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Oligonucleotide aptamers: promising and powerful diagnostic and therapeutic tools for infectious diseases

Qin Pan , Fengling Luo , Min Liu , Xiao-Lian Zhang *

State Key Laboratory of Virology and Department of Immunology School of Basic Medical Sciences, Medical Research Institute and Hubei Province Key Laboratory of Allergy Wuhan University School of Medicine, Donghu Road 185#, Wuhan 430071, PR China

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SUMMARY

The entire human population is at risk of infectious diseases worldwide. Thus far, the diagnosis and treatment of human infectious diseases at the molecular and nanoscale levels have been extremely challenging tasks because of the lack of effective probes to identify and recognize biomarkers of pathogens. Oligonucleotide aptamers are a class of small nucleic acid ligands that are composed of single-stranded DNA (ssDNA) or RNA and act as affinity probes or molecular recognition elements for a variety of targets. These aptamers have an exciting potential for diagnose and/or treatment of specific diseases. In this review, we highlight areas where aptamers have been developed as diagnostic and therapeutic agents for both bacterial and viral infectious diseases as well as aptamer-based detection.

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Introduction

Infections with a pandemic potential are major global risks, and infectious diseases in general are an important threat to public health. Preventing and controlling infectious disease are still associated with multiple challenges and obstacles. The increasing population and complexity of infectious disease are leading to synergistic effects. Novel pharmacological agents need to be developed for rapid diagnosis and cutting off emerging infectious diseases at the source. Exploration of novel molecular recognition elements specific for pathogens is the key strategy for development of anti-infective agents. New findings from the complete human genome sequence as well as genomes of some pathogens and on-going transcriptome and proteome projects offer novel targets for the fight against infectious diseases. Therefore, it is necessary to accelerate the discovery of anti-infective agents by biochemical methodologies and to develop novel biologically active compounds.

Although DNA and RNA are known as the most important biological macromolecules responsible for encoding and transmitting genetic information, they have a wide range of applications to genetic testing, molecular biology and medicine. Oligonucleotides not only can serve as probes for detecting complementary DNA or RNA targets, but they can also act as an affinity probes or molecular recognition elements for a variety of targets including small molecules, sugar moieties, lipids, peptides, proteins and even whole cells.

Nucleic-acid-based scaffolds that can bind to various targets are called aptamers (including single stranded-DNA [ssDNA] or RNA). Oligonucleotide aptamers fold into three-dimensional shapes and bind noncovalently to a variety of biological targets with high affinity.[1] Aptamers are functionally exploited as antagonists, agonists, or targeting ligands and are also called “chemical antibodies”. An anti-VEGF RNA aptamer (Macugen), the first aptamer therapeutic agent, was approved by the United Sates Food and Drug Administration (FDA) in 2005. From then on, a number of novel aptamer-based diagnostics and therapeutics have been undergoing clinical evaluation.[2,3]

Aptamers and the systematic evolution of ligands by exponential enrichment (SELEX) process

Aptamers are generated through a process of directed molecular evolution known as SELEX (Fig. 1).[4,5] In a conventional SELEX process, the initial oligonucleotide (ssDNA or RNA) pool has conserved primer-binding sites at each end and a random sequence (30–50 nt) in the middle to achieve a mixture of 10^{14}–10^{15} unique nucleic acid molecules. These oligonucleotides will fold into different secondary and tertiary structures, and they are then mixed with targets to form aptamer-target complexes. The target-bound aptamers are separated from unbound oligonucleotides by membrane filtration, magnetic beads, affinity columns, or capillary electrophoresis. The bound aptamers are amplified by PCR (DNA aptamers) or RT-PCR (RNA aptamers), and this enriched pool of aptamers is subjected to the next round of selection. Finally, the enriched aptamer sequences are identified by cloning and

* Corresponding author.
E-mail address: zhangxiaolian@whu.edu.cn (X.-L. Zhang).

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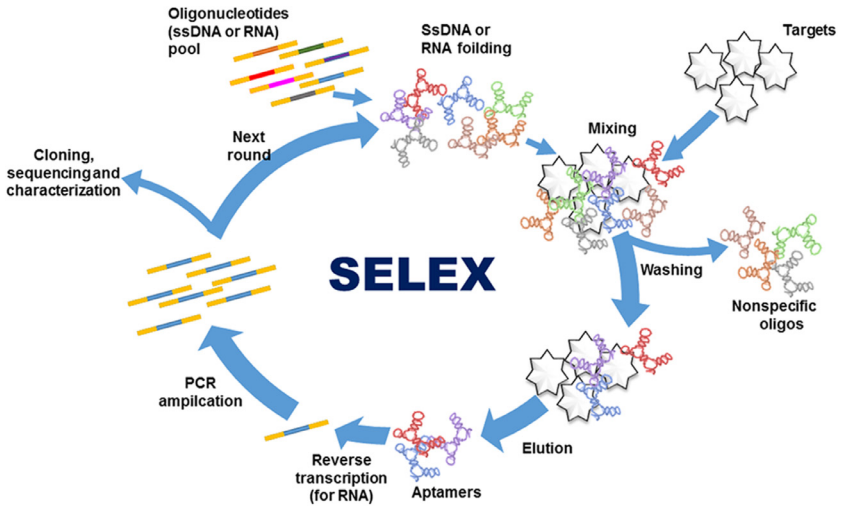

Fig. 1. Schematic diagram of the SELEX process. The initial ssDNA or RNA pool is incubated with targets to form aptamer-target complexes. The target-bound aptamers are separated from unbound oligonucleotides and then amplified by PCR (DNA aptamers) or RT-PCR (RNA aptamers). This enriched pool of aptamers is used in the next round of selection. After the final round of selection, the enriched aptamer sequences are identified by cloning and sequencing individual clones.

sequencing of individual clones, or alternatively, in recent years, high-throughput sequencing methods and bioinformatics analysis. During the SELEX process, several counterselections (negative-target selections) are added to eliminate nonspecific oligonucleotides. After 8-20 rounds of selection, specific aptamers that can bind to the target with high affinity will be obtained.

Optimization of SELEX methods

To identify nucleic acid-protein interactions, genomic SELEX has been developed. Unlike randomized-sequence nucleic acids in conventional SELEX process, the starting libraries in genomic SELEX are derived from the genome of the organism of interest. Whole-cell SELEX is a method developed and modified for creating aptamers binding to live cells including bacteria. Because the targets in whole-cell SELEX are live tumor cells or pathogenic bacteria, aptamers can find and more effectively bind to the surface molecules of target cells (than those in conventional SELEX approaches) and should have little cross-reactivity with the nontargets.

Aptamer vs. antibody

Although aptamers are similar to antibodies in terms of their affinity and specificity to targets, they offer several advantages over their antibody counterparts. First, aptamers are selected through an *in vitro* process and do not depend on animals, cells, or even *in vivo* conditions. Antibody generation requires the use of a live animal to stimulate an immune response, and the target molecule needs to be immunogenic as well as nontoxic. Second, ssDNA aptamers are stable at room temperature, whereas antibodies require refrigeration to avoid denaturation. Third, aptamers have shown low immunogenicity and toxicity thus far,[1,6] whereas antibodies may evoke a negative immune response. Finally, aptamers are small molecules and may effectively penetrate into tissue barriers and have effects on cells.

Aptamers are excellent non-protein-based alternatives to antibodies. In recent years, aptamers and the SELEX technology have received much more attention and been increasingly applied in the biomedical field, especially to the diagnosis and treatment of cancers and infectious diseases. In this review, we highlight areas where aptamers have been designed as diagnostic and therapeutic agents for major human infectious diseases.

Aptamers in diagnostics and therapeutics for bacterial infections

Aptamers targeting bacteria can be classified into the following two groups: (a) targeting predefined bacteria cell surface antigens or bacterial virulence factors; and (b) targeting whole cells with known or unknown molecular targets. To date, studies on antibacterial aptamers have been mostly focused on *Mycobacterium tuberculosis* (*M. tb*), *Salmonella, Listeria monocytogenes* (*L. monocytogenes*), *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) (Tables 1 and 2).

M. tb

Tuberculosis (TB) is the leading cause of deaths due to infectious diseases worldwide. Traditional diagnostic methods for TB, including tuberculin skin test, tuberculosis antibody detection and microscopic detection of *M. tb*, have some drawbacks, such as low sensitivity, poor specificity, lengthy procedure, and false-positive or -negative results. Bacterial culture, the “gold standard” of clinical tuberculosis diagnosis, required more than 10 days. Therefore, there is a clear need for new agents and strategies for TB diagnosis.

Aptamers targeting *M.tb* surface lipoglycan, proteins and whole bacterial cells have been generated as potential diagnostic tools. We selected ssDNA aptamer T9, which binds to mannose-capped lipoarabinomannan (ManLAM) from the predominant clinical epidemic *M.tb* strains of the Beijing genotype.[7] ManLAM is the major surface lipoglycan of *M. tb* and has immunomodulatory activity. Aptamer T9 detects ManLAM antigens in serum and sputum samples from patients with active pulmonary TB (aPTB) and patients with extrapulmonary TB (EPTB).[7] The 6 kDa early secreted antigenic target (ESAT6) and 10 kDa culture filtrate protein (CFP10) are secreted early by virulent *M. tb* and are not present in nonvirulent BCG. Both our group and Rotherham et al. have reported ssDNA aptamers against ESAT6 and CFP10 (CE protein).[8,9] These aptamers can detect ESAT6 and CFP10 antigens in serum samples from patients with aPTB or EPTB[8] or in sputum samples.[9] MPT64, which is a 24 kDa protein secreted by *M. tb* during bacterial growth, is also used as a diagnostic target for aptamers. Qin et al. selected ssDNA aptamers against MPT64 protein from *M. tb*.[10] They developed ssDNA aptamer MPT64-A1 against MPT64 antibodies, and reported that aptamer MPT64-A1 has the potential for the serological diagnosis of pulmonary TB, both in sputum smear-positive

Table 1
Summary of potential antibacterial aptamers for diagnosis.

Aptamer	Type	Organism	Target	Binding affinity	Detection limit	Ref
T9	DNA	*M.tb* Beijing strains	ManLAM	K_d 668 ± 59 nM	–	7
CE24 CE15	DNA	*M. tb* H37Rv	CE protein	K_d CE24: 0.375 μM CE15: 0.16 μM	–	8
CSIR 2.11	DNA	*M. tb*	CE protein	–	–	9
MPT64-A1	DNA	*M. tb*	MPT64	–	–	10,11
– (electrochemical aptasensor)	DNA					
	M. tb	MPT64		0.5 ± 0.2 fg/mL		12
– (electrochemical aptasensor)	DNA	*M. tb*	MPT64		0.2 fg/mL	13
G43 G78	RNA	*M. tb*	EsxG protein	K_d G43: 8.04 ± 1.90 nM G78: 78.85 ± 9.40 nM	–	14
MA1	DNA	*M. tb*	Whole-bacterium	K_d 12.02 nM).		16
Aptamer 1	DNA	*M. tb*	Whole-bacterium	K_d 37 ± 4 nM	100 CFU/mL	17
Aptamers 33 and 45	DNA	*S.*Typhimurium	Outer membrane proteins	–	3 CFU/ mL (Aptamers 33)	25,26
I-2	RNA	*S.* Typhimurium	OmpC protein	K_d 20 nM	–	28
C4	DNA	*S.* Typhimurium	Whole-bacterium	–	–	31
ST2P	DNA	*S.* Typhimurium	Whole-bacterium	K_d 6.33 ± 0.58 nM	25 CFU/mL	32
SAL 26	DNA	*S.* Typhimurium	Whole-bacterium	K_d 123 ± 23 nM	10^2 CFU/mL	33
Apt22	DNA	*Salmonella* Paratyphi A	Whole-bacterium	K_d 47 ± 3 nM	10^3 CFU/mL	34
S25	RNA	*S.* Enteritidis	Mixtures of ten strains of *S.* Enteritidis	–	–	38
Crn-1 and crn-2	DNA	*S.* Enteritidis	Whole-bacterium	K_d crn-1: 0.971 µM crn-2: 0.309 µM		35
$APT^{SEB}1$	DNA	*S. aureus*	SEB	–	–	42
C10	DNA	*S. aureus*	SEC1	K_d 65.14 ± 11.64 nM/L	6 ng/mL SEC1 in food samples	43
R12.06	DNA	*S. aureus*	Alpha toxin	K_d 93.7 ± 7.0 nM	200 nM alpha toxin in human serum samples	44
Antibac1 and Antibac2	DNA	*S. aureus*	Peptidoglycan	K_d Antibac1: 0.415 ± 0.047 μM Antibac2: 1.261 ± 0.280 μM	–	45
PA#2/8	DNA	*S. aureus*	Protein A	K_d 172 ± 14 nM for the recombinant Protein A 84 ± 5 nM for the native Protein A	–	46
SA20, SA23, SA31, SA34 SA43	DNA	*S. aureus*	Whole-bacterium	Median effective concentration, EC_{50} SA20: 70.86 ± 39.22 nM SA23: 61.50 ± 22.43 nM SA31: 82.86 ± 33.20 nM SA34: 72.42 ± 35.23 nM SA43: 210.70 ± 135.91 nM	682 CFU/mL in whole blood (SA20-conjugate-based assay)	47,48
SA17 SA61	DNA	*S. aureus*	Whole-bacterium	K_d SA17: 35 nM SA61: 129 nM	Single cell	49

(*continued on next page*)

Table 1 (*continued*)

Aptamer	Type	Organism	Target	Binding affinity	Detection limit	Ref
A8	DNA	*L. monocytogenes*	Internalin A	–	10^3 CFU/mL	55
LLO-3	DNA	*L. monocytogenes*	Listeriolysin O	–	4–61 CFU/100 μL	56
Lbi-17	DNA	*L. monocytogenes*	Whole-bacterium	–	60 CFU/500 μL	57
LMCA2 LMCA26	DNA	*L. monocytogenes*	Whole-bacterium	K_d LMCA2: 2.01×10^{-12} M LMCA26: 1.56×10^{-10} M	20 CFU/mL	59
E-5, E-11, E-12, E-16, E-17, E-18, E-19	DNA	*E. coli* O157	LPS	–	–	60
AM-6	DNA	*E. coli* O157	Whole-bacterium	K_d 107.6 ± 67.8 pmol		63
Apt B12	DNA	*E coli* K88	Whole-bacterium	K_d 15 ± 4 nM	1.1×10^3 CFU/mL in pure culture; 2.2×10^3 CFU/g in artificially contaminated fecal sample	64
E1, E2, E10, E12	DNA	*E. coli* KCTC 2571	Whole-bacterium	K_d E1: 12.4 nM E2: 25.2 nM E10: 14.2 nM E12: 16.8 Nm	8 CFU/mL (E1)	65,68
Ec3(31)	RNA	*E. coli* DH5α	Whole-cell	K_d 225 nM	2×10^4 CFU/mL	69
8.28A	DNA	*E. coli* DH5α	Whole-cell	K_d 27.4 ± 18.7 nM.		70

Table 2
Summary of potential antibacterial aptamers for therapy.

Aptamer	Type	Organism	Target	Binding Affinity	Detection limit	Ref
NK2	DNA	*M. tb* H37Rv	Whole-bacterium	K_{1a} $1.84 \times 10^5 (\pm 1.5 \times 10^4)$ M^{-1}; K_{2a} 7.65×10^6 $(\pm 6.0 \times 10^5)$ M^{-1}	–	18
ZXL1	DNA	*M. tb* H37Rv	ManLAM	K_d 436.3 ± 37.84 nM	–	19
BM2	DNA	BCG	ManLAM	K_d 8.59 ± 1.23 nM	–	20
Mtb-Apt1 Mtb-Apt6	DNA	*M. tb*	Acetohydroxyacid synthase	K_d Mtb-Apt1: 1.06 ± 0.10 μM Mtb-Apt6: 0.210 ± 0.05 μM	–	22
S-PS8.4	RNA	*S.* Typhi	Type IVB pili	K_d 8.56 nM	1 CFU	39,40
S3	DNA	*S. aureus*	SEA	K_d 36.93 ± 7.29 nM	–	51
AT-33 AT-36	DNA	*S. aureus*	α-toxin		Aptamers increased viability from 50–60% in cells treated with toxin alone up to about 85–90%.	52

and -negative patients.[11] Then several ssDNA aptamer-based electrochemical biosensors were devised for the detection of MPT64 and show the limit of detection in the fg mL^{-1} range.[12,13] Ngubane et al. demonstrated the suitability of surface plasmon resonance (SPR)-based SELEX for identification of two RNA aptamers, G43 and G78, which bind to the *M. tb* EsxG protein with high affinities.[14] The EsxG protein is both encoded within the ESX-3 locus of *M. tb* and secreted by the ESX-3 system, which is essential for the optimal growth of pathogenic *Mycobacteria* in low-iron environments.[14] They expected that these RNA aptamers would be candidate agents for the diagnosis of TB.[14] Additionally, Russell et al. generated slow off-rate modified aptamers (SOMAmers) with subnanomolar affinity for 18 *M. tb* protein targets and evaluated their utility for direct antigen detection.[15]

Two groups have selected ssDNA aptamers against whole cells for *M. tb* H37Rv detection.[16,17] Aimaiti et al. obtained species-specific aptamer MA1 against *M. tb* H37Rv and employed it in a sandwich enzyme-linked immunosorbent assay (ELISA).[16] Zhang et al. selected an ssDNA aptamer against whole cells of *M. tb* H37Rv and immobilized the aptamer on an Au interdigital

electrode (Au-IDE) of a multichannel series piezoelectric quartz crystal (MSPQC), then constructed a single-walled carbon nanotubes (SWCNT)/aptamer/Au-IDE MSPQC sensor for *M. tb* H37Rv detection[17].

Several groups have conducted studies on aptamers for potential therapeutic applications against TB. Our group has selected several aptamers specifically binding to *M. tb* whole cells[18] and surface lipoglycan.[19-21] We first implemented whole-cell SELEX and generated ssDNA aptamer NK2, which binds to virulent *M. tb* H37Rv with high affinity and specificity.[18] We then selected ssDNA aptamer ZXL1, which specifically binds to ManLAM from virulent *M. tb* strain H37Rv,[19,21] and significantly reduces the progression of *M. tb* H37Rv infections as well as bacterial loads in mice and rhesus monkeys.[19] Based on the different structures of ManLAM among different *Mycobacteria*, we next selected ssDNA aptamer BM2 that specifically binds to ManLAM from BCG.[20] Aptamer BM2 acts as an adjuvant enhancing immunoprotective effects of BCG against virulent *M. tb* infection in murine and monkey models.[20] To our knowledge, this was the first report regarding an aptamer serving as an adjuvant. Other groups have also reported aptamers potential for TB therapy. Baig et al. identified ssDNA aptamers Mtb-Apt1 and Mtb-Apt6 as a novel class of potent inhibitors of *M. tb* acetohydroxyacid synthase, which has been implicated in bacterial survival.[22] Ranjbar et al. designed and constructed a three-dimensional DNA origami box based on MPT64 ssDNA aptamer for tuberculosis treatment.[23] They expected that the MPT64 ssDNA aptamer-based drug delivery system would be capable of selectively interfacing with *M.tb* and would release an anti-TB drug near the bacteria.[23]

Salmonella

Salmonella infection arising from contaminated food has continued to be a concern for a decade because millions of cases of *Salmonella* infection occur worldwide annually. The detection of *Salmonella* before contaminated foods can be consumed is therefore an essential preventive measure for a public health system.[24] Joshi et al. chose outer-membrane proteins of *Salmonella* Typhimurium (*S.* Typhimurium) as the selection target and generated aptamers 33 and 45.[25] Aptamer 33 was conjugated to magnetic beads and used to capture *S.* Typhimurium seeded in whole-carcass chicken rinse samples, followed by detection via quantitative RT-PCR. They reported that 10–100 colony-forming units (CFUs) were successfully detected in 9 mL of the chicken rinsate in a pull-down assay.[25] Then, aptamer 33 was tested by Ma et al. to generate an electrochemical biosensor for *Salmonella* detection, and the detection limit turned out to be as low as 3 CFU/mL.[26] Aptamer 45 was chosen by Bagheryan et al. to develop a label-free impedimetric aptamer-based biosensor for *S.* Typhimurium detection.[27] Han et al. identified an RNase-resistant RNA aptamer I-2 specifically targeting outer-membrane protein C (OmpC) of *S.* Typhimurium. This selected aptamer specifically binds to *S.* Typhimurium but not to gram-positive bacteria (*S. aureus*) or to other gram-negative bacteria (*E. coli* O157:H7).[28]

Several groups have used whole-cell SELEX to identify ssDNA aptamers binding to *S.* Typhimurium, *Salmonella* Paratyphi and *Salmonella enterica serovar* Enteritidis (*S.* Enteritidis) for *Salmonella* detection.[29-35] These aptamers binding to the whole cell of *Salmonella* were conjugated to nanomaterials such as gold nanoparticles (AuNPs) and fluorescent nanoparticles as probes, magnetic nanoparticles (MNPs) as concentration elements, or loaded onto SWCNTs and reduced graphene oxide (rGO).[29,30,34,36,37] Oh et al. immobilized AuNPs-ssDNA aptamer conjugates onto a localized surface plasmon resonance (LSPR)-based sensor chip and reported a method for detection of S. *Typhimurium* in spiked pork meat samples with the detection limit of 1.0×10^4 CFU/mL.[30] Hyeon *et al.* tested mixtures of 10 strains of *S.* Enteritidis as targets and obtained RNA aptamer S25, which specifically binds to *S.* Enteritidis without any cross-reactivity with other *Salmonella* serovars.[38]

As for potential aptamers for treatment of *Salmonella* infection, we developed an effective RNA aptamer called S-PS8.4 for type IVB pili of *Salmonella enterica* serovar Typhi (*S.* Typhi).[39] RNA aptamer S-PS8.4 inhibits the entry of a piliated *S.* Typhi strain into THP-1 cells, indicating that it has possible applications as a therapeutic agent.[39] Based on S-PS8.4, Zelada-Guillén et al. reported a potentiometric biosensor for selective detection of a single CFU of *S.* Typhi, suggesting that the aptamer may also be applied to *S.* Typhi detection.[40] Yeom et al. developed an aptamer-based drug delivery system for the treatment of *S.* Typhimurium infection.[41] In this system, AuNPs are conjugated with an anti-*S.* Typhimurium ssDNA aptamer (AuNP-Apt), and antimicrobial peptides (AMPs) are loaded onto the AuNP-Apt. They reported antimicrobial activity of AMPs *via* AuNP-Apt conjugates during *S.* Typhimurium infection *in vitro* and *in vivo*.[41]

S. aureus

S. aureus is one of the major human pathogens that are responsible for a wide range of infections including hospital-acquired ones and life-threatening infections. In addition, the bacteria cause food-borne illnesses due to the excretion of several enterotoxins. Therefore, several ssDNA aptamers have been developed against staphylococcal enterotoxins (SEs). To develop potential diagnostic agents, aptamer APT^{SEB1} for staphylococcal enterotoxin B (SEB),[42] aptamer C10 for *S. aureus* enterotoxin C1 (SEC1),[43] aptamer R12.06 for α-toxin,[44] aptamer Antibac1 and Antibac2 for a peptidoglycan[45] and aptamer PA#2/8 for protein A[46] were generated for the detection of SEs. Among these aptamers, aptamer R12.06 for α-toxin was utilized in a modified sandwich ELISA and sensitive detection of 200 nM a-toxin in undiluted human serum samples was achieved.[44]

The first ssDNA aptamers specific to whole cells for *S. aureus* detection were introduced by Cao et al. in 2009.[47] They obtained a panel of ssDNA aptamers specific to *S. aureus* and have demonstrated that five aptamers (SA20, SA23, SA31, SA34 and SA43) recognize different molecular targets on *S. aureus* and have strong affinity, with K_d values in the nanomolar range.[47] SA20 was then chosen by Borsa et al. to develop a silica nanoparticle-aptamer conjugate, and they reported that their SA20-conjugate-based assay was successfully validated as a sensing platform for the specific detection of *S. aureus* cells at concentrations as low as 682 CFUs in 1 mL of whole blood.[48] Chang et al. also generated ssDNA aptamers SA17 and SA61 specifically for *S. aureus* with nanomolar affinity.[49] Aptamers SA61 and SA17 were conjugated with AuNPs and magnetic beads, respectively, and used in a resonance light scattering (RLS) spectral assay.[49] They reported that the limit of detection of the assay is as low as a single *S. aureus* cell in tested samples within 1.5 h.[49] Then, SA17 was employed by Wang et al. to develop a nanosystem for early sepsis diagnosis.[50] Their nanosystem is capable of simultaneous blood bacterial species identification and enrichment in a single step, and they reported successful diagnosis of *S. aureus* infection in mice by means of this nanosystem.[50]

Some studies have been conducted to select aptamers for the treatment of *S. aureus* infection.[51-54] Aptamer S3, which binds staphylococcal enterotoxin A (SEA) with K_d of 36.93 ± 7.29 nM, effectively inhibits SEA-mediated proliferation of human peripheral blood mononuclear cells and inflammatory secretion.[51] This result indicate that aptamer S3 is a potential agent for the treatment of *S. aureus* infection and SEA-induced diseases. α-Toxin is a major cause of *S. aureus* toxicity. SsDNA aptamers AT-33 and

AT-36 against α-toxin were generated by Vivekananda et al.[52] They reported that these aptamers significantly inhibit α-toxin-induced cell death and cytokine upregulation in Jurkat T cells.[52] Two research groups have employed anti-*S. aureus* ssDNA aptamers as the recognition elements in a drug delivery system.[53,54] Kavruk et al. designed aptamer-gated nanocapsules for the specific targeting of *S. aureus* with a controlled release of antibiotics.[53] Ocsoy et al. described the development of ssDNA aptamer-functionalized gold nanorods (Apt@Au NRs) for inactivation of methicillin-resistant *S. aureus* (MRSA) by targeted photothermal therapy (PTT).[54] They immobilized the aptamers onto Au NRs and demonstrated that Apt@Au NRs act as a targeting and photothermal agent that can selectively recognize and effiectively inactivate MRSA.[54]

L. monocytogenes

L. monocytogenes is a foodborne gram-positive bacterium that causes Listeriosis: a serious illness that can even cause death. These bacteria can survive and grow in a wide range of environmental conditions, including refrigeration temperatures (4 °C). The United Sates FDA and the European Union have all implemented zero-tolerance policies toward *L. monocytogenes* in ready-to-eat foods, thereby leading to a substantial effort to develop highly sensitive biosensors for foodborne pathogen detection. Thus far, all anti-*L. monocytogenes* aptamers in recent studies were developed for bacterial detection. Ohk et al. chose internalin A (an invasin protein of *L. monocytogenes*) as a target molecule to generate ssDNA Aptamer A8. Alexa Fluor 647-conjugated A8 was used in a fiber-optic sensor together with an antibody in a sandwich format for the detection of *L. monocytogenes* in food.[55] They demonstrated that A8 detects 10^3 CFU/mL *L.* monocytogenes in pure culture and in a mixture with other bacteria as well as *L. monocytogenes* from artificially contaminated (initial inoculation of 10^2 CFUs per 25 g) ready-to-eat meat products after 18 h of enrichment.[55] Listeriolysin O (LLO) is a cytolysin expressed in *L. monocytogenes*. Bruno et al. developed an ssDNA aptamer called LLO-3 for the LLO protein.[56] The aptamer was then applied to a fluorescent enzyme-linked DNA aptamer-magnetic bead sandwich assay for the detection of *Listeria*.[56] They reported that the assay was highly sensitive, with limits of detection in the range of 4 to 61 *L. monocytogenes* cells or the equivalent LLO produced by 4–61 cells on average per 100 μL sample in separate titration trials.[56]

Some studies were conducted for the generation of ssDNA aptamers for whole cells of *L. monocytogenes*.[57-59] Suh et al. reported that ssDNA aptamers for *L. monocytogenes* were identified after six rounds of positive and two rounds of negative selection.[57] These ssDNA aptamers have binding affinity for *L. monocytogenes* with a micromolar K_d value and only weak binding affinity for non-*Listeria* species.[57] One aptamer, Lbi-17, was chosen for conjugation to magnetic beads and was employed in a combined aptamer magnetic capture (AMC)-qPCR assay. They demonstrated that the pathogen could be detected at concentrations < 60 CFUs per 500 μL of a buffer, with capture efficacy of 26–77%.[57] One year later, Suh et al. demonstrated that they developed new ssDNA aptamers for *L. monocytogenes*.[58] The affinitiy of these new ssDNA aptamers was improved, with K_d values in the nanomolar range, and these aptamers were specific for *L. monocytogenes* in different growth phases.[58] Lee et al. adopted a whole-cell SELEX strategy, generated ssDNA aptamers LMCA2 and LMCA26, and then developed an aptamer-based sandwich assay platform for the quantification of *L. monocytogenes*.[59] They demonstrated that this platform yields a linear response over a wide concentration range of *L. monocytogenes* from 20 to 2×10^6 CFU/mL, thereby facilitating reliable detection of these bacteria at extremely low concentrations.[59]

E. coli

A lot of virulent strains of *E. coli* have been chosen as targets for the selection of specific aptamers because these bacteria are found ubiquitously in the intestinal flora of animals and humans, and their pathogenic variants cause major public health problems. *E. coli* O157:H7 causes several severe illnesses in human beings such as a gastrointestinal disease and bloody diarrhea (that is a root cause of hemolytic uremic syndrome). Bruno et al. generated ssDNA aptamers against *E. coli* O157 lipopolysaccharide (LPS),[60] and these aptamers were then used to develop biosensors with chemiluminescence[61] and color[62] for rapid detection. Amraee et al. selected ssDNA aptamer AM-6 with higher sensitivity and specificity for recognition of whole-cell *E. coli* O157:H7 and reported that the binding affinity of aptamer AM-6 for the target bacteria is 107.6 ± 67.8 pM.[63]

An ssDNA aptamer library specific for *E. coli* K88 whole cells was enriched by Peng et al. for *E. coli* detection. They also developed a sandwich detection system, in which K88 monoclonal antibody-conjugated magnetic beads capture the target bacteria, and the ssDNA aptamers serve as the reporter in a fluorescence assay. They reported that this aptamer-based system detects bacteria at 1.1×10^3 to 2.2×10^3 CFU/g in samples.[64] Kim et al. isolated and characterized ssDNA aptamers against *E. coli* KCTC 2571.[65] During the selection, they used other bacterial species (*Klebsiella pneumoniae, Citrobacter freundii, Enterobacter aerogenes*, and *Staphylococcus epidermidis*) for counter-selection to enhance the selectivity of ssDNA aptamers for target *E. coli* detection.[65] One of these aptamers (E2) was conjugated with fluorescent nanoparticles (A-FNPs) and was incorporated into an optofluidic particle-sensor platform for high-throughput real-time and single-cell detection of *E.coli*.[66] Aptamer E2 was then employed by Jin et al. to develop a detection platform based on fluorescence resonance energy transfer (FRET) for rapid, ultrasensitive and specific detection of bacteria.[67] Another aptamer against *E. coli* KCTC 2571, called aptamer E1, was applied to label-free and highly sensitive electro chemical detection of *E. coli* on the basis of rolling circle amplifications coupled peroxidase-mimicking DNAzyme amplification, with the detection limit of 8 CFU/mL.[68] Dua et al. generated a 2'F modified RNA aptamer Ec3 by whole-cell SELEX to target *E. coli* DH5α.[69] They developed an aptamer Ec3-based gap capacitance biosensor for electrochemical impedance spectroscopy (EIS); it has the detection limit of 2×10^4 CFU/mL for *E. coli* cells without any labeling and signal amplification techniques.[69] Renders et al. conducted whole-cell SELEX to select ssDNA aptamers with a tyrosinelike side chain against live *E. coli* DH5α cells.[70] They reported that aptamer 8.28A possesses high selectivity and affinity for the target cells and was greatly enriched for phenolmodified dU nucleotides.[70] This result highlights a means of increasing the chemical diversity of aptamers that will afford high affinity and specificity to potentially challenging targets.[70]

The reports on screening of aptamers as potential antibacterial or "antibiotic" agents against infection by virulent *E. coli* are scarce. DNA aptamers were developed against LPS from *E. coli* O111:B4 by Bruno et al.[71] They demonstrated that their polyclonal anti-LPS aptamers bind both LPS and *E. coli* with high affinity, and these aptamers were coupled to the human C1qrs protein and triggered destruction of *E. coli* O111:B4 and K12 strains.[71]

Aptamers in the diagnostics and therapeutics for viral infections

Specific aptamers can target the viral proteins involved in the stages of adsorption, penetration, replication, maturation and even release during a viral infection cycle. Currently, some aptamers have been are reported to have potential applications in the

Table 3
Summary of potential antiviral aptamers for diagnosis.

Aptamers	Type of aptamer	Organism	Target	Binding Affinity (K_d)	Inhibitory effect	Ref
RNA^{Tat}	RNA	HIV-1	Tat	120 ± 13 pM	Strong affinity for Tat even in the presence of a large excess of HIV TAR in cell culture	73-75
ZE2	DNA	HCV	E2 protein	1.05 ± 1 nM	Inhibition of HCV cell culture (HCVcc) infection *in vitro*	108
9-14 9-15	RNA	HCV	Core protein	9–14: 142 nM; 9–15: 224 nM	–	109
C4	DNA	HCV	Core Protein	–	Inhibition of HCV production LD: 3.3 pg/mL	110,111
A22	DNA	Influenza A virus (H3N2)	HA(91-261)	–	Inhibition of the hemagglutinin capacity of the virus and viral infectivity *in vitro*	128
Clone B	RNA	Influenza A virus (H3N2)	HA	200 pM	–	129
P30-10-16	RNA	Influenza A virus (H3N2)	Whole virus	188 pM	95% inhibition of viral fusion efficiencies in the presence of 5 μM aptamers	130
A10	DNA	Influenza A virus (H5N1)	HA	–	Inhibition of receptor binding	131
C7	DNA	Influenza A virus (H9N2)	HA(101-257)	–	55% inhibition of the viral infection at 1 nM in the cell viability assay	132
HAS15-5	RNA	Influenza A virus (H5N1)	HA	–	Inhibition of receptor binding	133
D-26	RNA	Influenza A virus (H1N1)	HA	67 fM	Complete inhibition of the agglutination of RBC in the presence of 200 nM aptamer	134
HA68	DNA	Influenza A virus (H3N2)	HA	7.1 nM	Inhibition of the agglutination of RBC in the presence of 2.5 μM aptamers, and detection of H3N2 variant isolated from human cases	135
8-3	RNA	Influenza A virus (H5N1 and H7N7)	HAs from H5N1 and H7N7	170 fM	Efficiently interferes with HA-glycan binding (EC_{50}: 25 nM)	137
ApI	DNA	Influenza A virus	HA1 subunit	ApI: 64.76 ± 18.24 nM	Binding to the HA1 subunit of subtype H1 (H1-HA1), but not to the HA1 subunit of subtype H5 (H5-HA1)	138
ApII ApIII				ApII: 69.06 ± 12.34 nM ApIII: 50.32 ± 14.07 nM		
A9	DNA	Influenza A virus (H9N2)	HA	A9: 46.23 ± 5.46 nM	Inhibition of H9N2 virus infection *in vitro*	139
B4				B4: 7.38 ± 1.09 nM		
–	DNA	Influenza A virus (H1N1)	Whole virus	K_d 55.14 ± 22.40 nM	–	142
IF22 IF23	DNA	Influenza A virus (H5N1 and H5N8)	Whole virus	–	–	144
HBs-A22	RNA	HBV	Surface antigen	–	Inhibition of receptor binding	147
G5α3N.4	RNA	HPV 16	E7 protein	1.9 μM	–	152

diagnostics and therapeutics for viral infections, *e.g.*, human immunodeficiency virus-1 (HIV-1), hepatitis C virus (HCV), influenza virus (Tables 3 and 4).

HIV-1

HIV-1 is the cause of the majority of HIV infections globally. HIV infection leads to chronic viral disease and eventually to acquired immunodeficiency syndrome (AIDS). People with chronic HIV-1 infection may have none of the HIV-related symptoms for many years. Therefore, early detection of HIV-1 infection is important for HIV/AIDS treatment. HIV accessory proteins include the trans-activator of transcription (Tat) protein, which is important for the transcription of viral RNA and enhances the amount of protein produced by attaching itself to the viral RNA.[72] The Tat protein is one of the most promising candidates for HIV screening because this protein is released in the body at an early stage of infection.[72] Yamamoto et al. first generated an RNA aptamer specific for the HIV-1 Tat protein.[73] The aptamer was then used to develop biosensors detecting the Tat protein.[74,75] Another research conducted by Rahim Ruslinda et al. yielded a split RNA aptamer as the detection probe for Tat.[76] They demonstrated that the effectiveness of a diamond-FET (field-effect transistor)-based RNA aptamer for the detection of a real sample of HIV-1 Tat protein is at concentrations down to 1 nM.[76]

Although combination antiretroviral therapy (cART) can suppress the HIV-1 virus and stop the progression of HIV-1 disease, it is not curative. Aptamers have been considered an alternative or adjuvant to the chemical antiviral agents in cART. Aptamers specifically targeting various parts of the HIV-1 genome and

Table 4
Summary of potential antiviral aptamers for therapy.

Aptamers	Type of aptamer	Organism	Target	Binding affinity (K_d)	Inhibitory effect	Ref
B40	RNA	HIV-1	Gp120	B40: 21 ± 2 nM	Inhibition of gp120-CCR5 interaction	78
B40t77				B40t77: 31 ± 2 nM		
A-1	RNA	HIV-1	Gp120	52 nM	Inhibition of HIV-1 infection in primary human peripheral blood mononuclear cells	80
BclON-mut	DNA	HIV-1	Gp120	143 ± 79 nM	IC_{50}: BclON-mut 0.08 ± 0.03 μg/mL	86
F-thio-BclON				86 ± 17 nM	F-thio-BclON 0.040 ± 0.003 μg/mL	
1.1	RNA	HIV-1	RT	5 nM	–	88
RT1t49	DNA	HIV-1	RT	1 nM	IC_{50}: 0.3 nM	89
4.20	DNA	HIV-1	RT	180 ± 70 pM	–	90
R12-2	DNA	HIV-1	RT	70 nM	Inhibition of the RNase H activity of intact HIV-1 RT; $IC_{50} < 100$ nM	91
37NT	DNA	HIV-1	RT	660 pM	Inhibition of primer-template binding	93
FA1	FANA aptamer	HIV-1	RT	Low pM range	Inhibition of HIV-1 RT primer extension	95
RNApt16	RNA	HIV-1	5′-untranslated region of HIV-1 genome	280 ± 60 nM	Inhibition efficiency in a human cell line: 85%	96
IV04	DNA	HIV-1	TAR RNA element	20 nM	Formation of RNA-DNA kissing complexes for disrupting TAR secondary structure	97
T30695	DNA	HIV-1	Integrase	0.5 ± 0.2 μM	$IC_{50} < 100$ nM	98,102
93del	ssDNA	HIV-1	Integrase	–	Inhibition of HIV-1 integrase in the nanomolar range	99-101
8-6	RNA	HIV-1	Nucleo-capsid protein	1.4×10^{-9} M	–	103
DP6-22	RNA	HIV-1	Gag protein	DP6-22: 100 ± 3.4 nM	Disrupting Gag-genomic RNA interaction and negatively affectiong genomic RNA transcription, processing, or stability	104
RBE(apt)	RNA	HIV-1	Rev protein	–	Conjugation with ribozyme targeting HIV Env for gene therapy	105
S3R3	RNA	HIV-1	Integrase	47 ± 3 nM	Anti-integrase aptamer expressed as an shRNA–aptamer fusion confered long term resistance to HIV-1 replication in T cells.	106
G6-16	RNA	HCV	NS3 protein	238 nM	IC_{50}: 3 μM	112
G9-I	RNA	HCV	Truncated protease domain of NS3 protein	10 nM	IC_{50}: 100 nM	113
G5	RNA	HCV	Helicase domain of NS3	25 nM	IC_{50}: 50 nM	116
3-07	RNA	HCV	IRES domains III–IV	9 nM	Inhibition of IRES-dependent translation	117
AP50	RNA	HCV	IRES	5 nM	Inhibition of IRES-dependent translation; IC_{50} value in the low μM range	118-119
HH-11	RNA	HCV	IRES domain IIIf and IV		IC_{50} value of 170 ± 20 nM	120
27v	DNA	HCV	NS5B	132.3 ± 20 nM	IC_{50}: 196 ± 16 nM	121,122
r10/43	RNA	HCV	NS5B	r10/43: 1.3 ± 0.3 nM	K_i r10/43: 1.4 ± 2.4 nM	123
r10/47				r10/47: 23.5 ± 6.7 nM	r10/47: 6.0 ± 2.3 nM	
R-F t2	RNA	HCV	NS5B	$2.62 \pm 0.90 \times 10^{-9}$ M	Blocking both the initiation and the elongation of RNA synthesis	124,125
HA12-16	RNA	Influenza A virus (H5N2)	Glycosylated HA	–	Preventing influenza infection by strongly binding to the glycosylated HA	145
PAN-2	DNA	Influenza A virus (H1N1, H5N1, H7N7, and H7N9)	Amino acid residues in the N-terminal of the PA_N of the influenza A virus polymerase	K_d 247 ± 11 nM	IC_{50}: around 10 nM	146
S9	RNA	HBV	Truncated P protein	–	Competitively inhibiting P protein binding and suppressing viral pgRNA packaging	148
Apt.No.28	DNA	HBV	Core protein	–	Inhibiting the assembly of the nucleocapsid; reducing extracellular HBV DNA	149

(*continued on next page*)

Table 4 (*continued*)

Aptamers	Type of aptamer	Organism	Target	Binding affinity (K_d)	Inhibitory effect	Ref
AO-01	DNA	HBV	Capsid	180 ± 82 nM	47% inhibition of virion production at 3 days post transfection *in vitro*	150
A2	RNA	HPV 16	E7 protein	107 nM	Inhibtion of E7-pRb interaction	151,153
F2	RNA	HPV 16	E6 protein	–	Inhibition of the interaction between E6 and PDZ-binding motif	154
NG8	DNA	SARS-CoV	Helicase	5 nM	IC_{50}: 91.0 nM	155
S15	DNA	DENV-2	Envelop protein domain III	200 nM	IC_{50}: 4.2 μM	156
GE54	DNA	RABV	Glyco-protein	307 nm	Inhibiting of viral replication and protecting mice challenged with RABV *in vivo*	157

HIV-1 proteins have been selected and shown to effectively inhibit viral fusion with the target cell and to suppress viral replication. There are some aptamers available for HIV-1 envelope glycoprotein gp120. [77-80] One of these RNA aptamers for gp120, called B40, blocks gp120 binding to its T cell co-receptor, CCR5 (C–C chemokine receptor type 5).[78,81] Its shorter variant, B40t77 and modified B40t77 (UCLA1) have been shown to strongly neutralize HIV-1 infectivity.[79,82,83] Another anti-gp120 aptamer, A-1, was applied to construct a therapeutic aptamer-siRNA chimera for HIV-1, and the chimera was reported to show therapeutic efficacy against of HIV-1 infection *in vivo*. [80,84,85] Recently, new potent anti-HIV-1 ssDNA aptamers that bind to gp120 were obtained by Prokofjeva et al.[86] They demonstrated that both G-quadruplex(G4)-forming aptamer (F-thio-BclON) and non-G4-forming aptamer (BclON-mut) inhibit lentiviral transduction in the low nanomolar range and thus can be considered for the development of microbicides against HIV-1.[86] For inhibiting the HIV-1 replication cycle, reverse transcriptase (RT) is one of the main drug targets in HIV/AIDS. HIV-1 RT catalyzes the conversion of the single-stranded genomic viral RNA into double-stranded proviral DNA, which in turn is integrated into the host genome.[87] To date, numerous RNA or DNA aptamers against HIV-1 RT have been generated, and their application to the inhibition of viral replication has been investigated.[88-95] Among these anti-RT aptamers, an ssDNA aptamer generated by capillary electrophoresis-SELEX and 2′-deoxy-2′-fluoroarabinonucleotide (FANA) aptamer FA1 can strongly bind HIV-1 RT, with low picomolar dissociation constants.[90,95] Besides anti-RT aptamers, highly specific aptamers—that target various parts of the HIV-1 genome, the trans-activation-responsive (TAR) RNA element, or HIV-1 integrase, nucleocapsid, Gag or Rev proteins—have been isolated and shown to effectively suppress viral replication.[96-106]

HCV

HCV is the common cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma.[107] There is currently no approved HCV vaccine, and aptamer research for the diagnosis and treatment in this area is at the initial stages. For inhibiting HCV initial attachment to host cells as well as to develop agents for the early diagnosis of HCV infection, our group carried out cell surface-SELEX and generated ssDNA aptamer ZE2 that binds specifically to HCV surface glycoprotein E2.[108] A good correlation was observed among HCV patients between the HCV E2 antigen-aptamer (ZE2) assay and the assays for HCV RNA quantities or HCV antibody detection.[108] Additionally, aptamer ZE2 specifically captures HCV particles and significantly inhibits HCV cell culture (HCVcc) infection of human hepatocytes (Huh7.5.1) *in vitro*.[108] We can speculate that ZE2 may be beneficial as a new molecular probe for HCV detection as well as anti-HCV therapy. Other research groups reported aptamers against HCV core (C) antigen for HCV detection.[109-111] Ghanbari et al. immobilized an anti-C antigen aptamer onto graphene quantum dots (GOD), and the aptamer-GOD was placed on the surface of a glassy carbon electrode.[111] They demonstrated that the electrochemical aptamer-based biosensor effectively detects the HCV C antigen with the detection limit of 3.3 pg/mL.[111] These aptamers against HCV E2 and C antigens are crucial for the early diagnosis of hepatitis C within the “diagnostic window period,” when serum antibodies have not appeared yet.

In the earlier studies on aptamers for anti-HCV therapy, most efforts had been focused on developing novel aptamers binding to the HCV NS3 protein or the internal ribosome entry site (IRES).[112-120] The NS3 protein possesses protease, nucleoside triphosphatase, and helicase activities. HCV IRES directs the binding of ribosomes in close proximity to the start codon of the viral open reading frame.[112,117] The aptamers blocking the activities of NS3 or IRES are expected to inhibit HCV replication and translation and hold promise as anti-HCV therapeutic agents.[112-115,117] Recently, Romero-López et al. designed multivalent aptamers against the HCV genome.[120] They reported that chimeric RNA aptamer called HH-11, with an IC_{50} value of 170 ± 20 nM, simultaneously targets HCV IRES domains IIIf and IV.[120] The blocking of RNA-dependent RNA polymerase (nonstructural protein 5B [NS5B]) is another strategy to develop anti-HCV therapeutic aptamers. Aptamers against NS5B strongly inhibit HCV polymerase activity and viral replication. These aptamers suppress formation of infectious virus particle *in vitro*.[121-125]

Influenza virus

Influenza viruses cause respiratory diseases in birds and mammals. The three major types of influenza viruses are influenza A, B, and C. The influenza A virus leads to seasonal epidemics as well as sporadic pandemics of influenza, and hence it is regarded as the major cause of this acute infectious disease and deaths. In recent years, influenza rapid diagnostic tests became available. Most of these diagnostic tests are based on detection influenza antigens by antibodies.[126] However, aptamers currently are viewed as equal or better tools compared with antibodies for antigen identification.[127] The hemagglutinin (HA) antigen is ubiquitously expressed on the surface of influenza viruses and serves as the major target for aptamers. The HA antigen also facilitates the first stage of the influenza virus infection: the binding to a species-specific host cell in the respiratory epithelium. Thus far, more than 10 aptamers against HA have been developed for diagnostic purposes.[128-139] RNA aptamer P30-10-16 binds to HA (human influenza A virus subtype H3N2) with more than 15-fold stronger affinity as compared with a conventional anti-HA monoclonal antibody.[130] Bai et al. developed a portable SPR aptasensor (an aptamer-based biosensor) based on the specific DNA aptamer for the rapid detection of avian influenza virus (AIV) H5N1. The total detection time was < 1.5 h,

which is faster than all conventional methods for AIV detection, such as virus isolation and identification (5–7 days), ELISA (3 h), and RT-PCT (3–5 h).[140] Wang et al. selected an ssDNA aptamer against HA from AIV H5N1, and the K_d value of this aptamer was 4.65 nM.[136] The anti-HA aptamer was later immobilized onto a gold interdigitated microelectrode for the development of a rapid H5N1 detection method, and the method had the detection limit of 1 hemagglutination unit (HAU) in the tracheal chicken swab samples spiked with the H5N1 virus.[141] Other aptamers for influenza virus detection target whole virus particles.[142-144] Lai et al. carried out screening and found an influenza A virus-specific aptamer,[142] which was later incorporated into an integrated microfluidic chip for H1N1 detection, and the limit of detection was found to be 0.032 HAU.[143]

As for potential therapeutic aptamers, Kwon et al. isolated an RNA aptamer specific to the glycosylated receptor-binding domain of the HA protein (gHA1) and demonstrated that the selected aptamer suppresses viral attachment to host cells by neutralizing the receptor-binding site of influenza virus HA.[145] Yuan et al. chose amino acid residues in the N-terminus of the PA subunit (PA_N) of the influenza A virus polymerase as a target to select ssDNA aptamers. They generated ssDNA PAN-2, which affords cross-protection against infections with H1N1, H5N1, H7N7, and H7N9 influenza viruses, with IC_{50} of ~10 nM.[146]

Other viruses

Although it has been more than 20 years since aptamers were first constructed, the studies on the exploitation of aptamers against many viral diseases are not advanced enough and are not designed systematically. However, recently, a few studies were conducted on aptamers against some viruses such as hepatitis B virus (HBV),[147-150] human papilloma virus (HPV),[151-154] severe acute respiratory syndrome coronavirus (SARS-CoV),[155] dengue viruses (DENVs)[156] and rabies virus.[157,158] Among these selected aptamers, several aptamers target viral surface antigens or virus-infected cells, *e.g.*, RNA aptamer HBs-A22 specifically binds to HBV surface Ag-expressing hepatoma cells,[147] ssDNA aptamer S15 targets DENV-2 envelope protein,[156] and ssDNA aptamer GE54 targets neurotropic rabies virus (RABV) glycoprotein-expressing cells and rabies virus-infected cells.[157,158] Some aptamers bind to viral enzymes to inhibit replication, *e.g.*, an RNA aptamer against HBV polymerase protein[148] and an ssDNA aptamer against SARS-CoV helicase.[155] Several aptamers target viral proteins thus blocking nucleocapsid formation, *e.g.*, ssDNA aptamers against the HBV core protein or matrix-binding domain.[149,150] Other aptamers target viral oncoproteins, *e.g.*, RNA aptamers binding to oncoproteins HPV16 E7 and E6.[151-154]

Aptasensor methods

Aptasensors are biosensors in which the biological recognition elements are DNA or RNA aptamers.[159] In recent years, more and more aptasensors have been designed for possible diagnostic applications. Major detection methods include (a) optical transduction, (b) electrochemical detection and (c) mass sensitive detection. In the following subsection, the basic principles of aptasensor-based detection are briefly discussed.

Optical transduction

Optical transduction can be subdivided into three major methods: absorbance assay, fluorescence detection and colorimetric detection. In an absorbance assay, an enzyme-linked oligonucleotide assay (ELONA) is usually implemented. In the direct method of ELONA, a plate is coated with the target molecule or cell, and a biotinylated aptamer binds to the target, followed by the addition of a streptavidin-horse radish peroxidase (HRP) conjugate and an enzyme substrate for color development (Fig. 2A).[7,8,19] In a sandwich assay of ELONA, the capture antibody or aptamer is immobilized on a plate, followed by addition of the target molecule, the HRP-linked aptamer or antibody, and an enzyme substrate.[7,8,20]

The principle of fluorescence detection is measurement of fluorescence signals (generation or quenching) when an aptasensor binds to a target (Fig. 2B). Aptamers can be labeled with fluorophores or conjugated with quantum dots (QDs), and the changes in the fluorescent signal will be detected after the target is bound by an aptamer.[20,108,160,161] Sometimes, a FRET-based technology has been used in aptasensors. In a FRET process, fluorescent particles are donors and quencher particles are acceptors. The fluorescence of the donors can be quenched by the acceptors if they come close to each other (< 10 nM).[162] A quencher is usually attached to the complementary sequence of the aptamer. Thus, the fluorescence of the aptamer can be quenched due to the good overlap between the fluorescence emission of the aptamer and the absorption spectrum of the complementary sequence. When an aptamer binds to the target, the quencher is moved away from the fluorophore owing to the conformational change of the aptamer and affords “signal on” detection.

AuNPs are widely used in aptasensor-based colorimetric assays. AuNPs have size-and-distance-dependent optical features. A well-dispersed AuNP solution is red, while the aggregated form of AuNPs is blue or purple due to the change in the surface resonance frequency of AuNPs.[36] Aptamers are modified with a mercapto group at the 5′ or 3′ end and are conjugated with AuNPs through Au–S bonding. The binding of aptamer-AuNP conjugates to targets cause aggregation of the AuNPs, resulting in absorbance spectral changes of the AuNP solution (Fig. 2C).[36,163] Sometimes, biotinylated aptamers are combined with streptavidin-coated AuNPs in reporter systems. In such a system, target bacteria are captured by a capture antibody or aptamer, and then, biotinylated aptamers and streptavidin-coated AuNPs are added develop color.[164]

Electrochemical detection

The principle of electrochemical detection is based on motoring the changes in electrical properties when an aptamer binds to a target. One strategy of the detection is to coordinate aptamers with SWCNTs for signal amplification (Fig. 3A). SWCNTs serve as effective ion-to-electron transducers because of their extraordinary ability to promote electron transfer between heterogeneous phases of SWCNTs.[40,165,166] In a SWCNT-based aptasensor system, aptamers are immobilized on SWCNTs. When the immobilized aptamers capture the target bacteria, a measurable change emerges in the conductance of the system because the SWCNT-aptamer hybrid material allows for a charge transfer between the SWCNT or a solution interface and the ions around the target bacterial wall.[40,165,166]

Chemically modified graphene oxide (GO) and reduced graphene oxide (rGO) have excellent electrochemical and transduction properties and can be incorporated into an electrochemical aptasensor to enhance signals. Aptamers are covalently or non-covalently attached onto the GO/rGO sheet (Fig. 3B).[167] The binding of the aptamers to targets causes the aptamers to leave the GO/rGO surface. Consequently, changes in the electromotive force (EMF) take place between the terminals of the GO/rGO aptasensor (as the working electrode) and the double junction reference electrode because of the separation of negatively-charged phosphodiester groups from the GO/rGO surface (Fig. 3B).[167]

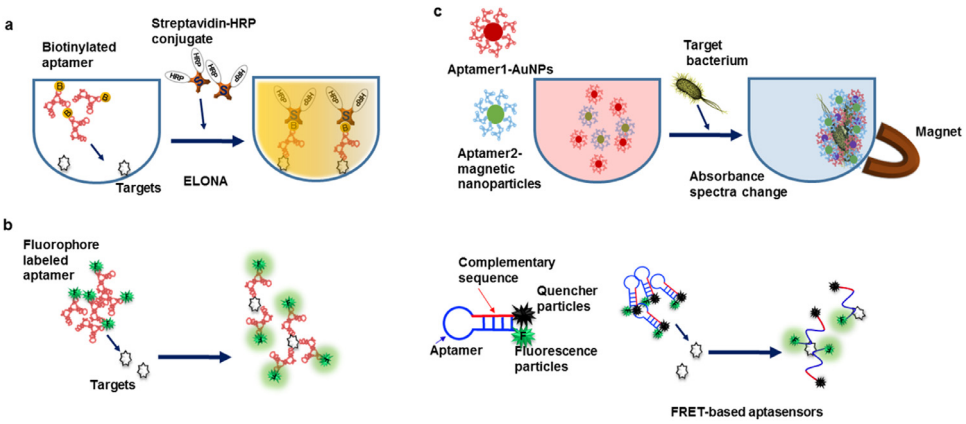

Fig. 2. Optical aptasensor. A: Direct ELONA. The target is coated onto the plate and the biotinylated aptamer binds to the target followed by the addition of streptavidin-HRP conjugate and enzyme substrate for color development. B: Fluorescent aptasensors. Left panel, aptamers are labeled with fluorophores and the changes in the fluorescent signal are detected upon targets bound by the aptamers. Right panel, FRET process. A quencher is labeled on the complementary sequence of the aptamer. When the aptamer binds to the target, the quencher is moved away from the fluorophore owing to the conformational change of the aptamer and affords "signal on" detection. C: Optical and AuNPs-based aptasensors. Aptamer1-AuNPs and Aptamer2-magnetic nanoparticles are mixed with the target bacteria. Aptamer2-magnetic nanoparticles-target-Aptamer1-AuNPs complexes are formed. In the magnetic field, the target bacteria are accumulated, and the changes in the absorbance spectrum are detected.

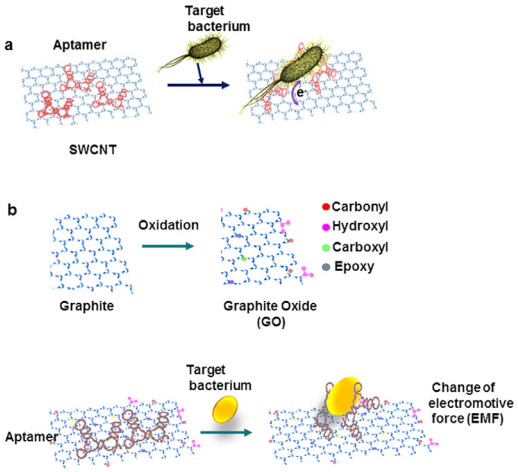

Fig. 3. Electrochemical aptasensor. A: SWCNT-based aptasensor. Aptamers are immobilized on SWCNTs. When the immobilized aptamers capture the target bacteria, a measurable change emerges in the conductance of the system. B: GO-based aptasensor. Aptamers are loaded onto the GO sheet. The binding of the aptamers to targets causes the aptamers to leave from the sheet surface. Consequently, EMF changes take place.

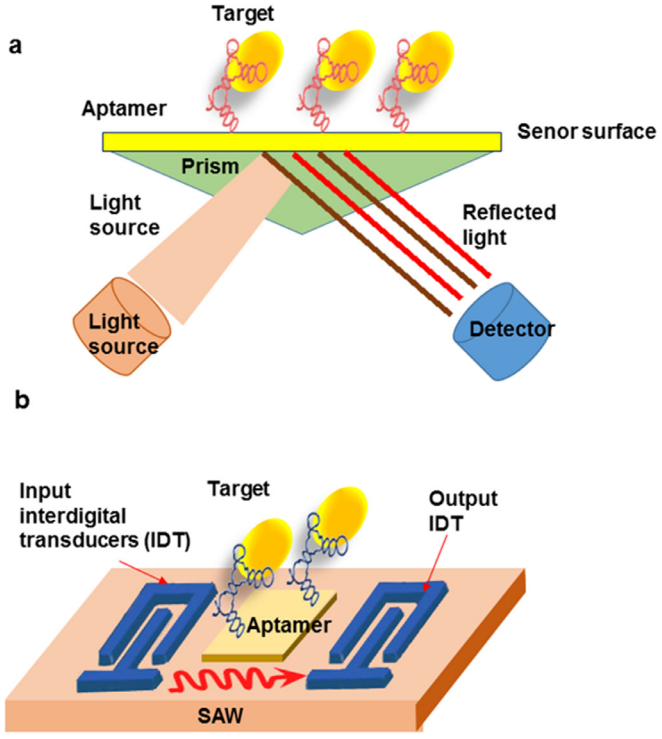

Fig. 4. SPR aptasensor and SAW aptasensor. A: SPR aptasensor. Aptamers are immobilized on the chip. The binding between targets and aptamers leads to a change in the plasmon resonance and plasmon angle. B: SAW aptasensor. The phase shift and amplitude of SAW changes because of the binding of targets to surface-immobilized aptamer.

Mass sensitive detection

Mass sensitive detection methods for pathogens include (a) an SPR aptasensor, (b) a surface acoustic wave (SAW) aptasensor and (c) a quartz crystal microbalance (QCM) aptasensor. These detection systems do not require additional labeling. In an SPR-based system, aptamers are usually labeled with biotin and immobilized on a streptavidin coated gold chip. The binding between targets and the aptamers leads to a change in the plasmon resonance and plasmon angle, which can be measured (Fig. 4A).[140] Both SAW aptasensors and QCM aptasensors are acoustic wave sensors. SAW aptasensors record the phase shift and amplitude change due to the binding of targets to surface-immobilized aptamers (Fig. 4B).[168] In a QCM aptasensor system, aptamers are loaded onto gold-coated quartz. The binding of targets to aptamers causes an increase of the mass on the surface of the crystal and generates a detectable decrease in the resonance frequency of the crystal.[169]

Challenges of aptamer-based diagnostic and therapeutic applications

Although aptamers are promising candidates for diagnostic and therapeutic applications, some challenges still remain.

In vitro *selection* vs. in vivo *targeting*

Most aptamers have been selected *in vitro*. Given that the structures of some target molecules are diverse and complex *in vivo*, the aptamers may not work *in vivo* as expected. Cross-binding to non-target molecules may limit the use of aptamers for detection in many real world complex samples, such as biological fluids and food matrices. Therefore, careful design of the selection process is necessary to improve specificity of the identified aptamers, which can better distinguish closely related molecules at low concentrations. High variation of bacteria and viruses and complex structures of targets are additional factors that may influence the performance of aptamers. It is important to continue to develop simpler and more efficient SELEX procedures (requiring less time in selection) to develop specific and/or universal aptamers against various bacterial pathogens.

Nuclease degradation

Unmodified aptamers (especially RNA aptamers) are generally susceptible to digestion by cellular nucleases present in body fluids or cells. Therefore, chemical modifications are often introduced into the oligonucleotides to increase *in vivo* stability of aptamers. These chemical modifications include capping the 3-end with inverted thymidine/biotin,[170-173] phosphorothioate/methylphosphonate substitution in the phosphate backbone,[174] 2′-sugar modifications with a fluoro (F), amino (NH_2) or O-methyl (OCH_3) groups and locked nucleic acid (LNA) modification.[175] The LNA modification is an intramolecular 2′-O to 4′-C methylene bridge, and LNAs show high resistance to ribozymes.[175] The most common modifications are a substitution of a sulfur atom for a non-bridging oxygen in the DNA or RNA phosphate backbone as well as the modifications of RNA aptamers at the 2′-position on the ribose ring. Modified RNA aptamers have half-life ranging from several to hundred hours but unmodified RNA usually has a half-life of seconds in human serum.[176]

Renal filtration

Because of small mass (6–30 kDa) and short diameter (< 5 nm) of aptamers, they are easily filtered through the glomerular capillaries. To extend the circulation time of aptamers, they are conjugated to compounds with high molecular mass, *e.g.*, polyethylene glycol (PEG),[124] cholesterol,[125] an antibody,[177] liposome[178] or other nanomaterials.[179] PEG has been widely been used to prolong circulation half-life of biological agents. Besides half-life extension in the blood circulation, conjugation of PEG can improve drug solubility and stability.[180] The first aptamer-based drug (Macugen) is based on the PEGylation.[180] This PEGylated aptamer has a half-life of 9.3 h according to the pharmacokinetic experiments performed on rhesus monkeys.[181]

Conclusion

Over the last two decades, there has been a growing number of studies on aptamers targeting pathogens. Although few aptamers against fungi have been reported,[182,183] a lot of aptamers that specifically target bacterial or viral virulence factors—as well as entire bacterial cells or viral particles—have been generated. For an example, ssDNA aptamer ZE2 against HCVsurface glycoprotein E2 has been used in several reports.[184-186] Oligonucleotide aptamers are promising candidates for the diagnosis and treatment of infectious diseases.

The development of aptasensors is becoming an increasingly diverse field. In aptasensors, DNA or RNA aptamers serve as biological recognition elements, and an enzyme-linked oligonucleotide assay, fluorescence, colorimetry, electrochemistry and mass sensitivity have been popular as readout formats. The many attractive features of aptasensors encourage researchers to continue to optimize their use for disease diagnosis and treatment. An increasing number of applications of aptasensor to the realm of medicine may be commercialized in the future.

Aptamers have several advantages over protein antibodies: lower immunogenicity, easier production, and smaller size. Both aptamers and antibodies can potentially serve as diagnostic and therapeutic tools. Since the first monoclonal antibody was produced in the 1970s, more widespread distribution and adoption of antibodies as well as a well-developed infrastructure have led to widespread application of antibodies to disease diagnosis. However, aptamers are more stable and easier to label and modify, and have a much lower cost of manufacture as compared with antibodies. Therapeutic aptamers have the advantages over antibodies in the treatment of antitoxin, antivenom and anti-multiple drug resistant bacterial and viral infections, but the therapeutic effects and safety of aptamers should be carefully studied. Recently, the first report of aptamer-specific resistance in cell culture has been published, and the utility of broad-spectrum aptamers for medical treatment and tracking of long-term effects of aptamers may be necessary.[187]

The aptamer field has probably touched only the tip of the iceberg. Researchers are focus on optimization of the SELEX methods, aptamer modifications, combinations of aptamers and targeted drug delivery. The aptamer technology continues to reveal its promising feature and the vast diagnostic and therapeutic potential for infectious diseases in the present and the future.

Conflicts of interest

The authors declare that they have no conflict of interest.

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