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# Application of Aptamer Based Biosensors for Detection of Pathogenic Microorganisms

WANG Yi-Xian, YE Zun-Zhong\*, SI Cheng-Yan, YING Yi-Bin

College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310058, China

**Abstract:** Aptamer is a kind of synthetic oligonucleotides discriminated by in vitro screening and systematic evolution of exponential enrichment technology (SELEX), which can bind to certain targets (small molecules, proteins, or even entire cells) with extremely high specificity. Owing to the advantages of simple preparation, easy modification and good stability, aptamers have been used to construct biosensors for the detection of pathogenic microorganisms. This paper presents the latest advances in SELEX for screening aptamers for pathogenic microorganisms, demonstrates some reported aptamers for pathogenic microorganisms (protozoa, viruses, bacteria), and reviews aptamer based biosensors for detection of pathogenic microorganisms. Finally, the new trends in aptamer based biosensors for detection of pathogenic microorganisms are also discussed.

**Key Words:** Aptamer; Pathogenic microorganism; Detection; Biosensor; Review

## 1 Introduction

Pathogenic microorganisms, such as protozoa, virus and bacteria, are a kind of microorganisms that can cause the various diseases of animals, plants and humans. The common pathogenic microorganisms include plasmodium, human immunodeficiency virus (HIV), Escherichia coli (*E. coli*), Salmonella typhi, and so on. Each year they have caused great harm to the health of humans, animals and plants, leading to huge economic losses<sup>[1]</sup>. Therefore, it is highly desirable to develop a rapid, sensitive and low cost detection method to monitor and control their spread in food and environment. The traditional methods for detection of pathogenic microorganism in the sample demand several steps, in which include isolating and culturing, and a series of complicated biochemical tests<sup>[2]</sup>. Although the detection method is of high sensitivity and specificity, it cannot be applied to the fast on-site testing because it requires a long time (usually 5 to 7 d). In recent years, the biosensor as a rapid, sensitive, highly specific, low-cost detection technique has been widely used for the

detection of pathogenic microorganism<sup>[3]</sup>. Biosensors convert the biological information produced by specific binding of bio-recognition elements and pathogenic microorganisms into physical signal output (like electrical, optical, and so on) to achieve the purpose of analysis/detection<sup>[4]</sup>. Antibodies most frequently used are the bio-recognition elements with the advantages of high affinity and high specificity in biosensors. However, the antibodies suffer from several drawbacks, such as requirement of a series of cumbersome and complex selection process, expensive, easy to degenerate and high environmental requirements for detection<sup>[5]</sup>. Therefore, new bio-recognition elements for detection of pathogenic microorganisms with high specificity and overcoming the shortcomings of antibodies are still needed to explore.

Aptamers are a kind of synthetic oligonucleotides discriminated by systematic evolution of exponential enrichment technology (SELEX) and in vitro screening, which can bind a certain targets (small molecules, proteins, or even entire cells) with extremely high specificity<sup>[6–8]</sup>. As bio-recognition elements, aptamers have some advantages over

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\* Corresponding author. Email: zzye@zju.edu.cn

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antibodies in fabrication of the biosensors: (1) Aptamers are obtained by in vitro screening. The selection SELEX cycle generally need 2 to 3 months, the fastest is just 2 weeks, while preparation of monoclonal antibody is at least 3 to 6 months and expensive; (2) Aptamers are chemically synthesized, thus it can be modified flexibly with a variety of groups; (3) Antibodies are proteins that are easy to degenerate. The instability of traditional immunosensor limit its application scope, but the aptamers have chemical stability in the pH range of 2 to 12, and have a certain thermal refolding; (4) The composition of aptamers is simple, generally has a few dozen nucleotides (less than 100 nt), and thus the design of the aptamers is relatively simple. Due to the advantages of aptamers, the use of aptamers to design biosensors for identification and detection of pathogenic microorganisms is of extremely importance in the field of analysis<sup>[9,10]</sup>.

## 2 SELEX screening method for aptamers of pathogenic microorganisms

SELEX technology is a common screening process for specific aptamers, including five main steps (Fig.1): combination, separation, elution, amplification and regulation. The first step is to use a random nucleic acid library (ssDNA or RNA) of  $10^{13}$  to  $10^{15}$  sequences to incubate with a target molecule, followed by the physical methods to separate the nucleic acid sequences combining the target molecule. Eluting and collecting the bound sequences, which are amplified by polymerase chain reaction (PCR) to generate the enriched library for the next cycle. For every target, 6 to 20 consecutive cycles are performed and the final enriched library is cloned and sequenced to obtain aptamers specifically recognizing the target molecule<sup>[11]</sup>. The SELEX process for screening aptamers of pathogenic microorganisms has two critical steps: selection of the targets for screening aptamers and separation of the binding sequences.

### 2.1 Targets for screening aptamers

Pathogenic microorganisms are biological macromolecules that have a complex target structures. Usually, the targets for screening aptamers of pathogenic microorganisms are the specific purified proteins that exist on cell surface<sup>[13]</sup>, or cell lysates<sup>[14]</sup>, or whole cells<sup>[15]</sup>.

Currently, the most targets for the aptamers selection of pathogenic microorganisms are the purified proteins with high water solubility. This is because the purified proteins ensure structural stability in each cycle, which improve the efficiency of SELEX screening<sup>[16]</sup>. However, by using purified protein as targets, there are some drawbacks. First, the purified proteins cannot maintain the native conformation in the whole cells, which lead to the binding constant between the screening aptamers and whole cells to decrease. Second, some pure target proteins are not easy to obtain, especially when the targets are unknown.

For the study of complex pathogenic microorganisms, it is necessity to develop a screening method for the composite targets. In recent years, more and more aptamers screening method for pathogenic microorganisms have used the whole cells as targets. However, this method is often difficult to screen the aptamers with high specificity due to the complex target sites. To solve this problem, the counter-selection cycle involves the incubation of a closely related cell or microorganism with the random nucleotide library to eliminate the sequences that are non-specific to the target<sup>[15,17]</sup>. Cao *et al.*<sup>[15]</sup> used *Staphylococcus aureus* as the targets, and used *Streptococcus* and *Staphylococcus epidermidis* for counter-selection to improve the specificity of aptamers. After several cycles, they obtained a group of 11 sequences of specific aptamers for *S. aureus*, which shows higher specificity than that of a single aptamer.

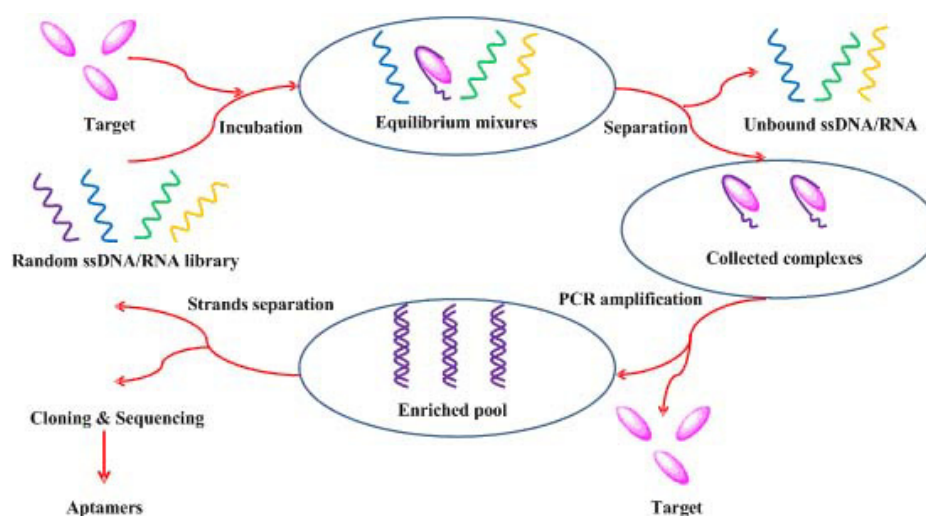


Fig. 1 Schematic of SELEX technology<sup>[11]</sup>

### 2.3 Separation of bound sequences

Effectively removing unbound oligonucleotides is the most critical step in aptamer selection process, which has a profound impact on the affinity and specificity of the screening aptamers. Good separation methods can effectively reduce the number of screening cycles and improve the specificity and affinity of screening aptamers. As the targets (proteins or whole cells) are biological macromolecules, commonly separation methods are filtering<sup>[8]</sup>, centrifugation<sup>[17]</sup>, magnetic beads separation. Although the above methods can remove the unbound oligonucleotides, they cannot get the affinity information between target-binding sequences and the pathogenic microorganisms, which need the followed testing process. Currently, a lot of new separation technologies such as capillary electrophoresis<sup>[19]</sup>, surface plasmon resonance (SPR<sup>[20,21]</sup> and flow cytometry, have been gradually used to separate target-binding sequences from non-specific oligonucleotides. These methods can not only remove the unbound oligonucleotides but also simultaneously evaluate the affinity of target-binding sequences and pathogenic microorganisms, which make the aptamer selection process relatively easier. The working principle of capillary electrophoresis is that the different charge-mass ratio between different components can result in the electrophoretic mobility differences. When the affinity between aptamers and targets is strong enough to form stable complex, relevant electrophoretic peaks for free oligonucleotides and the complex would be obtained in a suitable separation and detection condition<sup>[22]</sup>. The capillary electrophoresis has a great potential in this area owing to its advantages of rapidity, high resolution, simple operation, small amount of sample and low cost. In addition to capillary electrophoresis, SPR and flow cytometry have also been applied for aptamer screening. These methods not only efficiently separate the target-binding sequences but also simultaneously measure affinity information between targets and target-binding sequences, which simplify the steps in SELEX and are of great significance in the field of aptamer selection

## 3 Aptamers for pathogenic microorganisms

### 3.1 Protozoa

*T. cruzi* is an important kind of pathogenic microorganisms, and can be parasitized in a variety of warm-blooded and cold-blooded animals which caused great harm to the health of the human and animals. *Trypanosoma brucei* aptamer selection is one of the first reports for the screened aptamer for trypanosome. By using the whole-cell of *Trypanosoma brucei* as the targets, Homann *et al.*<sup>[18]</sup> selected a total of 22 sequences from three classes of RNA aptamers to specifically identify the flagellar proteins on the cell surface of *Trypanosoma*

*brucei*. Subsequently, Ulrich and co-workers selected another trypanosome-*Trypanosoma cruzi* as the aptamers and obtained a total of 23 sequences from four classes of RNA aptamers that specifically bound cell surface recognition sites that recognized the host of *Trypanosoma cruzi*<sup>[23]</sup>.

### 3.2 Virus

At present, the screened virus-specific aptamer are the aptamers for primary human immunodeficiency virus (HIV)<sup>[13,20,24,25]</sup>, hepatitis C virus<sup>[26–28]</sup>, influenza virus<sup>[17,21,29,30]</sup>, severe acute respiratory syndromes (SARS)<sup>[31]</sup> (Table 1). HIV is lentivirus genus with a very long incubation period retrovirus. Three kinds of aptamers of HIV were selected by using three different kinds of surface proteins of HIV as targets, including structural proteins RT (p50 reverse transcriptase)<sup>[13]</sup>, structural protein gp120<sup>[20]</sup>, regulatory proteins Tat<sup>[24]</sup>, respectively. The hepatitis virus that can cause viral hepatitis is another kind virus for aptamer screening. Fukuda and Biroccio respectively used non-structural protein NS3<sup>[26]</sup> and NS5<sup>[28]</sup> of hepatitis C virus (HCV) as the targets to select aptamers of HCV. Influenza virus is a kind of easy pandemic virus. Gopinath *et al.*<sup>[17]</sup> reported another aptamer selection process of another influenza A virus (H3N2). They used the whole-cell of H3N2 as targets to obtain the aptamers that specifically bound the HA protein on the surface of H3N2. The affinity between screening aptamers and HA proteins is 15-fold higher than the affinity between monoclonal antibodies and HA proteins.

### 3.3 Bacteria

For bacteria, the reported aptamers are mainly about *E. coli* (*E. coli*)<sup>[32–35]</sup>, *Bacillus anthracis* spores<sup>[36,37]</sup>, *Salmonella*<sup>[38,39]</sup>, *Staphylococcus aureus*<sup>[15]</sup>, *Mycobacterium tuberculosis*<sup>[39]</sup>, *Campylobacter jejuni*<sup>[41]</sup> (Table 1). *Bacillus anthracis* spores aptamer selection is one of the first reports for the screened aptamer for bacteria. Zhen *et al.* used *Bacillus anthracis* spores as the targets for aptamer screening and get 79 sequences from 13 different classes of DNA aptamers by 18 cycles<sup>[36]</sup>. *E. coli* is the main bacteria in human and animal gut, in which some specific serotypes are pathogenic for humans and animals, especially for infants and young animals (birds). In recent years, the *E. coli* aptamer selection has also been increasingly reported. Bruno used *E. coli* O111:B4 endotoxin LPS as targets for selecting specific aptamers of *E. coli* O111:B4<sup>[35]</sup>. Subsequently, the group also used *E. coli* 8739 outer membrane protein as targets and obtained 25 sequences of aptamers of *E. coli* 8739<sup>[33]</sup>. In addition, the aptamers of other types of *E. coli* (*E. coli* DH 5 $\alpha$ <sup>[32]</sup>, *E. coli* k88<sup>[34]</sup>) have also been reported. *Salmonella* is the main foodborne bacteria and there is also some reports about its aptamers screening<sup>[38,39]</sup>.

Table 1 Aptamers for pathogenic microorganisms

Target	Aptamer sequence (s)	Number of sequence (s)	Identification of target molecule (s)	Ref.
<b>Virus</b>				
Human immunodeficiency virus 1 (HIV-1)	AAACTGAAGAGGCACCGAAGGGGGG GTATT	25 sequences	Reverse transcriptase (RT)	[13]
HIV-1	ACGAAGCUUGAUCCCGUUUGCCGGU CGAUCGCUUCGA	4 sequences	Tat protein	[24]
HIV-1	CACCUACUAGACCACUUUUUGAGCCG GUUUUUUCGGGAACUUGCCAA	27 sequences	R5 SU glycoprotein (gp120)	[20]
Influenza A virus (H3N2)	GGGAGAAUCCGACCAGAAGGGUUA GCACUCGGCAUGGGGUACAGACAGA CCUUUCCUCUCUCCUCCUCUUCU	2 sequences	Hemagglutinin (HA)	[17]
Influenza B virus	GGGAGCUCAGCCUUCACUGCACUCCG GCUGGUGGACGCGGUACGAGCAAUU UGUACCGGAUGGAUGUUCGGGCAGC GGUGUGGCAGGGAUGAGCGGCACCA CGGUCGGAUCCAC	4 sequences	HA	[29]
Influenza A virus (H5N1)	GAATTCAGTCGGACAGCGGGGGGAA TTCTAGCAGTACACCTCGAGAATTATA GCCAGGATGGACGAATATCGTCTCCC	2 sequences	HA	[30]
Hepatitis C virus (HCV)	AUCCGACCAGAAGCUUCGGGAUUU GAGGGUAGAAUGGGACUACCUUCC UCUCUCCUCCUCUUCUGGGAGA	3 sequences	Non-structural protein 3 (NS3) protease	[26]
HCV	GCAGUAGUGUAUAGGC	4 sequences	NS3 helicase	[27]
HCV	CACGCGAGGCGUAUCGCAGCGUGGU	7 sequences from 3 classes	NS5 RNA polymerase	[28]
SARS coronavirus (SCV)	GCAGAAAAGGGGAAGAAGAGGGUG AUUCAGGCGAGAG	6 sequences from 3 classes	NTPase/Helicase	[31]
<b>Bacteria</b>				
<i>Escherichia coli</i> DH 5 $\alpha$	GGGAGAGCGGAAGCGUGCUGGGUCG CAGUUUGCGCGGUUCAACUUCUCUC AUCACGGAAACUAACCCAGAGGUC GAU	1 sequence	Non-identified	[32]
<i>Escherichia coli</i> 8739	ATCCGTCACACCTGCTCTGTCTGCGAG CGGGGCGCGGGCCCGGGGGATGC GTGGTGTGGCTCCCGTAT	25 sequences	Outer membrane proteins (OMPs)	[33]
<i>Escherichia coli</i> k88	GGCGACCCCGGGCTACCAGACAATG TACGCAGCAAGAGTGACGGTCTGATCC TCGGAGTC	4 sequences	Fimbriae protein	[34]
<i>Escherichia coli</i> O111: B4	ATCCGTCACCCCTGCTCTCGTCGCTAT GAAGTAACAAAGATAGGAGCAATCGG GTGGTGTGGCTCCCGTAT	20 sequence from 2 classes	Lipopolysaccharide (LPS)	[35]
<i>Salmonella typhimurium</i>	TATGGCGGCGTCACCCGACGGGACT TGACATTATGACAG	2 sequences	OMPs	[38]
<i>Staphylococcus aureus</i>	-	No sequences reported	Non-identified	[15]
<i>Streptococcus pyogenes</i>	TTCACGGTAGCACGCATAGGGCCCGA CACTCGTCCACCCGATACCTCTCATGT GTCCCCATCTGACCTCTGTGCTGCT	7 sequences	Non-identified	[18]
<i>Mycobacterium tuberculosis</i>	UCACUGUUAUCCGAUAGCAGCGCGG GAUGA	9 sequences	Membrane protein	[40]
<i>Bacillus anthracis</i> Sterne strain spores	-	No sequence reported	Non-identified	[37]
<i>Bacillus anthracis</i> Sterne strain spores	CAGGCCACGTGGGGTGTATAAATAAG TATGGG	79 sequences from 13 classes	Non-identified	[36]

Table 1 (Continued)

Target	Aptamer sequence (s)	Number of sequence (s)	Identification of target molecule (s)	Ref.
<i>Bacillus thuringiensis</i> spores	CATCCGTCACACCTGCTCTGGCCACTA ACATGGGGACCAGGTGGTGTGGCTC CCGTATC	1 sequence	Non-identified	[42]
<i>Francisella tularensis</i> bacterial protein lysate	-	No sequence reported	Non-identified	[14]

#### 4 Aptasensors for microbial detection

Biosensors are the analytical devices that combine bio-recognition elements and signal transducers to detect the target compounds<sup>[43]</sup>. Aptasensor is a new type of biosensor by using aptamers as bio-recognition elements. They convert the high-affinity reaction between targets and aptamers immobilized on the sensor substrate into physical, chemical, electrical or optical changes through sensor technology to detect the concentration of targets in the samples. Different transducers have a great influence on the performance of the biosensors. Therefore, according to the different transducers of the aptasensors for detection of pathogenic microorganisms, it contains three kinds of aptasensors, including electrochemical aptasensors, optical aptasensors and piezoelectric crystal aptasensors.

##### 4.1 Electrochemical aptasensors

Electrochemical aptasensors are formed by the electrodes immobilized with aptamers and recognition elements with electrochemical activity. First, under appropriate conditions the aptamers are immobilized on the electrode surface. After the interaction of aptamers with the target, a change in the structure of aptamers on the electrode surface occurs and thus the changes of electrochemical signal (potential, current, conductance or impedance) are observed, which can be used for the detection of the targets<sup>[44]</sup>. This kind of aptasensors combines the high specificity of binding of aptamers and target with the low cost and high sensitivity of electrochemical biosensors, so has considerable superiority.

Conventional potentiometric sensors for bacteria detection usually need bio-recognition elements to be labeled. This is because that the interaction of pathogenic microorganisms and bio-recognition elements cannot produce the electrochemical signal, so it needs to use markers for detecting potential changes. While Zelada-Guillen *et al.*<sup>[45,46]</sup> developed a label-free potentiometric sensor for detecting *Salmonella typhi* through using strong charge transfer capability of single-walled carbon nanotubes. A layer of the single-walled carbon nanotubes was modified on the electrode surface, and then the aptamers were immobilized on the surface of carbon nanotubes through self-assembled monolayer. When *Salmonella typhi* bound with aptamers, it led to

conformational change of aptamers. Conformational change of aptamers removed the phosphate on the surface of carbon nanotubes, which caused the potential changes on the surface of the electrode. The biosensor can be used to detect *Salmonella typhi* at the concentration range of 0.2–10<sup>3</sup> cfu mL<sup>-1</sup> [45]. The method has also been used to detect *E. coli* CECT 675 with the detection limits of 6 cfu mL<sup>-1</sup> and 26 cfu mL<sup>-1</sup> in milk and apple juice, respectively<sup>[46]</sup>.

Field-effect transistors (FETs) aptasensor as another new type of electrochemical aptasensors, with the advantages of miniaturation and fast response, has been widely used for the detection of various targets<sup>[47]</sup>. FETs aptasensor is based on the principle that the interaction of the aptamers immobilized on FETs and the target results in the changes of the distribution of charge on the FETs. Figure 2A is a typical scheme of FETs aptasensor. In comparison with antibodies, aptamers have the considerable advantages in fabrication of FETs aptasensors: (1) The size of aptamers is smaller (about 1 to 2 nm). The binding of the target to the aptamers occurs in the electrical double-layer, thus the resulting change of the charge distribution can be easily detected; (2) their small sizes and versatility allow efficient immobilization at high density on the FETs surface, thereby enhance the sensor sensitivity. So *et al.*<sup>[32]</sup> developed single-walled carbon nanotubes (CNT)-EFTs aptasensor for the detection of *E. coli*. The aptamers were immobilized on the surface of CNT. When bound with the aptamers, the *E. coli* has shielding effect on the negative charge of the aptamers, which led to significantly decrease of conductance.

##### 4.2 Optical aptasensor

According to the different transducers, optical aptasensors are classified into four types, surface plasmon resonance (SPR) aptasensor, surface-enhanced Raman aptasensor, chemiluminescence aptasensor and fluorescent aptasensor.

###### 4.2.1 Surface plasmon resonance aptasensor

SPR aptasensor is based on immobilizing the aptamers on the SPR chips. When the incident light with the critical angle of incidence enters into the resonator with two different refractive indexes, it leads to resonance of the free electrons of the metal. Due to electron absorbing light energy, the

reflected light is greatly reduced within a certain angle. When the binding of aptamers and microorganisms occurs, the displacement of the refractive index of the plasma resonance device is changed. The size and location of the displacement are related to the surface characteristics of the reaction layer<sup>[48]</sup>. Figure 2B is the scheme of the SPR aptasensor for the detection of pathogenic microorganisms. SPR aptasensor is label-free and has several advantages of miniaturization, automation and portability<sup>[5]</sup>.

Tombelli *et al.*<sup>[49]</sup> developed a SPR aptasensor to detect Tat protein of AIDS virus HIV-1. The aptamers were immobilized on the surface of the chip using the self-assembly method based on biotin-avidin linker. The different concentrations of HIV-1 Tat protein were injected to bind with the aptamers, which consequently induced the signal changes of refraction. The detection limit of this method is 0.12 mg L<sup>-1</sup>. Similar SPR aptasensor has also been used to detect apple stem hole virus (ASPV)<sup>[50]</sup>. Lautner *et al.* immobilized the aptamers of ASPV on the surface of chip by self-assembly method to detect the ASPV. The results showed that the biosensor was high sensitive and specific, and can be used for the detection of ASPV in real samples (For example, plant extracts).

#### 4.2.2 SERS aptasensor

When the light gets through the media, the incident photons and molecules collide with each other and the molecular vibrational/rotational energy and photon energy superimpose, which produced the scattering spectrum due to the change of frequency. That kind of scattering spectrum is called Raman spectroscopy. Surface enhanced Raman spectroscopy (SERS) is a enhanced Raman scattering produced by the molecules adsorbing on the surface of rough metal and nanoparticles<sup>[51]</sup>. Enhancement factor is up to 14–15 orders of magnitude, which can be used to study the molecular level information. Negri *et al.*<sup>[52]</sup> reported a SERS aptasensor for the detection of nucleoprotein of influenza virus. First, silver nanorods were deposited on SERS surface, and then the aptamers were immobilized on its surface, and final the nucleoprotein of influenza virus was added to bind with the aptamers to detect SERS signal. Using respiratory syncytial virus and 22 nt random sequence of DNA as negative control, the result showed that the biosensor had good specificity for detection of influenza virus nucleoprotein. Ravindranath *et al.*<sup>[53]</sup> reported a SERS aptasensor for simultaneously detecting multiple bacteria. The aptamers for *Salmonella typhimurium* and the antibodies of *Staphylococcus aureus* and *E. coli* O157:H7 were immobilized on the surface of gold, silver and silver core-shell three kinds of nanoparticles, respectively, and then Raman dye molecules were marked on them. The mixture was filtered to detect the Raman signal. The results showed that the biosensor had the advantages of good specificity and sensitivity, and can be used to simultaneously detect the

multiple bacteria in the mixture with a detection limit of 10<sup>2</sup> cfu mL<sup>-1</sup>.

#### 4.2.3 Chemiluminescence aptasensor

Chemiluminescence is the light radiation produced by some kind of molecule adsorbing chemical energy. Chemiluminescence method has the advantage of high sensitivity (detection limit of 10<sup>-12</sup>–10<sup>-21</sup> mol) because of its emitting photons detection and no interference of the scattered light background. In addition, it has the advantages of wide linear range (3 to 6 orders of magnitude), simple and inexpensive equipment demand and easy miniaturization<sup>[54]</sup>. Therefore, the chemiluminescence aptasensor has been attracted intense interest and become an important branch of the optical sensor.

Bruno *et al.*<sup>[37]</sup> developed a chemiluminescence aptasensor based on sandwich system to detect anthrax spores. First, the captured aptamer probes were immobilized on the magnetic beads, and then after *Bacillus anthracis* spores binding with the captured aptamers, Ru(bpy)<sub>3</sub><sup>2+</sup> labeled aptamer was added to react with the complex for chemiluminescent detection. Chemiluminescence intensity was related to the concentration of *Bacillus anthracis* spore. The chemiluminescence aptasensor has also been used to detect the nucleocapsid protein of SARS coronavirus (SARS-CoV)<sup>[55]</sup>. The target nucleocapsid proteins is bound on the surface of the aptamers, then antibodies were introduced to create aptamer-target-antibody sandwich structure, and then alkaline phosphatase conjugated anti-antibodies were added to bind with the sandwich complexes. Final, the surface was exposed to the substrate for chemiluminescent. The detection limit of the sensor was as low as 2 pg mL<sup>-1</sup>. This work provided a rapid, sensitive and effective method for the detection of SARS virus.

#### 4.2.4 Fluorescent aptasensor

Fluorescent aptasensors are mainly based on the fluorescence polarization or fluorescence intensity change produced by interaction of targets and fluorescent probe labeled aptamers for the detection of targets<sup>[41,56]</sup>. Ohk *et al.*<sup>[56]</sup> developed a fluorescent aptasensor to detect *Listeria monocytogenes* in food. The antibodies were immobilized on the waveguide surface to capture *Listeria monocytogenes* in food, and then the fluorescent probe labeled aptamers were used as markers to detect *Listeria monocytogenes* by detecting the fluorescence intensity. The detection limit of the biosensor for the detection of *Listeria monocytogenes* is 10<sup>3</sup> cfu mL<sup>-1</sup>. Xiao *et al.*<sup>[57]</sup> reported another fluorescent aptasensor to detect Nguyen viral protein (PrPC) (Fig.2C). The three guanines at 3' end of the aptamer can quench the 76.6% of fluorescence produced by the 5' end of the aptamer modified phosphor. When Nguyen viral protein (PrPC) combined with the aptamer, the guanines at the 3' end is far from the phosphor at



the 5' end resulting in fluorescence recovery. The detection range of fluorescent aptasensor was 1.1 to 44.7 mg L<sup>-1</sup> and the detection limit was 0.3 mg L<sup>-1</sup>.

Quantum dots (QDs) are spherical fluorescent nanocrystals, which has been widely used as the fluorescent probe. In comparison with the traditional organic fluorescent probes, QDs have many advantages such as high stability and multi signal detection<sup>[42]</sup>. Ikanovic *et al.*<sup>[42]</sup> used the aptamer-modified QDs to detect *Bacillus thuringiensis* spores. The detection limit of the method using zinc-coated CdSe QDs was 1000 cfu mL<sup>-1</sup>, which was six times lower than that of the method using the common fluorescent dyes. After the interaction of QDs-aptamers and bacterial spores, aptamer-QDs and spores conjugates were collected through the membrane, and were re-suspended in the buffer for measuring fluorescence signal. Roh *et al.*<sup>[58]</sup> used quantum dots labeled aptamers to detect the target protein of hepatitis C virus NS3 with a detection limit of 5 µg L<sup>-1</sup>. In recent years, quantum dots based aptasensors have been also used to detect *Campylobacter*<sup>[59]</sup>, *E. coli* O111:B4<sup>[60]</sup>, influenza A virus<sup>[61]</sup> and so on.

### 4.3 Piezoelectric crystal aptasensor

Quartz crystal microbalance (quartz crystal microbalance, QCM) is the most common piezoelectric crystal sensor due to its advantages of high sensitivity and simple device demand. As shown in Fig.2D), QCM aptamer sensor is based on the principle that aptamers are immobilized on the quartz crystal, and when the load increases, the oscillation frequency of the

chip would reduce under the external electric field, which is related to the mass adsorbed on the chip<sup>[62]</sup>. Minunni *et al.* used the QCM sensor to detect the Tat protein of AIDS virus HIV-1. The RNA aptamers of HIV-1 Tat protein were immobilized on the QCM gold electrode surface by self-assembly method and biotin-streptavidin linker to detect HIV-1 Tat protein with a detection limit of 0.25 mg L<sup>-1</sup>. The three proteins of BCL-2, hIgG, and HIV Rev were used to confirm the specificity. The result showed that the biosensor had a good specificity for the detection of HIV-1 Tat protein. Using sodium hydroxide and alcohol to regenerate, 15 times of regeneration cannot reduce the sensitivity of biosensor<sup>[63]</sup>.

## 5 Problems and prospects

In recent years, aptamers as a class of bio-recognition elements that possess many advantages such as high specificity and affinity, small size, easy synthesis, easy modification and relatively stability, have been increasingly used to construct biosensors to detect a variety of microorganisms. However, compared to wide variety kinds of antibodies, the types of aptamers for different pathogenic microorganisms are limited, which limits the development of the aptasensors for detection of pathogenic microorganisms. So it is necessary to develop more efficient screening methods for aptamers of different types of pathogenic microorganisms to screen out the high-affinity and high specificity of aptamers. Here, according to the development status of current aptasensors for detection of pathogenic microorganisms, we summed up the following trend of the aptasensors.

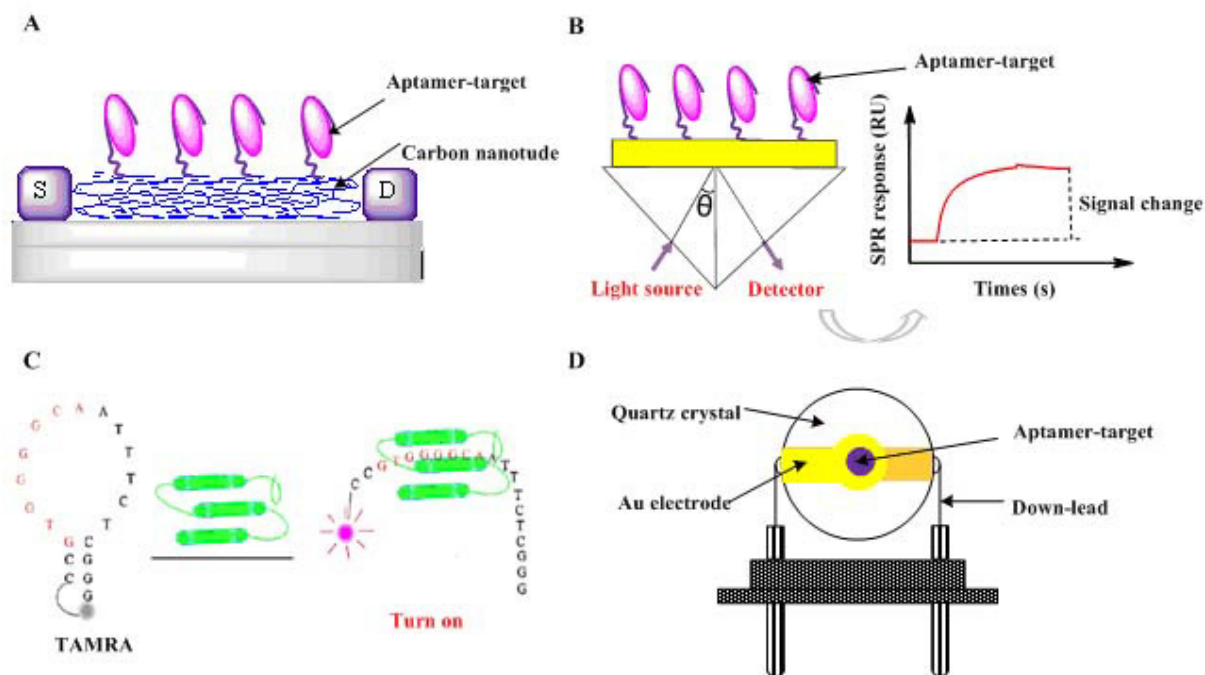


Fig.2 Schematic of aptamer based FETs biosensor (A), schematic of aptamer based SPR biosensor (B), schematic of aptamer based fluorescent biosensor for detection of prion protein (PrPC) (C) and schematic of aptamer based QCM electrode (D)



## 5.1 Nanomaterials

Nanomaterials have attracted intense interest because it can greatly improve the sensitivity and stability of the biosensors due to their excellent optical and electrical properties. Graphene<sup>[64]</sup> and carbon nanotubes<sup>[32]</sup> have excellent electrical conductivity, which are widely used in fabrication of electrochemical aptasensors. The gold nanoparticles<sup>[52]</sup>, carbon nanotubes<sup>[46]</sup> and other nanomaterials due to the biocompatibility and large specific surface area, are often used to immobilize aptamers to increase the surface density of aptamers in order to improve the sensitivity and stability of biosensors. The QDs<sup>[58–61]</sup> with high stability often replace traditional fluorescent dyes for detection. Metal nanoparticles<sup>[53]</sup>, magnetic nanoparticles<sup>[37]</sup> and other nanomaterials are widely used in optical and electrochemical aptasensor for signal amplification. Nanomaterials have played an increasingly important role in the field of aptasensors because of their remarkable performance. In particular, the emergence of new nanomaterials such as nano-composite materials (graphene-metal nanoparticles, metal nanoparticles modified carbon nanotubes, etc.) have brought new vitality for the development of the aptasensors.

## 5.2 Optimization of separation methods

In the SELEX aptamer selection process, the effective separation method of the nucleic acid sequences bound targets is the most critical step. In addition to the traditional separation methods including centrifugation, filtration, affinity chromatography, magnetic bead separation, some new separation technologies such as flow cytometry<sup>[65]</sup>, capillary electrophoresis<sup>[19]</sup>, surface plasmon resonance<sup>[20]</sup>, atomic force microscopy<sup>[66]</sup> are also used in separate binding sequences. These new technologies can improve the separation efficiency and reduce filtering cycles. The process can also obtain the affinity information of aptamers and the target, which greatly simplify the screening procedure and reduce the screening time. Combining these new high-performance separation methods with SELEX, the aptamer selection process to screening aptamers for a variety of pathogenic microorganisms is the future development direction in the field.

## 5.3 Microfluidics technology

Microfluidics technology is a kind of technology that used to control, operate and test complex fluids in the micro size. In recent years, microfluidic technology<sup>[67]</sup>, due to the advantages of trace, simplicity, automation, rapidity, high throughput, and portability, have been widely used in sample preparation, separation, detection process. However, the microfluidic technology used to construct the aptasensors to detect pathogenic microorganisms is rarely reported. Therefore, the

aptasensors combining with microfluidic technology can improve the sensitivity of detection of pathogenic microorganisms and realize the truly automated real-time online testing, which would be an important trend in future research.

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