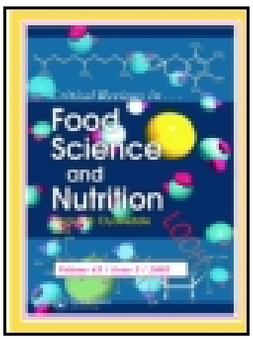

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REVIEW

Research advances of DNA aptasensors for foodborne pathogen detection

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ABSTRACT

Aptamers, referring to single-stranded DNA or RNA molecules can specifically recognize and bind to their targets. Based on their excellent specificity, sensitivity, high affinity, and simplicity of modification, aptamers offer great potential for pathogen detection and biomolecular screening. This article reviews aptamer screening technologies and aptamer application technologies, including gold-nanoparticle lateral flow assays, fluorescence assays, electrochemical assays, colorimetric assays, and surface-enhanced Raman assays, in the detection of foodborne pathogens. Although notable progress (more rapid, sensitive, and accurate) has been achieved in the field, challenges and drawbacks in their applications still remain to be overcome.

KEYWORDS

Aptamer; food safety; rapid detection; SELEX; *Escherichia coli*; *Salmonella*

Introduction

Foodborne pathogens, such as *Salmonella*, *Streptococcus* spp., *Escherichia coli*, and *Vibrio* spp., are harmful species that cause infectious diseases through food contamination and proliferation. Common infection complications, including acute gastroenteritis diarrhea, headache, vomiting, and even death, are called food poisoning. It is worth mentioning that foodborne pathogen infections have increased globally (Dallman et al., 2014; Deng et al., 2016; Gopinath et al., 2013). The WHO announced that food poisoning outbreaks are associated with billions of people annually worldwide. The CDC reported that there were 48,000,000 cases of foodborne illness, including 128,000 cases of inpatient and 3000 cases of death in the USA each year (Kirk et al., 2015). In April 2018, the USDA announced the recall of 53,000 pounds of standard meat products in Texas due to possible *Salmonella* contamination. In the same month, the FDA announced the recall of 207 million eggs with suspected *Salmonella* contamination in North Carolina (Hassan et al., 2018). In China, the greatest threat to food safety is also caused by foodborne pathogen contamination. According to the statistics data from National Health and Family Planning Commission of the People's Republic of China, it was reported that there were 3181 cases of foodborne pathogen poisoning in China in 2015, accounting for 53.7% of the total food poisoning cases (http://www.nhc.gov.cn/cms-search/xxgk/getManuscriptXxgk.htm?id=8d34e4c442c54d33909319954c43311c). The estimate indicated that foodborne pathogen poisoning presented a major burden on public health and heavy financial burden for local governments (Ralston, Kite-Powell, and Beet 2011). It can be seen that accurate and early diagnosis of foodborne pathogens will assist in controlling and preventing the spread of infection; therefore, it is crucial to identify foodborne pathogens with efficient methods.

In recent years, nucleic acid-based assays have been widely used for the accurate detection of foodborne pathogens (Ha, Zhang, and Lee 2016; Hancks et al., 2015; Patel et al., 2017). These assays can be classified into two main categories: nucleic acids as the detection marker and amplification template and nucleic acids as the probe. For the former category, PCR is the widely used and standard method for the detection of various pathogens due to its rapidity, sensitivity, and specificity compared to culture-based methods (Akyol, 2018; Baba et al., 2014; Hayashi et al., 2013; Yuet al., 2010). A potential drawback for PCR-based methods is the inability to discriminate viable from dead pathogens because DNA can be released from both alive and dead pathogen cells. Moreover, it is difficult to use PCR techniques in food sample analysis, as many PCR inhibitors in food samples would cause false positive or negative results. For the latter category, exogenous aptamers are the common probes used for pathogens (Davydova et al., 2016; Punnarak et al., 2012). For diagnostic purposes, aptamers are appropriate substitutes to antibodies (Acquah et al., 2019).

Aptamers are (25–90 nt) single-stranded nucleic acid molecules (DNA or RNA) generated from Systematic Evolution of Ligands by Exponential Enrichment (SELEX), an in vitro selection technique. As bio-recognition elements, aptamers

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can recognize various target ligands with high specificity, including ions, drugs, mycotoxins, pathogens or whole cells (Dong et al., 2015; Dupont et al., 2015; Meng et al., 2015; Tan et al., 2016). Aptamers exhibit good affinity to specific targets, within a dissociation coefficient (Kd value) from pM to mM. Compared to antibodies, aptamers show higher stability to temperature, pH and ionic strength, easier synthesis and modification, longer shelf life, and lower cost. Based on the superiority, aptamers have attracted a lot of attention in construction of aptamer-based assays and sensors, referred to as apta-assays and aptasensors, respectively (Chung et al., 2018; Ling et al., 2016). Until now, diverse aptasensors have been established for foodborne pathogenic detection, including *E. coli* (Zou et al., 2018), *S. aureus* (Qiao et al., 2018), *Salmonella* spp. (Shin et al., 2018), *Vibrio parahaemolyticus* (Wu et al., 2018), *Campylobacter* spp. (Bruno and Sivils, 2017), *Listeria* spp. (Liu et al., 2018c), and *Shigella flexneri* (Duan et al., 2013a). Aptasensors are simple and rapid, and do not require tedious washing and enrichment steps. In this review, we highlighted recent advances in aptasensors used for foodborne pathogens. We also assessed the superiority and limitations of reported aptasensors to determine a rational design for foodborne pathogen aptasensors.

Aptamer screening technologies for foodborne pathogen aptamer

Aptamers are obtained from DNA or RNA library of nucleic acids by in vitro selection. SELEX is employed to select aptamers recognizing various target, including small molecules, proteins, bacteria and viruses, cell lines and even whole cells. Cell-SELEX refers to in vitro method of aptamer selection using live cells (bacteria, viruses, cells) as targets. The advantages of cell-SELEX superior to other SELEX are as following: (1) The generation of aptamer probes by cell-SELEX could be in favor of accurate detection and disease diagnosis, due to using molecules on the cell surface as targets. (2) Unlike other protein or molecule-SELEX, it is not necessary to know the biomarkers for cell-SELEX method in advance, while high purity recombinant protein or molecules is necessary prior to aptamer selection. Always, a successful cell-SELEX of aptamers can discover new unknown biomolecules, which can be partitioned to identify the target as potential biomarkers. (3) In cell-SELEX, the target molecules are all in native conformation and it eases the complexity of dealing with target conformation. In addition, it is not needed to fix the cells on solid support, since the target molecules are no the cell surface in the cell-SELEX.

On the other hand, there are still also certain technical challenges in the cell-SELEX. (1) It is not easy to screen aptamers against surface proteins with low expression on the cell surface. (2) The used cells should be viable and normal growing, which could reduce nonspecific binding. (3) Compared to other SELEX, cell-SELEX requires more rounds of selection to obtain high selectivity and affinity aptamers, which cost more money and time. Fortunately, significant work has already done to address these challenges.

Generally, oligonucleotides in the libraries contain a randomized region of 20–60 nt flanked by two constant regions of ~20 nt each, which are used for primer binding and PCR amplification. Different from single-stranded DNA library, a promoter sequence for T7 RNA polymerase is introduced into the 5′-end region of ssDNA library to generate a RNA library (Dua et al., 2016). The screening technologies are classified into two types: SELEX and Non-SELEX.

SELEX for foodborne pathogen aptamer

The SELEX process for aptamer selection is a universal process containing repetition of 5 mains steps (binding, partition, elution, amplification, and conditioning). The scheme of SELEX is shown in Figure 1. First, a combinatorial DNA library is synthesized. The sequences of the nucleic acid library are composed of random sequences in the middle and flanked by fixed sequences as primer binding sites in two terminus. The random region is normally of 20–40 nt, in which contains sequences with library capacity of 10^{13} to 10^{15}. Second, specific target pathogens are incubated with under suitable conditions. Third, the weakly bound and unbound nucleic acids are partitioned from those bound nucleic acids specifically to the targets, and then they are eluted from the target and amplified as template by PCR or RT-PCR. Critically, partition is one of the most crucial step for aptamer selection and strongly affects binding constant of the aptamers to be selected. This whole SELEX process involves several repeated rounds in order to obtain nucleic acid with highest affinity and specificity to the target. The number of necessary rounds depends on many factors, such as target features and concentration, design of the starting random oligonucleotide library, selection conditions, or the efficiency of the partitioning method. The last SELEX round is terminated after the amplification step and the PCR

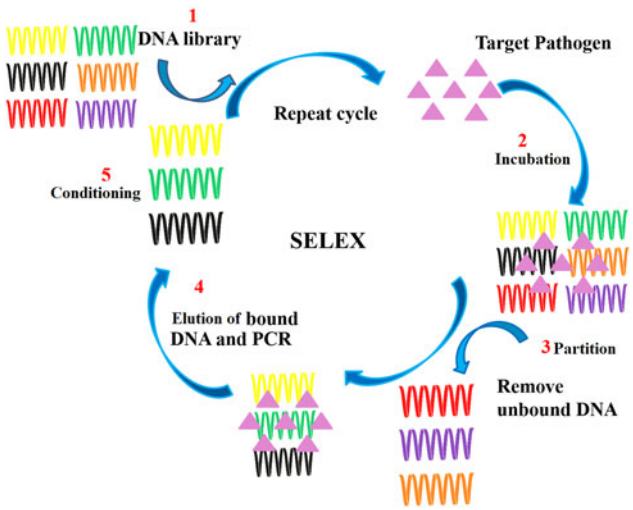

Figure 1. Schematic illustration of aptamer selection for foodborne pathogens using the SELEX. Briefly, five steps are carried out during SELEX. 1. Synthesized DNA library in vitro; 2. Incubation the target pathogens with the DNA library; 3. Partition of the unbound nucleic acid sequences; 4. Elution of bound nucleic acid sequences from the target ligands; 5. The target-bound nucleic acid sequences used as template for the NEXT PCR amplification. The amplified products are used as new DNA library for the next round of selection.

products are cloned to get individual aptamer clones, which are sequenced subsequently. For RNA aptamer SELEX, an additional step of RNA SELEX includes an additional step, that bound RNA should be reverse-transcripted to cDNA as subsequent PCR amplification (Amraee et al., 2017).

In addition to the selection process, a library is gradually enriched by sequences possessing higher affinity for the target. After 5-15 SELEX rounds, aptamers can be obtained, although in some cases, much faster enrichment can be achieved. Finally, the library is cloned and sequenced to confirm the sequences of individual aptamers. The structure analysis of aptamers can be used to evaluate the affinity of aptamers to their targets. To screen pathogen aptamers, whole-cell SELEX, magnetic bead-SELEX, and affinity chromatography SELEX are often applied (Bruno, Richarte, and Phillips 2014; Hedayati Ch et al., 2016; Wang et al., 2017b). In whole-cell SELEX, whole bacterial cells are used as targets that broaden the screening range and avoid target enrichment (Dwivedi, Smiley, and Jaykus 2013). In magnetic bead-SELEX, the sphere surface of the complex is beneficial to maximize target exposure and ease separation. The advantages of affinity chromatography SELEX include low cost, excellent reproducibility, and ease in determining the binding sites (Kowalska et al., 2014).

Non-SELEX for foodborne pathogen aptamer

Because of high-background, SELEX-based partitioning methods need rounds of amplification and partitioning, which is a time-consuming process. Thus, how to rapidly obtain desirable aptamers has been a key technology. Non-SELEX is a variation derived from the capillary electrophoresis (CE) without PCR amplification and strand separation at the end of each subsequent round first reported by Berezovski in 2006. Non-equilibrium capillary electrophoresis of equilibrium mixtures (NECEEM), a typical non-SELEX method, is a highly efficient method for partitioning. Non-SELEX was firstly used for h-RAS aptamer screening. Different from SELEX, the Non-SELEX is a more efficient screening method without PCR steps. After 2–3 rounds of separation and screening, the aptamer for targets can be achieved (Berezovski et al., 2006a, 2006b). Kim reported the aptamer specific to lipopolysaccharide in *E. coli O55:B5* by Non-equilibrium capillary electrophoresis screening method within 3 rounds of non-SELEX (Maeng et al., 2012). Common SELEX includes 8–15 screening rounds costing 1–3 months. However, it takes hours to days for aptamer screening, which is much shorter and easier than SELEX process. The limitation of Non-SELEX is the requirement of capillary electrophoresis, which is only suitable for macromolecular substances.

There are 3 major features of non-SELEX of aptamer. (1) Non-SELEX is rapid and simple: non-SELEX selection cost only 1 h and can be performed in an automated manner using commercial CE instrument. (2) It is available to accurately determine the aptamers in a naïve DNA library. (3) The most remarkable advantage of non-SELEX is its applicability to non-amplifiable DNA libraries, such as those of tag-modified DNAs obtained by DNA synthesis. Tags can be conjugated with magnetic beads or other functional nanoparticles to derive novel non-SELEX strategies. These features make non-SELEX a potentially indispensable tool for drug discovery.

No matter SELEX or non-SELEX, there is no standardized selection protocol for any target. The aptamer selection process has been modified over the years and become more efficient and less time-consuming, to reach higher specificity and affinity.

The aptamers for foodborne pathogens, such as *E. coli*, *Salmonella*, and *S. aureus*, have been screened by SELEX and non-SELEX (Table 1). These aptamers can be directly used for aptasensor establishment for rapid, sensitive, and specific foodborne pathogen detection.

Aptasensors for foodborne pathogens

Pathogen detection is crucial for food safety and public health. Three main application areas for foodborne pathogens are the food industry, environmental quality control, and clinical diagnosis. Great efforts have contributed to relative fundamental studies, method performance studies, and the development of new applied methods (Hung et al., 2017; Park et al., 2017; Poltronieri, Primiceri, and Radhakrishnan 2019).

Until now, aptasensors have been rarely applied in food safety and public health (Alamer et al., 2018; Li et al., 2018; Teng et al., 2016). The core reason for this might be the complexity of the aptasensors and processes including sample extraction, purification, enrichment and separation. Foodborne pathogen detection is a crucial factor affecting public health and food safety. The remaining efforts should be transferred to fundamental studies, method performance studies, and the development of new applied methods (Neethu et al., 2018; Xu et al., 2018). Aptasensors demonstrate numerous advantages in the recognition of biosensors over traditional immunological sensors. In addition, aptamers are small in size, chemically stable, and cost-effective. Based on these characteristics, nucleic acid aptamers are widely used in the field of biosensors. Therefore, numerous aptamer-based biosensors have been developed to detect foodborne pathogens (Oh et al., 2016, Wang et al., 2017a, Zarei et al., 2018). Hence, we review and classified the reported aptamer-based biosensors for foodborne pathogen detection (Figure 2).

Colorimetric aptasensors

Colorimetry is a process transforming the target signal into color changes, which has attracted extensive concerns due to its convenience of visual readout and simple procedures (Alhogail, Suaifan, and Zourob 2016; Oh et al., 2016). The key technology for colorimetry is the choice of a chromogenic substrate and corresponding catalytic enzyme. Typically, horseradish peroxidase (HRP) showed high substrate specificity and catalytic efficiency, which can catalyze oxidation of substrates, including 3,3′,5,5′-tetramethylbenzidine (TMB)

Table 1. Screened aptamers of foodborne pathogens.

Pathogen	Target	Screening method	Sequence (5′–3′)	Reference
Escherichia coli	*O157:H7*	Whole-cell SELEX	ACCAGTAGACTTTCAACTTTACTGCCATCGTGTGCCCTAA	(Yu et al., 2018; Amraee et al., 2017)
		Non-SELEX	CCGGACGCTTATGCCTTGCCATCTACAGAGCAGGTGTGACGG	(Guo et al., 2014)
	O111:B4	Magnetic bead-SELEX	ATCCGTCACCCCTGCTCTCGTCTATGAAGTAACAAAGATAGGAGCAATCGGGTGGTGTTGGCTCCCGTAT	(Bruno, Carrillo, and Phillips 2008)
	O111	Whole-cell SELEX	ATCCGTCACACCTGCTCTACTGGCCGGCTCAGCATGACTAAGAAGGAAGTTATGTGGTGTTGGCTCCCGTAT	(Luo et al., 2012)
	O55:B5	Non-SELEX	TAGCCGGATCGCGCTGGCCAGATGATATAAAGGGTCAGCCCCCCA	(Zhu et al., 2019a)
Salmonella	*Typhimurium*	Whole-cell SELEX	ACGGGCGTGGGGGCAATGCCTGCTTGTAGGCTTCCCCTGTGCGCG	(Moon et al., 2013)
		SELEX	TATGGCGGCGTCACCCGACGGGGACTTGACATTATGACAG	(Joshi et al., 2009)
	O8	Whole-cell SELEX	GATCCGGGCCTCATGTCGAACACCCCCCAACTAAAACAACAAAACACCACCGCCATTGAGCGTTTATTCTGAGCTCCCA	(Dwivedi, Smiley, and Jaykus 2013)
		SELEX	TGATCCGGGCCTCATGTCGAACACCCCCCAACTAAAACAACAAAACACCACCGCCATTGAGCGTTTATTCTGGCTCCCA	(Duan et al., 2013b)
	enteritidis	Magnetic bead-SELEX	GGGUUCACUGCAGACUUGACGAAGCUUGAGAGAUGCCCCCUGAUGTGCAUUCUUGUGUUGUUGCGGCAAUGGAU CCACAUCTACGAAUUC	(Zhang et al., 2018)
Staphylococcus aureus	*aureus*	Whole-cell SELEX	GCGCCCTCTCACGTGGCACTCAGAGTGCCGGAAGTTCTGCGTTAT	(Moon et al., 2015)
	Enterotoxin	SELEX	TTTGGTATTGAGGGTCGCATCCACTGGTCGTTGTCTGTTGTCTGTTATGTTGTTTCGTGATGGCTCTAACTCTCCTCT	(Huang et al., 2015)
	Teichoic acid	SELEX	GGGAGUUUUGAUACGGCUUCAUGCAGUAAUGUUUUUAU	(Maeng et al., 2012)
	Eenterotoxin B	SELEX	AGCAGCACAGAGGTCAGATGTATACTTCTAAAATTTGTTTGTATCTACGATGTTCTTCGTCCTATGCGTGCTACCGTGAA	(DeGrasse, 2012)
	Enterotoxin A	SELEX	TACTTATGCATTTCCTCCCCACGATCTTATTTGAGAGTGAC	(Wang et al., 2016b)
Vibrio parahaemolyticus		Whole-cell SELEX	ATAGGAGTCACGACGACCAGAATCTAAAAATGGGCAAAGAAACAGTGACTCGTTGAGATACTTATGTGCGTCTACCT CTTGACTAAT	(Song et al., 2019)
Listeria monocytogenes		Whole-cell SELEX	TGGGAGCTCAGAATAAACGCTCAACTTTGTTCTCTTTGCTTTTTTTTTCTTTTTTTGTTCGACATGAGGCCCGGATCA	(Suh et al., 2014)
Bacillus cereus	Spore	SELEX	CATCCGTCACACCTGCTCGGTGCAGACCCATAGGGGGGGCGTGCGGATGTAGGAGTAGGGTGTTGGCTCCCGTATC	(Fischer et al., 2015)
Shigella	*dysenteriae*	Whole-cell SELEX	CGGAACTAGCGTTTAAATGCCAGGACTGAAGTAGGCAGGG	(Duan et al., 2013a)
	sonnei	Whole-cell SELEX	ATTATGTTGTCTGAACTGCCATGGTCCCTCGTGTTTATTATGTTGTCTGACTGGCTGAGATTGCACTTACTATCT	(Song et al., 2017)
Pseudomonas aeruginosa		Whole-cell SELEX	CCCCCGTTGCTTTCGCTTTTCCTTTCGCTTTTGTTCGTTTCGTCCCTGCTTCCTTTCTTG	(Soundy and Day, 2017)

and 2,2- azino-bis (3-ethylbenzothiazo-line-6-sulfonicacid) diammonium salt (ABTS), to produce a colorimetric output signal. G-quadruplex is another well-known DNAzyme that is also commonly used in colorimetric assays due to its similar catalysis activity as HRP. Colorimetric aptasensors, especially those using G-quadruplex and HRP with peroxidase activity as signal amplification factors, have been applied for monitoring various targets because of simplicity, robustness, and low price (Wang et al., 2018a). Sun et al. constructed a colorimetric aptasensor for *V. parahaemolyticus* detection based on the colorimetry reaction catalyzed by G-quadruplex (Figure 3A) (Sun et al., 2019). The aptamer and its complementary sequence were fixed on magnetic beads. In the presence of *V. parahaemolyticus,* the complementary sequence in which G-quadruplex sequences were imbedded could dissociate from the magnetic beads. The greater amount of *V. parahaemolyticus*, the greater amount of complementary sequences with G-quadruplex that would enter the supernatant. The added TMB and H_2O_2 could be catalyzed by the G-quadruplex to produce a blue color, which is measured at 650 nm. Otherwise, without *V. parahaemolytic*us, the solution would not appear colorimetric signal. Based on the high catalytic activity of G-quadruplex DNAzyme, excellent sensitivity could also be obtained. Under optimal conditions, the detection limit could be as low as 10 cfu/ml with a wide linear detection range from 10^2 to 10^7 cfu/mL. This aptasensor also showed good recovery in *V. parahaemolyticus* detection in contaminated salmon samples.

These types of peroxidase have some disadvantages, such as difficulty in storage, and instable catalytic activity affected by external conditions. Therefore, substitutes for peroxidase would overcome the inferiors. In 2013, Woo found Fe_3O_4 nanoparticles exhibiting intrinsic peroxidase-like activity as HRP (Woo et al., 2013), and oxide nanomaterials as enzyme mimics have been widely applied due to their considerable surface areas and controlled catalytic activity. A variety of metal oxide nanoparticles have been synthesized as peroxidase mimetics, such as CeO_2 NPs, Co_3O_4 NPs, $ZnFe_2O_4$ NPs (Cheng et al., 2019; Li et al., 2017; Su et al., 2012). Wu et al. developed a new colorimetric aptasensor to detect *S. typhimurium* based on the $ZnFe_2O_4$-reduced graphene oxide ($ZnFe_2O_4$/rGO) nanoparticles (Wu et al., 2017). In the presence of *S. typhimurium*, a "sandwich-type" complex of aptamer (on micro-plate)-target-aptamer$ZnFe_2O_4$/rGO complexes could produce a blue color change. The limit of detection for *S. typhimurium* in buffer solution was 11 cfu/mL, and the detection range was 11 to 1.10×10^5 cfu/mL.

In addition, another type of label-free aptasensor is gold nanoparticle (AuNP) (Jiang et al., 2017). It did not require complicated preparation procedures or delicate instruments and provides a readout visible by the naked eye; the AuNP colloidal would turn from red to purple if the AuNPs aggregate in a certain case. Aptamers can be readily adsorbed on the surface of AuNPs, which protect AuNPs from ion-induced aggregation. Conversely, the aptamer dissociates from the AuNP surface and binds to the target in the presence of the target pathogen, leading to the color change from red to purple. Based on the principle, Ma reported

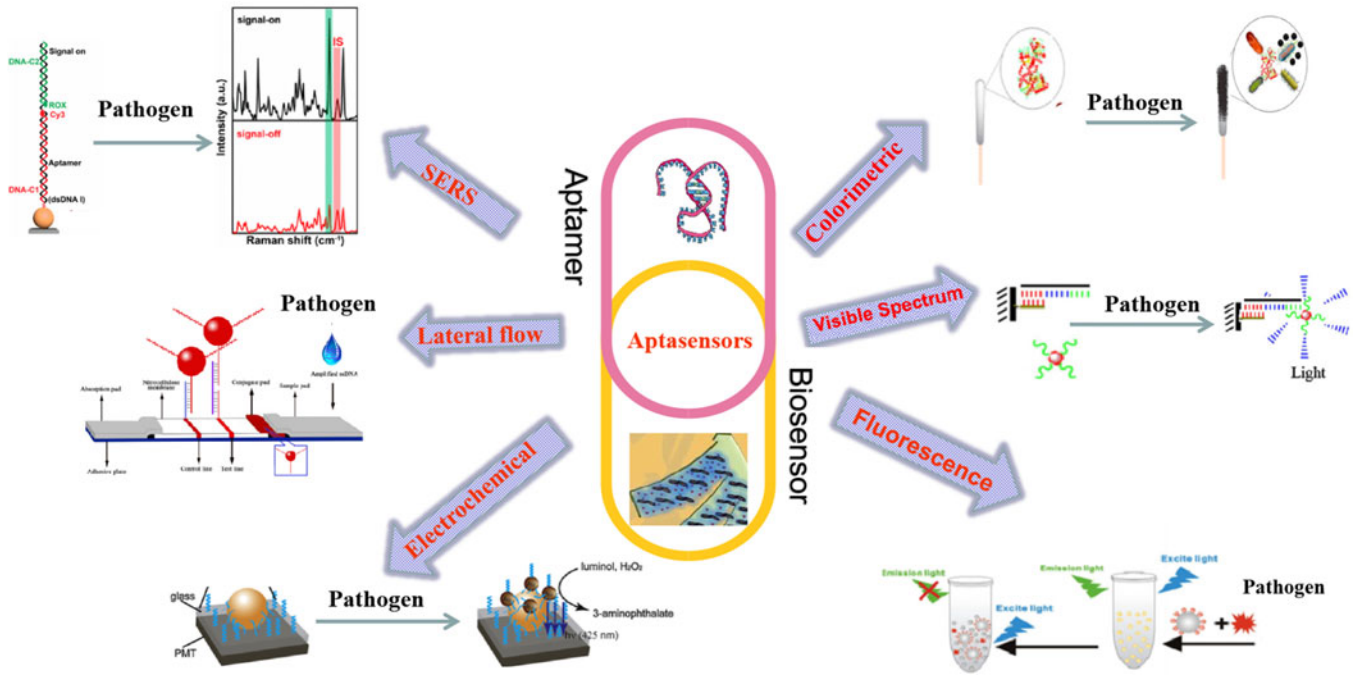

Figure 2. Schematic illustration of common aptasensors for pathogen detection.

a simple, fast, and convenient colorimetric aptasensor for *Salmonella typhimurium* (*S. typhimurium*) detection (Figure 3B) (Ma et al., 2017). In the presence of *S. typhimurium*, aptamers preferably combine with *S. typhimurium*, and the protective effect of the aptamers is broken. The exposed AuNPs aggregated to some extent with the addition of high-concentration NaCl. The color changed from red to purple to blue can be observed by the naked eye and a UV–Vis spectrophotometer. The linear range for detection of *S. typhimurium* was from 10^2 cfu/mL to 10^7 cfu/mL with a detection limit as low as 56 cfu/mL. Kim et al. recently reported a similar AuNP-aptasensing platform for the rapid and on-site detection of *Campylobacter jejuni (C. jejuni)* and *Campylobacter coli (C. coli)* in chicken carcass samples. The accuracy was superior to the conventional agar-based culture method ($p = 0.016$) in the detected 50 naturally contaminated chicken carcass samples. It is the first report about a colorimetric aptasensor targeting both live *C. coli and C. jejuni* detection in naturally contaminated samples. This aptasensor provided an excellent screening candidate, with a reduction in the detection time from 48 h to 30 min after enrichment to save time, labor, and cost (Kim et al., 2018).

Visible spectrum aptasensors

Various biosensors for pathogen detection have been fabricated by using mechanical, electrical, nuclear magnetic resonance (NMR), and optical assays (Lee et al., 2018; Liu et al., 2015; Weisshoff et al., 2018). Plasmonic biosensors, another type of visible specturm biosensors, offered superior sensitivity and multiplexing capability (Chavez, Hagen, and Kelley-Loughnane 2017; Lu et al., 2013). These distinct superiority have promoted the application of visible spectrum aptasensors, even some of which are currently sale on the market. For example, DuPont launched an optical strip for *E. coli O157, Salmonella, Listeria determination* based on antibody-cell interaction within 15 min (http://www.dupont.com). Moreover, visible spectrum sensors combination with microfluidic system has been commercially developed, such as LAMDAGEN (http://lamdagen.com/). These products were all dependent on the specific antibodies for target pathogens. The induction of aptamer as recognition elements would bring new concept for the development of commercial optical aptasensors for foodborne pathogen detection. The developed PCC systems drove by these forces make foodborne pathogen more efficient, convenient and inexpensive, which would be popularized in underdeveloped and developing countries.

It is more and more feasible to developing POC systems based on aptasensor contributing to recent advances in technologies and miniaturized instruments, which facilitates the integration of microfluidics and optics devices for data output. Chung et al. developed a detection method using fluorescent conjugated aptamer-modified nanoparticles (A-FNPs) for *E. coli* detection (Chung et al., 2015). In presence of *E. coli*, the A-FNP-labeled target bacterial complexes were loaded onto an optofluidic particle-sensing system, which provided rapid and continuous single-cell detection. This optofluidic device achieved a detection throughput of ~100 particles per second with high accuracy (~85%) in detecting single bacterial cells conjugated with A-FNPs (Figure 4A). Urmann et al. presented a simple, label-free and rapid optical biosensor enabling specific detection of protein A, specific biomarker for *Staphylococcus aureus (S. aureus)* (Urmann et al., 2017). Aptamer for Protein A was immobilized onto a nanostructured porous silicon thin film, as the optical transducer element. The aptasensor for *S. aureus* showed sensitivity with a linear detection range from 8 and 23 μM and the LOD with 3.17 μM. Moreover, a sandwich assay modified assay was used to amplify the optical signal.

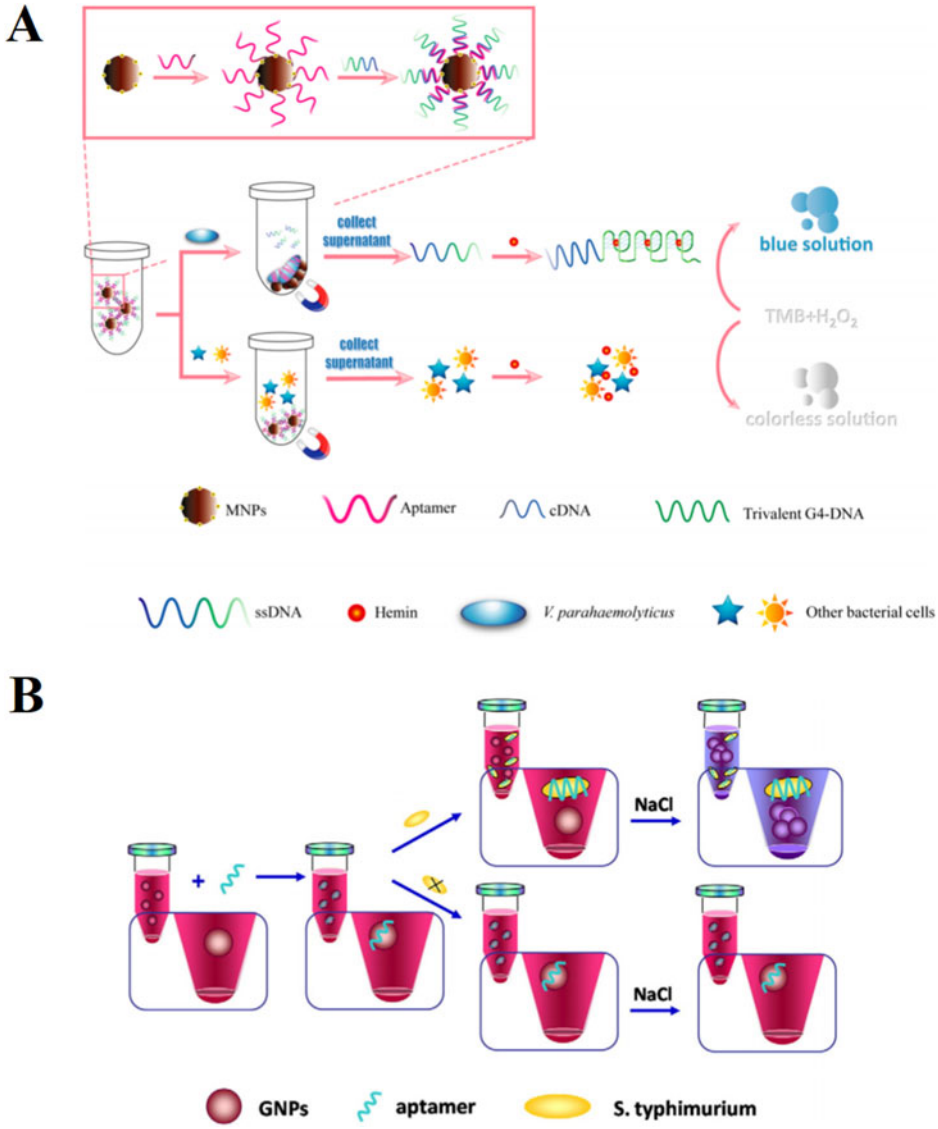

Figure 3. Different colorimetric aptasensors for foodborne pathogen determination. (A) G-quadruplex peroxidase based assay and (B) AuNP-based assay. Adapted from published papers (Wang et al., 2017a,b).

Using this approach, the LOD was improved for 3 times (Figure 4B). These miniaturized aptasensors can be immediately extended to the real-time, high-throughput detection of other targets by substituting different aptamers.

Fluorescence aptasensors

Fluorescence occurs when a valence electron is excited from its ground state to an excited singlet state. The excitation is produced by the absorption of light of sufficient energy (Zhu et al., 2019b). Fluorescence spectroscopy is a reliable method for sensitive detection of analytes with low concentration. Fluorescence dye molecules and fluorescence nanomaterials are two main used fluorescence signal sources (Belal et al., 2018; Li et al., 2015).

For the former one, the aptasensors are always integrated fluorescence molecules at sensing or response step at molecular level and homogeneous solution is used for sensing. Briefly, the specific aptamers are chemically modified with FITC, Cy3 or Cy5, etc., and the fluorescence intensity can be recorded. Huang et al. constructed a steganographically aptasensing fluorescence platform for multiplex detection of *Aeromonas hydrophila and Edwardsiella tarda* in vivo imaging of fish pathogens, which rely on the selective adsorption and fluorescence quenching capacities of graphene oxide (GO) (Figure 5A) (Zhu et al., 2019b). In this aptasensor, these elements can encode or decode a message through the attached fluorescenced aptamer or dissociated aptamer on GO, in which GO as a cover, target pathogens as a public key and aptamer-binding-pathogen as an encryption key. It is of guiding value in the development of multifunctional devices or machines at molecular-level.

For the latter one, fluorescent multifunctional nanoparticles (FNPs) have been synthesized and successfully used for analytical application for proteins, viruses, cells, and pathogens. Besides fluorescence characteristic, the FNPs are always electrostatic and magnetic. Tan et al. recently fabricated a set of FNPs with good fluorescence properties

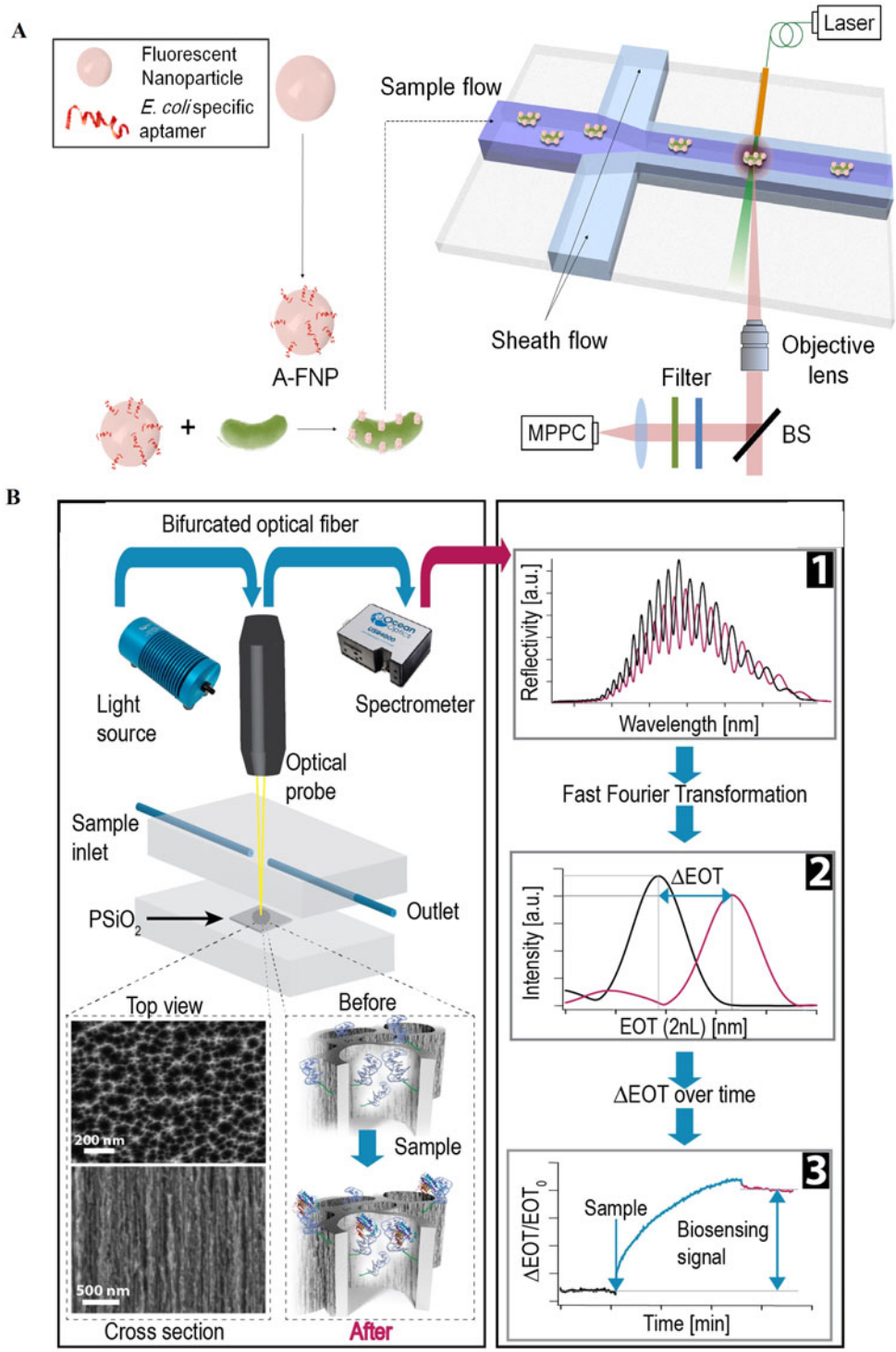

Figure 4. Different optical aptasensors for foodborne pathogen determination. (A) Optofluidic particle-sensing system-based aptasensor for *E. coli* and (B) Infrared spectroscopy-based aptasensor for *S. aureus*. Adapted from published papers (Kim et al., 2018; Urmann et al., 2017).

and variable magnetic response by employingγ-Fe_2O_3 nanoparticles and quantum dots (QDs) as magnetic and fluorescent components, respectively (Figure 5B) (Li et al., 2018). Specific aptamers to target pathogens *E. coli and S. typ* were conjugated with FNPs to yield apt-FMNPs nanoprobes for multiple pathogens assay. In the presence of *E. coli and S. typhimurium*, the pathogen@nanoprobes complex would produce fluorescence signal after magnetic separation with good linear ranges and detection limits within 1 h. Different FNPs were also been synthesized for pathogen detection. For example, Wang et al. Used aptamers labeled with multicolor lanthanide-doped time-resolved fluorescence (TRFL) nanoparticles to quantify *S. typhimurium* and *S. aureus*, within LOD of 15 and 20 CFU/ml for *S. typhimurium* and *S. aureus*, respectively (Wang et al., 2016c).

Electrochemical aptasensors

Electrochemical biosensors always exhibit privileged merits among various types of biosensors, which is cheap and easy

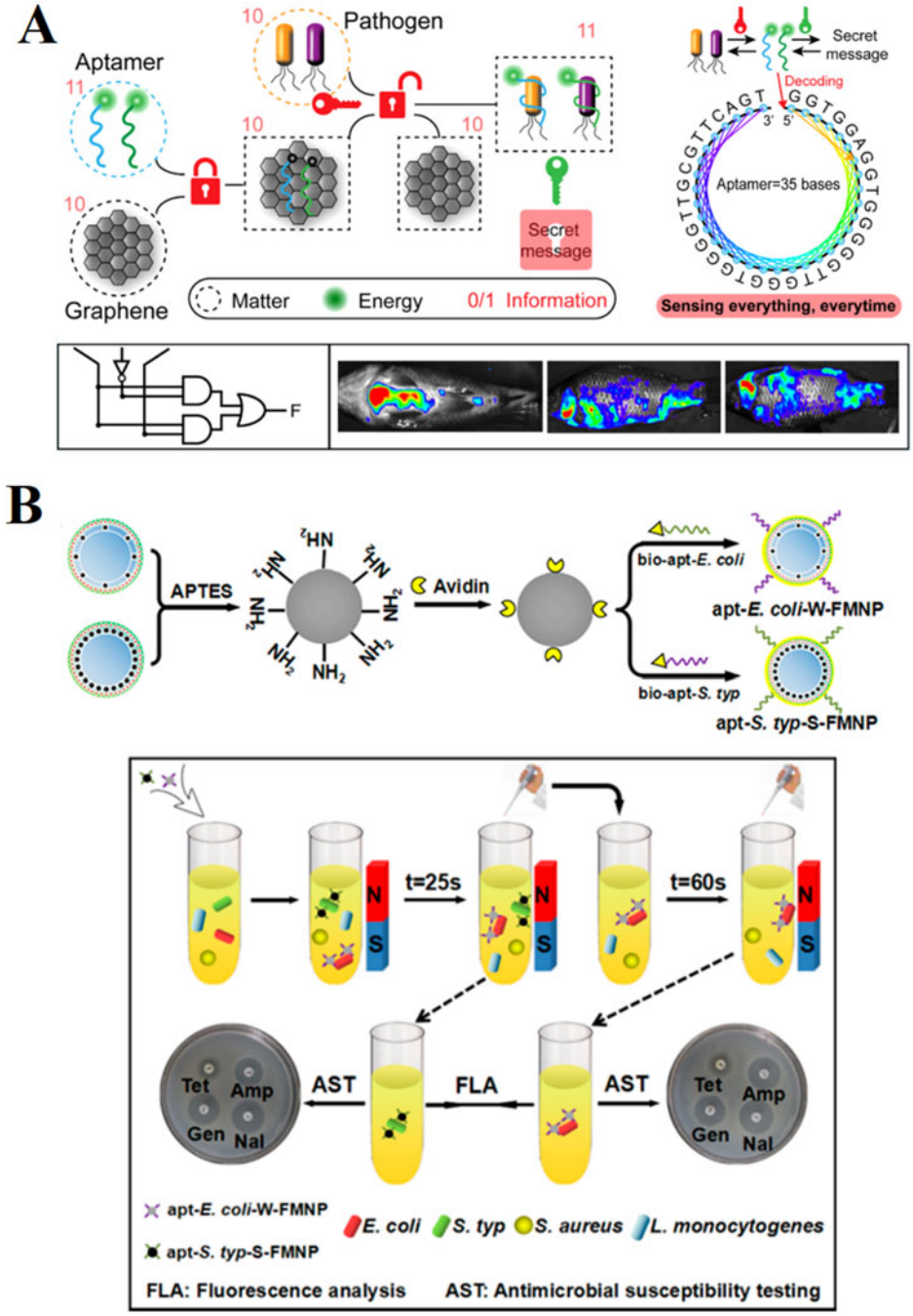

Figure 5. Different fluorescence aptasensors for foodborne pathogen determination. (A) Fluorescence-dye-based aptasensor for *Aeromonas hydrophila* and *Edwardsiella tarda* and (B) Fluorescent nanoparticle-based aptasensor for *S. typhimurium* and *S. aureus*. Adapted from published papers (Huang et al., 2015; Tan et al., 2016).

to operate (Muniandy et al., 2017). The most famous electrochemical biosensor is glucose meter, which can meet the household demand and fast record blood glucose in 15 s. Other electrochemical biosensors are also solid choice for POC determination of metabolites in short time, such as lactate and released toxin from pathogens. Therefore, electrochemical biosensors combination with aptamer has attracted more and more attention in this field. For example, Abbaspour et al. reported an electrochemical dual aptamer-based anodic stripping voltammetry method for sandwich detection of *S. aureus* as low as 1 CFU/mL (Abbaspour et al., 2015).

Label-free electrochemical detection is an attractive option in the electrochemical biosensor development. Special materials with excellent electronic characteristics have been applied for label-free detection method. Polypyrrole-based polymers have been used as valuable candidates for the fabrication of chemo/biosensors and functional surfaces, contributing to intrinsic chemical and electrical properties. Sheikhzadeh et al. reported a lable-free aptasensor for *S. Typhimurium* detection, on the combination of poly-[pyrrole-co-3-carboxyl-pyrrole] polymer and aptamer (Sheikhzadeh et al., 2016). Impedimetric measurements were facilitated by the electrical properties when the aptamer/target interaction on the intrinsic conjugation of the poly [pyrrole-co-3-carboxyl-pyrrole] copolymer. The aptasensor exhibited LOD of 3 CFU/mL of *S. Typhimurium* and in

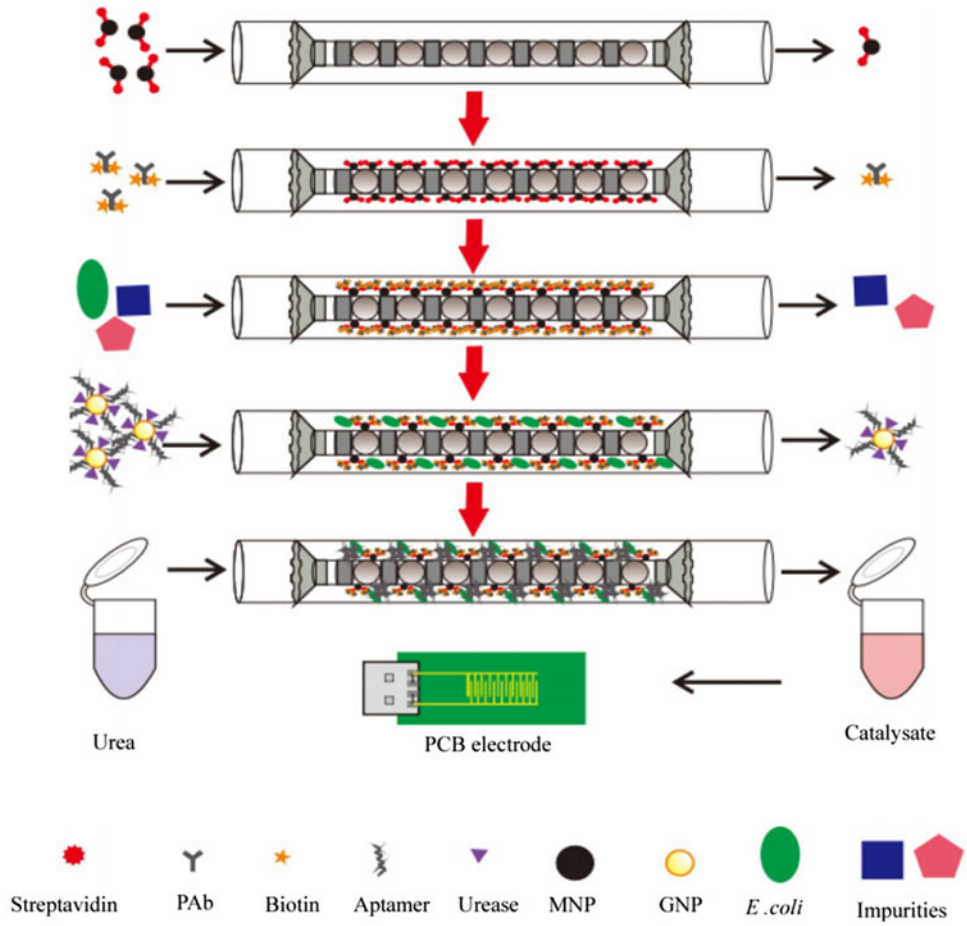

Figure 6. Electronical aptasensors for foodborne pathogen *E. coli* determination. Adapted from published papers (Lin et al., 2017).

a linear concentration range of 10^2–10^8 CFU/mL with high selectivity over other model pathogens. Special nanoparticles are also suitable for label-free detection. Wang et al. developed a novel electrochemical aptasensor for rapid and sensitive detection of *E. coli O157:H7* using magnetic nanoparticles (MNPs) for specific separation of the target bacteria (Figure 6). For signal production, the hydrolysis of urea by the urease produced electrons for amplification of the impedance signals, and a gold electrode for measuring the impedance change. Aptamer and urease was modified onto the gold nanoparticles, and injected into the capillary to react with the bacteria and form the MNP-PAb-bacteria-aptamer-GNP-urease complexes. The hydrolysis reaction of urea catalyzed by urease decreased the impedance of gold electrode. The impedance change was correlated with the concentration of the target. This aptasensor could detect *E. coli* as low as 10^1 CFU/mL within 3 h (Wang et al., 2017a).

Surface-enhanced Raman scattering aptasensors

In contrast to optical, colorimetric, fluorescent and electrochemical sensors, surface-enhanced Raman scattering (SERS) devices can differentiate the molecular spectral fingerprints of recognition molecules from analyte-binding complexes by directly acquiring the SERS spectrum of analytes without signal transduction (Pang, Labuza, and He 2014; Wu et al., 2013a; Yang et al., 2017). This unique property not only efficiently improves the selectivity of resistance to the sample matrix interference but also avoids using sensing labels in devices, which simplifies the design and reduces the cost. These advantages contribute to the widespread application of environmental pollutants and food safety monitoring. The aptamer-conjugated SERS platforms were first reported in 2008, and they were applied for adenosine and thrombin detection (Chen et al., 2008). SERS spectral changes would happen when the target captured by single aptamer monolayers (SAM). A simulated correlation equation was used to converse the conformational changes observed in the spectra of the aptamers upon target concentration. Similar to the protein mentioned above, *Bacillus anthracis* is a bacterium of high toxicity can be detected using this strategy. Using a SERS aptasensor, spores were detected as low as 10^4 CFU/mL. The aptamer also captured *B. mycoides* spores, but the SERS spectra of the spores were indistinguishable. Whole bacteria have also been detected by SERS aptasensors (Mazzaracchio et al., 2019). For *S. typhimurium*, an increase signal at 725 cm^{-1} was significantly different from other bacteria (Duan et al., 2016). For *S. aureus*, the sample was first incubated and immobilized to AgNPs though aptamer-bacteria-AgNPs complex. The induction of AgNPs greatly enhanced the SERS signal because one bacterium bound many aptamers and AgNPs. An increase signal in SERS

intensity at 735 cm^{-1} was observed, which positively correlated between 10 and 1×10^7 CFU/mL, and the LOD was as low as 1.5 CFU/mL. Moreover, it was easy to obviously distinguish *S. aureus* from a pathogen mixture, improving the selectivity in other similar analyses (Wang et al., 2016a).

Lateral flow aptasensors

Lateral flow strips (LFS), also called dipstick test strips, are a promising method for point-of-care detection in various fields. Our team previously developed different types of LFS for the visual detection of nucleic acids, metal ions, pathogens, and stem cells (Wang et al., 2018b; Wu et al., 2013b; Zhao et al., 2018). These biosensors were easy to use, sensitive and specific, without complex and expensive instruments. Most importantly, the readout of detection results can be observed by naked eyes. Lateral flow aptasensors (LFA) are LFS that a combine aptamers as recognition elements, which integrate the advantages of LFS, and aptamers in molecular diagnostics. In addition, the distinctive superiority of using aptamers is based on the nucleic acid property of aptamer, which can be used as template for signal amplification to improve the sensitivity.

Wu et al. constructed an LFA for simple and visual detection of the common seafood borne pathogen *Vibrio parahaemolyticus* (*V. parahaemolyticus*) (Figure 7) (Wu et al., 2018). In this study, two specific aptamers were modified for target capture and signal amplification. In the presence of *V. parahaemolyticus*, a complex of a-aptamer/target/c-aptamer/MBs was formed. Then, the "sandwich-type" complex was used as a template for isothermal amplification, and the amplified single-strand was loaded onto LFS. The entire process could be completed within 55 min; enrichment processing (20 min), isothermal amplification (30 min) and LFS determination (~5 min). Under optimized conditions, the LOD of *V. parahaemolyticus* was as low as 5.6 CFU/ml by calculation in a pure culture. No cross-reactivity with other *Vibrio* species and non-*Vibrio* species was observed. Without DNA extraction, the simplified operation and result readout rendered this LFA suitable for the rapid screening of *V. parahaemolyticus* in clinical, food, and environmental samples. In addition, this type of LFA could also be used for other analytes. Gu et al. produced a novel LFA system to detect vaspin, in which a primary V1 aptamer was modified with Biotin as a capturing probe and a secondary V49 aptamer conjugated with AuNPs as a signaling probe (Ahmad Raston, Nguyen, and Gu 2017). Using this LFA, vaspin was visually detected within the detectable concentrations of vaspin at up to 5 nM in both buffer and serum conditions. The sensitivity of the LFSA developed in this study ranged from 0.137 to 25 nM in buffer and from 0.105 to

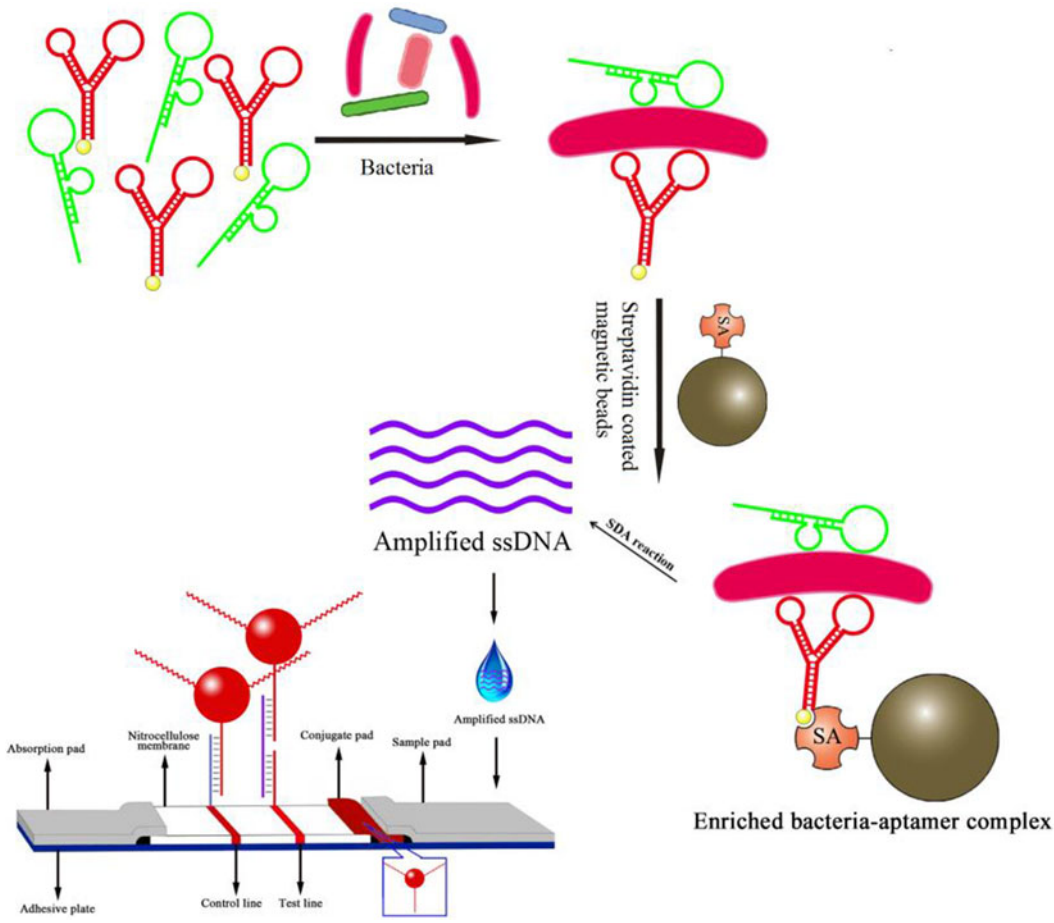

Figure 7. Lateral flow aptasensors for foodborne pathogen *V.parahaemolyticus* determination. Adapted from published papers (Wu et al., 2018).

Table 2. Comparison of analytical properties of the aptasensors for food-borne pathogen detection.

	Type of aptasensors					
Items	Visible spectrum	Lateral flow	Colorimetric	Surface plasmon resonance	Fluorescence	Electrochemical
Suitable occasion	POC testing and Commercial use	Visual and rapid detection in complex food matrix	Visual and rapid detection in complex food matrix	Non-destructive Testing for targets with trace concentration	High throughput for various samples	Difficult for sampling, Trace detection
Sensitivity	Moderate ~100 cfu/mL	High ~10 cfu/ml	High ~10 cfu/mL	High ~5 cfu/mL	Moderate ~10^3 cfu/mL	High ~1 cfu/mL
Specificity	High	High	High	High	High	High
Portability	Compact, portable device that can operate at the site of interest	Portable and setting up without technical skill and support	Relatively portable but setting up requires moderate technical skill and support	Unportable and needed special equipment	Relatively portable but setting up requires high technical skill and support	Unportable but setting up requires high technical skill and support
Temperature	RT	Thermal cycle and RT	RT or 37 °C	RT	RT	RT
Time of analysis	~1 h	~4 h	~3 h	~2 h	~2 h	~2 h
Sample processing	Without pre-enrichment	Easy-to-operate, pre-enrichment	Label free, minimal and easy	Label free and without pre-enrichment	Complex pre-enrichment	Label free and without pre-enrichment
Sensing method	Mechanical, electrical or NMR signal	Lateral flow strip	Enzymatic catalysis for colorimetry	Surface plasmon resonance signal	Fluorescence	Electrons captured by electrochemical platform
Skill of operator	User-friendly	No specialist training needed	No specialist training needed	Specialist training needed to use the assay	Specialist training needed to use the assay	Specialist training needed to use the assay
Cost	Feasible be economical and miniaturization	Low	Relative Low	Relative high	Relative high	Relative high
Interiority	Not sensitive and difficult to quantify accurately	Difficult to quantify accurately	Complex process, and difficult to quantify	Expansive instruments	Expansive materials and instruments	Poor repeatability

25 nM in spiked human serum. These studies have successfully demonstrated the efficiency and sensitivity of LFA in foodborne pollution detection. This system will be particularly applicable as a screening method for POC detection of any targets with a generated pair of aptamers.

Given that foodborne pathogen pollution is general and common, it is a matter of course to talking about microbiological poisoning. Aptasensors are usually categorized according to the transduction platforms (colorimetric, optical, fluorescent, electrochemical, SERS, LFA sensors). Particularly, electrochemical and lateral flow aptasensors are the most widely used aptasensors in foodborne pathogen determination, due to their sensitivity and visibility. The comparisons of the reported aptasensors are listed in Table 2.

Quality assurance/quality control (QA/QC)

Foodborne pathogens are harmful species that cause infections and contagious diseases in food consumption. Common pathogens and their complications include *E. coli* and *Salmonella* (food poisoning), *Helicobacter pylori* (gastritis and ulcers), and *S. aureus* (boils, cellulitis, abscesses, wound infections, toxic shock syndromes, pneumonia, and food poisoning). The foodborne pathogen pollution is regarded as the crucial threat to food quality and safety during processing and preservation (Liu et al., 2018b). Therefore, the establishment of a generally accepted QA/QC procedure for monitoring foodborne pathogens will be valuable to the food industry. Aptasensor integrates the superiority of biosensors and aptamers, which brings a new era in analytical methods. The QA/QC procedures using aptasensors can be divided into 3 key steps during the processing including raw materials procurement, sterilization, package and preservation (Liu et al., 2018a).

1. In the QA/QC procedure, the determination of foodborne pathogens in raw materials is the first step. On this occasion, rapid and accurate optical, LFA or SERS aptasensors can be recommended because fresh food is susceptible to pathogen proliferation, and a long time of QA/QC will sharply reduce seafood quality.
2. The second QA/QC step refers to the sterilization, also called germicidal effect evaluation. Various treatment such as high hydrostatic pressure, ultrasound, irradiation or chemical oils, have been applied in aquatic food sterilization. In this QA/QC case, it is recommended that sensitive electrochemical or fluorescent aptasensors could be used for pathogen monitoring, as the high sensitivity could meet the low-remaining pathogen monitoring.
3. The third QA/QC step is used in packaging and preservation, evaluating shelf life of products. Most of the package and preservation methods can significantly inhibit microbial inactivation and improve the safety of processed products (Oliveira et al., 2010). Colorimetric or LFA could be recommended on this occasion, as visual detection would not damage the package.

Strict QA/QC is as crucial as sterilization and preservation, because the accuracy in pathogen monitoring is prerequisites for food safety and quality in consumption.

Conclusions and future outlook

In the past decades, various types of aptasensors for the detection of different analytes have attracted increasing attention in this field. This review highlights that aptasensors have been extensively investigated for foodborne pathogen monitoring and updating the advances based on novel transducer signal production.

The most important challenge to the future of foodborne pathogen detection is to achieve a balance between the increasing foodborne pollution and the more and more strict environmental friendliness of analytical methods, also called Green Aptasensors. As we known, chemical analysis in aptasensors is a complex process. There are 6 important issues in the Green aptasensors, containing sample issue (minimum sample size), reagent issue (safe and nontoxic reagents), instrument issue (miniaturized and energy efficient instruments), transduction method issue (automated and in-situ signal readout), waste issue (rational disposal of waste) and operation issue (human-friendly interface) (Tobiszewski and Namieśnik, 2010 and 2012). The successful development of Green Aptasensor for foodborne pathogen would become more and more true in the light of Green Analytical Chemistry. In addition, these prospects will be a breakthrough in biosensor field.

There are still several limitations for real foodborne pathogen application of aptasensors. 1) Food samples usually contain a variety of interfering substances (proteins or saccharides) which could hinder the specific interaction between targets and the aptamers. Thus, aptamer-target capture through physical adsorption would suffer from poor stability and lower sensitivity. To solve this, it is necessary to modify the surface of transducer to reinforce the immobilization of aptamer with target capture, achieving a bacterial monolayer with very strong adhesion to the transducer surface but not losing biological function. 2) The concentration of some pathogens in food samples is too low for accurate detection. To solve this, it is beneficial to put future innovations on the improvement of sensitivity and selectivity, as well as increasing sample throughput. Various strategies have been made for signal amplification, resulting in better sensitivity and low detection limits. 3) Most of the reported aptasensors sense foodborne pathogen in vitro. It is necessary to design aptasensor for determining targets' concentrations *in vivo* and in complex matrices without pre-enrichment. Overall, although some obstacles remain to be overcome, aptasensors have great future potential in the fields of health, environment and food quality.

The prospective development is to integrating nanotechnology into aptasensors. Due to the advantages of nanotechnology, including good biocompatibility, high surface area and enhanced electron transfer property, novel nanomaterials have been constructed in biosensing. Aptamer modified magnetic nanoparticles could enhance the capture activity for targets to improve the selectivity. Aptamer modified with nanostructured electrodes or nanomaterials could significantly improve the sensitivity of sensors. Application of minimized electrodes or magnetic beads to fabricate single sensing devices provide new insight of how aptamer response to targets. In addition, lab-on-chip nanotechnology is an ideal research direction in pathogen biosensing, within minimal sample loading, micro-electromechanical sensing and high throughput.

Disclosure statement

No potential conflict of interest was reported by the authors.

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