Advancements in Biosensors: Fundamentals, Technologies, and Applications

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Perface

Biosensor technologies have emerged as essential instruments that revolutionise diagnostic, monitoring, and analytical processes in current science and engineering. The book chapters address current developments in biosensor technologies and their applications for the early diagnosis of various diseases. The first chapter focuses on the use of nucleic acid-based aptamers in recently developed electrochemical and optical biosensors, evaluating the performance advantages offered by these novel biorecognition elements. The second chapter covers the fundamental principles and application areas of paper-based biosensor technologies, which offer low-cost and portable solutions. The third chapter discusses biosensor design strategies for the early diagnosis of breast cancer, covering topics such as biomarker selection, sensor architecture, and diagnostic performance. The fourth chapter focuses on biosensor systems used in the diagnosis of prostate cancer, examining their scientific basis, technological advancements, and clinical implications. The final chapter discusses current biosensor designs developed for the early diagnosis of neurodegenerative diseases, and evaluates next-generation sensor platforms that enable the sensitive detection of biomarkers with potential for clinical use. This book provides a comprehensive overview of the potential use of biosensors across disciplines and their transformative role in diagnostic technologies.

Contents

Chapter 1

Recent Advances in the Use of Nucleic Acid Aptamers in Electrochemical and Optical Biosensors

Canan Özyurt[1]

Abstract

Nucleic acid aptamers are single-stranded DNA (ssDNA) or RNA oligonucleotides that have the ability to recognize different targets with their special secondary structures. Aptamers, which have high specificity and affinity, are gaining increasing importance in biosensor technologies as recognition elements that can be easily synthesized chemically and modified according to the need. The small size, stability, easy modification and low immunogenicity of aptamers make them an important alternative to traditional antibodies as biosensor recognition elements. In this book chapter, current electrochemical and optical approaches to aptamer-based biosensors are comprehensively discussed and systems based on various sensing principles are comparatively analyzed. Particular attention is paid to electrochemical, colorimetric, fluorescent, surface plasmon resonance (SPR) and lateral flow assay (LFA)-based aptasensor designs. This chapter compares different biosensor platforms in terms of sensitivity, selectivity and analytical parameters, and also includes technological advances that enable the development of new generation aptasensors. In this context, the study aims to be an up-to-date reference source for future research in the field of aptasensors.

1. Introduction

Nucleic acid aptamers are unique recognition elements that have shown great potential for use in numerous applications since they were first described by Ellington and Szostak (1). Nucleic acid aptamers, consisting

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of short-chain RNA or ssDNA sequences, are commonly developed against almost any target by the method called Systematic Evolution of Ligands by Exponential Enrichment (SELEX) (2). During the development of aptamers using the SELEX method, environmental conditions such as ionic strength, temperature, and pH can be adjusted as needed. This makes aptamers very advantageous as recognition elements, as they can be manipulated according to the desired conditions. The main advantages of aptamers over antibodies, which are traditionally the most preferred recognition elements in biosensors, are their chemical synthesizability, high specificity and affinity, small size, stability and durability, and easy modifiability. Moreover, the batch-to-batch variability of aptamers is lower than that of antibodies (3). In particular, their ability to be easily modified while preserving their secondary structures has brought aptamers to the forefront as recognition elements in biosensors over the past decade (4). Biosensors developed using aptamers are becoming increasingly important in modern diagnostic technologies due to their high specificity and sensitivity. Various sensing principles such as optical, electrochemical, colorimetric, fluorescence-based, surface plasmon resonance (SPR), and piezoelectric can be easily adapted to aptasensors (5, 6, 7, 8, 9). Aptasensors are widely used in the determination of many different analytes such as clinical biomarkers, food toxins, environmental pollutants, pharmaceuticals, and small molecules due to their relatively low cost, high sensitivity, and fast response time. The development of aptasensors using different measurement strategies is closely related to the advantages of aptamers such as stability, high selectivity, easy modifiability, and reusability.

This chapter presents a comparative analysis of current approaches to nucleic acid aptamer-based biosensors. The focus is on the application of aptamers in various fields such as clinical biomarker diagnosis, food analysis, and environmental detection. Technological developments used in the design of aptasensors based on different sensing principles are also included. Different platforms are compared in terms of sensitivity, selectivity, and ease of application, accompanied by current examples from the literature.

2. Aptasensors

2.1. Electrochemical aptasensors

Electrochemical-based biosensors are analytical devices based on the principle of converting biochemical interactions between biological recognition elements and the surface of electrodes or electroactive components into electrical signals via a transducer (10, 11). They generally

operate with high sensitivity to specific target analytes using biologically based recognition elements such as enzymes, antibodies, nucleotides, molecularly imprinted polymers (MIPs), aptamers or cells. Electrochemical-based approaches, which can have different measurement principles such as amperometric, potentiometric, voltammetric and impedimetric, are widely used for the detection of analytes in a wide range of scales by offering advantages such as high sensitivity, low detection limit and short analysis time. Thanks to advanced surface modification techniques, it has become possible to immobilize biomolecules on the electrode surface in a way that they maintain their stable and interaction-friendly structures. With these features, electrochemical biosensors have gained an important place in commercial applications as well as maintaining their popularity in academic research. In recent years, aptamers have begun to play an important role as recognition elements in electrochemical biosensor designs. Particularly, the fact that aptamers can be modified at different positions with many different functional groups such as amine, thiol, carboxylic acid, biotin (12) during the chemical synthesis stage offers significant advantages in terms of surface design. The ability to modify aptamers with different functional groups is quite useful in terms of controlled surface immobilization. However, different strategies are required to orient antibodies (13). Aptamers can be covalently immobilized and directed to surfaces with different functionalities using modifications. In particular, it is possible to add linker sequences in addition to these modifications to provide ideal rational freedom for secondary structures related to target binding. A schematic diagram depicting the most commonly used electrochemical aptasensor techniques is presented in Figure 1. This diagram represents aptamer-modified electrodes that interact with target molecules and the electrochemical methods used to measure this interaction, such as EIS, SWV, and DPV.

In a recent study conducted by Didarian et al., an impedimetric biosensor was developed using the PTH (53–84) aptamer, in particular, as a result of the selection of different segment-specific aptamers for parathyroid hormone (PTH) by the SELEX method. In the study where the interface impedance change formed on the Carbon Screen Printed Electrode (SPE) electrode surface as a result of the interaction of the developed PTH fragment-specific aptamer with the target molecule was monitored, pg/mL sensitivity was achieved. This study also provides insight into the usability of aptamer-based biosensors in the instantaneous evaluation of surgical processes, focusing on a clinically important area such as intraoperative PTH monitoring (14). In another study based on impedimetric measurement, cerium oxide nanoparticles functionalized graphene oxide (CeO_2-GO) was modified with

target-specific aptamer for the detection of breast cancer biomarker EGFR. The developed aptasensor showed an extremely low detection limit of 1.87 fg/mL in PBS medium. The large surface area and high electrochemical conductivity of CeO_2-GO nanocomposite facilitated aptamer binding, thus increasing the sensitivity of the sensor (15). The study by Moradi and his colleagues has brought the first electrochemical impedimetric aptasensor developed for the detection of tramadol (TRA) to the literature. It is one of the most recent and striking examples in the field in terms of visual and quantitative verification of aptamer-target interaction with molecular dynamics (MD) simulation. The determination of the aptamer binding site by MD simulations provides an innovative approach in terms of design (16). A dual-mode aptasensor based on electrochemical impedance spectroscopy (EIS) and electrochemical (EC) was developed for the determination of aflatoxin B1 (AFB1), an important food toxin. The sensor was constructed by loading platinum and gold nanoparticles (MXene\@Thi/PtAuNPs) onto thionine-functionalized Ti_3C_2Tx MXene, and both electron transfer efficiency and internal reference signal providing capacity were increased. This nanocomposite structure was modified with hairpin-shaped DNA (hDNA) and ferrocene-labeled AFB1 aptamer (Fc-Apt) to transform it into a platform that provides specific signal changes in the presence of target. The sensor detected AFB1 with high accuracy and selectivity between 10 pg/mL and 30 ng/mL in ratiometric EC mode and between 3 pg/mL and 30 ng/mL in EIS mode. The study also yielded successful results in real corn samples, and the selectivity, repeatability and stability of the system were found to be high (17). The AFB1 aptamer was used as an electroactive signal transmitter by labeling it with ferrocene. When the AFB1 target is bound, the ferrocene-labeled aptamer leaves the general structure, thus the ferrocene signal (IFc) at the electrode decreases while the thionine signal increases, and this creates a sensitive detection mechanism. Although redox active molecules such as ferrocene do not directly produce a signal in the EIS analysis, they affect the charge transfer resistance (Ret) through the steric and electrical changes they create on the electrode surface, leading to a change in the EIS signal. Since the binding or dissociation of the ferrocene-labeled aptamer changes the charge transfer properties on the surface, it also provides an indirect but significant effect on the presence of the target in the EIS measurement. In this way, ferrocene-labeled aptamers contribute to target-specific and reliable signal changes in both voltammetric and impedimetric systems. In a recent study, which is an important example of the successful detection of large targets such as pathogenic cells with electrochemical aptasensors, *Escherichia coli* (*E. coli*) was detected in buffer with a detection limit of 1.4

CFU/mL. The impedimetric based aptasensor developed for the detection of E. coli bacteria in real samples was formed by covalently binding the DNA aptamer called P12-55 to a gold electrode and gave a linear response in the range of 100–10^4 CFU/mL. The sensor also showed successful results in real samples such as urine and tap water, maintaining high specificity and sensitivity especially in urine. The absence of cross-reactivity against other bacteria such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* proved its bioselectivity (18). In a study conducted by Sharma et al., a specific and sensitive aptamer-based impedimetric biosensor was developed for the diagnosis of chikungunya virus (CHIKV). The electrode surface was coated with nanographene oxide (NG) composite modified with zinc oxide (ZnO) nanoparticles to improve electron transfer and provide more aptamer retention. When the aptamers immobilized on this modified surface bound CHIKV antigen, electron transfer was inhibited and this was successfully detected by electroimpedimetric measurements. The developed aptasensor can detect antigen at a low limit of 1 ng/mL and gives a linear response up to 10 μg/mL; its performance and stability were also demonstrated in human serum. The absence of cross-reactivity against DENV antigen confirms the high specificity of the aptamer and the clinical selectivity of the sensor. The electrochemical aptasensor developed in this study is built on a paper-based platform, the electrochemical paper-based analyzer (ePAD). Such platforms are highly suitable for diagnostic applications, especially in resource-limited areas, due to their low-cost production, biodegradability, portability, and simple use (19). In one of the interesting studies conducted recently, a high specificity aptamer for rapid and portable dose determination of acute radiation sickness was developed by the MCP-SELEX method. Aptamers with high affinity for p21 protein were integrated into SPEs to form an EIS-based aptasensor. The developed sensor provides a linear response to radiation dose in the range of 0–10 Gy, while providing much faster and portable measurements than traditional methods with a low detection limit of 0.38 Gy. The study showed that the p21 protein level increased directly proportionally to the radiation dose, proving that this sensor works effectively both in cell cultures and in human peripheral blood samples. As a result, this method offers a new radiation dose assessment approach that is faster, portable and applicable in the field compared to existing biodetection techniques (20). Measuring the level of a radiation-sensitive protein (p21) with an aptamer-based electrochemical biosensor as in this study is a very rare strategy.

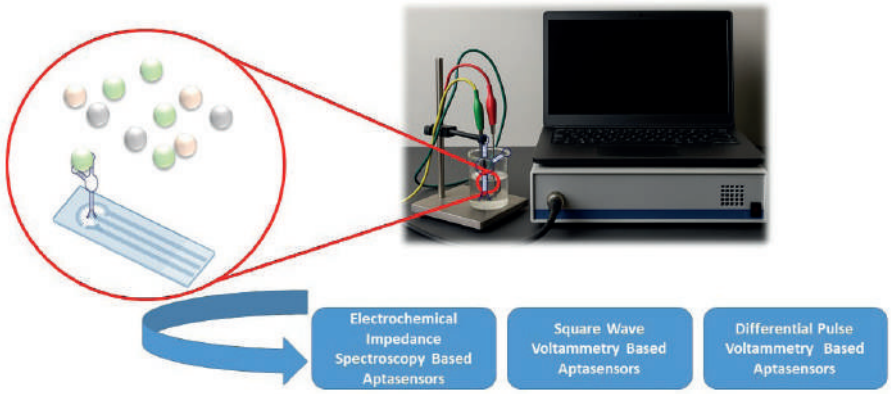

Figure 1. Schematic representation of electrochemical aptasensors. Target molecules bind to aptamer-modified electrodes, and the resulting signal is measured using electrochemical techniques such as electrochemical impedance spectroscopy (EIS), square wave voltammetry (SWV), and differential pulse voltammetry (DPV).

Square wave voltammetry (SWV) is an advanced electroanalytical technique characterized by high sensitivity and low background current, allowing rapid and selective detection of electroactive species depending on analyte concentration. A specific square wave voltammetry (SWV) based aptasensor for the hormone 11-deoxycortisol (11-DCL) was developed by comparing the MAB phase (MoAlB) and the multilayer MBene structures derived from it. MBene, obtained by removing aluminum from the MAB phase, provided a more suitable platform for aptamer immobilization by providing higher surface area and electron transfer capacity. The MBene-based sensor reached an extremely low detection limit of 0.014 pg/mL and showed high specificity against 11-DCL. The sensor gave minimal signals in cross-reactivity tests with similar hormones and achieved successful results in real serum samples. These findings indicate that MBene structures have high potential in electrochemical biosensors (21). MBene material, which has rarely been studied in the literature, was used for the first time in an electrochemical aptasensor platform in this study. While MXenes have been studied more traditionally, the use of MBenes in biosensor applications is limited. In this respect, the study pioneers the introduction of a new material to biosensor technologies. The effect of the MBene structure is evaluated in comparison with its direct precursor, the MoAlB MAB phase, and the advantages of MBene are demonstrated by electrochemical data. This approach provides a strong scientific contribution to the study from both mechanistic and engineering perspectives. In another study using the SWV method, DNA aptamers selected by the SELEX method were

used as recognition elements for the sensitive determination of the surface antigen SAG1 of Toxoplasma gondii. The sensor platform was created by immobilizing the SOK14 aptamer with high affinity specific to SAG1 onto screen-printed carbon electrodes modified with graphene quantum dots (GQD). Linear responses between 0.01–100 nM were obtained by square wave voltammetry and a low detection limit of 11.5 pM was achieved. Selectivity studies showed that there was no cross-reactivity with other pathogens such as influenza and RSV. High recovery rates were obtained in experiments with spiked serum samples, demonstrating the usability of the sensor in clinical samples (22). The SWV method was used in a biosensor developed for the detection of HER-2, a breast cancer biomarker. The biosensor was built on a nanohybrid-based electrode consisting of zinc oxide tetrapod (ZnOT) structures and potassium perylene tetracarboxylate (K4PTC) compound. ZnOT provides high surface area and electron conductivity, while the carboxylate groups on K4PTC allow stable binding of aptamers. The developed sensor has a linear response between 1 fg/mL and 10 μg/mL and an extremely low detection limit of 0.58 fg/mL (23). In another study conducted in 2025, a hydrogel-protected electrochemical aptamer-based biosensor (HP-EAB) was developed that can directly detect doxorubicin (DOX), a cancer drug, in whole blood samples. The aptamer, which is attached to the gold electrode with a thiol-modified tip and labeled with methylene blue (MB), changes conformation when bound to DOX, reducing the distance between the electrode and MB, which causes a significant increase in the reduction current measured by SWV. The thin agarose gel coating applied on the electrode surface prevents biofouling and increases the stability of the sensor by allowing the passage of small molecules while preventing large biomolecules (e.g. hemoglobin, albumin) from reaching the electrode. The results show that the sensor has a linear response between 0.1–50 μM and offers a detection limit of 25.9 nM. The modified form of the aptamer triggers the signaling mechanism by undergoing a structural transformation after target-specific binding, thus providing high selectivity and sensitivity (24). In a dual-electrode SWV-based electrochemical aptasensor study for the simultaneous detection of two antibiotics of high environmental importance, lincomycin and neomycin, the sensor surface was coated with a nanocomposite consisting of gold nanoparticles and carbon nanofibers providing high surface area and conductivity, and thiol-modified aptamers specifically designed for each antibiotic were immobilized. This binding mechanism of aptamers, which are immobilized on the surface by covalently binding to gold nanoparticles via thiol groups and prevent electron transfer with redox couples when the

target is bound via negatively charged phosphate backbones, causing signal decrease, made it possible to work with high selectivity and low pg/mL detection limits thanks to electrostatic interactions and three-dimensional structural specificity of the aptamer. The developed system has shown success with high recovery rates in tap water samples and is promising for environmental monitoring applications with its practical, sensitive, specific and multiple analysis capability (25).

Amperometric biosensors are typically designed to correlate the current generated by the reduction or oxidation of electroactive products—formed either in the presence of the analyte or via a mediator at the electrode surface—with the concentration of the analyte. The Differential Pulse Voltammetry (DPV) method is an electrochemical analysis method performed by adding short-term pulse signals to the linear potential sweep applied to the electrode. The current difference measured after each pulse gives a sensitive signal corresponding to the electrochemical activity of the analyte. This method offers high sensitivity and low detection limit for the determination of substances at low concentrations. Aptamer-based DPV and other amperometric biosensors have also been widely reported in the literature. Mu and co-workers developed a DPV-based biosensor for Adenosine Triphosphate (ATP) determination for use in the diagnosis of stomatological diseases. In the study, the approach of entrapping Pt\@Au nanoparticles with high electrocatalytic properties into a metal-organic skeleton structure (UiO-66) was adopted. Aptamer modification was carried out by conjugating ATP-specific DNA aptamers to the MOF surface, and this structure allowed electron transfer and particle collision by opening the "locked" sensor system when the target molecule was bound with ATP. This binding mechanism is based on the conformational change of the F-MOF structure upon binding of ATP to the aptamer, thus activating the electrocatalytic sites. The sensor stood out as an effective diagnostic platform for stomatological applications by exhibiting a very low lower detection limit of 0.046 pg/mL and a wide linear response range between 10 pg/mL – 50 μg/mL (26). In the design of a DPV based aptasensor developed for the detection of AFB1, aptamers were immobilized on the surface of N-doped carbon nanofibers/carbon fibers (N-CNFs/CFs) modified with gold nanoparticles (AuNPs) by thiol-gold chemistry. The gold-thiol interaction was both strong and directed, contributing to the conformational position of the aptamer. Since AFB1 is not electroactive, the Apt/AFB1 complex formed after binding limits the electron transfer and a decrease in the signal is observed; this makes the specific binding electrochemically detectable. The sensor showed a wide linear range between 10.0–10^8 pg/mL with a

LOD of 6.4 pg/mL and was effectively used in mixed samples of Chinese medicine with high recoveries (27). In a biosensor design, which is one of the examples of studies where different biosensor detection systems come together on a single analysis platform, a DNA self-assembly based, dual signal output biosensor was developed for the high sensitivity detection of thrombin. Both photoelectrochemical (PEC) analysis and DPV were used as measurement methods; thus, sensitivity and specificity were increased. The aptamer was modified by integrating it into the X-shaped structured DNA; DNA chains triggered by specific binding in the presence of thrombin ensure the binding of the GOx enzyme and the separation of the Fc-bearing DNA. This mechanism causes the photocurrent to decrease with the consumption of O_2 by GOx and the DPV signal to decrease with the decrease in the amount of Fc. Thus, thanks to the dual-mode system, highly sensitive thrombin detection was achieved with a femtomolar detection limit in the range of 10 fM - 10 nM (PEC: 6.89 fM, DPV: 5.86 fM) (28).

2.2. Optical aptasensors

Optical biosensors are systems that convert the interaction of biological recognition elements with the target analyte into optical signals. These sensors generally use optical principles such as fluorescence, absorbance, refractive index or surface plasmon resonance (SPR) to detect the presence of analytes. Optical-based approaches also exhibit high compatibility with aptamer-based designs due to their high specificity, fast response time and the possibility of labeled or label-free analysis. A schematic diagram depicting the most commonly used optical aptasensor techniques is presented in Figure 2. This diagram illustrates aptamer-functionalized platforms that interact with target molecules, along with optical detection methods such as colorimetry, FRET, SPR, and LFA used to monitor these interactions.

The Fluorescence Resonance Energy Transfer (FRET) method is one of the most widely used approaches in fluorescence-based aptasensor design. In the FRET method, aptamers undergo structural changes when bound to the target, affecting the distance between the donor and acceptor fluorophores; this makes it possible to correlate the signal change with the analyte concentration. The specific target recognition and conformational flexibility properties of aptamers provide significant advantages in FRET-based sensor design (29, 30). In an aptasensor developed for glucose detection, the FRET approach based on energy transfer between CdTe quantum dots (donor) and Cy3 dye (acceptor) was used. The specificity of the system for glucose was provided by the thiol-modified glucose aptamer, which binds to glucose with high affinity. While the aptamer was bound to CdTe QDs with the

TGA molecule, the FRET mechanism was activated by the inclusion of a short complementary sequence labeled with Cy3. When glucose was added to the system as a target molecule, it specifically bound to the thiol-glucose aptamer and disrupted the interaction with Cy3, which caused the cessation of FRET and the increase in the fluorescence signal. Thus, the binding mechanism is based on the control of FRET activity with the target-specific conformational change of the modified aptamer, and it gave successful results in human serum and urine samples with high sensitivity (LOD: 7.72 $\times$ 10^{-9} M) and selectivity (32). Wang et al. developed a turn-on FRET based aptamer biosensor for the simultaneous and sensitive detection of mycotoxins such as aflatoxin B1 (AFB1) and ochratoxin A (OTA). Dual fluorescent (Fam-DNA and Cy3-DNA) Fe_3O_4@TiO_2@Ag nanocomposites were used as the measurement method, and the fluorescent signal was reactivated in the presence of the target toxin and detection was achieved. Aptamers were conjugated with silver triangle nanoparticles (Ag TNPs) on the surface and when binding occurred, they changed their structures and detached from the surface, thus terminating FRET suppression and increasing the signal. This binding mechanism offers a unique design advantage based on the conformational transformation of aptamers. The developed system enabled the detection of AFB1 and OTA at low limits such as 0.26–0.94 ng/mL and was successfully applied in cereal/oil samples (33). Lu et al. developed a FRET-based ratio aptasensor for the determination of deoxynivalenol (DON). The measurement principle is based on the FRET mechanism, in which blue carbon dots (BCDs) are used as energy donors and graphene oxide (GO) as energy acceptors. Red carbon dots are also included as internal references in the system. The aptamer is covalently attached to BCDs via amidation to form the BCDs\@apt structure. These structures adsorb onto the GO surface via π–π interactions, while in the presence of DON, the aptamer binds specifically to the target, increases the distance between BCD and GO, and interrupts FRET; thus, the blue fluorescence increases again. The binding mechanism gains specificity due to the aptamer modification, thus the sensor exhibits high selectivity, low detection limit (14.7 pg/mL), and wide linear range (0.05–200 ng/mL) (34). A FRET-based aptamer biosensor was designed for the detection of the pesticide chlorpyrifos (CPF). The aptamer was conjugated to upconversion nanoparticles via amide bonds. The FRET mechanism relies on the overlap of the emission spectra of the energy donor, CIT-$NaYF_4$\:Yb^{3+},Tm^{3+}, and the absorption spectra of the acceptor, AuNPs. In this context, AuNPs bind to the complementary sequence of chlorpyrifos, establishing close contact. As demonstrated in this study, the use of

complementary sequences in FRET-based designs is a common strategy because they provide the sensor platform with a target-sensitive and reversible structure. In the absence of CPF, the aptamer and the complementary sequence hybridize, resulting in FRET, resulting in fluorescence quenching. However, in the presence of CPF, the aptamer binds to the target and the hybridized pair dissolves, thus decreasing FRET and reappearing fluorescence. The recovered fluorescence intensity is directly proportional to the chlorpyrifos concentration. The developed biosensor showed high sensitivity with a linear range of 0.1–200 ng/mL and a detection limit of 0.051 ng/mL (35). Aptasensors with FRET-based designs have also been developed for analytes containing relatively small and aromatic structures, including antibiotics. A recent example is a FRET aptasensor design based on DNAzyme and strand-displacement-based dual signal amplification, designed to simultaneously detect the antibiotics sarafloxacin (SAR) and enrofloxacin (ENR) in milk samples. The system triggers signal generation using Zn^{2+}-dependent DNAzyme arrays that are activated by binding to aptamers specific to the target antibiotics. The system generates strong FRET signals with a single excitation wavelength from FAM to ROX/JOE. The high selectivity of the aptamers prevents cross-reactivity, and the sensor stands out with both its specificity and low detection limits (1.95 pM for SAR and 5.01 pM for ENR) (36). He and colleagues developed an innovative aptamer-based FRET aptasensor based on a self-reproducing hybridization chain reaction for the detection of the antibiotic sulfamethoxazole (SME), which is present at low levels in human serum. Upon binding to SME, the aptamer folds into a specific conformation, releasing the initiator I strand that initiates HCR-1. This, in turn, triggers HCR-2, leading to the formation of long-branched DNA nanostructures that significantly enhance the signal through a feedback loop. A unique feature of this design is that the aptamer initiates a signal-enhancing DNA reaction after target-specific recognition. This allows the aptasensor to exhibit both high sensitivity and a wide linear range (0.5–2000 nM) with a low LOD of 0.301 nM (37). It is seen that the FRET method was used in an aptasensor developed for the determination of sulfadimethoxine (SDM), another sulfonamide group antibiotic. The FRET method was used in an aptasensor developed for the determination of sulfadimethoxine, another sulfonamide antibiotic. In this study, an aptamer-based FRET aptasensor was developed using a ZrFe bimetallic metal-organic framework (MOF) and ferrofluids. When the aptamer specifically binds to the target molecule, SDM, it reduces the MOF's peroxidase-like activity by allowing the complementary sequence to bind to the MOF surface. This results in a

decrease in DAP production and, consequently, FRET-induced quenching, while also increasing MOF fluorescence. The aptamer's binding to the target, the MOF's catalytic activity, and the FRET mechanism lead to measurable changes in the fluorescence signal, enabling the determination of SDM (38). While energy transfer in FRET-based methods occurs depending on the distance between two fluorophores, the fluorescence method used in some studies relies on the direct interaction of the fluorogenic aptamer with the fluorophore after target binding. In one study exemplifying this approach, Grob and colleagues developed an RNA aptamer-based fluorescent biosensor operating in *E. coli* to monitor intracellular burden. Eight different aptamers were tested in two different *E. coli* strains with various promoters, and the tRNA-Broc aptamer emerged as the most prominent, providing a high context-independent signal. However, due to their complex structures, some of these fluorogenic aptamers negatively impacted bacterial growth and created additional cellular burden. Consequently, the tRNA-Broc aptamer was identified as the most suitable biosensor, offering both low cellular efficacy and high responsiveness, and was shown to be capable of distinguishing burdens caused by different genetic constructs (39). In a 2025 study by Wang et al., an aptamer-based turn-on fluorescent biosensor was developed to image the SIRT2 protein in living cells and screen for its modulators. Twelve aptamers with high affinity for SIRT2 were selected using the magnetic bead-based SELEX (MB-SELEX) method; Aptamer 45, with the highest binding capacity, was used as the sensor's recognition element. The fluorescence measurement principle is based on the interaction of the FAM-labeled aptamer with BHQ1, a quencher, quenching fluorescence. Upon binding to SIRT2, BHQ1 is displaced, reactivating the fluorescence ("turn-on"). This system, delivered via Au nanoparticles, enabled the in vivo visualization of SIRT2 in three different cell types, and three modulators capable of increasing SIRT2 levels were identified. This approach will also allow the screening of other protein modulators by replacing the aptamer with the appropriate target protein (40). Fluorescence-based aptasensors are used to detect nucleic acid-based analytes such as RNA. In the study by Yang et al., a novel fluorescent aptamer biosensor based on ring-opening polymerization (NCA ROP) was developed for the detection of Tobacco Mosaic Virus (TMV) RNA. In the biosensor, aptamers with sequences specific to TMV RNA were immobilized on magnetic beads to enable recognition of the target RNA. Subsequently, a second aptamer (probe 2) modified with NCA-derived fluorescent peptides was hybridized with the target to amplify the signal. This fluorescence method produced fluorescence signals that increased depending on the amount of target RNA,

enabling target detection at concentrations as low as 0.085 pM (41). In a study developed by Zhang and Zhao, a fast and sensitive fluorescence biosensor based on a short DNA aptamer labeled with double-end pyrene was designed for the detection of chloramphenicol (CAP). When the CAP target molecule binds to the aptamer, it causes it to fold into a hairpin structure, causing two pyrene molecules to approach each other and form a pyrene excimer. As a result of excimer formation, a characteristic fluorescence signal was observed at 485 nm. This structure-modification-based mechanism enabled CAP determination with low background signal and high selectivity, with a detection limit of 24.4 nmol/L. The aptamer used in this study was selected using the Capture-SELEX method, and the final structure was optimized by modifying both ends with pyrene molecules (42). Wu and colleagues developed a magnetically driven dual-channel fluorescent biosensor (MDDCF) for the simultaneous detection of breast cancer-specific exosomal proteins (MUC1 and HER2). The biosensor's unique feature lies in the integration of HER2 and MUC1 aptamers with logic gates; this structure generates a signal output dependent on the recognition of both targets. The fluorescent signal is enhanced by plasmon-enhanced fluorescence (PEF) generated by the interaction of dye-labeled short sequences released after the aptamer-target interaction with silica-coated silver nanoparticles (SiO_2@Ag NPs). Unlike other designs, this system enables highly sensitive and spectrally interference-free multi-target measurement by integrating both magnetic enhancement and a dual aptamer logic gate, increasing its potential for clinical application, particularly for molecular subtype discrimination (43). Aptamer-based fluorescence sensor designs significantly increase the sensitivity and selectivity of various fluorescence mechanisms, particularly FRET, thanks to their high target-specific binding affinity and structure-modifying capabilities. The recent studies above demonstrate that the modifiable structures of aptamers offer flexible design possibilities that allow for the simultaneous and reliable measurement of both single and multiple targets. These properties provide strong advantages in fluorescence-based biosensors in terms of specificity, low detection limit, and applicability to biological samples.

While fluorescence-based aptasensors attract attention with their high sensitivity, another powerful technique that does not require labels and offers the advantage of real-time analysis is surface plasmon resonance (SPR)-based aptasensors. Santa and colleagues developed a split-aptamer-based SPR biosensor for real-time monitoring of low-molecular-weight drugs such as vancomycin. To address the signal weakness of traditional single-aptamer approaches, the aptamer was split into two fragments, one

immobilized on the surface and the other released into solution to form a ternary complex in the presence of vancomycin. This design, a notable example of the use of split aptamers, not only increased the SPR signal approximately 10-fold but also ensured the sensor's reversibility and stability throughout its exposure time. Furthermore, this sensor was tested in biological samples under various pH and ion conditions, demonstrating its functionality in biological environments. Using a split-aptamer offers reversible and continuous monitoring capabilities, whereas conventional SPR systems typically operate with a full aptamer structure and more limited reversibility. However, overstabilization of split-aptamer pairs can lead to issues such as increased background signal and slow sensor resetting. Additionally, signal attenuation due to surface biofouling is a potential risk during long-term measurements (44). An aptamer-based, highly sensitive optical SPR sensor was developed for the label-free and selective detection of berberine hydrochloride, a plant-derived alkaloid. A gold film was coated on the surface of an MSM (multimode–singlemode–multimode) optical fiber, and thiol-modified aptamers were immobilized via covalent Au–S bonds. To increase sensitivity, the surface was modified with gold nanoparticles (AuNPs), which amplified the electromagnetic field via local surface plasmon resonance (LSPR). Thus, the sensor operated in the range of 0.1–500 μM, and the LOQ was reduced to 0.0467 μM with the inclusion of AuNPs. This biosensor offers potential for portability and system integration because it offers a fiber-based platform with a smaller volume compared to conventional SPR sensors. Additionally, surface functionalization with AuNPs increased the electromagnetic field intensity, thus enhancing the SPR signal. However, due to the high precision required for fiber assembly, coating, and functionalization, the fabrication process is complex and can reduce reproducibility. Compared to existing SPR sensors, this approach offers higher sensitivity and selectivity, but improvements in automation and cost-effectiveness are necessary for widespread use in clinical applications (45). In recent years, significant advances have been made in the structural and optical components of SPR biosensors. In addition to traditional gold and silver surfaces, the use of alternative plasmonic materials such as 2D nanomaterials, metal nitrides, and carbon-based structures, which enable precise control of the refractive index, is increasing. Furthermore, miniaturized optical fiber-based and waveguide systems are improving portability and multiplex analysis capabilities. This trend is supporting the use of SPR technology, which typically relies on relatively sophisticated instrumentation, in laboratory settings and beyond. Alternatives are being proposed to overcome the limitations imposed by antibodies, such

as stability, difficulty of production, and cost, through the integration of aptamers onto the SPR sensor surface. Artificial intelligence-assisted data processing algorithms are making SPR systems more suitable for multiple target analysis and high-throughput screening, and it is anticipated that these approaches will increase in the near future (46).

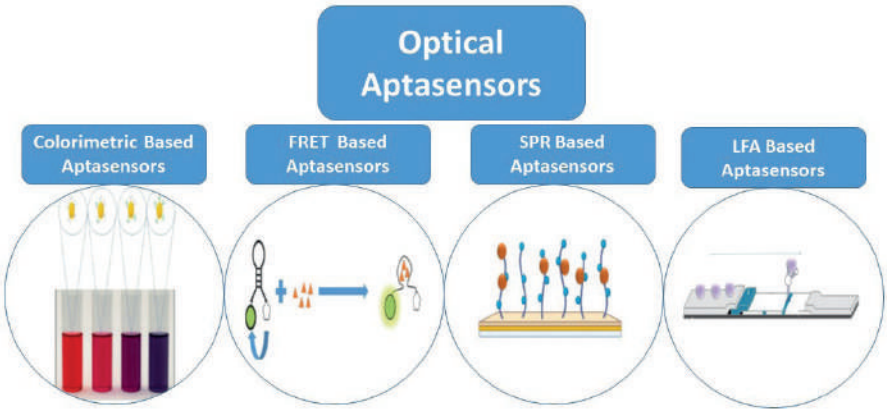

Figure 2. Schematic representation of optical aptasensors. Target molecules bind to aptamer-functionalized platforms, and the resulting signal is detected using optical techniques such as colorimetry, Fluorescence resonance energy transfer (FRET), surface plasmon resonance (SPR), and lateral flow assay (LFA).

Colorimetric biosensors offer rapid and practical analysis thanks to their simple readout systems and low-cost hardware requirements. Integrating these platforms with aptamers, thanks to their unique binding capacity and easily chemically modifiable structures, both increases selectivity and enables the development of user-friendly designs. In the study by Wei et al., a colorimetric biosensor was developed for the detection of synthetic His-tagged proteins and peptides using split aptamers as specific recognition elements and mannan oligosaccharide-coated positively charged gold nanoparticles (AuNPs-MOS) as signal transduction elements. A unique design strategy was employed to obtain split aptamers with high affinity, and a dissociation constant of 132 nM was achieved with the 6H7-Sp1/Sp2 aptamer pair derived from binding site-enriched sequences. The AuNPs, whose nanozyme activity was controlledly attenuated by MOS, produced a color change in the presence of the target molecule, enabling visual and quantitative detection. The developed biosensor demonstrated successful performance in terms of both selectivity and sensitivity, and its applicability to real bioprocess samples was confirmed (47). FRET-based systems have

also been frequently used in viral analyses, including HIV detection (31, 48). A dual-mode RNA-splitting aptamer biosensor targeting the Tat peptide was developed for the early diagnosis of HIV infection. The biosensor combines both colorimetric and fluorescent detection modes using gold nanoparticles (AuNPs) and FAM-labeled RNA aptamers. In the presence of the Tat peptide, the aptamers bind to the target and prevent its adsorption to the AuNPs surface, triggering ThT-mediated aggregation, resulting in a color change and recovery of the fluorescent signal due to the removal of the FRET effect. The developed method provided a low detection limit of 0.28 nM and a wide linear range between 0.5 and 60 nM, enabling successful measurement in human serum. Thus, the study demonstrates a low-cost, practical, and convenient platform for early diagnosis that combines both sensitivity and visual agility (48). Liu and colleagues developed a high-affinity aptamer (Apt-C5) capable of simultaneously recognizing *Cronobacter sakazakii*, *C. malonaticus*, and *C. turicensis*, thus designing an innovative biosensor to address a significant public health problem. The novelty of the study was the successful application of a multiple target selection strategy, Multiple Bacteria Alternate-SELEX (MBA-SELEX), resulting in an aptamer with cross-recognition against three different Cronobacter species. The resulting aptamer was shown to bind to lipopolysaccharides with high specificity, demonstrating that the interaction mechanism relies on the outer membrane structure. The detection mechanism is based on the color change principle, which is based on the binding-destabilization relationship of the aptamer with the peroxidase-like activity of Pd/Au biconic nanozymes. When the aptamer binds to the nanozyme, catalytic activity is suppressed. In the presence of target bacteria, the aptamer binds to the bacteria and dissociates from the nanozyme surface, resulting in the recovery of the color signal. With this strategy, the detection limit for each of the three pathogens was determined to be in the range of 15–26 CFU/mL, and successful detections were achieved in infant formula. This study makes a significant contribution to the literature regarding both the aptamer selection process and nanomaterial integration, while also highlighting the need for more extensive field validation (49). A study published by Chang and colleagues in 2025 developed an aptamer-based microneedle biosensor platform aimed at early assessment of cardiovascular disease risk by measuring C-reactive protein (CRP) levels. In the study, microneedle arrays, manufactured from polylactic acid and functionalized with CRP-specific aptamers via gold coating, were designed to capture biomarkers directly from the skin or blood. The sensing mechanism begins with the binding of the target protein to the aptamer at the microneedle tip, followed by a color (TMB-

based) colorimetric analysis using primary and HRP-conjugated secondary antibodies, providing the user with visible detection. The developed system enabled CRP determination in the range of 0–10 mg/L and was specifically designed to consider the clinical significance of levels above 3 mg/L in terms of cardiovascular disease risk. Unlike similar microneedle-based sensors in the literature, this study is both disposable and suitable for home use due to its portability and low cost. Furthermore, aptamers are more stable and reproducible than antibodies, making the system more reliable. However, the sensor design still has some limitations; for example, skin penetration depth can be affected by individual differences, and because the system does not establish a definitive correlation between CRP in interstitial fluid and blood levels, its clinical validity is not yet fully established. Nevertheless, the study demonstrates that the aptamer-microneedle combination is a powerful and innovative approach for field diagnostic applications (50). Colorimetric aptamer-based sensors are platforms that provide practical and cost-effective detection of analytes both qualitatively and quantitatively with advanced techniques through color changes visible even to the naked eye. These designs are often integrated with optically active nanomaterials, such as gold nanoparticles, and utilize the principle of aggregation or dissociation due to target-aptamer interaction. Specific aptamer binding to the target alters the stabilization equilibrium on the nanomaterial surface, resulting in a color change. Such systems reduce equipment dependency and offer user-friendly solutions for rapid field screening. At the same time, aptamer design, surface chemistry, and analysis environment conditions must be carefully optimized to achieve low detection limits.

In optical approaches based on lateral flow assays (LFA), there are biosensor studies that utilize both traditional labels such as AuNPs and innovative methods such as fluorescence-based techniques. In a study published by Wang et al. in 2025, an aptamer-based LFA platform was developed for the rapid and sensitive detection of AFB1, one of the most potent mycotoxins that threaten food safety, in different agricultural products. Eight aptamers identified in the literature were compared in detail using molecular dynamics (MD) simulations and the SYBR Green I fluorescence assay. Aptamer 7, with the highest binding energy and structural stability, was selected for further studies. The detection mechanism is based on AFB1 competing with the target molecule, preventing the interaction of the gold nanoparticle (AuNP)-supported aptamer with the capture probe on the test line. Thus, the red color intensity on the test line decreases with increasing AFB1 concentrations. This competitive sensor design exhibited a linear response between 1–200 ng/mL and a very low detection limit of

0.23 ng/mL. A notable aspect of the study is that the aptamer selection was guided by detailed MD-based analyses prior to the experimental steps. This approach provides a rational and cost-effective strategy for sensor design (51). In one study that exemplifies the creative use of aptamer-based systems in the literature, an "indirect lateral flow" approach was adopted, using a commercial LFA kit to detect not the target analyte directly, but a molecule (morphine) released upon interaction with it. The detection mechanism relies on the dissociation of the capping aptamer from mesoporous silica nanoparticles capped with an aminoglycoside aptamer upon encountering the target antibiotic, kanamycin, thereby releasing the loaded morphine molecules. The released morphine is visually detected by a commercial MOP LFA assay, indirectly detecting the presence of kanamycin. A notable aspect of this approach is the use of the aptamer without any marker modification and the integration of commercially available kits for signal generation, rather than designing a new LFA kit. This novel design is particularly promising for low-cost and portable diagnostic systems; however, controlling potential false-positive sources such as morphine may require additional specificity assessments. Additionally, the need for interaction time as long as 4 hours may be a limiting factor for real-time POCT applications (52). The aptamer-based fluorescent LFA biosensor, developed for the detection of *Staphylococcus aureus* (*S. aureus*), goes beyond traditional color-change-based assays and generates a signal based on the binding of a Cy5-labeled aptamer to the target. The interaction mechanism is based on specific binding competition between the target bacteria and the aptamer; in the presence of the target, the aptamer binds to the target and displaces the complementary sequence, resulting in a decrease in the fluorescence signal in the test line. The system, which boasts high specificity and a low detection limit (1.65 CFU/mL), has been successfully tested in both milk and chicken meat samples. However, due to its dependence on fluorescence readers and the complex optimization process, the method requires additional engineering solutions for widespread use in field applications (53). A study conducted for cadmium detection presents a striking innovation in the design of an aptamer-based LFA developed for the detection of small molecules, with its signal-on mechanism. Unlike traditional signal-off approaches, the formation of a signal in the presence of Cd^{2+} simplifies visual interpretation and prevents false negatives. Small molecules often struggle to generate sufficient signal with aptamers due to their limited epitope surface area and low molecular weight. This problem was overcome by utilizing the principle that the aptamer undergoes a conformational change upon binding to Cd^{2+}, preventing hybridization. In the presence of Cd^{2+}, the aptamer forms a hairpin-like structure, closing the

binding site, allowing the AuNP conjugates to reach the test line, generating a positive signal. This results in both increased specificity and a system that can be easily assessed with the naked eye, providing a highly useful and practical solution for environmental monitoring applications (54). Table 1 shows some parameters of electrochemical and optical based aptasensors.

Table 1. Current electrochemical and optical based aptasensors based on different methods and some of their properties.

Targets	Detection method	Aptamer modification method	Linear range	Reference
PTH fragment	EIS	5′-NH_2	20 to 600 pg/mL	14
EGFR	EIS	Unlabeled	10 fg/mL to 100 ng/mL	15
TRA	EIS	5′-NH_2	50 pM to 1.3 nM	16
AFB 1	EC/EIS	5′-SH and 3′- FC	10 pg/mL and 30 ng/mL in ratiometric EC mode and between 3 pg/mL and 30 ng/mL in EIS	17
Escherichia coli	EIS	5′-NH_2	100–10^4 CFU/mL	18
CHIKV	EIS	Unlabeled	1 ng/mL to 10 μg/mL	19
P21 protein	EIS	5′-SH	0.47 – 1.88 μg/mL	20
11-DCL	SWV	5′-SH	0.01 – 100 pg/mL	21
SAG1	SWV	Unlabeled	0.01–100 nM	22
HER2	SWV	Unlabeled	1 fg/mL – 10 μg/mL	23
DOX	SWV	5′-SH and 3′-MB	0.1–50 μM	24
Lincomycin and Neomycin	SWV	5′-SH	Lincomycin: 0.02 pg/mL Neomycin: 0.035 pg/mL	25
ATP	DPV	Unlabeled	10 pg/mL to 50 μg/mL	26
AFB 1	DPV	5′-SH	10.0–10^8 pg/mL	27
Thrombin	PEC and DPV	Unlabeled	10 fM – 10 nM	28
Glucose	FRET	5′-SH	0.1 μM – 1.0 μM	32
AFB 1 and OTA	FRET	Both aptamers are unlabeled, other DNAs are FAM and Cy3 labeled	AFB 1 25–250 ng/mL OTA 0–100 ng/mL	33
DON	FRET	5′-COOH	0.05–200 ng/mL	34
CPF	FRET	5′-NH_2	0.1–200 ng/mL	35
SAR and ENR	FRET	Unlabeled	25 pM – 100 nM	36

SME	FRET	Unlabeled	0.5–2000 nM	37
SDM	FRET	5′-NH_2	10 pM – 10 nM	38
Cellular burden in *E. coli*	Fluorescence via fluorogenic RNA aptamers	Unlabeled	Not defined	39
SIRT2	Fluorescence "turn on"	5'-FAM and 3'-BHQ1	0.1–5 μg/mL	40
Tobacco Mosaic Virus RNA	Fluorescence	5′-NH_2	pM – 10 nM	41
CAP	Fluorescence	5′ and 3′ pyrene	24.4 nmol/L – 50 μmol/L	42
Exosomal MUC1 and HER2	Plasmon-amplified dual-channel fluorescence	Unlabeled	10^2–10^7 particles/μL	43
Vancomycin	SPR	Split-aptamer	0.05–100 μM	44
Berberine hydrochloride	SPR	5′-SH	0.1 – 500 μM	45
His-tag, His-tagged proteins, His-tagged peptides	Colorimetric assay	Split-aptamer	14.60 – 3750 nM (for 6×His-tag)	47
HIV Tat peptide	Dual-mode – colorimetric assay (UV-Vis) and fluorescence spectroscopy	3'-FAM	For the fluorescence method: 0.5–25 nM and for the colorimetric method: 2–60 nM	48
Cronobacter sakazakii, Cronobacter malonaticus, Cronobacter turicensis	Nanozyme-based colorimetric assay	5'-FAM and Unlabeled	10^2 – 10^8 CFU/mL (for all three *Cronobacter* species and mixtures)	49
CRP	Colorimetric assay	5'-SH and 5'-SH/3'-FITC dual-labeled ssDNA	0–10 mg/L	50
AFB 1	LFA	Unlabeled	1–200 ng/mL	51
Kanamycin	LFA	Unlabeled	10 nM to 350 nM and 500 nM–1500 nM	52
S. aureus	LFA	5'-Cy5	2.8×10^1 to 2.8×10^8 CFU/mL	53
Cadmium ion	LFA	5'-SH	1.0–5000 ppb	54

3. Conclusion

This chapter presents an analysis of the performance parameters of current electrochemical and optical approaches using nucleic acid aptamers as recognition elements. Current methodologies are assessed, encompassing the benefits and drawbacks of aptamers in comparison to conventional recognition elements. The significant rise in the utilization of aptamers in biosensor designs, particularly during the past five years, is undeniable. This document provides a detailed explanation of the labeling and application of aptamers to biosensor surfaces. Additionally, the evaluation of novel design strategies that can enhance field application has been conducted, specifically for biosensors developed in the past year. This evaluation clearly demonstrates the potential offered by aptamer-based electrochemical and optical biosensors in terms of performance criteria such as selectivity, sensitivity, stability, and portability. Electrochemical approaches stand out with their low-cost hardware and miniaturization capabilities, while optical methods offer high sensitivity, particularly in fluorescence-based systems. The ability to chemically modify aptamers has enabled the development of platforms that enable multiple target detection. However, several technical challenges, such as matrix effects, surface modifications, and integration with portable devices, remain unresolved. Future work will focus on the development of low-cost, high-accuracy systems that increase field usability and accelerate the integration of these technologies into real-time and widespread diagnostic applications.

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Chapter 2

Advances in Paper-Based Biosensing Technologies: From Principles To Practical Applications

İnci Uludağ Anıl[1]

Abstract

Paper-based biosensors have become of significant interest in recent years owing to their affordability, mobility, and eco-friendliness. Platforms like dipstick assays, lateral flow assays (LFA), and microfluidic paper-based analyzers (μPADs) offer swift and accessible detection capabilities in healthcare, food safety, and environmental monitoring. This study examines the latest advancements in paper-based biosensors, highlighting the enhanced sensitivity and specificity attained by the incorporation of aptamers, CRISPR/Cas systems, and isothermal nucleic acid amplification methods (RPA, RAA, MIRA). Moreover, the utilization of functional nanomaterials, including gold nanoparticles and upconversion nanoparticles, has markedly improved signal amplification and detection efficacy. Although these sensors were originally intended for qualitative assessments, the shift towards quantitative detection is intensifying due to the demand for precise measurements in clinical and food applications. However, challenges such as variability in paper substrates, environmental conditions, and matrix effects remain critical issues. Future perspectives include advancements including smartphone integration, multi-parameter detection, biodegradable materials, and 3D paper-based biosensors.

1. Introduction

Biosensors are analytical instruments that facilitate the rapid, precise, and sensitive identification of specific analytes by combining biological recognition components (such as enzymes, antibodies, aptamers, DNA, and cells) with a physical or chemical transducer. These technologies

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transmute alterations from a biological interaction into electrical, optical, or mechanical signals, facilitating quantitative or qualitative examination. In recent years, biosensors have made substantial advancements in several domains, including medical diagnostics, environmental monitoring, food safety, and biotechnological applications. Paper-based biosensors (PBBs) signify a substantial transformation in biosensor technologies. Paper microfluidic devices (μPADs), created in 2007 by Martinez and colleagues, have expedited research in this field (1). Paper, being a cost-effective, biodegradable, and readily processable material, has emerged as an optimal substrate for biosensor platforms. Paper-based biosensors were created to enhance healthcare accessibility and facilitate quick diagnostic methods, especially in resource-constrained areas. They are also notable for their user-friendly designs, requiring minimal training, and rapid analysis times. The principal factors contributing to the increasing significance of paper in biosensor design are its low manufacturing cost, high mobility, and environmental sustainability. The cellulose-based structure promotes capillary liquid transfer, allowing analyses to occur without external pumps or energy sources. Moreover, paper may be effortlessly cut, folded, and incorporated into diverse designs. Its biodegradability and recyclability provide substantial benefits, especially for the advancement of eco-friendly technologies (2,3).

Paper-based biosensors have established their utility across several applications. In the healthcare industry, they are extensively utilized for early diagnosis of infectious diseases, chronic disease monitoring, and point-of-care (POC) testing (4). In food safety, they have emerged as a crucial instrument for pathogen identification, toxin assessment, and quality assurance. Moreover, they are regarded as an efficient medium for identifying heavy metals, herbicides, and contaminants in environmental surveillance. These adaptable applications establish paper-based biosensors as prominent technologies for the future in biotechnology and analytical chemistry. This chapter will outline the fundamental categories and overarching attributes of paper-based biosensors and synthesize the existing literature on each biosensor type. The main aim of this review is to thoroughly assess the benefits, existing limits, and possible solutions of these biosensor platforms across diverse application domains (healthcare, food safety, environment, etc.). Furthermore, the potential future research and application opportunities for paper-based biosensors will be discussed.

2. Fundamentals Of Paper-Based Biosensors

Paper is a porous, hydrophilic material composed primarily of cellulose fibers. This porous design facilitates the capillary action characteristic of paper. The fiber network of the paper creates a microfluidic environment by enabling the spontaneous movement of liquids driven by surface tension. This feature establishes an optimal medium for the conveyance and distribution of liquids without need on an external pump or energy source. Moreover, the flexibility, lightweight nature, and biodegradability of paper facilitate the efficient manufacture and transportation of microfluidic systems.

The types of paper utilized in paper-based biosensors different based on the device's performance and intended application. Cellulose-based papers are natural and extensively utilized. They possess elevated capillary activity, facilitating fast absorption and conveyance of liquids. They are both economical and ecologically sustainable. Nitrocellulose membranes are typically favored for lateral flow biosensors. Their elevated protein binding ability renders them useful in immobilizing biomolecules and is typically appropriate for color or optical signals (4). Modified papers are papers that have undergone chemical alteration by the introduction of functional groups or polymer coatings. Performance can be improved by including attributes such as targeted analyte interactions, hydrophobicity regulation, or electrical conductivity.

3. Dipstick Biosensors

Dipstick biosensors represent one of the most straightforward paper-based analytical systems, functioning by immersing a paper strip into a sample solution. The liquid traverses the paper through capillary action, engaging with pre-immobilized biosensing molecules, resulting in a visual reaction, typically a color change. The primary benefits of these sensors encompass inexpensive manufacturing expenses, convenient mobility, straightforward operational principles, and swift responsiveness. Moreover, they necessitate no user training, rendering them appropriate for practical applications. Nonetheless, the constraints of dipstick biosensors encompass inadequate analytical sensitivity, semi-quantitative outcomes, and frequently restricted specificity. Moreover, the characteristics of the sample matrix (pH, ionic strength, etc.) can adversely affect analytical performance.

Belsare et al. have created an economical and accessible paper-based dipstick biosensor for the monitoring of gestational diabetes. This technique emphasizes the quantification of glycosylated albumin, an intermediary biomarker, to address the shortcomings of glucose and HbA1c assessments.

In this study involved the invention of two dipstick colorimetric biosensors utilizing aptamers: one tailored for glycosylated albumin and the other for total serum albumin. Gold nanoparticles served as signal generators, while aptamers were chosen as biological recognition elements. The created paper-based dipstick biosensor system is an advanced platform that provides quantitative analytical capabilities, surpassing a mere qualitative test reliant on visual color change. Subsequent to the testing, the paper strips were digitally scanned utilizing a flatbed scanner, and the resultant images were meticulously evaluated employing ImageJ software. Consequently, quantitative analysis of glycosylated albumin and total serum albumin was conducted with excellent precision within physiological concentration ranges. Additionally, limit of detection (LOD) values were determined, and the method's sensitivity was quantitatively validated. Both glycosylated and non-glycosylated albumin forms were quantified within the pertinent physiological concentration ranges—50 μM–300 μM with a LOD of 6.5 μM for glycosylated albumin, and 500 μM–750 μM with a LOD of 21 μM for non-glycosylated serum albumin (5). This method ensures long-term stability at ambient temperature, great specificity, and an extensive dynamic range, in addition to being cost-effective and portable. This study exemplifies the utility of aptamer-based colorimetric sensors, especially in point-of-care (POC) diagnostics for conditions like gestational diabetes.

Nucleic acid amplification-based biosensors are an effective instrument for the swift and sensitive identification of target pathogens, particularly in low concentrations. Isothermal amplification methods, like as Recombinase-Aided Amplification (RAA), are commonly employed in these systems. The RAA method is a swift and economical isothermal DNA amplification approach functioning at low temperatures (37–42°C). Amplification products are frequently discernible by lateral flow dipstick (LFD) tests. This method provides a straightforward, expedient, and equipment-free solution, rendering it especially appropriate for field applications and accessible due to its visual outcomes. In this regard, the RAA-LFD-based biosensor created by Lin et al. presents a novel method for the swift and visible identification of Plasmodium species, the etiological agent of malaria. Its originality consists of being among the initial methods to integrate RAA with LFD for Plasmodium detection, yielding very sensitive findings in about 20 minutes. The assessment is wholly qualitative and depends on the creation of color bands; outcomes are visually evaluated as "positive" or "negative." Moreover, the sensitivity of the approach was thoroughly examined in the study. The LOD for recombinant plasmid DNA was established at 1 copy/mL; in clinical samples, sensitivities of 0.1 pg/mL were attained for

Plasmodium falciparum, 10–100 pg/mL for P. vivax and P. ovale, and 100 pg/mL for P. malariae. Moreover, sensitivities of 0.5 parasites/mL were attained with cultivated parasites. The results indicate that the procedure is a reliable alternative for applications such as screening blood donors or asymptomatic persons in resource-limited settings.

Zheng et al. integrated the reverse transcription recombinase-assisted amplification (RT-RAA) technique with a dipstick platform for the quick, economical, and field-compatible identification of SARS-CoV-2. This work demonstrated nucleic acid amplification in under 30 minutes at a constant temperature of 39°C, with findings interpretable visually by a straightforward dipstick. The new technique has exceptional analytical sensitivity, with a LOD established at 1 copy/mL, surpassing the sensitivity of most current quick testing methods. The measurement approach, although visually impactful as a qualitative test, is semi-quantitative owing to the established LOD and sensitivity analysis. The study demonstrated complete concordance with RT-qPCR in a comparative examination of 100 clinical samples, with specificity and sensitivity rates reported at 100% (6). This platform, characterized by its quick reaction, minimal equipment needs, and elevated sensitivity, has considerable promise for the early detection of infectious illnesses like COVID-19 in field settings or resource-constrained environments.

CRISPR (clustered regularly ınterspaced short palindromic repeats) technology has facilitated groundbreaking advancements in biotechnology, especially in genome editing, and has recently arisen as a potent instrument in the creation of highly specific nucleic acid-based detection systems. These technologies, particularly when integrated with isothermal amplification techniques, has the capability to develop quick, portable, and user-friendly diagnostic platforms. The ERA (enzymatic recombinase amplification)-CRISPR/Cas12a-based lateral flow dipstick biosensor for porcine parvovirus (PPV), created by Wei et al., is significant in this context. This study is unusual since it integrates CRISPR/Cas12a technology with a lateral flow dipstick for the first time, allowing for the detection of PPV within 30 minutes at a constant temperature as low as 39°C. The devised technology facilitates a clearly discernible hue change. The measurement approach relies on visual observation, rendering it fundamentally qualitative; yet, the study explicitly defined the LOD and exhibited high sensitivity. The LOD was recorded at 3.75×10^2 copies/mL, indicating a notable sensitivity superiority compared to PCR. Additionally, no cross-reactivity was detected in specificity assays involving several porcine viruses. In clinical specimens, test outcomes demonstrated complete agreement with qPCR (7). The

approach presents considerable promise for identifying viral infections, especially in resource-constrained regions, due to its rapidity, portability, affordability, and appropriateness for field use. The combined application of Recombinase Polymerase Amplification (RPA) with the CRISPR/Cas12a system facilitates extremely selective and visually discernible detection due to target-specific cleavage activity following amplification. Furthermore, Multienzyme Isothermal Rapid Amplification (MIRA) and lateral flow dipstick (LFD) techniques provide considerable benefits in field applications due to their affordability and swift colorimetric results. In this context, in the study conducted by Li et al., two distinct visual biosensor systems, RPA-CRISPR/Cas12a and MIRA-LFD-based, were created for the swift and precise detection of the quarantine pest Bactrocera correcta. The study's innovation is in the simultaneous optimization of both methodologies, yielding quick, portable, and cost-effective diagnostic platforms applicable across several domains. Both systems yield entirely qualitative results; the RPA-CRISPR/Cas12a system differentiates positive from negative by producing a green fluorescent signal under UV light, whereas the MIRA-LFD system differentiates positive from negative by creating a colored line on the lateral flow strip. This work thoroughly examined the sensitivity of both approaches, establishing the LOD for each system at 1.0×10^{-1} ng/μL DNA. Moreover, due of the expedited DNA extraction methodology, these techniques have been effectively utilized across larval, pupal, and adult phases, yielding results within 10 to 30 minutes (8). This study exemplifies the advancement of biosensor-based, portable, and user-friendly systems for detecting agricultural and quarantine pests.

4. Microfluidic Paper-Based Biosensors

Microfluidic paper-based devices (μPADs) provide regulated fluid movement across paper through hydrophobic and hydrophilic zones established by precise patterning methods. These devices employ capillary action to convey fluid to various analysis zones, facilitating the concurrent detection of many analytes. Principal advantages encompass multiplexed analysis, minimal sample volume requirements, cost-effectiveness, and the absence of pump operation. Moreover, their modular architecture facilitates the creation of tailored platforms. Nonetheless, the intricacy of the patterning procedures in the manufacturing process and restricted sensitivity are considerable disadvantages. Moreover, the efficacy of quantitative measurements may be constrained in certain applications, and concerns related to long-term stability may emerge (9–11).

Colorimetric analyses, a prevalent technique in paper-based microfluidic biosensors, are particularly advantageous for field applications owing to their simplicity, cost-effectiveness, and minimal equipment needs. In these systems, color alterations due to enzymatic processes are evaluated either visually or via software analysis. Distance-based approaches, specifically, create a direct correlation between analyte concentration and the extent of color change, minimizing user-induced errors and yielding visually accessible quantitative findings. These methodologies have secured a significant role in biosensors, especially for the identification of analytes at minimal concentrations. In this regard, the research by Allamah et al. (12) involved the development of a distance-based μPAD for glucose detection in tear samples, created using laser cutting. This study's innovation is in a device, constructed by a CO_2 laser cutting method, tailored for low glucose levels and offering visual detection through distance-based quantitative assessment. The measurement method is a quantitative system reliant on the length of color change, with the color field length exhibiting a strong correlation with glucose concentration. The study reported the LOD as 0.1 mM. The assay utilizes merely 10 μL of material, with results readily observable to the unaided eye within 3–5 minutes, and necessitates no complicated apparatus. The developed sensor emerges as a formidable alternative, especially in resource-constrained settings and for applications like minimally invasive tear-based diabetes monitoring.

Fluorescence-based biosensor systems are extensively utilized in food safety, environmental monitoring, and biological diagnostics owing to their exceptional sensitivity, rapid reaction times, and minimal detection limits. Platforms utilizing Förster resonance energy transfer (FRET) can produce optical signals directly correlated with analyte concentration via fluorescence quenching and recovery processes. Aptamers in these approaches offer excellent specificity for target molecules, allowing for quantitative measurement of signal changes. In biosensor systems, fluorescence methodologies, along with portable devices and basic imaging systems, have emerged as potent analytical instruments for field applications. Tong et al. (13) incorporated multicolor fluorescent carbon dot (CD) aptamer sensors (mCD-μPAD aptasensors) onto a laser-printed paper-based microfluidic chip, facilitating the concurrent and visual identification of three distinct antibiotic residues (sulfamethazine, oxytetracycline, and chloramphenicol) in seafood. This study's innovation lies in its capacity to visually and concurrently quantify three distinct antibiotics in a single assay utilizing multicolor carbon dots and MoS_2 nanosheets, demonstrating great specificity. The measuring method employed is quantitative; fluorescent

color alterations were captured with a smartphone, and the RGB values were assessed using a color recognition tool to determine the analyte concentration based on the gray value. The determined LOD were 0.47 ng/mL for sulfamethazine, 0.48 ng/mL for oxytetracycline, and 0.34 ng/mL for chloramphenicol. The test lasted about 15 minutes and was intended for field applications utilizing a portable 3D-printed apparatus.

Chemiluminescence-based immunosensors are significant in biosensor technology because to their exceptional sensitivity, extensive dynamic range, and minimal background signal. These approaches often transform the particular binding of a target analyte, usually by enzyme-labeled antibodies, into a chemiluminescent signal, facilitating quantitative assessment. When integrated with μPADs, these methodologies provide swift analysis in the field, characterized by cheap cost, mobility, and user-friendliness. Lazzarini et al. (14) created a novel chemiluminescent immunosensor for detecting ovalbumin (an egg allergy) utilizing magnetic microspheres and an origami-based paper microfluidic substrate. The revolutionary aspect is the automation of the multi-step analytical procedure on paper by an origami folding technique, together with the incorporation of all pre-dried reagents within the device. The sensor functions by an immunoassay method that relies on competition between ovalbumin fixed on magnetic microspheres and ovalbumin present in the sample. Chemiluminescent signals were quantitatively assessed utilizing a portable CCD camera and ImageJ software. The reported LOD in the study is very low, at around 1 ng/mL. This work serves as a notable example for the advancement of portable, sensitive, and user-friendly food allergy detection systems.

5. Lateral flow assay (LFA) biosensors

Lateral flow assay (LFA) biosensors are one of the most widely used paper-based biosensor types today and are particularly well-known for pregnancy tests. These devices employ capillary action to convey fluid to various analysis zones, facilitating the concurrent detection of many analytes. Principal advantages encompass multiplexed analysis, minimal sample volume requirements, cost-effectiveness, and the absence of pump operation. Moreover, their modular architecture facilitates the creation of tailored platforms. Nonetheless, the intricacy of the patterning procedures in the manufacturing process and restricted sensitivity are considerable disadvantages. Moreover, the efficacy of quantitative measurements may be constrained in certain applications, and concerns related to long-term stability may emerge (15–17).

The incorporation of CRISPR-Cas systems, especially with LFA-based biosensors, facilitates the creation of accessible, fast, and visually interpretable diagnostic tools. The Bio-SCAN platform, created by Ali et al. (18), integrates the CRISPR/dCas9-specific binding mechanism with isothermal amplification (RPA) and lateral flow technology, providing a novel method for detecting SARS-CoV-2. This study's innovation is in the visual detection of FAM-tagged target DNA on a lateral flow strip utilizing solely biotin-labeled dCas9, so obviating the necessity for intricate apparatus. This offers a technology with elevated sensitivity akin to PCR, yet appropriate for field applications. The measurement approach is fundamentally qualitative, producing visual "positive/negative" outcomes; yet, the established LOD value of 4 copies/μL demonstrates considerable sensitivity. In clinical samples, a positive concordance of 96% and a negative concordance of 100% were attained, with sgRNAs adjusted to guarantee variant specificity. Su and colleagues (19) developed the OC-MLFA (Orthogonal CRISPR-Mediated Multiplexed Lateral Flow Assay) platform by merging orthogonal CRISPR-Cas12a and Cas13a systems, enabling the simultaneous detection of two distinct SARS-CoV-2 genes (ORF1ab and N) on a single lateral flow strip. This study's innovation is in the simultaneous optical detection of two targets on the same strip without cross-talk, thereby surpassing the single-target constraint commonly observed in CRISPR-Cas systems. This approach is qualitative, producing results through color change in various lines on the test strip. The LOD was documented as 10 copies per test, attaining 100% accuracy in clinical specimens. Moreover, it demonstrated the capacity to differentiate from analogous viruses such as SARS-CoV and MERS-CoV with great specificity, underscoring its relevance in the domain. Both research illustrate that CRISPR-based biosensors may be included into lateral flow platforms, thereby converting them into efficient, portable, and sensitive diagnostic instruments. They assert that CRISPR-based biosensors can significantly contribute to the identification of infectious illnesses.

Nanoparticle-based LFA devices have made considerable advancements in biosensor technology, especially for colorimetric and fluorescence-based investigations. In these techniques, metal or fluorescent nanoparticles function as both signal amplifiers and carriers of specialized recognition elements. Nanoparticles, due to their extensive surface area, tailored functionalization, and enhanced optical characteristics, provide markedly higher sensitivity and quantitative measuring capabilities relative to traditional colored gold nanoparticle (AuNP)-based systems. The incorporation of various nanoparticles onto LFA systems has emerged as a crucial approach for quick, portable, and extremely sensitive diagnostic

applications. A study by Yue and colleagues (20) created a fluorescent lateral flow assay platform based on gold nanoclusters (AuNC) for the detection of melamine. AuNCs stabilized with the 6-Aza-2-thiothymine (ATT) ligand generated a robust fluorescent signal via the aggregation-induced emission (AIE) mechanism due to their interaction with melamine. The measurement method employed was quantitative, and the rise in fluorescence was shown to be directly related to the concentration of melamine. The determined LOD value was 217 nM, and significant linearity was attained within the range of 1–100 μM. This technology is distinguished by its simplicity, speed, and cost-effectiveness, functioning exclusively through chemical recognition without reliance on biomolecules. Jin et al. (21) created a LFA utilizing upconversion nanoparticles (UCNPs) for the differential diagnosis of monkeypox virus (MPXV) clades. This sensor functions in both qualitative and quantitative modes because to the robust anti-Stokes fluorescence characteristics of UCNPs. The dual-T-track construction facilitated the concurrent detection of two distinct MPXV clades. This system, readable with a smartphone camera or a benchtop fluorescence analyzer, attained LOD in the picomolar (pM) range, with data produced in eight minutes. This approach exemplifies an effective strategy for diagnosing viral diseases in field settings. In the LFA system created by Ai et al. (22), a mesoporous silica@upconversion nanoparticle@polydopamine (MSUD)-based fluorescence-colorimetric hybrid nanoparticle system was employed for the detection of methamphetamine (MATM). In this instance, UCNPs incorporated into magnetic mesoporous silica (MS) with a substantial surface area were enveloped in a polydopamine (PDA) layer, yielding robust fluorescence and colorimetric signals. This technology possesses a bi-modal reading capability, facilitating qualitative analysis visually and quantitative analysis via a smartphone. The LOD established for methamphetamine were notably low, measuring 1.047 x 10^4 pg/mL for the colorimetric analysis observable by the naked eye and 47.25 pg/mL for the fluorescence-based analysis utilizing a smartphone. It has been evaluated with excellent precision in authentic urine and hair specimens. Gold nanoclusters improved chemical selectivity and fluorescence intensity, whilst UCNPs offered elevated sensitivity and signal-to-noise ratio due to their robust near-infrared-induced fluorescence. The MSUD architecture incorporated fluorescence and color signals, facilitating dual-mode analysis. These investigations illustrate that nanoparticles serve as crucial elements in LFA-based biosensors, functioning as both signal enhancers and facilitators of particular recognition and diverse readout modalities. Research indicates that gold nanoclusters improve chemical selectivity and fluorescent signals,

whilst UCNPs offer elevated sensitivity and a superior signal-to-noise ratio due to their robust NIR-induced fluorescence. The MSUD architecture incorporates fluorescence and color signals, facilitating dual-mode analysis. These investigations illustrate that nanoparticles serve as pivotal elements in LFA-based biosensors, functioning as both signal enhancers and facilitators of particular recognition and diverse readout modalities.

Antibodies are typically employed as biorecognition components in conventional lateral flow assay techniques. Nonetheless, the constraints of antibodies, including their elevated manufacturing costs, restricted stability, temperature sensitivity, and variability during the production process, are amplifying interest in alternative biorecognition agents. Aptamers are distinguished by their exceptional properties. Synthetic DNA or RNA sequences, such as aptamers, have great specificity and affinity, can be chemically produced, demonstrate significant thermal stability, and are readily modifiable due to their modular architecture. Moreover, their target-specific binding affinities are analogous to those of antibodies, enhancing repeatability in biosensors. The Aptamer Sandwich Lateral Flow Assay (AptaFlow) platform, created by Yang et al. (23), clearly illustrates that aptamers can serve as a formidable substitute for antibodies in lateral flow assays. This study utilized two aptamers (SNAP1 and SNAP4) that exhibit high specificity for the N-terminal domain (NTD) of the SARS-CoV-2 spike protein to construct a fully aptamer-based sandwich structure, eliminating the necessity for antibodies. This dual-aptamer technology facilitated both capture and signal production, while aptamer-functionalized AuNPs delivered visual and quantitative signals through colorimetric changes. This method has garnered interest not only for its total neutralization of antibodies but also for its capacity for swift adaptability to emerging variations due to the chemical versatility of aptamers. Moreover, the AptaFlow platform's minimal production expenses and appropriateness for field applications indicate that aptamer-based LFAs may become more prevalent as a practical, cost-effective, and easily scalable diagnostic instrument in the future. This study convincingly illustrates that the incorporation of aptamers into LFA systems is not merely a technological option but also a potential catalyst for a paradigm shift in bioanalytical diagnostic methodologies.

6. Conclusion and Future Perspectives

Paper-based biosensors have achieved significant prominence in biosensor technologies due to its affordability, portability, straightforward working principles, and eco-friendly design. Their swift proliferation, especially in health, food safety, and environmental monitoring, is significant. In recent

years, sophisticated biotechnological methods, including CRISPR/Cas systems, isothermal nucleic acid amplification, aptamers, and nanoparticles, have been effectively incorporated into diverse paper-based platforms, such as lateral flow assays (LFA), microfluidic paper-based devices (μPADs), and dipstick systems. These devices facilitate swift diagnosis through visual or digital output, while providing the benefits of little user training and compatibility in field conditions. Nonetheless, inadequate sensitivity, restricted shelf life, prolonged durability concerns, and absence of standardization persist as considerable constraints in many applications. Moreover, there exists a possibility of false-positive or false-negative results, as well as a potential loss of quantitative precision, particularly in intricate biological samples.

For many years, qualitative assessments have constituted the principal emphasis of paper-based biosensors. Although visual outcomes derived from color changes in lateral flow assays have benefits of simplicity and rapidity, the demand for quantitative analysis is progressively becoming more prominent. In numerous domains, ranging from clinical diagnosis to food safety, merely identifying the presence is inadequate; precise and reproducible quantification of a given analyte's concentration is essential. Nonetheless, quantitative assessment on paper-based technologies continues to pose considerable obstacles. The precise assessment of color intensity or signal strength may be influenced by factors including lighting circumstances, imaging equipment, user error, and discrepancies in paper composition. Moreover, the intricacy of biological sample matrices might adversely affect quantitative precision. Consequently, the advancement of quantitative paper-based biosensors necessitates extensive enhancements in sensor design, signal amplification techniques, and the incorporation of digital technology for data interpretation. Despite this, interest in paper-based biosensors is rising swiftly. The integration of smartphones is a leading focus in contemporary research trends. This facilitates immediate examination of color alterations or fluorescence signals, digitization of outcomes, and cloud-based data transmission. Moreover, multiparameter detection (multiplexing) capabilities are increasingly prevalent, especially in lateral flow and microfluidic systems, facilitating the concurrent identification of multiple biomarkers with high specificity. The advancement of entirely biodegradable systems is becoming increasingly significant due to the rising need for eco-friendly analytical devices and provides benefits regarding waste management. Moreover, advanced designs, like 3D paper-based biosensors, are garnering significant attention in clinical and environmental

applications, facilitating intricate flow control and multi-stage analysis inside a single device.

The significance of paper-based biosensors will continue to escalate in the future. They are anticipated to proliferate as readily available, cost-effective, and user-friendly platforms in domains such as field diagnostics, personalized health monitoring, food contamination management, and environmental surveillance. Aptamer-based biosensors, CRISPR integrations, and nanoparticle-assisted multiplex readout modalities enable the creation of versatile and sensitive diagnostic instruments that surpass conventional antibody-based assays. With the acceleration of standardization, stability enhancement, and industrial scalability, these technologies are anticipated to secure a prominent position in both academic research and as commercial products within the global health, agriculture, and environmental sectors.

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Chapter 3

Biosensor Design Strategies For Early Diagnosis of Breast Cancer

Burcu Eroğlu[1]

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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Chapter 4

Biosensor Systems For Prostate Cancer Diagnosis: Principles, Advances and Clinical Perspectives

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Abstract

Prostate cancer remains one of the most prevalent malignancies among men worldwide, necessitating the development of rapid, sensitive, and reliable diagnostic methods. This chapter provides a comprehensive overview of biosensor systems developed for prostate cancer diagnosis, emphasizing their working principles, biomarker integration, and technological evolution. Key prostate cancer biomarkers—including prostate-specific antigen (PSA), PCA3, TMPRSS2-ERG gene fusion, microRNAs, sarcosine, and creatine kinase—are discussed in detail, highlighting their clinical relevance and application in biosensing platforms. The chapter also explores recent advancements in biosensor technologies, ranging from electrochemical and optical sensors to cutting-edge CRISPR/Cas-based, artificial intelligence (AI)-integrated, and smartphone-enabled biosensors. Particular attention is given to the design innovations that enhance sensitivity, specificity, and usability. Biosensors are anticipated to substantially improve the accuracy and availability of prostate cancer diagnoses, facilitating early intervention and superior patient outcomes.

1. Introduction

Prostate cancer (PCa) remains one of the most prevalent malignancies affecting men worldwide and represents a significant cause of cancer-related morbidity and mortality (1). Early diagnosis is critical for improving survival rates and treatment outcomes, yet current clinical diagnostic methods face several limitations. Prostate-specific antigen (PSA) testing, the most widely

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used biomarker for PCa, suffers from poor specificity and is prone to false-positive results due to benign conditions such as prostatitis and benign prostatic hyperplasia (BPH) (2,3). Moreover, PSA-based screening often leads to overdiagnosis and overtreatment, highlighting the urgent need for more specific, sensitive, and non-invasive diagnostic tools. In recent years, biosensors have emerged as promising diagnostic platforms capable of detecting prostate cancer biomarkers with high sensitivity, specificity, and rapid turnaround times (4). These devices integrate biorecognition elements with physicochemical transducers to convert biological interactions into measurable signals. Electrochemical, optical, and piezoelectric biosensors have been extensively explored for PCa diagnosis, targeting a range of biomarkers such as PSA, PCA3, miRNAs, and various protein markers (2,3,5). Advanced biosensors, including those based on nanomaterials, CRISPR/Cas technologies, and fiber-optic systems, have demonstrated remarkable performance enhancements, enabling the detection of biomarkers at extremely low concentrations (5,6). Among the innovative developments, electrochemical biosensors employing aptamers, gold nanoparticles, and screen-printed electrodes have shown excellent sensitivity in detecting PCA3 and PSA in biological fluids like urine and serum. Optical biosensors, including fiber-laser-based systems and quartz crystal microbalance (QCM) platforms, have been optimized for real-time, label-free detection of PCa markers, offering compact designs and potential for point-of-care applications (2,6,7). Furthermore, CRISPR/Cas12a-based biosensors have introduced a new dimension in prostate cancer diagnostics by enabling highly sensitive and specific detection of miRNAs associated with tumor progression (5). Recent studies emphasize the importance of multiplexed biosensing approaches and the integration of machine learning algorithms to improve diagnostic accuracy, especially in distinguishing between aggressive and indolent PCa (1,4,8). These advancements underscore the transformative potential of biosensors in clinical settings, paving the way for personalized medicine and more precise disease monitoring. As research progresses, the development of biosensor technologies targeting prostate cancer continues to evolve, driven by interdisciplinary innovations in nanotechnology, molecular biology, and data science (9). This chapter aims to provide a comprehensive overview of biosensor systems for prostate cancer diagnosis, focusing on fundamental principles, recent technological advancements, and their clinical perspectives.

2. Principles of Biosensors and Key Biomarkers

2.1. Biosensors: Basic Concepts and Functional Principles

Biosensors are analytical devices that integrate biological recognition elements with transducer systems to selectively detect target analytes by converting biochemical interactions into measurable electrical or optical signals (10,11). In electrochemical biosensors, enzymes play a crucial role by catalyzing highly specific reactions with the analyte, producing electroactive species such as hydrogen peroxide or enabling oxygen consumption, which can be detected by electrodes. These biosensors generally consist of integrated enzyme reactors, membranes, and electrode systems, where the catalytic reaction initiates a measurable electrical signal. However, challenges such as interference from other redox-active species and changes in physical parameters can impact selectivity. Redox mediators are frequently implemented to optimise biosensor performance and facilitate electron transfer, thereby facilitating enhanced sensitivity and a broadened detection range, thereby mitigating these challenges (10). Beyond electrochemical systems, other transduction mechanisms such as optical, piezoelectric, and thermal techniques are increasingly utilized in biosensors for biomedical analysis. Optical biosensors, for instance, convert biochemical interactions into measurable optical signals through changes in absorbance, fluorescence, or refractive index, allowing for rapid and label-free detection (11). These alternative platforms, alongside electrochemical systems, expand the versatility of biosensors across a wide range of clinical applications.

2.2. Prostate Cancer Biomarkers: Current Trends and Novel Targets

The most frequently employed clinical biomarker in prostate cancer (PCa) diagnostics is prostate-specific antigen (PSA). PSA testing enables early cancer detection but is limited by its low specificity, as PSA levels may also be elevated in benign prostatic hyperplasia (BPH) or prostatitis, leading to unnecessary biopsies and overtreatment (1,3,12).

Several alternative biomarkers have been investigated to enhance diagnostic accuracy. One of the most promising is prostate cancer antigen 3 (PCA3), a non-coding RNA highly specific to prostate cancer, detectable in urine samples. PCA3 testing, particularly when combined with PSA or TMPRSS2-ERG gene fusion analysis, significantly enhances diagnostic specificity (2,13). TMPRSS2-ERG, a common gene fusion event in prostate cancer, has emerged as another powerful biomarker that complements PCA3 and PSA to improve early detection rates (3,12).

MicroRNAs (miRNAs) such as miR-21, miR-141, and miR-375 have also been identified as potential non-invasive biomarkers due to their stability in body fluids and their role in cancer-related pathways (5,14). These miRNAs show strong potential for distinguishing prostate cancer from benign conditions and may also aid in prognosis and therapeutic monitoring (15). While its clinical value was initially debated, recent studies highlight sarcosine's potential for distinguishing aggressive prostate cancer cases, particularly in combination with genomic or transcriptomic biomarkers (13,16).

Creatine kinase has also been proposed as an emerging biomarker, offering insights into metabolic alterations associated with prostate cancer progression (9). Collectively, these biomarkers—when used in combination—offer promise for the development of multiplexed diagnostic panels, enabling more precise, non-invasive, and personalized prostate cancer detection strategies (4,13).

2.3. Biomarker Recognition and Interaction Mechanisms in Biosensors

Biosensors function through the selective interaction between a biological recognition element and a specific biomarker, triggering a measurable physicochemical signal. In prostate cancer diagnostics, biomarkers such as PSA, PCA3, TMPRSS2-ERG, miRNAs, and sarcosine serve as molecular targets that bind specifically to bioreceptors immobilized on the biosensor surface (17,18). These bioreceptors may include antibodies, aptamers, molecularly imprinted polymers (MIPs), or nucleic acid probes, depending on the biomarker's molecular characteristics and the detection strategy employed (19,20). The interaction between the biomarker and the bioreceptor results in a biochemical event, such as antigen-antibody binding, nucleic acid hybridization, or specific enzymatic reactions. This event induces detectable changes in electrical, optical, or mechanical properties at the biosensor interface (21,22). For example, in electrochemical biosensors, electron transfer or impedance changes occur upon biomarker binding, while in optical sensors, refractive index shifts or fluorescence changes are monitored (23,24). These signals are directly proportional to the biomarker concentration and are converted into quantifiable outputs through the transducer component of the biosensor. Critical to the performance of biosensors is the efficient immobilization of bioreceptors on the biosensor surface, maintaining their bioactivity and ensuring high specificity with minimal non-specific adsorption (25,26). Moreover, biosensors designed for prostate cancer diagnostics often incorporate surface modification

strategies, such as nanostructured coatings or antifouling layers, to enhance binding affinity and reduce background noise, thereby improving sensitivity and reliability in clinical settings.

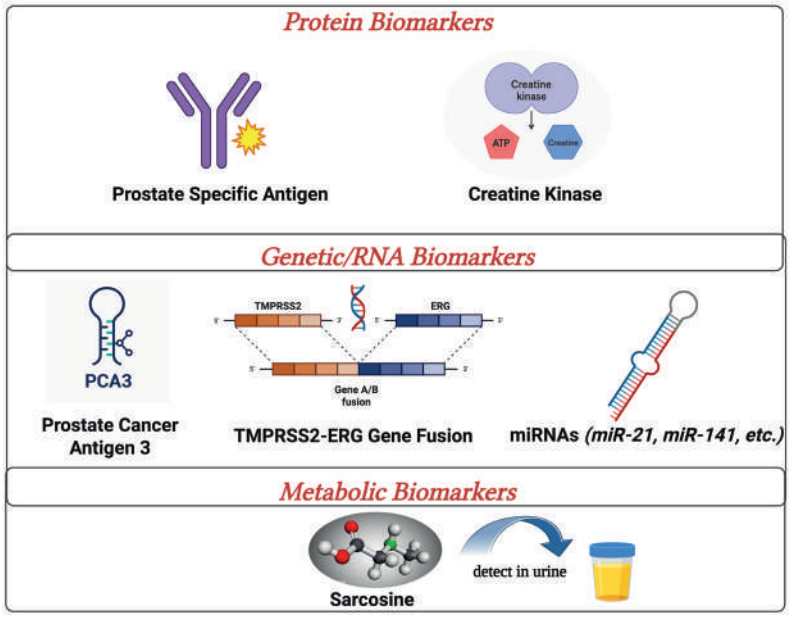

Figure 1. Classification of major prostate cancer biomarkers based on molecular category.

3. Recent Advances in Biosensor Technologies for Prostate Cancer

Biosensor technologies for prostate cancer diagnosis have evolved remarkably, transitioning from traditional electrochemical designs to advanced multi-modal and AI-integrated platforms. Early developments predominantly focused on electrochemical biosensors, particularly targeting PSA. These systems offered simple design, cost-effectiveness, and relatively high sensitivity, enabling point-of-care diagnostics for early-stage disease monitoring (3,7).

Subsequent innovations introduced aptamer-based biosensors with improved specificity and lower detection limits for PSA and emerging biomarkers such as PCA3. Notably, impedimetric aptasensors demonstrated exceptional label-free detection performance for PCA3, achieving sensitivities in the nanomolar range (2). These biosensors enhanced analytical accuracy while maintaining miniaturized, portable formats.

Simultaneously, microfluidic-integrated biosensors gained traction, combining immunosensing with lab-on-a-chip technologies to offer rapid, multiplexed detection of multiple prostate cancer biomarkers in a single assay. Such systems significantly reduced assay time and sample volume requirements, thus facilitating on-site diagnostics (6).

Nanomaterials and plasmonic nanostructures further revolutionized the field. Platforms employing silver nanocrystals and gold nanospikes enabled enhanced signal amplification for PSA detection, achieving unprecedented sensitivity levels suitable for early-stage cancer detection (4,7). Additionally, nanopore-based biosensors were developed for the precise identification of microRNAs such as miR-141-3p, offering label-free, high-resolution biomarker analysis at ultra-low concentrations (5).

Recent breakthroughs include artificial intelligence (AI)-integrated biosensors, capable of real-time prostate cancer screening by synergizing electrochemical transduction with machine learning algorithms. These systems enable automated result interpretation and enhanced diagnostic accuracy (27). Similarly, CRISPR/Cas-based biosensors have emerged as disruptive tools, enabling ultra-specific nucleic acid detection with rapid signal amplification, paving the way for next-generation prostate cancer diagnostics (28).

Moreover, nanozyme-assisted smartphone-integrated biosensors represent the forefront of personalized diagnostics, allowing highly sensitive, point-of-care detection of metabolic markers such as sarcosine with user-friendly interfaces (29). These devices demonstrate remarkable portability and accessibility, aligning with the global shift toward decentralized healthcare solutions.

Collectively, these innovations underscore the dynamic evolution of prostate cancer biosensors-from conventional electrochemical devices to cutting-edge AI-augmented and molecularly engineered platforms- each bringing new dimensions of sensitivity, selectivity, and clinical utility.

Table 1. Comparison of Recent Biosensor Technologies for Prostate Cancer Diagnosis

Biosensor Type	Target Biomarker(s)	Detection Method	Immobilization Method	Limit of Detection (LOD)	Reference
Electrochemical Biosensor	PSA	Amperometric	Carbon Nanotube-modified Electrode	0.1 ng/mL	(3)
Impedimetric Aptasensor	PCA3	Impedimetric	Aptamer + Gold Nanoparticles	1 fM	(2)
Microfluidic Biosensor	PSA, PCA3	Electrochemical + Microfluidics	Lab-on-a-chip, D-shaped Fiber	Multiplexed Detection	(6)
Optical Plasmonic Biosensor	PSA	Optical (Plasmon Resonance)	Gold Nanospikes	0.01 ng/mL	(7)
CRISPR/Cas12a Biosensor	miRNA (miR-141, miR-21)	Fluorescence, Nucleic Acid Ampl.	CRISPR/Cas12a with Dual Amplification	34 aM	(5)
AI-Integrated Electrochemical	PSA	Electrochemical + AI	Hybrid AI Algorithm with Electrochemical Sensor	0.01 ng/mL	(27)
Smartphone-based Nanozyme Biosensor	Sarcosine	Colorimetric	His@Co-NC Nanozyme + Smartphone Interface	5 μM	(29)

4. Conclusion

This chapter has comprehensively reviewed the principles, technological advancements, and clinical perspectives of biosensor systems developed for prostate cancer diagnosis. The discussion covered the fundamental mechanisms of biosensors, the most significant prostate cancer biomarkers, and their integration into various biosensing platforms. In particular, the growing shift from traditional electrochemical biosensors to innovative technologies such as aptamer-based biosensors, microfluidic devices, plasmonic systems, CRISPR/Cas platforms, AI-integrated biosensors, and smartphone-enabled nanozyme biosensors has been highlighted. The expanding importance of biosensors in prostate cancer diagnostics is evident from their ability to deliver rapid, highly sensitive, and non-invasive detection of both classical and emerging biomarkers. These technologies offer significant potential to overcome the limitations of conventional diagnostic methods, providing valuable tools for early diagnosis, risk stratification, treatment monitoring, and ultimately improving patient

outcomes. Looking forward, the future of prostate cancer biosensors lies in the continuous convergence of nanotechnology, artificial intelligence, and molecular diagnostics. The development of next-generation, cost-effective, and portable biosensors—capable of multiplexed detection and real-time analysis—holds promise for advancing personalized medicine and enhancing global access to reliable prostate cancer screening and monitoring tools.

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Chapter 5

Current Biosensor Designs and Applications For Early Detection of Neurodegenerative Diseases

Nur Tarımeri Köseer[1]

Abstract

The most common neurodegenerative diseases worldwide are Alzheimer's and Parkinson's. These diseases occur when the brain and peripheral nervous system gradually lose their function or result in the death of nerve cells. Thus, a healthy life depends on the early detection of neurodegenerative illnesses. Because of the high expense, limited sensitivity, and drawbacks of conventional diagnostic techniques, currently available biosensor technologies enable the development of alternative techniques for the early identification of neurodegenerative illnesses. Biosensors make it possible to quickly, sensitively, and usually non-invasively identify biomarkers of certain neurodegenerative disorders by using bioreceptors and transducers (optical, piezoelectric and electrochemical techniques). Together with these biomarkers, exosomal microRNA and Tau oligomer biomarkers also make effective detection possible. A thorough analysis of research employing biosensor technology for the early detection of neurodegenerative illnesses is provided in this section. These include discussion of several biosensor kinds, their operation, clinical uses with various immobilization techniques, and upcoming advancements.

1. Introduction

Neurodegenerative diseases include Alzheimer's disease (AD), Parkinson's disease (PD), prion disease, motor neuron disease (MND), Huntington's disease (HD), spinocerebellar ataxia (SCA), and spinal muscular atrophy (SMA) (1). Affecting 55 million people worldwide, the most common are Alzheimer's and Parkinson's disease. Statistics show that these diseases increase with age. Among individuals aged 65-85, the prevalence of these diseases doubles every five years (2,3). Genetic factors

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should also be considered. Types of neurodegenerative diseases and their causes are illustrated in Figure 1 and 2.

Symptoms observed in individuals with neurodegenerative diseases include

- Physical and behavioral decline
- Decreased mental function
- Behavioral problems

Biosensors are systems that find specific substances using a biological part and a sensor, giving quick and accurate results without needing extra chemicals (4). Electrochemical biosensors offer various applications for easy-to-use, low-cost, and portable devices for medical diagnosis (5). Piezoelectric biosensors are systems that utilize biological components as coating materials and incorporate piezoelectric crystals (6). Optical biosensors are sensor systems that transform light by refraction, absorption, and reflection (7). Biosensor designs developed with current technology have many applications. Biosensors are used in many areas, including the military, food, the chemical industry, healthcare, and environmental analysis (8).

This chapter examines new biosensor technologies for early detection of neurodegenerative diseases, focusing on biomedical engineering, operating principles, applications, and future prospects.

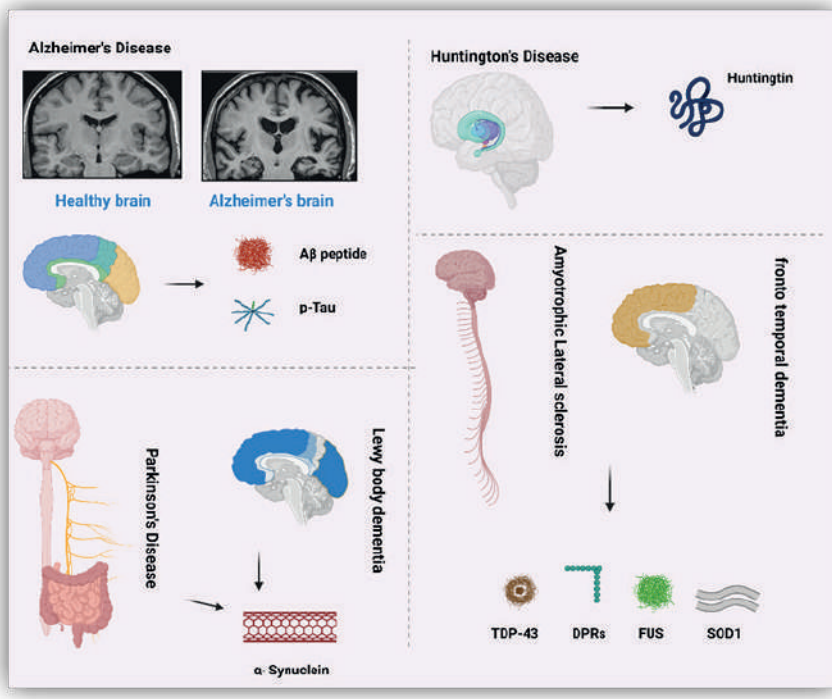

Figure 1: Neurodegenerative diseases and their effects (Created in https://BioRender.com)

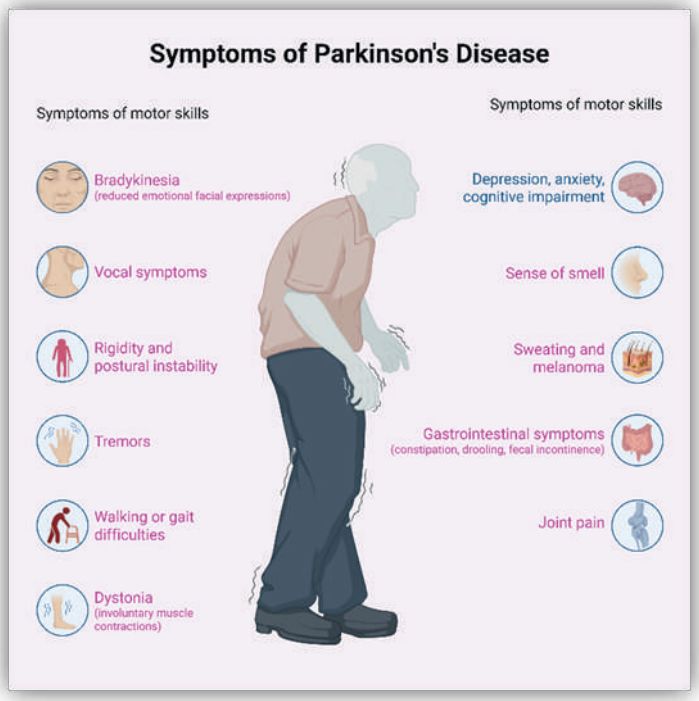

Figure 2: Symptoms of PD, a neurodegenerative disease (Created in https://BioRender.com)

2. Biosensor systems for early diagnosis of neurodegenerative diseases

Biosensors are recognition systems designed to identify bioreceptors and analytes. When transducers interact with receptors, a measurable signal is generated. These transducers transmit signals by assessing the conductivity of surfaces after receiving electrical, optical, or electrochemical inputs. The efficiency of signal transfer depends on the surface conductivity. A large surface area and the capacity to detect conductivity are the primary reasons why carbon materials are preferred in biosensor studies for this purpose. Additionally, graphene-based nanomaterials may be advantageous for signal conversion and sensor surface binding. However, enzymes can only achieve surface binding through physical adsorption (9).

Nanotubes, AuNPs, quantum dots, and GO are among the numerous nanomaterials that are included in the category of biosensors, as indicated by numerous studies. Researchers extensively employ biosensors in the

early diagnosis of neurodegenerative diseases and in the detection of AD in cerebrospinal fluid (CSF) (10). Recent advancements in nanomaterials, biorecognition components, and microfabrication techniques have advanced the area, paving the way for the development of biosensors that are more useful and efficient in clinical settings.

Haşvin et al. (11) investigated protein misfolding to investigate the causes of AD and PD. They designed optical and electrochemical biosensors using carbon nanotubes and gold nanoparticles. They used them to detect Aβ40/Aβ42, tau, ApoE, and miRNA markers in CSF and blood. In different study, Karaboğa et al. (12) designed a disposable electrochemical biosensor for the early diagnosis of Parkinson's-type dementia using alpha-synuclein protein. The study used an ITO-PET electrode modified with AuNPs and glutamic acid as the working electrode. Furthermore, the study was expected to aid in disease diagnosis by sensitively detecting the protein in CSF. The biosensor's linear detection range was 4-200 pg/mL, and the limit of detection was 0.135 pg/mL.

Jose et al. (13) developed an electrochemical biosensor using the misfolding method of tau protein. This study aimed to determine how tau protein in solution binds to immobilized tau protein, using surface characterization data to assess electrostatic changes. While the charge transfer resistance (Rct) of tau protein was determined to be 2.9 ± 0.6 kΩ, it was observed that the resistance (Rct) formed after Tau-Tau binding decreased to 0.3 ± 0.1 kΩ. A linear relationship was observed between (Rct) and the solution tau concentration (0.2–1.0 μM).

Aminabad et al. (14) designed an electrochemical biosensor system for the determination of alpha-synuclein protein. In this study, they created an AuNP-supported dimethylglyoxime layer on a GCE surface to facilitate antigen-antibody interaction. The linear detection range of the designed immunosensor was 4-128 ng/mL, and the limit of detection value was determined to be 4 ng/mL.

In different study Karaboğa et al. (15) developed an immunosensor for the selective and practical analysis of α-synuclein protein. This study's working electrode represents an innovative approach to modern technology. The working electrode used was gold-coated QTF electrodes. QTFs were obtained by conjugation with 4-ATP (aminothiophenol). The linear detection range was 1-500 ng/mL, and the limit of detection was 0.098 ng/mL. Since the QTF uses a mass-sensitive biosensor system, it was able to recover 92%-104% of the samples taken from CSF for the designed sensor. Tao et al. (16) used a GCE to immobilize anti-α-synuclein antibodies by

creating a composite of polyglycosamine, AuNPs, carbon nanotubes, and reduced GO. In this work, a square-wave voltammetry method was used to create an immunosensor. The sensor was found to have a linear range of 0.05-500 fM and a limit of detection of 0.03 fM. Human plasma samples were used for testing the developed sensor.

Chandra et al. (17) designed short microRNA (mRNA) sensors associated with Parkinson's disease. Using miR133b as a biomarker, the ssDNA sequence was tagged, and the process of oxidation and reduction was observed with tris phosphine hydrochloride. The optimum sensor was determined to have a linear range of 10 fM-520 pM and LOD of 168 aM. In different study Raquel et al. (18) designed an electrochemical microRNA sensor using carbon SPE electrodes with two gold nanostructures and an anti-miR-34a OP. The linear range of this biosensor was determined to be 100 pM-1μM, and the LOD value was determined to be 93 aM. In another study, Wang et al. (19) developed an electrochemical biosensor that supports antigen-antibody formation on gold microstrip electrodes coated with protein G. The EIS technique was used for analysis in the study. The study demonstrates that the designed biosensor can detect tau protein levels as low as 0.03 pM.

Table 1. The biosensor systems for early diagnosis of neurodegenerative disease

Targets	Measurement method	Modification method	Linear range	Reference
Aβ40/Aβ42, tau, ApoE, and miRNA	Electrochemical & Optical	Modification with carbon nanotubes and gold nanoparticles.	-	(11)
α- synuclein	Electrochemical	Modification with AuNP and glutamic acid in ITO-PET	4-2000 pg mL^{-1}	(12)
Tau	Electrochemical	Tau-Tau	0.2–1.0 μM	(13)
α- synuclein	Electrochemical	AuNP-supported dimethylglyoxime layer on a GCE surface	4-128 ng mL^{-1}	(14)
α- synuclein	Electrochemical	Modification with 4ATP in QTF	1-500 ng mL^{-1}	(15)
α-synuclein	Electrochemical	Modification with nanocomposite polyglycosamine, AuNPs, carbon nanotubes, and reduced graphene oxides	0.05 -500 fM	(16)
miR133b	Electrochemical	Ss DNA	10fM-520 pM	(17)
miR-34a	Electrochemical	Carbon SPE	100 pM-1μM	(18)
Tau	Electrochemical	The gold microband electrodes	0.03 pM	(19)

3. Conclusions

The challenge of researching the central nervous system leads to neurodegenerative illnesses. The quality of life for these individuals will be greatly enhanced by facilitating this difficulty and increasing the success of early diagnosis and treatment. Biomarkers used in the diagnosis of targeted neurodegenerative diseases through the integration of biosensors, bioreceptors, and transducers can be detected rapidly, sensitively, and cost-effectively. Electrochemical, optical, and piezoelectric biosensors are proving highly successful in the diagnosis of new-generation biomarkers and their use in various blood, such body fluids, brain, spinal fluid, and tears. In addition, while the designed biosensors have strong potential for early diagnosis, clinical validation is ongoing due to the unexplored areas of the brain and nervous system.

In conclusion, biosensor technologies will not only expand the possibilities of early diagnosis in neurodegenerative diseases through routine clinical application but will also contribute to the development of patient- and disease-specific treatment options.

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