Chapter

Current and Prospective of Breast Cancer Biomarkers

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Abstract

Biomarkers have shown great promise over the past decade the process of drug development more effective and have become an integral part of diagnosis of diseases. Biosensors were integrated with biomarker detection and point-of-care detection for signal amplification, high specificity and sensitivity, rapid response time, low cost, simplicity and multi-analytical testing. In order to detect more sensitively, these particular biomarkers have been explored with the possibility of real-time measurements in order to develop simple and compact systems which can analyze complex specimens. Various biosensors including electrochemical biosensors have recently been developed based on disease-specific biomarkers in the diagnosis of cancer disease. The main objective of the book chapter is to review research with new materials/methods in electrochemical biosensing techniques to detection of breast cancer biomarkers and evaluating latest techniques for detection of important analytes in real samples. In this book chapter, the recent development of electrochemical biosensors of breast cancer biomarkers will be reviewed. Furthermore, recent and future trend application of breast cancer biomarkers will be discussed.

Keywords: biomarkers, breast cancer, electrochemical immunosensors

1. Introduction

Cancer is considered a complex disease because several factors are involved the disease outbreak [1]. Breast cancer (BCa) is among the most common cancers in women, accounting for about 14% of deaths from cancer in women around the world. In both the developed and less developed world, breast cancer is the most common cancer in women. About 508,000 women worldwide are estimated to have died from breast cancer in 2011 [2].

One of the most important methods is the detection and analysis of cancer biomarkers. The development of cancer has the maximum potential for therapeutic intervention. Recent developments in molecular biology have made it clear that biomarkers of cancer play a major role in the treatment, prognosis and insight into cancer etiology. The National Cancer Institute describes a biomarker as a "biological molecule contained in blood, other body fluids or tissues that is a sign of a normal or abnormal process or disease or disorder."

Cancer markers are one of the most valuable tools for early detection, diagnosis, treatment, progression tracking and evaluation of chemotherapy resistance. There are over 200 different cancer-related diseases impacting various parts of the human

body. Tumor markers are usually found at low levels in the absence of a tumor. After tumor formation, level changes and therefore cancer marker clinical tests must be fast, selective and sensitive sufficiently distinguish slight changes in marker levels in complex biological fluids.

Eventually, the extensive use of tumor markers in healthcare would rely on the identification of many highly selective and sensitive tumor markers. However, traditional immunoassays such as enzyme-linked immunosorbent assay (ELISA) have some drawbacks including time-consuming, extensive incubation procedures are performed for the antibody-antigen interaction to reach equilibrium and performed by highly skilled personnel using costly and sophisticated instruments.

1.1 Electrochemical immunosensors: definitions and methods

Over the past decade, a growing number of researchers have focused on developing fast and simple-to-use biosensor technology-based techniques to detect specific biomarkers. Under the proposed International Union of Pure and Applied Chemistry (IUPAC) concept, a biosensor combines two important components of the bioreceptor and the transducer component for target detection. The chemical biosensors have enticing instruments that can be measured to convert biological interactions into electric signals. Nevertheless, the conversion of biological signal to a measurable signal is difficult due to the limitations of the biological environment and the extremely low tumor marker rates in biological samples.

The development of effective biosensors with sensitivity and selectivity has thus gained significant emphasis not only in clinical medicine, as well as in basic medicine and biomedical engineering. It is not surprising that due to their high potential in the bioassay region, the emphasis has been shifted to electrochemical biosensors as shown in **Figure 1**.

In addition to, they promote the reuse of molecules in biorecognition and prevent a time lapse among sample preparation and analysis. Moreover, biosensors have high potential to detect multiple biomarkers simultaneously. The use of electrochemical biosensors is particularly noteworthy due to their low cost, usability, sensitivity, specificity and suitability to detect low levels of molecular biomarkers

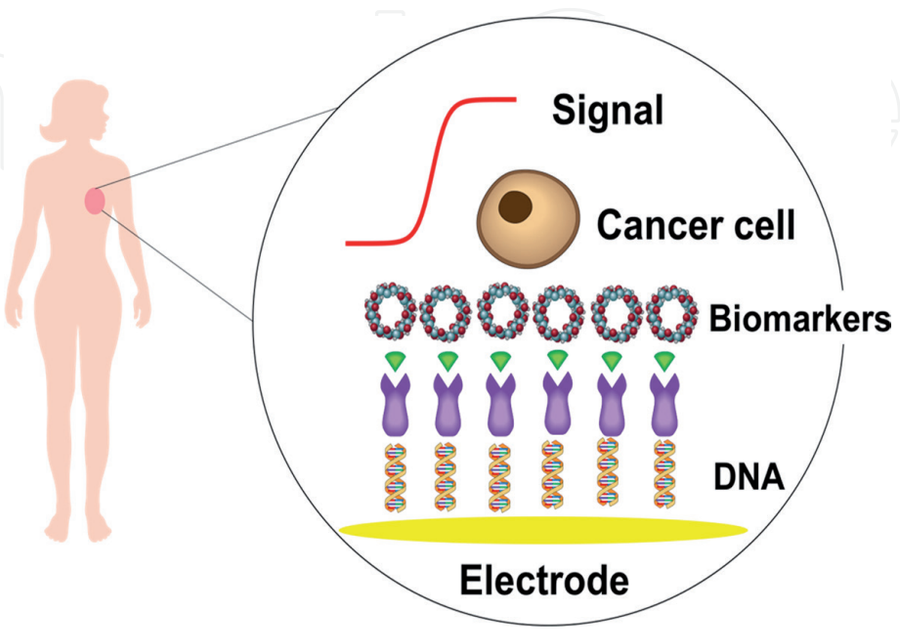

Figure 1.
The construction of breast cancer biomarkers-based biosensors.

using a range of available techniques like cyclic voltammetry (CV); differential pulse voltammetry (DPV); square-wave voltammetry (SWV) and electrochemical impedance spectroscopy (EIS). On the other hand, the electrochemical biosensors play a major role in moving towards simpler point-of-care (POC) research.

In this chapter, we addressed the molecular changes throughout cancer that have been developed and related biomarkers. Currently designed biosensor platforms and manufacturing methods to identify these biomarkers of cancer are discussed. Compared to the previous studies, this analysis highlights these biosensors analytical performance in terms of sensitivity, linear detection range and detection limit obtained with various manufacturing techniques.

2. Immunoassays with biomarkers for breast cancer

Cancer biomarkers are molecules in the presence of cancer in the body that are overexpressed. It can apply to a secreted enzyme triggered by a tumor or a specific body response to cancer. It is important to use a wide range of genomic, epigenetic, glycomic, proteomic and imaging biomarkers to recognize the point, prognosis, and epidemiology of cancer. Despite the fact that there are still multiple obstacles in turning the analysis of biomarkers into a therapeutic platform; several biomarkers dependent on genes and proteins have now been used. These biomarkers are reviewed in the following sections.

2.1 Carcinoembryonic antigen (CEA)

One of the first tumor antigens to be reported was carcinoembryonic antigen (CEA), defined in 1965. CEA is a glycoprotein of the immunoglobulin family found by radioimmunoassay or enzyme-linked immunosorbent assay in the serum of patients with cancer. A strong false positive rate in common communities and a poor test sensitivity and accuracy reduce the therapeutic benefit of CEA identification. The elevated CEA level is not unique to breast cancer because CEA can be present in many various neoplasia forms. CEA is more prevalent in ductal than in lobular carcinomas in breast tumors. During the testing process, the FDA also identified CEA as an acceptable serum biomarker for colon cancer [3]. For detection of CEA, numerous electrochemical immunosensors have been developed [4–7].

Rizwan et al. [4] fabricated the nanocomposite of gold nanoparticles (AuNPs), carbon nano-onions (CNOs), single-walled carbon nanotubes (SWCNTs) and chitosan (CS) (AuNPs/CNOs/SWCNTs/CS) for the modification of GCE (glass carbon electrode) and development of highly sensitive label-free electrochemical immunosensor for the detection of carcinoembryonic antigen (CEA), clinical tumor marker using $[Fe(CN)_6]^{3/4-}$ as mediator solution. By using layer by layer fabrication of the immunosensors were observed using CV and SWV methods. When CEA antibody combines with CEA antigen, the formed immunocomplexes formed. The decrease in the electrical signals of the immunosensor has a linear relationship for the quantitative detection of CEA ranging from 100 fg mL^{-1} to 400 ng mL^{-1} with a low detection limit of 100 fg mL^{-1}. Interestingly, a novel label-free electrochemical immunosensors [5] for detecting CEA based on gold nanoparticles (AuNPs) and Nile blue A (NB) hybridized electrochemically reduced graphene oxide (NB-ERGO) as shown in **Figure 2**. The NB-graphene oxide (NB-GO) composite was developed by the π-π interaction. The linear range of the proposed immunosensor was estimated at 0.001–40 ng mL^{-1} under optimal conditions using DPV technique and the detection limit was estimated at 0.00045 ng mL^{-1} for CEA. In addition, a silver nanoparticle (AgNPs) decorated with thionine/infinite coordination polymers as sensing platforms

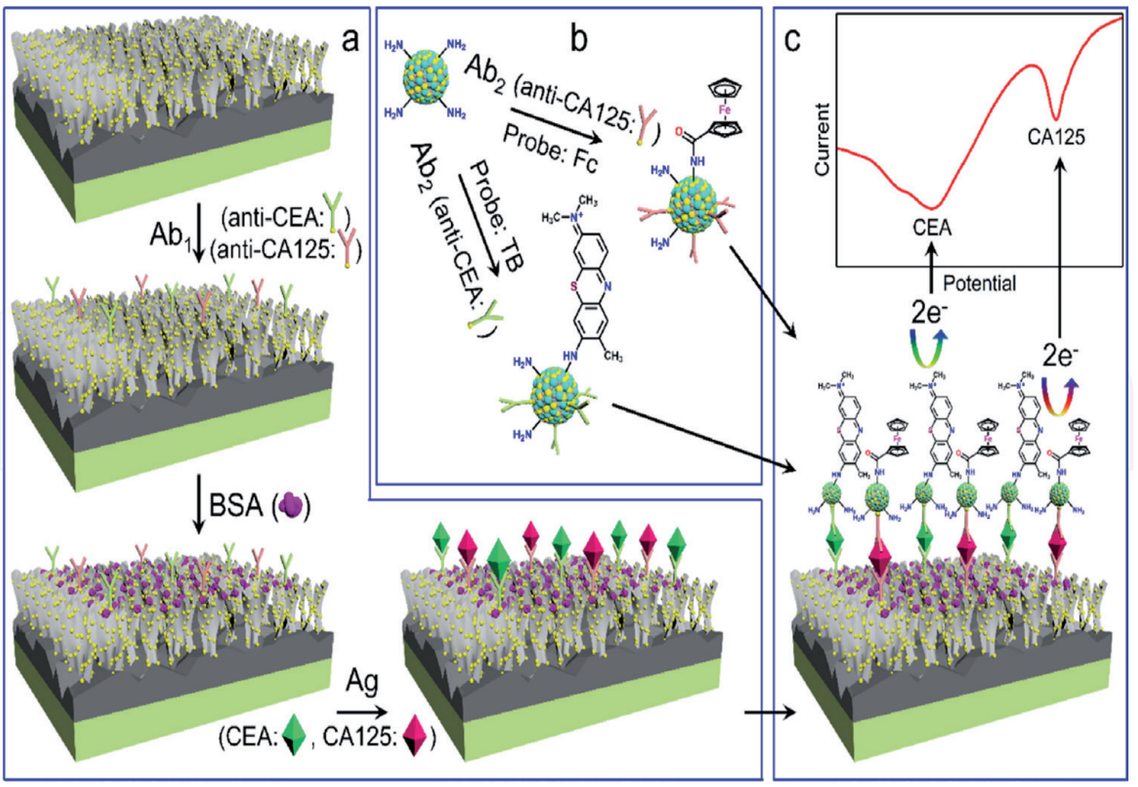

Figure 2.
Schematic of the fabrication of a sandwich-type immunosensor with a Au-VBG/BDD sensing electrode. Reprinted with permission from Ref. [5].

for detection of CEA. However, this type of glycoprotein associated with the development of breast, ovary, pancreas, lung and colon cancer. The sensor showed a detection limit of 0.5 fg mL^{-1} and a wide linear range of 50 fg mL^{-1}–100 ng mL^{-1} [6].

Additionally, simultaneous detection of carcinoembryonic antigen (CEA) and the carcinoma antigen 125 (CA125) constructed with the immunosensor containing vertical boron-doped graphene (VBG) and Boron-doped diamond (BDD) composite film by chemical vapor deposition method. These process characteristics add a wide unique surface area and strong electrocatalytic activity to the vertical BG (VBG)/BDD film resulting increase electroactive surface area. Hence, the Au-VBG/BDD signal amplification device immunosensor demonstrated strong selectivity, specificity and excellent stability for the simultaneous identification of the CEA and CA125 at 0.5–100 pg mL^{-1} and 0.5–100 mU mL^{-1} concentrations, respectively, with detection limits of 0.15 pg mL^{-1} and 0.09 mU mL^{-1} respectively [7].

2.2 Cancer antigen 15-3 (CA15-3)

CA15.3 is among the most widely accepted common biomarkers linked with breast cancer and part of the mucins family with a glycoprotein [8]. The normal level of CA15-3 is under 30 U mL^{-1} in human serum [9]. The concentration of CA15-3 is also linked to the postoperative condition, recurrence rate and monitoring of metastases of the patients. However, the serum level of this tumor marker is an important indicator for the early detection of breast cancer and the determination of disease severity. Several electrochemical biosensors focused on nanoparticles have been designed for the identification of CA15-3. According to recent studies, we can explain that the design of biosensors includes a number of distinct nanostructures among the most commonly utilized signal enhancers.

Using sandwich based electrochemical was fabricated for detection of CA15-3. According to this works, Qin and coworkers [10] used the sensitive sandwich

electrochemiluminescence (ECL) immunosensors which were modified by graphene oxide-PEI carbon quantum dots (CQDs)-Au nanohybrid to detect CA15-3. Firstly, nanocomposite has been synthesized through dopamine and Ag^+ redox reaction formed in Ag nanoparticles and polydopamine (AgNPs-PDA). The high-surface nanocomposite can provide an efficient substrate for initial antibody (Ab1) immobilization. Carbon quantum dots (CQDs) are attached by amide bonds on polyethyleneimine functionalized graphene oxide (PEI-GO). Gold nanoparticles are modified on CQDs-decorated PEI-GO substrates. Then, the secondary antibody (Ab2) was immobilized by AuNPs/CQDs-PEI-GO composite. According to this report, this ECL sensor showed good linear concentration range of CA15.3 from 0.005 to 500 U mL^{-1}, with a relatively low detection limit of 0.0017 U mL^{-1}. Further, an ultrasensitive label-free electrochemical immunosensor configured with highly conductive dendritic Au@Pt core-shell nanocrystals (Au@Pt NCs) uniformly dispersed with ferrocene-grafted-chitosan (Fcg-CS) was prepared by Wang et al. [11]. Au@Pt NCs were developed using hexadecyl dimethyl benzyl ammonium chloride (HDBAC) as a growth-directing agent through a simple wet-chemical one-pot technique. According to this report, the proposed immunosensor had a low detection limit of 0.17 U mL^{-1} and worked well over a linear range of 0.5–200 U mL^{-1}. The technique designed for the immunosensor presents a feasible approach for clinical diagnostic applications.

In another study, Cobalt sulfides/graphene nanocomposite and AuNPs (CoS_2-GR-AuNPs) was used to detect CA15-3. In this composite, AuNPs act as the immobilization site for binding of antibodies. CoS_2-GR nanocomposite exhibits excellent electrocatalytic activity against catechol oxidation and also yields large surface area that enhances the amount of CA15-3 antibody immobilized as shown in **Figure 3**. The developed immunosensor showed a wide linear range of 0.1–150 U mL^{-1} and a low detection limit of 0.03 U mL^{-1}. The immunosensor demonstrated good precision, reliability, specificity and was successfully applied in serum samples for CA15-3 detection [12].

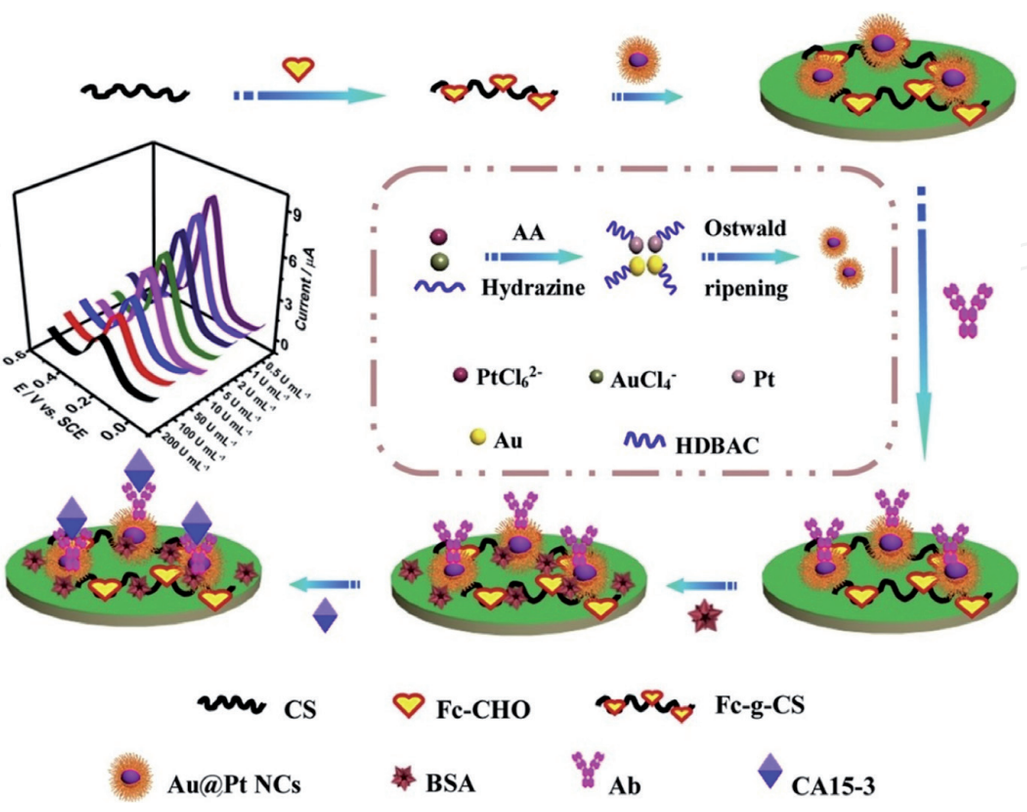

Figure 3.
Formation strategies of Fc-CS and Au@Pt NCs, along with constructing a label-free electrochemical immunosensor. Reprinted with permission from Ref. [11].

2.3 Human epidermal growth factor receptor 2 (HER2)

Anti HER2 is a monoclonal antibody (mAb) with molecular weight 185-kDa, that can bind HER2 protein and has been recognized as one of the biomarkers of breast cancer. HER2 is a receptor tyrosine kinase, a part of the cellular signaling pathways associated with the epidermal growth factor receptor (EGFR) family, which can complexes occurred in HER2 and alike proteins (such as erbB1, erbB3, and erbB4). Trastuzumab (Herceptin®), an antibody designed to treat breast cancer patients with a HER2 receptor, was the first genetically-guided medication authorized by the FDA [13], and included evaluations for its applicability before it could be used for both the diagnosis of HER2 positive gastric cancer and breast cancer [14]. HER2 overexpression in some breast cancer patients is often used as a main prognostic predictor and important treatment goals for the detection of breast cancer in adult females. The normal range in the blood of healthy women is between 2 and 15 mg L^{-1}. Since HER2 is linked with breast cancer, the development of highly sensitive biosensors to identify low levels of HER2 biomarkers is great importance.

For the detection of HER2, a disposable screen printed carbon electrode was modified with gold nanoparticles [15]. The gold nanoparticles allow rapid movement of electrons and provide a biocompatible surface to immobilize small fragments of antibodies in a directed manner, resulting in enhanced binding antigen performance. The proposed immunosensor showed a wide dynamic range of 0.01–100 ng mL^{-1} with detection limit of 0.01 ng mL^{-1}. The fabricated immunosensor with HER2-avian single chain variable fragment (ScFv) has outstanding durability with a retention rate of more than 95.6% up to 22 days. Recently, Shamsipur et al. [16] designed the label free immunosensor, functionalization of 3-aminopropyltrimethoxysilane coated magnetite nanoparticles with antibody (antiHER2/APTMS-Fe_3O_4), as a platform bioconjugate (PB), and deposited on a bare GCE. However, the PB was covered with magnetic gold nanoparticles self-assembled by thiolated antibodies (antiHER2/Hyd@AuNPs-APTMS-Fe_3O_4) containing chemically reduced silver ions, as a bioconjugate (LB) label. Under optimized conditions, using DPV, the level of HER2 was determined obtained in the range of 5.0×10^{-4}–50.0 ng mL^{-1} with a LOD of 2.0×10^{-5} ng mL^{-1}.

Furthermore, an electrochemical sandwich-based immunosensor for HER2 was designed using a lead sulfide quantum dots anti-HER2 antibody as a label (Ab2-PbS QDs) [17]. The presence of amine and hydroxyl groups from secondary anti-HER2 and coated PbS QDs are covalently linked together by carbonyldiimidazole (CDI) in the bioconjugation of PbS QDs. Using SWV signal, the proposed immunosensor, the level of HER2 was determined the linear range from 1 to 100 ng mL^{-1} with a limit of detection of 0.28 ng mL^{-1}. Recently, Freitas et al. [18] developed a quantum dots (QDs) as electrochemical label for electrochemical immunosensing strategy established on in situ detection of HER2-ECD in human serum samples. By using the screen-printed electrode, core/shell CdSe@ZnS to provide immobilization of bioreceptor functional groups as shown in **Figure 4**. The analytical performance was tested in spiked human serum samples, demonstrating an excellent performance in a wide linear range (10–150 ng mL^{-1}) with a limit of detection of 2.1 ng mL^{-1}.

2.4 MIRNAs

MIRNAs are a class of small non-coding RNA components that control the post-transcription expression of target genes by either translational repression or mRNA degradation. Mature miRNAs are made up of β22 nucleotides and are generated through 70–100 nucleotide hairpin substrate molecules. The human genome has

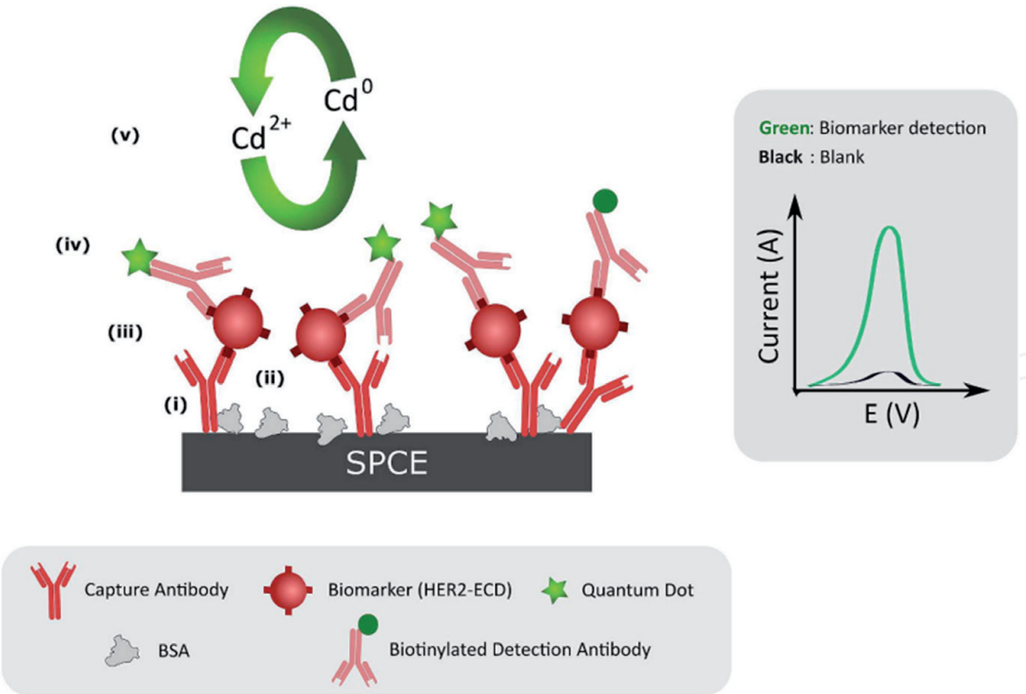

Figure 4.
Representation of the immunosensor construction and detection strategy of biomarker HER2. Reprinted with permission from Ref. [19].

been reported to be capable of encoding over 1000 miRNAs, which can control up to 60% of mammalian genes. MIRNA detection has produced a number of electrochemical sensors, which have been reviewed elsewhere.

In recent study, highly sensitive ultrasensitive electrochemical biosensor detection is of microRNA-222. In this works, the biosensor was fabricated as follows. The gold nanoparticles-modified graphene oxide (rGO/Au NPs) with capture probe (cDNA) through a thiolated group was immobilized. The modified electrode (cDNA/rGO/Au NPs/GCE) was linearly hybridized with microRNA-222 and signal probe, resulting in a sandwich structure of the modified electrode cDNA-microRNA signal probe on the surface. Under optimized conditions, using DPV technique, the biosensor of microRNA showed wide linear response range (0.5 fM to 70 nM) and a low limit of detection of 0.03 fM [19]. Further, a novel label free and simple electrochemical biosensors strategy was developed for detection of mRNA-21. From this research works, firstly confirmed with excellent ability of AuNPs superlattices for electron transport and tunable structures, a remarkable improvement was made in the immobilizing quantity of probe molecules on the electrode surface and a major improvement in the substratum's electrical signal. Moreover, toluidine blue (TB) and micro-RNA interaction established with negatively charged backbone phosphate groups increasing electrostatic interaction. By using this technique, microRNA with a relatively low detection limit of 78 aM can be identified in a linear range from 100 aM to 1 nM. The proposed electrochemical nano biosensor could be clinically valuable in early breast cancer diagnosis through direct identification of serum microRNA-21 in real clinical samples without sample testing [20].

In addition, Azimzadeh et al. [21] developed a novel electrochemical nano biosensor for the detection of miRNA-155. From this works, this methodology based on thiolated probe-functional gold nanorods (GNRs) on a graphene oxide (GO) layer on a glass carbon electrode surface as shown in **Figure 5**. Nevertheless, the authors used Oracet Blue (OB) for the first time in the proposed electrochemical nano biosensor as an electroactive miRNA label. A novel intercalating label Oracet Blue, the reduction signals were measured using the method of differential pulse

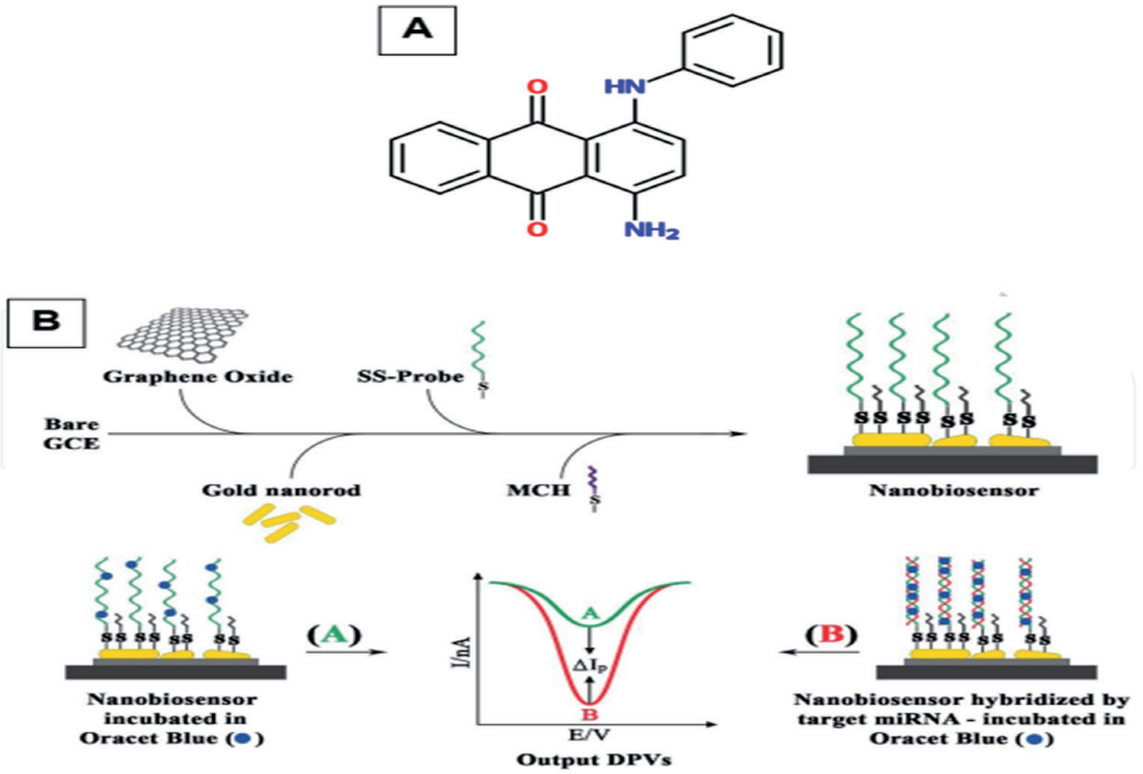

Figure 5.
(A) Molecular structure of the Oracet Blue molecule, (B) schematic illustration of the assembling and working procedure of the proposed electrochemical nanobiosensor for miR-155 detection. Reprinted with permission from Ref. [22].

voltammetry technique. This electrochemical nano biosensor method provided a linear range between 2.0 and 8.0 fM and a limit of detection of 0.6 fM. In addition, the versatility of the proposed nano biosensor has been demonstrated by direct detection of the miR-155 in plasma without the need for sample extraction and/or amplification, which can be applied in clinical applications such as early detection and/or as a predictor of drug response and prognostic trends in patients with breast cancer. Lastly, the proposed electrochemical nano biosensing method could also be used to detect any sequence of miRNAs, simply by changing the capture probe.

2.5 Cancer antigen 125 (CA125)

The cancer antigen 125 also known as mucin 16 (MUC 16) is a therapeutic tumor marker present in many ovarian cancer cells on the surface. However, some malignant diseases such as breast cancer, mesothelioma, non-Hodgkin's lymphoma can lead to increased CA125 levels. Usually, the normal blood CA125 concentration is less than 35 U mL^{-1} (units per milliliter) [22, 23]. A number of electrochemical immunosensors for detection of CA125 have been developed so far.

Recent study, Fan et al. [24] developed paper-based electrochemical immunosensor to detect cancer antigen 125 (CA125) by screen-printing method. The reduced nanocomposites of graphene oxide/thionine/gold (rGO/Thi/AuNPs) were compounded and coated for CA125 antibody (anti-CA125) immobilization and identification signal amplification on the paper electrode of the immunosensor. The principle of detection was based on the premise that the immunocomplex produced by the binding of CA125 antibody and antigen could reduce the current thionine reaction, which was corresponding to the corresponding CA125 antigen concentration. The results of the immunoassay demonstrate that the linear range of CA125 was between 0.1 U mL^{-1} and 200 U mL^{-1} with a detection limit of 0.01 U mL^{-1}.

In addition, Ravalli and coworkers [25] developed the analytical performances of a label-free impedimetric immunosensor for the detection of tumor marker CA125 using modified screen-printed graphite electrode in gold nanoparticles. In this works the immunoassay is focused on a self-assembled monolayer (SAM) of electrodeposited gold nanostructures with corresponding monoclonal antibody immobilization on screen-printed graphite electrodes as shown in **Figure 6**. The development of immunosensor each steps are characterized using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) techniques. The response of the immunosensor ranged linearly (r^2 = 0.996) with antigen concentration varying from 0 to 100 U mL^{-1}. The approximate detection limit was found to be 6.7 U mL^{-1}. Also, the immunoassay was tested on serum samples, resulting in promising results for use with real samples.

Recently, Baradoke et al. [26] constructed Screen-printed carbon electrodes were electroplated with gold or platinum nanostructures and used as an antibody immobilization platform for CA125. In this works, nanostructured surfaces have been used as a tool for the design of immunosensors and analyses in electrochemical kinetics such as antibody immobilization, electrode surface blocking and antigen binding. In addition, the detection of CA125 was demonstrated on the coated Au and Pt nanostructured interfaces with a LOD of 419 ± 31 ng mL^{-1} and 386 ± 27 ng mL^{-1}.

2.6 Breast cancer type 1 and 2 susceptibility proteins (BRCA1 and BRCA2)

In regards to breast cancer, some hereditary gene mutations were linked with the development of cancer, mainly identified with BRCA1 and BRCA2 tumor suppressor genes [27]. In specific, the BRCA1 gene encodes a protein of 1863

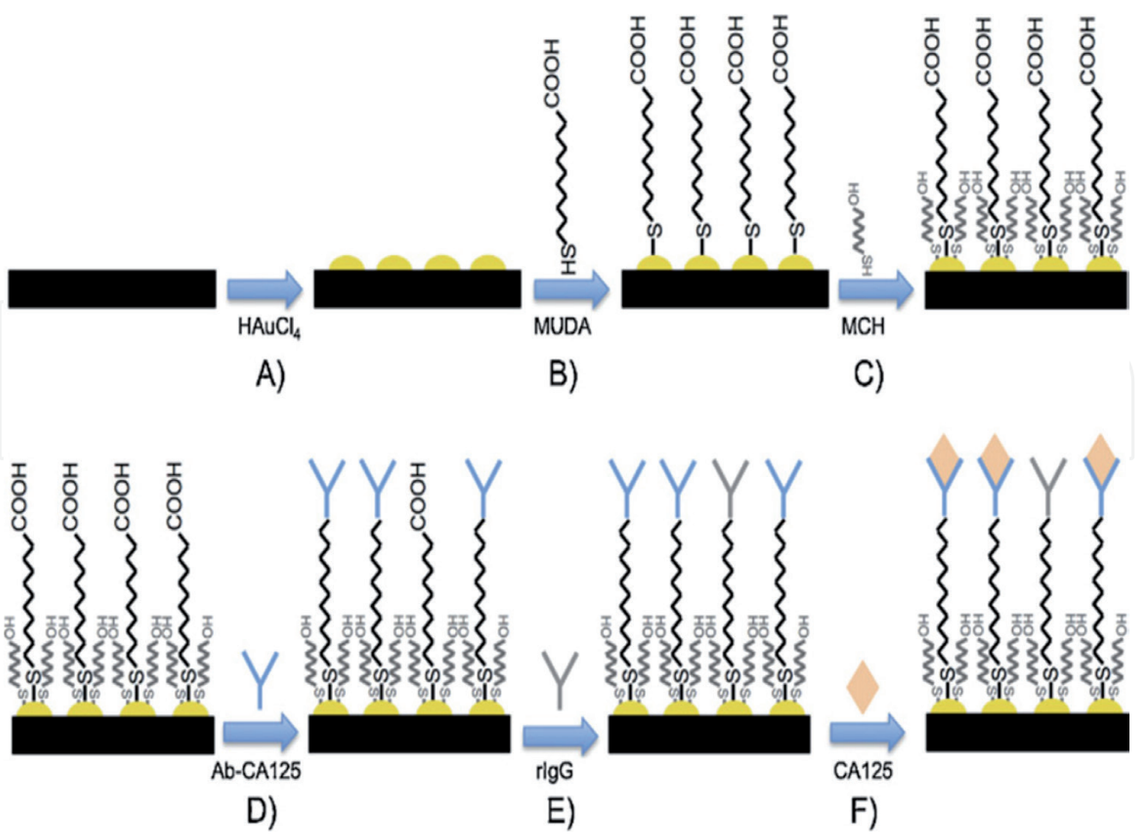

Figure 6.
Scheme of CA125 immunosensor developed: (A) electrodepositing of AuNPs on SPGE, (B) functionalization of gold nanoparticles with MUDA, (C) mixed SAM formation with MCH, (D) activation of COOH groups with EDAC/NHS and Ab-CA125 immobilization, (E) blocking step with rIgG, (F) Ab-Ag affinity reaction. Reprinted with permission from Ref. [26].

amino acid implicated in genomic stability. Mutations in this gene are characterized as closely associated with and early onset of family breast cancer syndrome. These are also responsible for controlling and managing the checkpoints and cell division of the cell cycle. Mutations in the BRCA1 and BRCA2 genes are linked to increase of breast cancer and are essential for about 21–40% of hereditary cases of breast cancer [28]. BRCA1 protein expression was reported to be decreased in 30% of sporadic cases of breast cancer [29]. The extent of the BRCA1 protein reduction depends on the extent of the breast cancer and is inversely to the expression of BRCA2 protein used as a tool for the treatment of sporadic breast cancer [30]. Furthermore, BRCA2 can be used for breast cancer as both a prognostic and a screening biomarker.

Currently, Shahrokhian and Salimian [31] developed an ultrasensitive label-free electrochemical DNA (E-DNA) sensor based on conducting polymer/reduced graphene-oxide platform has been developed for the detection of BRCA1 gene. An electrochemical technique was used as a simple and easy to control method for the electrochemical reduction of graphene oxide and also for the electropolimerization of the monomer of pyrrole 3 carboxylic acid. The signal produced from the E-DNA sensor uses CV, DPV and EIS methods to detect the redox probe's electrochemical behavior. This sensor allows BRCA1 to be quantitatively determined in the linear range of 10 fM to 0.1 μM with a low detection limit as 3 fM. In addition, the modified electrode was effectively used in blood plasma samples to accurately determine the trace amounts of the DNA target.

Furthermore, recently developed novel immunoassay based on multiple polymer signal amplification technique for BRCA1 protein recognition. The developed immunoassay processed by poly (dopamine-beta cyclodextrine-cetyl trimethylammonium bromide) doped by silver nanoparticles (P[DA-β-CD/CTAB])-AgNPs and functionalized mesoporous silica matrix (MCM-41-SO_3H) produced on the glassy carbon electrode with a large surface area that has been designed to provide a new device for the immobilization of primary antibodies and outstanding conductivity. MCM-41-SO_3H provides the appropriate volume of pores and functional groups to detect further horseradish peroxidase-labeled antibodies and improve conductivity to further amplify the electrochemical signal. The experimental immunoassay indicates adequate analytical efficiency for BRCA1 screening with a linear range of 0.01565–10 pg mL^{-1} (DPV) and 0.625–20 pg mL^{-1} (SWV) and a low quantification value of 0.003 pg mL^{-1} [32].

In another study, label free DNA biosensor on a modified magnetic bar carbon paste electrode for BRCA1 mutation detection. In this research works, firstly, Fe_3O_4-RGO nanoparticles were synthesized, accompanied by physical adsorption of the synthesized nanoparticles composite to the built magnetic bar carbon paste electrode (MBCPE) as shown in **Figure 7**. Using PANHS leads to decreasing electrode preparation, possessing an excellent selectivity for determination of BRCA1. However, the composite of the nanoparticles are linked with using 1-pyrene butyric acid-N-hydroxy succinimide ester (PANHS) as a detection of DNA sequence also (BRCA1 5382 insC mutation detection) strands were immobilized on the surface of the electrode for exact incubation time. By using EIS technique the linear range (1.0×10^{-18} mol L^{-1} –1.0×10^{-8} mol L^{-1}) and the low detection value of 2.8×10^{-19} mol L^{-1} [33].

2.7 Interleukin-6 and -8 (IL-6 and IL-8)

Interleukin-6 (IL-6) is a leukocyte-secreted 21 kDa glycoprotein, related to as both a pro- and an anti-inflammatory cytokine because it has roles in both directions. Interleukin (IL)-8 is an inflammatory chemokine contained in the subfamily C-X-clinically relevant rates of IL-6 in physiologically normal situations

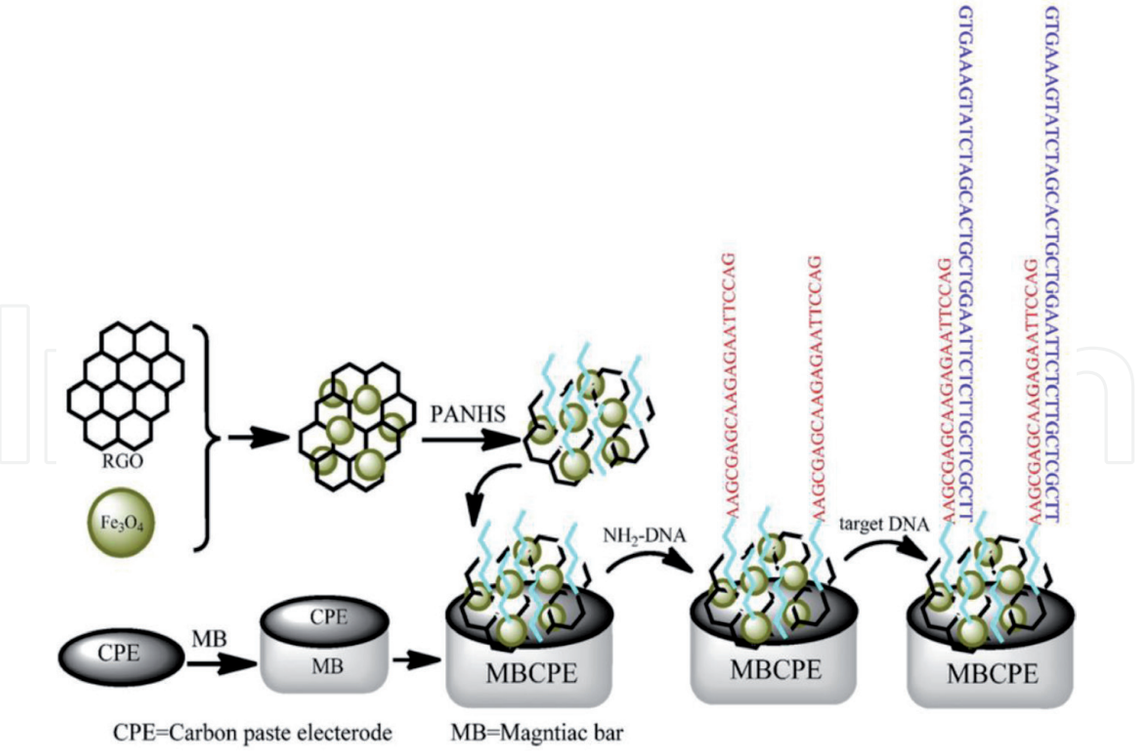

Figure 7.
Schematic representation of the modified electrochemical biosensor based on MBCPE/Fe_3O_4-RGO/PANHS platform. Reprinted with permission from Ref. [34].

have recently been recorded as 5–25 pg mL^{-1} and up to 1000 pg mL^{-1} in sepsis patients [34]. IL-8 is expressed by certain cell types, including activated monocytes and macrophages, a wide range of epithelial cells and fibroblasts [35].

For the detection of interleukin 1β in human serum and saliva, new impedimetric immunosensor was prepared using semi-conductive poly (2-thiophen-3-yl-malonic acid) (P3-TMA) as matrix material for immobilization and anti-IL-1β antibody as a component for biorecognition. P3-TMA added a lot of antibody binding in the presence of carboxyl groups. EIS and CV techniques were used d to monitor the detection of IL-1β antigen concentration in the range of 0.01–3 pg mL^{-1} with the detection value of 3 fg mL^{-1} [36].

Using a similar principle, an impedimetric immunosensor for highly sensitive detection of IL-8 is in human serum and saliva samples. 6-phosphonohexanoic acid (PHA) was used to label the anti-IL-8 antibody. In addition, anti-IL8 antibody interaction to IL8 antigen was observed using SFI (single frequency impedance) technique as shown in **Figure 8**. EIS technique was applied for the interrogation of IL-6 level in the linear range of 0.02–3 pg mL^{-1} with a detection value of 6 pg mL^{-1} and good stability (7 weeks) [37].

2.8 Vascular endothelial growth factor (VEGF)

Specific tyrosine kinase receptors divided into subtypes of VEGFR-1, VEGFR-2, and VEGFR-3 are vascular endothelial growth factor receptors (VEGFRs). VEGFR-2 activates most angiogenic processes among the three forms. VEGFR-2 is an important biomarker for breast cancer with a blood level of >15 ng L^{-1} indicating the presence of tumors of the breast cancer [38].

For detection of VEGFR-2, a sandwich immunoassay was designed to detect VEGFR-2, by immobilizing anti-VEGFR-2 Ab1using chitosan/rGO/thionin-modified GCE as shown in **Figure 9**. An HRP-labeled Ab2 was used to identify antibody,

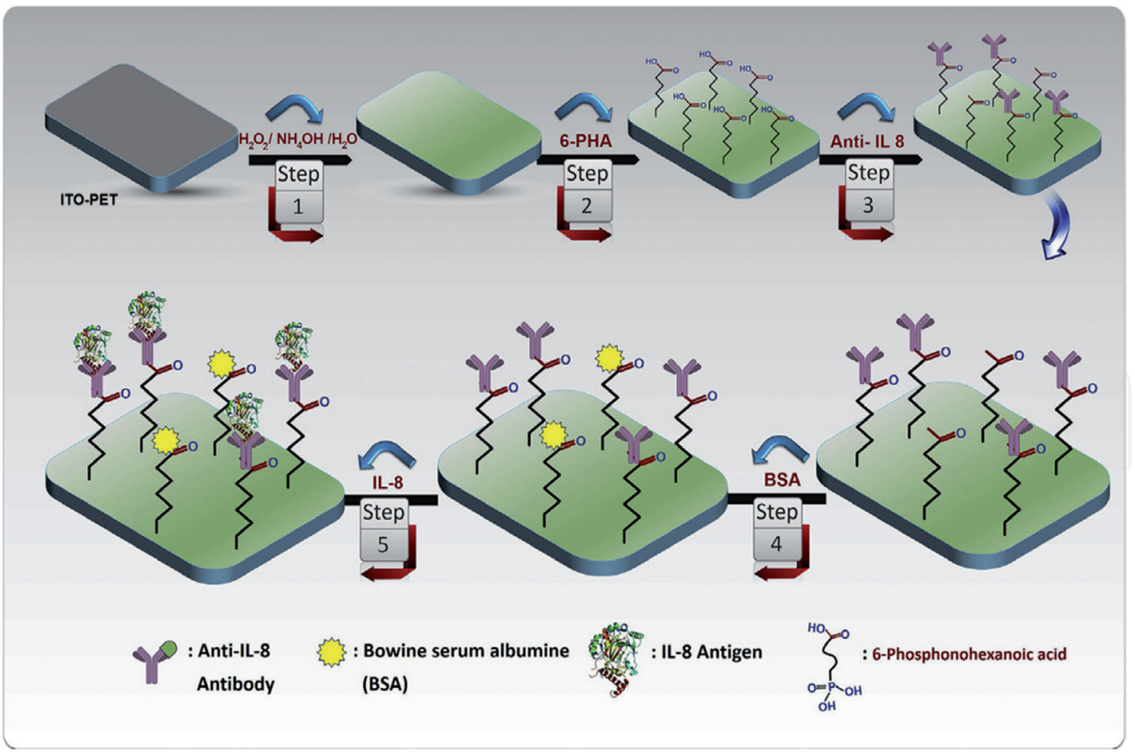

Figure 8.
Schematic representation of the impedimetric immunosensor IL-8 detection. Reprinted with permission from Ref. [38].

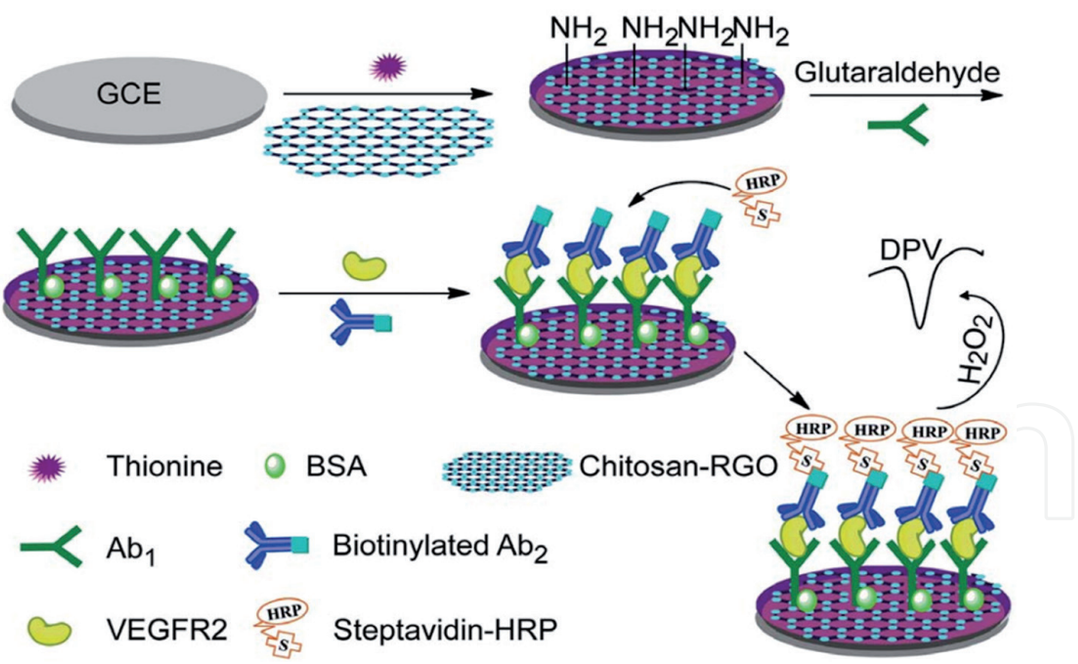

Figure 9.
Schematic representation of the electrochemical biosensing for VEGFR2 detection. Reprinted with permission from Ref. [40].

catalyzing thionine oxidation by H_2O_2. VEGFR-2 was quantified by DPV with a detection limit of 0.28 pM in the linear concentration range of 0.4–86.0 pM [39]. For its detection, VEGFR, sensitive label-free impedimetric sensor fabricated on molecular impressed polymer (MIP) as a biomimetic receptor coupled with screen-printed electrodes. Next, o-phenylenediamine (oPD) electropolymerization was conducted on graphite-screen-printed electrodes in the presence of VEGF

molecules through cyclic voltammetry. The single-use MIP-based sensor demonstrated good analytical efficiency for VEGF detection from 20 to 200 pg mL^{-1} with a detection limit of 0.08 pg mL^{-1} using with EIS technique [40].

2.9 Cluster differentiation 146 Ag (CD-146)

Cluster differentiation 146 Ag (CD-146) is a molecule of cell adhesion that belongs to the superfamily of immunoglobulins. It is identified as a progression marker for melanoma (melanoma adhesion molecule antigen) and breast cancer. The normal level of CD-146 in blood serum of healthy individuals is generally 309 μg L^{-1} [41].

For the identification of CD-146, a sandwich-based amperometric immunosensor was manufactured in which rGO-tetra ethylene pentaamine (TEPA) enhanced GCE antibody (Ab1) was immobilized as shown **Figure 10**. This improvement offered the electrode a large number of amino groups to improve the loading potential of antibodies. The secondary Ab was controlled with colloidal sphere TiO_2 and nanoparticles Au/Pd and assay was conducted by calculating the amperometric reaction to electrocatalytic reduction of H_2O_2. However, the immunosensor displayed a wide linear range in 0.0050–20 ng mL^{-1}, a low limit detection value of 1.6 pg mL^{-1} [42].

Furthermore, biomimetic mussel-inspired polydopamine coating photoelectrochemical biosensing chip was constructed to detect CD146. The CdS/TiO_2-ITO chip was designed using the electrodeposition process to deposit CdS on the TiO_2-ITO chip. In addition, the PDA (polydopamine), developed by DA (dopamine) self-polymerization, was anchored on the CdS/TiO_2-ITO (cadmium sulphide/titanium dioxide-indium tinoxide) chip surface through its strong adhesivity and specific interactions such as electrostatic attractions or covalent bindings. Also, without using external crosslinkers, PDA/CdS/TiO_2-ITO chips could be used for direct immobilization of antibodies. By measuring the photocurrent responses to different concentrations of CD146, quantitative determination of CD146 was achieved based on this principle. The photocurrent decreased linearly from 1 pg mL^{-1} to 20 ng mL^{-1} with an increase in CD146 concentration and a detection value of 0.3 pg mL^{-1} [43].

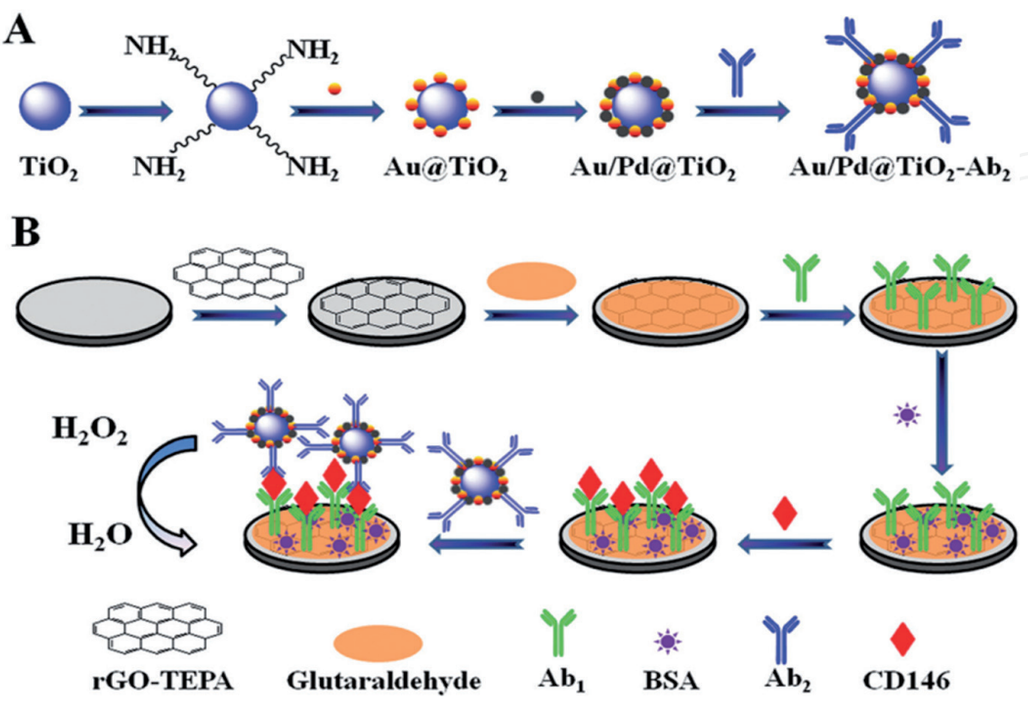

Figure 10.
Schematic representation of the preparation of Au/Pd@TiO_2-Ab2 (A) and immunosensor (B). Reprinted with permission from Ref. [43].

2.10 Other important biomarkers

Biomarkers such as tenascin-C (TN-C); p53 tumor suppressor protein (p53); DNA methyltransferase (DNA-MTase); estrogen receptor (ER); matrix metallopeptidase 9 (MMP-9); apurinic/apyrimidinic endonuclease 1 (APE1); mucin-like carcinoma-associated antigen (MCA); murine double minute 2 (MDM2); nuclear factor kappa B (NF-κB) are other important biomolecules that are known as tumor markers for breast cancer [44].

Tenascin-C is a large extracellular matrix protein newly expressed during tissue remodeling processes including angiogenesis, inflammation, and tumor growth. Tenascin-C is especially overexpressed in tumor tissues [45]. For the detection of TN-C a chemiluminescence (CL) based voltammetric immunosensor was prepared using carboxylated carbon nanoparticles (cCNPs) and conjugated with magnetic beads (MBs). In addition, an aptamer labeled with the CL reagent N-(4-aminobutyl)-N-ethylisoluminol was adsorbed to form labeled aptamer modified cCNPs-MBs on the surface of carboxy-modified magnetic carbon nanoparticles. When the tenascin-C sample is applied, it will associate with the labeled aptamer to build a complex with the labeled aptamer. This complex is measured by CL whose amplitude is linearly linked to the tenascin C concentration by 1 pM to 1 nM and the detection value as 0.4 pM respectively [46].

Tumor suppressor protein (p53) is an important transcription factor in regulating cellular responses to stress factors. Loss of p53 activity induces tumor activation and gene mutation, resulting in a conformational alteration in the p53 protein structure [47]. The level of p53 protein in serum samples from cancer patients varies from 0.52 ± 0.23 ng mL^{-1} to 1.03 ± 0.59 ng mL^{-1} [48]. Another, nanocomposite based ultrasensitive electrochemical immunosensor for p53 was designed using polycysteine/graphene quantum dots/gold nanoparticle. In this study, p53-antibody was immobilized on a green and biocompatible nanocomposite comprising L-cysteine (P-Cys) as a conductive matrix and gold nanoparticles (GNPs) as a dual amplification component of graphene quantum dots (GQDs). This approach facilitated the linear analysis of p53 in the range from 0.0244 to 0.369 pM (SWV technique) and 0.195–50 pM (DPV technique) with a lower limit of 12.1 fM [49].

DNA methyltransferase (DNAMTase) is a widespread epigenetic alteration in both prokaryotes and eukaryotes and plays a crucial role in controlling gene expression, genomic stability and cell growth. Most tumors such as thyroid, liver, heart, prostate and breast cancer have over-expression of DNAMTase [50]. Further, DNAMTase was detected by label-free electrochemical biosensor for identification and inhibitor screening of methyltransferase activity based on graphene quantity and enzyme-catalyzed reaction. However, in this work, the modified HRP catalyzed the 3, 3′, 5, 5′-tetramethylbenzidine hydrogen peroxide-mediated oxidation resulting in an electrochemical signal production. The proposed biosensor achieved sensitivity detection of MTase activity within the range of 1–40 U mL^{-1} with a detection limit of 0.3 U mL^{-1} [51].

The biomarker of the estrogen receptor alpha (ERα) is a receptor and transcription factor with nuclear hormones. It controls gene expression and inhibits the proliferation and differentiation of cells in the target tissue. The occurrence of elevated ERα rates in the breast epithelium that implies an increased risk of breast cancer, indicating both the function of ERα in initiation and cancer progression [52]. For ERα detection, a electrochemical magneto immunosensing platform was developed taking an anti-human ERα antibodies modified SPCE. The antibody acquisition was immobilized on modified magnetic beads in carboxylic acid compounds, while the biotinylated antibody was labeled with a streptavidin-HRP conjugate. In addition, determining the target ERαprotein with a detection limit of 19 pg mL^{-1} evaluated the applicability of the integrated disposable magneto immunosensor [53].

Matrix metalloproteinase 9 (MMP-9) is an extracellular 92 kDa protease belonging to a family of endopeptidases dependent on zinc and calcium. There was an improvement in expression of MMP-9 in a number of different tumors relative to healthy subjects, with an overall positive association between tumor aggressiveness and activity levels of MMP-9 [54]. For detection of MMP-9, SERS (surface-enhanced Raman scattering) nano-tags integrated magnetic-separation biosensor was designed with whole blood. The silica coated Ag SERS nano-tags built as labels were used to identify MMP-9 in unprocessed blood samples in a quick and accurate MSB immune sensor. The results showed the sensitive and reproducible response constructed in whole blood to the concentration of MMP-9 in the range up to 100 ng mL^{-1} with a detection limit of 1 pg mL^{-1} [55].

Human apurinic/apyrimidinic endonuclease 1 (APE1) is an intracellular multifunctional enzyme, also known as the redox impact factor 1. For detection of APE1, a novel electrochemical biosystem was designed by immobilizing antibody-APE1 modified on gold electrode. The technique skillfully incorporates immunoassay through an intricate template of enzyme activity study. The APE1 biosensor detection limit is as low as 0.00518 U mL^{-1}. The technique will monitor enzyme activity controlled by an APE1 inhibitor and its isozyme discrimination [56].

Mucin 1 (MUC1) protein is a membrane-associated glycoprotein containing 31 amino acids in the hydrophobic domain, 69 amino acids in the cytoplasmic domain and 20 identical amino acids per repeat in the extracellular domain. MUC1 is also a well-known tumor marker present in a range of malignant tumors [57]. For detection of MUC1, carboxylic group of disposable electrochemical immunosensors rich in graphene oxide for the identification of biomarkers with methylene blue using with human serum samples. The authors investigated highly conductive surfaces of carboxylic group rich graphene oxide on screen-printed carbon electrodes in this process. The established immunosensor demonstrated good detection range (0.1–2 U mL^{-1}), for MUC1 with outstanding linearity with a detection limit of 0.04 U mL^{-1} by using the differential pulse voltammetry (DPV) technique [58].

3. Future prospects and challenges

In recent years, the development of biosensors for biomarkers for breast cancer has received a lot of attention. However, the developments of biomarkers and the innovation of diagnostic tools for early detection of breast cancer are still in their early stages. While electrochemical immunoassays were very successful bio transducers, biomarkers established for breast cancer are needed to test their specificity, responsiveness and efficiency against the diagnostic standards created. The production and progression of these advanced cancer screening systems will aid in the early stages of accelerated clinical cancer diagnoses. Nonetheless, proposed detection approaches for biomarker detection of cancer necessarily require standardization of pre- and post-analytical protocols such as sample preparation, storage and optimization of experimental conditions for true validity of assays and more genuine output of the biosensor produced. Although very low LODs have been obtained by electrochemical biosensors, they typically convey multi-step mark strategies that complicate the experimental activity. For future works, the development another problem is that owing to its low accuracy and reliability, few portable electrochemical instruments are in clinical usage. Therefore, robust biosensor-based POC devices are required of ultrasensitive electrochemical label-free methods will be a great potential. Researchers must train the electrochemical biosensor to solve their reliability problems with a significant number of clinical samples. The development of wireless micro/nano electrochemical biosensors is an ideal option for in vivo

detection, as they can work in an invasively style. The approachable properties of electrochemical instruments improve the performance of cancer diagnostics and therapy monitoring. With further advancement and funding, these handheld instruments are anticipated to improve cancer diagnosis, rendering diagnostic findings accessible in a matter of minutes at the patient bedside or practitioner's office.

4. Conclusion

Novel electrochemical techniques for the detection of biomarkers of breast cancer have been established using the remarkable progress in nanotechnology and biosensor techniques. Effective electrochemical detection systems are combined with immunology guidelines to host different antibody-complex reactions on the transducer surface and quantitatively identify the biomarkers. Current electrochemical biosensors, such as DNA or immunosensors, have extraordinary sensitivity that is important for early detection of cancer. The integration of electrochemical instruments with nano-scale materials provides a unique multiplexing mechanism for various cancer marker simultaneous measurements. However, before marketing the developed biosensors for actual clinical practices for the detection of biomarkers for breast cancer, important gaps and approaches need to be addressed and implemented.

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Conflict of interest

The authors declare no conflicts of interest to disclose in relation to this book chapter.

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