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Electrochemical aptamer-based biosensors for disease biomarkers

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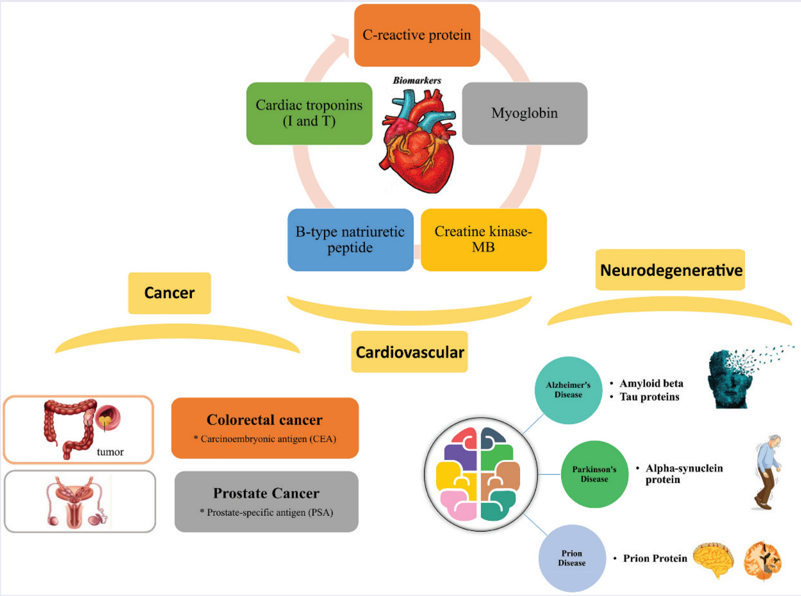
ABSTRACT

The increasing prevalence of diseases such as neurodegenerative conditions, cardiovascular disorders, and cancer represents a significant public health challenge. Timely diagnosis plays a crucial role in improving patient outcomes and alleviating the overall societal burden posed by these disorders. Recently, there has been increasing interest in using electrochemical aptasensors in diagnosing and prognosis neurodegenerative diseases, cardiovascular disorders and cancer. This comprehensive review categorizes and examines the latest advances in electrochemical aptasensors for evaluating these diseases. The aptasensors under investigation target specific analytes appropriate for each disease group. For cardiovascular diseases, analytes such as Cardiac troponins (cTns), myoglobin (Mb), C-reactive protein (CRP), creatine kinase-MB (CK-MB) and B-type natriuretic peptide (BNP) have been in focus. AβO, Tau, Prion protein and Alpha-synuclein (α-synuclein) were examined in neurodegenerative diseases. Carcinoembryonic antigen (CEA) and Prostate-specific antigen (PSA) biomarkers are of particular interest in the context of cancer. The review includes a comprehensive analysis of the development of these aptasensors over the last 5 years under three main headings, taking into account the advantages and disadvantages of these diagnostic tools and issues such as linear ranges, detection limits and preferred nanomaterials. In addition, this review focuses on an overview of the current problems and achievable solutions of aptasensors in detecting common diseases, and future trends are also predicted.



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1. Introduction

An aptamer is single stranded RNA/DNA engineered to bind specifically to a specific target component, such as a protein, small molecule, or even another RNA. Aptamers are short oligonucleotides, ranging in length from 20 to 80 nucleotides. In contrast to antibodies, aptamers offer the advantages of being rapid, repeatable, and mass-produced.[1] Scientists worldwide have shown

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significant interest in aptamers and are currently performing investigations to learn about their potential applications in several fields. Aptamer is generated through known in vitro selection or systematic evolution of ligands by exponential enrichment (SELEX).[2] SELEX is a well-known and significant method for screening aptamers. SELEX is a procedure that involves generating a substantial collection of randomized RNA/DNA sequences and then using the process of amplification and selection to select nucleotides that attach to the target molecule of interest.[3]

1.1. SELEX techniques

The SELEX process (Figure 1) enables the production of aptamers with superior specificity to a target molecule. An oligonucleotide (ssDNA or RNA) library is a crucial essential component of SELEX. The ssDNA or RNA molecules that make up the oligonucleotide library range in length from 20 to 80 nucleotides. The random region determines the affinity for the target, while the constant regions facilitate primer annealing, enabling amplification in subsequent steps. Initially, a diverse nucleic acid library with random sequences flanked by fixed primer-binding sites is synthesized.[4] This library is incubated with the target molecule, allowing binding interactions to occur. Bound sequences are then separated from unbound ones through methods like affinity chromatography, filtration, or magnetic bead separation, followed by the elution of the bound aptamers. These sequences are amplified using polymerase chain reaction (PCR) to generate an enriched pool for subsequent rounds of selection. The process is repeated multiple times, typically 8-15 rounds, with increasing selection stringency to enrich for high-affinity aptamers. After several rounds, the enriched aptamer pool is cloned and sequenced to identify individual sequences. High-throughput sequencing may be employed to assess the diversity and frequency of the sequences.[5,6] The selected aptamers are then characterized for binding affinity, specificity, and functionality using techniques such as surface plasmon resonance (SPR) or enzyme-linked oligonucleotide assays (ELONA). Key considerations in SELEX include the diversity of the initial library, the nature of the target, the stringency of selection conditions, and the fidelity of amplification. SELEX-derived aptamers find applications in therapeutics, diagnostics, and research tools due to their ability to bind and inhibit specific targets or serve as sensitive molecular probes.[7]

Aptamers have many advantages as biorecognition elements, including remarkable accuracy and sensitivity, and the capacity to bind to a variety of target molecules.

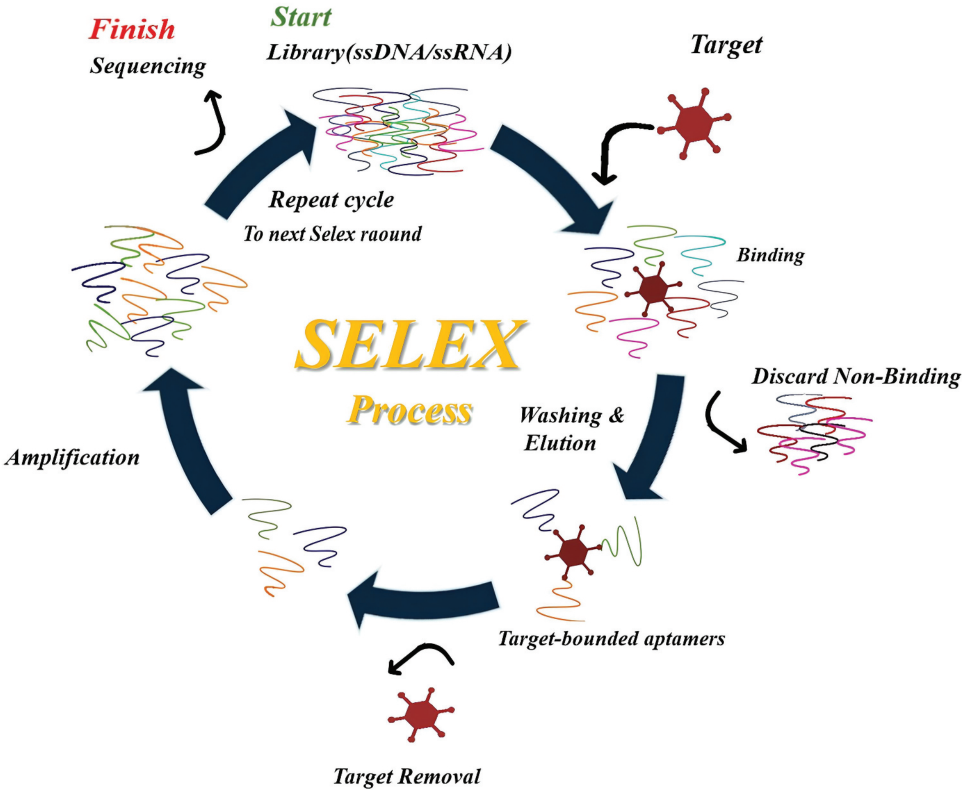

Figure 1. Diagram showing the SELEX procedure used to identify aptamers.

These properties make aptamers useful in a vast array of applications, including diagnostics, treatments, and biosensing. In addition to the above advantages, aptamers have some other advantages such as being smaller than antibodies, they are also nuclease resistant, which makes them stable in biological samples and can be used in vivo. However, there are some disadvantages to using aptamers as biorecognition elements, including the high cost and complexity of production, limited stability, and potential for immune response.[8] It is crucial to emphasize that these drawbacks and advantages may vary based on the particular practical application and the optimization of aptamers through the selection process. Also, it is important to keep in mind that the field of aptamers is a rapidly evolving field and new technologies and developments may address some of these limitations and expand the use cases even further.

The increasing prevalence of diseases, such as neurodegenerative, cardiovascular, and cancer diseases, is a major concern for public health.[9] Early detection is crucial to keep improving the results of patients and minimize the overall burden of these diseases on society. Aptamers have been widely employed since their debut in the 1990s for the determination of a variety of biomarkers, including those related to cardiac, cancer, viral, and bacterial diseases.[10] The number of publications related to aptamers in biosensing has increased from 6.4% in 2012 to 17.7% in 2020, indicating a growing interest in this field of research.[8] As the technology continues to evolve, it is expected that biosensors will become an increasingly important tool for improving public health.

Biosensor is a tool that unifies biological components with a physical transducer to produce a measurable output. They may be divided into groups according to the kind of biological component and transducer they employ and the kind of output they generate. Biosensors may be categorized into the following groups based on the type of transducer they use: mass-based, optical, thermal, and electrochemical biosensors.[11] In addition, enzyme linked immunosorbent assay (ELISA), piezoelectric immunoassay, fluorescence, electrochemical and optical techniques have been advanced for disease detection. Among these detection methods, electrochemical-based biosensing offers advantages like short reading time, portability, low cost, rapidness, high sensitivity, and a small sample volume.

1.2. Electrochemical aptasensors

Electrochemical aptasensors are biosensors that use aptamers as the recognition element to detect target analytes. There are different approaches used to design electrochemical aptasensors, including label-free, label-based, competitive, and sandwich configurations.[12]

1.2.1. Label-free approach

In this approach, the aptamer is immobilized onto the electrode surface, and the binding between the aptamer and the target analyte is detected directly by monitoring changes in the electrical properties of the electrode. Label-free detection does not require any labeling reagents or tags, making it a simpler and faster approach.

1.2.2. Label-based approach

This approach involves the use of labeling reagents or tags to detect the target analyte. The aptamer is labeled with a signal-generating moiety such as an enzyme or a fluorescent molecule, and the binding between the aptamer and the target analyte is detected by measuring the signal generated by the label. This approach provides enhanced sensitivity and specificity compared to the label-free approach.[12]

1.2.3. Competitive approach

In this approach, the aptamer is immobilized on the electrode surface, and a labeled analogue of the target analyte is added to the sample. The labeled analogue competes with the target analyte for binding to the aptamer, resulting in a decrease in the signal generated by the label. This approach is particularly useful for detecting small molecules with low molecular weight.

1.2.4. Sandwich configuration approach

This approach involves the use of two different aptamers that bind to different regions of the target analyte. One of the aptamers is immobilized on the electrode surface, and the other is labeled with a signal-generating moiety. The target analyte is sandwiched between the two aptamers, resulting in an amplified signal. This approach provides enhanced sensitivity and specificity compared to the other approaches.[13]

The sandwich technique is a widely used approach in the design of electrochemical aptasensors. In general, the sandwich technique in electrochemical aptasensors provides high sensitivity and specificity to detect target molecules; This makes it a popular approach to biosensor development for applications as diverse as clinical diagnostics, food safety, environmental monitoring, and drug discovery.[14] Both labeled and label-free techniques have advantages and disadvantages, and the choice of technique depends on the specific requirements of the assay. Labeled techniques tend to be more sensitive but require more complex instrumentation, while label-free techniques are simpler but less sensitive.[15]

This review article collected and discussed the mechanism and recent developments of various electrochemical aptamer-based biosensors for the diagnosis of common diseases. The aptasensors under examination target specific analytes pertinent to each disease group. For cardiovascular diseases, analytes such as cTns, Mb, CRP, CK, and BNP have been the focus, whereas neurodegenerative diseases have seen the scrutiny of AβO, Tau, Prion protein, and α-synuclein. In the context of cancer, CEA and PSA biomarkers are of particular interest. In the last part of this review, a specific analysis was made about the components and important agents used in the studied aptasensors, which can be used in future research. Also, each biosensor's analytical capability is mentioned, and the concluding section addresses achievements and challenges of aptamer-based electrochemical biosensors for detecting cardiac, neurodegenerative, and cancer diseases. Within the scope of this review, possible solutions to the problems encountered in aptasensor design and future expectations are discussed based on studies on diseases for use in the health sector. Thus, collecting these problems, deficiencies, and solutions under a single compilation provides an important data opportunity for aptasensor researchers who will develop biosensors for the health field and shorten research times. Additionally, it is the first review to consider the development of electrochemical aptamer-based biosensors for diagnosing cardiac, neurodegenerative, and cancer diseases in the healthcare field under a single title.

2. Aptasensor for biomedical applications

2.1. Cardiovascular diseases

According to the latest health data, cardiovascular illness is one of the primary reasons people pass away. Cardiac disease refers to any disorder that affects the heart or the blood vessels in the heart. Some common types of cardiac disease include, arrhythmias, heart failure, and coronary artery disease. These illnesses are the leading reason for disability and premature death worldwide.[16] Levels of cTnI and cTnT are recognized as concentrations below 0.04 ng/mL in a healthy person, while its concentration rises to 100 ng/mL in sick individuals.[17]

Treatment for cardiac disease can include lifestyle changes, medication, and in some cases, surgery. Although the electrocardiogram is the most successful and widely used diagnostic approach, it has disadvantages due to non-diagnostic signals or vague readings that sometimes interfere with diagnosis.[18,19] Electrochemical aptasensor offer several advantages over traditional analytical methods, including high sensitivity, rapid response, and the ability to operate in situ. Biomarkers play a crucial role in the diagnosis, prognosis, and therapy of illness. CRP, cTns, CK-MB and Mb, have now been recognized as effective diagnostic biological markers for heart disease.[20]

This part offers an exhaustive summary of the progress made in using both label-free and labeled electrochemical aptasensors to detect biomarkers associated with cardiovascular disease.

2.1.1. *Cardiac troponin I*

High levels of TnI in the blood may indicate that a person is having a heart attack or experiencing heart failure. It is a protein present in cardiac muscle cells that enter the circulatory system when the heart is damaged, such as in a heart attack.[21] Increased cTnI levels can be detected within hours of a heart attack and may remain elevated for several days. This makes cTnI a valuable tool for quickly detecting and monitoring the problems of heart attack patients.[22]

Huang et al. aimed to construct an aptasensor for the determination of TnI biomarker by atomic layer deposition (ALD) method.[23] The reason for using the ALD method is to deposit NbS_2 nanoflakes directly on carbon fiber paper. Au nanoparticles were used to combine the cTnI aptamer (A-TnI) probe. The immobilization steps of the designed sensor consist of MCH/A-TnI/Au/NbS_2NFs/CFP. Cyclic voltammetry (CV), differential pulse voltammetry (DPV), and electrochemical impedance spectroscopy (EIS) were utilized to specify the most suitable electrochemical method to design the TnI sensor. It has been demonstrated that the 2D NbS_2 nanoflake structure and the CFP substrate's wide surface area and strong conductivity contribute to the increased sensibility of the cTnI sensor. As a result of the evaluation of the efficiency of the biosensor, it was confirmed that different concentrations of cTnI were detected between 10^{-10} and 10^{-15} M. Linear range of 1 fM-0.1 nM and limit of detection (LOD) 0.32 fM was reported. The successful creation of this biosensor hinged on the unique structure of the 2D NbS_2 nanoflakes and the extensive surface area and strong conductivity offered by the carbon fiber paper substrate. This work highlights the potential of the ALD method, combined with innovative materials and aptamer-based design, for creating highly sensitive and efficient biosensors for biomarker detection. It has the promise of significant contributions to the field of medical diagnostics and disease monitoring.

Label–free detection methods offer several benefits, including preserving active binding epitopes and avoiding steric hindrance. Additionally, these methods significantly simplify the development process and reduce experimental artifacts and costs. As a result, the utilization of label-free sensing methods for the determination of different biomarkers, using single-receptor aptamers have been considerable research. One of the most recent studies, the aptasensor designed by Sharma and coworkers, is dependent on an electrochemical aptamer-based technology to detect TnI.[24] The sensor was made of reduced graphene oxide, modified with a polyethyleneimine chemical. This modification made the sensor highly sensitive and could detect small amounts of cMb. The sensor is also label-free, meaning it does not require additional chemicals.

Another label–free electrochemical aptasensor study using gold nanoparticles (AuNPs) to detect TnI was done by Eshlaghi et al. [25] Aptasensor was constructed using conductive polypyrrole (PPy)-AuNPs substrate and aptamer probe. The combination of AuNPs and PPy, which is a polymer that conducts electricity, on the electrode's layer improved the ability to conduct electricity. The aptasensor has been reported to have a LOD 25 pg/mL. While it has a relatively higher LOD compared to Sharma et al. sensor, it offers several noteworthy advantages. The immobilization method modified with PPyAuNPs provided a promising biosensing platform with enhanced detection selectivity, reduced assay time, reproducibility, sensitivity, and reproducibility.

Likewise, Xu et al. developed a label–free TnI determination technique utilizing a cubic Au nanoparticle/indium oxide composite-based aptasensor and a Fe redox probe.[26] This aptasensor identified cTnI with an LOD of 60 pg/mL once tested with human serum samples. While it may not be as sensitive as sensor developed by Sharma et al. it offers the distinct advantage of being able to detect TnI in human serum samples, which is a more complex and real-world matrix. This could be particularly relevant for clinical applications. Another study by Negahdary and coworker developed a label–free aptasensor utilizing gold nanodumbbells-adapted gold electrodes to immobilize TnI-specific aptamers by a SAM mechanism.[27] (Figure 2(a)) This aptasensor showed high selectivity and discriminated in 0.05 to 500 ng/mL. These studies share the advantage of being label-free, simplifying the detection process by eliminating the need for additional chemicals.

In summary, sensor developed by Sharma et al. stands out for its exceptional sensitivity, making it suitable for early diagnosis applications. The sensor designed by CXu et al. is particularly relevant for clinical settings where TnI detection in complex matrices like human serum is required. Negahdary and coworkers' aptasensor offers excellent selectivity and a wide dynamic range, making it versatile for different applications. The choice between these aptasensors would depend on the specific requirements of a given application.

Kaicha and colleagues developed the sandwich method-based cTnI electrochemical aptasensor to diagnose Acute Myocardial Infarction (AMI).[28] To increase its sensitivity, two materials, Au loaded zirconium-carbon (Au\ZrC) as label substance, and Pt-Cu-Ni catalyst were used as the base material in the sensor design, respectively (Figure 2(b)). The process of identifying reactions was carried out by gradually exposing aptamer-1- TnI and finally aptamer-2-Pt-Cu-Ni to an electrode modified with Au\ZrC. The Au\ZrC electrode material was formed through a reduction and simple heating procedure. Zr-C material has a unique structure with many pores and good conductivity, which is further enhanced by adding Au. This makes it a suitable substrate for making a particular type of sensor. A catalyst called Pt-Cu-Ni was also made and found effective at reducing H_2O_2 and the Pt-Cu-Ni catalyst improves its catalytic activity and stability. The sensor can detect cTnI at very low levels with a wide sensitivity range and good selectivity, reproducibility, stability and recovery. The sensor could be utilized for medical diagnostics.

A 2D substance called molybdenum disulfide (MoS_2) has many benefits for biosensor systems. One of the primary advantages is its high sensitivity, which makes it an ideal material for detecting biomolecules.[29] Because of its enormous surface area and rapid transport of charge carriers, MoS_2 can detect biomolecules in even low quantities. Additionally, MoS_2-based biosensors can be engineered to be highly selective for specific biomolecules, such as DNA or proteins. This selectivity is achieved through the functionalization of MoS_2 with specific binding molecules that can capture the target biomolecules. Another advantage of using MoS_2 in biosensor systems is its stability and biocompatibility.[30] Since MoS_2 can tolerate extreme conditions and is resistant to deterioration, it is a dependable material for environmental and medical monitoring applications. Its biocompatibility also makes it well-suited for use in biological systems, as it does not interfere with the activity of cells or other biomolecules.[31] Overall, the combination of MoS_2 with other materials offers several advantages for biosensor systems, making it a material with great potential for various uses. In 2021, a group of scientists created a combination with MoS_2, copper-nanowires and reduced graphene oxide to fix it onto a glassy carbon electrode (GCE).[32] This biosensor was utilized to detect cTnI without labels. The results demonstrated that this method is sensitive and can detect small amounts of biomarkers due to the combined effects of different components in the

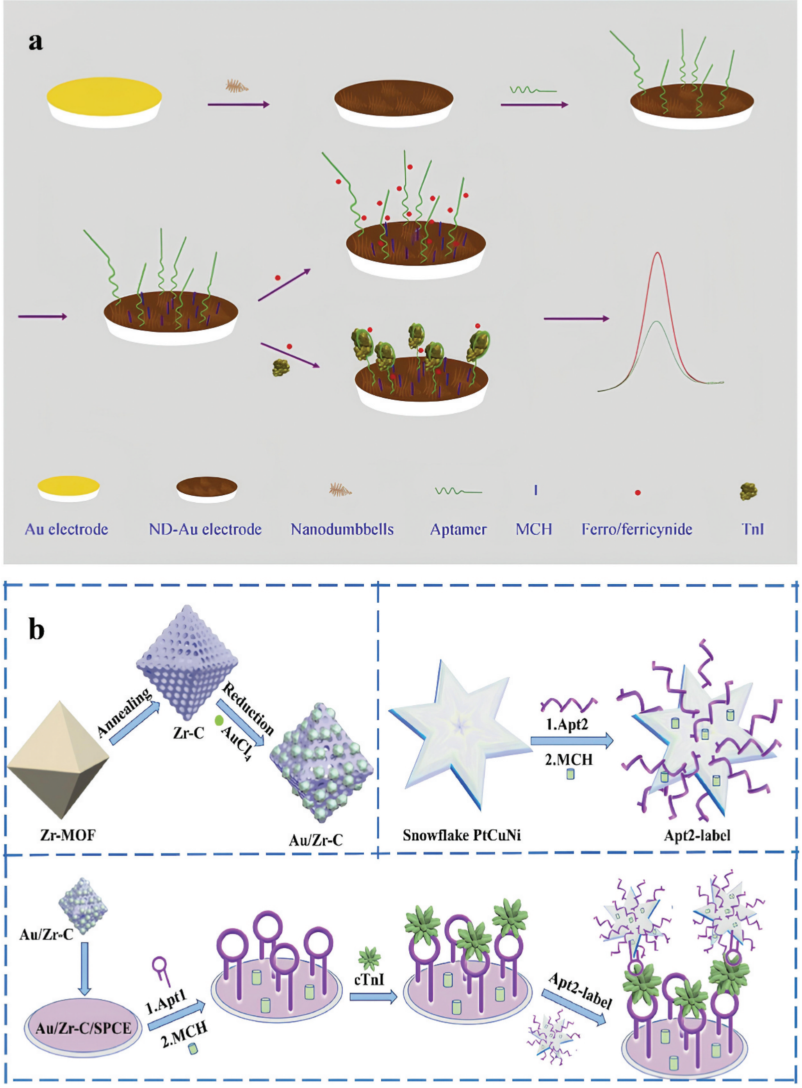

Figure 2. (a) Fabrication protocol of TnI aptasensor using gold nanodumbbells [27] (b) Schematic representations of the production procedures of the cTnI electrochemical aptasensor using Au\ZrC substrate material.[28] Reprinted from ref. [27,28] with permission.

nanocomposite. In another MoS_2 study, Qiao et al. constructed a novel approach for TnI determination depended on aptamer MoS_2 nanoconjugates, tested by the EIS technique.[33]

In a recent study, Huang and colleagues proposed a new way to detect TnI using an electrochemical aptasensor.[34] A special technique called DNAzyme-driven walker-walker serial amplification was used on a special type of electrode made of $Au/NiCo_2S_4$ nanocomposites. This technique involves joining two probes, HP I and HP II, on the electrode. When cTnI binds to HP I, it cleaves a specific HP II sequence of the DNA promoter, causing it to release a specific DNA. This DNA is then used to activate more DNA walkers, creating a chain reaction that amplifies the signal, making it easier to detect. With this method, the researchers reported, they avoided the problems of short distances or limited available propulsion in other single-leg DNA walkers. In addition, the sensor used a type of electrode material that is not only conducive but also stable and has a large surface area. This sensor could detect TnI in highly diluted samples, demonstrating its potential for use in medical testing and analysis. In summary, the proposed sensor is easy to use, efficient, and low-cost.

In summary, each of these studies offers unique advantages. Kaicha and colleagues' sensor prioritizes exceptional sensitivity, making it suitable for early detection applications. The emphasis on MoS_2 by Jiao et al. draws attention to its enormous potential for environmental and medical monitoring applications in terms of sensitivity, selectivity, stability, and biocompatibility. Huang and colleagues present a highly efficient and cost-effective approach to cTnI detection that emphasizes ease of use and signal amplification.

2.1.2. Cardiac troponin T

Commercial TnI detection sensors are less sensitive than TnT sensors.[8] However, to date, the electrochemically based troponin T (TnT) aptasensor for diagnosis of heart disease has been less explored than the TnI aptasensor.

Negahdary and colleagues presented a novel TnT aptasensor in 2019 by immobilizing a TnT-specific aptamer on the surface of PEG-electrodeposited AuNPs.[35] DPV technique was used to monitor the binding of TnT. As the concentration of TnT was increased, this lowered oxidation current of the redox couple was produced. This can be ascribed to the TnT insulating barrier layer's role in reducing the electron transport kinetics. The proposed sensor allowed detection in the wide range of 0.05–5.0 ng/mL in human serum. DPV is a sensitive electrochemical method widely used for quantification, and the use of AuNPs improves the electrochemical signal. This approach offers a wide detection range in human serum, a valuable feature for clinical applications. However, the method does not stand out with its originality, as it is based on established electrochemical principles.

In another similar study, Mitali et al. It immobilized thiol functionalized aptamers on the Au working electrode layer.[36] Various optimizations such as sequential modification with glutaraldehyde, cysteamine and avidin were made and the performance of the aptasensor was examined in detail by EIS technique. EIS provides information on charge transfer resistance and provides information on the kinetics of electron transport, making it a valuable analytical tool. However, the study lacks details regarding specific results or the sensitivity and selectivity values achieved.

2.1.3. Myoglobin

Myoglobin is a heme-containing biomolecule that is expressed in skeletal muscle and cardiac. Although it has limited specificity as a diagnostic marker for AMI, it is highly sensitive for early detection due to its rapid release from infarcted myocardium, typically occurring within 2-3 hours post-infarction. In 9-12 hours, the blood Mb concentration hits its peak, and in 24 hours, it has returned to normal.[37]

Optimizing the performance of conventional electrode surfaces by incorporating functionalized nanomaterials, nanocomposites, and nanohybrids with a high surface area is essential for maximizing the immobilization of aptamers. In addition to graphene, the utilization of various 2D layered materials has opened up exciting possibilities for the development of nanoscale biosensing systems. Materials like graphitic carbon nitride, boron nitride, and molybdenum disulfide have emerged as promising candidates for their unique properties.[38] For instance, Adelle conducted a study in 2019 that involved the modification of boron nitride nanolayers (BNNSs) with AuNPs and their deposition on a fluorine-doped tin oxide (FTO) electrode.[39] BNNSs, similar to graphene but composed of nitrogen and boron atoms, provided an excellent platform for the facile deposition of AuNPs. The resulting detection system, known as Apt-AuNPs-BNNSs-FTO, demonstrated remarkable performance with a high signal response and a LOD as low as 34.6 ng/mL for Mb, a key biomarker (Figure 3). These innovative modification have been shown to significantly enhance sensitivity and achieve lower detection limits in the electrochemical detection of Mb, thus advancing the field of biosensing.

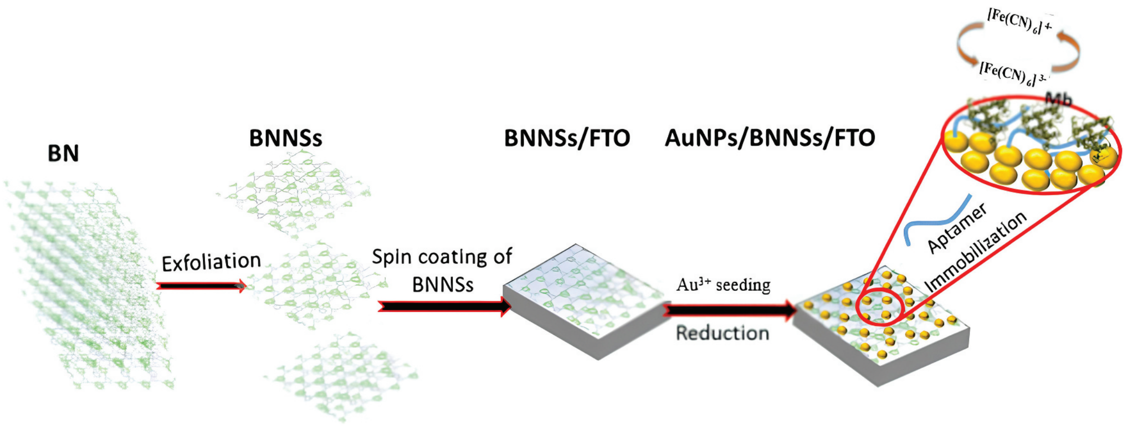

Figure 3. Use of boron nitride nanolayers for aptamer-based electrochemical detection of Mb.[39] Reprinted from ref. [39] with permission.

Furthermore, this trend of utilizing 2D materials for biosensing applications extends to other layered materials as well, such as MoS_2 and graphitic carbon nitride (g-C_3N_4). MoS_2, known for its large surface area and rapid charge carrier transport, has shown high sensitivity in detecting biomolecules, making it an ideal candidate for biosensors.[40] Similarly, g-C_3N_4's unique properties, like its excellent stability and biocompatibility, have rendered it valuable for biosensor development.[30] These materials hold great promise in expanding the capabilities of nanoscale biosensing systems for a wide range of applications, from clinical diagnostics to environmental monitoring and beyond. As research in this area continues to evolve, it is likely that even more 2D materials will be explored, each offering its distinct advantages for enhancing the sensitivity, selectivity, and stability of biosensors.

2.1.4. C-reactive protein

Blood plasma contains a protein called C-reactive protein, which has a large molecular weight of 125 kDa. The concentration of CRP in the serum is typically below 2 mg/L under normal circumstances. Elevated levels of CRP above 2 mg/L may indicate the presence of cardiovascular disease.[41] However, rapid fluctuations in CRP concentration in blood can also indicate other illnesses like cancer, infection and injury. As such, when used in conjunction with other cardiac biomarkers can be a promising biomarker for the cardiac diagnosis.[42] Researchers have yet to focus much on the electrochemical aptasensor for the diagnosis of CRP.

Microspheres, which generally act as carriers to fix biomolecules on the electrode surface, have received intense attention recently. They are preferred because they can be easily functionalized and have a large surface area.[43] Wang and colleagues developed the aptasensor using microspheres on the GCE electrode.[44] The synthesized silica microspheres were functionalized with AuNPs, providing a large surface area and acting as a signal enhancer and carrier. The electrochemical transduction was made by square-wave voltammetry (SWV). Reliable and sensitive determination of CRP (0.0017 ng/mL detection limit) was accomplished by the aggregation of RNA aptamers via Au-S bond on the electrode surface (Figure 4). The procedure of the aptasensor is simple and the results are quite satisfactory when tested with human serum samples spiked with the target analyte.

Given that RNA-based aptamers have their own set of restrictions, such a high propensity to be degraded by nucleases, these molecules are rarely used. The typical half-life of RNA oligonucleotides in blood is a few minutes, relying on the conformational shape and oligonucleotide content. In order to further improve the bioreceptor layer's stability, Marta et al. utilized the DNA counterpart of an RNA aptamer since it is less susceptible to being degraded by nucleases in their recognition layer. The developed aptasensor has been

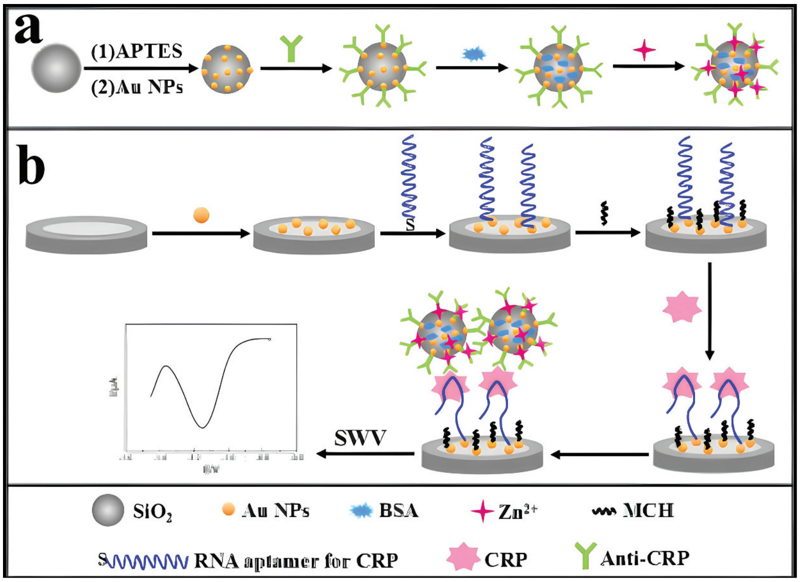

Figure 4. Fabrication procedure of aptasensor using composites of silica microspheres coated with gold nanoparticles. a) Preparation of immunoprobe Zn^{2+}\Ab\Au NPs@Si-MSs. b) Fabrication of AuNP\RNA\MCH-modified electrode [44] Reprinted from ref. [44] with permission.

successfully used in the concentration range of 0.23×10^{-3} to 23×10^{-3} ng/mL, creating a detection tool that is an alternative to existing techniques for CRP analysis. Similarly, a DNA-based aptasensor was prepared for CRP detection using the EIS technique in the wide range of 0.23 ng/L-2.3 mg/L using Au electrode modified with rhodium NPs.[45]

The studies presented by Kim et al. and Marta et al. both address the challenges associated with using RNA-based aptamers in biosensors. RNA aptamers have limitations due to their susceptibility to degradation by nucleases, resulting in a short half-life in blood. To overcome this issue, Marta et al. opted for DNA aptamers, which are known for their enhanced stability against nucleases in the bioreceptor layer. One key advantage of these approaches is the increased stability and durability of the DNA-based aptasensors. In both studies, the developed aptasensors exhibited successful detection of CRP in a wide range of concentrations, offering alternatives to existing techniques for CRP analysis. However, it's essential to note that the two studies employ different methodologies, with one utilizing the DNA counterpart of the RNA aptamer and the other employing the EIS technique on an Au electrode modified with rhodium nanoparticles. The choice of methodology may depend on specific application requirements and the need for sensitivity, cost-effectiveness, or ease of use. Overall, these studies showcase the adaptability and advantages of DNA-based aptasensors in addressing the limitations of RNA aptamers, providing more robust and stable bioreceptor layers for analytical purposes.

2.1.5. B-type natriuretic peptide

The heart produces the hormone BNP, while N terminal pro BNP (NT/proBNP) is a prohormone generated from the same molecule as BNP. Elevated levels of both BNP and NT/proBNP are considered biomarkers of heart failure and are used for both the diagnosis and prognosis of the condition.[46] Studies have shown that individuals with BNP concentrations above 80 pg/mL are at a higher risk of death. Additionally, NT/proBNP levels above 300 pg/mL have been found to exhibit a high degree of sensitivity (99%) and specificity (60%) in the assessment of heart problems.[47]

The use of a biocompatible conductive material interface with the biological sensing element is of great importance for building a biosensor. Platinum is a top contender due to its position as a noble metal, low absorbency, biocompatibility, and high conductivity for binding to the electrode of proteins compared to other noble metals such as gold.[48] Most biosensor studies developed on the platinum surface have preferred to use enzymes and antibodies as fixatives commonly. It uses platinum electrodes with material interfaces such as carbon nanocomposites/nanotubes, gold, graphene, polymers, silica, and chitosan. As a result of a comprehensive examination of the current literature, only one study develops aptasensors using the Pt interface. In this study, Mitali and groups used a Pt wire modified with glutaraldehyde, avidin and cysteamine sequentially to immobilize a biotinylated aptamer to determine BNP.[49] The binding event of the biomarker was examined by a label-free impedimetric detection.

In another report, pretty simple label-free methods for the determination of BNP-32 have been proposed by Grabowska and groups.[50] The aptasensor was developed using a gold SPE modified with polyethyleneimine/reduced graphene oxide films. The serum response of the developed BNP-32 aptasensor is linear, ranging from 1 pg/mL to 1 g/mL. This alteration resulted in robust and highly sensitive electrochemical platforms designed to detect BNP in serum, achieved without labeling agents. The researchers efficiently utilized a branched polyethyleneimine (PEI) version containing NH_2 groups to anchor BNP-specific ligands, particularly DNA aptamers. Their work showcased the remarkable achievement of sub-pM BNP detection limits in serum without the need for an amplification strategy. Notably, this methodology is well-suited for simultaneously detecting multiple analytes, offering adaptability for other biomarkers like cTnI by simply substituting the utilized aptamer.

Table 1 summarizes the design and analysis capabilities of the electrochemical aptasensors reported throughout this section. Electrochemical aptasensors for TnI and TnT have achieved remarkable sensitivity and specificity, with detection limits reaching pM levels. Recent studies have utilized nanomaterials such as gold nanoparticles, carbon nanotubes, and graphene to enhance signal transduction. Some aptasensors have been designed for simultaneous detection of both Mb and TnT, enabling comprehensive cardiac monitoring. Studies designed to detect several biomarkers simultaneously are limited. Techniques such as differential pulse voltammetry and cyclic voltammetry have been the most frequently used techniques. Different types of electrodes such as carbon fiber paper, SPE, and GCE are used, each offering unique benefits in terms of conductivity and surface properties. Label-free detection is a significant advantage, seen in several studies, as it simplifies the sensor design and reduces costs. Aptamers for TnT often use similar sequences and thiol groups for immobilization. Novel materials such as gold nanostructures and AuNPs/BNNSs composites are utilized to enhance detection capabilities.

Table 1. Electrochemical based aptasensor studies for diagnosis of cardiovascular diseases.

Disease	Biomarker	Sequence	Immobilization Strategy	Technique	Label free	Electrode	LOD	Linear range	Ref.
Cardiovascular	TnI	5′ -SH-$(C)_6$ CGTGCAGTACGCCAACCTTTCTCATGCGCGCTGCCCCTCTTA-3′	Au/NbS_2NFs	CV, CC, EIS	No	Carbon fiber paper	0.32 fM	1-0.1 nM	[23]
Cardiovascular	TnI	–	PPy-AuNPs	CV, SWV	Yes	SPE	25 pg/mL	50 – 500 pg/mL	[25]
Cardiovascular	TnI	Apt1, 5′-SH -(C_6)- CGTGCAGTACGCCAACCTTTCTCATGCGCTGCCCCTCTTA Apt2, 5′-SH -(C_6)- CGCATGCCAAACGTTGCCTCATAGTTCCCTCCCCGTGTCC	Au/Zr–C	CV, EIS, CC	No	SPCE	1.24×10^{-3} pg/mL	0.01 pg/mL-100 ng/ mL	[29]
Cardiovascular	TnI	5′ -SH-$(C)_6$ CGTGCAGTACGCCAACTTCTCATGCGCTGCCCCTCTTA-3′	CuNWs/MoS_2/rGO nanocomposite	CV, DPV	No	GCE	1×10^{-13} g/ mL	5×10^{-13} – 1×10^{-10} g/mL	[32]
Cardiovascular	TnI	–	Au/$NiCo_2S_4$/Nafion /GCE	CV, DPV	No	GCE	0.26 pg/mL	0.01-60 ng/mL	[34]
Cardiovascular	TnI	5′ -SH-$(C)_6$ TAGGGAAGAGAAGGACATATGATCACGGCATGTTGACTAGTACATGACCACTTGA-3′	GO@AuNP	CV, EIS	Yes	SPE	0.001 pg/mL	–	[100]
Cardiovascular	TnI	5′ -(SH)-$(CH_2)_6$-AGTCTCCGCTGTCCTCCCGATGCACTTGACGTATGTCTCACTTTCTTTTCAT TGACATGGGATGACGCCGTGACTG-3′	Gold nanodumbbells	DPV	Yes	SPE	8 pg/mL	0.05–500 ng/mL	[27]
Cardiovascular	TnI	5′ -NH_2-TTTTTTCGTGCAGTACGCCAACCT TTCTCATGCGCTGCCCCTCTTA-3′	GC/N-prGO	DPV	Yes	GCE	1 pg/mL	–	[101]
Cardiovascular	TnI	5′ -SH-(C_6)-CGTGCAGTACGCCAACCTTTCTCATGCGCTGCCCCTCTTA-MB-3′	Au@SiO_2@Au	SWV, EIS	Yes	GCE	1.23 pM	10 pM-10 µM	[33]
Cardiovascular	TnT	5′-(SH)-$(CH_2)_6$-CGTGCAGTACGCCAACCTTTCTCA TGCGCTGC CC CTCTTA-3′	–	DPV	No	SPGE	0.01 ng/mL	0.05-5 ng/ mL	[102]
Cardiovascular	TnT	5′ -(SH)-$(CH_2)_6$-CGTGCAGTACGCCAACCTTTCTCATGCGCTGCCCCTCTTA-3′ .	Gold nano structure	DPV	No	GCE	23 pg/mL	0.05- 5 ng/mL	[35]
Cardiovascular	TnT and Mb	5′-(SH)-$(CH_2)_6$-CCCTCCTTTCCTTCGACGTAGATCTGCTGCGTTGTTCCGA-3′	AuNPs/BNNSs	DPV	Yes	FTO	34.6 ng/mL	0.1–100 µg/mL	[39]
Cardiovascular	TnT	5′ -ATCCGTCACACCTGCTCTTAATTACAGGCAGTTCCACTTAGACAG ACACACGAATGGTGTTGGCTCCCGTAT-3	PEI–rGO	CV, EIS, DPV	Yes	ITO	2.1 pg/mL	–	[24]
Cardiovascular	CRP	5′ -SH-$(CH_2)_6$-GCCUGUAAGGUGGUC GGUGUGGCGAGUGUG UUAGGAGAGAUUGC-3′ .	AuNPs@Si-MSs	SWV	Yes	GCE	0.0017 ng/mL	0.005- 125 ng/mL	[44]
Cardiovascular	CRP	5′–GCCTGTAAGGTGGTCGGT GTGGCGAGTGTGTTAGGAGAGATTGC-3′	–	CC	No	Gold disk	–	1-100 pM	[103]
Cardiovascular	CRP	5′ -CGAAGGGGATTCGAGGGGTGA TTGCGTGCTCCATTTGGTGTGCTG GAACTCCCCGACTGC-3′	–	EIS	Yes	Au micro-gap electrode	0.349 pM	1 pM – 100 nM	[45]
Cardiovascular	CK	5′ -N_3-TTT-TTT-GGCGATTCG TGATCTCTGCTCTCGGTT TCGCGTTCGTTCG-3′	SPE/rGO/PEI	DPV	Yes	SPGE	–	1 pg/mL- 10 ng/mL	[50]
Cardiovascular	BNP	–	–	EIS, CV	Yes	Pt electrode	–	–	[49]

2.2. Neurodegenerative diseases

A set of conditions known as neurodegenerative diseases (NDs) are distinguished by death or the gradual deterioration of nerve cells in the spinal cord and brain.[51] Diagnosing neurodegenerative diseases can be challenging, as the symptoms and progression of these disorders can be similar among different conditions. A thorough neurologist or other specialist evaluation is typically required to make a diagnosis.

Since NDs have become more common in recent years, diagnosing them quickly and accurately has become increasingly difficult. Biosensors, and in particular aptasensors, have the potential to provide a sensitive, specific and noninvasive way to detect biomarkers associated with neurodegenerative diseases, thereby aiding early diagnosis.[52] However, it is significant to point out that the development of biosensors for the diagnosis of neurodegenerative diseases is still in the early stages and further research is needed to realize their full potential.

There are several types of neurodegenerative diseases, each with its own specific set of symptoms and progression. Some of the most common types of NDs include: Prion diseases, Parkinson's disease, and Alzheimer's disease. This part offers an exhaustive summary of the progress made in using both label-free and labeled electrochemical aptasensors to detect biomarkers associated with neurodegenerative diseases.

2.2.1. Alzheimer's disease

Alzheimer's disease (AD) is a neurodegenerative illness that progressively deteriorates mental faculties over time. There is now no treatment, although it is the leading cause of dementia in the elderly. Currently, there is no cure for Alzheimer's disease, but there are treatments and interventions that can help manage the symptoms and slow the progression of the disease.[53] Amyloid-beta (Aβ) and tau proteins that can be measured in the cerebrospinal fluid (CSF) are potential biomarkers in Alzheimer's disease.[54]

2.2.1.1. Amyloid beta. Aβ is a small peptide associated with Alzheimer's. It is a small piece of protein from a larger protein called amyloid precursor protein.[55] The normal range of Aβ1-42, which is considered the most toxic form of amyloid beta, in the CSF of healthy individuals is typically between approximately 200 to 600 pg/mL. High levels of Aβ1-42 in the CSF are considered a biomarker of Alzheimer's disease.[56]

Due to its potential application in electrochemical biosensors, metal-organic frameworks (MOFs), a family of biomaterials, have attracted much attention lately. MOFs comprise clusters or metal ions linked together by organic binders to produce highly organized, porous structures with vast surface. One way MOFs are used in electrochemical biosensors is to use a matrix to immobilize enzymes, proteins or other biomolecules. The large surface area of MOFs allows for the immobilization of high-density biomolecules, which can increase the selectivity and sensitivity. Furthermore, the porous nature of MOFs can also increase the bulk transport rate of analytes to the sensing surface, further increasing the sensitivity of the biosensor. Another way MOFs are used in electrochemical biosensors is as a material to create working electrodes.[57]

Considering these properties of MOFs, Zhou and his group developed a DPV-based aptasensor for the detection of AβO, using gold nanoflowers (AuNFs) as substrates and Cu-MOFs loaded with AuNPs as signal detectors. The findings indicated that the high electrical conductivity of AuNFs and the large number of Cu^{2+} ions in the AuNPs\Cu\MOFs nanocomposite caused a strong electrochemical reaction.[58]

The utilization of nanoelectrode material is one of the key factors for more selective and sensitive biosensor system design. Based on the ability of polyadenine to be activated by a hybridization chain reaction and absorb AgNPs, an electrochemical sensor was developed that could detect AβOs with LOD of 1.94 pg/mL.[59] In another electrochemical sensor study, SnS_2 nanolayers synthesized via a chemical vapor deposition process were used to detect AβO (Figure 5). A 56.9 fg/mL LOD value was reported on the detection platform prepared with aptamer immobilization, designated as CC/SnS_2/SH-MPTMS/Apt/BSA.[60]

A combination with a labeled signal-displaced probe (SDp) and unlabeled aptamer was thought to be a promising strategy to achieve simultaneous electrochemical signal amplification. Inspired by this, Wang et al prepared an electrochemical aptasensor using AuNPs \CuMOF\SDp immobilization to determine AβO with high sensitivity.[61]

2.2.1.2. Tau proteins. Tau proteins are a group of microtubule-associated proteins that play an important role in maintaining the structural stability of brain cells called neurons. In AD, tau proteins become abnormal and form aggregates called neurofibrillary tangles inside the nerve cells, which are thought to contribute to the degeneration and death of these cells.[62] The normal range of tau protein in the CSF of healthy individuals is typically between approximately 10 to 50 pg/mL. Increased tau protein concentrations in the CSF are considered a biomarker of AD.[63]

There are several commonly applied ways to improve the detection sensitivity of electrochemical sensors. One

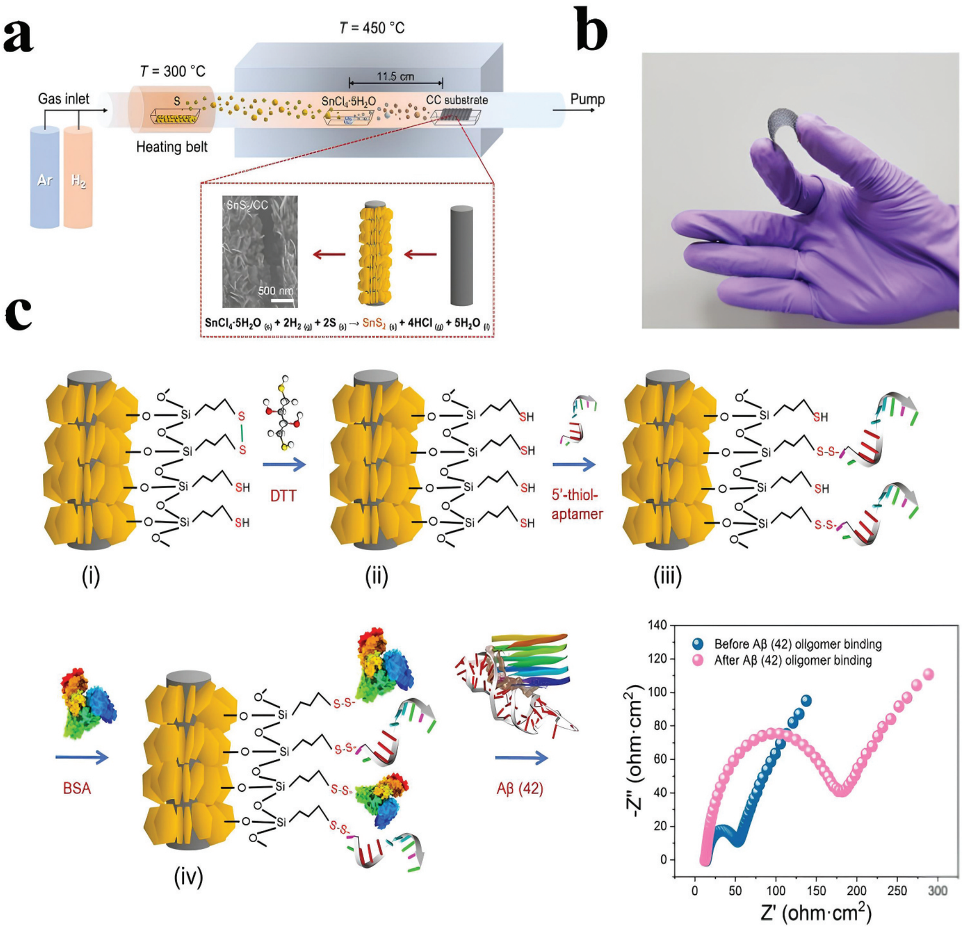

Figure 5. Graphic depiction of the biosensor creation process, including the synthetic pathway, chemical modification, and biosensor measurement [60] Reprinted from ref. [60] with permission.

approach is to modify the electrode surface by adding a thin layer of conductive or catalytic material, which increases the surface area and enhances electron transfer. Another method is to enhance the redox reaction kinetics by using catalysts to increase the reaction rate or by modifying the solution conditions, such as pH and temperature, to improve reaction kinetics. In addition, increasing the analyte concentration by pre-concentrating or enriching the sample prior to measurement can also amplify the analyte signal. Recently, scientists have used gold- or carbon-based nanomaterials,[64] an Ab-Apt sandwich assay,[65] MWCNT,[66] or integrated vertical graphene and AuNPs,[67] aimed at amplifying the signals of aptasensors targeting the Tau protein.

2.2.2. Prion diseases

Prion proteins play a critical role in numerous NDs, such as creutzfeldt-jakob disease and mad cow disease. In these conditions, abnormally shaped prion proteins can cause the normal form of the protein to change its conformation and aggregate, leading to damage and death of brain cells. Prion protein detection is therefore an important tool in the detection of these diseases, especially at the beginning when other symptoms may not yet be present.[68] This is usually done by testing tissue samples, such as brain biopsy or spinal fluid, for the presence of abnormal prion proteins. The accurate diagnosis of prion diseases is crucial for patient management, as well as for disease surveillance and control efforts.[69] The development of electrochemical aptasensors for the diagnosis of prion disorders has been slow throughout the past decade.

PAMAM (polyamidoamine) dendrimers are commonly utilized in the design of biosensors because of their special characteristics, which make them ideal for such applications.[70] Their small size and uniform structure allow for precise targeting of specific biological molecules, making them useful for the development of novel biosensors. Additionally, the high surface area-to-volume ratio of PAMAM dendrimers provides ample space for functionalization with recognition elements. Furthermore, PAMAM dendrimers are biocompatible, nontoxic, biodegradable, and can safely interact with biological systems, making them suitable for in vivo applications. Lastly, PAMAM dendrimers are versatile and can carry various functionalities, such as fluorescence or electroactive groups, and can be functionalized with multiple recognition elements, making them suitable for multiplexing and broadening their potential applications.[71]

Inspired by this, Miodek et al. reported two different immobilization methods using modified polypyrrole (PPy)-PAMAM,[72] and MWCNT–PAMAM conjugates [73] to develop voltammetric aptasensor based on prion proteins. Yu and groups put up an alternative strategy for building aptasensors. A labeled electrochemical platform was designed to control the interaction between two competitive redox probes. A composite of GCE modified with MWCNT\β-cyclodextrin was utilized.[73]

Current electrochemical aptasensor advancements for prion disease diagnosis include using novel materials and techniques. For example, researchers have developed aptamer-functionalized nanomaterials like carbon nanotubes, AuNPs and graphene, to enhance the sensitivity and selectivity of electrochemical aptasensors. These nanomaterials have large surface areas and unique electronic properties that could amplify electrochemical response and enhance binding efficiency of aptamer. Another development is the integration of microfluidics and electrochemical aptasensors, which enables fast and automated identification of prions in biological tissues. Microfluidics allows precise control and manipulation of fluids at the micrometer scale, which can improve the accuracy and reproducibility of electrochemical aptasensors.

2.2.3. Parkinson's disease

In recent years, Parkinson's disease (PD) has become a serious health concern, second only to AD among neurodegenerative disorders. PD is a progressive disorder of the nervous system that affects movement. It is triggered by the brain's loss of dopamine-producing cells.[74] Symptoms include tremors, stiffness, slow movements and balance difficulties. Although the exact cause of PD is unknown, a combination of genetic and environmental influences are thought to be involved.[75]

Currently, the diagnosis of PD is primarily dependent on symptoms such as tremors, bradykinesia, and rigidity. However, new advances in biosensor technology have opened up the possibility of developing objective markers for PD. Biosensors, which use biological molecules to detect specific biomarkers, can detect changes in biochemical processes associated with PD in real-time. For example, biosensors can detect changes in neurotransmitter levels or alterations in protein expression patterns, which are hallmark characteristics of PD. The use of biosensors for diagnosing PD can provide a more accurate and earlier diagnosis, which could lead to earlier initiation of treatments and improve patient outcomes.[76] One of the most studied biomarkers in the diagnosis of PD is alpha-synuclein.

2.2.3.1. Alpha-synuclein protein. Alpha-synuclein is a protein that is strongly linked with Parkinson's disease, a neurodegenerative disorder that affects movement control. α-synuclein is known to play a significant role in the development of PD because it is found in abnormally high amounts in the brains of people with the disease, and mutations in the α-synuclein gene have been associated with an enhanced risk of PD.[77]

Recently, electrochemical based aptasensors have been designed to detect α-synuclein with high sensitivity and specificity.[78,79] For example, some aptasensors have been improved for α-synuclein, including the RNA aptamer AS1411, DNA aptamer AS69, and the RNA aptamer S1.

The electrochemical aptasensors for α-synuclein detection have been developed based on several different transduction techniques, including electrochemical EIS, CV, and amperometry. For instance, a recent study by Wang et al. revealed the development of an electrochemical aptasensor for the detection of α-synuclein based on EIS. The aptasensor consisted of a gold electrode modified with graphene oxide and AS1411 aptamer. The sensor demonstrated high selectivity and sensitivity toward α-synuclein with a LOD of 0.15 pg/mL. Another study by Li et al. reported a novel electrochemical aptasensor for the detection of α-synuclein based on CV. The aptasensor was constructed by immobilizing the AS69 aptamer onto a gold electrode modified with polydopamine and gold nanoparticles. Although the sensor did not have high sensitivity with a LOD of 0.06 ng/mL, it showed high selectivity for α-synuclein.[80]

Furthermore, an electrochemical aptasensor based on amperometry for the detection of α-synuclein was reported by Zhang et al. The aptasensor was constructed by immobilizing the S1 aptamer onto a gold electrode modified with 3D nitrogen doped graphene. The sensor showed high sensitivity toward α-synuclein with a LOD of 0.07 pg/mL.[81]

Table 2 summarizes the design and analysis capabilities of the electrochemical aptasensors reported throughout this section. Differential pulse voltammetry has been the most frequently used technique. Use of nanomaterials has been a common trend across all types of aptasensors, significantly enhancing their performance in terms of sensitivity, specificity, and stability.

Table 2. Electrochemical based aptasensor studies for diagnosis of various diseases.

Disease	Biomarker	Sequence	Immobilization Strategy	Technique	Label free	Electrode	LOD	Linear range	Ref.
Alzheimer’s disease	AβO	5 -SH-GCCTGTGGTGTTGGGGCGGGTGCG- 3′	Aptamer–AuNFs/Cu-MOF	DPV	No	GCE	0.45 nM	1 nM – 2 mM	[58]
Alzheimer’s disease	AβO	Apt1: 5′ -AAAAAAAAAAGAGAGCCTGTGTTGGGGCGGGTGCG-3′ Apt2: 5′ -AGAGAGCCTGTGTTGGGGCGGGTGCGGTTATTAATGTGTGATGT-3′	AgNPs/HCR/APt$_2$/AβO/MCH/Apt1/GE	LSV, EIS	Yes	Gold electrode	430 fM	1 pM – 10 nM	[59]
Alzheimer’s disease	AβO	5′ SH-(CH$_2$)$_6$ TTTTTTGCCTGTGGTGTTGGGGCGGGTGCGTTTTTT-3′	AuNPs@CuMOF/SD	DPV	No	GCE	0.25 fM	0.5-500 fM	[61]
Alzheimer’s disease	AβO	5′ –NH$_2$–GCCTGTGTTGGGGCGGGTGCG-3′	Thionine – functionalized MWCNT	DPV	No	GCE	10 fM	0.0443–443.00 pM	[104]
Alzheimer’s disease	Tau	5′ ′ –NH$_2$-GCGGAGCGTGG CAGG-3′	Carboxyl graphene/thionin/gold nanoparticle	EIS, CV, DPV	Yes	GCE	0.70 pM	1.0 pM – 100 pM	[64]
Alzheimer’s disease	Tau	5′ - NH$_2$-GCGGAGCGTGGCAGG-3′	MPA modified electrode	DPV	No	Gold electrode	0.42 pM	0.5 pM – 100 pM	[65]
Alzheimer’s disease	Tau	5′ - NH$_2$-CGCCTCGCACCGTCC-3′	MWCNT	EIS	No	EIS electrode	1 fM	1 fM–1 nM	[66]
Alzheimer’s disease	Tau	5′ -SH-(CH$_2$)$_6$-CAGCACCGTCAACTGAATGGGTTGGCCGGGCAGCGGGGGGTAGGCTGGT GATGCGATGGAGATGT-3′	VG@AuNPs	DPV	No	Paper based electrode	0.034 pg/mL	0.1 pg/mL- 1 ng/mL	[67]
Prion disease	Prion protein	5′ -CGGTGGGGCAATTTCTCCTAC TGT dT15-3′ -Biotin	MWCNT-PAMAM	CV	No	Gold electrode	0.5 pM	1 pM –10 µM	[71]
Prion disease	Prion protein	5′-CGGTGGGGCAATTTCTCCTAC TGT dT15-3′-Biotin	Ppy-PAMAM	CV, DPV	No	Gold disc	0.8 pM	-	[72]
Parkinson’s disease	α-synuclein	5′-TTTTTGGTGGCTGGAGGGGGCGCGAACG-3′	CoMnZIF@CNF-based aptasensor	EIS	No	Gold electrode	45.7 fM	52.6 fM – 0.1 nM	[78]
Parkinson’s disease	α-synuclein	5′ -SH-(CH$_2$)$_6$-TTTTTGGTGGCTGGAGGGGGCGCGAACG-3′	–	EIS	Yes	Gold electrode	1 pM	2 µM – 1.4 nM	[105]
Parkinson’s disease	α-synuclein	5′ -SH-TTCCCGTTCGCGCCCCCTCC-3′	–	CV	Yes	SPGE	10 pM	60 pM – 150 nM	[106]

2.3. Colorectal and prostate cancer

Early cancer detection is crucial for effective treatment and better patient outcomes. When cancer is diagnosed early, the chances of successful treatment and recovery are higher. However, if the cancer is not detected until it has spread to other body parts, it becomes difficult to treat and can be life-threatening. Aptasensors are a promising technology for early cancer detection. When the aptamer binds to its target, it induces a signal that the sensor can detect.[82] Aptasensors can detect multiple cancer biomarkers simultaneously, providing a more comprehensive diagnosis, and can be presented as a powerful alternative to existing clinical diagnostic devices. This section discusses two common types of cancer.

2.3.1. Colorectal cancer

Carcinoembryonic antigen produced by certain tissues is a glycoprotein. In the case of colorectal cancer, breast, and lung cancer CEA is one of the most promising tumor indicators. While the CEA amount of 5.0 ng/mL is considered normal in healthy serum, the CEA level is present in abnormally high levels in the patient's serum.

A component of carbon compounds called graphene quantum dots (GQD), are in high demand for modifying electrodes in the development of electrochemical biosensors.[83,84] For the purpose of detecting CEA, some researchers created a brand-new aptasensor dependent on Pb^{2+} based DNAzyme-assisted signal amplification and GQD-IL-NF composite film.[85] GQD-IL-NF composite films, which have strong conductivity, great biocompatibility, and low toxicity, were used as the carrier for DNA immobilization through non-covalent- stacking interaction (Figure 6(b)). The developed aptasensor showed promise in clinical diagnosis, responding over a wide linear range 0.5 fg/mL-0.5 ng/ mL in serum samples.

In order to obtain low detection and quantization limitations, signal amplification and low noise are essential. With good redox properties, thionine is utilized as a mediator indicator system for electrochemical analysis. Inspired by this, the thiolated CEA aptasensor was prepared by immobilizing it on AuNP-deposited GCE.[86] In this study, the CEA aptamer, with 8 bases, was constructed as 5′-ATACCAGCTTATTCAATT-3′. A short nucleic acid sequence like this might be useful for inexpensively detecting CEA biomarkers (Figure 6(b)). In another similar study, an aptasensor based on thiolated methylene blue labeled hairpin DNA, and ferrocene modified aptamer DNA was developed. The technique showed a detection limit of 1.9 pg/mL.

As a hybrid nanocomposite, hemin functionalized reduced graphene oxide (Hemin-rGO) composites exhibit exceptional features dependent on good adsorption performance, high specific surface area and excellent catalytic activity when modified by metal nanoparticles. The sandwich type electrochemical aptasensor is gaining more and more attention, although there are some limitations. Xu et al. developed a novel sandwich-type aptasensor based on Hemin–rGO–AuNPs to detect CEA, capable of detecting a wide range of detections such as 100 fg/mL-100 ng/mL.[87]

Similarly, many sandwich-based aptasensors have been reported for determination of CEA, using the superior properties of various materials like nitrogen, quantum dots, platinum, gold [88–91]

2.3.2. Prostate cancer

Prostate-specific antigen is a protein produced by the prostate gland, and an elevated PSA level can be an indication of prostate cancer. Prostate cancer is one of the most prevalent types of cancer in men.[92] It is typically slow-growing and may not cause any symptoms in the early stages. However, as cancer progresses, it can cause symptoms such as difficulty urinating, blood in the urine or semen, and pain in the lower back, hips, or thighs.[93]

Recent advances in electrochemical aptasensors for PSA detection have focused on increasing the selectivity and sensitivity of sensors, as well as improving their simplicity, cost-effectiveness, and suitability for POC applications.

One approach to enhance the sensitivity of electrochemical aptasensors for PSA detection is to use signal amplification strategies.[94] For example, the researchers developed aptamer-functional AuNPs that can act as electrocatalysts to enhance the electrochemical signal of PSA binding. In this approach, AuNPs catalyze the reduction of H_2O_2 produced by the reaction between the aptamer and PSA, resulting in a higher electrochemical signal proportional to the PSA concentration.

Another strategy to increase the selectivity of electrochemical aptasensors for PSA detection is to use aptamer cocktails consisting of multiple aptamers targeting different regions of the PSA protein.[95] This may increase the specificity of the sensor by reducing the likelihood of false positive or false negative results due to differences in the structure or expression of PSA in different individuals.

In addition, studies have been carried out to develop electrochemical aptasensors suitable for POC applications.[96] For example, researchers have developed paper-based electrochemical aptasensors that can

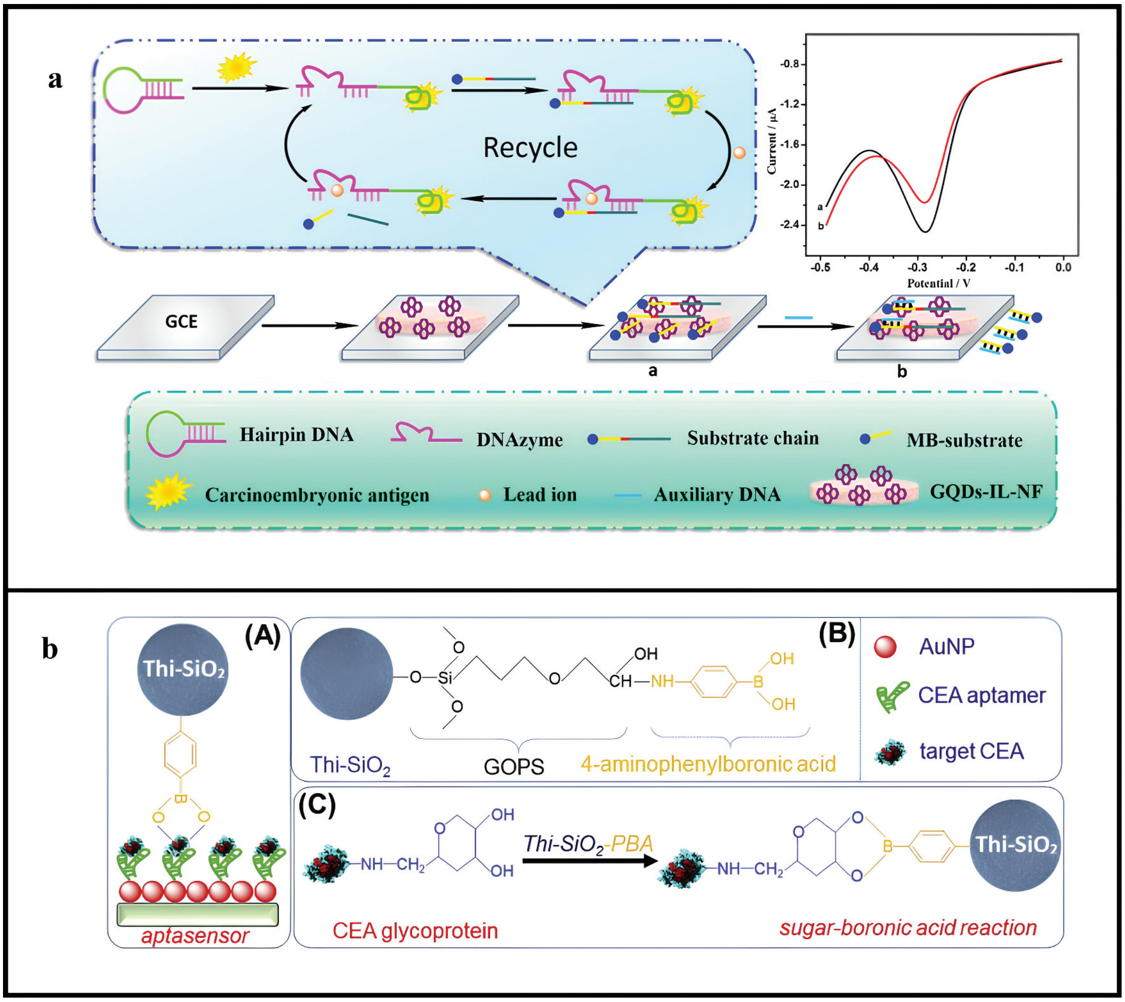

Figure 6. The schematic for the sensitive electrochemical detection of CEA using a GQD-IL-NF nano matrix and DNAzyme-assisted recycling of the target-aptamer complex.[85] Schematic representation of the electrochemical aptasensor platform capable of detecting CEA using thionine-doped nanometer-sized silica (Thi-SiO_2) [86] Reprinted from ref. [85,86] with permission.

be used for quantitative PSA detection in whole blood samples without requiring complex instrumentation or special training. These sensors use screen-printed electrodes and wax-printed paper, and results can be read using a smartphone camera and simple colorimetric analysis. For the detection of aptamer-based PSA, various components such as graphene oxidase [97,98] and AuNP [99] have been widely preferred to increase signal and sensitivity.

In conclusion, this section aims to highlight the main trends in this area, mostly by discussing the limited number of examples reported in the last few years. Table 3 summarizes the design and analysis capabilities of the electrochemical aptasensors reported throughout this section. In recent years, electrochemical aptasensor studies developed for the diagnosis of colorectal and prostate cancers have made significant progress in biomarker detection. In CEA aptasensors developed for colorectal cancer, extremely low detection limits have been achieved with EIS and DPV techniques using nanomaterials such as graphene quantum dots and AuNP-Thi-SiO2. In particular, these sensors immobilized on the GCE provide high sensitivity with wide linear ranges. At the same time, HRP-Cu3$(PO_4)_2$-HNF-AuNPs and Fc-Apt/hpDNA complexes show similar high performance as DPV and SWV techniques. In PSA aptasensors for prostate cancer, low detection limits and wide linear ranges were obtained with CV, SWV, EIS and DPV techniques by using nanohybrid materials and polymers such as Nafion/RGO-Au, Au/GO-nanohybrid and PANI/AuNPs. In general, these studies offer high sensitivity and specificity thanks to the combination of nanomaterial and electrochemical techniques, but have some limitations due to factors such as production and usage difficulties and cost. These shortcomings need to be addressed and further research needs to be done before these sensors can be widely used in clinical applications.

Table 3. Electrochemical based aptasensor studies for diagnosis of colorectal and prostate diseases.

Disease	Biomarker	Sequence	Immobilization Strategy	Technique	Label free	Electrode	LOD	Linear range	Ref.
Colorectal cancer	CEA	Hairpin DNA: 5′ - CATCTCTTCTCC GAGCCGGTGAA ATAGTGAGTATA CCAGCTTATTCAATTA AGAGATG-3′ Substrate Chain: 5′ - ACTCACTATA GGAAGAGATG-MB-3′	Graphene quantum dots	EIS	No	Carbon electrode	0.34 fg /mL	0.5 fg/mL- 0.5 ng/mL	[84]
Colorectal cancer	CEA	5′ –SH–$(CH_2)_6$-ATACCAGCTTATTCAATT-3′	AuNP-Thi-SiO_2	EIS	No	GCE	0.49 pg/mL	1.0 pg/mL- 10 ng/mL	[86]
Colorectal cancer	CEA	Apt1: 5′ -SH-$(CH_2)_6$-ATACCAGCT TATTCAATT-3′ Apt2: 5′ -NH2-$(CH_2)_6$-AGGGGGTGA AGGGATACCC-3′	HRP-Cu $_3$ (PO $_4$$^)$ ^{2} $^-$HNF-AuNPs	DPV	No	GCE	29 fg/mL	100 fg/mL-100 ng/mL	[107]
Colorectal cancer	CEA	5′ -MB-CGTGTATGCGTTGCACAATTTTTTTTTTAATTGAAT AAGCCGCATACACG–(SH)-3′ 5′ -Fc-ATACCAGCTTATTCAATT-3′	Fc-Apt/hpDNA complex	SWV	No	Gold electrode	1.9 pg/mL	10 pg/mL-100 ng/mL	[108]
Prostate cancer	PSA	5′ -SH-TTTTTTTTTTATTAAAGCTCG CCATCAAATAGCTGC-3′	Nafion/RGO-Au	CV, SWV, EIS	Yes	GCE	50 pg/mL	-	[98]
Prostate cancer	PSA	5′ -SH-TTTTTTTTTTATTAAAGCTCGCCATCAAATAGCTGC-3′	Au/GO-nanohybrid	CV	No	GCE	0.007 ng/mL	-	[97]
Prostate cancer	PSA	5′ -NH_2-TTTTTAATTAAAGCTCGC CATCAAATAGCTTT-3′	PANI/AuNPs	DPV	No	ITO	0.085 pg/mL	0.1 pg/mL-100 ng/ mL	[109]

3. Conclusion and future prospects

Although many electrochemical biosensors are under development and testing, some of these biosensors have reached the stage of commercial use. Thanks to today's technological developments, they are used in various fields. Due to the public's interest in human health, rapid diagnosis of diseases is of particular importance. The development of aptasensors for the most common cancer markers is a promising area of research. Electrochemical aptasensors typically target a biomarker. A sensor system targeting several indicators will increase disease diagnostic accuracy. This improvement also reduces diagnostic time and expense.

Biotin, amine, carboxyl and thiol are functional groups that interact with aptamers. The most frequently used functional group in aptasensor development was thiol, while the least preferred carboxyl group was. As a result, aptamer array contacts are predominantly formed with gold and nanomaterials, which can be tightly formed via Au-S covalent bonds. Such strong and stable bonds provide conformational flexibility for analyte identification. Aptamer-modified Au surfaces have been an attractive platform for electrochemical research due to the ease of preparation of recognition layers and the availability of pre-synthesized probes. To design an aptasensor with selectivity and sensitivity, it is important to apply appropriate signal amplification strategies in the detection platform. A wide variety of nanomaterials (polymers, metal oxides, nanocomposites, carbon materials, precious metals) are preferred in the design of electrochemical aptasensors from past to present. The use of functional nanomaterials such as metal oxide nanomaterials, polymer nanomaterials, graphene, carbon nanomaterials or gold nanomaterials as surface modifiers improves the binding and stability of these biological molecules. The extremely large surface area of these nanostructures also accelerates the movement of electrons, making detection more efficient. By immobilizing these biological receptors on the nanomaterial-modified surface, it allows target molecules in biological fluids to bind and subsequently be identified through changes in potential, resistance, and current.

Electrochemical biosensors described to date for the determination of biomarkers mainly include label-free, label-based, competitive and sandwich configuration approaches. Single aptamer receptors are also referred to as aptamer/biomarker/aptamer, aptamer/biomarker/antibody and antibody/biomarker/aptamer.

There are several commonly applied ways to improve the detection sensitivity of electrochemical sensors. One approach is to modify the electrode surface by adding a thin layer of conductive or catalytic material that increases the surface area and increases electron transfer. Another method is to improve redox reaction kinetics by using catalysts to increase the reaction rate or by changing solution conditions such as temperature and pH to improve reaction kinetics. In addition, increasing the analyte concentration by preconcentrating or enriching the sample prior to measurement can also amplify the analyte signal. Immobilization of aptamers should result in a receptor layer that is robust and reproducible, sensitive to the analyte, and inhibits non-specific adsorption of the matrix component. The interface behaviors, states, binding processes and complex structures of each new aptamer-protein pair should be extensively analyzed by researchers.

Despite extensive research in the past and present in the development of electrochemical biosensors, there are still problems in producing more reliable and advanced devices. They can be affected by temperature, pH and other environmental factors that can affect their accuracy. All three types of biosensors have disadvantages and advantages depending on the application. Amperometric biosensors are sensitive, but may be prone to interference by other species in the sample and require frequent calibration. Potentiometric biosensors are relatively insensitive, but very selective and can be used to measure ions in complex samples. Conductometric biosensors are relatively insensitive, but easy to manufacture, relatively inexpensive, and can be used to detect a wide variety of biomolecules. They also require frequent calibration and may require special transport and storage conditions to maintain their precision and stability.

As a result, aptasensors, which have many advantages over conventional antibodies, including inexpensive cost, high stability and specificity, are poised to take leading roles in the field of biomarker detection in the near future. Aptasensors with such advantages should be more widely integrated into clinical practice.

Abbreviations

SELEX	systematic evolution of ligands by exponential enrichment
ELONA	enzyme-linked oligonucleotide assays
ELISA	enzyme linked immunosorbent assay
CRP	c-reactive protein
cTns	cardiac troponins
CK-MB	creatine kinase-MB
Mb	myoglobin
ALD	atomic layer deposition
AuNPs	gold nanoparticles
MoS_2	molybdenum disulfide
GCE	glassy carbon electrode
BNNSs	boron nitride nanolayers

AD	alzheimer's disease
CSF	cerebrospinal fluid
Aβ	amyloid-beta
PAMAM	polyamidoamine
PD	parkinson's disease
CEA	carcinoembryonic antigen
GQD	graphene quantum dots
PSA	prostate-specific antigen

Disclosure statement

No potential conflict of interest was reported by the author(s).

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