Chapter 3

Biosensor Design Strategies For Early Diagnosis of Breast Cancer

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Abstract

Breast cancer is among the most prevalent cancers in women, and early detection is pivotal for enhancing treatment efficacy. Biosensor technologies emerge as prospective alternatives for early diagnosis of breast cancer due to the limitations of commercial diagnostic procedures, including invasiveness, high cost, and low sensitivity. Biosensors provide rapid, sensitive, and typically non-invasive identification of specific breast cancer biomarkers through the use of biological recognisers (such as enzymes, antibodies, DNA, and aptamers) and physical transducers (including electrochemical, optical, piezoelectric, and calorimetric methods). These technologies enable the successful detection of classical biomarkers such as Human epidermal growth factor receptor 2 (HER2), Cancer antigen 15-3 (CA 15-3), and Cancer antigen 125 (CA125), as well as novel biomarkers like microRNAs, circulating tumour cells (CTC), and exosomes. This chapter provides a detailed examination of biosensor technologies for the early diagnosis of breast cancer, including several biosensor types, their operational principles, bioengineering design methodologies, clinical applications, and prospective advancements.

1. Introduction

Breast cancer contributes to a substantial number of cancer diagnosis and fatalities annually, making it one of the most prevalent causes of cancer-related mortality among women worldwide (1). The global burden of breast cancer is on the rise, despite the improvement in treatment options. This underscores the urgent need for effective early detection strategies. Consistently, numerous studies have demonstrated that early diagnosis

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significantly enhances patient prognosis, allowing for less aggressive treatment approaches, improving quality of life, and substantially increasing survival rates (2,3). Mammography, ultrasound, and magnetic resonance imaging (MRI) are currently the most common methods for screening for and diagnosing breast cancer (4). By allowing for earlier detection, these imaging techniques—which are easily accessible—have been instrumental in lowering mortality rates from breast cancer. Nevertheless, their diagnostic efficacy and accessibility are limited by a number of issues. There are a number of major issues with certain procedures, such as mammography, including high procedural costs, low sensitivity, and exposure to ionizing radiation (5). In addition, the potential for false-positive and false-negative results, as well as the necessity for specialized apparatus and trained personnel, can result in patient anxiety, delayed diagnoses, and unnecessary biopsies. In light of these obstacles, there has been a growing need for the creation of innovative diagnostic technologies that are not only highly sensitive and specific, but also cost-effective, expeditious, and minimally invasive. The primary objective of these technologies is to enhance early detection rates, decrease diagnostic errors, and increase access to screening, particularly in low-resource settings. As potential alternatives or complements to conventional imaging techniques, biosensors, liquid biopsy platforms, and other advanced molecular diagnostics have garnered significant attention, providing promising opportunities for more personalized and precise breast cancer diagnosis (6).

In the analysis of breast cancer biomarkers, biosensing systems have emerged as promising platforms by integrating biological recognition elements with physicochemical transducers (7,8). The diagnostic capabilities of these devices are rapid, sensitive, and point-of-care, rendering them appropriate for personal health monitoring and clinical applications (9).

Biosensors are detection systems that are the result of the combination of biological recognition elements with physical transducers, allowing the determination of a specific target analyte. Each biosensor has certain static and dynamic properties. The biosensor's performance shows that these features have been improved. Selectivity describes how effectively a biosensor distinguishes the target analyte from other components in a sample. Reproducibility refers to the ability of a biosensor to provide consistent and reliable results under the same experimental conditions during repeated measurements. The limit of detection (LOD) or sensitivity of a biosensor is the lowest possible amount of analyte that it can detect. Linearity shows how accurate the measured response is for a set of measurements that use different metrics (10).

Electrochemical biosensors are widely applicable in sectors such as pharmaceuticals, medical or environmental monitoring due to their portability, cost-effectiveness, and specificity, which surpass thermal, optical, and piezoelectric alternatives. Electrochemical biosensors offer a convenient diagnostic technology that can determine biomarkers in body fluids such as blood, sweat, urine or feces. A wide variety of electrochemical techniques can be used to characterize biosensors or measure response signals during biorecognition events. Therefore, electrochemical based biosensing approach can be classified as voltammetric, conductometric, impedimetric, amperometric and potentiometric. A potentiometric biosensor is a device that has a biological sensing element and an electrochemical potential transducer connected to it. Potentiometric biosensors are usually based on biochemical reactions that lead to a simpler chemical species followed by electrochemical detection (NH_4OH, CO_2, pH, H_2O_2…). During a redox reaction, amperometric biosensors measure the flow of current between electrodes. Amperometric biosensors may detect heavy metal ions, antigens, antibodies, proteins, DNA fragments, pH changes, and more. The glucose biosensor is the amperometric biosensor system that has been studied the most. In this system, glucose oxidase (GOx) allows glucose react with oxygen to generate gluconolactone and hydrogen peroxide. Voltammetry is an electro-analytical method that gets information about the analyte by changing the potential and then measuring the current that comes out of it. Consequently, it is an amperometric technique. Cyclic voltammetry is one of the most common voltammetric procedures. The chemical rate constant and other electrochemical reaction rates, as well as the redox potential of analyte solutions, can be determined using this method. Conductometric biosensors measure how an electrochemical reaction changes the conductivity of an analyte by measuring the difference in conductivity between two electrodes. To observe metabolic activities in real-time, biological systems frequently employ impedimetric and conductometric biosensors. Impedimetric biosensors evaluate changes in charge conductivity and capacitance at the device's surface when the target binds to the sensor (11).

Optical biosensors are analytical instruments that integrate optical transducer systems with biological recognition elements. The primary function of an optical biosensor is to generate an optical signal that correlates with the concentration of the target analyte. Biosensors based on optical principals such as fluorescence, chemiluminescence, surface plasmon resonance (SPR), and fiber optics are the most popular types of optical biosensors. The SPR method can detect the change in refractive index that occurs when molecules interact with a metal surface. SPR is a label-free

method for studying the dynamics of biomolecular interactions. Fluorescence is an optical effect that uses labeling to find a molecule or analyte. The development of optical biosensors based on fluorescence has paid a lot of attention to this phenomena. Because it is very selective, sensitive, and has a quick response time, this type of biosensor is one of the most studied for use in medical diagnostics and monitoring the quality of food and the environment. There are three primary types of chemiluminescent-based sensors: (1) bio-chemiluminescent-based, (2) thermo-chemiluminescent-based, and (3) electrochemiluminescent-based. Biochemiluminescent-based sensors are particularly sensitive when employed in biological immunoassay systems; they can detect concentrations as low as attomoles. Chemiluminescent technology is utilized a lot for chemicals that are crucial to biology. A biological recognition element and an optical fiber or optical fiber bundle are both used in optical fiber-based biosensors (12).

The piezoelectric effect is the ability of some crystals to create an electric charge when they are put under mechanical stress, and the opposite happens when an electric field is applied. Changes in the voltage applied to the surface of a piezoelectric substance cause oscillation and mechanic stress. The adjustments are in line with the mass. The determination of a wide variety of macromolecular substances and microorganisms can be accomplished with the help of piezoelectric immunosensors. The piezoelectric immunosensors were the subject of extensive research and have a variety of promising applications. When it comes to the fundamental concept behind their application, piezoelectric biosensors that incorporate molecularly imprinted polymers are extremely similar to piezoelectric immunosensors. An affinity reaction is the means by which they directly react with an analyte, and the affinity reaction is responsible for the decrease in the oscillation frequency that is recorded (13).

Thermal biosensors, or calorimetric biosensors, have been created by integrating a biomaterial with a physical transducer, such as a thermometer (14). Thermal-based calorimetric biosensors can be employed in enzyme activity assessments, clinical monitoring, process regulation, non-aqueous environment evaluations, and environmental surveillance by tracking changes in temperature. (15).

Biosensors are easy-to-use analytical tools that can quickly and accurately find a large range of biological analytes (16). These devices have both a biological recognition element and a physical transducer, which lets them turn biochemical interactions into signals that can be monitored. Biosensors are becoming more and more popular in many areas (most importantly

clinical diagnostics). This is because they are easy to use, cheap, and can give data in real time. In the medical field, biosensors have gotten a lot of attention since they are very important for the early diagnosis, prognosis, and therapy monitoring of many diseases, including cancer. They are especially useful for analysis of biomarkers that are specific to a condition, which makes it possible to get medical help quickly and make treatment plans that are tailored to each person (17). As biotechnology, nanotechnology, and microfabrication processes are becoming better, biosensors are getting stronger, easier to carry, and more useful for point-of-care applications.

2. Biosensor systems for early diagnosis of breast cancer

2.1. Electrochemical biosensors for breast cancer

Electrochemical biosensing systems have emerged as highly desirable instruments for the analysis of breast cancer biomarkers. This is mostly due to the fact that these systems possess high sensitivity, rapid response, and the capability to perform tests at the point of care (18). Quantitative and targeted biomarker identification is made possible by these biosensors, which transform biological interactions into quantifiable electrical signals (19). Conductive nanomaterials are critical in the biosensor fabrication process. Sadrabadi et al. (20) fabricated a biosensor that is capable of analysis of cancer-associated microRNA 155. Functionalised metal-organic frameworks and carbon nanostructures constitute the principal elements of the biosensor. The limit of detection was 0.08 fM and the linear range was from 0.2 fM to 500 pM. In another sudy, Foroozandeh et al. (21) designed an electrochemical nanobiosensor that was capable of detecting CA125 with precision and selectivity. In order to maintain the stability of the aptamer strands on a modified glassy carbon electrode, the nanobiosensor made use of MoS_2/g-C_3N_4/PANI. This agent is molybdenum disulfide, graphitic carbon nitrides and polyaniline. The aptasensor was utilized to analysis the labeled CA125 using electrochemical techniques with label-free ferrocyanide and methylene blue.

The HER2 tumor marker is a well-established indicator of malignancy. In this regard, Sadeghi et al. (22) developed an electrochemical nano-biosensor and tested HER2 biomarker detection utilizing FGO (2D functionalized graphene oxide). Electrochemical biosensors provide an innovative approach due to its combination of great sensitivity, fast analysis, and portability. Recent advances in nanomaterials, biorecognition components, and microfabrication methods have propelled the field forward, paving the

way for the creation of biosensors that are both more effective and more applicable in clinical settings.

2.2. Optical biosensors for breast cancer

Optical biosensors detect molecular interactions and convert them into measurable signals by utilising light-based principles. Their capacity to offer quick, label-free, and multiplexed detection renders them highly desirable for clinical diagnostics and point-of-care applications. Optical biosensors exhibit high diagnostic accuracy in the early detection of breast cancer biomarkers, thereby facilitating opportune clinical intervention (23).

Hossain et al. (24) introduced a straightforward hybrid design and numerical evaluation of the graphene-coated fiber-optic SPR biosensor. This biosensor can detect early onset BRCA1 and BRCA2 genetic breast cancer. BRCA is breast cancer gene. Li et al. (25) created an electrochemiluminescence (ECL) biosensor to identify miRNA221 in another work. This biosensor is made up of MQDs-GSH (glutathione-capped MXene-derived quantum dots and magnetized biomimetic vesicles). The results of the tests show that an ECL biosensor could be used to find triple-negative breast cancer (TNBC).

Optical biosensors has effective potential for the early identification and clinical management of breast cancer. Ongoing research and interdisciplinary collaboration are crucial to overcome existing hurdles and expedite the practical application of optical biosensor technologies. Future improvements are expected to position these devices as significant in personalised medicine and precision oncology for breast cancer patients.

2.3. Piezoelectric biosensors for breast cancer

Piezoelectric biosensors generate measurable variations in frequency or acoustic waves by detecting changes in mass or mechanical features at the sensor surface. A piezoelectric biosensor was created by Han et al. (26) to identify miR-106b, which was isolated from cancer cells. The surface acoustic wave (SAW) biosensor showed that it could detect concentrations of miR-106b (with a limit of detection of 0.0034 pM) within a range of 0.1 pM to 1.0 μM. Furthermore, it exhibited a good regression coefficient of 0.997 as a result of its detection capabilities. Additionally, miR-106b was found in the total RNA that was taken from the exosomes that were isolated from the MCF-7 tumor cell line.

In real-time, QCMs have been acknowledged as an instrument for detecting biomolecular interactions without the use of labels (enzyme-conjugated secondary antibodies, fluorescent dye etc.). Shang et al. (27)

fabricated a platform for the detection of Herceptin in solutions. This platform is based on a durable synthetic peptide derived from HER2 Mimotope that is immobilised on the surface of a gold quartz electrode. When testing for Herceptin in human serum, piezoimmunosensor or QCM tests were employed with HER2 mimetope standing in for the HER2 receptor protein. Its linear range was (0.038 - 0.859 nM), and its limit of detection was 0.038 nM, according to the piezoimmunosensor assay.

A class of biosensors called piezoelectric microcantilever sensors (PEMS) can be activated and detected electrically via mechanical resonance. These sensors have a highly piezoelectric layer connected to a nonpiezoelectric layer. Capobianco et al. (28) developed a piezoelectric microcantilever sensor (PEMS) based on a lead zirconate-lead titanate (PZT)/glass composite for detection of HER2 biomarkers in diluted human serum. By utilizing the first longitudinal extension mode of the sensor and functionalizing its surface with an H3 single-chain variable fragment (scFv) via a silanization agent (3-mercaptopropyltrimethoxysilane) insulation layer, they achieved sensitive HER2 detection within the concentration range of 6–60 ng/mL (0.06–0.6 nM). Moreover, the dissociation constant (Kd) of the HER2-H3 interaction was determined, showing good agreement with values obtained via BIAcore SPR analysis. This study highlights the potential of piezoelectric microcantilever sensors for a label-free determination of breast cancer biomarkers.

2.4. Calorimetric biosensors for breast cancer

Early identification is crucial for increasing longevity and treatment results in breast cancer. In this regard, calorimetric biosensors stand out as an innovative and encouraging strategy. A label-free, direct, and extremely sensitive approach to biomarker detection is provided by these biosensors, which function by detecting heat changes linked to biochemical events or molecular interactions. A colorimetric biosensor for detecting BRCA1 mutations in breast cancer was described by Bai et al., (29) who used a three-step multiple signal amplification technique. There is a strong linear correlation between the DNA concentration and the reaction kinetics constant within a range of 10^{-12}-10^{-18} M, and the detection limit can be as high as 10^{-18} M. With its many benefits, including label-free detection, high sensitivity, and little sample preparation, calorimetric biosensors hold great promise as a tool for the early diagnosis and treatment of breast cancer. The miniaturisation of devices, surface functionalisation, and microfluidic integration that have been achieved in the field of biomedical engineering have greatly enhanced the potential of these sensors for use in clinical settings.

Table 1. The biosensor systems for early diagnosis of breast cancer

Targets	Measurement method	Modification method	Linear range	Reference
Cancer-associated microRNA 155	Electrochemical	Modification with MOFs and carbon nanostructures	0.2 fM-500 pM	(20)
CA125	Electrochemical	Modification with g-C3N4/MoS2/ PANI	2 $U.mL^{-1}$-10 $U.mL^{-1}$	(21)
HER2	Electrochemical	Modification with two-dimensional (2D) functionalized graphene oxide (FGO)	0.5 ng/mL - 25 ng/mL	(22)
BRCA1 and BRCA2	Optical	Graphene-coated fiber-optic SPR biosensor		(24)
miRNA221	Optical	Modification with GSH-MQDs	10 fM - 10 nM	(25)
miR-106b (exosomal miRNA)	Piezoelectric	SAW biosensor	0.1 pM - 1.0 μM	(26)
Herceptin	Piezoelectric	Synthetic peptide-based QCM	0.038nM–0.859 nM	(27)
HER2	Piezoelectric	Longitudinal extension mode of a lead zirconate-lead titanate (PZT)/glass PMS	6ng/mL–60 ng/ml	(28)
BRCA1	Calorimetric	Bi_2Se_3-AuNPs	10^{-12}-10^{-18} M	(29)

3. Conclusion

Breast cancer is still one of the most common and deadly cancers in women around the world. Identifying breast cancer early on greatly improves the chances of survival and the prognosis. This makes early detection a key part of good cancer management. In this situation, biosensor technologies have become very useful for diagnosing breast cancer since they can quickly, accurately, and cheaply analysis the specific biomarkers, frequently

with minimally invasive. Biosensors can determine a large range of breast cancer biomarkers with great accuracy by combining biological recognition components such antibodies, aptamers, enzymes, or nucleic acids with physical transducers. Some of these are well-known protein biomarkers including HER2, CA 15-3, and CEA. Others are new next-generation biomarkers like microRNAs (miRNAs), circulating tumor cells (CTCs), and exosomes. Electrochemical, optical, piezoelectric, and calorimetric biosensors have all shown good results for detecting these biomarkers. This makes it possible to diagnose them early and keep better track of treatment. Biosensor technologies have a lot of potential, but there are still a few significant issues that need to be solved before they can be widely used in clinical settings. These problems include technical issues with sensitivity, specificity, and long-term stability; the lack of standard procedures for making and testing biosensors; possible interference from complicated biological matrices; and the need for a lot of clinical testing to show that the biosensors are accurate and reliable. In the future, biosensors are likely to work even better as nanotechnology, surface chemistry, and microfabrication continue to improve. In conclusion, if biosensor-based diagnostic tools are successfully integrated into clinical practice, they could significantly transform the landscape of breast cancer diagnosis and treatment. These kinds of technologies not only have the potential to increase early detection, but they additionally provide the possibility to the creation of customized medicine methods, which allow for treatment plans that are tailored to each patient and improve their health and quality of life.

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