuNPs as electrochemical reporters (see Fig. 7.5c). This process avoids tedious and time consuming labors and has shown a limit of detection of 52 U/mL. The same group have developed an optical ELISA for the analysis of the same antigen useful for the follow-up of the medical treatment of breast cancer. AuNPs were used as carriers of the signaling antibody anti-CA15-3 – HRP (horseradish peroxidase) in order to achieve an amplification of the optical signal. The developed assay resulted in higher sensitivity and shorter assay time when compared to classical ELISA procedures while working between 0 and 60 U CA15-3/mL (Ambrosi et al. 2009a).

Zhong et al. [110] have reported the detection of CEA through nanogold-enwrapped graphene nanocomposites as enhancer probes of electrochemical immunodetection. An immunocomplex capture antibody – CEA is reported by antibody decorated nanocomposites yielding an amplified signal with detection limit as low as 10 pg/mL. Myung et al. [111] have performed a graphene-encapsulated nanoparticle-based biosensor for the selective detection of HER2 and EGFR. Antibody decorated graphene encapsulated nanoparticles are patterned as gate of a field effect transistor where biorecognition events are detected electrically. This biosensor has shown high specificity and sensitivity (1 pM for HER2 and 100 pM for EGFR). These novel procedures have a promising potential in clinical diagnosis.

Qian and colleagues [112] have proposed a simultaneous detection of CEA and IgG by using QDs coated silica. Two different types of QDs (CdSe and PbS) are capped with two different types of detection antibodies respectively. Capture antibodies against the two target analytes are deposited onto gold substrates so as to perform immunocomplexes. After assay steps, since the selected QDs have a different electrochemical response, the target analytes are measured by voltammetry with a detection limit of 50 pg/mL. Jokerst and colleagues [113] have developed a microfluidic biosensor [114] for the multiplexed screening of CEA, CA125 and HER2 based on detection antibody decorated QDs and capture antibody capped agarose beads. QDs are used as fluorescent probes of specific biorecognition events performed onto an array of localized agarose beads (see Fig. 7.5d). Notably, ELISA method and the employment of organic dyes as fluorescent probes in the same platform were compared with this approach. The best limit of detection was achieved by taking advantage of the powerful optical properties of the QDs. For example, real samples of saliva and serum were assayed with a detection limit of 0.02 ng/mL CEA

and 0.27 ng/mL HER2. These systems could be applied to multiple tumor markers screening in clinic samples.

Since cancer cells can quickly infect their surrounding cells and the disease can spread subsequently, the detection of a tiny amount of infected cells is vital towards early diagnosis [115]. De la Escosura-Muñiz et al. [116], have designed a rapid and simple tumor cell detection device based on the specific binding between cell surface proteins and antibody decorated AuNPs. AuNPs are employed as electrochemical probes (based on the enhancement of hydrogen catalysis) achieving a detection limit of ca. 4×10^3 cells/0.7 mL. Deng and colleagues [117] have developed a system based on aptamer decorated near infrared fluorescence methylene blue nanoparticles for leukemia infected cells detection (see Fig. 7.5e). Cancer cells have been detected quickly without the need of the complicated separation steps in whole blood samples by using the fluorescence properties of the nanoparticles. They have been able to detect from 4×10^3 to 7×10^4 cells/mL in a linear range. Gold nanoparticles have also been used, through a simple electrochemical approach, for cancer cell monitoring by Maltez-da Costa et al. [118]. This platform has achieved a limit of detection around 8.3×10^3 cells/mL. Oghabian and co-workers [119] and Rasaneh et al. [120] have proposed strategies based on antibody decorated MNP as contrast agent for tumor screening by magnetic resonance imaging. Antibodies against HER2 were used for the specific detection of tumor mice cells. These strategies could be considered for further research as an MRI contrast agent for the detection of tumors in human.

7.6 Conclusions and Future Perspectives

We have described the basic principles of the nanobiosenors and we have discussed several nanobiosensing approaches for biomarker detection towards medical applications in different disease categories. Nanobiosensors offer powerful capabilities to diagnostic technology. They can enable to reduce cost, sample amount and assay time. These novel sensors can exhibit high selectivity and unprecedented sensitivity. Despite these advantages, nanobiosensors possess some potential weaknesses; for example, some of the nanomaterials production technologies are still expensive and the inherent toxicity of these materials overall while being applied for in-vivo analysis is little known yet. The exposed nanobiosensors are successfully applied as research devices and they are far from being applied in the public domain; nevertheless, close consensus with regulatory agencies (such as the European Medicines Agency or the U.S. Food and Drugs Administration) to develop comprehensive standards for nanobiosensors and procedures will ensure the operative and realistic transition of nanobiosensors to common medical devices.

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