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REVIEW

Diverse applications and development of aptamer detection technology

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Abstract

Aptamers have received extensive attention in recent years because of their advantages of high specificity, high sensitivity and low immunogenicity. Aptamers can perform almost all functions of antibodies through the combination of spatial structure and target, which are called "chemical antibodies". At present, aptamers have been widely used in cell imaging, new drug development, disease treatment, microbial detection and other fields. Due to the diversity of modifications, aptamers can be combined with different detection technologies to construct aptasensors. This review focuses on the diversity of aptamers in the field of detection and the development of aptamer-based detection technology and proposes new challenges for aptamers in this field.

Keywords Aptamer · Aptasensor · Detection technology · Application of aptasensor

Introduction

Systematic Evolution of Ligands by Exponential Enrichment (SELEX) technology was first proposed by Ellington and Tuerk et al. [1, 2]. The basic principle of this technology was to obtain RNA pools from a DNA library consisting of fixed sequences at both ends and random sequences in the middle by transcription and then reverse transcription after incubation with the target and obtain secondary pools by PCR amplification. After cyclic operation, oligonucleotide sequences that can bind to the target, called aptamers, are obtained. The characteristic of aptamer binding to a target is to use the tertiary structure of single-stranded RNA to fold into a spatial conformation for binding. Two years later, Ellington and Szostak published the selection method of DNA aptamers again [3]. They proposed that RNA aptamers and DNA aptamers have different binding abilities even if their sequences are the same. Since the stability of DNA sequences is better than that of RNA sequences but the structural richness is weaker than that of RNA sequences (RNA secondary structures include the most common non-Watson-Crick pairs [4]), these characteristics lay the foundation for the later different applications of aptamers.

The spatial three-dimensional configuration of the aptamer easily forms spiral, hairpin, stem ring, convex ring, clover, pseudoknot and other structures, which can easily bind with various targets. The known binding targets include small peptides, proteins, bacteria, viruses, cells, metal ions, and pathological sections [5]. Aptamers are widely used in various fields due to their low synthesis cost, easy modification, high affinity, low immunogenicity and strong specificity. The fields that have been reported thus far include cell imaging, new drug research and development, disease diagnosis and treatment, bacteria and virus detection, etc. According to the application function category of aptamers, they can be divided into aptamers for detection and disease treatment.

The application principle of aptamers in disease treatment is currently based on the following strategies: aptamers, as blockers or activators, mainly combine with functional proteins or receptors on the cell membrane surface to play a role or target specific cells or tissues as drug delivery carriers to deliver therapeutic drugs [6]. Pfizer/Eyetech has obtained

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FDA approval for pegaptanib (Macugen) [7, 8], a nucleic acid adapter drug for AMD, which is the only aptamer drug on the market at present. Although there are still some aptamers in clinical development [9], the overall application of aptamers as therapeutic drugs is still not optimistic. The reason may be related to the business background, investment and other factors, as stated by Zhou [6]. At the same time, we cannot ignore the common bottleneck problems faced by some small molecule drugs. Nimjee and He have expressed some opinions on the challenges faced by the application of aptamers in the field of disease treatment in their review [10, 11]. Obviously, how to deliver the aptamer into cells or target specific tissues is a bottleneck problem, which is also a common problem to be solved by nucleic acid drugs, antibodies and small peptide drugs.

In contrast, the application of aptamers in different detection fields is much more mature. It has been reported that aptamers can be used to detect tumor-related proteins, viruses, bacteria, metal ions, pathological sections, cells, etc., and there are many kinds of detection targets. With the development of technology, the combination of aptamers with nanomaterials, electrochemical sensors, fluorescent materials and other technologies has greatly expanded the application scope and detection sensitivity of aptamers. This review will introduce and discuss these issues to summarize the new advantages of the application of aptamers and discuss the future development prospects and problems of aptamers.

Biomarkers

Disease markers include tissue sections, highly expressed proteins in diseased cells or diseased cells, and proteins with elevated correlation in body fluids after lesions. The accurate detection of markers plays an important role in the early diagnosis, disease monitoring and efficacy evaluation of diseases. Currently, aptamer-based assays are the focus of current attention, as conventional assays are unable to effectively detect trace markers.

Tumor markers

Carcinoembryonic antigen (CEA) is a structural antigen on the surface of tumor cells that can be detected in a variety of body fluids. The CEA detection method based on aptamers has very important clinical significance in the diagnosis, treatment effect and prognosis of tumor diseases [12]. Electrochemical aptasensor for CEA detection based on the amplification driven by polydopamine functionalized graphene and Pd-Pt nanodendrites (PDA@Gr/Pd-PtNDs), conjugated hemin/G-quadruplex (hemin/G4), makes use of Pd-Pt nanodendrite's large specific surface area to provide a larger aptamer and signal molecular load, which can be combined with two aptamers to generate signal amplification, thus improving the detection sensitivity of CEA [13]. The report shows that the response range of CEA in serum with standard protein is 50 pg/mL-1.0 μg/mL, and the detection limit is 6.3 pg/mL. The CEA detection method based on the porosity and high load capacity of DNA-functionalized metal-organic frameworks (MOFs) is an immobilization-free photoelectrochemical (PEC) biosensor [14]. When the aptamer combines with CEA, the pores of MOFs are opened, resulting in a significant photocurrent. The detection limit in serum supplemented with standard was reduced to 0.36 fg/mL, and the linear range was 1.0 fg/mL-10 ng/mL. Magnetic mesoporous silica nanohybrids (MMSNs) were used to load a large amount of 4-nitrophenol (4-NP), thus realizing highly sensitive detection of CEA in human serum samples, with a detection limit of 46 pg/mL [15]. The results of CEA detection in human blood by this method are consistent with those of commercial kits. Ji et al. not only provided more loading space for aptamers and signal molecules through the complex structure of the 3D DNA nanostructure but also introduced the exonuclease III-assisted signal amplification strategy to further improve the sensitivity [16]. The detection limit of the electrochemical biosensor for CEA in human blood reached 4.88 fg/mL, and the detection linear range was 10 fg/mL-50 ng/mL. In addition, some researchers have screened the RNA aptamer of CEA, but it has not been used for detection [17, 18].

Human epidermal growth factor receptor-2 (HER-2) is a member of the human epidermal growth factor receptor family, which is involved in the regulation of tumor growth, differentiation and even tumor angiogenesis [19–21]. By reacting with molybdate to form oxidation−reduction molybdophosphate and generate electrochemical current through the phosphate ion and phosphate group on the MnO_2 nanosheets and the aptamer, HER-2 can be determined in the concentration range of 0.1-500 pg/mL, with a detection limit as low as 0.05 pg/mL [22]. Zhang and Xu et al. used the aptamer to encapsulate ferrocene (Fc) or 3,3′, 5,5′–tetramethylbenzidine and methylene blue into zeolitic imidazolate frameworks (ZIFs) to achieve dual signal enhancement. For the supersensitive detection of HER-2, the limit of detection (LOD) was 155 fg/mL, and the detection linear range was 0.5-1000 pg/mL [23, 24]. The electrochemical aptasensor of gold nanoparticles conjugated to bimetallic manganese (II) and iron (II) Prussian blue analogs can not only detect low levels of serum HER-2 but also stably detect HER-2 within 500-5×10^4 cell/mL MCF-7 living cells [25].

Mucin 1 (MUC 1), the first identified membrane-binding mucin tumor marker, plays an important role in the occurrence and development of tumors. The detection of blood MUC 1 has important clinical significance in early diagnosis, efficacy monitoring and prognosis evaluation

of cancer [26, 27]. The unmarked dual signal electrochemical aptasensor uses the reversible redox ability of hemin as the electronic medium as the first signal, catalyzing H_2O_2 with the peroxidase-like ability of hemin, as a second signal [28]. The linear ranges of mucin 1 in the two signals were 8 pg/mL-80 ng/mL and 0.8 pg/mL-80 ng/mL, respectively, and the detection limits were 2.5 pg/mL and 0.25 pg/mL. In addition, the combination of aptamer and highly sensitive liquid crystal biosensor technology resulted in an LOD of 0.47 fg/mL and a detection range of 1–10^4 fg/mL for MUC 1. The ion channel designed by Pan et al. can detect MUC 1 at 0.0364 fg/mL by immobilizing aptamers in the channel [29]. Although specific aptamers combined with highly sensitive techniques have enabled the identification and detection of MUC 1 protein targets at the fg level, most studies have only calculated detection limits by adding standard samples, and the efficiency of detection for actual biological samples needs to be further explored.

As a glycoprotein, prostate-specific antigen (PSA) is a reliable biomarker for the diagnosis of prostate cancer, evaluation of chemotherapy response and prostate function[30]. The detection limit of the electrochemiluminescence aptasensor manufactured for PSA is 0.14 pg/mL and has a very wide detection range [31]. The signal strength increases with the concentration of PSA in the linear range of 1 pg/mL to 100 ng/mL. Zhao used an aptamer combined with surface-enhanced Raman scattering (SERS) technology to detect PSA [32]. The detection range of this technology for PSA is 0.01-100 ng/mL, and the LOD is 6 pg/mL. Although the sensitivity of this technology has not been improved compared with the previously reported PSA aptamer biosensor, it is one of the few aptasensors that can be directly used for the detection of clinical samples, and the results are very close to the gold standard.

Not only for a single tumor marker. Man reported a time-resolved chemiluminescence enzyme-linked immunosorbent assay for simultaneous quantitative detection of vascular endothelial growth factor (VEGF) and CEA in human serum samples [33]. The linear range of the VEGF calibration curve was 0.5-80 ng/mL, and the detection limit was 0.1 ng/mL. The linear range of the CEA calibration curve was 0.5–160 ng/mL, and the detection limit was 0.1 ng/mL. The developed functional liquid crystal (LC) aptasensor can also rapidly detect CEA, PSA and alpha-fetoprotein (AFP) at the same time [34]. These methods are simple in operation, short in detection time, small in sample consumption, and consistent with the results of commercial ELISA kits, which has inspired the future high-throughput detection of multiple markers.

Tumor tissue section

Aptamers can also directly identify diseased tissues or cells. In this case, aptamers do not simply target known pathogens expressed in tissues or cells but directly and specifically bind to known or unknown natural conformations on the surface of tissues [35–37]. Numerous published studies have shown that by screening for aptamers that target tissue sections, it is possible not only to differentiate between diseased and normal tissue but also to observe the movement or distribution of target molecules [38, 39], target therapy [40] and discover new biomarkers [41].

At present, cell-SELEX or tissue-SELEX technology is used to screen aptamers for application. Although the use of aptamers can improve the portability of detection and reduce the cost, it does not improve the detection efficiency compared with other detection methods. Through tissue-SELEX screening technology, the aptamer XQ-2 had 82.5% recognition ability for clinical tissue samples of pancreatic ductal adenocarcinoma [42]. According to the same technology, the selected aptamer SW1 can identify liver cancer tissue, with a detection rate of 72.7% [43]. REase-mediated SELEX (REase-SELEX) was used to obtain an aptamer with high affinity (K_d = 1.4 nM) [44]. Four types of cancer cells with PD-L1 expression levels ranging from low to high showed unprecedented high specificity. Modification of the structure of the aptamer or combination with other molecules to improve the performance of the aptamer may be a breakthrough point worthy of research. Studies have shown that by adding isothymidine (isoT) modification at both ends of the sequence of the aptamer BC-15, it can exhibit stronger biological activity and stability [45]. In addition, Li et al. confirmed that when the aptamer C6-8 targeting ROS 17/2.8 (rat osteosarcoma) cells were combined with fluorescent carbon nanodots, they could freely enter a variety of live tumor cell lines (HepG2, MCF-7, H1299 and HeLa) [46]. This study increases the potential of aptamers in cancer diagnosis and therapy.

Other disease markers

Circulatory tumor cells (CTCs) are tumor cells that enter the blood or lymphatic circulation after tumor tissue is shed. They are common in the early stage of tumor metastasis and are important biomarkers for cancer monitoring and diagnosis. The detection of CTCs is helpful to clarify the types of cancer cells and evaluate the treatment as early as possible [47, 48]. Zhang et al. detected CTCs by synthesizing aptamers, and the results were not significantly different from those using antibodies [49]. Shen developed a CeO_2@Ir electrochemical aptasensor for signal amplification using nanorods and an enzyme-free DNA walker [50]. Because nanomaterials have the advantage of signal amplification,

this electrochemical aptasensor has the advantage of high specificity and sensitivity when detecting CTCs, and the detection range is 2–2 × 10^6 cells/mL, with a detection limit as low as 1 cell/mL. This method can separate and detect CTCs at the same time, which has great practical application potential and good prospects in cancer diagnosis.

Exosomes, which are emerging as valuable cancer biomarkers, can serve as early indicators of cancer cell invasion. Zhang et al. developed an innovative method to directly detect exosomes in plasma by combining fluorescence polarization (FP) with a suitable ligand. This approach eliminates the need for sample processing and can be completed in under 30 minutes [51]. Similarly, Chen successfully utilized exosome aptamers coupled with Au-modified polymorphic carbons (CoMPC@Au-Apt) to capture urinary exosomes for patients with early gastric cancer [52]. Furthermore, researchers have identified nuclease-resistant RNA aptamers that specifically recognize exosomes through the novel differential SELEX strategy of Exo-SELEX [53].

C-reactive protein (CRP) is an early indicator of cardiovascular disease and infectious inflammation [54, 55]. An electrochemical aptasensor, which increases the specific surface area through a zeolite-like imidazole ester skeleton material [56], increases the conductivity through the carbonization of ZIF and increases the number of signal molecules through an enzyme-catalyzed reaction. After optimization, the LOD was 0.44 pg/mL, and the detection range was 10 pg/mL-10 μg/mL. The highlight of this study is that the detection time is only 5 minutes, which fully reflects the portability of detection after the combination of the aptamer and electrochemical technology.

Zhang built a silver nanovillus-based sandwich-type surface-enhanced Raman scattering noninvasive sensor similar to small intestinal villi based on the principle of bionics [23]. The detection limit of the aptasensor for 25-hydroxy vitamin D_3 is 1 pg/mL, which is far lower than the concentration lacking. However, this study only detected 25-hydroxy vitamin D3 in human serum with a standard and did not discuss the detection efficiency in clinical samples.β-amyloid protein (Aβ) is a biomarker for the early clinical diagnosis of Alzheimer's disease. By comparing Aβ, early monitoring is conducive to the prevention of AD. Xu prepared CFP/AuPt nanocomposites with a large specific surface area and hydrophobic surface [24] combined with electrochemical aptamer sensors and showed a low detection limit of 0.16 pg/mL amyloid-β oligomers and the ability to resist nonspecific adsorption after immersion in serum for 1 week.

Singh assembled the aptamer into a tree or bridge structure, thus realizing the low concentration detection of insulin, and the LOD reached 0.8 pM. The detection range is 0.8–250 pM [57]. In addition, this technology combined with the SPRi array platform can monitor the serum insulin level of 24 patients in real time.

Virus

The outbreaks of influenza virus, hepatitis virus, AIDS virus and other infectious diseases, as well as the ongoing coronavirus disease (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), pose a huge threat to the safety of humans and animals around the world. Early diagnosis is essential for the prevention and control of a global pandemic. Routine diagnostic methods of viruses include virus isolation and culture, real-time quantitative polymerase chain reaction (RT−qPCR), serological methods, antigen detection, enzyme-linked immunosorbent assay (ELISA), etc. These methods are usually effective but laborious and time-consuming. Therefore, there is an urgent need for effective strategies to quickly, accurately and sensitively diagnose various viruses.

The N-protein aptamer of SARS-CoV-2 was fixed on the surface of the gold electrode with the double-stranded DNA hybrid obtained by hybridization of its fc-labeled complementary chain, and an electrochemical aptasensor with complementary oligonucleotides as a probe was prepared, with a detection limit of 1 fM [58]. Similarly, electrochemistry combined with CRISPR/Cas12a was used for signal amplification to detect that the LOD of SARS-CoV-2 nucleocapsid protein (Np) was as low as 16.5 pg/mL [59]. Kurmangali developed an aptasensor based on electrochemical impedance spectroscopy (EIS), used an interdigital gold electrode (IDE) to detect SARS-CoV-2 spike (S) glycoprotein, and obtained an LOD as low as 0.4 pg/mL in buffer solution [60]. Han et al. developed a clamp-type impedimetric aptasensor by assembling a pair of Np protein aptamers into a biomimetic clamp, with an LOD of 0.31 pg/mL and rapid detection within 11 min [61]. Chen et al. found that in the presence of the N gene of SARS-CoV-2, the fixed aptamer combines with it and changes the chain conformation, thus increasing the contact area or distance between the aptamer and the SPR chip, leading to a change in the signal [62]. The lower LOD for the N gene was 4.9 pg/mL. In addition to the N gene of SARS-CoV-2, the specific aptamer of the spike glycoprotein of SARS-CoV-2 was also screened out [63], and its LOD was approximately 37 nM combined with SPR technology.

An ultrasensitive aptasensor based on a graphene quantum dot (GQD) nanocomposite can accurately detect the concentration of HCV core antigen in human serum samples [64], with a detection limit of 3.3 pg/mL and two linear concentration ranges of 10-70 pg/mL and 70–400 pg/mL. Biosensor composed of the aptamer and graphene oxide coated molybdenum disulfide (GO-MoS_2) hybrid nanocomposite based on SERS of the selected MERS-nanovesicle (NV) specific aptamer, and detected MERS-NV,

LOD = 0.525 pg/ml in 10% diluted saliva [65]. Kang et al. successfully developed a heterologous sandwich immunosensor that boasts a detection limit of 1.02 ng/mL for influenza A virus—a sensitivity ten times higher than that of traditional ELISA methods [66]. On the other hand, Kushwaha et al. used the competitive non-SELEX (SELCOS) approach to screen aptamers and developed an electrochemical sensor in DEPSOR-mode that can accurately distinguish between different subtypes of influenza virus with an LOD of 0.4 μg/mL [67]. Chen et al. prepared a three-dimensional (3D) nanopopcorn plasma substrate. Cy3-labeled aptamer DNA was released from the surface of the nanopopcorn substrate, and the interaction between aptamer DNA and A/H1N1 virus led to a decrease in Raman peak intensity; thus, the quantitative evaluation of A/H1N1 virus also enhanced the repeatability. By assembling a sandwich structure sensor, the primary aptamer is attached to the metal particles of the SERS substrate, and the influenza virus is captured and combined with the secondary aptamer labeled with a Raman-active molecule [68]. It realized rapid, low-cost and strain-independent influenza virus detection. A novel aptamer-based sandwich platform with gold nanorods enhanced SPR for the detection of norovirus capsid protein [69]. In addition, a pair of aptamers are used to recognize and combine with the target to form a sandwich complex, which can detect norovirus capsid protein within the aM range.

Bacteria

Bacteria are one of the pathogens of various infectious diseases. The common pathogens in clinical practice are Staphylococcus aureus, Brucella, Salmonella typhimurium, etc. There are two main methods for laboratory detection of pathogens. The first is traditional methods, including smear microscopy and immunological detection. The second is molecular detection technology, including PCR, sequencing, mass spectrometry, etc. Traditional methods have low sensitivity and are easy to miss, while molecular detection technology is expensive and requires high operators and laboratory equipment.

The aptamer sensor designed by Cheng can effectively and accurately detect Escherichia coli O157:H7, with a detection limit of 26 CFU/mL [70]. The detection limit of the electrochemical aptamer biosensor for nanoporous gold can reach 1 CFU/mL, and the detection result could be obtained in 40 minutes [71], while the electrochemical aptamer biosensor could shorten the detection time to 10 minutes, but the detection limit was 67 CFU/mL, and the production cost was high [72]. An ultrasensitive aptasensor for the determination of Mycobacterium tuberculosis antigen MPT64 in human serum by voltammetry [73]. The MOF material was used as the carrier of aptamers and AuNPs, and horseradish peroxidase was used to make signal nanoprobes. The two aptamers with synergistic effects on the target substance MPT64 were modified on the gold electrode surface, and the LOD was 10 fg/mL. It is also a choice to detect Mycobacterium tuberculosis antigen MPT64, a GO@Fe_3O_4@Pt mixed nanomaterial that has excellent peroxidase-like activity and recyclability. The linear range of Mycobacterium tuberculosis is 5–1 × 10^6 fg/mL, and the LOD is 0.34 fg/mL [74]. Wang reported a magnetically assisted SERS biosensor for single-cell detection of S. aureus on the basis of aptamer recognition with a linear range of 10–10^5 cells/mL [75]. Ma et al. designed a gold/silver nanodimer SERS probe based on the aptamer [76], which can simultaneously detect Salmonella typhimurium and Staphylococcus aureus. When the target bacteria appear, the aptamer combines with the target bacteria to form a sandwich composite structure "SERS signal probe target magnetic capture probe", and then the SERS method is used to realize the simultaneous detection of the two bacteria.

Residual harmful substances in food/environment

Aptamers not only recognize various targets in the biomedical field but are also often used in the fields of food safety and environmental safety to detect various toxins, chemical organics and even metal ions in food, soil and water environments.

Ochratoxin is another mycotoxin that has attracted worldwide attention after aflatoxin. For a label-free electrochemical aptasensor based on double signal amplification of Nafion dispersed multiwall carbon nanotubes (Nafion-MWCNTs) and Au nanoparticles [77], the detection limit of ochratoxin A (OTA) is as low as 1 pg/mL, and the linear range is 1 pg/mL-10 ng/mL. Additionally, an electrochemical aptasensor has been built for detecting OTA [78]. Through the specific identification of the aptamer, OTA can be detected with super sensitivity, the detection range is 20 fg/mL-2 ng/mL, and the detection limit is 14 fg/mL. Tetrodotoxin is a neurotoxin classified as an alkaloid found in the bodies of pufferfish and other organisms. Using the high performance of gold nanoparticles (AuNPs) embedded in MOF nanocomposites combined with SERS, the detection limit for tetrodotoxin was 8 pg/mL, and high detection sensitivity was obtained [79]. Deoxynivalenol is a prevalent food-contaminating mycotoxin that occurs widely in nature. A bionic magnetic SERS adaptive sensor based on a "double antenna" nanosilver can detect the fg level of deoxynivalenol with a detection limit of 32 fg/mL [80]. Citrinin is a mycotoxin that can cause nephrotoxicity and is carcinogenic. A simple and highly sensitive electrochemical aptamer sensor

Table 1 Comparison of the performance of reported aptamer detection technology

Target	Type	Sensor type	Detection method	Limit of detection(LOD)	Detection ranges	Detection Time	Application	Advantage	References
Disease markers									
CEA	DNA	Electrochemical detection	PDA@Gr/Pd-PtNDs	6.3 pg/mL	50 pg/mL-1.0 μg/mL	40 min	Spiked in human serum samples	Stable: can store at 4°C for 14 days	[13]
CEA	DNA	Photoelectrochemical (PEC) biosensor	MOFs	0.36 fg/mL	1.0 fg/mL-10 ng/mL	50 min	Spiked in human serum samples		[14]
CEA	DNA	Spectrometric assay	4-nitrophenol (4-NP)-loaded magnetic mesoporous silica nanohybrids (MMSNs)	46 pg/mL	0.1-100 ng/mL	50 min	Real human serum samples	Accurate: The detect results are consistent with commercial kits	[15]
CEA	DNA	Electrochemical detection	3D DNA nanoprobe	4.88 fg/mL	10 fg/mL-50 ng/mL	40 min	Spiked in human serum samples	Stable: can store at 4°C for 20 days	[16]
CEA	RNA						It has not been used for real sample detection		[17]
CEA	RNA						It has not been used for real sample detection		[18]
CEA	DNA	Chemiluminescence assay	Chemiluminescent enzyme-linked immunoassay (CLEIA)	0.1 ng/mL	0.5-160 ng/mL	30 min	Real human serum samples	Accurate: The detect results are consistent with commercial kits	[32]
CEA	DNA	Liquid crystal (LC) sensors	Magnetic beads sorting	0.31 ng/mL	0.46-3 ng/mL	50 min	Spiked in human serum samples	Multiplex detection of tumor markers in blood	[33]
HER-2	DNA	Electrochemical detection	ELONA	0.05 pg/mL	0.1-500 pg/mL	90 min	Spiked in human serum samples		[22]
HER-2	DNA	Electrochemical detection	MOFs	3.4 fg/mL	0–10 pg/mL	30 min	Spiked in human serum samples		[23]
HER-2	DNA	Electrochemical detection	MOFs	155 fg/mL	0.5-1000 pg/mL	50 min	real human serum Samples	Stable: can stored at room temperature for 12 days	[24]
HER-2	DNA	Electrochemical detection	AuNPs	0.247 pg/mL	0.001-1.0 ng/mL		Spiked in human serum samples	Stable: can store at 4 °C for 15 days	[25]
Mucin 1	DNA	Electrochemical detection	Hemin/graphene@PdPtNPs nanocomposite (H-Gr@PdPtNPs)	0.25 pg/mL	0.8 pg/mL-80 ng/mL	40 min	Spiked in human serum samples	Stable: can store at 4 °C for 28 days	[28]

Table 1 (continued)

Target	Type	Sensor type	Detection method	Limit of detection(LOD)	Detection ranges	Detection Time	Application	Advantage	References
PSA	DNA	Electrochemiluminescence (ECL) biosensor	CdS/Chito/g-C_3N_4 composite material	0.14 pg/mL	1 pg/mL-100 ng/mL	90 min	Real human serum samples	Accurate: The detect results are consistent with commercial kits	[30]
PSA	DNA	Surface-enhanced Raman spectroscopy (SERS)	Polystyrene colloidal sphere @Ag shell (PS@Ag)	6 pg/mL	0.01-100 ng/mL		Spiked in human serum samples	Stable: can store at 4 °C for 14 days	[31]
PSA	DNA	Liquid crystal (LC) sensors	Magnetic beads sorting	0.07 ng/mL		50 min	Spiked in human serum samples	Multiplex detection of tumor markers in blood	[34]
Tumor tissue	DNA	Fluorescence assay	Confocal microscopy imaging of Cy5-labeled aptamers			60 min	82.5% detection rate		[42]
Tumor tissue	DNA	Fluorescence assay	Confocal microscopy imaging of Cy5-labeled aptamers			90 min	72% detection rate		[43]
Tumor tissue	DNA		Confocal microscopy imaging of Cy5-labeled aptamers				It has not been used for real sample detection		[44]
CTCs	DNA	Fluorescence assay	Fluorescence in situ hybridization(FISH)				73.3% positive rate		[49]
CTCs	DNA	Electrochemical detection	CeO_2@Ir nanorods and DNA walker	1 cells/mL	2-2 × 10^6 cells/mL	30 min	Spiked in human whole blood samples	This aptasensor can simultaneously isolate and detect CTCs	[50]
CRP	DNA	Electrochemical detection	MOFs	0.44 pg/mL	10 pg/mL-10 μg/mL	5 min	Spiked in human serum samples	Stable: can store at 4 °C for 15 days	[56]
Vitamin D3	DNA	Surface-enhanced Raman spectroscopy (SERS)	MOFs	0.001 ng/mL	0.001–100 ng/mL		Spiked in human serum samples		[23]
Aβ	DNA	Electrochemical detection	MOFs	0.16 pg/mL	0.5–10000 pg/mL		Spiked in human serum samples	Excellent resisting nonspecific adsorption performance in biological samples	[24]
Insulin	DNA	Surface plasmon resonance (SPR)	Aptamer-insulin-antibody sandwich	0.8 pM	0.8-250 pM		Real human serum samples		[57]

Table 1 (continued)

Target	Type	Sensor type	Detection method	Limit of detection(LOD)	Detection ranges	Detection Time	Application	Advantage	References
Virus									
SARS-CoV-2	DNA	Electrochemical detection	Sequence conformational changes lead to electrical signal changes	1 fM	10 fM–100 nM		Spiked in human serum samples		[58]
SARS-CoV-2	DNA	Electrochemical detection	CRISPR/Cas12a	16.5 pg/mL	0 pg/mL-100 ng/mL	30 min	Spiked in human serum samples	Accurate: The detect results are consistent with commercial kits	[59]
SARS-CoV-2	DNA	Electrochemical impedance spectroscopy(EIS)	Interdigitated gold electrode (IDE)	0.4 pg/mL	0.2-0.8 pg/mL	25 min	Spiked in human serum samples		[60]
SARS-CoV-2	DNA	Electrochemical detection	DNA biomimetic clamp	0.31 pg/mL		11 min	Spiked in real samples	Promising for early diagnosis and water environment monitoring	[61]
SARS-CoV-2	DNA	Surface plasmon resonance (SPR)	Nb_2C MXene quantum dot	4.9 pg/mL	0.05–100 ng/mL		Spiked in real samples	Sample diversity: seawater, seafood, and human serum	[62]
SARS-CoV-2	DNA	Surface plasmon resonance (SPR)	D-shaped plastic optical fiber (POFs) probe	36.7 nM	25–1000 nM		Spiked in human serum samples		[63]
HCV	DNA	Electrochemical detection	Graphene quantum dots (GQD)	3.3 pg/mL	10–400 pg/mL	40 min	Spiked in human serum samples		[64]
MERS	DNA	Surface-enhanced Raman spectroscopy (SERS)	Graphene oxide encapsulated molybdenum disulfide (GO-MoS_2)	0.525 pg/ml	1 pg/mL-100 ng/mL		Spiked in diluted 10% saliva		[65]
A/H1N1	DNA	Surface-enhanced Raman spectroscopy (SERS)	3D nanopopcorn substrate	97 PFU/mL		20 min		Three orders of magnitude more sensitive than that determined by the ELISA	[68]
Norovirus	DNA	Surface-enhanced Raman spectroscopy (SERS)				12 min		Achieves 10^6 –10^9 times signal amplification	[69]
Bacteria									
Escherichia coli O157:H7	DNA	Electrochemical detection		26 CFU/mL					[70]
Escherichia coli O157:H7	DNA	Electrochemical detection	Nanoporous gold (NPG)	1 CFU/mL	6.5×10^2-6.5×10^8 CFU/mL	40 min	Spiked in real samples such as egg		[71]

Table 1 (continued)

Target	Type	Sensor type	Detection method	Limit of detection(LOD)	Detection ranges	Detection Time	Application	Advantage	References
Escherichia coli O157:H7	DNA	Electrochemical detection	Multiwalled carbon nanotubes (MWCNTs)	10 CFU/mL		10 min	Spiked in real samples		[72]
Mycobacterium tuberculosis	DNA	Electrochemical detection	AuNPs & MOFs	10 fg/mL	0.02-1000 pg/mL	180 min	Spiked in human serum samples		[73]
Mycobacterium tuberculosis	DNA	Electrochemical detection	Nanocomposite of type GO@Fe_3O_4@Pt	0.34 fg/mL	5-1 × 10^6 fg/mL	4 h	Spiked in human serum samples	Stable: can store at 4 °C for 10 days	[74]
Staphylococcus aureus	DNA	Surface-enhanced Raman spectroscopy (SERS)	Ag–coated magnetic nanoparticles (AgMNPs)	10 cells/mL	10-10^5 cells/mL	45 min			[75]
Staphylococcus aureus	DNA	Surface-enhanced Raman spectroscopy (SERS)	Sandwich-like composite structures "SERS signal probes-target-magnetic capture probes"	96 CFU/mL	3.2 × 10^2-3.2 × 10^7 CFU/mL		Spiked in real samples such as milk		[76]
Salmonella typhimurium	DNA	Surface-enhanced Raman spectroscopy (SERS)	Sandwich-like composite structures "SERS signal probes-target-magnetic capture probes"	50 CFU/mL	10^2-10^7 CFU/mL		Spiked in real samples such as milk		[76]
Residual harmful substances in food/environment									
Ochratoxin A (OTA)	DNA	Electrochemical detection	Nafion dispersed multiwalled carbon nanotubes (Nafion-MWCNTs)	1 pg/mL	1 pg/mL-10 ng/mL	1 h	Spiked in real samples		[77]
Ochratoxin A (OTA)	DNA	Electrochemical detection	AgPt bimetallic nanoparticles decorated iron-porphyrinic metal-organic framework (PCN-223-Fe)	14 fg/mL	20 fg/mL-2 ng/mL	1 h	Spiked in real samples		[78]
Tetrodotoxin	DNA	Surface-enhanced Raman spectroscopy (SERS)	Gold nanoparticles (AuNPs)- embedded MOF nanohybrids (AuNPs@MIL-101)	8 pg/mL	0.01–300 ng/mL		Real samples	Accurate: the detect results are consistent with commercial kits	[79]
Deoxynivalenol	DNA	Surface-enhanced Raman spectroscopy (SERS)	"Dual antennae" nanosilver	32 fg/mL	0.0001-100 ng/mL		Spiked in real samples	Stable: can store at 4 °C for 45 days	[80]
Citrinin	DNA	Electrochemical detection	MOFs	0.45 ng/mL	0.1–1 × 10^4 ng/mL		Spiked in real samples	Stable: can store at 4 °C for 9 days	[81]

Table 1 (continued)

Target	Type	Sensor type	Detection method	Limit of detection(LOD)	Detection ranges	Detection Time	Application	Advantage	References
Carbendazim	DNA	Electrochemical detection		8.2 pg/mL	10 pg/mL-10 ng/mL	30 min	Spiked in real samples	Accurate: The detect results are consistent with commercial kits	[82]
Carbendazim	DNA	Electrochemical detection	Carbon nanohorns & gold nanoparticles	0.5 pg/mL	1–1000 pg/mL		Real samples	Stable: can store at 4 °C for 7 days	[83]
Mercury (Hg)	DNA	Electrochemical detection	Cu@carbon nanoneedles (Cu@CNNs)	3.7 fM	10 fM–10 μM		Pretreatment of water		[84]
Mercury (Hg)	DNA	Electrochemical detection	Gold modified thiol graphene (Au@HS-rGO)	0.16 nM	1 nM–1 mM		Spiked in real samples		[85]
Mercury (Hg)	DNA	Surface-enhanced Raman spectroscopy (SERS)	Recycling amplification & magnetic separation	0.11 fM	0.2 fM-0.125 pM		Spiked water solution		[86]
Cadmium (Cd)	DNA	Electrochemical detection	AuNPs	46.23 pM	0.1 nM-1000 nM	15 min	Spiked real samples	Stable: can store at -20 ◦C for 5 days low-cost (less than $1)	[87]
Lead (Pb)	DNA	Fluorescence assay	Upconversion nanoparticles & magnetic Fe_3O_4-modified (MNPs) gold nanoparticles (GNPs)	5.7 nM	25–1400 nM		Spiked real samples		[88]
Arsenic (As)	DNA	Electrochemical impedance spectroscopy (EIS)	Chitosan-Nafion (Chit-Naf) composite	0.78 nM	1–50 nM, 100–500 nM	45 min	Spiked real samples		[89]

for the detection of citrinin was developed by increasing the specific surface area of materials with MOF materials [81]. The LOD was 0.45 ng/mL, and the linear range was 0.1-1 × 10^4 ng/mL. The response of carbendazim aptamer by using electrochemical impedance spectroscopy. The detection limit of this method is 8.2 pg/mL [82]. An impedance sensor based on a carbon nano angle/gold nanoparticle composite was used for the detection of carbendazim in lettuce and orange juice [83]. Under the optimum conditions, the linear response range of the sensor to carbendazim is 1–1000 pg/mL, and the detection limit is 0.5 pg/mL. Metal nanomaterials have been used as signal enhancers, combined with an adaptive electrochemical sensor, to detect the fM level of mercury ions in the environment [84–86]. Similarly, metal ions such as cadmium [87], lead [88] and arsenic [89] can be detected at the lowest pM level through specific recognition of the aptamer and different signal amplification strategies (Table 1).

Conclusions

Obviously, these technologies will bring hope for the application prospect of aptamers and add new feasibility to detection methods in different detection fields. However, from the perspective of practical application, we find that aptamers also face some challenges. For example, the current detection methods based on aptamers cannot replace the gold standard methods in various fields. We believe that the advantages of enhanced aptamer-based assays fundamentally include two aspects. On the one hand, the combination of current new technologies, including enzyme-catalyzed reactions, hybridization chain reactions [90], electrochemical signal amplification, and CRISPR/Cas [91], amplifies the binding signal of aptamers and improves their detection limits and portability. Among them, the CRISPR/Cas system, as an emerging technology that can be effectively used in the field of biosensing, has shown powerful capabilities not only for the detection of nucleic acids but also for the diagnosis of nonnucleic acid targets [92, 93]. In addition, it is necessary to increase the specificity of the binding between the aptamer and the target, eliminate the false positive rate and increase the sensitivity, which is still lacking at present. Most of the research reports are aimed at improving the sensitivity of aptamer detection by taking inclusive advantage of aptamers that can cooperate with different technologies. They have proven the establishment of the method but lack an evaluation of the actual application effectiveness or a comparative evaluation with the previous method and the gold standard detection method.

Enhancing the modification or structural stability of an aptamer can improve its specificity [94]. The essential reason is that the combination of aptamer and target plays a role by using its own fold tertiary structure. The stability of the structure is determined by the hydrogen bond of complementary pairing of nucleotide bases. It may be a feasible method to solve the structural stability of aptamers by increasing the complementary stability of their own bases. Interestingly, Shu et al. found that the a three-way junction (3WJ) structure of the phi29 pRNA sequence core from the phage DNA assembly motor has very strong structural stability [95]. However, the stability decreased after DNA sequence replacement. The implication of this phenomenon for the application of aptamers is whether more RNA aptamers should be used instead of DNA aptamers to increase the physical and chemical stability of the tertiary structure of the aptamers and resist the complexity of the detection environment. In the past, RNA aptamers were easily degraded by nuclease when detected in the serum environment, but at present, 2'F modification of RNA in vitro has solved this problem very well [96], so it is theoretically feasible to establish detection methods with RNA aptamers. In recent years, the development of locked nucleic acids (LNAs) has been very rapid, and LNA modification of aptamers can also increase their physical and chemical stability [97]. Modification of aptamers by LNA can reduce the sequence length of aptamers at the same time, which is also an effective solution for the application of aptamers in the field of disease treatment to overcome the delivery problem. In addition, it is also necessary to consider the selection method for selecting suitable aptamers with different targets [98]. Qiao's research found that the binding force of the aptamers screened by the MCP-SELEX method is increased compared with the MB-SELEX method [99].

In addition to the problems of the aptamer detection method itself, there are also problems such as the investment of related companies and related factors of market share from a commercial perspective. It is undeniable that, in terms of the overall development trend of aptamers, they have development prospects that cannot be underestimated.

Data availability The authors confirm that the data supporting the findings of this study are available within the article.

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