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Aptamer-based biosensors for *Pseudomonas aeruginosa* detection

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ABSTRACT

Pseudomonas aeruginosa possesses innate antibiotic resistance mechanisms, and carbapenem-resistant *Pseudomonas aeruginosa* has been considered the number one priority in the 2017 WHO list of antimicrobial-resistant crucial hazards. Early detection of *Pseudomonas aeruginosa* can circumvent treatment challenges. Various techniques have been developed for the detection of *P. aeruginosa* detection. Biosensors have recently attracted unprecedented attention in the field of point-of-care diagnostics due to their easy operation, rapid, low cost, high sensitivity, and selectivity. Biosensors can convert the specific interaction between bioreceptors (antibodies, aptamers) and pathogens into optical, electrical, and other signal outputs. Aptamers are novel and promising alternatives to antibodies as biorecognition elements mainly synthesized by systematic evolution of ligands by exponential enrichment and have predictable secondary structures. They have comparable affinity and specificity for binding to their target to antibody recognition. Since 2015, there have been about 2000 journal articles published in the field of aptamer biosensors, of which 30 articles were on the detection of *P. aeruginosa*. Here, we have focused on outlining the recent progress in the field of aptamer-based biosensors for *P. aeruginosa* detection based on optical, electrochemical, and piezoelectric signal transduction methods.

1. Introduction

Pseudomonas aeruginosa is rod-shaped gram-negative. The opportunistic bacterium is capable of causing nosocomial infections, including burn wound infections, bacteremia, hospital-associated- and ventilator-associated Pneumonia, moreover infection in cystic fibrosis [1]. Considering intrinsically antibiotic resistance of *Pseudomonas aeruginosa*, it can survive on various surfaces, especially in clinical care settings and facilities. This makes *Pseudomonas aeruginosa* an immense challenge in hospitals due to its difficult diagnosis and treatment [2]. The innate antibiotic resistance of *Pseudomonas aeruginosa* resulting from the low permeability of its outer membrane leads to a second

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resistance mechanism through applying enzymatic modification to antibiotics [3,4]. This feature of *Pseudomonas aeruginosa* makes it a crucial hazard due; to 2017 WHO published the list of prior antibiotic-resistant bacteria with the urgent need for developing new antibiotics. *Pseudomonas aeruginosa* is considered priority number one in this list based on its resistance to carbapenem [5].

Early diagnosis of *Pseudomonas aeruginosa* is essential to prevent possible challenges in the treatment procedure. Therefore, diagnostic methods for *Pseudomonas aeruginosa* include bacterial culture methods and following assays to identify colonies [6,7], Immunological assays such as Enzyme-linked immunosorbent assays (ELISA) [8–10], immunofluorescence methods [11,12], and molecular methods [13–15]. Along with all these methods, various biosensor platforms have recently been developed for *Pseudomonas aeruginosa* detection.

Biosensors are analytical devices with the ability to convert a response following interaction between bioreceptor and biomolecules into a measurable electrical signal [16]. Biosensors generally consist of two main parts, including 1) a bioreceptor that detects the target molecule and 2) a transducer that is responsible for converting biological response into an electrical signal [17]. Biosensors are classified into optical-, electrochemical-, and piezoelectric-based [18]. Another classification of biosensors is in regard to the bioreceptor component. Biosensors can be enzyme-, antibody-, aptamer-, and whole cell-based (Fig. 1) [19].

Aptamer-based biosensors have grown rapidly in recent years. Aptamers are single-stranded DNA or RNA molecules with 20–60 nucleotides in length and the ability to bind to a variety of targets from inorganic structures to biological molecules such as proteins due to their three-dimensional structure [20]. Aptamers are considered similar to antibodies in terms of the type of performance but with more benefits. Advantages of aptamers over antibodies consist of smaller size without batch to batch variation, low-cost production, low immunogenicity, higher stability, and the ability to be labeled without losing affinity for the target molecule [21,22]. Aptamers are selected through the Systematic evolution of ligands by exponential enrichment (SELEX). An initial library of random sequences is used to incubate with a pure target molecule, and after 10–15 rounds, specific sequences with high specificity are selected as the final aptamer. SELEX was first developed by two research teams separately in 1990, Tuerk and Gold [22]and Ellington and Szostak [23]. However, in recent years, various modifications have been applied to simplify the SELEX procedure with higher efficiency, such as Cell-SELEX [24], whole bacteria-SELEX [25], Capillary Electrophoresis-SELEX (CE-SELEX) [26], Microfluidic-SELEX [24], Hight Throughput sequencing-SELEX (HTS-SELEX) [27].

Biosensors using aptamers as their bioreceptor element are termed aptasensor. In this article, we will review various aptasensors which were developed for *Pseudomonas aeruginosa* diagnosis.

2. *Pseudomonas aeruginosa* pathogenicity

Multiple virulence factors are generated from *Pseudomonas aeruginosa* that mediate its pathogenesis. They are essential for *Pseudomonas aeruginosa* motility, adhesion to the extracellular matrix, and disturbing host cell signaling pathways. Lipopolysaccharide (LPS), which exists in the outer membrane of *Pseudomonas aeruginosa*, consists of Lipid-A, core oligosaccharide, and O-antigen. LPS interacts with receptors on the host cell surface, mediates antimicrobial resistance, and inhibits the host defense system. Differences between O-antigen among *Pseudomonas aeruginosa* strains are the basis of its serotyping [28]. It has been shown that antibody-dependent cellular cytotoxicity against *Pseudomonas aeruginosa* functions by T-cells that are using antibodies against O-antigen [29].

Among *Pseudomonas aeruginosa* secreting systems, the Type III Secretion System (T3SS) is the most important. In order to generate and regulate this secretion system, thirty-six genes in five different operons which are clustered together are involved. ExoS, ExoT, ExoU, and ExoY are identified among *Pseudomonas aeruginosa* toxins that use T3SS to penetrate host cells [30,31].

Quorum-sensing (QS) is responsible for population density regulation which is necessary for *Pseudomonas aeruginosa* survival following infection [32]. QS-dependent gene expression regulates genes that are involved in antibiotic resistance, biofilm production, motility, etc. This regulation can occur either via transcriptional or post-transcriptional pathways [33].

Moreover, the Flagellum is responsible for its swimming motility, and type IV pili exhibit adhesion characteristics in *Pseudomonas aeruginosa*. In a study, Jacinta C. Conrad et al. proposed that Flagellum mediates near-surface swimming and surface-anchored spinning, and type IV pili mediate horizontally oriented crawling and vertically oriented walking [34].

3. Common methods for *Pseudomonas aeruginosa* detection

Considering the ability of *Pseudomonas aeruginosa* in causing long-term chronic disease, early detection of the infection is critical for treatment strategy. Methods such as immunological assays [35], Flow Cytometry [36], and molecular tests [37] are commonly used in *Pseudomonas aeruginosa* diagnosis.

Immunological assays are based on the interaction between antibody and antigen, and the diagnostic procedure can be applied quantitative or qualitative. Among immunological assays, the Enzyme-linked immunosorbent assay (ELISA) is the most common immunological test to

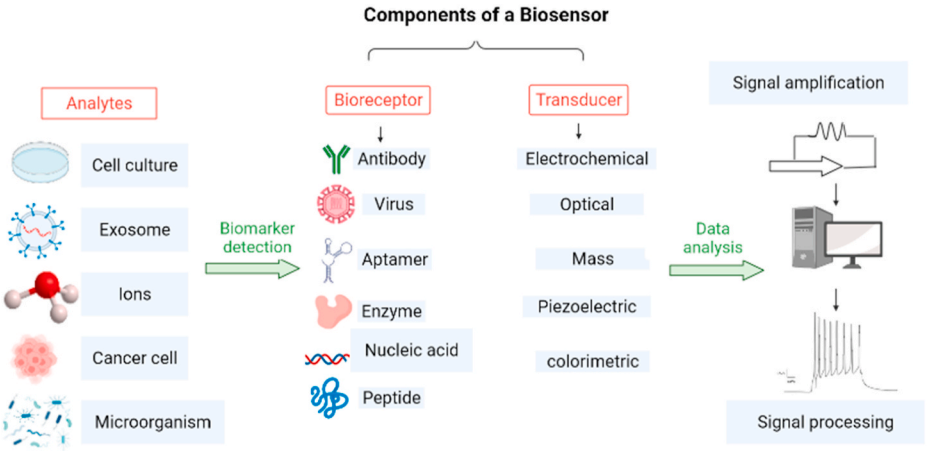

Fig. 1. Biosensor elements and selected components.

screen *Pseudomonas aeruginosa*, which is based on immobilizing antigen or antibody on a solid phase. Following enzyme-catalyzed action specifies the antibody-antigen attachment. Various ELISA platforms have been developed through the years to screen *Pseudomonas aeruginosa* [38–40].

Utilizing a fluorescent probe for a target-specific molecule or cell function, such as substrate uptake or enzyme activity, provides a suitable condition to characterize the viability of bacteria [41]. Consequently, detection of *Pseudomonas aeruginosa* was done using flow cytometry [36,42].

Molecular assays are sensitive and widely used to diagnose bacteria in clinical samples, especially when the query pathogen is difficult to detect with culture-based methods [43]. Various molecular techniques have been applied to detect *Pseudomonas aeruginosa*, such as conventional PCR for screening of 16srRNA [44], Real-time PCR for targeting the gyrB gene [45], and multiplex PCR (Fig. 2) [46].

4. Screening of *Pseudomonas aeruginosa* biosensor *by* SELEX technique

SELEX technique is a common method for selecting a specific aptamer against the desired target. Initially, a random library of 1013–1015 single-stranded DNA or RNA sequences is used to screen the binding ability of the target molecule. After the first incubation of the sequence pool with a target molecule, bounded sequences are separated from unbound. Following amplification of bounded sequences, the cycle will repeat to select sequences with higher affinity to the target. Almost 10–15 cycles are needed to obtain the most specific sequence (Fig. 3) [47]. Since conventional SELEX is labor and time consuming, various modifications were applied to reduce the time of screening with higher efficiency, including such as Cell-SELEX [24], whole bacteria-SELEX [25], Capillary Electrophoresis-SELEX (CE-SELEX) [26], Microfluidic-SELEX [24], Hight Throughput sequencing-SELEX (HTS-SELEX) [27].

Several efforts with different purposes employed the SELEX technique to select aptamer against *Pseudomonas aeruginosa* and its molecules [48–50]. Due to their structure and function, Aptamers have numerous applications, including diagnostic and therapeutic [51]. Aptamers have been widely adopted in diagnostic platforms, especially in biosensor development (Table 1) [52]. Therefore, several biosensors

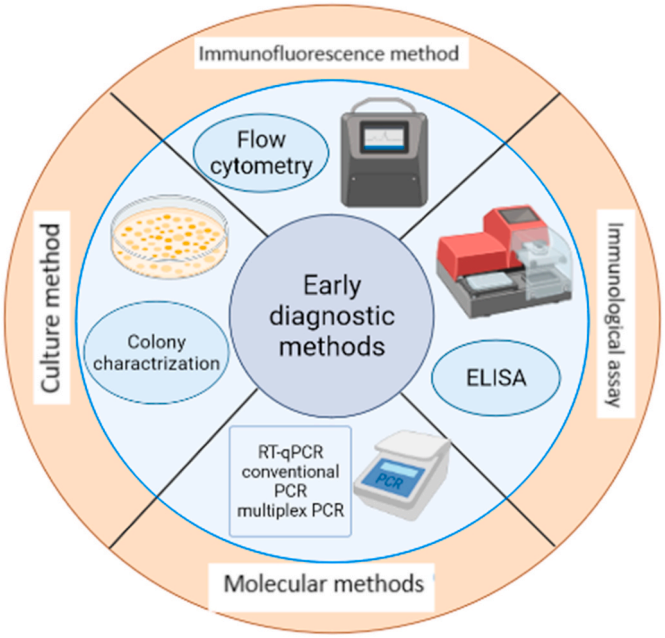

Fig. 2. Evaluation of early detection diagnostic strategies.

are developed with the purpose of *Pseudomonas aeruginosa* diagnosis.

5. Biosensor and nanobiosenor

Biosensors are robust analytical tools capable of capturing a target molecule through a biological reaction and converting this response into a measurable electrical signal. Biosensors are fabricated from two main components [63]: Bioreceptor and transducer. Bioreceptor can be an enzyme, antibodies, aptamers (Table 2), and whole-cell, and based on transducer elements, optical, electrochemical, and piezoelectric can be developed [64]. Biosensors can be used to detect query targets in various samples, such as blood, urine, saliva, water, etc., to target screening, drug development, food industry, and environmental applications [65].

The concept of nanobiosensors is achieved when nanomaterials are used with the aim of increasing the sensitivity and specificity of biosensing. Several nanomaterials, from gold and magnetic nanoparticles to carbon-based nanostructures and nanotubes, have been employed in recent years to increase the efficiency of biosensing [66–70] (Fig. 4).

5.1. Optical aptasensor for pseudomonas aeruginosa detection

Integrating an aptamer as a bioreceptor with an optical transducer creates an optical aptasensor. Optical aptasensors are classified into fluorescence (Fig. 5) [71], surface plasmon resonance (SPR) [72], localized surface plasmon resonance (LSPR) [73], colorimetric [74], and surface-enhanced Raman scattering (SERS)-based aptasensors [75]. Following recognition of the target molecule with aptamer, a refractive index alteration will happen in optical aptasensors [76].

Optical aptasensors utilize aptamers which are labeled with fluorescence material, nanoparticles, enzymes, and luminophores [77]. Most aptasensors that are developed for *pseudomonas aeruginosa* detection are based on fluorescence. But several label-free detection methods, including Surface plasmon resonance and localized plasmon resonance, are established for *pseudomonas aeruginosa* Aptasensing.

A fluorescence-based aptasensor was recently developed using fluorescein isothiocyanate (FITC) and quantum dot (QD) to label aptamer for the detection of *pseudomonas aeruginosa*. This platform is capable of detecting *pseudomonas aeruginosa* whole cell in drinking water with a limit of detection of 5.07 cells/mL, limit of quantification of 5.64 cells/mL, and limit of linearity of 100 cells/mL [78].

Next fluorescence aptasensor was published, in view of aptamer hybridization with carboxyfluorescein-labeled complementary DNA (FAM-cDNA) in blend with magnetic separation [57]. In the lack of *pseudomonas aeruginosa*, FAM-cDNA hybridizes with the aptamer, which is covalently bound to the surface of magnetic nanoparticles (MNPs). In the existence of *pseudomonas aeruginosa*, FAM-cDNA will be replaced from the aptamer by the bacterium and so released from the MNPs. The quantification of *pseudomonas aeruginosa* could then be defined from the fluorescence intensity of the FAM-cDNA in the supernatant after magnetic dissociation. The evaluation has a prominent linear response to the logarithm of *pseudomonas aeruginosa* concentrations ranging from 10 to 108 CFU mL^{-1}, with a LOD as low as 1 CFU mL^{-1}. The whole detection process could be completed within 1.5 h. Gao et al. [61] in addition, designed a fluorescent aptasensor based on the same DNA hybridization principle, with graphene oxide quantum dots (GOQDs) helping as the quencher. In addition to *P. aeruginosa*, the aptamer specifically binds to it as a bio-recognition component. FAM-cDNA likes to hybridize with the aptamer, outcomes in the desorption of FAM-cDNA from GOQDs and recovery of FAM fluorescence. The aptasensor shows a linear response to *pseudomonas aeruginosa* concentration in the range from 1.28 × 103 to 2.00 × 107 CFU mL^{-1}, with a LOD of 100 CFU mL^{-1}. This system could supplement all detection steps within 2 h and has been applied to detect *pseudomonas aeruginosa* in drinking water, juice, and ice cream samples [61].

Another aptasensor for *pseudomonas aeruginosa* diagnosis was developed utilizing photoluminescent carbon dots (CDs), which are

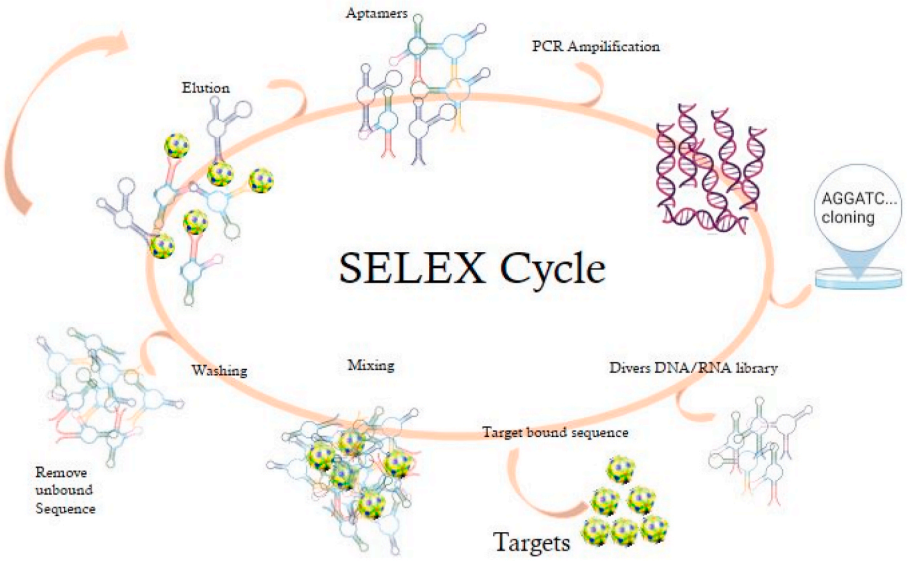

Fig. 3. The cell-SELEX process is depicted in a simplified schematic image.

fluorescent probes, and for the quenching element, graphene oxide (GO) was employed. This aptasensor can detect *pseudomonas aeruginosa* in water samples with a limit of 9 CFU mL^{-1} and a wide detection range from 10 to 10^7 CFU mL^{-1} [50].

An aptasensor based on fluorescent and DNA hybridization was developed to detect *pseudomonas* an aptasensor based on fluorescent and DNA hybridization was developed to detect *pseudomonas aeruginosa* in water, orange juice, and popsicles. Without bacteria in the query sample, 5-carboxyfluorescein-labeled complementary DNA (FAM-cDNA) will hybridize with a segment on aptamer sequence. Therefore, the fluorescence signal will be quenched due to the presence of graphene oxide quantum dots (GOQDs) as a quencher. This aptasensor has a linear range from 1.28×10^3–2.00×10^7 CFU/mL [61].

LSPR is a promising label-free biosensing method. An LSPR-based aptasensor was developed to detect whole-cell *Pseudomonas aeruginosa* strain PAO1. The linear rage for this aptasensor is from 10 – 10^3 CFU mL^{-1} and limit of detection (LOD) of 10 CFU mL^{-1}. Due to the presence of aptamers at the sensor's surface, bacterias are pulled down and follow an emergence of a redshift in wavelength [79].

An integrated LSPR-based aptasensor was developed for multiplex detection of *Lactobacillus Acidophilus, Salmonella typhimurium, and Pseudomonas aeruginosa* with a detection limit of 10^1–10^9 CFU mL^{-1} [80].

Hu and others [58] published aptamer-based LSPR sensing platform for whole-cell identification of *pseudomonas aeruginosa strain* PAO1. They applied nanosphere lithography to made a sensor surface with a hexagonal array of Au nanotriangles. By immobilizing the aptamers on the P sensor, aeruginosa cells are taken from the solution on the surface of the sensor, resulting in a redshift of the LSPR extinction maximum [58]. The method is very sensitive, with a low LOD 10 CFU ml^{-1}. Yoo and others [80] used three specific aptamers as detection elements and introduced another array of LSPR sensors for multiplex detection of L. acidophilus, *S. typhimurium*, and *pseudomonas aeruginosa.* This system uses a gold-capped nanoparticle multi-point array chip that consists of a dielectric layer consisting of a thin layer of gold (Au) a layer of glass absorbed by nanoparticles (NPs) of silicon dioxide (Si). And the presence of targets shown as a change in the LSPR peak chip incubation intensity with sample volume of only 3 ml. Despite the fact that LSPR-based aptasensors can be implemented detection of *pseudomonas aeruginosa with* high susceptibility and properly specified, they require expensive optical systems and also long time to prepare [80].

In an approach, Zhengzong Wu et al. applied a bimodal aptasensing platform that uses surface-enhanced Raman spectroscopy (SERS) and colorimetry to detect *Pseudomonas aeruginosa* quantitatively. An aptamer against *Pseudomonas aeruginosa* and a complementary DNA (cDNA) conjugated on the surface of two different gold nanoparticles (AuNPs) were employed. AuNPs with aptamer is responsible for colorimetric detection, and cDNA-AuNPs act as SERS signaling probes [70]. When *Pseudomonas aeruginosa* is not in the sample, aptamer-AuNPs and cDNA-AuNPs are complemented together. Following *Pseudomonas aeruginosa* presence in query samples, aptamer-AuNP separate from cDNA-AuNPs and bind to the target bacterium. Ultimately, after centrifugation SERS signal is decreased. The limit of detection for SERS mode was considered to be 20 CFU mL^{-1} and for colorimetric mode evaluated 50 CFU mL^{-1}. The linear range for SERS mode were 10^2–10^7 CFU mL^{-1} [81].

5.2. Electrochemical aptasensor for pseudomonas aeruginosa detection

Electrochemical aptasensors represent ultrasensitive detection even in turbid samples. The basis of this type of nanobiosensor is conducting an electrode and the conjugated bioreceptor (which here is aptamer) onto the surface of the electrode (Fig. 6). Subsequently, the attachment of the target sequence to the aptamer results in converting the biological reaction into an electrical signal using electrochemical methods. Electrochemical biosensors are capable of detecting a target in samples without preparation [82–84].

Their advantages, such as real-time analysis, ease of use, cost-benefit, and miniaturization, are widely used in disease diagnostics strategies, environmental contaminants, and then the food industry [85]. Recently, several electrochemical nanobiosensors were developed for *Pseudomonas aeruginosa* detection utilizing aptamers as a recognition element.

An electrochemical aptasensor using nanosized chitosan particles (NCs) was developed for ultrasensitive detection of *Pseudomonas aeruginosa*. The Limit of detection is 3 CFU mL^{-1} and its linearity range is from 10^1 to 10^7 CFU mL^{-1}. Aptamers against *Pseudomonas aeruginosa* are covalently attached to the surface of glassy carbon electrodes (GCE), which are altered with NCs. Aptasensing in this platform is measured through electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) methods [59].

Another approach to electrochemical Aptasensing of *Pseudomonas aeruginosa* was developed using modified glassy carbon electrodes (GCE) with silver nanoparticles (AgNPs). An aptamer covalently fixed on the surface of AgNP/GCE and using cyclic voltammetry, electrochemical

Table 1
Aptamers against *Pseudomonas aeruginosa.*

Name	Sequence (5′-3′)	Recognition site	Follow work	Ref
Decoy-SELEX Method (MREs) (Magnetic beads)	5′-TGTACCGTCTGAGCGATTCGTAC-3′ 5′-Biotin-GCACTCCTTAACACTGACTGGCT-3′	Exotoxin A		[53]
MRSw aptasensor	5′-NH2-CCC CCG TTG CTT TCG CTT TTC CTT TCG CTT TTGTTC GTT TCG TCC CTG CTT CCT TTC TTG-3′	Whole-cell (Particle aggregation)		[54]
Vertical-type aptamer – functionalized (aptasensor)	5'-CCC CCG TTG CTT TCG CTT TTC CTT TCG CTT TTG TTC GTT TCG TCC CTG CTT CCT TTC TTG-(CH2)3'-SH-3'	Whole- cell		[55]
Optical Colorimetric Electrochemical Amperometric	F23 (5′CCCCCGTTGCTTTCGCTTTTCCTTTC GCTTTTGTTCGTTTCGTCCCTGCTTCCTTTCTTG3′)	Whole cell		[56]
Optical Fluorescence	5′-CCC CCG TTG CTT TCG CTT TTC CTT TCG CTT TTG TTC GTT TCG TCC CTG CTT CCT TTC TTG-C6-NH2-3′ 5′-6-FAM-AC GAA CAA AAG CGA-3′	Whole cell		[57]
Optical Localized Surface Plasmon Resonance sensor	(Bt-aptamer, 5′[Bt]-ATACCAGCTTATTCAATTCCCCCGTTGCTTTCGCTTTTCCTTTCGCTTTTGTTCGTTTCGTCCCTGCTTCCTTTCTTGAGATAGTAAGTGCAATCT-3) (6FAM-aptamer-Bt, 5′[6-carboxyfluorescein-ATACCAGCTTATTCAATTCCCCCGTTGCTTTCGCTTTTCCTTTCGCTTTTGTTCGTTTCGTCCCTGCTTCCTTTCTTGAGATAGTAAGTGCAATCT-[Bt]3′)	Whole cell		[58]
glassy carbon electrode (GCE) was modified with nano-sized chitosan particles (NCs) or [aptamer/Glu/NC/GC electrode and [Fe(CN)6]3]	5′-NH2-CCC CCG TTG CTT TCG CTT TTG TTC GTT TCG TCC CTG CTTCCT TTC TTG-3′	Whole cell		[59]
Electrochemical biosensor, HZIFs (Zeolitic imidazolate framework)	5′-NH2 CCC CCG TTGCTT TCG CTT TTC CTT TCG CTT TTG TTC GTT TCG TCCCTG CTT CCT TTC TTG-3′	Whole cell		[60]
s fluorescent aptasensor [graphene oxide quantum dots (GOQDs)]	5′-CCC CCG TTG CTT TCG CTT TTC CTT TCG CTT TTG TTC GTT TCG TCCCTG CTT CCT TTC TTG-3′ 5′-6-FAM-AC GAA CAA AAG CGA-3′	Whole cell		[61]
PA-ap1[Singlewalled carbon nanotubes (SWNTs)]	5′- CCCCCGTTGCTTTCGCTTTTCCTTTCGCTT TTGTTCGTTTCGTCCCTGCTTCCTTTCTTG 3′	Biofilm		[62]

Table 2
Aptamer-based biosensors for *Pseudomonas aeruginosa* detection.

Detection method	Platform	Recognition motif	LOD	Linear range	ref
Fluorescence	FITC		5.07	5.64–10^2	[78]
	FAM-cDNA, MNPs		1	10^1–10^8	[57]
	FAM-cDNA, GOQDs		100	1.28 × 103–2 × 107	[61]
	CDs, GO		9	10^1–10^7	[50]
	QDs and UCNPs, cDNA-MB		25	10^2–10^6	[91]
	(PDA-PEI) copolymer dots		1	10^1–10^7	[92]
Colorimetric	AuNPs, cDNA		50	10^2–10^6	[93]
SPR and LSPR	Au nanotriangle array		10	10^1–10^3	[58]
	MG-NPA		10^4	10^4–10^9	[80]
SERS	AuNPs, cDNA		20	10^2–10^7	[93]
Electrochemical	GNPs		60	6 × 10^1–6 × 10^7	[56]
	Cu-ZrMOF/Super P, AuNPs		2	10^1–10^6	[94]
	HZIFs-8, GCE, Fc-GO		1	1.2 × 10^1–1.2 × 10^7	[60]
	GCE, AgNPs		33	10^2–10^7	[87]
	NC modified GCE		3	10^1–10^7	[59]
Piezoelectric	Au IDE-MSPQC MB, polyadenylated-DNA,		9 (buffer) 52 (spiked sample)	8.1 × 10^1–8.1 × 10^5 (buffer) 1.9 × 10^2–10^6 (spiked sample)	[90]

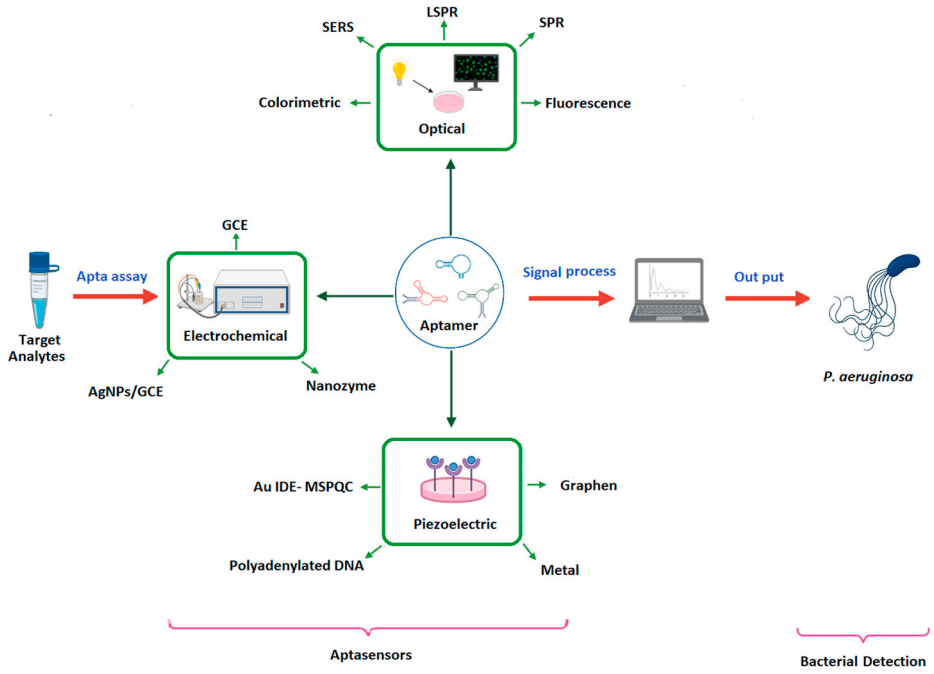

Fig. 4. Aptamer-based biosensors (aptasensors) for *Pseudomonas aeruginosa* detection are fabricated from two main components: aptamer and transducer (optical, electrochemical and piezoelectric). AgNPs: silver nanoparticles, Au IDE-MSPQC: gold interdigital electrode-multichannel series piezoelectric quartz crystal, GCE: glassy carbon electrodes, LSPR: localized surface plasmon resonance, SERS: surface-enhanced Raman scattering, SPR: surface plasmon resonance.

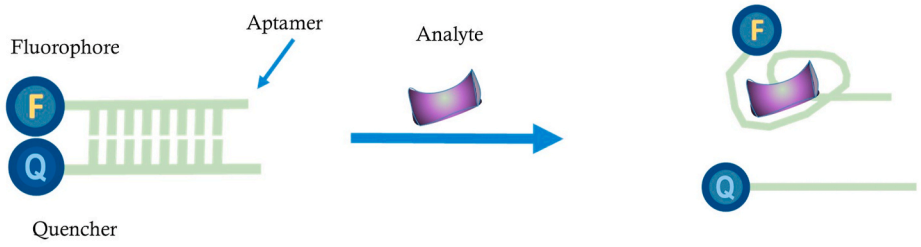

Fig. 5. A schematic view of optical aptasensor based on fluorescence.

impedance spectroscopy, and electrochemical properties were studied to detect *Pseudomonas aeruginosa*. The Limit of detection of this setup is 33 CFU mL^{-1} and the linearity range is from 10^2 to 10^7 CFU mL^{-1} [86].

In another study, a highly sensitive NanoZyme-based electrochemical Aptasensensor for rapid detection of *Pseudomonas aeruginosa* in water was developed with a limit of detection of ~60 CFU mL-1 and a

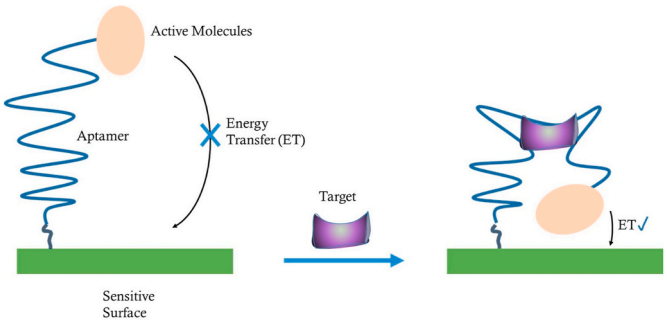

Fig. 6. A schematic view of electrochemical aptasensor.

linear range from 60.0 to 6.0 × 107 CFU mL^{-1}. In this nanosensor platform, the peroxidase-like NanoZyme feature of gold nanoparticles was employed and was integrated with specific aptamers against *Pseudomonas aeruginosa*. Using disposable carbon screen-printed electrodes, electrochemical Aptasensing was carried out [56].

Roushani and others [87], founded a glassy carbon electrode (GCE) impedimetric aptasensor modified with silver nanoparticles (AgNPs). The free amino groups of the aptamer are covalently attached AgNP/GCE surface. The interaction between *pseudomonas aeruginosa strain* and aptamers significantly reduces the charge transfer exacyanoferrate redox system on the electrode surface, and quantification of *pseudomonas aeruginosa strain* is done by monitoring the change in charge transfer resistance using a hexacyanoferrate redox system as an electrochemical probe. This test is used to measure *pseudomonas aeruginosa strain* in spiked serum samples with a low LOD of 33 CFU ml^{-1}. Sarabaegi and Roushani [59] proposed an electrochemical aptamer with a specific aptamer covalently bonded to the surface of GCE and hexacyanoferrate redox system as an electrochemical probe. They use nano-size chitosan particles (NC) for shape-sensitive GCE modification layer and to improve performance. Aptasensor has less LOD 3 CFU ml^{-1} and used to detect *pseudomonas aeruginosa strain* in serum samples. These impedimetric aptasensors were developed and have been identified as promising analytical tools for *pseudomonas aeruginosa* strain detection. However, they require a lot of time and expense modeling electrode processes. More suitable materials are available at need to produce impedimetric aptasensors [59].

5.3. Piezoelectric aptasensor for pseudomonas aeruginosa detection

Following the attachment of query target to the aptamer, the bioreceptor, mechanical stress is applied on a specific material or vice versa. This phenomenon generates a voltage that is the framework of piezoelectric aptasensors. The sensing procedure can be performed without labels and in real-time [88,89].

A piezoelectric aptasensor was developed to detect *Pseudomonas aeruginosa* with a limit of detection of 9 CFU mL^{-1} in buffer and 52 CFU mL^{-1} in simulated blood sample. Aptamer in this system is conjugated to magnetic beads, and a complementary polyadenylated DNA is bound to the aptamer. Following *Pseudomonas aeruginosa* attachment to the aptamer due to the presence of the target sequence, complementary DNA separates from the aptamer. Free complementary DNA can absorb onto gold (Au) interdigital electrode, which is connected to multichannel series piezoelectric quartz crystal (Au IDE-MSPQC) system, and ultimately, a frequency shift will result. The linear range is shown to be from 8.1×10^1 to 8.1×10^5 CFU mL^{-1} [90].

6. Conclusion

In summary, aptasensors are used widely in *Pseudomonas aeruginosa* detection. With recent advancements in biosensor technologies, point-of-care diagnostics can be delivered faster, more accurately, and more economically than conventional methods. Currently, modern biosensors make use of microfabrication and nanofabrication technologies as well as diverse sensing strategies such as optical, electrical and mechanical transducers to make measurements. Few notable examples of biosensors in clinical applications have been translated from research laboratories to clinical settings despite clinical need. The preparation of samples, the effects of matrixes, and the integration of systems are among the challenges to overcome. Considering the advantages of using aptamers, such as low cost, no batch-to-batch variation, easily produced and modified, high stability, and low immunogenicity, they are an effective candidate to be employed in the fabrication of nanobiosensors. However, aptamers have some limitations compared to antibodies in specificity to accurately distinguish different pathogens, and there is an urgent need to develop more specific aptamers for diagnostic strategies. Among all aptasensors for *Pseudomonas aeruginosa* detection, fluorescence- and electrochemical-based aptasensors were the most popular platforms. Nanomaterials, including nanoparticles and nanostructures, have revolutionized the biosensing field by increasing detection sensitivity. Despite the benefits of existing nanomaterials in the fabrication of nanobio sensors, developing novel nanomaterials to increase sensitivity in sensing procedures can be helpful in detecting pathogens like *Pseudomonas aeruginosa.*

Finding

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Declaration of competing interest

There is no conflict of interests.

Data availability

No data was used for the research described in the article.

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