Analytica Chimica Acta 1209 (2022) 339893

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Aptamer-based signal amplification strategies coupled with microchips for high-sensitivity bioanalytical applications: A review

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ARTICLE INFO

Keywords:
Aptamer
Microchips
Signal amplification strategies
Biochemical applications

ABSTRACT

With their advantages in specificity, high stability and easy screening, aptamers are becoming increasingly popular recognition elements for biosensor platforms. At the same time, microchips as the new analytical detection platforms have achieved significant growth in the past decades. At present, with the intersection of aptamer and microfluidic technology, aptamer-based high-sensitivity bioanalysis on microchips exhibits a great application potential in biomedical science and environmental fields. In this review, we highlight the recent progress in high-sensitivity bioanalytical applications based on aptamer signal amplification strategies on microchips. Furthermore, the main challenges in the practical application are discussed, and the development in the future is prospected.

1. Introduction

Nucleic acid aptamers are oligonucleotide chains with specific recognition functions screened by exponential enrichment system [1]. Since RNA aptamers were firstly isolated by Szostak [2] and Tuerk [3] in 1990, more and more aptamers have been screened, and their targets have covered small molecules [4,5], macromolecules [6], microorganisms [7–9] and cells [10,11]. Aptamers can fold into various structural shapes such as hairpins, stem-loops, pseudoknots, bulges, and G-quadruplexes, and they can bind to the targets through complementary pairing between bases, hydrogen bonding, van der Waals force, electrostatic interaction, etc. [12]. These conformational changes provide a structural basis for aptamers binding to specific targets.

The affinity of the aptamer to its target molecule is thought to resemble that of the antibody. Moreover, aptamers have many remarkable advantages that make them become a more effective option than antibodies [13]. Firstly, the screening of aptamers is relatively simple from a synthetic library without cell lines or animals [14]. Secondly, aptamers can easily modify functional groups for different purposes without changing their intrinsic characteristics [15]. Thirdly, aptamers are much more stable than antibodies, and aptamer-target binding is generally reversible through environmental parameters such as pH and temperature. They can sustain reversible denaturation [16]. These excellent biochemical properties make aptamers ideal candidates as recognition elements in developing biosensor platforms. Furthermore, aptamers can achieve signal amplification aided by their own characteristics (e.g., chain reaction, functionalization combined with nanomaterials), which exhibit their own unique advantages in the fields of biological sensing and disease diagnosis. At present, a variety of aptamer-based signal amplification methods have been developed for the detection of trace substances, which have been widely applied because of their simple operation and high sensitivity.

Over the past decades, microchips, as potential biosensor platforms, have received great attention in fields of biochemical sensing, medical diagnostics, and environmental monitoring. Various kinds of microchip platforms have sprung up, which mainly include microfluidic chips, paper-based microchips, and microarray chips. They have their own intrinsic advantages for different applications. For instance, microfluidic chips can easily achieve multi-unit integration and automatic operation. Paper-based microchips own the advantages of easy fabrication and

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https://doi.org/10.1016/j.aca.2022.339893
Received 18 March 2022; Received in revised form 19 April 2022; Accepted 28 April 2022
Available online 2 May 2022

portability for in-field detection. Microarray chips have a potential advantage in high-throughput bioassay. However, to these microchip platforms, the high-sensitivity detection is still a leading challenge for the biological samples, especially for those with only nL-pL volume. With the rapid development of recognition of aptamer along with a range of signal amplification strategies, the aptamer-conjugated microchip exhibits a promising solution for high-sensitivity bioanalysis [17].

At present, there have been several excellent reviews on aptamer with different emphases, such as aptamer screening techniques [18,19], aptasensors and detection techniques [20,21], signal amplification strategies [22,23], and applications [24–26]. But as far as we know, the review on the aptamer-based signal amplification for high-sensitivity bioanalysis on microchips has not been reported. This review, as shown in Fig. 1, firstly briefly discusses the characteristics of aptamer-based microchip platforms, which mainly include microfluidic chips, paper-based microchips, and microarray chips. Then we focus on aptamer-based signal amplification for high-sensitivity bioanalysis on microchips, which includes the aptamer-based signal amplification strategies on microchips and the high-sensitivity biochemical applications (Table 1). Finally, the main challenges and the development trends in the practical application are discussed.

2. Aptamer-based microchips

At present, aptamer, either used as an affinity probe or an affinity stationary phase on microchips, makes full use of its own high affinity and stability characteristics for biochemical applications. Microchips, as special platforms of aptamer reaction, have been widely reported, which mainly include microfluidic chips, paper-based microchips and microarray chips [27–30].

2.1. Aptamer-based microfluidic chips

It is convenient to manipulate the processes of extraction, separation and enrichment for biological samples on the microfluidic chip. Meanwhile, the microfluidic chip can transport a precise amount of biological samples, which can make effective use of reagents, especially for those enzymes or hormones poorly present in the practical sample. Aptamer-based microfluidic chip makes it possible to separate the complex sample quickly and accurately because of the affinity interaction between aptamers and analytes [31]. Nguyen et al. [32] introduced a microfluidic chip to immobilize the aptamer onto the beads filled in the microchamber, which could purify and enrich adenosine monophosphate (as a model analyte) from the salt-contaminated mixture. They employed thermally induced reversible characteristics of aptamer-analyte binding to realize the capture and release of analytes by controlling chip temperature. The aptamer-based microfluidic chip coupled with mass spectrometric assay could successfully detect adenosine monophosphate at 10 nmol L^{-1} complex sample. The aptamer-based microfluidic chip exhibits a great potential to address the requirements of detecting low abundance analytes.

In addition, the microfluidic chip can integrate multiple-operation units, which allows many operation processes to be automated [33,34]. Sinha et al. [35] proposed an automatic microfluidic chip that integrated with micropumps, micromixers and reagent storage chambers. The cardiac troponin I (cTnI) biomarker from six untreated clinical serum samples was simultaneously detected by using an aptamer-based ELISA-like assay on the microfluidic chip. The whole detection process of cTnI could be performed automatically within 35 min including reagent mixing, transport, and washing, and the detection limit was 0.01 ng mL^{-1} (Fig. 2A1). Man et al. [36] proposed a microfluidic colorimetric sensor for detecting salmonella in vegetables by using a smartphone imaging APP. Based on the binding between aptamer and salmonella, the visible color was changed by the aggregation of thiolated polystyrene microspheres and gold nanoparticles (AuNPs). The processes of mixing, enrichment and detection of salmonella were completed on the microfluidic chip in less than 45 min, and the detection limit reached 6.0×10^1 cfu mL^{-1} (Fig. 2A2).

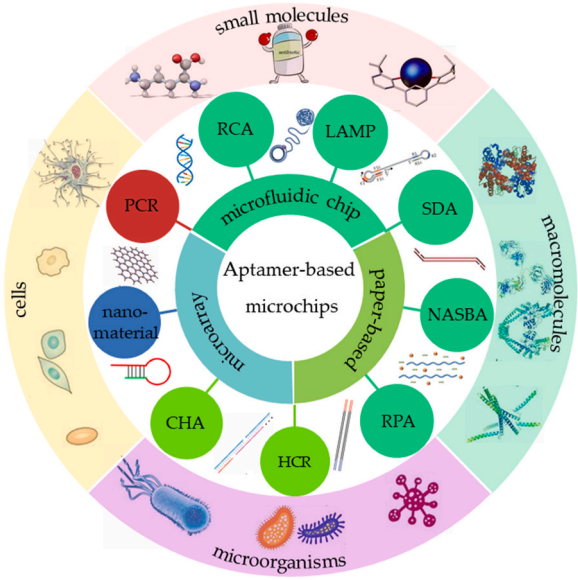

Fig. 1. Schematic illustration of aptamer-based signal amplification techniques and biochemical applications on microchips.

In recent years, the microdroplet analysis platforms have been widely reported, which can efficiently realize droplet generation and droplet-based microreaction. Based on this platform, a cell membrane-anchored fluorescence aptasensor was proposed for the detection of cytokine secretion at a single-cell level [37]. The two ends of an IFNγ-specific aptamer, as a recognition element, were modified with the fluorophore and quencher respectively. The extended aptamer could spontaneously form a hairpin structure, which brought both ends in proximity and resulted in fluorescence quenching. When binding to the target, the aptamer probe formed a tertiary structure, which separated the fluorophore from the quencher, and caused the restoration of fluorescence. The response of the cholesterol-linked aptamer to IFNγ was demonstrated with a detection limit of 10.0 nmol L^{-1}. The droplet-based microfluidic platform combined with the aptamer sensor exhibits a great potential to study immune response at a single-cell level.

2.2. Aptamer-based paper chips

Paper, as a porous cellulose fiber with a high surface area, has been used in a wide variety of biochemical assays since firstly proposed by Whitesides' group [38]. Paper has its own advantages of portability and ease of store compared with other materials. Furthermore, the porous microstructure of paper will help to immobilize protein macromolecules. Currently, there are three main aptamer-functionalized modes on the paper-based microchip for biochemical analysis: (i) as a signal recognition element for the qualitative or semi-quantitative assay [13], (ii) amplifying and triggering a high-efficiency aptamer chain reaction to achieve a high-sensitivity quantitative assay, and (iii) building aptamer-based cross-linking gel by capturing and releasing analytes to develop an equipment-free quantitative assay [24].

Due to its inherent white background, the low background interference and portability enable the paper-based microchip to realize qualitative or semi-quantitative detection. He et al. [39] constructed upconversion nanoparticles (UCNPs) on the paper-based microchip for field detecting cocaine combined with a smartphone. (Fig. 2B1). The luminescence of UCNPs fixed on the paper was quenched by aptamer-modified AuNPs, which could quantify the cocaine with a detection limit of 10 nmol L^{-1}. Moreover, this paper-based microchip

Table 1
Summary of bioanalytical applications based on aptamer signal amplification strategy on microchips.

Applications	Target	Microchip	Signal amplification	Linear range	LOD	Ref
small molecules	kanamycin	microfluidic chip	HCR	1 pg•mL^{-1}-10 ng mL^{-1}	0.29 pg mL^{-1}	[118]
	kanamycin	microfluidic chip	RCA	0.8–10^{4} ng mL^{-1}	0.3 pg mL^{-1}	[159]
	kanamycin	microfluidic chip	RCA	0.001–10 ng mL^{-1}	0.32 pg mL^{-1}	[123]
	kanamycin	microfluidic chip	MAJR	–	0.41 pg mL^{-1}	[101]
	kanamycin	microfluidic chip	CHA	0.001–10 ng mL^{-1}	0.7 pg mL^{-1}	[117]
	kanamycin	microfluidic chip	HCR	5.89 × 10^{-4}-10 nmol L^{-1}	1.76 × 10^{-4} nmol L^{-1}	[122]
	oxytetracycline	microfluidic chip	CHA	0.001–10 ng mL^{-1}	0.9 pg mL^{-1}	[117]
	chloramphenicol	microfluidic chip	MAJR	0.001–40 ng mL^{-1}	0.52 pg mL^{-1}	[101]
	erythromycin	Paper-based	RPA	250–500 pmol L^{-1}	10 pmol L^{-1}	[83]
	mercury ions	microfluidic chip	nanomaterials	10^{-6}-10^{-11} mol L^{-1}	10 pmol L^{-1}	[120]
	lead ion	microfluidic chip	HCR	4.29 × 10^{-4}-3.5 nmol L^{-1}	1.29 × 10^{-4} nmol L^{-1}	[124]
	lead ions	microfluidic chip	nanomaterials	0.48 nmol•L^{-1}-1.2 μmol L^{-1}	0.64 nmol L^{-1}	[121]
	lead ions	paper-based	nanomaterials	10 nmol L^{-1}-1 mmol L^{-1}	0.7 nmol L^{-1}	[122]
	17 β-estradiol	microfluidic chip	RCA	0.02–2 ng mL^{-1}	6.8 pg mL^{-1}	[123]
	17 β-estradiol	microfluidic chip	HCR	3.94 × 10^{-4}-3.5 nmol L^{-1}	1.18 × 10^{-4} nmol L^{-1}	[122]
	aflatoxin M1	microfluidic chip	RCA	3–50 pg mL^{-1}	0.95 pg mL^{-1}	[123]
macromolecules	VEGF	microfluidic chip	RCA	10–250 pg mL^{-1}	10 pg mL^{-1}	[133]
	PSA	microfluidic chip	HCR	0.001–100 ng mL^{-1}	0.31 pg mL^{-1}	[135]
	exosome	microfluidic chip	CHA	–	10^{3} exosomes•μL^{-1}	[96]
	exosome	paper-based	HCR	1.7 × 10^{4}–3.4 × 10^{8} particles/mL	5 × 10^{3} particles/mL	[137]
	peptide	paper-based	HCR	20 pmol•L^{-1}-50 nmol L^{-1}	8.33 pmol L^{-1}	[139]
	AFP	microfluidic chip	CHA	–	0.1 pg mL^{-1}	[125]
	CA125	microfluidic chip	CHA	–	0.2 pg mL^{-1}	[125]
	CEA	microfluidic chip	CHA	–	0.15 pg mL^{-1}	[125]
	PDGF-BB	microarray	nanomaterials	16 pg•mL^{-1}-250 ng mL^{-1}	1.4 ng mL^{-1}	[46]
	PDGF-BB	microarray	nanomaterials	16 pg•mL^{-1}-250 ng mL^{-1}	7.8 ng mL^{-1}	[46]
	PDGF-BB	microfluidic chip	PCR	62 fmol L^{-1}-1 nmol L^{-1}	62 fmol L^{-1}	[59]
	c-reactive protein	microarray	nanomaterials	4–200 nmol L^{-1}	5 nmol L^{-1}	[47]
	thrombin	microfluidic	RCA	0.100–50.000 pg mL^{-1}	0.083 pg mL^{-1}	[61]
	thrombin	paper-based	HCR	50 fmol•L^{-1}-1000 pmol L^{-1}	16.7 fmol L^{-1}	[143]
	thrombin	paper-based	RCA	–	240 pmol L^{-1}	[144]
	N-glycan	paper-based	HCR	100-10^{7} cells•mL^{-1}	21 cells•mL^{-1}	[90]
microorganisms	*E. coli* O157:H7	microfluidic chip	CHA	2 × 10^{2}–2 × 10^{5} cells•mL^{-1}	75 cells•mL^{-1}	[116]
	E. coli O157:H7	microfluidic chip	RCA	–	80 cells•mL^{-1}	[63]
	E. coli O157:H7	microfluidic chip	RCA	10^{2}-10^{5} cells•mL^{-1}	10^{2} cells•mL^{-1}	[62]
	E. coli O157:H7	microfluidic chip	HCR	500–5 × 10^{7} CFU mL^{-1}	250 CFU mL^{-1}	[148]
	E. coli O157:H7	microfluidic chip	RCA	–	–	[149]
	H1N1 viruses	microfluidic	LAMP	–	3 × 10^{-4} HAU/reaction	[75]
	HCV	microfluidic	LAMP	–	2 copies•μL^{-1}	[76]
	HBV	microfluidic	LAMP	–	2 copies•μL^{-1}	[76]
	HIV	microfluidic	LAMP	–	2 copies•μL^{-1}	[76]
	noroviruses	paper-based	nanomaterials	13 ng mL^{-1}-13 μg mL^{-1}	3.3 ng mL^{-1}	[153]
	Vibrio parahaemolyticus	Paper-based	SDA	10–10^{7} CFU mL^{-1}	5.6 CFU mL^{-1}	[79]
cells	CTCs	microfluidic	nanomaterials	–	–	[154]
	CTCs	microfluidic	nanomaterials	–	–	[155]
	IL-8	microfluidic	RCA	7.5–120 pg mL^{-1}	0.84 pmol L^{-1}	[156]
	MCF-7	paper-based	nanomaterials	180–8 × 10^{7} cells•mL^{-1}	8 × 10^{7} cells•mL^{-1}	[157]
	HL-60	paper-based	nanomaterials	210–8 × 10^{7} cells•mL^{-1}	7 × 10^{7} cells•mL^{-1}	[157]
	K562	paper-based	nanomaterials	200–8 × 10^{7} cells•mL^{-1}	7 × 10^{7} cells•mL^{-1}	[157]

can be extended to other targets by replacing the target aptamer, and it holds great application potential in the field of drug abuse detection. At present, paper-based colorimetric detection coupled with aptamer signal amplification has gained popularity because of its simple operation. Zhang et al. [40] proposed naked-eye quantitative aptamer-based assays for detecting adenosine on the paper microchip. When the target existed, a short DNA strand covalently modified on the super-paramagnetic particle (SP) could trigger hybridization chain reaction (HCR) for signal amplification. As a result, many hairpin probes were captured onto the SP to bind glucose oxidase tags, and further cause the oxidation-reduction reaction on the paper microchip. The paper length of the colored region and the number of colorless microzones based on the oxidation-reduction reaction could respectively quantify the adenosine with the naked eye, which demonstrated the sensitive, specific aptamer-based assay.

In addition, some efforts have been made for equipment-free quantitative or semi-quantitative aptamer-based assays on the paper microchip. Wei et al. [41] proposed an aptamer-cross-linked hydrogel paper microdevice which was used as a flow regulator for target-response to mediate fluid flow and signal readout. The degree of hydrogel formation could affect the travel length of the solution in the flow channel. When there was no target in the sample, the hydrogel was formed in the flow channel, which prevented the colored indicator from reaching the observation spot, thus showing a "signal off". In contrast, the "signal on" was generated without forming hydrogel in the presence of a target. The colored indicator corresponding to "signal on/off" readout could simultaneously detect adenosine, cocaine and Pb^{2+} in the complex sample. For achieving quantitative analysis and further improving the detection sensitivity, they designed a crosslinked sweet hydrogel paper microdevice with target-responsive aptamer for visual quantitative readout, and the cascade enzymatic reactions were used for signal amplification [42] (Fig. 2B2). The "sweet" hydrogel with the aptamer cross-linker trapped glucoamylase (GA). When the target was presented, the "sweet" hydrogel collapsed and released GA into the sample, which resulted in the generation of glucose because of GA-catalyzed amylolysis reaction and further induced a cascade reaction on the enzyme/substrate-modified paper microchip to generate a distance signal of stained stripe. There was a positive correlation between the

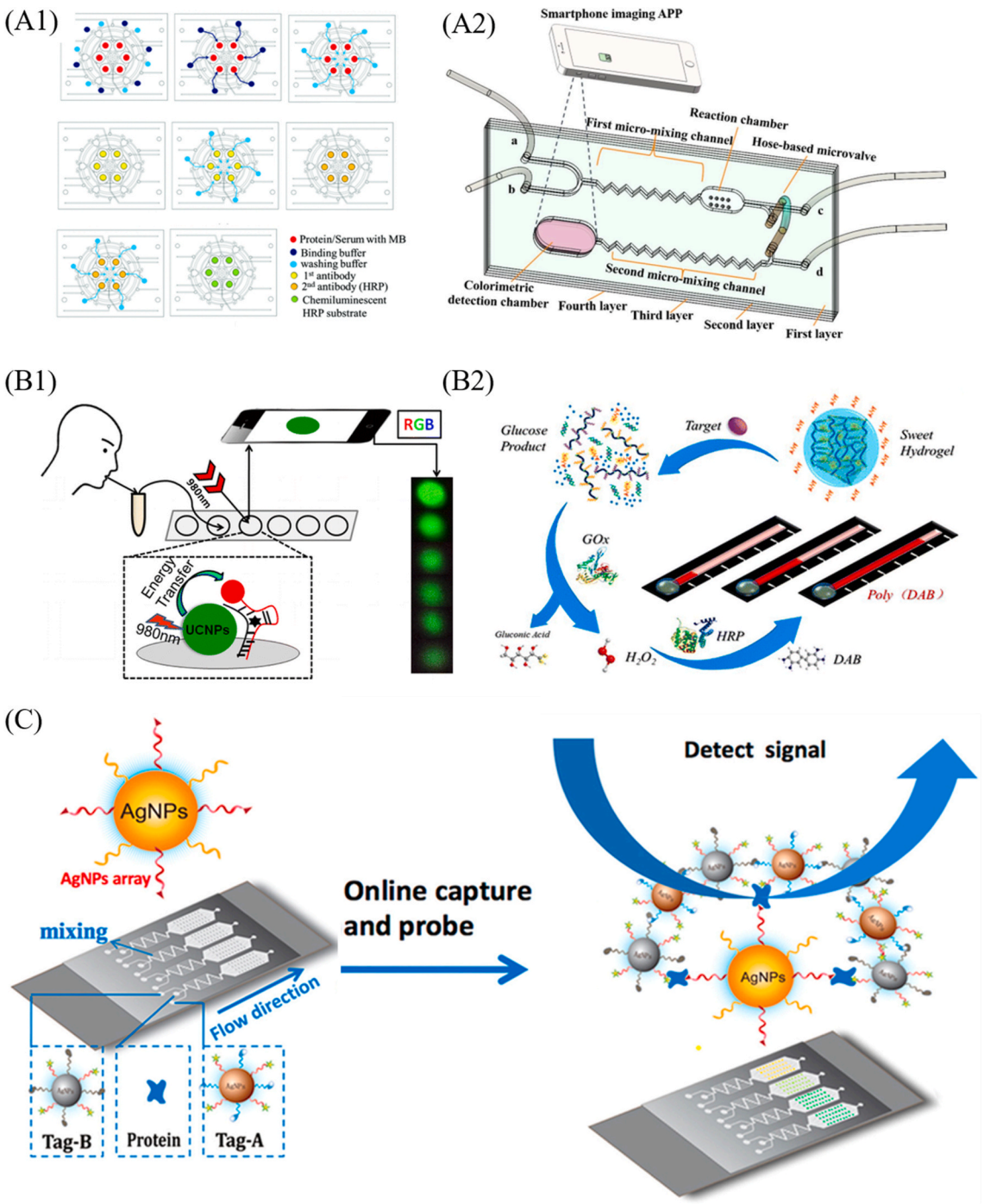

Fig. 2. Schematic illustrations of aptamer-based microchips. (A1) The automatic aptamer-based ELISA-like assay on the microfluidic chip. Adapted with permission from Ref. [35]. Copyright (2019) IEEE. (A2) 3-dimension structure of the microfluidic colorimetric biosensor for the detection of *Salmonella typhimurium* by smartphone imaging APP. Reproduced with permission from Ref. [36]. Copyright (2021) Elsevier. (B1) The UCNPs-based paper microdevice for on-site testing cocaine. Reproduced with permission from Ref. [39]. Copyright (2016) American Chemical Society. (B2) Paper-based microdevice integrated the distance readout sweet hydrogel for the visual quantitative analysis of cocaine. Reproduced with permission from Ref. [42]. Copyright (2016) American Chemical Society. (C) The aptamer-based sandwich assay in the microarray chip for detecting protein. Reproduced with permission from Ref. [46]. Copyright (2018) Elsevier.

distance of the stained stripe and the concentration of targets, which could realize the quantitative detection of cocaine using the naked eye.

2.3. Aptamer-based microarray chips

Microarray can offer several advantages in terms of high speed, miniaturization, and automation for biochemical analysis [43]. Phung et al. [44] developed a microarray prescreening platform coupled with a lateral flow assay for the detection of C-reactive protein. An aptamer binding C-reactive protein was immobilized on microarray chips to achieve the parallelized and fast screening for binding conditions. At the same time, the aptamer-based microarray chip can also allow high-precision capturing of different targets for multiple detections in a single device [45]. Liu et al. [46] developed an aptamer-based sandwich assay in a microarray chip to detect two biomarkers simultaneously by constructing a multivalent aptasensor array along with silver aggregated

amplification strategy (Fig. 2C). The aptamers-modified silver nanoparticles dotted in array could specifically identify target proteins. Meanwhile, the target proteins could also gather the aptamers-modified silver nanoparticles in the microarray, which amplified the fluorescence signal through the sandwich structure. The detection limit of platelet-derived growth factor-BB reached 1.4 pg mL^{-1}.

Aptamers can be modified on the microarray chips to construct aptamer arrays for high-throughput detection. However, the assembly processes of microarray biomolecules are laborious. To simplify the operation steps, Sathish et al. [47] proposed a two-step patterned method to create an aptamer-based immunoassay in the microchip. Aminosilanes were patterned covalently on the device substrate to construct an aptamer array, and the target molecules bound with aptamer array could withstand the high shear stresses of flow in the device and maintain their biological function. Using this covalent coupling method, aptamer-based immunoassays for different biomolecules can be created in the microarray chip, which exhibits a great potential for high-throughput multiplexed bioassays in the future.

3. Aptamer-based signal amplification strategies on microchips

At present, a range of aptamer-based high-sensitivity bioanalysis on microchips have been developed and coupled with various detection techniques such as colorimetry [40], chemiluminescence [48], fluorescence detection [49], electrochemistry [50], Raman [51], surface plasmon resonance [52], etc. The aptamer-based signal amplification strategy is an important factor in improving the detection sensitivity for bioanalysis. In this section, we focus on discussing the aptamer-based signal amplification strategy on microchips, which is defined broadly as the aptamer-based chain reactions (enzyme-mediated, non-enzyme-mediated) and aptamer-functionalized nanomaterials.

3.1. Enzyme-mediated signal amplification on microchips

Polymerase chain reaction (PCR), as the "gold standard" for enzyme-mediated aptamer chain reaction, has been widely used in the fields of diagnostics, biochemistry and molecular biology [53–58]. Csordas et al. [59] developed a microfluidic platform coupled with aptamer-based PCR to achieve high-sensitivity detection of proteins. They employed high-gradient magnetic field to directly capture target-aptamer complexes in serum samples. The aptamer was used as an affinity and amplification reagent for real-time PCR, which significantly increased the assay sensitivity and multiplexing capability. The cancer biomarker PDGF-BB could be detected from 62 fmol L^{-1} to 1 nmol L^{-1} in complex serum background. However, PCR is also complex and time-consuming, and usually involves temperature cycles and expensive equipment. Now, aptamer-based isothermal amplification, as a promising alternative to PCR amplification technology, is emerging on microchips. And it includes rolling circle amplification (RCA), loop-mediated isothermal amplification (LAMP), strand displacement amplification (SDA), nucleic acid sequence-based amplification (NASBA) and recombinase polymerase amplification (RPA), etc.

RCA can produce long single-stranded RCA products composed of abundant tandem repeats complementary to a circular template under the action of DNA polymerase, which can achieve signal amplification [60] (Fig. 3A). To aptamer-based RCA on microchips, the aptamers are generally modified in the microchannels, which selectively capture analytes (e.g., thrombin) and then amplify the signal by RCA and G-quadruplex catalyzed ABTS-hemin-H_2O_2. This method exhibited high selectivity for thrombin and the detection limit reached 0.083 pg mL^{-1} [61]. In addition, the products of RCA can also be loaded with some signal molecules such as fluorescent probes [62,63] or quantum dots [64–66]. Jiang et al. [62] employed RCA amplification to capture *E.coli* O157:H7 cells on the microfluidic chip. Poly (amidoamine) dendrimer was immobilized on the PDMS channel for further ligating aminated aptamers. The RCA products loaded with numerous fluorescent probes could enhance detection signals by up to 50 times. Now, RCA is generally divided into linear RCA [67], multi-primer RCA [68] and hyperbranched RCA (HRCA) [60,69]. HRCA introduces multiple primers based on linear RCA to achieve exponential amplification and significantly improve the amplification efficiency. By combining HRCA and the specificity of an aptamer to the target, Zhang et al. [70] developed a fluorescent aptamer sensor for the detection of ochratoxin A, and the detection limit reached 1.2 fg mL^{-1}. At present, in the RCA reaction process, the ring is used as a template, which is cumbersome and time-consuming [71]. Furthermore, RCA is susceptible to background signal interference in the amplification process, which may cause false positive or false negative cases [72].

In 2000, Notomi et al. [73] put forward the concept of LAMP for the first time, which has been developed to amplify nucleic acids with rapidity and high efficiency. Currently, LAMP employs DNA polymerase with strand replacement activity along with four groups of primers at a constant temperature (60–65 °C) within 40–45 min (Fig. 3B). Huang et al. [74] firstly introduced LAMP into the microfluidic chip, and they explored the suitable reaction conditions of LAMP in the microenvironment, thereby successfully developing a real-time fluorescence detection system based on isothermal amplification. A 1 nL efficient reaction volume was carried out on the microfluidic chip. For the rapid detection of the H1N1 virus, a reverse transcription LAMP (RT-LAMP) was proposed on the self-driven microfluidic chip, which realized sensitive and efficient detection of H1N1 virus in 40 min with a detection limit of 3×10^{-4} hemagglutinating units (HAU)/reaction [75]. To realize multiplex detection, Xie et al. [76] proposed a LAMP-based self-driven microfluidic chip to detect hepatitis C virus (HCV), hepatitis B virus (HBV) and human immunodeficiency virus (HIV). Due to the air permeability of PDMS, there was a pressure difference between the reaction chamber and the vacuum chamber in the microfluidic chip. Using this characteristic, the self-drive sample loading was completed within 12 min and LAMP primers were placed in different reaction rooms in advance to achieve multiple detections. 2 copies/μL of HBV, HCV and HIV target nucleic acids could be detected by a smartphone. LAMP on the microchip presents a promising application for further molecular diagnosis and genetic analysis. However, it requires many primers, which increases the possibility of primer-primer interactions, thereby causing false-positive results [77].

In addition, there are several reports of SDA, NASBA and RPA on the microchips, which are also enzyme-mediated isothermal amplification. Walker et al. [78] firstly proposed SDA in 1992, which used DNA polymerase with chain displacement activity to unlock DNA double strands and strip the old downstream strands while extending the new strands. They achieved 10^7-fold amplification of a genomic sequence from *Mycobacterium tuberculosis* within 2 h at 37 °C. In order to further improve the sensitivity and simplify the operation, Wu et al. [79] combined SDA with the lateral flow biosensors (LFB) for the visual detection of Vibrio parahaemolyticus. Under the optimum conditions, the detection limit reached 10 cfu ml^{-1}. The SDA/LFB platform showed great application potential for the rapid screening of Vibrio parahaemolyticus contamination in food and environmental samples. NASBA is also a primer-dependent nucleic acid amplification technology. A single-stranded RNA is used as a template, which is catalyzed by three enzymes of AMV reverse transcriptase, RNase H and T7 RNA polymerase. The reaction can continuously amplify the template RNA about 10^9-10^{12} times within 2 h [80]. Won et al. [81] developed an Fc region-specific aptamer as a reporter probe to characterize breast tumor cells. On the microfluidic chip platform, the aptamer was simply amplified by NASBA, and further quantified the marker protein of tumor cells. Compared with the other amplification techniques, RPA can complete the amplification process at room temperature and the target amplification product can be obtained in less time (about 20 min). RPA generally requires three main enzymes including a recombinase, an ssDNA-binding protein, and a polymerase for DNA synthesis from primer-paired target DNA [82]. Du et al. [83] combined RPA

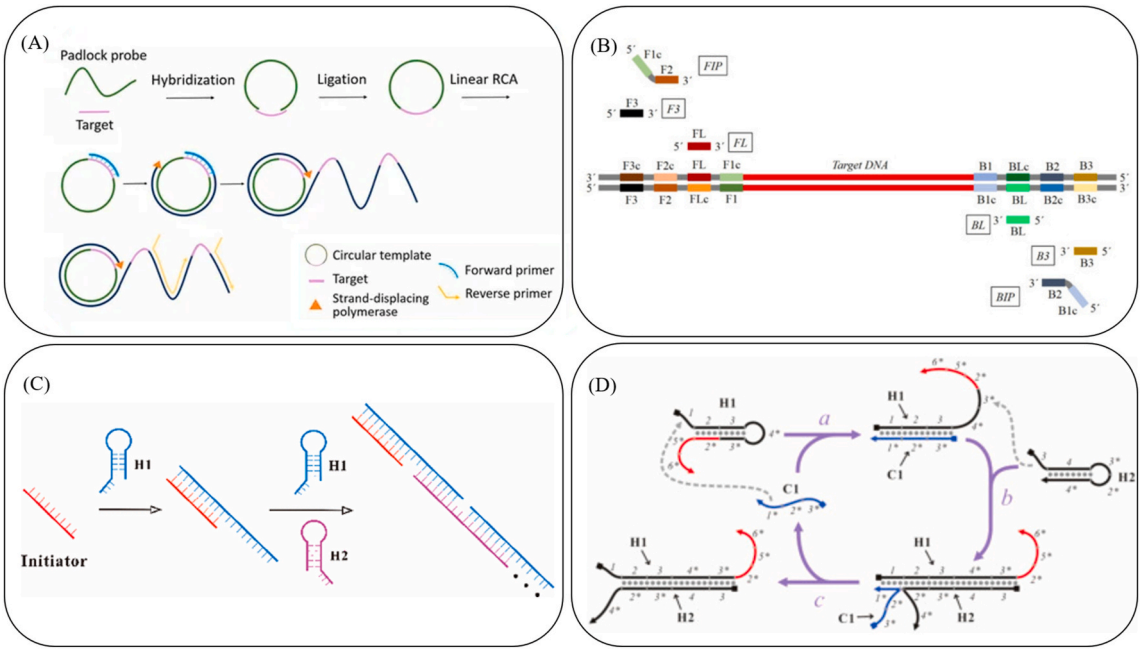

Fig. 3. (A) Schematic illustrations of typical RCA mechanism. Reproduced with permission from Ref. [113]. Copyright (2021) MDPI. (B)Typical maps of primer set location and corresponding sequence homology of LAMP. Reproduced with permission from Ref. [114]. Copyright (2020) MDPI. (C) Basic working principle of HCR. Reproduced with permission from Ref. [91] Copyright (2020) American Chemical Society. (D) Schematic illustrations of CHA circuit. Reproduced with permission from Ref. [115] Copyright (2021) Elsevier.

amplification with LFB to realize the portable rapid detection of erythromycin. They utilized a novel aptamer Ery_06 as a sensing element to capture erythromycin, and the signal was further amplified by RPA on the lateral flow strip to be visible within 15 min, and the detection limit reached 3 pmol L^{-1}.

3.2. *Non-enzyme-mediated signal amplification on microchips*

Compared with enzyme-mediated aptamer chain reaction, HCR, catalyzed hairpin assembly (CHA), and multi-arm junctions recycling (MAJR) as new aptamer chain reactions without enzyme participation, only depend on the intermolecular hydrogen bonding and simple temperature control, which greatly reduce experimental cost and improve the reproducibility [84,85].

A typical HCR system generally contains an initiator (I) and a pair of complementary DNA hairpins with sticky ends (H1, H2). The target can be amplified via the repeated production of the target strands based on the assembly of H1 and H2 (Fig. 3C). The traditional HCR sequence assembly usually takes several hours and even exists insufficient amplification, which limits its applications [86]. Chen et al. [87] used a three-dimensional chip to realize HCR assembly and signal amplification in 15 min. Because the three-dimensional chip was a compact multichannel structure with a large surface area, it could realize the sensitive sensing and multifunctional integration, which greatly reduced the reaction time. The biosensing system has the detection limits of 4 and 8 cfu mL^{-1} for *Staphylococcus aureus* and *Salmonella enterica* Typhimurium, respectively. For realizing the more sensitive detection, HCR combined with hemin/G-quadruplex was employed to detect the hlyA gene, where the HCR products could serve as the signal tag with a highly efficient catalytic ability benefiting from the associated generation of a great deal of hemin/G-quadruplex DNAzyme molecules, and the detection limit reached 1.1 fmol L^{-1} [88]. Furthermore, HCR long chain can combine with many luminescent materials to induce signal amplification [89]. Ge et al. [90] developed an ultrasensitive paper-based biosensor by combining multibranched HCR (mHCR) with graphene quantum dots (GQDs)-induced signal amplification strategies. They realized the in-situ detection of N-glycan expression on the cell surface. Currently, several new HCR signal amplification strategies are developed including multibranched HCR, localized HCR, migration HCR, netlike HCR, in situ HCR, etc [91]. The new HCR strategies have the characteristics of simplicity, rapidity, high sensitivity and strong specificity. However, the HCR hairpin probe is still crucial to improving signal amplification, and the complexity and imperfection of hairpin probe design remain to be solved [92].

CHA is a novel DNA self-assembly method by free energy-driven firstly proposed by Pierce's group [93] in 2008. CHA is developed on the basis of HCR, and its target chain acts as a catalyst throughout the reaction process, which can be replaced and recycled for the next round of CHA reaction [94] (Fig. 3D). Li et al. [95] firstly attempted to introduce CHA into multiple detection modes including colorimetry, fluorescence and electrochemistry, and obtained a high catalytic efficiency, which achieved 100-fold signal amplification in a few hours. In order to further improve the sensitivity, an agarose-based microfluidic chip combined with CHA was proposed to allow high-sensitivity detection of exosomes [96]. Exosomes could be rapidly enriched in less than 15 min, and the detection limit reached ~10^3 exosomes in a 1-μL sample. At present, the CHA strategy can be easily extended for developing various biomolecule assays because of its higher amplification efficiency and lower background signal [97–99].

The non-enzyme-mediated signal amplification strategy is based on DNA toehold-mediated strand-displacement, which is a metastable reaction. That usually suffers a plague of signal leakage because the hairpins may be opened in the absence of target. Recently, to amplify DNA fragments and minimize the leakage, a non-enzymatic MAJR signal amplification strategy with a high energy barrier was proposed [100]. Due to the complementary relationships between the hairpins, numerous four-arm and three-arm DNA branched junctions could form based on a cascade assembly of the hairpins in the presence of triggers. Zhang et al. [101] developed a stir-bar assisted MAJR signal amplification strategy for the simultaneous detection of chloramphenicol and kanamycin based on microchip electrophoresis. The aptamers on the stir-bar could specifically bind chloramphenicol and kanamycin and generated different single-stranded DNA primers, which triggered MAJR to produce numerous four-arm and three-arm DNA junctions

corresponding to the targets. And then the signals were amplified accordingly. They employed microchip electrophoresis to separate and detect the DNA multi-arm junctions, thereby quantifying the target with low detection limits of 0.41 pg mL^{-1} kanamycin and 0.52 pg mL^{-1} chloramphenicol. However, to MAJR signal amplification strategy, numerous hairpin probes are required for multi-arm junctions, which can cause a more complex analysis process. That will hinder its wide application to some extent.

3.3. Functionalized aptamer combined with nanomaterials on microchips

Since nucleic acid aptamer is composed of synthetic single-stranded oligonucleotide sequences, it is easy to modify different functional groups without affecting their intrinsic properties. Functionalized aptamers can be directly conjugated with nanomaterials to achieve signal amplification [102], which mainly embodies the following four aspects: (i) the nanomaterials have large surface area, which could be loaded with more aptamers and signal molecules to achieve signal amplification [103–105]; Zhao et al. [106] proposed a sandwich-type electrochemical aptamer sensor for detecting thrombin based on the aptamer-AuNPs-horseradish peroxidase (HRP) conjugates. AuNPs have large specific surface area which can load much more aptamers. By using the large surface area of AuNPs and catalytic reactions of HRP, a remarkable signal amplification was achieved with a detection limit of 30 fmol L^{-1}. (ii) many nanomaterials have the properties of mimic enzymes, so they can be used as bridges between aptamer and signal molecules for signal amplification [107,108]. Aptamer can inhibit the nano-enzyme activity of AuNPs by surface passivation. In the presence of target, the aptamer binds the target to leave the surface of AuNPs and reactivates the enzyme properties of AuNPs [109]. Based on this approach, Weerathunge et al. [109] developed a new colorimetric biosensing assay by combining an acetamiprid-specific S-18 aptamer and inherent peroxidase-like nanozyme activity of AuNPs. They realized the detection of 0.1 ppm acetamiprid within 10 min (iii) many two-dimensional nanomaterials have excellent fluorescence quenching effects. When the aptamer labeled with fluorophore interacts with the nanomaterial, a fluorescence response system can be constructed by fluorescence resonance energy transfer (FRET) [110]. Chang et al. [111] constructed a graphene-based FRET aptasensor for the detection of thrombin. The functionalized aptamer was conjugated to graphene oxide (GO) through π-π stacking, which could induce fluorescence quenching. The fluorescence was restored in the presence of thrombin because the specific binding between aptamer and thrombin kept the fluorescence group away from GO. The detection limit of the graphene-based FRET aptamer sensor was 31.3 pmol L^{-1} (iv) a colorimetric sensor was designed by combining the color characteristics of nanoparticles with the specificity of aptamer. Han's group [112] constructed a simple and ultrasensitive colorimetric sensor based on AuNPs-aptamer to detect saxitoxin (STX). The aptamer on the AuNPs could specifically bind STX and result in the aggregation of AuNPs and the color change. The biosensor could realize ultrasensitive STX detection with a detection limit of 10 fmol L^{-1}.

4. Aptamer-based biochemical applications on microchips

4.1. Small molecules

Antibiotics as small molecules are widely researched because the residual antibiotics in the environment can enter the human body through the food chain and cause harmful effects on human health. However, high-sensitivity detection of antibiotics remains a challenge due to low concentration and severe matrix interference in the sample. Many researchers have carried out the detection of antibiotics based on aptamer-based signal amplification techniques on microchips [101,116]. Wang et al. [117] developed a high-throughput microchip electrophoresis platform for multiplex antibiotic detection. In the presence of the target, the binding of aptamer and target could trigger the target CHA amplification and produce the strong signals which represented the concentrations of kanamycin and oxytetracycline, respectively. With the aid of CHA amplification, the signal could be amplified 300 times compared with the non-amplified system. Under optimal conditions, the detection limits of the microchip electrophoresis platform for kanamycin and oxytetracycline were 0.7 pg mL^{-1} and 0.9 pg mL^{-1}, respectively. In order to efficiently capture the antibiotics from the complex matrix, Zhang et al. [118] used a stirring bar carrying a gold-labeled aptamer to construct a universal detection system for antibiotics, which integrated microfluidic chip electrophoresis and HCR signal amplification. The aptamer on the stirring bar could unwind the duplex DNA and release the complementary chains (cDNA), which triggered HCR in the presence of source strands (H1 and H2 beacons). The amount of H1 and H2 was decreased in the reaction, and the decreased signal of H1/H2 was used for the quantification of kanamycin. The detection range for kanamycin was 1 pg mL^{-1} to 10 ng mL^{-1} with a detection limit of 0.29 pg mL^{-1}. By using the stir bar assisted sorptive extraction and RCA for signal amplification, He et al. [119] developed a microfluidic chip electrophoresis-based ratiometric aptasensor for kanamycin detection. The primer (hybrid duplex DNA, one primer to trigger RCA combined with the aptamer of kanamycin) labeled on the stir bar was displaced and released into the supernatant in the presence of kanamycin. The primer could react with the circular DNA templates (CDT), and it could also trigger RCA to produce many single-stranded products (RCA product strand, RPS). CDT and RCA were separated by microfluidic chip electrophoresis and the ratio of signals was employed to quantify kanamycin. The detection process could be completed within 3 min and the detection limit reached 0.3 pg mL^{-1} for kanamycin.

In addition, several environmental contaminants such as heavy metal ions (e.g., mercury ions [120], lead ions [121]), aflatoxin M1, 17 β-estradiol [122,123] have all gained the attention of aptamer-based microdevice researchers. Chung et al. [120] combined microdroplets with surface-enhanced Raman spectroscopy for the detection of mercury (II) ions, and the aptamer-functionalized nanomaterials were used as high-performance sensing probes. In the presence of mercury ions, aptamers were released from the surface of nanoparticles to restore the Raman enhancement effect of nanoparticles and the detection limit reached 10 pmol L^{-1}, which was three orders below the maximum contaminant level defined by the Environmental Protection Agency. Park et al. [121] proposed a microfluidic sample pretreatment platform with aptamer-linked photoluminescence graphene oxide quantum dots (GOQD) for the quantitative detection of lead ions (Fig. 4A). In the presence of lead ions, aptamers on GOQD surface could specifically capture lead ions and induce FRET between GOQD and lead ions under UV light irradiation. The system exhibited high sensitivity for lead ion detection with a detection limit of 0.64 nmol L^{-1} and a detection range from 1 to 1000 nmol L^{-1}. Although these methods can obtain high sensitivity in metal ions detection, most of them need a cumbersome operation and large equipment. Fakhri et al. [124] designed a simple paper-based microchip to detect lead ions in drinking water. They employed a colorimetric assay based on AuNPs aggregation-based aptamer. In the presence of lead ions, the aptamer disassociated from the surface of AuNPs, which caused the aggregation of AuNPs and the color change under the action of NaCl. With this colorimetric quantitative method on the nylon filter paper, the detection limit of lead ions reached 0.7 nmol L^{-1}.

There are usually multiple pollutants in a single food or environmental sample. Therefore, the methods based on specific recognition molecules for simultaneously detecting multiple chemical contaminants are urgently needed. In order to detect multiple targets in complex samples, Chen et al. [122] developed a multianalysis method coupled with magnetic encoded aptamer probes on the microfluidic chip, which realized the simultaneous detection of kanamycin, 17 β-estradiol and lead ion (Fig. 4B). The coding probe based on magnetic beads (MB) was used to selectively capture multiple targets, and then single-chain

primers were generated, which could trigger mHCR signal amplification. Finally, different lengths of complementary strands (cDNA) were hybridized with the arm of mHCR to form three multi-branched DNA nanostructures. The decreased signals of cDNA were used to quantify the targets. The detection limits of three targets reached 1.76×10^{-4} nmol L^{-1} (kanamycin), 1.18×10^{-4} nmol L^{-1} (17 β-estradiol) and 1.29×10^{-4} nmol L^{-1} (lead ions), respectively. In order to improve the detection sensitivity and analysis speed, He et al. [123] developed a microfluidic chip aptasensor, which replaced HCR with RCA based on the above similar methods. The magnetic tripartite DNA assembly structure probes were used to recognize targets for quantifying the targets in the sample. Kanamycin, aflatoxin M1 and 17 β-estradiol could be simultaneously detected within 3 min and the corresponding detection limits reduced to 0.32, 0.95 and 6.8 pg mL^{-1}.

4.2. Biological macromolecules

Aptamers can bind a huge range of biomacromolecules, among these, tumor markers [125], such as proteins [126], antigens [127] and miRNAs [128], have potential clinical value for the early-stage diagnosis of cancer [129]. The integration of aptamers and microchips can be a light on biomarker diagnosis that is significant in early-stage diagnosis and treatment of cancer. For example, vascular endothelial growth factor (VEGF) leads to the growth of many solid tumors, and therefore it could be served as a typical marker of liver and oral cancer [130,131]. Lin's group [132] developed a G-quadruplex specific probe complex 1 (iridium (III) complex 1) for the quantitative analysis of $VEGF_{165}$ from cell metabolism on the microchip. And they could achieve the dynamic analysis of aptamer-protein interaction using luminescence spectrum. Based on the RCA reaction and sandwich immunoassay structure, their group constructed an open-space microfluidic chip with fluid walls to integrate cell culture for on-line detecting VEGF with fluorescence imaging, and the detection limit reached 10 pg mL^{-1} [133]. In order to monitor bioaffinity adsorption of VEGF, Chen et al. [134] developed an RNA aptamer microarray by the surface transcription reaction of T7 RNA polymerase on a microfluidic chip. The nonspecific adsorption (<0.2%) was observed on the RNA aptamer microarray with surface plasmon resonance imaging. Prostate-specific antigen (PSA) is regarded as a vital marker for the diagnosis of prostate cancer. A magnetic controlled photoelectrochemical (PEC) sensing system was constructed for the quantitative detection of PSA [135], which used a reduced GO-functionalized $BiFeO_3$ as the photoactive material and target-triggered HCR for signal amplification. Under optimal conditions, the detection limit toward target PSA was 0.31 pg mL^{-1}. Additionally, exosomes are also recognized as promising biomarkers for noninvasive diagnosis and prognosis [136]. However, because of their low concentration and small size (30–150 nm), efficient and rapid detection of exosomes remains a challenge. Qian et al. [96] achieved simultaneous in-situ detection of exosomal miRNAs and intact exosomes on the agarose-based microfluidic chip, which had an on-line enrichment function based on strong water permeability and the capillary effect. They integrated the CHA strategy into the agarose-based microfluidic

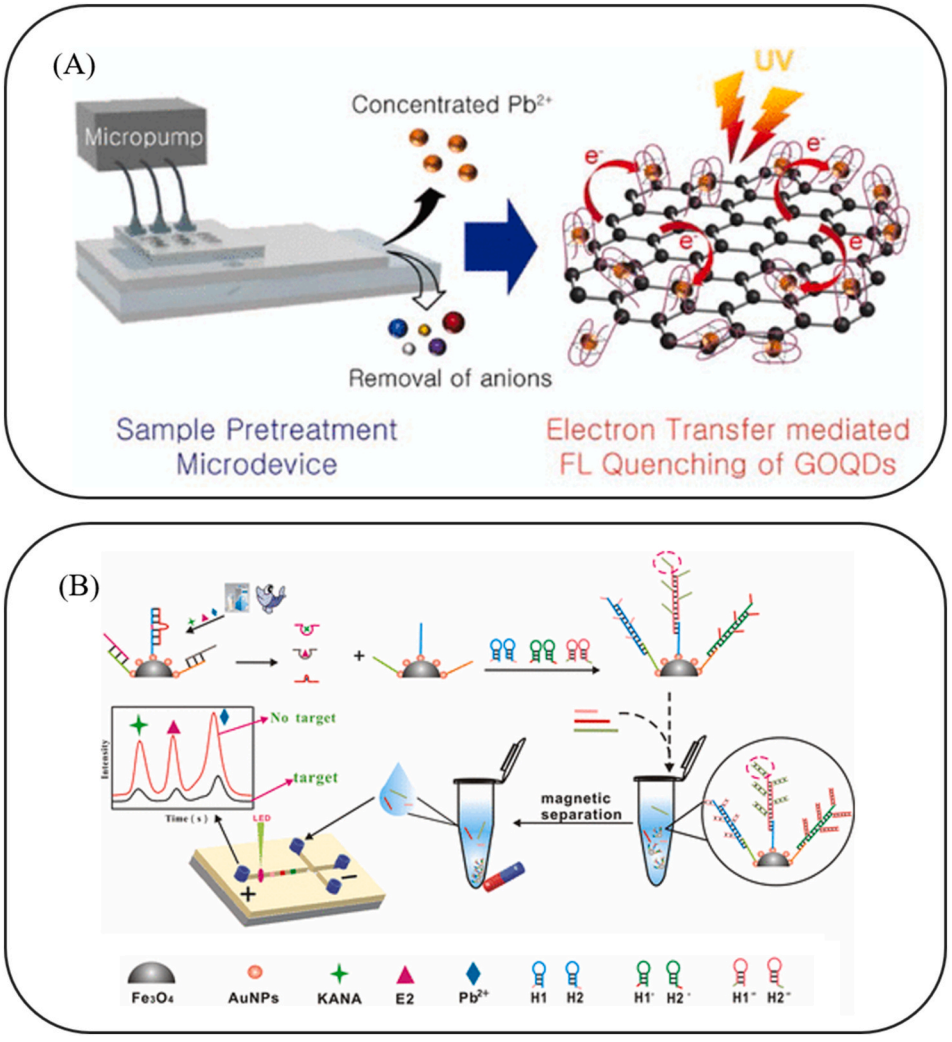

Fig. 4. Small molecule assays on aptamer-based microchips. (A) Schematic illustration of the microfluidic sample pretreatment platform with aptamer-linked GOQD for the quantitative detection of lead ions. Adapted with permission from Ref. [121]. Copyright (2015) American Chemical Society. (B) Schematic illustration of simultaneous determination of multiple chemical contaminants by coupling aptasensor and HCR. Adapted with permission from Ref. [122]. Copyright (2019) American Chemical Society.

chip, and the target exosomes could be automatically enriched at the end of the microchannel. The high detection sensitivity with ~10^3 exosomes in a 1-μL sample could be achieved. Benefiting from the powerful signal amplification of HCR, a paper-based electrochemical sensor for ultrasensitive detection of exosome was also proposed by Liu et al. [137]. By recognizing exosomes based on Zr-metal-organic frameworks and aptamer, HCR was triggered to form G-quadruplex DNAzymes, which converted the exosome detection to DNAzyme-catalyzed signal amplification. The paper-based biosensor could realize the ultrasensitive exosome assay with a detection limit of 5 × 10^3 particles/mL.

In recent years, more and more attentions have been paid to peptides, which are also important clinical indicators [138]. Ma et al. [139] proposed a paper-based microchip for peptide electrochemiluminescence (ECL) detection based on aptamer specific recognition along with HCR signal amplification method (Fig. 5A). The aptamer/initiation strand (AP/IS) was used to recognize the target peptide. When the target peptide bound the AP, the AP/IS could release IS. HCR could allow the target peptide to induce IS and form long dsDNA polymers. Numerous ECL probes embedded into the grooves resulted in a significant signal output. The paper-based ECL microchip could achieve a detection range of 25 pmol L^{-1} to 50 nmol L^{-1} with a detection limit of 8.33 pmol L^{-1} for MUC1 (a model peptide).

Thrombin, as a vital macromolecule, plays a key role in cardiovascular disease, senile dementia, and the regulation of immune and inflammatory reactions [140–142]. Lin et al. [61] constructed the aptamer-modified microchannels which could specifically capture thrombin. The aptamer-based specific recognition microchip along with the dual-amplification strategy of RCA and hemin/G-quadruplex system could achieve high-throughput and ultra-sensitivity colorimetric assay. The detection range for thrombin was 0.100–50.000 pg mL^{-1} with a detection limit of 0.083 pg mL^{-1}. Meanwhile, the HCR signal amplification method combined with a paper-based electrochemical detection microdevice also exhibited high sensitivity and excellent specificity for thrombin with a detection limit of 16.7 fmol L^{-1} [143] (Fig. 5B). To simplify operation and reduce the complexity, a paper-based RCA inhibition assay system for thrombin detection was proposed [144]. Aptamer was used as a primer to initiate RCA reaction and bind fluorescent dyes (SYBR Gold or QuantiFluor dyes). When thrombin existed, aptamer could specifically bind thrombin and inhibit RCA reaction. Otherwise, in the absence of thrombin, the aptaprimer could initiate RCA and produce long DNA products which were detected by binding fluorescent dyes. The paper-based RCA inhibition assay system could realize the detection of thrombin in 15 min with a detection limit of 240 pmol L^{-1}.

4.3. Microorganisms

Rapid and high-sensitivity detection of foodborne pathogens is greatly important to food safety and public health because it can lead to foodborne illnesses and even death. *E. coli* O157:H7, as a highly threatening pathogen, is commonly detected by ELISA or PCR. However, the traditional detection methods are usually tedious, time-consuming, and cause large expenses. Recently, aptamer-based detection methods

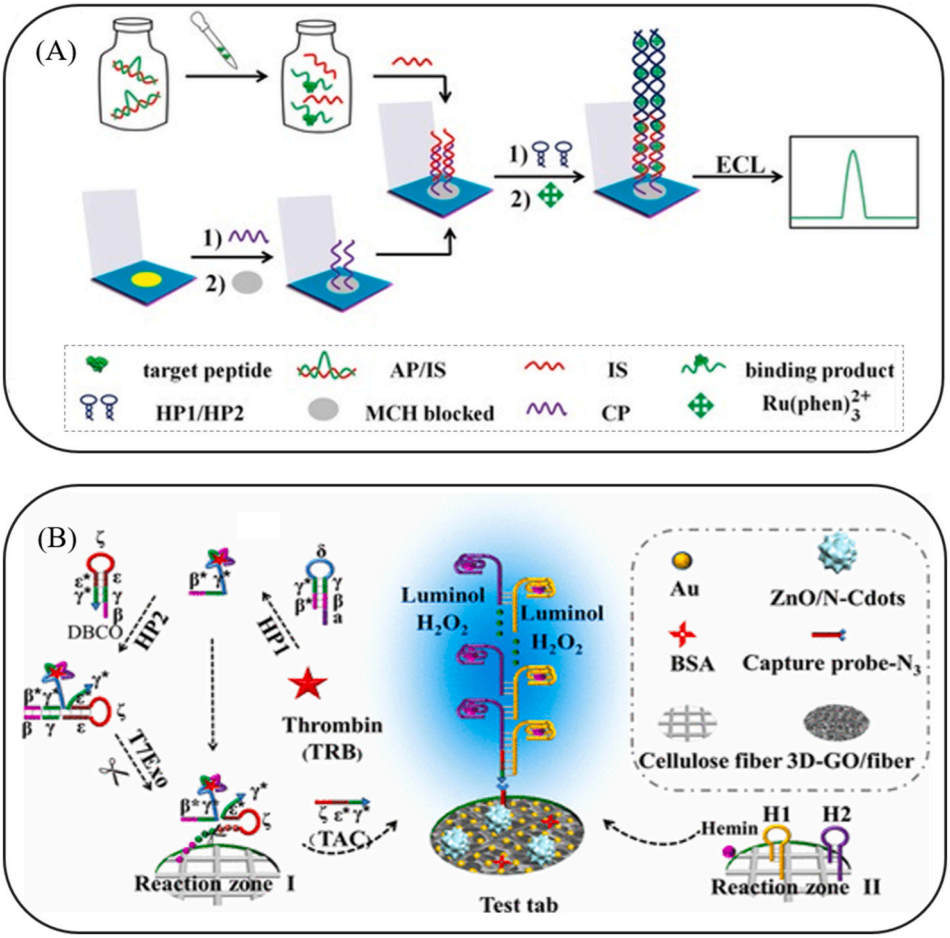

Fig. 5. Macromolecule assays on aptamer-based microchips. (A) Schematic illustration of the aptamer-based paper microchip for the detection of peptides. Adapted with permission from Ref. [139]. Copyright (2017) John Wiley and Sons. (B) Schematic illustration of HCR signal amplification method combined with a paper-based electrochemical detection microdevice. Adapted with permission from Ref. [143]. Copyright (2017) American Chemical Society.

of *E. coli* O157:H7 have been reported due to their rapid, high-sensitivity, cost-effective analysis [145–148]. For example, a dendrimer-aptamer modified microfluidic chip was employed to detect *E. coli* O157:H7 cells [62]. Poly(amidoamine) dendrimer was immobilized on the PDMS channel and further modified with *E. coli* O157:H7 capturing aptamer. Based on the sandwich structure of capturing aptamer | bacterium (*E. coli* O157:H7) | aptamer-primer, when the target bacteria were present, the RCA reaction was triggered to enhance the fluorescence signals, which resulted in a 50-fold increase in signal intensity than that of without RCA. The detection limit was reduced to 100 cells•mL^{-1}. Meanwhile, in situ capturing RCA aptamer surface modification approach in microfluidic channels could also improve the capturing performance of *E. coli* O157:H7 [149], which allowed for rapid and sensitive pathogen detection. Furthermore, a double RCA signal amplification system, including capturing RCA (cRCA) and signaling RCA (sRCA), could further cause the fluorescence signal enhancement by up to 250 times compared with the system without RCA (Fig. 6A) [63]. The detection limit of 80 cells•mL^{-1} was achieved.

Currently, the highly infectious viruses, such as H1N1 viruses, human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV) [76], etc, have caused a huge threat to human health and the social economy. H1N1 viruses, as one of the most infectious diseases in the past decade, the specificity and sensitivity of detection are still relatively low. A reverse transcription LAMP approach on a self-driven microfluidic chip was introduced to detect H1N1 viruses [75] (Fig. 6B). The microfluidic chip integrated virus isolation, lytic virus, LAMP, and colorimetric detection, and the whole detection process was only 40 min. The limit of detection reached 3×10^{-4} hemagglutinating units/reaction. Noroviruses are the major causes of acute gastroenteritis, which can be usually detected with many methods, including enzyme-linked immunosorbent assay (ELISA) [150], western blot [151], and nucleic acid-based methods [152], etc. However, rapid, simple, and ultra-sensitive detection methods are still urgently need as an alternative to the traditional assays. An aptamer-based fluorometric detection method for norovirus on the paper-based device was reported [153]. The norovirus-specific aptamer was labeled with 6-carboxyfluorescein, which could be quenched by multiwalled carbon nanotubes (MWCNT) or GO. When noroviruses were present, the fluorescence was restored due to the good specificity of the aptamer. The quantitative detection of norovirus could be successfully performed about 10 min, and the detection limits reduced to 4.4 ng mL^{-1} (using MWCNT) and 3.3 ng mL^{-1} (using GO), respectively.

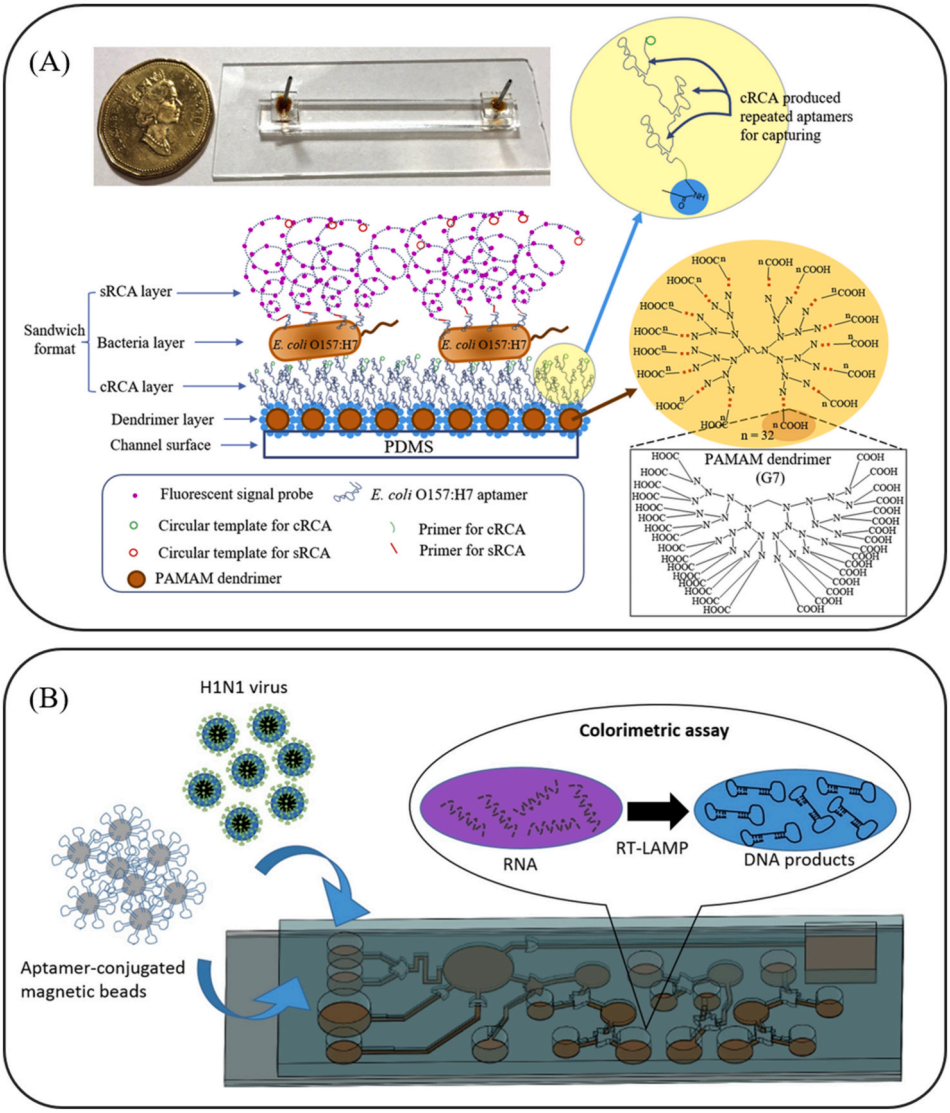

Fig. 6. Microorganism assays on aptamer-based microchips. (A) Schematic illustration of a dual-RCA for detecting *E. coli* O157:H7 cells. Adapted with permission from Ref. [63]. Copyright (2020) Elsevier. (B) Schematic illustration of a reverse transcription LAMP for detecting H1N1 viruses on a self-driven microfluidic chip. Adapted with permission from Ref. [75]. Copyright (2019) Elsevier.

4.4. Human cells

The combination of aptamer and microchip in cell detection has the advantages of high sensitivity and high accuracy. Circulating tumor cells (CTCs), as a key biomarker for cancer diagnosis, follow-up and treatment monitoring, exist in various solid tumors. However, the numbers of CTCs in complex samples are extremely low, and the detection faces great challenges. Sheng et al. [154] developed an AuNPs-aptamer-mediated cell microfluidic device, which was used to improve the capture efficiency of CTCs. The microchannel was firstly coated with avidin and then bound the biotinylated aptamer-conjugated AuNPs by biotin-avidin interaction. Aptamers, as efficient affinity carriers, could capture the target cancer cells when the sample passed through the channel. The binding affinity of AuNP-aptamer conjugates was 39 times higher than that of aptamers alone, and the cell capture efficiency was increased from 49% to 92% in the laminar flow flat microchannel. Furthermore, biomimetic nanoparticles combined with a microfluidic chip could also achieve efficient and specific capture of CTCs [155] (Fig. 7A). Leukocyte membrane fragments were camouflaged onto Fe_3O_4 magnetic nanoclusters, which were modified with aptamer SYL3C to load into the microfluidic channel with the help of magnets. The device could rapidly detect 90% rare CTCs in the blood within 20 min.

In addition to improving cell capture efficiency by combining functional aptamers with nanomaterials, detection sensitivity can also be improved through the isothermal amplification of nucleic acid aptamers. Zhang et al. [156] proposed a dual-function microfluidic chip for cell culture and online interleukin 8 (IL-8) detection simultaneously. The microfluidic chip was designed with two high microchannels connected by several narrow microchannels. One microchannel was used to culture cells and the other microchannel was used to immobilize capture the antibody for IL-8 detection. By building the sandwich structure of antibodies, IL-8, and aptamers, the immunoassay of IL-8 based on the biotin-streptavidin linkage and RCA was successfully performed in tumor-derived endothelial cells and human umbilical vein endothelial cells. The detection range for IL-8 was 0.84 pmol•L^{-1}-13.44 pmol L^{-1} with a detection limit of 0.84 pmol L^{-1}.

Although the above methods have high sensitivity, accuracy and specificity in the detection of cell, the fast, simple, multiplex detection methods are still needed for clinical diagnosis. Liang et al. [157] combined GO and aptamer to realize multiple detections of cancer cells by FRET effect based on the paper-based microdevice (Fig. 7B). The mesoporous silica nanoparticles coated with quantum dots were modified to the aptamer chain, and the fluorescence of MSNS/QDs-aptamer bioconjugates could be quenched by GO. When the target cells existed, the fluorescence was restored, and the changes in color could be directly observed using the naked eye. On the paper-based microdevice, the three-color fluorescence output signals based on three cancer cells corresponding to different colored quantum dots were achieved for the rapid, simple, high-sensitivity and multiplex detection of cancer cells. The detection limits reached 62, 65 and 70 cells•mL^{-1} for MCF-7, K562 and HL-60 cells, respectively.

5. Conclusions

In recent years, aptamer, as a recognition element in the microchips, is gaining widespread attention, and aptamer-based signal amplification techniques on microchips are evolving at a rapid pace. Since the traditional signal amplification methods based on enzyme-mediated have some drawbacks such as high cost, strict temperature control and complex operation. Several aptamer-based non-enzyme-mediated signal amplification techniques have been proposed to achieve aptamer chain

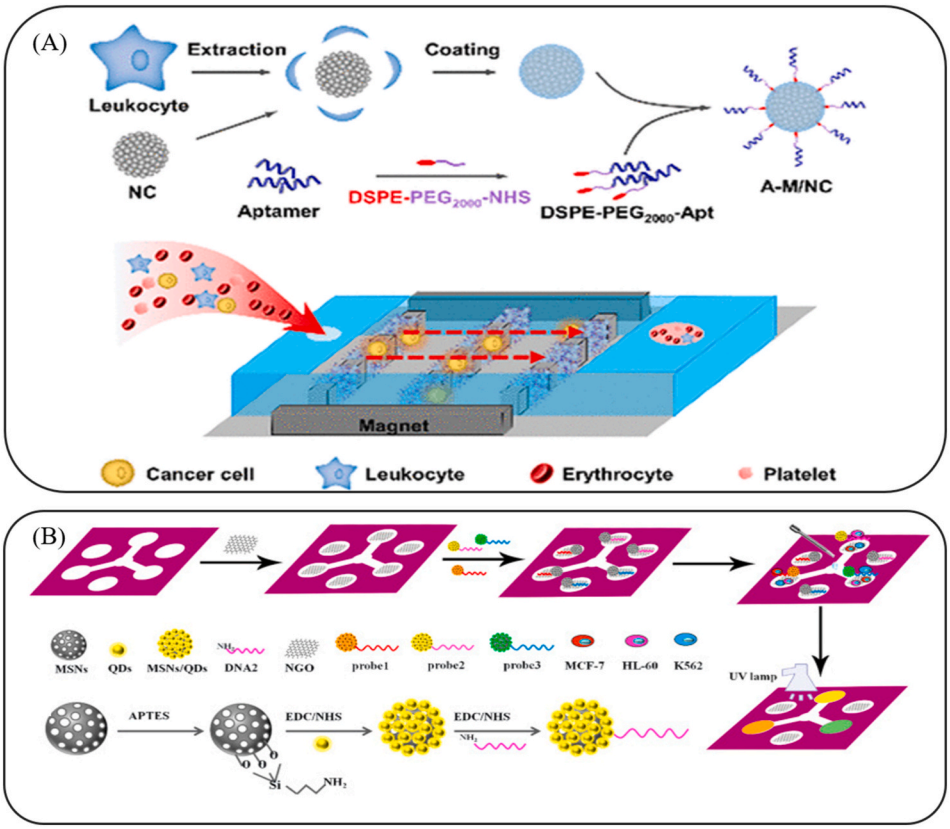

Fig. 7. Cell assays on aptamer-based microchips. (A) Construction of aptamer-magnetic nanoclusters and the schematic illustration of a biomimetic microfluidic system for capturing tumor cells. Adapted with permission from Ref. [155]. Copyright (2019) American Chemical Society. (B) The detection process of cytosensor by FRET based on paper microchips. Adapted with permission from Ref. [157]. Copyright (2016) Elsevier.

reaction with characteristics of constant temperature, easy operation, stable reaction and specificity [158]. Meanwhile, the signal amplification can also be achieved by combining functionalized aptamers with nanomaterials aided by the size effect, magnetism and enzyme catalytic activity of nanomaterials. Based on the integration of aptamers and microchips, ultrasensitive trace bioassays can be realized. It exhibits a promising application in the detection of small molecules, macromolecules, microorganisms and cells.

However, aptamer-based signal amplification on microchips will still face many challenges as well as opportunities for researchers: (i) the aim of ultra-trace bioanalysis is to discover the key biomarkers to realize early-stage diagnosis and therapy. However, at present the real applications of aptamer-based signal amplification strategy on microchips are still relatively few. Firstly, the sufficient sensitivity and specificity of these methods in clinical settings still need to be validated further. Secondly, there are still many important biomarkers that do not have corresponding aptamers with high specificity and affinity. Thirdly, the fabrication process of traditional microchips is still complex. Simplifying the production process of microchips or constructing a new production system is desirable. The above three aspects will be future important development directions; (ii) Although these techniques can achieve high-sensitivity detection, they require a long waiting time to realize enough binding and signal amplification between the aptamer and target molecule, making them unsuitable for the demands of rapid and high-throughput assay on microchips. Improving the efficiency of signal amplification reaction process and reducing the reaction time are very essential in bioanalysis; (iii) an effective and fast non-target removal process will be beneficial to the aptamer-based microchips in achieving high sensitivity. However, most of the microchips (especially the paper-based materials) are difficult to be washed thoroughly in the process of experiment. Thus, the background interference will degrade the detection sensitivity, which needs to be further resolved [21]; (iv) the aim of developing aptamer-based microdevices is to make them become the powerful analysis tools in biomedical science and environmental fields. Therefore, the intelligent levels of the whole microdevice need further improvement, which achieves its potential application to the maximum extent.

Although challenges abound at present, many researchers have devoted efforts to the development and innovation of aptamer recognition along with signal amplification approaches on the microchips. We believe that there is a promising future for aptamer-based microchips across biomedical science, environmental monitoring, and a wide range of other fields.

CRediT authorship contribution statement

The manuscript was written by Ranran Xu and Yongqiang Cheng, along with the structural and linguistic contributions provided by all authors. All authors have given approval to the submission of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Financial supports from Natural Science Foundation of Shandong Province (No. ZR2021MB082), Fundamental Research Funds of Shandong University (No. 2020GN022), Natural Science Foundation of China (No. 21207080), National Key Technology R&D Program of the Ministry of Science and Technology (No. 2012BAF14B04) are gratefully acknowledged.

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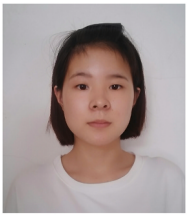

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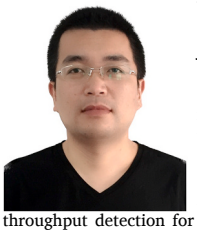

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