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Biosensors Designed for the Detection of Breast Cancer Biomarkers by Different Electrochemical Methods

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ABSTRACT: Today, breast cancer is one of the leading causes of cancer deaths, especially in women, as it is a life-threatening type of cancer that is most frequently diagnosed in women worldwide, rarely seen in men. Most deaths from breast cancer are not caused by the tumor itself, but by metastasis to other organs in the body. Therefore, as in all cancer types, early diagnosis of breast cancer can reduce the incidence of metastatic disease and prolong disease-free and survival time. Breast ultrasonography, mammography, breast MRI, positron emission tomography and fine needle biopsy techniques are the most commonly used and the most effective techniques in the diagnosis of breast cancer. However, these methods are limited because they are costly to set up, some methods are painful for the patient, and are not suitable for all ages. Recently, electrochemical methods have become very common because they are fast, sensitive, selective, low-cost, easily prepared and interpreted devices. Accordingly, biosensors are preferred more and more day by day. In this review, it is aimed to summarize the biosensor studies designed for the detection of biomarkers used in the early-stage diagnosis of breast cancer.

Key Words: Breast cancer, biosensor, electrochemical determination.

1 INTRODUCTION

Worldwide, breast cancer is the most common type of cancer diagnosed in women, and it occurs at a rate of about one percent in men. Men have worse outcomes than women due to delays in diagnosis [1,2]. Cancer is defined as a disease that occurs with the development of a tumor as a result of the uncontrolled division of the cell. Cancerous cells belonging to the tumor tissue enter the vascular circulation closest to it, reach other body organs and cause cancer to the spread, in other words to metastasize [3]. Early diagnosis and treatment are of great importance in order to prevent the spread of cancer to other parts of the body. Early diagnosis provides more opportunities for successful treatment [4,5]. General screening technologies such as MRI, X-Ray imaging, ultrasound,

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mammography, CT-scan and biopsy are used in the diagnosis of breast cancer [6,7]. However, biopsy diagnosis method is an invasive procedure, requires qualified personnel, long-term examinations, and breast X-Ray is not an appropriate diagnostic method in young girls [8]. Therefore, advances in technology are needed to develop sensitive detection methods. Biosensors are integrated transducer devices that have overcome many challenging determinations by producing fast andselective quantitative or semi-quantitative analytical information. It is also a suitable method for early diagnosis due to its rapid, selective, portable, cost-effective and high sensitivity. New easily detectable platforms can be designed for breast cancer diagnosis with tumor-specific biomarker with the developments in biosensor technologies [9,10]. Because of these features of electrochemical biosensors, designed and/or newly designed biosensors are promising for early-stage diagnosis of cancer. In this review, a review of biosensor studies designed for the determination of biomarkers used in the early-stage diagnosis of breast cancer is presented.

1.1 Breast Cancer Biomarkers

Biomarkers are cellular, biochemical, or molecular changes used to measure and evaluate pharmacological responses that occur in normal or pathological conditions. Biomarkers can be used to differentiate between normal and pathological conditions. Biomarkers are classified according to their structural and functional functions in a cell as genomic (DNA), proteomics (proteins), transcriptomics (mRNA) and metabolomics (metabolites). The classification of these biomarkers is important for the early-stage detection and treatment of breast cancer, grading, monitoring and evaluation. Changes in BRCA1 and BRCA2, which are defined as tumor suppressor genes, are the most frequently reported genomic cancer biomarkers in the diagnosis of breast cancer [11,12].

mRNA known as messenger RNA in tumor cells, provides information for cancer diagnosis. MicroRNA-21, microRNA-34a, and microRNA-155 are important transcriptomic biomarkers for breast cancer diagnosis [13–15]. The proteomic biomarker, which is the most widely used in the diagnosis of breast cancer, plays an important role in the diagnosis of the disease because it is relatively rich in RNA and DNA. Various proteomic biomarkers such as human epidermal growth factor receptors (EGFR)/(HER) family, carcinoembryonic antigen (CEA), cancer antigen 15-3 (CA15-3), cancer antigen 15-9 (CA15-9), cancer antigen 19-9 (CA19-9), cancer antigen 27.29 (CA27.29), cancer antigen 125 (CA125), cancer antigen 242 (CA242), Tumor suppressor (p53), EGF and VEGF are available for breast cancer diagnosis [16–22]. The metabolome consists of small

molecules found in body tissues, cells, organs and fluids. When the human body gets sick, changes occur in the amount of these metabolomes in biological fluids depending on factors such as age, gender, diabetes and kidney disease. Considering these changes, it is used as a metabolomic biomarker in the diagnosis of the disease [23–26].

1.2 Biosensors Designed for the Detection of Breast Cancer Biomarkers

Electrochemical biosensors are preferred because of their many advantages such as low cost, sensitive, fast, simple, easy to transport and miniaturization. There are various biosensors used for monitoring breast cancer biomarkers [27–29]. Biosensors designed with different electrochemical techniques for the determination of breast cancer biomarkers are combined with their analytical parameters in Table 1.

Due to the increased risk of cancer, genome-wide association studies have led to the recognition of associated genes. Most breast cancers are associated with specific mutations in the BRCA1 anti-oncogene sequence, known as the tumor suppressor gene. Guixiang Wang et al. designed a sensor for BRCA1, a genomic biomarker for breast cancer diagnosis, with citrate ion doped poly (3,4-ethylenedioxycythiophene) (PETOD), fixed to the GCE surface, then zwitterion peptides and nickel cations fixed to this modified GCE. Afterwards, the peptide-modified PEDOT was then fixed to a DNA probe to create the sensor designed for BRCA1. Guixiang Wang et al. designed a DNA sensor with a limit of detection (LOD) of 0.03 fM for BRCA1 using the DPV technique [30]. In another study for the BRCA1 biomarker, Wenting Wang et al. used the electrochemical impedance spectroscopy (EIS) technique. A biosensor was designed by fixing gold nanoparticles after modifying the glassy carbon electrode (GCE) surface with amine group containing polyethylene glycol (PEG). The LOD value of the designed biosensor was 1.72 fM. [31].

There are studies using different electrochemical methods because transcriptomic biomarkers containing various types of RNA, which are among the important biomarkers used in the diagnosis of breast cancer, constitute an important target in medical diagnosis and prognostics. Torrente-Rodríguez et al used a H_2O_2/hydroquinone (HQ) system with disposable screen-printed carbon electrodes (SPCE) modified with a specific DNA-RNA antibody as a capture bio receptor for micro ribonucleic acid (miRNA) detection. A biosensor was constructed for electrochemically detection of miRNA with a limit of detection (LOD) of 2.4 pM using the amperometric method [32]. A sandwich-based construct with two complementary DNA probes was designed by Daohong et al. for the detection of the circRNA biomarker, which is

Table 1. Analytical Parameters of Electrochemical Biosensors Used in the Determination of Breast Cancer Biomarkers.

Biomarker	Method	Linear Range	LOD	References
BRCA1	DPV	1.0×10^{-16} - 1.0×10^{-10} M	0.03 fM	30
BRCA1	EIS	50 fM - 1.0 nM	1.72 fM	31
miRNA	Amperometric	8.2–250 pM	2.4 pM	32
circRNA	CV	0.5–10 pM	0.22 pM	33
miRNA-34a	EIS	0–7.5 $\mu g.mL^{-1}$	0.95 $\mu g.mL^{-1}$	34
miRNA-21/miRNA-155	SWV	0.1 fM - 10 nM	18.9 aM / 39.6 aM	35
miRNA-155/miRNA-21/miRNA-16	DPV	1 fM - 10 nM	0.98 fM / 3.58 fM / 0.25 fM	36
EGFR	CV	0.001–100 $ng.mL^{-1}$	1 $pg.mL^{-1}$	37
EGFR	DPV	0–1000 $pg.mL^{-1}$	0.05 $pg.mL^{-1}$	38
EGFR	EIS	$1.0 \times 10^{-6} - 1.0 \times 10^{-10}$ $g.L^{-1}$	0.37 $pg.L^{-1}$	39
HER2	CV	0.001 - 0.5 $ng.mL^{-1}$	34.9 $pg.mL^{-1}$	40
HER2	DPV	0.01–1.00 $pg.mL^{-1}$	3.00 $fg.mL^{-1}$	41
HER2	SWV	1–100 $pg.mL^{-1}$	0.047 $pg.mL^{-1}$	42
HER2	EIS	1 $pg.mL^{-1}$ - 100 $ng.mL^{-1}$	172 $pg.mL^{-1}$	43
CA15-3	DPV	2×10^{-5} - 40 $U.mL^{-1}$	5×10^{-6} $U.mL^{-1}$	44
CA15-3	SWV	1.0–1000 $U.mL^{-1}$	0.95 $U.mL^{-1}$	45
CA125	SWV	0.0001–1000 $U.mL^{-1}$	0.00125 $U.mL^{-1}$	46
CA125 / CA15-3 / CEA	DPV	0.05–20 $U.mL^{-1}$/0.008–24 $U.mL^{-1}$/0.020–20 $ng.mL^{-1}$	0.002 $U.mL^{-1}$ / 0.001 $U.mL^{-1}$ /7.0 $pg.mL^{-1}$	47

circular RNA. CircRNA was measured at the sensor designed by adding ferrocene capped gold nanoparticle/streptavidin conjugates to the biotinylated probe using the CV method. The oxidation current of ferrocene was proportional to the circRNA concentration and a limit of detection (LOD) was 0.22 pM. In addition, the applicability of the methods by detecting circRNA in PCR products produced from blood samples taken from breast cancer patients and healthy women was demonstrated in this study [33]. Another biomarker, miRNA-34a is a biomarker used in many diseases such as Alzheimer's disease. An impedimetric biosensor for miRNA-34a was designed by Congur et al. An electrochemical platform was developed using the EIS method and electrode modified with a polyamidedoamine dendimer. The LODs for miRNA-34a detected in phosphate buffer and fetal bovine serum were 950 and 520 $mg.mL^{-1}$, respectively [34]. A DNA loop capture probe was designed by Sai Xu et al. for simultaneous analysis of miRNA-21 and miRNA155 biomarkers. The modified electrode was fabricated by applying the tetrahedron DNA structure. Ferrocene and methylene blue were immobilized with a DNA probe for multiple detection of biomarkers. miRNA-21 was conjugated with ferrocene, and miRNA-155 was bound with methylene blue, resulting in two different responses. In order to the simultaneous detection of miRNA-21 and miRNA-155 LODs were 18.9 aM and 39.6 aM, respectively in the linear range of 0.1 fM-10 nM at the sensor using the SWV method [35]. Another study for the simultaneous detection of micro ribonucleic acids (miRNAs) was performed by Pimalai et al. A multiplex biosensor was designed for the detection of miRNA-16, miRNA-21 and miRNA-155 using the DPV method by modifying reduced graphene oxide/poly (2-aminobenzylamine)/gold nanoparticles (rGO/P2ABA/AuNPs) to SPCE. As a result of this study, the LOD values for miRNA-16, miRNA-21 and miRNA-155 with a linear range of 1 fM and 10 nM were determined as 0.98 fM, 3.58 fM and 0.25 fM, respectively [36].

Biosensors have been designed using different electrochemical techniques for the human epidermal growth factor receptors (EGFR/HER) families, which are used as biomarkers in the diagnosis of breast cancer. An immuno-biosensor for EGFR was designed by Vasudev et al. In this study, the detection of EGFR was achieved by immobilizing anti-EGFR antibody (anti-EGFRab) and dithiobissuccinimidyl propionate (DTSP) as a self-assembled monolayer (SAM) on the gold electrode. The LOD value was measured as 1 pg/mL using the CV method at the designed EA/Anti-EGFRab/DTSP/Au immuno-biosensor [37]. A chip-format sandwich electrochemical immunoassay biosensor was designed by Omidfar et al. for the

determination of EGFR biomarker using DPV technique. The developed sensor was constructed using the Fe_3O_4/N-trimethyl chitosan (CHS)/AuNPs composite (as a tracking tag to label the anti-EGFR antibody) for the modification of a commercial screen-printed platinum electrode. LOD was calculated as 0.05 $pg.mL^{-1}$ at this developed nanoimmunosensor [38]. A peptide-based biosensor was developed by Li et al., for EGFR determination using the EIS technique. It was a peptide-based biosensor obtained by immobilizing sulfhydryl and ferrocene-modified peptide ligands (with Au-S interaction) on the gold electrode. The LOD value was 0.37 $pg.L^{-1}$ at the peptide-based biosensor [39].

Similarly, biosensors designed using different electrochemical techniques for the HER2 biomarker from the EGFR/HER family, which are human epidermal growth factor receptors, are available in the literature. An immunosensor was designed for HER2 using the CV technique by Hartati et al. Biomarker detection was achieved by forming a cerium oxide-monoclonal antibody bioconjugate. Cerium oxide-HER2 bioconjugate was obtained by adding antibodies (anti HER2) on cerium oxide reacted with 3-aminopropyl trimethoxysilane and polythene glycol-n-hydroxide succinimide-maleimide (PEG-NHS-Maleimide). Then, the screen printed carbon (SPC) electrode surface was modified with gold nanoparticle (AuNPs) using the amine bonding system with the obtained bioconjugate. It has been observed that the produced bioconjugate interacts with HER2, preventing an electron transfer and a decrease in peak current in direct proportion to different concentrations of HER2. As a result, an immunosensor was designed with a LOD of 34.9 $pg.mL^{-1}$ [40]. A sandwich-type sensitive voltammetric immunosensor for HER2 was developed using the DPV method by Yola. The modified sensor was designed using a copper-organic framework, platinum doped graphite carbon nitride and quaternary chalcogenide. Gold nanoparticles/Cu-organic framework (AuNP/Cu-MOF) composite was synthesized by amidation reaction between AuNPs with amino groups and Cu-MOFs containing carboxylic acid. Cu_2ZnSnS_4 nanoparticle and quaternary chalcogenide with platinum (Pt)-doped g-C_3N_4 (CZTS NPs/Pt/g-C_3N_4) composite was obtained by hydrothermal method as a sensor platform after conjugation of primary HER2 antibody and antigen HER2 protein to AuNP/Cu-MOF. The LOD value was obtained as 3.00 $fg.mL^{-1}$ with the designed immunosensor [41]. A sandwich-based aptasensör was developed for HER2 detection using the SWV method by Shen et al. In the study, an aptamer was used both as a ligand and to generate a signal that allowed current to be generated. The aptasensor was formed when a DNA primer on the HER2 aptamer self-

assembled on the electrode to form long, one-dimensional DNA. This obtained DNA then reacted with molybdate to form an electrochemical current. The limit of detection (LOD) for this sensor, whose sensitivity was greater than that of non-DNA self-assembly receptors, was 0.047 $pg.mL^{-1}$ [42]. Additionally, two detection platforms were developed on gold screen-printed electrodes using EIS technique for HER2 determination by Ferraira et al. The first platform was constructed from a self-assembled monolayer (SAM) consisting of a mixture of thiolated DNA aptamers specific for HER2 and 1-mercapto-6-hexanol. The second platform was constructed from a triple SAM of 1,6-hexanethiol using the same aptamer. Both platforms were further passivated with 1-mercapto-6-hexanol, and blocked with bovine serum albumin. It has been observed that the triple SAM platform minimizes non-specific adhesion on the electrode surface due to the antifouling property of 1,6-hexanethiol. The LOD was 172 $pg.mL^{-1}$ for the designed aptasensor [43]. Electrochemical sensor designs are also available for the detection of CA15-3, another breast cancer biomarker. A bilayer immunosensor was obtained by S. Ge et al. The electrochemical treatment was based on the immunosensor application of a nanoporous gold (NPG) and graphene (GN) hybrid composite with horseradish peroxidase-encapsulated liposomes in the presence of CA15-3. It was observed that the catalytic current increased with the increase of CA15-3 concentration in the sample at this immunocomplex-based immunoser. The designed immunosensor had an LOD of 5.10^{-6} $U.mL^{-1}$ for the CA15-3 test. An electrochemical immunosensor was designed by Rebelo et al for the determination of the CA15-3 biomarker using the SWV technique. First, a self-forming monolayer of mercaptosuccinic acid was formed on the gold screen-printed electrode surface. Then, the CA15-3 antibody was covalently attached to the carboxylic groups at the electrode interface. The LOD was calculated as 0.95 $U.mL^{-1}$ at the immune sensor [45]. Another study for the determination of CA125 by SWV technique was the immunosensor designed by Zhao et al. The immunosensor they produced was based on a polyaniline-polythionine hydrogel (PANI-PTi gel). This hydrogel was easily synthesized by electropolymerization, and good conductivity was obtained. A high current signal was exhibited by the produced AuNPs/PANI-PThi gel, and this signal was amplified by the catalytic oxidation of H_2O_2. The LOD value was 0.00125 $U.mL^{-1}$ at the designed sensor [46]. A platform for multiple determinations of breast cancer biomarkers CEA, CA125 and CA15-3 was established by Cui et al. Graphene sheet (GS) was used to increase the surface area and fix different antibodies onto the electrode surface. Firstly,

capture antibodies (Ab1) were fixed to GS-SPCE. Second antibodies (Ab2) were then fixed onto the surface of a mesoporous Pt electrode. As a result, GS/Ab1/Ag/M-Pt-Ab2 was developed onto the SPCE surface. Three antibodies (cancer antigen 125, cancer antigen15-3 and carcinoembryonic antigen) were fixed on a biosensor to identify three target analytes simultaneously. LOD values in the linear range of 0.05-20, 0.008-24 and 0.020-20 $U.mL^{-1}$ for CA125, CA15-3 and CEA biomarkers were determined at the designed biosensor as 0.002 $U.mL^{-1}$, 0.001 $U.mL^{-1}$ and 7.0 $pg.mL^{-1}$, respectively [47].

2 CONCLUSION

Early diagnosis of breast cancer in women before it reaches an advanced stage increases the hopes of women to hold on to life, together with a more successful response in treatment. Thus, the belief in surviving the cancer disease increases. Biomarkers offer an important avenue for early detection of breast cancer. Existing methods are limited and laborious due to reasons such as expensive, requiring expert personnel, not suitable for different age groups, and painful procedures. Research for the early-stage diagnosis of breast cancer is increasing rapidly and new methods are being discovered. Voltammetric methods are the most preferred methods recently due to their advantages such as wide, fast, easy, specific, sensitive, simultaneous determination of several analytes with high sensitivity, procedures that do not require pretreatment, and high sensitivity. Therefore, designs for breast cancer biomarkers that are easily portable and do not require specialized personnel have been of interest. Biosensors developed for breast cancer biomarkers can be extremely useful in diagnosis, can become more sensitive with the use of different nanomaterials, be accurate and selective for early diagnosis. Studies on biosensor techniques that can instantly sense and detect for early-stage diagnosis of breast cancer are continuing.

2.1 Suggestions and Future Perspectives

Devices based on electrochemical biosensors are rarely used in clinical patients. Among the various electrochemical measurements, DPV and SWV techniques provide a general and sensitive possibility for the detection of biomarkers. In addition, these methods also provide the opportunity to perform simultaneous multi-analyte analysis in a short time. The literature review showed that breast cancer biomarkers have the potential to be individually identified, but some important considerations should be considered for future research. Taking advantage of these advantages, biosensors for the simultaneous analysis of breast cancer biomarkers for multiple determination should be developed. The clinical use of these developed biosensors should be facilitated and increased by transforming them into a portable, easy-to-

carry minimal and high-reliability device for in vivo detection.

3 AUTHOR CONTRIBUTIONS

Hypotesis: Y.N., M.B.; Design: Y.N.Z., Y.Y.; Literature review: B.A., İ.A. K.A.; Data Collection: Z.Ö., K.B.; Analysis and/or interpretation: C.V.Z., M.A.A.; Manuscript writing: Y.Y., M.B.A., Y.N.Z.

4 CONFLICT OF INTEREST

In the conflict of interest section, if there is no conflict of interest, "Authors declare that there is no conflict of interest." statement should be included.

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