

Biochemical Biomarkers and Electrochemical Biosensing Strategies for the Early Detection of Breast Cancer: A Critical Integrated Review

Pakeeza Riaz

Lecturer Biology, Higher Education Department, Lahore, Pakistan

Email: Pakeezacheema524@gmail.com

Syedah Sara Batool

M.Phil. Scholar in Public Health, University of Lahore, Pakistan

Email: ssarabatool97@gmail.com

ABSTRACT

Breast cancer remains one of the leading causes of cancer. The morbidity and mortality among women need the development of reliable strategies for early detection. Although breast cancer is still among the most prevalent diseases associated with morbidity and mortality in women globally. There is a need to develop an effective approach to detect this type of cancer at an early stage. It is necessary because early diagnosis can increase survival rates and reduce the need for treatment. Traditional imaging technologies used in the detection process. This includes mammography, but it's not sufficiently sensitive to early molecular changes. It indicates only tumor development. In recent years, significant progress has been achieved. The biochemical biomarker approaches are used for diagnosing breast cancer. Among them are various protein markers, such as CEA, CA15-3, MUC1, and HER2. Genetic factors (BRCA1/2), epigenetic modifications (DNA methylation), nucleic acids (circulating DNA, cfDNA, ctDNA), microRNAs, and immune-related parameters. This includes autoantibodies. All these biomarkers exhibit different levels. Based on specificity and sensitivity. However, no biomarker offers sufficient specificity and sensitivity needed for the effective early diagnosis of breast cancer. Thus, indicating the need to combine several biomarkers to create a multiplex biomarker panel. At the same time, remarkable advances in electrochemical biosensors and nanomaterial-based biosensing techniques could be a great achievement for humanity.

Keywords: Breast Cancer, Early Detection, Biochemical Biomarkers, Electrochemical Biosensors, Liquid Biopsy, CA15-3, CEA, BRCA1, MUC1, DNA.

Introduction

Breast cancer is one of the most widespread and life-threatening diseases. It's affecting women worldwide and represents a significant global health burden. The most common and fatal form of cancer amongst women is breast cancer. It is associated

with a significant health burden worldwide. Not only does it affect individuals' health, but it also has various social, psychological, and clinical impacts. As far as breast cancer is concerned, early diagnosis is the best method of reducing deaths. Epidemiological evidence among women the earlier the tumor is detected, the better it is for treatment purposes, and less invasive treatment is needed [1].

Traditional diagnostic methods have always been essential components in the diagnosis of breast cancer. Especially those that include imaging techniques like mammography. Nevertheless, they all suffer from certain shortcomings such as low sensitivity among women with dense breasts, incapability of detecting molecular changes, and subjectivity [1]. Additionally, most imaging-based techniques cannot diagnose tumors until the patient has experienced significant physical changes, which means they lack the capacity to conduct early diagnoses. Therefore, considerable effort has been devoted to developing a biomarker-based diagnostic approach. Biomarkers are biological markers detectable in body fluids and tissues. They indicate the physiological condition or the presence of an abnormal process like cancer development [2]. They play a very important role in early detection, diagnosis, predicting outcomes, and monitoring treatment progress. Blood-based biomarkers offer many advantages over traditional diagnostics due to their ease of access and noninvasiveness [1].

New and traditional biochemical biomarkers are used in the diagnosis of breast cancer. It includes tumor-associated proteins like CEA, CA15-3, and MUC1. Genetic and epigenetic biomarkers. It includes BRCA1/2 mutations, ctDNA, and DNA methylation profiles. These are also used [1, 2]. The emerging use of biomarkers such as microRNAs has attracted significant. The interest from researchers due to their stability in body fluids and their role in regulating gene expression associated with tumor formation it preferred. MicroRNA-based diagnostic techniques have shown immense potential for detecting early breast cancers. For instance, electrochemical biosensors have enabled the precise identification of miR-21. An oncomir, at femtomolar and even attomolar levels its being used [3, 4]. Also includes the application of nanobiosensors based on graphene oxide and gold nanorods for miR-155 diagnosis. which plays a critical role in tumor development. It has provided remarkable accuracy [5, 6]. This shows that nucleic acid biomarkers are becoming increasingly important in cancer diagnostics. In tandem with biomarker discovery, considerable progress has been made.

The result is the development of analytical detection methods witnessed. Electrochemical biosensors have proven efficient due to their high sensitivity. Its fast response times, cost-effectiveness, and suitability for point-of-care testing are the cause of this success. It includes immunosensors, genosensors, and aptasensors. This permits the detection of trace amounts of biomarkers. They use electrochemistry in this process. Even though there have been significant improvements in this field, many problems persist regarding achieving adequate sensitivity, specificity, and clinical validation for the accurate detection of cancers [2]. By Combining several biomarkers with advanced electrochemical techniques offers an exciting opportunity for more reliable diagnoses. In this way the early-stage cancer detection become easy. In this review discusses the significance of biochemical biomarkers for breast cancer diagnosis and the use of advanced electrochemical sensing systems. Recent

developments in electrochemical biosensors have significantly improved. This design and functionality of diagnostic systems for breast cancer biomarkers [7, 8].

Clinical Need for Early Detection

Early detection is crucial for identifying cancer early. To reduce its burden and improve survival outcomes, it's required. The ideal early detection test must be minimally invasive. They must be tissue-specific, affordable, reproducible, and sensitive enough to have a low false-positive rate. During the process, all these things are needed. Blood tests are especially advantageous. Since they can be used repeatedly, they help patients to accept screening procedures. More easily used and convenient for laboratory use. Mammography is a common tool used in early detection. A combination of imaging methods, like biomarker-based methods, can be considered. Biomarkers might detect changes in tumor biology before anatomic alterations do [7].

Biomarkers in Breast Cancer: Concept and Classification

It was supported by various research studies. A biomarker is a quantifiable biological marker found in blood. In the blood tissue, or other bodily fluids, that indicate either pathological conditions, normal physiological conditions, or reactions to treatment [1, 2]. When it comes to breast cancer, the biomarkers have an important function. It is not just in diagnosis but also in predicting prognosis and therapeutic efficacy. Tracking of the progression or recurrence of the disease. At its earliest stages, for disease diagnosis, biomarkers should be highly sensitive. It is a specific in non-invasive biological materials, such as blood or plasma [1].

The biomarkers can be grouped into various categories depending on their biological source and function. Some biomarker types include genetic, protein, epigenetic, circulating nucleic acid, and autoantibody biomarkers. They all indicate an immune response [2]. The four biomarker types reflect the biological characteristics of tumor growth through proliferation. Genetic instability, epigenetic changes, and immunological reactions from the host organism. According to the existing literature, there is no single biomarker that meets all the requirements of an ideal breast cancer. The screening test, as it falls short in terms of sensitivity, specificity, and consistency. Hence, these studies have focused on creating multiplexed biomarker panels [1].

Major Biochemical Biomarkers for Early Breast Cancer Detection

Protein-Based Biomarkers

Protein biomarkers represent the earliest and most extensively studied type of cancer biomarkers. They are detectable in serum and associated with tumor burden, progression, and metastasis. Several clinically relevant protein biomarkers have been investigated for breast cancer detection, although most exhibit limitations in sensitivity and specificity for early-stage diagnosis.

Human Epidermal Growth Factor Receptor 2 (HER2)

The human epidermal growth factor is named receptor 2 (HER2). It belongs to the family of transmembrane tyrosine kinase receptors. These are essential for cell proliferation and differentiation. HER2 overexpression is seen in 15% to 20% of

breast cancers. A higher rate of tumor aggressiveness and poor patient outcomes [9]. HER2 is a biomarker for diagnostics and a predictor of trastuzumab treatment. In recent studies, electrochemical biosensors have demonstrated high sensitivity. For HER2 detection using both antibody and aptamer sensing, enabling precise, rapid detection in biofluids [9]. The use of nanomaterials, including gold nanoparticles, with graphene has enabled the biosensor. Moreover, it is effective due to its increased signal amplification and electron-transfer capabilities. They are making it a potential tool for early detection applications.

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Carcinoembryonic Antigen (CEA)

According to the literature, CEA levels can be elevated in other cancers. Such as colorectal, stomach, and lung cancers. Thereby restricting its specificity for breast cancer diagnosis. The importance of CEA in clinical practice cannot be overstated due to its usefulness. In disease monitoring and detection of its recurrence. Electrochemical biosensors have enhanced sensitivity for detecting CEA. Nanomaterial-based sandwich-type immunosensors have achieved a detection limit of 2 picograms per milliliter [2]. Carcinoembryonic antigen is a glycoprotein involved in cell-to-cell adhesion. It is expressed at very low levels. In healthy adult patients, but can be elevated. In some cancers, such as breast cancer, without any specificity to tumors, it can also work [1]. According to the literature, CEA levels can be elevated in other cancers. Such as colorectal, stomach, and lung cancers. Thereby restricting its specificity for breast cancer diagnosis. The importance of CEA in clinical practice cannot be overstated due to its usefulness. In disease monitoring and detection of its recurrence. The electrochemical biosensors has led to enhanced sensitivity in detecting CEA. Nanomaterial-based sandwich-type immunosensors have achieved a detection limit of 2 picograms per milliliter [2].

Cancer Antigen 15-3 (CA15-3)

The normal level of CA15-3 in serum is usually less than 30 U/mL. Whereas its levels are high in about 30-50% of breast cancer patients [2]. The CA15-3 test does not have sufficient sensitivity or specificity. that to be used for early diagnosis. Especially among asymptomatic patients its common. The conventional technique for detecting CA15-3. This includes ELISA and radioimmunoassay, and usually takes time. It involves complicated sample preparation procedures. Electrochemical biosensors represent alternative means. That is for detection, which provides quick, sensitive, and inexpensive analysis. These concern the use of electrochemical detection in antigen-antibody reactions [2]. Cancer antigen 15-3 (CA15-3) is another widely studied tumor-associated glycoprotein, commonly used in clinical practice to monitor treatment response and detect recurrence in breast cancer patients. The

normal level of CA15-3 in serum is usually less than 30 U/mL. Whereas its levels are high in about 30-50% of breast cancer patients [2]. The CA15-3 test does not have sufficient sensitivity or specificity, that is to be used for early diagnosis. Especially among asymptomatic patients its common. The conventional technique for detecting CA15-3. This includes ELISA and radioimmunoassay, and usually takes time. It involves complicated sample preparation procedures. Electrochemical biosensors represent an alternative means. That is for detection, which provides quick, sensitive, and inexpensive analysis. These concern the use of electrochemical detection in antigen-antibody reactions [2].

Mucin 1 (MUC1)

Mucin-1 aptasensors have demonstrated excellent sensitivity. Its specificity is due to the high affinity of aptamers for the targeted protein. Based on the application of gold nanoparticles, the electrochemical method uses methylene blue as a signal generator. It has been successful in quantitatively measuring the amount of MUC1 present in biological samples [2]. Mucin 1 (MUC1) is a high molecular weight transmembrane protein. They have a significant glycoprotein nature and help protect epithelial tissues from various insults. MUC1 expression is highly increased in breast cancer patients. It has an abnormal glycosylation pattern. Therefore, MUC1 can serve as a potential biomarker for breast cancer diagnosis [2]. Mucin-1 aptasensors have demonstrated excellent sensitivity. Its specificity is due to the high affinity of aptamers for the targeted protein. Based on the application of gold nanoparticles, the electrochemical method uses methylene blue as a signal generator. It has been successful in quantitatively measuring the amount of MUC1 present in biological samples [2].

Genetic Biomarkers

Genetic biomarkers are associated with inherited or acquired mutations that contribute to breast cancer development. Among these, the BRCA1 and BRCA2 genes are the best-known tumor suppressor genes involved in DNA repair. BRCA1 and BRCA2 proteins play a crucial role in maintaining genomic stability by repairing DNA double-strand breaks. Mutations in these genes significantly increase the risk of developing breast cancer, making them important biomarkers for hereditary cancer susceptibility [2]. However, these mutations are primarily used for risk assessment rather than direct early detection of active disease. Electrochemical genosensors have been developed for detecting BRCA gene mutations with extremely low detection limits in the femtomolar range. These sensors rely on DNA hybridization principles and nanomaterial-enhanced signal amplification, demonstrating high analytical sensitivity and potential for early-stage detection [2].

Circulating Nucleic Acid Biomarkers

Circulating nucleic acids in blood have emerged as powerful tools for non-invasive cancer detection, forming the basis of liquid biopsy approaches.

Cell-Free DNA and Circulating Tumor DNA

Cell-free DNA (cfDNA) and circulating tumor DNA (ctDNA) are released into the bloodstream through apoptosis and necrosis of tumor cells. These nucleic acids carry

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tumor-specific genetic and epigenetic alterations, including mutations and DNA methylation patterns [1]. Unlike total cfDNA concentration, which shows significant variability, tumor-specific features provide greater diagnostic value.

DNA methylation changes, such as hypermethylation of tumor suppressor genes, have been identified as early events in breast carcinogenesis. Studies have demonstrated that methylation markers can be detected in plasma DNA even before clinical diagnosis, highlighting their potential for early detection [1]. However, variability in methylation patterns across patients necessitates the use of multigene panels to improve diagnostic accuracy.

MicroRNAs

MicroRNAs (miRNAs) are small non-coding RNA molecules. These regulate gene expression at the post-transcriptional level. Dysregulation of miRNAs is closely associated with cancer development. Its progression and metastasis. Their remarkable stability in blood and resistance to degradation make them highly promising. Biomarkers are a cause for early cancer detection. Numerous studies have demonstrated ultrasensitive detection of miRNAs, such as miR-21 and miR-155, using electrochemical biosensors, with detection limits in the femtomolar to attomolar range [3–5]. These biosensors often employ nanomaterial-based amplification strategies, including gold nanoparticles, graphene oxide, and catalytic complexes, to enhance detection sensitivity. The microRNA (miRNA) is an RNA molecule that regulates gene expression post-transcriptionally.

The potential contribution of miRNAs towards the onset and progression of cancer has been extensively studied. There is a high degree of association between miRNA dysregulation and the onset of cancer. The stability and non-degradability of miRNAs make them excellent biomarkers. That can be used to diagnose cancer. Numerous reports on the ultra-sensitivity of miRNA detection are explored. Specifically, that of miR-21 and miR-155, via the use of electrochemical biosensors with detection levels from femtomolar to attomole [3-5], is available.

MicroRNA-Based Electrochemical Biosensors

MicroRNA-based electrochemical biosensors represent a rapidly advancing field in cancer diagnostics, combining the biological relevance of miRNAs with the analytical advantages of electrochemical detection. These biosensors enable rapid, sensitive, and label-free detection of miRNAs in biological fluids. Advanced sensing platforms have been developed using techniques such as bio-barcode amplification, enzyme-assisted signal amplification, and nanomaterial-enhanced electrochemical detection. For example, bio-barcode-based systems and iridium(III)-catalyzed platforms have demonstrated extremely low detection limits for miR-21, reaching femtomolar levels [3, 4]. Similarly, graphene oxide-based nanobiosensors have been successfully employed for detecting miR-155 in plasma samples for early breast cancer diagnosis [5]. Despite their excellent analytical performance, challenges such as standardization, reproducibility, and clinical validation remain significant barriers to the widespread clinical adoption of these technologies. Future research should focus on integrating multiple biomarkers into multiplexed biosensing platforms and validating their performance in large-scale clinical studies.

Autoantibodies

Autoantibodies directed against tumor-associated antigens. It may arise early in tumorigenesis and sometimes before a tumor. It becomes clinically apparent. Immune amplification may increase detectability. Autoantibody signatures have attracted interest as early warning biomarkers. But their specificity and reproducibility across populations remain important.

Table 1

MicroRNA-based electrochemical biosensors for breast cancer diagnosis

Biosensor Specification		Target miRNA	Linear Range	Detection Limit	Ref.
A.	Anti-miR probe/GPE	miR-21	—	6 pM	[10]
B.	HRP/AuNPs-barcode/miR-	miR-21	0.01–700 pM	6 fM	[11]
C.	NAH/AuNPs/graphene/Au electrode				
D.	Hemin/miR/DNA-Au probe/AuNPs/Au	miR-21	0.01–500 pM	6 fM	[3]
E.	Electrode				
F.	ALP/APBA-biotin-AuNP/miR/DNA	miR-21	10 fM–5 pM	3 fM	[12]
G.	probe/Au electrode + redox cycling				
H.	HRP-antiMBP-p19-dsRNA-MBs/SPCE	miR-21	1.4–1.0 nM	4.2 fM	[13]
I.	Anti-miR/CP-AP/PDA/GCE	miR-21	100 aM–1 nM	30 aM	[14]
J.	ALP/DNA probe/Au electrode + redox cy-	miR-21	0.5 fM–1 pM	0.2 fM	[15]
K.	Cling				
L.	ALP/miR/DNA probe/Au electrode + redox	miR-21	0.5 fM–1 pM	0.2 fM	[16]
M.	Cycling				
N.	Ir(III)/MB-DNA probe 2-AuNP/miR/DNA	miR-21	5 fM–1 pM	1.6 fM	[4]
O.	probe 1/Au electrode				
P.	Anti-miR/MWCNT-COOH/GCE	miR-21	1.0–500 pM	84.3 fM	[17]
Q.	ALP/DNA probe 2/miR/DNA probe 1-	miR-21	0.01 fM–100 pM	0.05 fM	[18]
R.	AuNPs/WO ₃ -graphene/GCE + redox cy-				
S.	clinging				
S.	P19 captured dual argonaute complex on	miR-205	2–10 nM	0.6 nM	[19]
T.	SPCE				
U.	Anti-miR probe/GNR/GO/GCE	miR-155	2.0 fM–80 pM	0.6 fM	[5]
V.	Anti-miR probe/AuSPE	miR-155	10 aM–1.0 nM	5.7 aM	[6]

Epigenetic Biomarkers and DNA Methylation

Epigenetic alterations like aberrant DNA methylation represent one of the most promising molecular approaches for early detection. The genetic mutations and epigenetic changes do not alter the DNA sequence. It regulate gene expression through reversible chemical modifications. Amongst these, hypermethylation of promoter regions in tumor suppressor genes has been identified. Its as a key early event in breast carcinogenesis [1]. DNA methylation changes can be detected in

various biological samples, including ductal lavage fluid, tumor tissues, and circulating cell-free DNA (cfDNA) in plasma, making them highly suitable for minimally invasive diagnostic approaches [1]. Several genes, such as *RASSF1A*, *RAR-β*, *APC*, and *GSTP1*, have been extensively studied as methylation biomarkers due to their frequent epigenetic silencing in breast tumors. Notably, abnormal methylation patterns have been detected even in asymptomatic individuals with normal imaging results, indicating their potential for very early detection [1]. As non-invasive biomarkers for detecting early breast cancer [20]. While individual methylation biomarkers exhibit biological variation, a panel of optimized multi-gene methylation biomarkers greatly enhances diagnostic accuracy and reliability in the context of liquid biopsy [20]. A comparison of the key properties of the most important biochemical biomarkers reviewed herein is provided. The major advantage of epigenetic biomarkers lies in their ability to capture tumor-specific molecular alterations at an early stage of disease. One of the main strengths of epigenetic biomarkers is their ability to detect molecular changes. These are associated with tumors at an early stage, without the need for large amounts of target molecules. Some individual methylation biomarkers demonstrate poor reproducibility between patients. In the case of tumor heterogeneity and biological variation. Therefore, contemporary studies focus on applying multi-gene methylation profiles. These comprise several epigenetic biomarkers [1]. Systematic investigations have demonstrated the promising clinical utility of DNA methylation profiles. As non-invasive biomarkers for requiring detecting early breast cancer [20]. While individual methylation biomarkers exhibit biological variation, a panel of optimized multi-gene methylation biomarkers greatly enhances diagnostic accuracy and reliability in the context of liquid biopsy [20]. A comparison of the key properties of the most important biochemical biomarkers reviewed herein is provided in Table 2.

Table 2

Comparison of major biochemical biomarkers for early breast cancer detection

Biomarker	Type	Detection Sensitivity	Sample Type	Limitations
CEA	Protein	Low (ng/mL range)	Serum/Plasma	Poor specificity, varied in multiple cancers
CA15-3	Protein	Moderate (U/mL range)	Serum	Limited sensitivity for early-stage detection
MUC1	Protein	Moderate-High	Serum/Tissue	Requires specific detection platforms
HER2	Protein	High (pg/mL range with biosensors)	Tissue/Serum	Overexpressed only in a subset of patients
BRCA1/2	Genetic	Very High (mutation detection)	Blood/Tissue	Indicates risk, not active disease

Biomarker	Type	Detection Sensitivity	Sample Type	Limitations
cfDNA/ctDNA	Circulating DNA	High (mutation-specific)	Plasma	Low abundance, requires amplification
DNA Methylation	Epigenetic	High (gene-specific)	Plasma/Tissue	High variability, requires panels
MicroRNA (miRNA)	Circulating RNA	Very High (fM-aM range)	Plasma/Serum	Standardization challenges
Autoantibodies	Immune-response	Moderate	Serum	Limited specificity and reproducibility

Electrochemical Biosensing Strategies for Breast Cancer Biomarkers

Why Electrochemical Biosensors?

Electrochemical biosensors have emerged as powerful analytical tools. Electrochemical biosensors have proven effective tools for diagnosing cancer. The numerous benefits associated with them. These include high sensitivity, fast response time, lower cost, and the ability to miniaturize and be portable. Recently, the development of electrochemical biosensors has focused on increasing detection capabilities. By implementing nanoparticles, magnetic separation, and surface modification [8]. Electrochemical biosensors generate an electrical signal. These are based on the chemical reaction that occurs between the analyte and the receptor immobilized on the sensor. Electrochemical biosensors are capable of detecting even ultra-low concentrations of the biomarkers[2]. A major advantage of electrochemical biosensors is that they can be used for POC care. But conventional laboratory analysis methods, such as electrochemical biosensors, do not require sample pretreatment. They can provide real-time results. This makes them highly suitable for early-stage cancer detection. The use of electrochemical biosensors reduces the sample volume required. The patient compliance and repeated measurements are facilitated [2]. Recently, the development of electrochemical biosensors has focused on increasing detection capabilities. By implementing nanoparticles, magnetic separation, and surface modification [8].

Major Types of Electrochemical Biosensors

In terms of electrochemical biosensing platforms for breast cancer biomarker classification can be divided into four major categories based on their recognition mechanisms:

Immunosensors: Specific antigen and antibody interactions

Genosensors: Nucleic acid hybridization principles

Aptasensors: Oligonucleotide ligands

Enzyme-based biosensors: Catalytic amplification mechanisms.

Each of these platforms offers distinct advantages. It depends on the type of biomarker being targeted. These are selected and guided by factors such as sample type, sensitivity requirements, and clinical application [2].

Immunosensors

Immunosensors are among the most widely used electrochemical platforms for detecting protein biomarkers such as CEA and CA15-3. These systems typically operate in a sandwich assay configuration. Which a capture antibody immobilized on the electrode surface binds the target antigen. It's followed by the introduction of a labeled secondary antibody. Its purpose is to generate a measurable signal. Recent advancements have incorporated nanomaterials. This includes graphene, gold nanoparticles, and conductive polymers, to enhance signal amplification and electron-transfer efficiency. These improvements have enabled detection. It limits in the picogram-per-milliliter range, significantly enhancing analytical performance [2].

Genosensors

The principle of genosensors is to detect nucleic acid markers, such as BRCA mutations. tumor cell DNA, and methylated DNA. A genosensor works through probe-target hybridization, which induces electrochemical changes. Nanostructured materials, such as graphene and carbon nanotubes, are commonly used to enhance surface properties and improve electron transfer. Genosensors have detection capabilities at the attomolar to femtomolar level, making them an effective tool for early cancer diagnosis [2]. Genosensor technology is based on the detection of nucleic acid markers, such as BRCA mutations, cancer cell DNA, and methylated DNA. Genosensors function by hybridizing a probe with the target and causing electrochemical effects. Materials such as graphene and carbon nanotubes have been used to nanostructure surfaces and enhance electron transport. Detection of biomarkers is possible at attomole to femtomole levels, making genosensors useful for cancer diagnosis.

Aptasensors

The aptasensor uses aptamers, which are artificial single-stranded DNA/RNA molecules that bind specifically to biomolecules such as MUC1. Compared with antibodies, aptamers offer several benefits, including enhanced stability, greater synthetic capacity, lower cost, and greater adaptability for modification. It has been shown that electrochemical aptasensors exhibit outstanding efficiency in detecting breast cancer biomarkers owing to their high binding affinity and modifiability during sensor development [2]. The use of electrochemical aptasensors in combination with various nanomaterials can further enhance their capabilities, making them highly promising for the development of next-generation biosensors. An overview of the key electrochemical biosensors for breast cancer biomarker detection is provided in Table 3. The working principle of an aptasensor involves aptamers, which are synthetic oligonucleotide single strands of DNA or RNA capable of binding specifically to target molecules, such as MUC1. Compared with antibodies, aptamers offer certain advantages, such as greater stability, ease of synthesis, lower cost, and the ability to be modified. It has been observed that electrochemical aptasensors exhibit superior sensitivity for detecting breast cancer biomarkers owing to their excellent binding affinity and ease of modification during fabrication. Using electrochemical aptasensors in conjunction with nanomaterials enhances their functionality, making them a promising choice for biosensors of the future. The key electrochemical

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biosensors used to detect breast cancer biomarkers are summarized in Table 3.

Role of Nanomaterials in Sensor Enhancement

The application of nanotechnology has been critical to the development of electrochemical biosensors. These are major causes of breast cancer. Materials like graphene, gold nanoparticles, reduced graphene oxide, carbon nanotubes, and mesoporous silica. These conductive polymers possess unique physicochemical properties that enhance sensor efficiency [2].

These contribute to biosensor functionality through multiple mechanisms:

Signal amplification via catalytic and redox-active properties

Electron transfer due to high conductivity

Increased area for biomolecule immobilization

Biocompatibility and stability,

Multi-label detection strategies.

According to the latest research, nanomaterials act as electron mediators. The enzyme carriers and electrochemical markers enhance the sensitivity and reduce the limit of detection [2]. Though, nanomaterials offer numerous benefits. These are in terms of analysis, obstacles such as complicated processing methods, expensive manufacturing, and poor stability hinder. Their widespread use in clinics also high.

Table 3

Comparison of electrochemical biosensing platforms for breast cancer detection

Sensor Type	Target Biomarker	Detection Limit	Advantages	Limitations
Immunosensor	CEA, CA15-3	pg/mL	High specificity established method	Antibody cost and instability
Genosensor	BRCA, ctDNA	fM-aM	High sensitivity, mutation-specific	Complex preparation
Aptasensor	MUC1, HER2	pM-fM	Stable, reusable, cost-effective	Limited clinical validation
miRNA Sensor	miR-21, miR-155	fM-aM	Ultra-high sensitivity	Standardization issues
Nanomaterial-based sensor	Multiple biomarkers	fM-aM	Signal amplification, fast response	Fabrication complexity
Multiplex sensor	Multi-target panels	Variable	Comprehensive detection	Data complexity, calibration

Representative Biomarker–Sensor Systems

BRCA1 Detection

Genosensors have been developed to test variations. These variations in the BRCA1 gene sequence. They exhibit excellent sensitivity to advances in signal amplification

using nanomaterials. In genosensors and gene mutations are identified through impedance. They can also identify current generated by DNA hybridization. While effective in analytical procedures and their primary application in medicine is genetic testing [2].

MUC1 Detection

The overexpression of the antigen on the surface of breast cancer cells has led to its emergence as MUC1. This is one of the most promising targets in electrochemical sensing. Due to the exceptional properties of aptamers used in detecting MUC1 in many biological specimens. Especially those involving gold nanoparticles and enzyme amplification methods. MUC1 detection is enabled by amplification using nanomaterials and aptamer binding [2].

CEA Detection

The electrochemical immunoassay for CEA is considered one of the most significant investigated systems. This is used for breast cancer diagnosis. Various methods using nanomaterials, including graphene, magnetic nanoparticles, and bimetallic catalysts, have been developed. These methods to reach ultra-sensitive detection levels via amplified signals. However, it should be noted that CEA lacks high specificity as a biomarker [2].

Advantages of Combining Biomarkers with Electrochemical Detection

The combination of biochemical biomarkers with electrochemical biosensing technologies offers significant advantages for cancer diagnostics:

Invasive sample collection

Rapid analysis and real-time detection

Low sample volume requirements

High analytical sensitivity and specificity

Potential for multiplex biomarker detection

Reduced cost and portability for point-of-care applications.

It is therefore clear that the use of such electrochemical biosensors could be instrumental. In the development of new-age diagnosis approaches that could aid in detecting early-stage breast cancer. The combination of advances in biomarker identification and sensor fabrication is making numerous possibilities a reality [2].

Challenges and Limitations

Despite all the progress made in developing biomarkers and electrochemical biosensing. There remain many obstacles preventing their use in practical medicine. The first problem concerns poor biological performance. Protein biomarkers such as carcinoembryonic antigen and cancer antigen 15-3 lack good specificity. It is considered specificity and sensitivity in this field. Especially when detecting early stages of breast cancer. These biomarkers usually become elevated at the later stages of cancer. Moreover, they may be present in benign cases. They may also be in other tumor types. The use of single biomarkers is not sufficient. For diagnosing the disease in its early stages, it needs multiple tests. The second challenge is the difference between laboratory analytical performance and clinical performance of biosensors.

Despite achieving ultralow detection limits. The electrochemical biosensors are often limited by the matrix. The effects that interfere with accurate measurements. SThe matrix often limits the electrochemical biosensors pecifically, the complexity of biological matrices, such as plasma, makes the detection of specific biomarkers difficult. It becomes problematic due to matrix effects. Protein and salt interference, as well as nonspecific adsorption of substances, can occur on electrode surfaces. In addition, the reproducibility and stability of biosensing devices remain very important. Electrode processing and environmental factors can result in variations. From one biosensor platform to another or even in an individual experiment. Stability is especially crucial for portable devices. Unfortunately, most biosensors degrade. Biomolecular recognition units, such as antibodies or enzymes, may also cause.

In addition, the use of nanomaterials increases sensitivity and amplification. Signal detection poses certain challenges. It may include costs, scalability, and the difficulty of fabricating nanomaterials. Some advanced nanomaterials, such as gold nanoparticles, graphene oxide derivatives, and nanocomposites, require complex synthesis and functionalization techniques. Furthermore, their inability to scale up due to issues. These issues include batch-to-batch variations and the unavailability of standardized protocols. Which pose certain challenges for their clinical application. Another important issue with most biosensor-based cancer diagnostics is the absenteeism of clinical validation tests. Many of the diagnostic systems have been validated. In vitro, using small sample sizes, it does not give a proper picture of their performance. In actual clinical situations, it is needed. Thus, there is an urgent need for randomized control trials. To compare these new diagnostic tools with traditional approaches. It may include mammography and histopathology. In conclusion, promising results have been achieved in detecting biomarkers using biosensors. But certain limitations still persist. There is an acute requirement for a multidisciplinary approach. In this way, we overcome the existing issues in biomarker biology, nanomaterial design, device engineering, and clinical research.

Future Perspectives

Prospects for breast cancer diagnosis can be found in the fusion of state-of-the-art. Biomarker identification technology and novel biosensor technology can be used. Some important trends that will guide the future of breast cancer are diagnostic technologies. Firstly, multiplex biomarker identification. It is anticipated to emerge as a preferred option over conventional single-target identification. Breast cancer is heterogeneous in nature. Using multiple types of biomarkers, such as protein-, nucleotide-, and epigenetic-based biomarkers, can greatly enhance diagnostic efficiency.

The second area is liquid biopsy technologies. In the field, circulating biomarkers, like ctDNA, cfDNA, and microRNAs, are anticipated to be of great importance for early diagnosis. It's considered continuous disease monitoring. In contrast to traditional tissue biopsies, this is a type of diagnostic tool. It offers numerous benefits by enabling real-time examination of tumor behavior. Third design of portable and point-of-care biosensor devices. It will provide the means of decentralized diagnostics. It allows conducting early tests. These tests will be conducted under limited resource conditions. Electrochemical sensors are characterized by their

simplicity and rapid response. These are very suitable for such tasks.

Fourthly, the use of artificial intelligence. Machine learning algorithms will enable data analysis. AI-driven models can process multimarket information. They detect patterns and improve diagnostic decision-making and its accuracy. The validation and standardization of biosensing systems are important for their implementation. The implementation of standardized methods for sample collection and sensor fabrication is necessary. In conclusion, it is important to consider not only analytical but also practical aspects in the development of future diagnostic systems, with the main aim of creating highly sensitive sensors. When detecting substances at very low concentrations, the primary focus should be on practical applicability.

Conclusion

The diagnostic tests for breast cancer are an integral part of oncological research. It demands the development of detection methods. That can reliably indicate cancer without being invasive. Biomarkers are indispensable. In this area, they enable researchers to study tumor development and detect diseases at the molecular level. Protein-based biomarkers like CA15-3 and CEA have helped monitor the progression of diseases. Unfortunately, these biomarkers cannot be used effectively. To detect early stages of breast cancer, they lack both the sensitivity and specificity required to perform this task well. In this area, some new biomarkers, like MUC1, DNA methylation patterns, circulating nucleic acids, and autoantibodies, show promising results. Electrochemical biosensor technology can help diagnose breast cancer at an early stage. Due to its high sensitivity, speed, and cost-effectiveness, this method of testing patients is ideal. The introduction of nanomaterials enabled the detection of trace amounts of target biomarkers. This greatly increased the effectiveness of electrochemical biosensors. Still, there is room for improvement before it can be used in practical applications. It appears that convergence of biomarker biology. Biosensors are the best approach to early diagnosis. In conclusion, it will be necessary to integrate a combination of strong biomarker biology and biosensor technology. In order to achieve the best possible strategy for early diagnosis of breast cancer. This technology is a promise for the future because it is a multiplex detection technology. That is coupled with point-of-care systems and AI-based data analysis techniques.

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