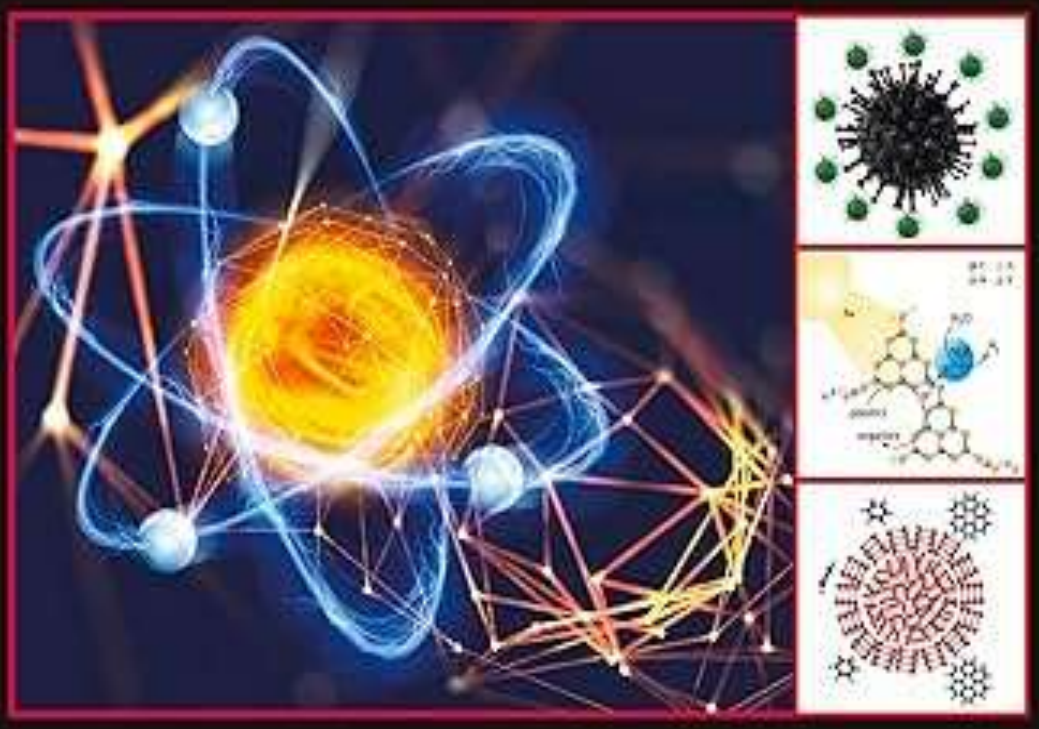
Progress in Biochemistry and Biotechnology

ADVANCES IN NANO AND BIOCHEMISTRY

Environmental and Biomedical Applications



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CHAPTER 16

Advanced functionalized nanomaterial–based electrochemical biosensors for disease diagnosis

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16.1 Introduction

An electrochemical biosensor is a device that detects the biochemical response on the surface of the sensing electrode and converts it into an electrical signal by measuring changes in conductance, resistance, or potential. Such electrochemical recognition system comprises of (a) a biologically sensitive material/recognition element/probe/receptor molecule that detects the biomarker (biomolecule indicative of specific disease or body condition)/target/analyte and generates a stimulus, (b) a transducer system that converts biological binding or biomolecule identification response as a stimulus into an electrical signal, and (c) an output signal processing system that displays the measured signal [1,2]. The rapid response, excellent selectivity, sensitivity, low-cost testing, portability of the device, low detection limits, and reduced use of sample volumes are some of the advantages which stimulated the use of electrochemical sensing methods [3,4]. A electrochemical biosensor is designed depending on the type of biomarker recognition and signal transduction. The biorecognition element is immobilized on the biosensing substrate and responsible for selectively capturing the biomarker. Biosensors can be broadly classified based on electrochemical recognition process. The two categories of biosensors are, one using biocatalytic probe (enzymes, tissues, and cells) and the other based on bioaffinity interactions. The bioaffinity-based biosensors are mainly immunosensors which use antigen–antibody binding, genosensors using nucleic acids as probes, and aptamer-based sensors employing synthetic nucleotides as receptors [5]. Based on signal transduction, various electrochemical sensing methods exist such as amperometric/coulometric biosensors, potentiometric biosensors, and impedimetric biosensors.

Nanomaterials as electrodes for biosensors provide a high-performance sensing strategy imputable to the unique conductivity and catalytic properties of the nanomaterials. Additionally, nanomaterial acts as support for immobilization of receptor molecules and further functionalization of these nanomaterials improves electrode–analyte interaction, enhancing biosensing efficiency. Among other nanomaterials, extensive research has been conducted on metal nanoparticles, carbon, and metal oxide–based nanobiosensors.

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Nanomaterial synthesized with a range of shapes and sizes such as nanotubes, nanowires, nanocubes, nanoparticles having different surface to volume ratio and electron transport property can exhibit varied sensing properties. So also, one-dimensional nanorods and nanowires, two-dimensional nanofilms, nanosheets and nanoplatelets, and three-dimensional nanoparticles offer wide possibilities of tuning the electrode interfaces. Therefore, utilizing the improved sensitivity conferred by these materials, nanomaterial-based electrochemical biosensors have made significant advancement in the diagnosis of fatal cardiovascular diseases, cancers, and other disorders by enhancing the performance of biosensors and lowering the detection limit.

16.2 Fundamental techniques of biosensing and nanomaterial-based diagnostic tools

16.2.1 Electrochemical biosensing techniques

In the electrochemical biosensing technique, a receptor molecule/material is immobilized on the electrode surface. When a biomolecule binds to the electroactive receptor system, the physicochemical transducer system detects the change and converts it into a measurable signal.

16.2.1.1 Types of electrochemical biosensors for sensing biomarkers

The electrochemical biosensors can be mainly categorized into four types, namely, voltammetric, potentiometric, impedimetric, and amperometric biosensors. The difference in these techniques is based on the difference in transduction principle, that is, detection of type of output signal (voltage, current, impedance) generated as a result of biorecognition event (binding of analyte to the receptor) [6].

16.2.1.1.1 Voltammetric biosensors

In voltametric biosensors an applied potential is varied over a range and the resulting signal in the form of current is measured. The current is proportional to the analyte concentration. This is the commonly used electrochemical technique owing to high sensitivity and its ability to detect multiple analytes. The voltametric biosensors are further classified into four main voltametric sensing tools (Fig. 16.1):

(a) Linear sweep voltammetry (LSV): In LSV, voltage is varied linearly at a constant rate and the resulting current is measured.

(b) Square wave voltammetry (SWV): In SWV, the potential between reference and working electrode is applied at a constant frequency in the form of square wave pulse in which forward waveform coincides with the staircase potential step. The magnitude of the current versus potential peaks is proportional to the analyte concentration.

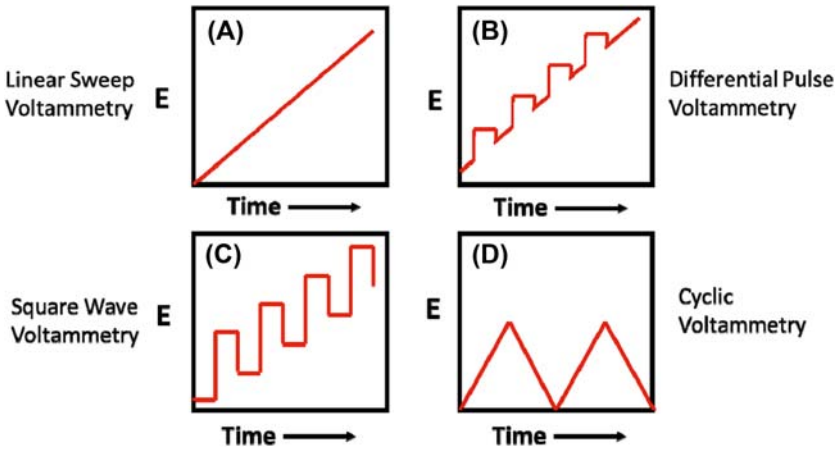

Figure 16.1 Types of voltammetry techniques based on variation of the applied potential: (A) linear sweep voltammetry (LSV), (B) differential pulse voltammetry, (C) square wave voltammetry (SWV), (D) cyclic voltammetry (CV).

(c) Differential pulse voltammetry (DPV): In DPV, voltage is applied in the form of series of short pulses, having a fixed amplitude generally ranging between 10 and 100 mV. The waveform of these pulses is superimposed on the changing linear base potential. The difference in current measured at two points of each pulse is plotted against the base potential.

(d) Cyclic voltammetry (CV): This technique is mainly used to monitor redox species. The linear potential is applied in backward and forward direction and the resulting current is measured for a cycle.

16.2.1.1.2 Amperometric biosensors

In amperometric biosensors current is measured at constant applied potential. The current is produced at the working electrode due to interaction of the electroactive species, i.e., reduction–oxidation reactions occurring on electrode surface when a constant potential is applied. Thus, the measured current is proportional to the concentration of the target analyte species. Amperometry is also one of the sensitive, precise, and rapid electrochemical technique.

16.2.1.1.3 Potentiometric biosensors

In potentiometric biosensors the change in potential between the working and reference electrode is measured. This difference in potential at the working electrode arises due to accumulation of charge owing to interaction between the analyte and the biorecognition element.

16.2.1.1.4 Impedimetric biosensor

These biosensors measure the resistive and capacitive properties at the electrode surface. A small sinusoidal signal is applied and the perturbation of the system is measured in the form of in-phase or out-of-phase current as a function of applied signal frequency [7].

16.2.2 Advanced nanomaterial–based electrochemical biosensor platforms

Remarkable properties such as high surface to volume ratio, high electrical conductivity, biocompatibility, and nontoxicity, bestowed by the nanoparticles, make them advanced materials in the biosensing field [8]. The use of these advanced nanomaterials has revolutionized the nanotechnology-based biosensing platforms by achieving high speed of detection, real-time sensing, accuracy in measurement, and thus reliability on such nanosensors. The improved sensitivity can only be attributed to the decrease in size of the particles down to subnanometer range equating to the dimensions of sensing biomolecules which leads to efficient signal transduction at the nanoscale range [9–11]. Moreover, the ease of synthesis, tunability, stability, and storage make these materials more robust as sensors over other prevailing sensor technologies. Taking into account their unique physicochemical properties, the most commonly used nanosensors are based on metal nanoparticles, metal oxides, and polymers.

The general protocol for fabrication of nanomaterial-based electrochemical biosensors involves

(a) Fabrication of transducer for electrochemical biosensor

The transducer converts the signal from the binding event between the biomarker and receptor to a measurable electrical signal, thus playing a very sensitive and selective role in the functioning of electrochemical biosensor. The first step in the fabrication of transducer involves constructing a electrode followed by surface modification of the electrode by nanoparticles (Fig. 16.2).

(b) Immobilization of biomolecule on the electrode surface

In this step the biomolecule is anchored on the nanoparticle surface. The active sites of these biomolecules are accessible for the analyte to bind [12].

(c) Electronic signal generation system connected to the transducer for data collection.

16.2.2.1 Noble metal nanosensors

Noble metal nanoparticles are in the forefront of nanosensor devices taking into account the following attributes: (i) good biocompatibility owing to surface functionalization of biomolecules, high surface energy, excellent bioactivity, and stability (ii) the superior conductivity of the metallic nature leads to fast electron transfer thus enhancing the electrochemical activity at the electrode interface and also amplification of electrochemical signal, and (iii) excellent catalytic activity due to large number of surface-active sites. Gold (Au) nanoparticles and silver (Ag) nanoparticles are the most explored nanoparticles due to their distinct physicochemical properties [13–15]. The binding of the target

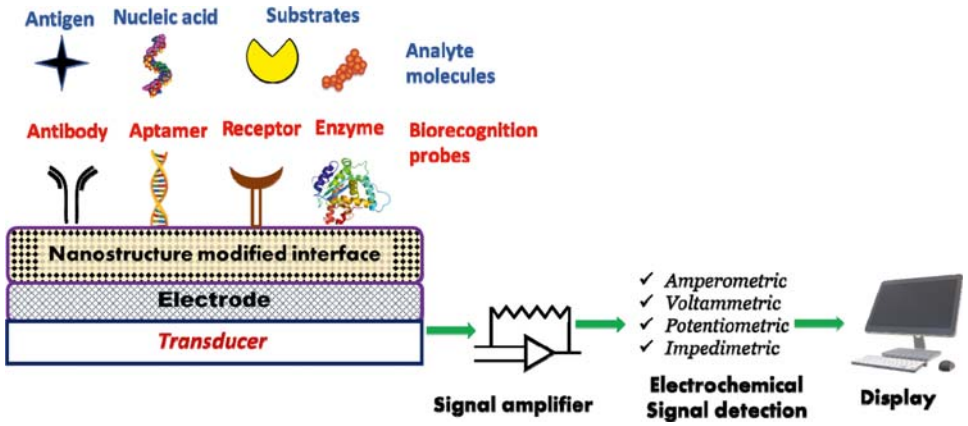

Figure 16.2 Components of nanomaterial-based electrochemical biosensors. The electrode surface is modified with nanostructured entities. The modified electrode is further functionalized with receptor probes. These recognition elements capture the analyte molecules leading to binding event on the electrode surface. The transducer converts this recognition event into a measurable signal.

molecule causes change in the electrochemical properties of nanoparticle biosensor leading to specific concentration-dependent detection of analyte. In order to be used as biosensor materials the NPs should possess unique properties of high adsorption, monodispersity, and high surface area [16]. These properties are exclusively dependent on the method of synthesis and modulation of these NPs. Nanoparticles fabricated with desired interfaces, composition, controlled size, and stability endow them with specific selectivity and sensitivity which determines the performance of biosensors [17]. Among the chemical methods of synthesis which involves reduction of metal ion followed by nucleation to form the NP, citrate-based synthesis is commonly employed. Synthesis of Au NPs by sodium citrate reduction of chloroauric acid $HAuCl_4$ using stabilizing agents is the most common method. Synthesis of monodispersed Ag NPs is challenging due to its aggregation and corrosion in electrolyte [18]. The size of NPs depend on pH, temperature, reducing agent, and the surfactant. Au and Ag NPs are also fabricated by using laser ablation and evaporation-condensation, both of which generate high purity NPs. The laser ablation is a photoinduced technique generating NPs from the nucleation of gaseous species produced by laser vaporization of bulk metal. In biological synthesis of NPs, the proteins and amino acids of fungi, bacteria, and plants acts as reducing and stabilizing agents for the formation of NPs [19–21].

For improved electrochemical signal transduction, electrode surface modification using noble metal NPs is crucial toward immobilization efficiency of biomolecules and acceleration of charge transfer on electrode surface. Depending on direct assembly of noble metal NPs on the electrode surface or formation of complex nanostructures in integration with other materials, four surface modification methods are known. These modification

methods include self-assembled monolayer formation, hybridization, layer-by-layer assembly, and sol–gel-based modification. The self-assembled monolayers are formed by amphiphilic molecules whose head group is attached to the electrode surface and the end part exposed to the environment which defines the functionalization or attachment point for the NPs or probes (Fig. 16.3A) [22]. The predominantly used surfaces for noble metal–based electrochemical biosensors are thiol/amine bonded noble metal surfaces. Such array of S-/NH- metal bonded surfaces owing to their good biocompatibility are used to immobilize receptor biomolecules. Layer-by-layer is a complex technique of formation of highly ordered structures with high control over number of layers, thickness of overall film and composition (Fig. 16.3B) [23]. It consists of variety of components including proteins and enzymes, polymers, biomolecules, noble metal NPs, other nanomaterials such as carbon nanotubes, TiO_2 nanotubes, etc., to provide high specificity and flexibility to the biosensor. For example, nanoporous films of Au were prepared on electrode surface by depositing Ag NPs and Au NPs in layer-by-layer manner using 1,5-pentanedithiol as cross-linker. The modified surface with immobilized cytochrome *c* for electron transfer was used as H_2O_2 (a product of oxidative reactions) biosensor [24]. So also, Au–Pt bimetallic alloy/glutaraldehyde/acetylcholinesterase (AChE)/choline oxidase (ChOx) multilayer was prepared on electrode surface which showed notable electrocatalytic activity toward detection of H_2O_2 [25]. Hybridization involves modification of the electrode surface by hybridizing noble metal NPs with other organic, inorganic, or polymer species. Multidimensional carbon nanomaterials, graphene, and carbon nanotubes are commonly used as hybridizing materials for biosensors. For

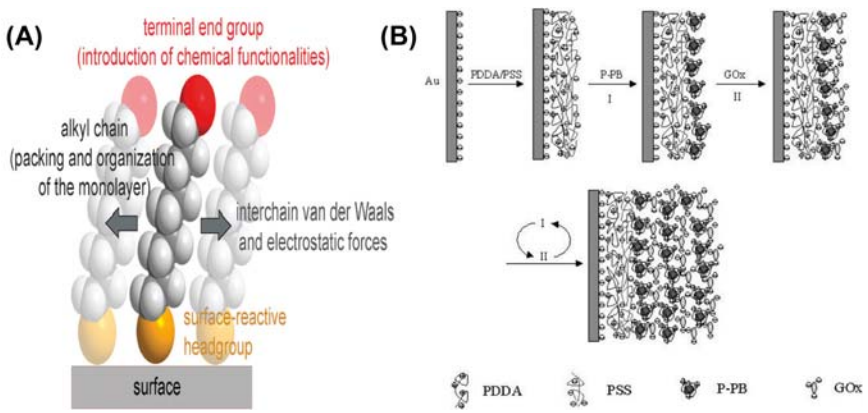

Figure 16.3 (A) Arrangement of the self-assembled molecule on the electrode surface; (B) layer-by-layer surface modification technique depicting the deposition of various polymers (PDDA, PSS, P-PB) followed by glucose oxidase enzyme as recognition element. *((A) Reproduced with permission from J.J. Gooding, S. Ciampi, Chem. Soc. Rev. (2011), Royal Society of Chemistry Copyright 2011; (B) Reproduced with permission from W. Zhao, J.J. Xu, H.Y. Chen, Electroanalysis (2006).)*

example, graphene/AuNPs/chitosan nanocomposites are used as hybrid support matrix on electrode for immobilization of protein. The collective effect of Au NPs and graphene in the biosensor led to remarkable electrocatalytic activity toward O_2 and H_2O_2 [26]. The sol–gel technique forms a 3D molecular network of noble metal nanoparticle encapsulated in polymer matrices or forming nanocomposite with other nanomaterials such as silica and alumina. This molecular network is immobilized on electrode surface for further functionalization with biomolecules [27].

Noble metal NPs modified electrodes are mainly used for immobilization of redox enzymes and proteins which need connectors to facilitate electron transfer from redox centers to electrode surface. Au and Ag NPs-based electrochemical biosensors can be categorized into enzyme biosensors, immunosensors, and DNA biosensors [29]. Enzyme biosensors include detection of glucose, hypoxanthine, and peroxidase by immobilization of glucose oxidase (GOx), xanthine oxidase and horseradish peroxidase (HRP), respectively. Immunosensors involve transducing the electrochemical signal of the binding event between antigen and antibody immobilized on the electrode. DNA biosensors involve immobilization of single stranded DNA to form double stranded oligonucleotide (DNA hybridization) based on complementary pairing principle and generating electrochemical signal upon the recognition event. Pt-based electrochemical biosensors have also shown advancement in detection of glucose, glutamic acid, and hormones [29].

16.2.2.2 Metal oxide nanosensors

Metal oxides have emerged as highly competitive nanomaterials in electrochemical biosensing field. Their unique physicochemical interfacial properties, high chemical stability, morphological flexibility, modified surface work function, high catalytic activity, nontoxicity, and ease of nanocomposite formation have gained considerable attention for their application in biosensors. In addition, their energy band alignment is suitable for biosensors owing to ease of electrochemical charge transfer. The major advantage offered by metal oxide NPs is their feasible and low-cost synthesis methodologies which include sonochemical synthesis, co-precipitation, hydrothermal or sol–gel, and thermal oxidation. These synthesis methods also allow formation of diverse morphologies in the form of nanospheres, nanosheets, porous nanoparticles, and flower- or star-like nanostructures.

For the functioning of effective metal oxide–based bionanosensor, an important step involves selection of specific metal oxide NPs for immobilizing selective biomolecule. This step largely affects the bionanosensor performance as the binding interface between the biomolecule and metal oxide NP will decide the efficiency of signal transduction. The metal oxide NP binds with the biological probe either by physical adsorption (e.g., physisorption, van der Waals, electrostatic) or by chemical binding (covalent bond formation). The electron transfer rate during recognition event depends on the

properties of this nanobiointerface. Antibodies, enzymes, and nucleic acids are common receptor units immobilized on metal oxide nanostructure modified electrodes.

Antibody immobilized metal oxide–based immunosensors binds antibodies as biorecognition probes. The fragment crystallizable (Fc) terminal of the antibody bind the metal oxide surface and fragment antigen binding (Fab) terminal is free to capture the antigen from the test sample with high specificity. The antibodies that are generally bound to metal oxide for specific biosensor development include antibiotin antibody for horseradish peroxidase (HRP) labeled and unlabeled biotin detection, monoclonal antibodies for pathogen detection, antiprostrate cancer antigen (PSA), anti-α-fetoprotein (AFP), and anticarcinoembryonic antigen (CEA) for cancer detection.

Since enzymes cannot be attached directly to the electrode surface for redox reaction, metal oxides provide biocompatible electroactive surface for superior electron transfer. Glucose oxidase (GOx) for glucose biosensing, cholesterol oxidase (ChOx) for cholesterol detection, urease/glutamate dehydrogenase for urea biosensor, and HRP for H_2O_2 detection are most commonly employed enzymes for construction of metal oxide bionanosensors. Nucleic acid–based biosensors which immobilizes single-stranded DNAs specific to DNA sequences of pathogens and tumor-specific antigens, mainly use DNA hybridization principle to fabricate biosensors for detection of viral/bacterial infections and cancers. The most commonly focused metal oxides for development of electrochemical biosensors include CuO, ZnO [30], TiO_2 [31], and Fe_3O_4 [32,33].

16.2.2.3 Conducting polymeric nanosensors

Conducting polymers (CPs), organic materials possessing electrical conductivity and redox activity, are excellent materials for biosensors due to their difference in properties with that of other nanomaterials. Polymers are chains of monomers bound together via π covalent bonds. This unique π orbital structure endow them with higher sensitivity arising from a slight alteration in chain conformations [34]. This property can be used in biosensing mechanism wherein binding of the target to the probe can cause perturbations in chain conformation of the CP, thus converting this recognition event to an electrical signal. The important conducting polymers are poly(acetylene), poly(aniline) (PANI), poly(3,4-ethylenedioxythiophene) (PEDOT), polyacrylic acid (PAA), poly(-thiophene), and poly(pyrrole) (PPy). The CPs can be synthesized chemically or electrochemically which is thoroughly discussed elsewhere [35–37]. The predominant functional groups on CPs, namely, amine ($-NH_2$) and carboxylic acid (−COOH), are used in biosensing devices as covalent attachment centers for immobilizing the biomolecules as recognition probes.

The various methods that are used to immobilize the recognition probes on CP include physical adsorption, covalent binding, electrochemical entrapment, and affinity interactions (Fig. 16.4) [38,39]. Physical adsorption is based on the electrostatic attraction between cationic CPs and anionic biomolecules and also interactions due to van der

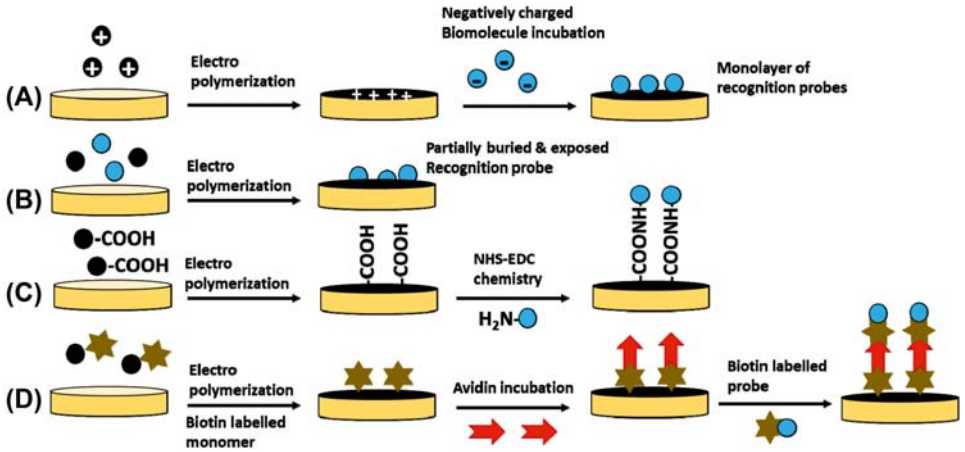

Figure 16.4 Bioprobe immobilization strategies on conducting polymers by (A) physical adsorption, (B) electrochemical entrapment, (C) covalent bonding, and (D) affinity interactions.

Waals forces and hydrophobic forces [40]. Such physical adsorption of glucose oxidase on PPy [41], enzymes [42–44], and oligonucleotides is explored [45]. Electrochemical entrapment increases the binding efficiency of the biomolecule with the polymer by partially embedding the recognition probe into the bulk polymer matrix [46,47]. This is achieved by electropolymerizing the biomolecule in presence of the biorecognition molecule. Generally, PPy is considered as the most suitable polymer for this technique. Similar methods are used for immobilizing enzymes [48,49], antibodies [50], cells [51–53], and oligonucleotides [54]. Through covalent binding the recognition probes are bound to the electrode surface by coupling to $-COOH$ and $-NH_2$ groups on CP film [55]. Enzymes such as glucose oxidase [56], urease [57], and pyruvate oxidase [58] can be functionalized on CP surface through covalent linkage. Aptamers have also been attached to CP surface using this method [59–61]. Similar strong binding is also provided through affinity interactions which relies on providing the geometric arrangement for specific binding of the probe. Avidin-biotin affinity system has strong interaction and is widely used [62,63]. Affinity binding using avidin-biotin involves electrodepositing the biotinylated monomers on surface of electrode, binding of avidin on biotin present on polymer electrode surface, and finally attaching the biotinylated probes onto the biotinylated polymer composite [64,65].

Depending on type of detection species, the CP-based electrochemical biosensors are categorized as enzyme-based, immunosensors, and DNA-based sensors. The common example of enzyme-based CP electrochemical biosensor is of glucose detection using GOx as recognition probe [46]. The anchored enzyme catalyzes glucose oxidation using O_2 and produces β-gluconic acid and H_2O_2, the reduction of H_2O_2 is detected electrochemically. The electron transfer rate of CP-based glucose sensors was increased by

incorporating noble metal nanoparticles in the polymer [66,67]. So also, the sensor performance was improved by integrating redox active molecules within CPs such as Prussian blue [68,69], doping PAA and PEDOT with poly(4-lithium styrenesulfonic acid) (PSSLi), and anthranilic acid (AA) to improve the efficiency of glucose oxidation [70]. CP-based hydrogel matrix was also used to increase the sensitivity and electron transfer rates of the biosensors [66,71]. Other than GOx, HRP-based CP biosensors for detection of H_2O_2 exhibited improved detection limit [72–74].

CP-based immunosensors using immunoglobin antibodies are composed of two main fragments, Fab (facing the analyte solution for antibody binding) and Fc (immobilized on CP surface) [75,76]. In order to decrease the number of nonspecific binding sites on CP surface, the sites are blocked using antifouling materials [77,78] or bovine serum albumin [79]. On account of functionalization of CPs with different groups, various antibodies can be immobilized on CPs making them a widely used platform for immunosensors. The typically employed functionalization include biotin/avidin binding [80], creatinine functionalization [81], and alpha fetoprotein (AFP) functionalization [82].

Construction of CP-based DNA sensors should take into account three parameters, namely, retaining the probe affinity during immobilization, hydrophilicity of CP for fast electron transfer in aqueous solution, and preventing DNA oxidative damage during measurement. Oligonucleotides as probes are incorporated in CPs using electrochemical entrapment. For example, PPy along with $(oligo(dG)_{20})$, $(oligo(dA)_{20})$ were electrodeposited on glassy carbon electrodes [54]. KCl acted as dopant to increase the electrochemical activity and conductivity [83]. Due to the leakage of probe from CP film, covalent attachment technique was preferred over electrical entrapment for immobilization of the probes. The covalent binding involved attaching the probe to the nitrogen of pyrrole ring [84,85] or attachment to $-NH_2$ or $-COOH$ groups. Click chemistry method was also used to covalently bind DNA probes with acetylene terminal groups [38,39,86]. Recently, miniaturized nanoscale–based CP (conducting polymer nanowires) label-free biosensors have displayed higher electrochemical sensitivity.

16.2.3 Functionalized nanomaterials for disease diagnostic electrochemical biosensors

16.2.3.1 Biosensors for cancer diagnosis

Cancer can be regarded as group of diseases in which cells of particular part/tissue of the body divide uncontrollably leading to formation of malignant tumors which can spread to the other body organs or attack neighboring tissues. Cancer is a second most deadly disease and a common cause of death worldwide posing a challenge to global healthcare. The cause of cancer is mainly attributed to genetic (DNA) mutations within the cells. Depending on this, around 200 different types of cancers specific to the body parts are known, such as cancer of bone, ovary, blood, lung, neuron, colon, breast, etc. Thus, an early and precise detection of cancer-specific biomarkers is of utmost importance.

Cancer biomarkers are substances or secreted molecules that are overexpressed in presence of cancerous cells in the body. Detection assays that can recognize these cancer-specific protein biomarkers is a challenge due to diversity of cancers and range of biomarkers available for each type of cancer [87]. Today this challenge has been addressed to considerable extent by designing nanomaterial-based electrochemical biosensors and development of variety of molecular recognition units functionalized on nanostructures for ultrasensitive cancer biomarker detection. A biosensor is developed by targeting a specific DNA/RNA sequence, for which a probe having an alternate sequence to that of target species is immobilized on the nanoparticle substrate [88]. The advancement in such robust diagnostic tools has led to rapid and early, sensitive, portable, selective, and noninvasive diagnosis of range of cancers resulting in timely treatment of the disease. Although the classical method of testing such as ELISA (enzyme-linked immunosorbent assay) which is a common laboratory test employing antibody detection in blood sample as a tool for diagnosis is a highly sensitive method, it involves complex techniques and time-consuming procedures [89].

The receptor units or affinity binding receptors for the detection of cancer biomarkers are generally categorized into antibodies, enzymes, and aptamers (artificial single stranded DNA/RNA fragments or oligonucleotides) depending on which biosensors are classified as immunobiosensors, enzymatic biosensors and genobiosensors, respectively. A range of functionalized nanostructures can be designed for electrochemical biosensors which can target a range of biomarkers specific to the type of cancers, the few important ones being, prostate-specific antigen (PSA), Carcinoembryonic antigen (CEA), Carcinoma antigen 125 (CA125), MicroRNAs (miRNA).

Prostate-specific antigen (PSA) with molecular weight 33 kDa is the first cancer biomarker approved by Food and Drug Administration (FDA) which is used for the diagnosis of prostate cancer. It is produced by prostatic epithelial cells. It is also known to be a possible biomarker for breast cancer [90]. A range of electrochemical affinity biosensors are designed for the detection of PSA with an aim to achieve lower detection limit [91–95]. The general fabrication protocol for PSA biosensor involves modifying the working electrode by deposition of the nanomaterial as substrate followed by functionalization of the nanoparticles with anti-PSA antibody which can capture the antigen. Upon binding of the antigen to the antibody, a resultant change in the current is observed indicative of the successful detection. Electrochemical biosensors with functionalized antibody on Au [96–101], Fe_3O_4 [102,103], and g-C_3N_4 [104,105] are designed. The limit of detection (LOD) of 23 a.m. was observed in case of biosensor fabricated from silicon nanowires using DNA aptamers as probes which operated on the principle of memristive effect [106].

Carcinoembryonic antigen (CEA) having molecular weight 180 kDa is an important oncomarker for colon cancer and other malignancies of lung, pancreas, liver, etc. It is the most observed oncomarker in human serum and low-cost method for detecting presence

of cancerous cells. Knowing the levels of this oncomarker in serum helps to monitor the stages of cancer in patient namely diagnostic stage, prognosis, and response to treatment. The electrochemical biosensor for ultrasensitive detection (LOD 4.88 fg/mL) was developed which employed a DNA nanostructure self-assembled 3D-DNA nanoprobe as recognition unit. Once the target CEA was recognized, the DNA nanotweezers opened and released one of the self-assembled oligonucleotide strands. This strand was captured by signal DNA probe immobilized on electrode and labeled with ferrocene. The capturing occurred according to DNA hybridization (base complementary pairing) principle. The Exonuclease III digested the signal probe thus leading to decline in the electrochemical signal due to loss of large number of signal probes [107]. Many other Au NPs and polymer-based biosensors are developed in which anti-CEA antibodies as probes are immobilized on the electrode surface along with formation of sandwich complex after binding of the antigen which resulted in signal amplification [108–111].

Carcinoma antigen 125 (CA125) having molecular weight 200 kDa is an ovarian cancer biomarker, also known as mucin 16 (MUC16). The standard concentration of CA125 in blood is 35 units/mL and an increased level of it can also be associated with breast cancer, mesothelioma, and gastrointestinal tract. Recently, electrochemical biosensors based on Au NPs [112–114] showed sensitive and efficacious detection of CA125 oncomarker. The AuNPs were immobilized with antibody which acted as a receptor for the antigen binding resulting in decrease in current after recognition event [115]. Using glassy carbon as electrode modified by Chitosan-Au NPs/Multiwalled carbon nanotube/Graphene oxide detection of CA125 was achieved with Au NP-lactate oxidase (LOx) as signal probe. Antibody molecules were immobilized on the electrode surface. Upon binding of the antigen, the H_2O_2 was electrocatalytically oxidized which served as a signal response [116].

MicroRNAs (miRNAs) are short noncoding RNAs of 18–24 nucleotides which play significant role in regulation of gene expression. They are an important class of biomarkers for cancers and other diseases. Due to presence of low concentration of these oncomarkers in real samples, miRNA biosensors with amplification strategies are developed for high sensitivity [117]. miRNA biosensors target miRNA sequence and work on the principle of hybridization of complementary probe on electrode. The signal amplification is achieved by accumulation of redox markers using electrocatalytic process. The cationic $[Ru(NH_3)_6]^{3+}$ is added to the analyte solution which electrostatically binds to the negatively charged phosphate backbone of miRNA nucleotide [118]. After binding of the miRNA to the probe on the electrode, the $[Ru(NH_3)_6]^{3+}$ is coupled to $[Fe(CN)_6]^{3-}$ of solution where the electrocatalysis occurs. The reduced $[Ru(NH_3)_6]^{2+}$ is oxidized by $[Fe(CN)_6]^{3-}$ to regenerate $[Ru(NH_3)_6]^{3+}$ [119,120]. This electrocatalytic cycle leads to the signal amplification in detection of miRNAs. The detection of miRNAs was also achieved by simple, cheaper, and easier nonamplified biosensors which

showed improved specificity for the binding target molecule resulting in remarkable efficiency of the biosensors [121–123].

16.2.3.1 Glucose detection for diabetic management

Diabetes mellitus is globally a major health problem which is caused by deficiency of insulin leading to hyperglycemia which is a condition of increased level of glucose in blood sugar. The role of insulin is, it signals the liver, muscle, and fat cells to absorb glucose from the blood and generate energy. Millions of glucose diabetic tests done daily using the amperometric biosensors have made glucose biosensors to dominate 85% of the global biosensors' market. The self-testing easy-to-use glucose strips which are screen-printed enzyme electrodes along with the pocket-size testing meters have captured $5 billion/year disease diagnosis market [124]. These biosensors generate signal in 5–10 s with 0.5–10 μL of blood thus turning as useful point-of-care device for glucose detection. With this advancement in glucose biosensing, diabetes patients can now be monitored accurately in a cost-effective manner. Presently, the research on glucose biosensors is basically focused considering it as a model disease to test the efficacy of the biosensors and to develop novel electrochemical sensing concepts. Thus, considering this established broad field of electrochemical glucose biosensors, the basic principles of operation of these sensors and their development over the years is discussed.

The first generation of glucose biosensors were based on the reduction of flavin group (FAD) of the GOx enzyme [125,126]. This reduced form upon oxidation with the molecular oxygen as an electron acceptor generated H_2O_2 which was detected electrochemically using a three-electrode system, with Ag/AgCl as working electrode [127,128]. The major issue with the first-generation glucose sensors was monitoring the oxygen tension and limitation of oxygen in the sample. Thus, in second-generation glucose biosensors, oxygen was replaced by other nonphysiological electron acceptor as mediators (M) which can act as redox centers to transfer electron from the enzyme to the electrode surface (Fig. 16.5). The reduced GOx was oxidized by M_{ox} and the reduced M_{red} was reoxidized by the electrode showing a current signal in proportion with the glucose concentration. The mediators could be ferrocene and phenazine derivatives, organic salts, quinones, and transition-metal complexes [129–132]. Wired enzyme electron relays and nanomaterials as connectors also played the role of mediators to facilitate the electron transfer. In wired enzyme electrodes, the enzyme was wired to a polymer backbone covalently linked to metal-complex electron relays. Such 3D network of redox-polymer/enzyme resulted in efficient electron transfer with high current output [133,134]. Considering the similar size of nanoparticles and the enzymes, nanomaterial especially Au NPs and carbon nanotubes (CNTs) were used as electrical connectors between the electrode surface and the redox center on GOx. The redox part of the enzyme was functionalized on these nanoconnectors to promote electron transportation. The third-generation glucose biosensors removed the mediator to allow the transfer of

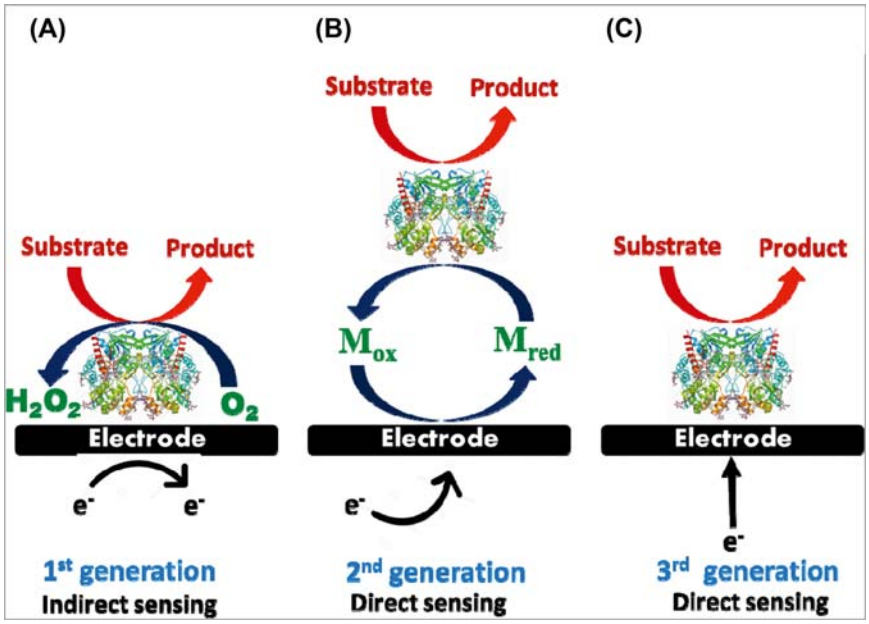

Figure 16.5 Evolution from first to third generation of electrochemical glucose biosensors, (A) first-generation indirect glucose sensing through in situ production of H_2O_2, (B) second-generation direct glucose sensing by use of mediators (M) which act as redox centers for electron transfer from glucose to electrode, and (C) third-generation direct glucose sensing allowing direct electron transfer from glucose to electrode surface.

electron directly from glucose to electrode through the enzyme-active site. The present home testing electrochemical glucose biosensors is based on testing strips. These strips are screen-printed enzyme electrodes. Both working and reference electrodes are printed on the strip with working electrode printed with reagents such as mediators, enzymes, stabilizers, etc. The filters incorporated in the test strip purify the sample by separating blood cells and provide a better sample coverage. These strips are associated with control meter which chronoamperometrically detects the signal [135].

Presently, a lot of research has been focused on developing efficient mediators for glucose biosensors which are based on functionalized nanostructures [136]. Apart from gold nanoparticles which has dominated the field of glucose biosensors, silver nanoparticles are equally found to enhance the electrochemical signal [137,138]. The improvement in the electron transport doesn't entirely depend on nanomaterial conductivity, but depend on well-defined arrangement of nanomaterial for ordered functionalization of the enzyme on its surface [139,140]. Carbon-based and metal oxide—based nanohybrids and nanocomposites are also widely explored. Carbon nanotubes and graphene oxide nanohybrids have displayed direct and faster electron transfer kinetics [141,142], whereas graphene, in case of Gox-based sensors, is known to overcome

protein hindrance which causes blockage of enzyme redox active centers and thus allow direct electron movement [143,144]. In order to improve the electrocatalytic activity of the carbon-based nanomaterials, chemical doping of heteroatoms such as nitrogen, sulfur, boron, etc., into carbon matrix is found promising [145–147]. Surface area of the carbon nanomaterials was also increased to increase the number of enzyme sites for interaction with the biomolecules, by coupling porous polyoxometalates (POM) with carbon-based materials forming nanohybrids [148,149]. Metal oxide–based nanohybrids employed in glucose biosensing produced a synergistic effect in electrochemical glucose sensing by associating the properties of two or more metals in a single nanohybrid [150–152]. They have also proven to be stable and efficient electrocatalytic centers for generated H_2O_2 decomposition [67]. In addition to metal oxides- and carbon-based nanohybrid materials, polymer-based nanocomposites have also shown potential for glucose electrochemical biosensors [143].

16.2.3.3 Cardiovascular disease diagnostic biosensor

Analysis of the mortality trends has shown that cardiovascular disease (CVD) is the most cause of death worldwide followed by cancer. CVDs are group of noncommunicable disorders of heart and blood vessels. The early diagnosis of CVD is highly important to save the lives of people especially suffering with coronary artery disease resulting in cardiac arrest which is a silent killer. There are various biomarkers of CVD that can be detected in blood serum samples or plasma [153,154], among which C-reactive protein (CRP) and cardiac troponins (cTnI and cTnT) are the FDA-approved important biomarkers.

Cardiac troponins are important biomarkers of myocardial injury and indicative of acute myocardial infarction (AMI) commonly known as heat attack. Both cTnI and cTnT is considered as a "gold standard" for specific indication of cardiac injury. The electrochemical immunosensor developed for the detection of troponins is based on the principle of immobilizing the antibody on nanostructure surface and generation of electrochemical signal upon the recognition event. In the fabrication of carbon nanotube–based immunosensors, the antibodies are immobilized on carbon through amide linkage by forming bonds with amine groups of carbon nanotubes. The Fc terminal of anti-cTnT antibodies is attached to carbon, whereas Fab exhibits affinity toward antigen epitopes [155–157]. The detection of troponins is based on use of ferricyanide redox probe which enhances the detection sensitivity of antigen–antibody binding. Some nanoparticle-based impedimetric immunosensors used 3,3′,5,5′-tetramethylbenzidine (TMB) as a signal generator for label-free detection methods. One of the two amine groups of TMB is attached to –COOH functionalized surface and other is for immobilization of probe biomolecule. Such TMB-based nanoparticle immunosensors showed detection of troponins at fM concentration in dilute serum samples [158–160]. Other immunosensors based on Au, Ag, and metal oxide nanostructures have also shown high specificity and sensitivity toward the recognition event [160–166]. Recently, highly

sensitive microfluidic sensor chips based on nanocomposites as transducers are also fabricated for immunodiagnosis of cardiac troponins [167–169].

C-reactive protein (CRP) is a biomarker for acute inflammatory processes and is used for early detection of CVD. Patients with concentrations of more than 3 mg/mL of this biomarker are considered to be at high risk of CVD or inflammatory diseases. Aptamer-based sandwich immunoassays are designed for CRP detection with signal amplification. For example, design of RNA-aptamer based CRP biosensor with Au NPs@Silica microspheres as signal enhancers is reported. In this biosensor, glassy carbon electrode was modified with Au NPs, and RNA aptamers were immobilized on Au NPs via Au–S bond. Zn^{2+}/Antibody/Au NPs@Si microspheres were used as immunoprobes. In presence of CRP, aptamer-CRP-immunoprobe sandwich structure was formed, thus resulting in change in detection current. The role of Zn^{2+} immobilized on AuNPs was to amplify the sensing signal [170]. Similar aptamer-based sandwich assays were developed as a promising tool for prediction of CRP level with lower detection limit [171–173]. Apart from such complex models for biosensing, a simpler DNA aptamer–based biosensor is also recently developed [174].

As an advancement in the detection of cardiac markers, a simultaneous multitarget (CRP and cardiac troponins) analyzing biosensor based on two different nanostructured electrodes are developed. The two electrode materials were used to immobilize two different recognition elements, each specific to cardiac biosensors. The biosensors used sandwich immunobinding principle for detection of the analyte [175,176].

16.2.3.4 Alzheimer's disease diagnosis

Alzheimer's disease (AD) is so far untreatable, progressive neurodegenerative disease which is associated with loss of memory, thus causing inability to remember the events and ultimately can lead to cognitive impairment. AD is a common cause of dementia which is caused due to brain shrinkage and death of brain cells. The main cause of AD is building up of abnormal proteins, within and around the brain cells. The two main proteins involved are amyloid and tau, one forms plaques and other forms tangles around and within brain cells, respectively. Thus, beta-amyloid (Aβ) and tau proteins are important biomarkers for the diagnosis of AD.

Utilization of proper functional nanomaterials for electrochemical biosensing is crucial for immobilization of biomolecules for high-performance and cost-effective detection devices [177,178]. Different functionalized nanostructures of metal oxides [179], metals [180–186], metal organic frameworks (MOFs) [187,188], polymers [189–191], and carbon-based materials have been developed as materials for electrode modification which have enabled low-abundance AD biomarker quantification leading to early diagnosis of the disease (Fig. 16.5). On account of their high electrical conductivity and large surface area, functionalized carbon nanomaterial–based electrochemical biosensors are widely used among other nanomaterials. Carbon nanotubes (CNTs)

film-based field effect transistor fabricated with metal semiconductor structure showed a high sensitivity for Aβ at the level of 1 pg/mL [192]. So also, 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonate) and poly (diallyldimethylammonium chloride) bifunctionalized single walled CNTs (ABTS-PDDA/SWCNTs) modified carbon electrode could simultaneously detect two biological species Aβ and tau [193]. A CuO decorated CNT-modified electrode was used for redox probe-free detection of Aβ42 which showed improved sensitivity with lower LOD (0.4 pg/mL). A plastic antibody, MPan (obtained by electropolymerization of aniline), was immobilized on the modified electrode to form MPan/Aβ-42/CNT-Cu/C-SPE. This mediator-free biosensor using plastic antibody displayed potential applications for using other biorecognition elements [194,195].

Similarly, graphene and its derivatives, i.e., graphene oxide (GO) and reduced GO (rGO) modified electrodes, were employed for fabrication of AD biosensors [196–198]. A recently developed graphene-based biosensor showed a remarkable sensitivity toward Aβ detection, wherein LOD was 41.4 fg/mL. The highly active Co_9S_8 was functionalized on graphene along with Pd nanoparticles due to its superior electrocatalytic activity toward H_2O_2 reduction. The anti-Aβ antibodies were immobilized on the modified electrode, to form G/Co_9S_8-Pd/Anti-Aβ surface which captured the target Aβ [199]. In contrast to wide use of carbon-based nanomaterials for detection of Aβ, few carbon nanomaterial biosensors are reported for quantification of tau biomarker [200–203]. In order to target multiple biomarkers of AD and to increase the diagnostic accuracy and reliability, biosensors for multiplex detection are designed. CNT-based biosensor for simultaneous detection of Aβ42, Aβ40, t-Tau, and p-Tau181 was constructed which recorded lowest LOD in femtomolar range (2.13–2.72 fM) [204].

16.3 Latest research and development in the biosensing field

The recent developments in electrochemical biosensing are mainly aimed at lowering the sample response time of the electrochemical sensor, developing a cheaper, portable ultrasensitive device and ability of sensor multiplexing to measure numerous analytes on a single device. The diagnostic procedures for detection of chronic diseases such as viral infections and cancers which require high sensitivity and specificity are generally conducted in centralized clinical laboratories. Such diagnostic testing involves high-cost sample processing and analysis tools. The latest research targets the transfer of such complex diagnostic techniques to the point-of-care which includes process of receiving the diagnostic information from a device in fraction of time at the location where the patient is receiving the care. Such places include hospital bedside and doctor's clinic. The most common example of point-of-care testing in day-today life is blood glucose sensor.

The two important advancements in the direction of developing highly sensitive and low-cost portable biosensors are constructing lab-on-chip devices using nanostructured

microelectrodes and photoelectrochemical biosensors. The development of these rapid biosensing tools has led to the detection of attomolar concentration of analytes within fraction of minutes.

16.3.1 Nanostructured microelectrodes for lab-on-chip devices

The lab-on-chip is a tiny (few cm^2 size) device made of nanostructured microelectrodes which can act as biological detector analyzing several parameters on a single chip. Such miniaturized micro-Total Analysis System (μ-TAS) monitoring multicomponents on a single device achieves high selectivity, real-time testing due to device compactness and rapid signal output [205]. The components on LOC system consist mainly of sensing elements in form of apertures grown with nanostructures for sensing biomolecular analytes, connected to microfluidic channels for the sample transportation to the sensing point. The microfluidic channels are network of microchannels embedded in a chip linked to the holes of varied dimensions which open on the surface of the chip. The testing fluid is injected through these holes, the fluid travels through the microchannels and reaches the testing electrochemical system integrated on to the chip (Fig. 16.6A). In high-throughput LOC devices the microfluidic channels are designed for mixing, separation, and manipulation of fluid to achieve multiplexing. The sensing nanostructured microelectrodes are deposited as thin films using masking method [206], templated electrodeposition [207], lithography [208], or by laser ablation [209]. The LOC devices are fabricated with cost-effective and high-performance substrates such as silicon [210], borosilicate glass [211], polymers (plastics), ceramics [212,213], and also paper [214,215]. Among other nanoelectrode deposition techniques, templated electrodeposition is widely used for patterning nanostructured electrodes on borosilicate glass substrates [216]. The electrodes can be uniformly grown on small apertures on surface of the

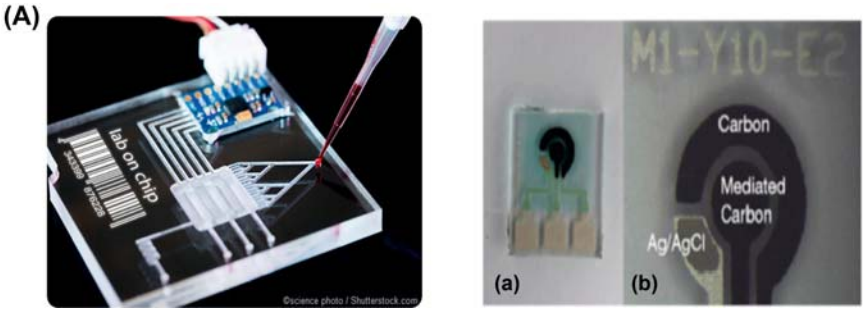

Figure 16.6 (A) Network of microfluidic channels in LOC device. The blood sample is injected through a hole which flows through microchannels and reaches the electrochemical sensing system for multiplexing analysis, (B) screen printed three electrode systems. *(Reproduced with permission from H.Y. Tan, W.K. Loke, N.T. Nguyen, S.N. Tan, N.B. Tay, W. Wang, S.H. Ng, Biomed. Microdevices (2014), Springer Copyright 2014.)*

borosilicate glass. In addition, these cheap and commonly available glass substrates provide flatness and rigidity for small aperture sizes. Plastic or polymer substrates are although the cheaper alternatives produce low resolution apertures, large background currents, thus leading to poor electrochemical performance. Printed circuit boards are other substrates with electrical functionalities for deposition of nanostructured electrodes. These substrates have ordered arrangement of electrodes with apertures that can be used to grow the nanostructures [217].

The LOC system is a three-electrode electrochemical sensing system (Fig. 16.6B). The reference electrode is generally Ag/AgCl [218–220], and gold, aluminum, carbon, and copper are commonly used counter electrode materials. The working electrode is the actual point of detection on the chip where the electrochemical sensing process occurs. The fabrication of the microchip with nanostructured mircoelectrodes as working electrodes is accomplished as follows: the surface of the chip substrate (glass/silicon) is passivated and thick films of gold are patterned on the substrate using electrode beam assisted evaporation or laser ablation or photolithography [206,208]. These gold layers in the form of metal pads connected to μm wide gold wires serve as templates for electrodeposition of metal nanostructured electrodes at the tip of the wires. These gold layers also serve as external contacts at the metal pads to off-chip instrumentation. The gold layers are further passivated to create half-micron apertures at the tip of the gold wires using photolithography. These patterned openings individually serve as templates for electrodeposited growth of nanostructured metal electrodes which are further functionalized with probes to detect the analyte [221,222]. The morphology of the nanostructures can be regulated by varying the potential applied for electrodeposition, deposition time, metal ion concentration, and electrolyte concentration. The surface morphology of the nanostructured microelectrode has an effect on the detection sensitivity. In case of palladium microelectrodes, the finely nanostructured surface showed a stronger detection signal than smooth surfaced morphology [207]. Also, the LOD for nanostructured Pd electrode was lower (1 fM) than smooth microelectrode (100 fM). This is because high surface area in case of nanostructured surfaces supports dense probe coverage, thus increasing the efficiency of the electrode.

The LOC device with nanostructured microelectrodes as sensing tool is predominantly used for analysis of nucleic acids which act as biomarkers for diagnosis of infectious diseases and cancers. The electrochemical detection of nucleic acid from the test solution is achieved through the process of DNA hybridization that occurs on the surface of the nanostructured microelectrode. The microelectrode is functionalized with single stranded oligonucleotide and upon binding of the target sequence (complementary nucleic acid strand), the DNA hybridization occurs causing increase in detection current [223]. This DNA hybridization detection is an electrocatalytic process which employs electrocatalytic reaction between $Ru(NH_3)_6^{3+}$ and $Fe(CN)_6^{3-}$. Ru(III) binds to the anionic phosphate backbone of the nucleic acid immobilized on the electrode surface

[224]. On DNA hybridization, the concentration of Ru(III) at the electrode increases due to increase in the negative charge from the bound nucleic acid phosphate backbone. With the potential sweep applied during the current-voltage scan, Ru(III) is reduced at the microelectrode which is reoxidized by Fe(III) [225]. Thus, binding of the DNA or RNA (biomarker) at the working electrode which results in the double stranded complex in turn causes detectable changes in electrocatalytic signal at the surface of the nanostructured microelectrode generating higher current.

16.3.2 Photoelectrochemical biosensors

Photoelectrochemical (PEC) bioanalysis is an emerging detection technique owing to its excellent selectivity, high sensitivity, and low background signal. Photoelectrochemistry is a branch of electrochemical science which explores the effect of light on class of materials (called photoactive materials) that absorb the light and convert into electrical energy which in turn can be converted to chemical energy. The photoactive materials upon absorbing the photons convert the energy to generate electron−hole pairs (excitons) resulting in electrical signal. When the target molecule (biomarker) interacts with the probe immobilized on the photoactive material, the generated specific recognition events alter the electrical signal through redox reactions, thus leading to the detection of the target species [226] (Fig. 16.7). Thus, the difference in energy forms of excitation source (light) and detection signal (current) allows higher sensitivity in case of PEC biosensing system compared to conventional electrochemical biosensing techniques [227]. Unlike electrochemical sensing, PEC doesn't depend on the applied potential as the generated

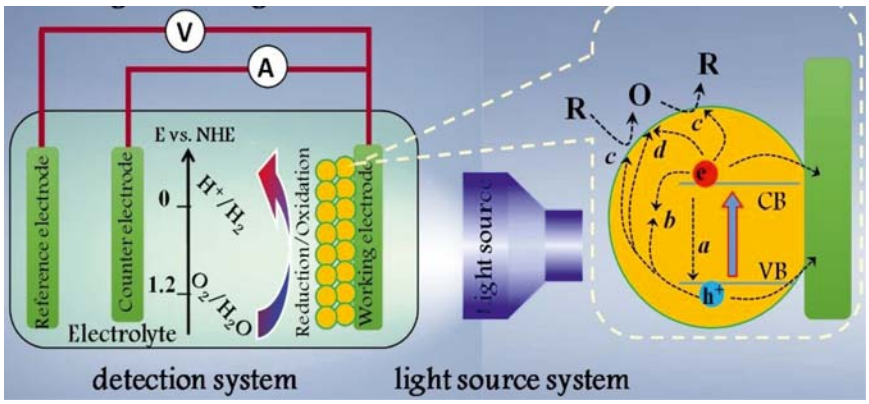

Figure 16.7 Photoelectrochemical sensing system and generation of photocarrier and their recombination pathways. The photoactive material deposited on the electrode absorbs the light generated by electron/hole pairs (excitons). These excitons or photocurrent generated contributes to the signaling mechanism upon binding of the analyte. The alteration in the detected photocurrent signifies the occurrence of recognition event. *(Reproduced with permission from J. Shu, D. Tang, Anal. Chem. (2020), American Chemical Society Copyright 2020.)*

excitons hold strong redox activity to produce measurable signal at specific potential. In addition, separation of the light source and detected electrical signal lowers the background noise [228]. The PEC sensing system is mainly composed of (i) the excitation light source, (ii) working electrode (photoelectrode) which is the transducer converting the biological signal into PEC signal, modified with photoactive material, and (iii) signal detection and display device. The photoactive materials are semiconductors acting as transducers play a vital role in PEC detection system [229]. The important semiconducting materials that have been excessively utilized as materials for photoelectrodes can be categorized into metal oxides [230–234], metal sulfides [235–237], carbon-based 2D materials especially graphitic carbon nitride [238–241], transition metal chalcogenides [242–244], and quantum dots [245–249].

PEC biosensors can be built based on the type of signaling mechanisms or strategies which are a result of recognition event on the surface of the photoactive material and its effect on the PEC reaction. There are mainly three types of recognition event triggered signaling approaches [228], reactant determinant–based mechanism, electron transfer, and energy transfer-based signaling. The reactant determinant–based signaling mechanism employs the principle of change in photocurrent which is in response to the binding event of the reactant on the photoelectrode. The alteration in the photocurrent is either due to change in the concentration of photoactive species [250–252], electron donor/acceptor, or steric hindrance caused at the photoelectrode surface resulting in poor diffusion of electron donor/acceptor species to the photoactive material. The change in concentration of photoactive species could be attributed to the increase in distance between the photosensitizer and photoelectrode upon binding of the target [253], release of the photoactive material from the electrode [254], and in situ generation of the photoactive species due to binding event [255–257]. In case of electron donor/acceptor species concentration, the detection of the target is governed by reaction of photoactive species with generated or consumed electron acceptor O_2 [258,259], electron donor H_2O_2 [260,261], and electron donor ascorbic acid [262,263] at the time of recognition event, to produce the photocurrent. The steric hindrance causes a drop in the photocurrent due to inability of the quencher molecules to diffuse to the electrode surface owing to the formation of complex entities as a result of recognition event [264–267]. The steric hindrance signaling mechanism which indicates the drop in the photocurrent as a signal to the binding of the target could also result from the formation of insoluble product on the electrode surface inhibiting the diffusion of the electron donor/acceptor species [268]. In addition, special type of semiconductor@metal-organic framework (MOF) heterostructures as photoactive materials show remarkable molecular size selective biosensing activity based on H_2O_2 and ascorbic acid as electron donor/acceptor species. A rise and decline in photocurrent is observed upon addition of H_2O_2 and ascorbic acid, respectively, displaying size selective signal for specific biomolecules detection [234].

The electron and energy transfer—based signaling strategy exploits the electrical property of the photoactive material and applied potential as factors affecting the photocurrent response. The PEC biosensors can be constructed based on electron transfer mechanism by surface sensitization or elemental doping as signal generation tags, whereas energy transfer mechanism uses surface plasmon resonance effect. Each of these signal generating mechanisms is discussed herein. As UV light is damaging to the biosystems, using visible to near infrared spectral range as PEC light source is inevitable. In this regard, the wide band gap of semiconductors as photoactive materials should be extended for absorption of visible light. Thus, surface sensitization plays a key role. A sensitizer is a material or molecule which has a higher excited state energy level than the photoactive material's conduction band. So also, through sensitization mechanism, rapid recombination of electron—hole pair is prevented by allowing charge transfer process between the sensitizer and the semiconductor. To exemplify the sensitization mechanism as signaling mode for PEC biosensor, role of CdSe quantum dots (QDs) functionalized on TiO_2 is discussed. CdSe due to its narrow band gap was photoexcited by irradiating under visible light. The generated electrons were transported from the conduction band of CdSe to TiO_2 conduction band, thus preventing the charge recombination and providing increased stable photocurrent as contrast to only TiO_2. The probes for the detection of protein (PCKS6) promoting rheumatoid arthritis were immobilized on the nanohybrid which upon binding the target led to the decrease in the detected photocurrent signal [269]. A similar sensitization effect can be observed in case of ZnO/$CH_3NH_3PbI_3$/NCQDs (nitrogen-doped carbon QDs). NCQDs sensitized ZnO/$CH_3NH_3PbI_3$ and improved its electron transfer efficiency and inhibited charge recombination. The detection of fibroblast-like synoviocyte cell was achieved by observing a decrease in photocurrent upon binding of these cells to the probe CD95 antibody immobilized on the electrode surface [270]. In addition to semiconductors, organic molecules can also be used as sensitizers for PEC biosensors [271—274].

The element-doped nanohybrid photoactive material can be a signal generation tag introducing localized electronic states which are impurity energy levels in between the conduction and valence band of the host material. Such introduced impurity levels allow for two-step photoexcitation of the parent semiconductor thus improving the photogenerated charge separation and increasing the charge recombination period [275]. To illustrate, the commonly studied case of Mn-doped CdS QDs functionalized on g-C_3N_4 [276,277], the lifetime of photogenerated electrons was increased by preventing quick recombination occurred due to jumping of these electrons from CdS energy levels to Mn energy levels. The increased lifetime promoted PEC conversion efficiency and facilitated the redox reactions. The detection of target prostate-specific antigen by g-C_3N_4/CdS:Mn functioning as PEC system biosensor was monitored as a decrease in strong photocurrent upon binding of the target to the probe immobilized on the photoelectrode [249].

The surface plasmon resonance (SPR)-based PEC biosensing technology has become an important field of sensing in biochemistry [278–280]. The SPR effect arises from the plasmonic metal nanoparticles (NPs) (Pt, Au, and Ag) which are immobilized on the semiconductor photoelectrode. According to this effect, when light photon is incident on the metal surface, the photon energy couples with the electrons on the metal surface and move under excitation. This movement of electrons is called plasmon which propagate in parallel to the metal surface. The role of SPR in PEC biosensors is enhancement of the photocurrent [281]. The influence of the target binding on this intensified photocurrent is evaluated in PEC biosensors based on SPR signaling mechanism. To understand the role of SPR in PEC biosensor, a typical example of Au deposited TiO_2 can be illustrated. The Acetylcholinesterase (AChE) acts as a probe to the (R)-NMSal neurotoxin which is a biomarker for Parkinsonian syndrome. The AChE-Au-TiO_2 nanohybrid system exhibited stronger PEC response than AChE-TiO_2 system owing to the plasmonic Au nanoparticles. Upon binding of the target to AChE probe a resultant decrease in the photocurrent was observed [282]. Similar SPR-enhanced PEC immunosensor was studied. Loading Au nanoparticles on TiO_2 showed increase in photocurrent density. Au nanoparticles functionalized with ganglioside acted as cell membrane receptor of cholera toxin unit. Upon binding of the analyte molecules on the Au nanoparticle surface recognition unit, attenuation in electrochemical field coupling effect between Au and TiO_2 was observed demonstrating a decline in photocurrent [283].

16.4 Conclusion and future perspectives

In this chapter we have summarized electrochemical biosensors as remarkable tools for disease diagnosis by instantaneous analysis of biological sample through direct conversion of biological event into an electrical signal. Over past decades, numerous advancements occurred in the design and operation of these sensors. The use of functionalized nanomaterials has revolutionized the biosensing field on account of distinct properties exhibited by the nanostructures. Various classes of nanomaterials ranging from metal nanoparticles, metal oxides, carbon-based materials, polymers to metal sulfides and metal-organic frameworks, have been explored to test their potential in biosensing. Finally, the latest advancement in the biosensing field is focused on research and development in the area of LOC devices for their application in point of care diagnostics. Besides reaching the landmarks of accuracy, rapidness in testing, precision, low cost, and stability, the LOC devices still need to unravel the challenges of biocompatibility, low toxicity, and powerless operation. Although these requirements have been resolved for glucose sensors, which is an abundant analyte in the blood sample, the analysis of low concentration biomarkers in the test solution remains a challenge. Seeing functionalized nanomaterials as potential contender to address these challenges, the nanomaterials also need to be researched for their stability in high ionic strength buffers, and varying

temperatures. The limitation in tailoring the surface chemistry of nanomaterials also adds constraint in designing multifunctional nanomaterials. For large-scale production, to advance the technology from lab bench to commercial level, successful integration of the developed nanomaterials as transducers in LOC devices is very crucial. Other morphologies and dimensionalities of nanomaterials such as nanowires/nanoneedles which are known for efficient electron transport are still unmapped.

In order to boost up and strengthen the field of LOC devices, following strategies needs to be implemented while designing the electrochemical biosensors: (a) increasing signal-to-noise ratio, (b) promoting direct interaction of nanomaterial with the biological fluid, (c) avoiding nonspecific interactions, (d) improving electron transport on the electrode surface, and (e) developing strategies through multiplexing.

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