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Aptamer-based biosensor: a promising platform for the detection of pathogenic microbes

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ABSTRACT: Aptamers are short single-stranded artificial DNA or RNA nucleotide sequences. They can be developed by systematic evolution of ligands by exponential enrichment (SELEX) *in vitro* or generated *in-silico* using computational approaches such as molecular docking and molecular dynamics (MD) simulations. These aptamers recognise and bind numerous specific targets with high sensitivity including pathogenic microbes and their biomarkers. Due to their unique properties, they serve as excellent recognition elements in biosensors and enable the detection of a variety of molecules. In this review, the principles of aptamer-based biosensing along with various available aptamer sensor platforms and their applications in the detection of pathogenic microbes were discussed using specific examples. It also explores the integration of aptamers with various transduction techniques such as electrochemical, electrical, surface-enhanced Raman scattering (SERS) and surface plasmon resonance (SPR) methods among others. In addition, recent advances and developments of aptamer-based biosensors were highlighted, such as multiplex detection, CRISPR associated (CRISPR/Cas) system, as well as development of point-of-care testing (POCT) devices. Furthermore, the strengths and limitations of this detection method and its feature prospects as a diagnostic method for infectious disease pathogens of public health significance are described.

KEYWORDS: aptamer, biosensors, pathogen detection, sensor platform

INTRODUCTION

In recent decades, the rapid and accurate detection of pathogenic microbes has become increasingly vital in various fields including healthcare, food safety, and environmental monitoring [1–3]. Early detection and accurate diagnosis of infectious pathogens are essential for controlling their spread and treating patients effectively [4]. Common detection methods such as culture-based techniques, Enzyme-linked Immunosorbent Assay (ELISA), Polymerase Chain Reactions (PCR) and sequencing are often laborious and require time-consuming procedures and specialised equipment, leading to delays in diagnosis [5]. Consequently, there is a pressing need for alternative detection platforms to combat the increasing threat of infectious diseases. As a result, aptamer-based biosensors have emerged as a promising solution to address these challenges.

Aptamers are synthetic oligonucleotides that can be designed to bind specific targets with high affinity [6] and these target molecules may include pathogenic microbes and/or their biomarkers [7]. Advances in nanotechnology and signal transduction techniques have enabled aptamer-based biosensors to offer exceptional sensitivity, selectivity, and portability, making them an attractive platform for pathogen detection [8].

Several aptamer-based sensors have been developed, which include but are not limited to electrochemical sensors, lateral flows, SERS and fluorescence sensors, among others. These sensors have proven to be effective in rapidly detecting a wide range of microorganisms and their biomarkers, which include pathogenic microbes [9–11].

While several reviews have reported the detection of pathogens using aptamer-based biosensors [10–12], most of these studies focus primarily on bacterial detection because bacteria represent the most common group of microorganisms associated with diseases in humans, animals, and plants [13]. However, other pathogen groups, including fungi, parasites, and viruses, are also of significant public health concern as they cause a wide range of infectious diseases [14–16]. Therefore, this review discusses the application of aptamer-based biosensors for detecting a broad spectrum of pathogens, including bacteria, fungi, parasites, and viruses. In addition to describing the principles of aptamer selection and design, the review is primarily organised according to biosensor transduction mechanisms, while examples within each platform highlight the different pathogen types and molecular targets detected (e.g., whole cells, surface proteins, toxins, and nucleic acids). Furthermore, recent advances,

challenges, and future perspectives in sensor fabrication and detection strategies are discussed.

To ensure that this review reflects recent developments in the field, relevant literatures were primarily sourced from major scientific databases including Scopus, Web of Science and PubMed. Emphasis was placed on studies published within the last five years (2020–2025), particularly those reporting innovative aptamer-based biosensing strategies and real-sample applications. Earlier foundational studies were included where necessary to provide background context. Articles were selected based on their relevance to pathogen detection, type of biosensing platform, analytical performance, and applicability to real-world diagnostics.

APTAMERS

Aptamers, first discovered in 1990 [17], are short single-stranded nucleic acid sequences that can effectively bind to several target molecules, including proteins, bacterial cells, viruses, cancer cells, and even tissues [3, 18]. Aptamers are deliberately designed oligonucleotide sequences that differ from naturally occurring ligands. They possess the unique ability to selectively recognise and bind to different targets through a combination of molecular interactions such as van der Waals forces, hydrogen bonding, and electrostatic interactions [19]. Most aptamers are screened and obtained *in vitro* by SELEX, which can fold into secondary and three-dimensional conformations from random sequence nucleic acid pools. This enables the generation of DNA aptamers that recognise proteins by applying a DNA library to an affinity column containing the protein target [20]. They can also be alternatively designed *in silico* by using computational algorithms such as molecular docking and MD simulations to predict and optimise sequences capable of recognising and binding to a particular target molecule. These simplify the selection process and enhance their efficacy [21].

Aptamers are often considered as "chemical antibodies" due to their biomimetic properties and simple synthetic processes. They exhibit high molecular interactions towards their targets, with dissociation constants (Kd) typically in the pico to nanomolar range [17, 22]. Compared to antibodies, aptamers have advantageous properties in several respects, including *in vitro* production for broader ligand binding, low-cost, modification flexibility, non-immunogenicity for *in vivo* applications and high stability under harsh conditions [7, 20, 21]. These collective properties make aptamers valuable tools that can be used in a wide range of fields, particularly in the detection of pathogens and offer significant potential for the development of advanced sensing platforms.

APTAMER-BASED BIOSENSORS

A biosensor typically comprises two fundamental components; a recognition element and a signal transducer. The recognition element can be an antibody, antigens, nucleic acids, enzymes or aptamers [22]. Meanwhile, the signal transducer utilises various detection methods such as fluorescence, electrochemistry, colourimetry or chemiluminescence to convert the biological response into a measurable signal, providing a diagnostic indicator tailored to specific applications and sensitivity requirements [23].

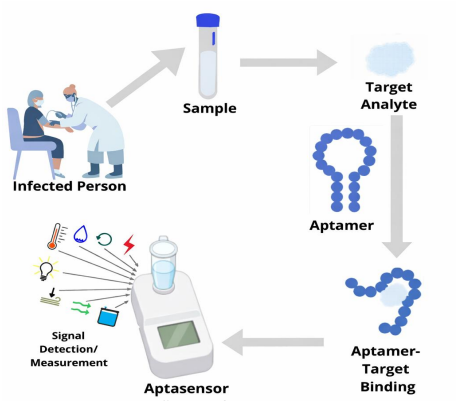

Fig. 1 A typical workflow of aptamer-based biosensors for pathogen detection from clinical sample.

Aptamers, known for their remarkable capability to interact with diverse targets, ensure accurate detection even at low concentrations [24]. Their unique binding properties make them highly effective recognition elements in biosensors, supporting various applications in medical diagnostics, therapeutics, environmental monitoring, and food safety [23, 25, 26]. Biosensors incorporating aptamers as biological recognition elements are referred to as aptasensors. In a typical aptasensor-based assay, clinical samples are collected from infected individuals and may contain the target pathogen. The specific aptamer recognises and binds to the pathogen, if present. This binding event is then transduced into a measurable signal through suitable detection platforms, such as electrochemical, SPR or fluorescence among others, thereby enabling rapid, sensitive, and accurate pathogen identification (Fig. 1) [23, 27].

APTAMER BIOSENSOR PLATFORMS

Aptamer-based biosensors are emerging as promising platforms for pathogen detection due to their efficiency, broad applicability, and cost-effectiveness. In these systems, aptamers act as recognition elements and are integrated with different sensor platforms to specifically bind microbial targets. The resulting binding event is converted into a measurable signal through various transduction mechanisms.

In this review, aptamer biosensor platforms are primarily categorised based on their signal transduction principles, which determine how the aptamer–target interaction is translated into a detectable signal. These

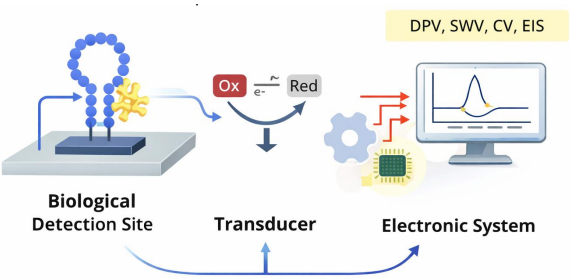

Fig. 2 Schematic illustration of a typical electrochemical aptasensor platform for target detection.

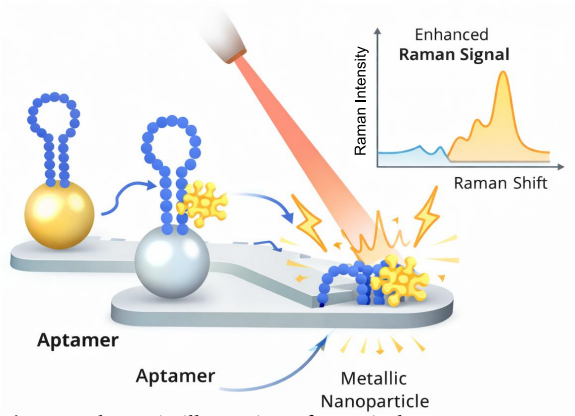

Fig. 3 Schematic illustration of a typical SERS aptasensor platform for target detection.

platforms include electrochemical, SERS, fluorescence, SPR, electrical sensing strategies as well as enzyme-mediated colorimetric assays such as enzyme-linked oligonucleotide assay (ELONA). In addition, device-oriented formats such as lateral flow systems are also discussed, as they represent widely used point-of-care (POC) implementations that typically integrate optical signal detection within a portable assay format.

Accordingly, the major platforms discussed in this section include:

Electrochemical platform

This platform in general, measures the changes in electrical properties (current, voltage, impedance) when the aptamer is bound to the target. It usually consists of a biological detection site, a transducer, and an electronic system that includes a signal amplifier, a processor, and a display. It detects a target analyte by using a selective biological element to recognise a biological process at its interface and converting it into a detectable electrochemical signal that is quantitative or semi-quantitative [28]. These signals can be detected by differential pulse voltammetry (DPV), square wave voltammetry (SWV), cyclic voltammetry (CV) or electrochemical impedance spectroscopy (EIS) (Fig. 2) [23].

Electrochemical biosensors are cheaper and more feasible for in-field identification requiring minimal reagents [28]. This is possibly why they are the most widely used, as various microbial pathogens have been detected using this platform. For instance, the antigen of the Chikungunya virus (CHIKV) was detected using an electrochemical aptamer sensor based on a flexible substrate using zinc oxide (ZnO) with an estimated detection limit of 1 ng/ml [29]. In another study [30], dengue virus non-structural protein 1 (NS1) was also detected with an electrochemical aptasensor using AuNPs aggregated with polyethylenimine with a detection limit of 0.3 ng/ml. The platform was also used for the detection of bacterial pathogens in various food samples. These include *Listeria monocytogenes* with a detection limit of 5 CFU/ml [31], *Shigella dysenteriae* with a sensitivity of 8.09 CFU/mLl [31] and *Salmonella typhimurium* with detection limits of 3 CFU/ml [32], 10.01 CFU/ml [33] and 3.50 CFU/ml [34]. Fungi and protozoa whose routine detection is laborious and expensive were also detected using the electrochemical sensing platform. Aflatoxin M1 from *Aspergillus spp*. was detected in milk using DNA-aptamer recognition and EIS with a detection limit of 1.15 ng/l [14]. *Plasmodium falciparum* lactate dehydrogenase [35] and *Giardia intestinalis* trophozoite protein [36] were also detected electrochemically with a detection limit of 50 parasites/µl and 0.35 pg/ml, respectively.

Despite their high sensitivity, low cost and suitability for point-of-care applications, electrochemical aptasensors may face challenges such as electrode fouling, non-specific adsorption particularly when analysing complex biological samples and issues with reproducibility due to surface modification variability. Improving sensor stability, surface chemistry and robustness remain essential for reliable real-world application.

SERS platform

SERs aptamer sensors for pathogen detection leverage the unique enhancement of Raman signals by metallic nanostructures [37]. In general, aptamers are immobilised on these nanostructures (e.g. gold or silver nanoparticles). When a specific target binds to the aptamer, it brings the target molecule close to the metal surface, amplifying its Raman signal due to the localised SPR [38]. This results in a significant increase in Raman scattering intensity and enables the detection of the pathogen [39] (Fig. 3). Several pathogens have already been detected with the SERS aptasensor.

A study by Kukushkin et al [40] reported the development of a highly sensitive SERS aptasensor using a pair of aptamers in a sandwich format. This sensor successfully detected influenza virus with a detection limit of 1×10^{-4} hemagglutination units per probe. Gu et al [41] also achieved the detection of SARS-CoV-2 using aptamers-gold nanoparticles (SCAP) SERS

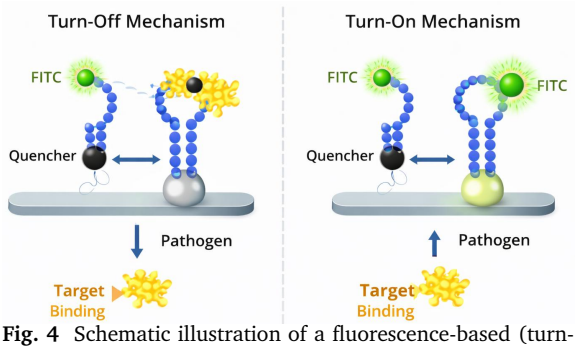

Fig. 4 Schematic illustration of a fluorescence-based (turn-on and turn-off mechanisms) aptasensor platform for target detection.

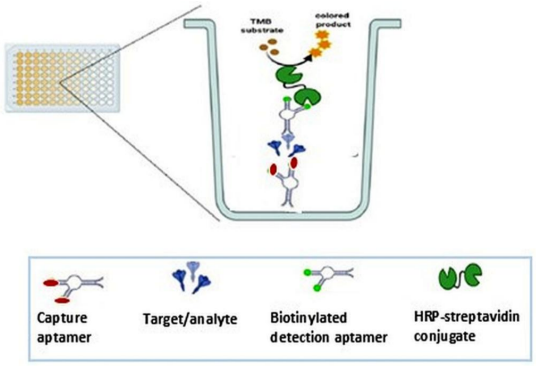

Fig. 5 Schematic illustration of a typical sandwich ELONA aptasensor platform for target detection.

substrate with a detection limit of 0.8 transduction unit/ml. It was also used for the detection of *Klebsiella pneumoniae* [25], *Escherichia coli* [39] as well as aflatoxin B1 from *Aspergillus* spp. [43] with detection limits of 10 cells/ml, 5.9×10^3 CFU/ml and 3.6 pg/ml, respectively.

SERS-based aptasensors offer ultra-high sensitivity and specificity due to plasmonic signal enhancement. However, their practical application is constrained by the complexity and cost of fabricating reproducible metallic nanostructures. Signal variability arising from substrate heterogeneity and inconsistent hotspot formation can also affect analytical reliability. These challenges may limit routine clinical implementation and large-scale commercialisation without further standardisation and cost reduction.

Fluorescence platform

A fluorescent aptamer sensor for pathogen detection utilises the selective binding properties of aptamers and the effectiveness of fluorescence detection. Normally, aptamers are labelled with fluorescent dyes such as fluorescein (FITC), rhodamine, Cyanine 3 and Cyanine 5 so that a fluorescent donor and a quencher are conjugated to both ends of the aptamer. When the target molecule is present in a sample, it binds to the aptamer and causes a conformational change in the aptamer structure [44]. This change can either bring the fluorescent dye close to a quencher molecule, reducing fluorescence (turn-off mechanism), or separate them, increasing fluorescence (turn-on mechanism). The resulting change in fluorescence intensity correlates directly with the presence and concentration of the target molecule, enabling detection and quantification of the target (Fig. 4) [45].

Many studies have used fluorescent aptasensors in detecting various pathogens. For example, Wang et al [46] recently used a fluorogenic RNA aptamer for molecular detection of as low as 4 copies of Monkeypox virus (MPV) DNA. A fluorescence microplate-based assay was also used to detect 5 oocysts of *Cryptosporidium parvum* in 300 µl of wastewater [26]. In addition, another fluorescent aptasensors were employed to detect *Vibrio cholerae O139* [44], *S. typhimurium* [47] and aflatoxin B1 from *Aspergillus* spp. [48] with detection limits of 426 CFU/ml, 2.37×10^3 CFU/ml and 0.35 ng/m, respectively.

Fluorescence-based aptasensors provide high sensitivity and real-time detection with relatively simple signal readout. However, their performance may be affected by photobleaching, background fluorescence interference, and complex sample matrices. The need for fluorescent labelling and quencher systems may also increase assay complexity and cost, potentially limiting applicability especially in resource-limited or POC settings.

ELONA platform

Usually, the aptamer-based ELONA biosensor can be carried out in direct or sandwich form like the usual ELISA. In sandwich ELONA, an immobilised captured aptamer is used to specifically bind/capture an analyte or antigen in a sample. A detection aptamer labelled with biotin then detects the target. A reporter enzyme such as horseradish peroxidase (HRP) or alkaline phosphatase conjugated with streptavidin is then added. Upon addition of a substrate such as ABTS or TMB, the enzyme generates a detectable signal that enables quantitative measurement of the target or analyte on a multi-well microplate (Fig. 5) [49]. It is the most commonly used ELONA technique and has been used to detect the Zika virus NS1 protein with a detection limit of 0.1 ng/ml [50], the Toxoplasma ROP18 protein with a detection limit of 1.56 µg/ml [10] and the SARS-CoV-2 spike protein from infected nasopharyngeal samples with a sensitivity of 80% [16].

In direct ELONA, however, a target molecule is directly immobilised on a surface and a single aptamer conjugated to a reporter enzyme such as HRP binds directly to the target. Upon addition of substrate (TMB), the enzyme catalyses a reaction and generates a measurable signal [51]. This technique has only been used

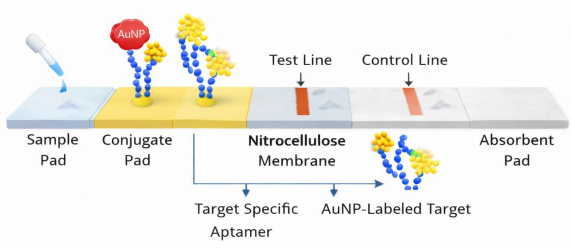

Fig. 6 Schematic illustration of a typical lateral flow aptasensor platform for target detection.

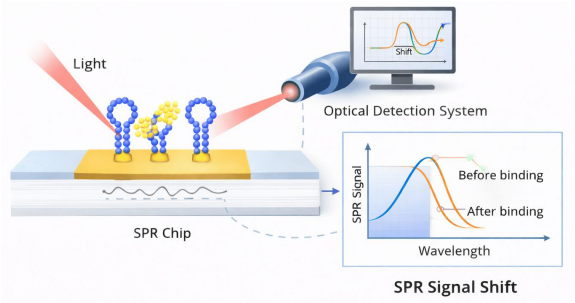

Fig. 7 Schematic illustration of a typical SPR aptasensor platform for target detection.

in a few studies to detect pathogenic microorganisms. This could be due to the fact that coating real samples onto microplates is always challenging due to their complex and heterogeneous nature, which can lead to uneven immobilisation and non-specific binding, ultimately reducing signal accuracy [52]. However, one of the few detected targets using this technique is the SARS-CoV-2 spike protein from clinical samples with a sensitivity of 91% [53].

No doubt, ELONA-based aptasensors provide high specificity and quantitative detection using well-established assay formats. However, the multi-step procedure, including several washing steps and reliance on enzyme labelling, increases assay time and complexity. In addition, non-specific binding and challenges associated with immobilising targets from complex samples in direct ELONA may affect accuracy. These limitations reduce their suitability for rapid and POC applications.

Lateral flow platform

The aptamer sensor for POCT detects pathogens using the lateral flow immunoassay principle in general with aptamers designed for specific target recognition. The device consists of a sample pad, a conjugate pad, a nitrocellulose membrane, and an absorbent pad [29]. Target-specific aptamers on the test and control lines of the membrane capture pathogens from the sample. If present, the pathogens bind to the labelled aptamers on the conjugate pad and form a complex that generates a visible signal on the test line. The control line captures excess conjugate, ensuring proper flow, and functionality (Fig. 6) [54]. This platform is simple, rapid, and user-friendly enabling effective pathogen detection at the POC.

Aptamer lateral flows are used for pathogen detection due to their high specificity, stability, and ease of production. For example, in the detection of SARS-CoV-2, a pair of aptamers that bind to different sites on the S protein were used in an aptamer sandwich Lateral Flow Assay (LFA) that detected the SARS-CoV-2 virus at concentrations as low as 10^6 copies/ml [55] and with a limit of detection of 51.81 ng/ml [56]. The avian influenza virus H5N2, was also detected using the same approach, with a detection limit of 2.09×10^5 EID_{50}/ml [57]. In another development, an aptamer-based lateral flow test strip was developed and used for the simultaneous detection of *S. typhimurium*, *E. coli* and *Staphylococcus aureus* with a visual detection limit of 10^3 CFU/ml, 10^4 CFU/ml and 10^4 CFU/ml, respectively, in just 10 min [58]. In addition, Dirkzwager et al [59] also detected the malaria parasite biomarker *P. falciparum* lactate dehydrogenase (PfLDH) with a limit of 2 ng/ml using the aptamer-based POCT they developed.

Unlike most other platforms, the lateral flow aptasensors offer rapid, user-friendly, and cost-effective detection, making them highly suitable for POC applications and use in resource limited settings. However, their sensitivity is often lower than that of laboratory-based methods, and quantitative analysis can be challenging. In addition, limited multiplexing ability and variability in visual signal interpretation may affect reliability. These constraints can restrict their application in settings requiring high sensitivity and precise quantification.

SPR Platform

SPR-based aptamer biosensors detect targets by monitoring changes in the refractive index at the metal-dielectric interface that occur when aptamer-target binding takes place on the sensor surface. In this platform, an aptamer specific to the target analyte is immobilised on an SPR chip, typically coated with a thin metal layer such as gold. Upon interaction between the aptamer and its target, a measurable shift in the SPR signal is generated due to changes in the local refractive index near the sensor surface [60]. These changes are recorded by the optical detection system and enable sensitive and real-time monitoring of the presence and concentration of pathogens in the sample (Fig. 7) [61].

SPR-based aptasensors have been successfully applied for the detection of various pathogenic microbes and their biomarkers. For instance, Luo et al [62] developed an SPR-based sensor using gold nanoparticles functionalised with a T-shaped aptamer for the

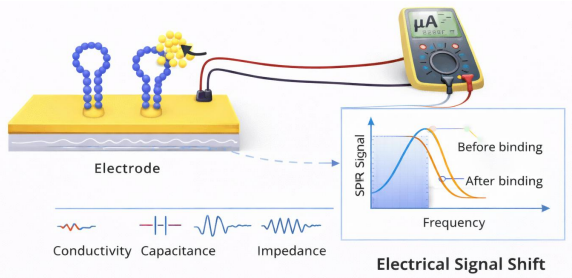

Fig. 8 Schematic illustration of a typical electrical aptasensor platform for target detection.

detection of the N protein of SARS-CoV-2 from clinical samples, achieving a sensitivity of 92.3%. Similarly, SPR aptasensors have been used for the detection of *Helicobacter pylori* [61], *P. falciparum* PfLDH [63] and ochratoxin A (OTA) produced by *Aspergillus* spp. and *Penicillium* spp. [64], with detection limits of 45 CFU/ml, 57 pg/μl and 0.01 ng/ml, respectively.

SPR-based aptasensors offer several advantages, including label-free detection, high sensitivity, and the ability to monitor biomolecular interactions in real time. However, their performance can be influenced by environmental factors such as temperature fluctuations and variations in refractive index. In addition, the requirement for sophisticated instrumentation and technical expertise may increase operational costs and limit portability, which can restrict their applicability in POC diagnostic settings.

Electrical platform

Electrical aptamer sensors in broad terms detect pathogens by measuring changes in electrical properties when a target analyte binds to an aptamer immobilised on an electrode. The specific binding event alters the electrical properties of the sensor, such as conductivity, capacitance or impedance [65]. These changes are monitored by electrical measurement methods such as impedance spectroscopy or conductivity measurements (Fig. 8). The magnitude of the electrical signal shift correlates with the presence and concentration of the target analyte and enables its detection and quantification (Fig. 8) [60].

Despite their advantages in terms of label-free and real-time detection abilities, electrical aptasensors are less commonly used for pathogen detection due to challenges with signal interpretation, interference from complex samples and the need for specialised technical expertise and equipment [61]. Nevertheless, an electrical aptasensor was used to detect the Zika virus from human serum with a detection limit of 38.14 pM [66]. In addition, another electrical aptasensor targeting the S protein of SARS-CoV-2 was also used to detect up to 100 copies/μl of the virus in clinical samples [67].

In spite of the increasing application of aptamer-based biosensors for the detection of a wide range of targets, there are limited studies utilising electrical aptasensors for the detection of other microbial groups, such as bacteria, fungi, and parasites. This highlights a significant gap in the field and calls for further research to expand the utility of electrical aptamer-based biosensors for the detection of diverse pathogenic microbes beyond viruses.

Other platforms

Aptamer-based biosensors for the detection of pathogens are the focus of research due to their efficiency, broad applicability, and cost-effectiveness. While common platforms such as electrochemical, SERS, fluorescence, ELONA, lateral flow, and SPR platforms are widely used, other platforms such as magnetic, thermal, field effect, and photonic crystal sensors are less common. However, they offer unique advantages.

Magnetic sensors, for instance, enable the rapid separation and concentration of target pathogens from complex samples enhancing detection sensitivity. They have already been used to detect up to 1×10^2 CFU/ml *E. coli* from wastewater [68]. Thermal sensors can measure minute temperature changes resulting from the interaction between aptamer and target, providing a label-free detection method that has also been used to detect up to 180 CFU/ml *E. coli* [69]. Field effect transistors offer high sensitivity and real-time detection by monitoring changes in electrical charge and have been used to detect *P. falciparum* PfGDH with a detection limit of 48.6 pM [70]. Photonic crystal sensors on the other hand can achieve highly sensitive detection through changes in light diffraction patterns and have been used to detect SARS-CoV-2 in saliva samples by targeting the consensus receptor-binding domain of all variants of concern of the virus with a detection limit of 12.7 ng/ml [71]. These platforms could be adopted in specific applications where their unique properties can improve the sensitivity, specificity and robustness of pathogen detection.

To enhance clarity and enable direct comparison among the major aptamer-based biosensing platforms, a summary of their detection principles, advantages, limitations, and POC suitability is provided in Table 1.

RECENT ADVANCES IN APTAMER-BASED BIOSENSORS

Aptamer-based biosensors have made remarkable strides in the detection of pathogens, providing accurate and reliable performance as well as rapid response times. These advances are particularly crucial in addressing global health challenges, especially in the context of emerging infectious diseases. Here are some notable recent advances and their specific relevance in pathogen detection:

Table 1 Comparison of the main Aptamer-Based Biosensing Platforms for Pathogen Detection.

Number	Platform	Principle of Detection	Key Advantages	Major Limitations	POC Suitability
1	Electrochemical	Changes in electrical signals (current, voltage, impedance) upon aptamer–target binding at electrode surface [28].	High sensitivity, low detection limits, cost-effective, easy miniaturisation [28].	Electrode fouling, surface instability, interference from complex samples [32, 34]	Highly suitable [28].
2	SERS	Enhancement of Raman signals via localised SPR when target-bound aptamers bring analytes close to metallic nanoparticles [39, 40].	Ultra-high sensitivity, molecular fingerprint specificity, multiplexing capability, low detection limits [37].	Requires precise nanostructure fabrication, signal variability, expensive instrumentation; complex data interpretation [42, 43].	Moderate [40].
3	Fluorescence	Variation in fluorescence intensity following aptamer–target interaction [44].	High sensitivity, real-time detection, simple signal readout [45, 47].	Photobleaching, background fluorescence, labelling complexity, higher cost [46].	Moderate [26].
4	ELONA	Enzyme-mediated colorimetric signal generated after aptamer–target binding and substrate conversion [49].	High specificity, quantitative detection, well-established assay format [49].	Multi-step procedure, time-consuming, enzyme labelling dependence, non-specific binding [10, 53].	Limited suitability [49].
5	Lateral Flow	Capillary-driven migration with visual signal generation (colorimetric/nanoparticle-based) upon target binding [54].	Rapid, user-friendly, portable, low cost [56, 59].	Lower sensitivity, only qualitative results, limited multiplexing [54].	Highly suitable [55].
6	SPR	Changes in optical properties such as refractive index, absorbance, or plasmon resonance after aptamer–target binding [60].	Label-free detection, real-time monitoring, high sensitivity via optical signal amplification [60].	Expensive instrumentation, environmental sensitivity, limited portability [62, 63].	Moderate to limited suitability [61].
7	Electrical	Changes in electrical properties (e.g., conductivity, impedance, capacitance) upon target binding to aptamer-functionalised electrodes [65].	Label-free detection, rapid response, simple instrumentation, easy miniaturisation, low cost [60].	Non-specific signals, surface fouling, interference from complex samples [66].	Highly suitable [67].

SERS: Surface-enhanced Raman Scattering, ELONA: Enzyme-Linked Oligonucleotide Assay, SPR: Surface Plasmon Resonance.

Multiplex detection

One of the most important recent developments in aptamer-based biosensors is the emergence of multiplex detection systems capable of simultaneously detecting multiple pathogens or pathogen strains within a single assay. Multiplex capability is typically achieved through device design strategies that incorporate multiple sensing regions or transducers, each functionalised with a distinct target-specific aptamer [58]. In such systems, different capture probes are immobilised on spatially separated sensing sites, such as individual electrodes in multi-electrode arrays, discrete test lines in lateral flow devices or microarray spots, allowing simultaneous and differential detection of multiple analytes within the same sample.

For example, da Silva et al [72] reported an aptasensor capable of simultaneously detecting Dengue and Zika viruses. Similarly, Tao et al [73] developed a multiplex aptasensor for the simultaneous detection and differentiation of *S. aureus*, *E. coli* and *S. typhimurium*, while Effah et al [25] reported multiplex detection of *Acinetobacter baumannii* and *K. pneumoniae*. Such multiplex capability allows rapid differentiation of infections with similar clinical symptoms and improves diagnostic efficiency.

Nanomaterials integration

Recent advances in aptamer-based biosensors have increasingly focused on the integration of emerging nanomaterials and advanced nanostructures that enhance sensitivity, signal amplification and analytical stability. Materials such as two-dimensional nanomaterials, metal-organic frameworks (MOFs) and hybrid nanocomposites provide high surface area, tunable surface chemistry and improved catalytic activity, enabling efficient aptamer immobilisation and enhanced signal transduction.

For example, a recent electrochemical aptasensor based on a black phosphorus/Ti_3C_2 MXene nanohybrid combined with magnetic covalent organic frameworks was developed for norovirus detection, achieving an ultralow detection limit of 0.003 copies/ml, demonstrating the strong signal-amplification capability of MXene-based nanostructures [74]. Similarly, MOF-based aptasensors have attracted attention due to their porous architecture and high aptamer loading capacity. A recently reported aptamer-functionalised $Ni_3(HITP)_2$ MOF field-effect transistor biosensor enabled sensitive detection of *Mycobacterium tuberculosis* biomarker with improved responsivity and reduced detection limits compared with conventional graphene-based sensors [75]. In another study, magnetic MOFs were used as signal-enhancing platforms for electrochemical aptasensors targeting the SARS-CoV-2 spike protein, demonstrating improved sensitivity and simplified sensor fabrication suitable for miniaturised diagnostic devices [76].

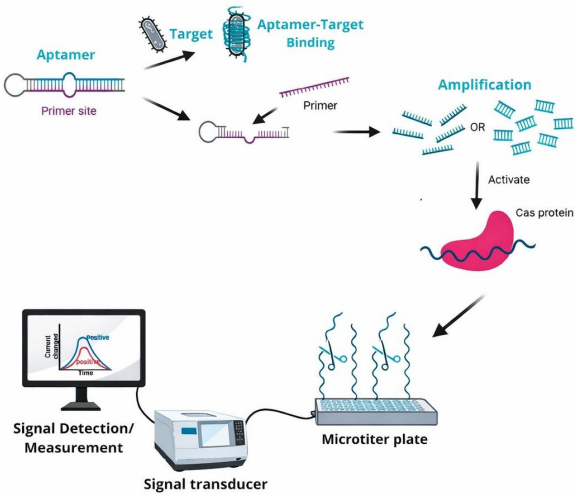

Fig. 9 Schematic representation of an aptamer-based CRISPR/Cas biosensor for the detection of microbial pathogens.

These emerging nanomaterials continue to expand the capabilities of aptamer-based biosensors by improving analytical sensitivity, stability and signal amplification, thereby supporting the development of rapid and highly sensitive pathogen detection platforms.

Clustered regularly interspaced short palindromic repeats (CRISPR/Cas) system

The integration of CRISPR/Cas systems with aptamer-based biosensors represents another emerging direction in pathogen detection. CRISPR-associated nucleases such as Cas9, Cas12a, Cas12b, Cas13a, and Cas13b provide highly specific target recognition and signal amplification through collateral cleavage activity [77]. In these systems, aptamers serve as the primary recognition elements, while CRISPR/Cas components amplify the detection signal. Upon recognition of the target nucleic acid, activated Cas proteins cleave reporter molecules, generating measurable optical or fluorescent signals (Fig. 9) [78]. This strategy has been applied in recent times to the detection of several pathogens, including SARS-CoV-2 [71], *Pseudomonas aeruginosa* [79], *Bacillus cereus* [80] and *E. coli* O157:H7 [78], demonstrating the potential of CRISPR–aptamer hybrid biosensors for ultrasensitive pathogen detection.

Portable and POCT devices

The development of portable and POCT devices has significantly expanded the real-world applicability of aptamer-based biosensors. These systems enable rapid, on-site detection without the need for complex laboratory infrastructure, which is particularly important in outbreak situations and resource-limited settings [54]. Examples include lateral flow test

strips, handheld electrochemical readers, and portable fluorescence-based sensors. For instance, a carbon ink–printed glove-based aptasensor was developed for rapid POCT detection of Chikungunya virus [29], while an aptamer-tethered enzyme capture POCT system has been reported for the detection of *P. falciparum* [59]. Other POCT platforms include fluorometric aptasensors for rapid detection of *Candida species* [82] and aptamer-based lateral flow strips for simultaneous detection of *S. typhimurium*, *E. coli*, and *S. aureus* [58]. These developments demonstrate the growing importance of aptamer biosensors in decentralised diagnostics.

Digital biosensing

Recent advances in digital technologies have enabled the development of digital aptasensors capable of real-time signal processing, data analysis and remote monitoring. In these systems, aptamer-target interactions are converted into digital signals that can be analysed using electronic devices or smartphone-based platforms [83]. For instance, Lee et al [84] reported a portable aptasensor known as the "Virus Pod" (V-Pod), which combines designer DNA nanostructures with a pocket-sized fluorimeter connected to a smartphone for rapid detection of SARS-CoV-2 in saliva samples. The system achieved clinically relevant detection limits comparable to laboratory-based PCR methods. Similarly, Liu et al [85] developed a digital aptasensor integrating aptamers with single-molecule array technology for ultrasensitive detection of T-2 toxin produced by *Fusarium oxysporum*, achieving a detection limit of 20.8 fg/ml. These innovations highlight the potential of digital aptasensors for rapid and accessible diagnostics.

In-silico aptamer design

Computational approaches have recently emerged as powerful tools for accelerating aptamer development. Techniques such as molecular docking, MD simulations, and structure prediction algorithms enable the identification and optimisation of aptamer–target interactions prior to experimental validation [46]. This strategy can significantly reduce the time and cost associated with traditional SELEX-based selection processes [21]. Several studies have successfully applied computational approaches for aptamer development. For example, Lin et al [86] used molecular docking to identify aptamers targeting the SARS-CoV-2 spike protein, while Sabri et al [87] designed aptamers for hepatitis B surface antigen using MD simulations. Other studies have predicted aptamer interactions with *Streptococcus agalactiae* surface proteins [88] and aflatoxin B1 produced by *Aspergillus* species [89]. These computational strategies are expected to further accelerate the development of high-affinity aptamers for pathogen detection.

CHALLENGES, LIMITATIONS, AND FUTURE PERSPECTIVES

Despite the significant progress achieved in the development of aptamer-based biosensors for pathogen detection, several technical and practical challenges still limit their widespread adoption in clinical diagnostics and field applications. Addressing these limitations will be critical for translating laboratory-scale technologies into reliable and commercially viable diagnostic tools.

One of the primary challenges lies in the performance of aptamers under complex biological conditions. Although aptamers generally exhibit high affinity and specificity toward their targets, binding performance may be affected by variations in pH, ionic strength, and temperature. In addition, aptamers selected through traditional SELEX or computational approaches may demonstrate excellent performance under controlled experimental conditions but may show reduced selectivity in complex biological matrices due to non-specific interactions and matrix interference [43, 90].

Another important limitation is the susceptibility of nucleic acid-based aptamers to degradation by nucleases present in biological fluids. Such degradation can significantly reduce aptamer stability and sensor lifespan during real-world applications. Chemical modifications, including phosphorothioate backbones, locked nucleic acids or PEGylation, have been proposed to enhance nuclease resistance; however, these modifications may sometimes affect the structural conformation and binding efficiency of the aptamer [91].

The development and optimisation of aptamer-based biosensors can also involve technical complexities. Although the integration of nanomaterials and advanced detection strategies improves analytical sensitivity and signal amplification, it often introduces challenges related to nanomaterial functionalisation, reproducibility, and preservation of aptamer bioactivity on sensor surfaces. These processes may require sophisticated instrumentation and expertise, which can limit large-scale production and practical implementation [8].

Furthermore, the selection and development of high-affinity aptamers remain a time-consuming process. Traditional SELEX procedures involve multiple iterative rounds of selection and amplification, which can extend the development timeline for new aptamers, particularly when targeting emerging pathogens. Although computational approaches and *in-silico* screening methods are beginning to accelerate this process, further refinement of these techniques is required to improve prediction accuracy and experimental validation efficiency [21, 46].

In addition to technical challenges, several key translational bottlenecks continue to hinder the real-world deployment of aptamer-based biosensors. A

major challenge is the clinical translation gap, where promising laboratory-scale results often fail to translate into robust performance in clinical settings due to sample complexity, variability, and limited large-scale validation studies. Furthermore, the lack of standardised protocols and uniform performance metrics, including variations in detection limits, sample preparation methods and validation procedures, makes it difficult to compare results across studies and assess clinical reliability. Moreover, regulatory approval remains a significant barrier, as aptamer-based biosensors must undergo rigorous analytical and clinical validation, quality control, and reproducibility assessment before acceptance by regulatory bodies [8]. Addressing these issues will be essential for ensuring consistency, reliability, and widespread adoption of these technologies in real-world diagnostic applications.

Looking forward, several emerging strategies are expected to overcome current limitations and further advance aptamer-based biosensors. Integration with microfluidic lab-on-chip systems and portable diagnostic platforms may enable automated, miniaturised devices capable of rapid on-site pathogen detection [68]. Advances in artificial intelligence and machine learning are also expected to accelerate aptamer development and optimise aptamer-target interactions through predictive modelling. In addition, artificial intelligence/machine learning approaches are increasingly being applied to biosensor signal processing, pattern recognition and data interpretation, enabling improved noise reduction, enhanced detection accuracy, and real-time analysis of complex biosensing data [21]. Furthermore, the development of wearable or implantable aptamer-based sensors could allow continuous monitoring of pathogen-related biomarkers in biological fluids [9, 92]. The convergence of biosensing technologies with digital health platforms, wireless connectivity, and Internet-of-Things (IoT) systems may further enable real-time disease surveillance and rapid outbreak response. Moreover, integrating aptamer-based biosensors with targeted therapeutic delivery systems could support responsive diagnostic, treatment strategies, such as controlled antimicrobial release in response to detected infection levels.

In general, continued interdisciplinary research integrating molecular biology, nanotechnology, computational modelling, and biomedical engineering will play a critical role in overcoming existing limitations and advancing aptamer-based biosensors toward practical diagnostic applications.

CONCLUSION

In conclusion, aptamer-based biosensors provide a state-of-the-art platform for the detection of pathogenic microbes and offer remarkable efficiency and wide-ranging applications. Their unique features, including high affinity, specificity, ease of chemical synthesis and ability to undergo structural modifications, make them versatile recognition elements. Compared with antibody-based assays and nucleic acid amplification methods, aptasensors offer distinct advantages such as lower cost, improved stability, and rapid adaptability to variety of targets. Recent innovations, including the integration of nanomaterials, CRISPR/Cas systems and advanced transduction techniques, have further enhanced their sensitivity, multiplexing capacity and potential for POC testing. Despite these advantages, challenges remain in large-scale clinical validation, standardisation of assay protocols, and transition to regulatory approval and commercialisation. Continued interdisciplinary efforts across molecular biology, materials science and engineering are essential to overcome these limitations. With sustained progress, aptamer-based biosensors hold strong promise as next-generation diagnostic tools for the timely and accurate detection of infectious disease pathogens.

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