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Applications of aptamers for chemistry analysis, medicine and food security

Rongrong Huang[1], Zhijiang Xi[1,2] & Nongyue He[1*]

[1]*State Key Laboratory of Bioelectronics; School of Biological Science and Medical Engineering, Southeast University, Nanjing 210096, China*
[2]*College of Life Science, Yangtze University, Jingzhou 434025, China*

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Since aptamer and its *in vitro* selection process called SELEX were independently described by Ellington and Gold in 1990, extensive research has been undertaken and numerous isolated aptamers for various targets have been applied. Aptamers can bind to a wide range of targets that include small organic molecules, inorganic compounds, haptens and even whole cells with high binding affinity and specificity. Aptamers for a wide range of targets have been selected currently. In addition, aptamers are thermo stable and can also be regenerated easily within a few minutes denaturation, which makes them easy to store or handle. These advantages make aptamers extremely suitable for applications based on molecular recognition as analytical, diagnostic and therapeutic tools. In this review, the recent applications of aptamers for chemistry analysis, medicine and food security, along with the future trend will be discussed.

aptamers, applications, chemistry analysis, medicine, food security

1 Introduction

The word "aptamer" is derived from a linguistic chimera composed of the Latin word "*aptus*", which means to fit, and the Greek suffix "-*mer*". Aptamers can be considered as nucleic acid analogue of antibodies. They can possess strong affinities to target molecules, with dissociation constants down to nanomolar or picomolar range. Currently, aptamers have been selected for a wide range of targets [1], including peptides, nucleotides, amino acids [2], antibiotics, proteins, vitamins, low-molecular organic or inorganic compounds and even whole cells.

Aptamers are generated by an *in vitro* selection process called systematic evolution of ligands by exponential enrichment (SELEX) [3,4]. Through this process, functional oligonucleotides against a specific target can be isolated from a random single strand DNA or RNA library. These oligonucleotides are folded into unique 3D structures [5]. Besides, cell-based SELEX has recently been reported which enables to isolate aptamers directly from living cells.

Aptamers are an emerging class of molecules with several important advantages. First, aptamers are efficient at binding both large molecules and small molecules. Unique folded structure of the aptamer is needed to adapt to its target [6]. Thus, aptamers show a high binding affinity and specificity to their targets. For example, an anti-*L*-arginine RNA aptamer which exhibits a 12000-fold affinity reduction toward *D*-arginine has been selected [7]. Second, aptamers are isolated and chemically synthesized *in vitro*. Therefore, they can be performed under non-physiological condition including extremely high or low temperatures. Furthermore, *in vitro* selection process makes it possible to obtain aptamers for a wide range of targets, including toxic or non-immunogenic molecules. Third, aptamers can be amplified through chemical synthesis which is cost-effective and has minimal batch to batch difference in activity [8]. Forth, aptamers are thermo stable and can also be regener-

*Corresponding author (email: nyhe1958@163.com)

ated easily within a few minutes denaturation, which makes them easy to store or handle. Finally, due to their small size and simple structure, aptamers enable easy control of bioavailability and delivery durability.

These advantages make aptamers an excellent choice for biomedical applications. Aptamers have been used in numerous investigations as therapeutic or diagnostic tools, for the development or delivery system of new drugs. For the first time, an aptamer has been approved by the US Food and Drug Administration (FDA) for the clinical treatment of age-related ocular vascular disease [9]. Moreover, aptamers are capable to be excellent chemistry biosensors and play an important role in the public health, environmental monitoring and food security.

One limitation that has yet to be addressed for the practical applications of aptamers is that nucleic acid aptamers could be vulnerable to nuclease degradation [10]. It has been shown that the stability of such aptamers can be improved by chemical modification of the ribose ring at the 2′-position [11]. Therefore aptamers with enhanced stability in biological fluids can be selected from libraries containing modified pyrimidines with 2′-amino and 2′-fluoro [12].

2 Applications of aptamers for chemistry analysis

2.1 Small molecules analysis

Aptamers hold a promise as molecule recognition tools when cooperated with affinity chromatography, capillary electrophorus, biosensors or mass spectrometry [13–15].

In comparison to the commonly-used antibodies in affinity chromatography, aptamers have better stability and longer shelf life. It is easy to modify aptamers with functional groups for immobilization onto a solid support. As aptamers are smaller than antibodies, the density of immobilized aptamers on the solid support can be higher. Furthermore, the aptamer-selection process can be carried out under well controlled conditions [16]. This unique feature benefits easy elution of the captured targets in affinity [17,18]. Han and co-workers [17] developed a DNA aptamer based high-performance affinity chromatography for selective extraction and screening of a basic protein lysozyme with high efficiency, low cost and ease-of-operation.

Aptamers can act as bioactive components in biosensors. This provides several advantages such as high sensitivity, rapidity in detection, cost-effectiveness and the ease of miniaturization. Zhang and co-workers [19] developed an electrochemical sensing strategy for highly sensitive detection of small molecules based on switching structures of aptamers from DNA/DNA duplex to DNA/target complex (Figure 1). Similarly, Zhu and co-workers [20] developed a highly sensitive and selective electrochemiluminescent (ECL) biosensor for the determination of adenosine.

Biosensors based on aptamers which are modified with a variety of organic dyes, carbon nanotubes, Ag colloidal nanoparticles and other nanoparticles have been used to detect digoxigenin, vitamin D, and folate, biotin (Figure 2) [21,22] etc. Furthermore, through the design of arrayed chips,

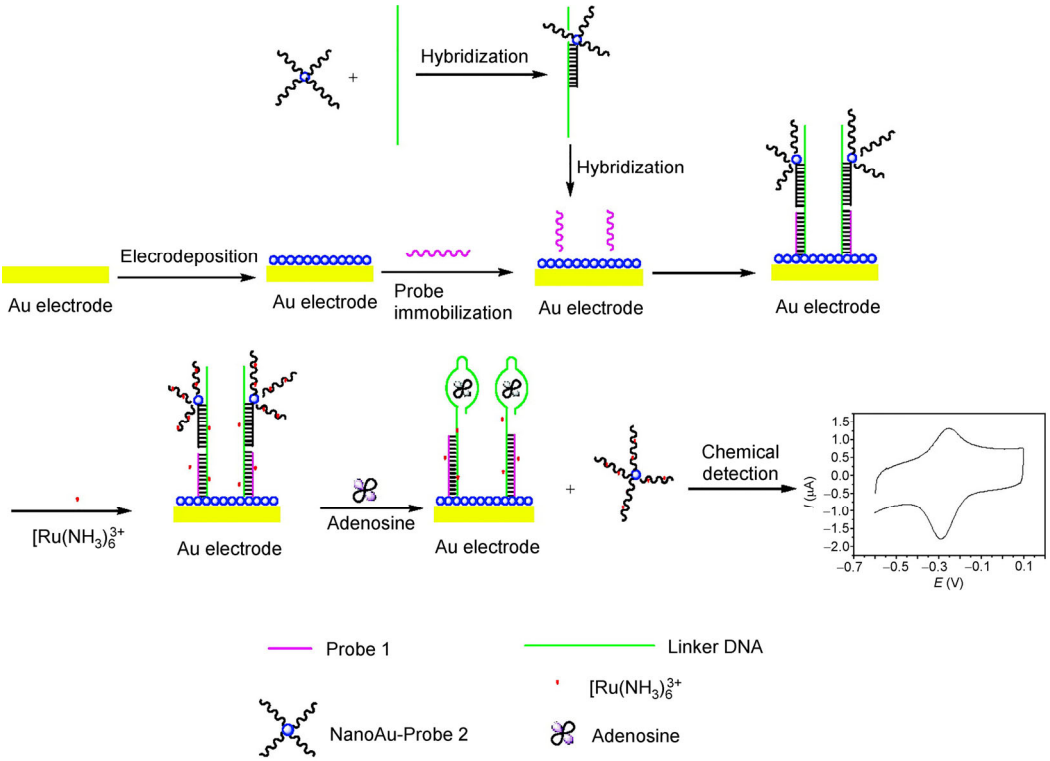

Figure 1 Schematic representation of the adenosine sensing: Probe 1 as the capture probe and Probe 2 as the report probe [19].

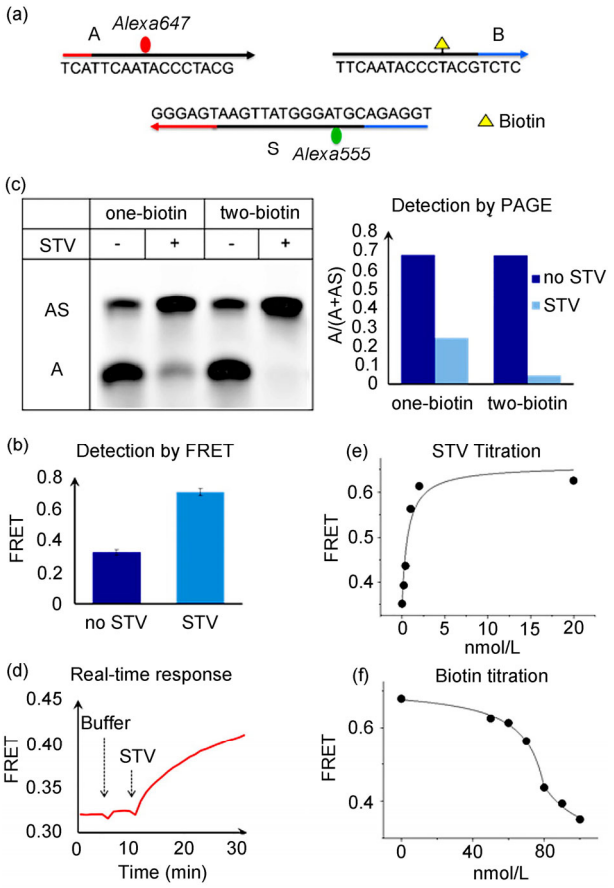

Figure 2 Detection of streptavidin (STV) and biotin as a model system [22].

aptamer-based biosensors are suitable for multitarget detection [23].

Besides, aptamers have been applied in affinity preconcentration, extraction, and purification of targets [24]. Aptamer immobilized on solid-phase supports column and magnetic nanoparticles (MNPs) [25] have shown some promise in this application.

The *L*-enantiomers of aptamers which can show similar selectivity and binding affinity to the mirror image of the targets have been used to separate molecules and their related compounds. By using a partial filling technique in combination with micellar electrokinetic chromatography, Huang and co-workers [26] developed a simple and reproducible method for enantioseparation and determination of *DL*-tryptophan (*DL*-Trp). Later, the same group reported a simple approach for rapid preparative separation of enantiomers, in which the separation of *DL*-tryptophan (*DL*-Trp) is demonstrated as an example to show the feasibility of the approach [27].

2.2 Protein analysis

Aptamers can not only bind their cognate protein but also inhibit its function efficiently. And therefore aptamers are promising in protein and protein-DNA interaction analysis. Given both chemical and structural approaches were used to improve stability, binding affinity and biological activity of a known thrombin-binding aptamer, Olga and co-workers [28] compared directly those two approaches to aptamer optimization and analyzed their relative advantages and disadvantages as well as their potential in drug design and fundamental studies.

Aptamer-based affinity capture approaches holds potential for analysis, sensing, purification and preconcentration of proteins [29,30]. An aptamer-based affinity purification for His-tagged proteins for comparison of purification efficiency with the conventional Ni^{2+}-based affinity chromatography was reported by Lim and co-workers [31].

Recently, an immobilized trypsin reactor that was based on aptamers and applied for the first time for proteomic digestion was reported by Xiao and co-workers [32]. Compared with in-solution digestion, the aptamer-based trypsin reactor exhibited similar results for protein identification but used a much shorter digestion time (approximately 30 min).

Aptamer-conjugated magnetic beads and quantum dots (QDs) have been successfully used in Western blots to detect the tracer proteins. Shin and co-workers [33] synthesized RNA aptamer-functionalized QDs, and demonstrated their application to specific protein detection, as an alternative to the conventional Western blot analysis. Their system could harness the high brightness, stability and reusability to quantitatively detect aptamer-recognizable proteins.

The detection of proteins was also reported by using quartz crystal microbalance. Tibor and colleges detected the binding of thrombin to an aptamers by quartz crystal microbalance (QCM) method in flow measuring cell [34].

3 Applications of aptamers for medicine

3.1 Diagnosis

Aptamers can be widely used in the diagnosis of certain disease such as cancer, angiocardiopathy, etc. There are several diagnostic applications and assay formats in which aptamers have proven their value as diagnostic tools.

Several aptamer-based assays have been proposed for early and accurate detection of proteins, such as cytokines, nucleolin, growth factors or cell surface receptors that act as oncogenes and cause cellular transformation, which have a particular emphasis in clinical and medical research [35]. Aptamers can serve as cancer diagnostic tools by detecting circulating cancer cells which are kinds of specific biomarkers, or imaging diseased tissue. So far, a variety of aptamer-based assays for cancer cell detection and early cancer diagnosis have been developed [36]. Among them, aptamer-conjugated nanoparticles (ACNPs) have become a promising agent as biosensors [37,38]. For example, a label-free and turn-on aptamer strategy for cancer cell detection based on the recognition-induced conformation alteration of

aptamer and hybridization-induced fluorescence enhancement effect of DNA-silver nanoclusters (DNA-Ag NCs) in proximity of guanine-rich DNA sequences was presented by Yin and co-workers [37]. This cancer cell detection strategy is simple, rapid, sensitive, universal, and specific, which may provide a new insight into the detection of cancer cells. Magnetic nanoparticles (ACMNPs) are another kind of nanoparticles used in this kind of strategy. Bamrungsap and co-workers [40] designed magnetic relaxation switches based on aptamer-conjugated magnetic nanoparticles. As can be seen in Figure 3, only cells treated with target ACMNPs showed bright fluorescence signal. The ACMNPs could detect as few as 10 cancer cells in 250 μL of sample.

Meanwhile, aptamer-based probes have shown great promise in molecular imaging applications. Noninvasive imaging with aptamer-based probes has been applied for lesion detection, patient stratification and monitoring using techniques include fluorescence imaging [41], molecular magnetic resonance imaging, targeted ultrasound, radionuclide-based imaging such as singlephoton emission computed tomography, and positron emission tomography (PET) [42–44]. For example, Rockey and co-workers [45] conjugated an RNA aptamer that bound specifically to a prostate specific membrane antigen (PSMA) to the radiolabeled chelator for targeted molecular imaging of prostate cancer by PET. And recently, Taleat and co-workers [46] reported an electrochemical immunoassay based on aptamer-protein interaction and functionalized polymer for cancer biomarker detection.

As thrombin plays a central role in a number of cardiovascular diseases, and is thought to regulate many processes in inflammation and tissue repair at the vessel wall, many assays, mainly biosensors, based on the thrombin-binding aptamer for the detection of thrombin have been developed and some of them have been used as analytical method for the detection of thrombin in real samples [47–49]. Bai and co-workers [50] developed a sensitive and selective sensor which showed good selectivity for thrombin without being affected by some other proteins, such as bovine serum albumin (BSA). Another method for rapid determination of thrombin spiked in whole blood was reported by de la Escosura-Muñiz and co-workers [51] by taking advantage of both aptamer-based recognition and the use of a nanoporous membrane.

Other proteins related to certain diseases can also be targeted by aptamers [52]. Aptamers which can detect three adipokines for diagnosing type 2 diabetes have been used by Lee and co-workers [53]. The sensitivities of this method for all three adipokines were enhanced by at least 20-fold to up to 68-fold higher than that of the surface plasmon resonance (SPR) system, which only used aptamers as capturing probes.

3.2 Therapy

Aptamers can not only detect proteins but also bind to specific sites on biological molecules which include cell factors, enzymes, hormone, toxin or membrane-bound proteins. This property can be used for therapeutic purposes in many diseases and have been slowly reaching the marketplace [54,55]. A quantity of high-affinity aptamers that target a broad of biological molecules including HIV-1 regulator of virion protein expression (Rev), selectin, acetylcholine receptor (AChR), integrin, cytohesin, interferon, angiopoietin, protease, heat shock factor 1 (HSF1), hormone, peptide, anti-amylin, plasminogen activator inhibitor, orphanin and staphylococcal enterotoxins have been found for therapeutic applications.

3.2.1 Aptamers binding to membrane-bound proteins

Many researches on aptamers which target to membrane-bound proteins including surface receptors, cell adhesion molecule have been done during the decades. For example, Integrin αvβ3 is a crucial factor involved in a variety of physiological processes, such as cell growth and migration, tumor invasion and metastasis, angiogenesis, and wound healing. Therefore, inhibiting the function of αvβ3 integrin represents a potential anti-cancer, anti-thrombotic, and anti-inflammatory strategy. Lim and co-workers [56] developed aptamer$_{\alpha v\beta 3}$-conjugated magnetic nanoparticles (Apt$_{\alpha v\beta 3}$-MNPs) to enable precise detection of integrin-expressing cancer cells.

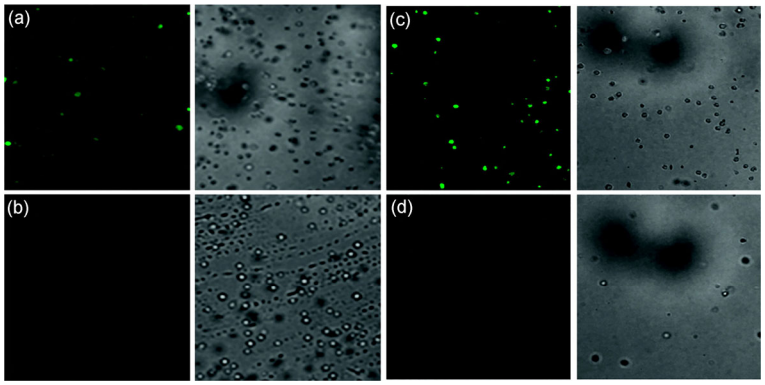

Figure 3 Specific recognition of the magnetic nanosensor to their target cancer cells. Left is fluorescence image and right is transmission image [40].

Besides, aptamers have provided a new therapeutic agent for sickle cell disease. Adhesive interactions between circulating sickle red blood cells (RBCs), leukocytes, and endothelial cells are major pathophysiologic events in sickle cell disease (SCD). A new therapeutics that efficiently inhibiting adhesive interactions was developed by Gutsaeva and colleges [57] by generated an anti-P-selectin aptamer. The anti-P-selectin aptamer inhibited the adhesion of sickle RBCs and leukocytes to endothelial cells. It also increased microvascular flow velocities and reduced the leukocyte rolling flux.

Aptamers can target to certain cells and form a novel targeted drug-delivery platform. Boyacioglu and co-workers [58] identified a new DNA aptamer to prostate-specific membrane antigen (PSMA), which is of interest for selective delivery of therapeutics for cancer treatment as a consequence of its elevated expression on the apical plasma membrane of prostate cancer cells and in endothelial cells of vasculature from diverse malignancies, to develop dimeric aptamer complexes (DACs) for specific delivery of Dox to $PSMA^+$ cancer cells [58].

3.2.2 Aptamers binding to cell factors

Cell factors, include interleukin, colony stimulating factor, interferon, tumor necrosis factor, transforming growth factor-β family, growth factor and chemokine family, have a wide range of biological functions [59]. For example, Liu and co-workers [60] developed an electrochemical aptasensor for simultaneous detection of two important inflammatory cytokines, interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α).

Furthermore, aptamers can offer new ways to treat diseases. For example, Siller-Matula and co-workers [61] used an aptamer that was specifically inhibit anti-Von Willebrand Factor (VWF), which plays an important role in the initiation of platelet adhesion and aggregation and shear-dependent thrombogenesis and its levels are heightened in patients experienced adverse cardiac events that are linked to a poorer prognosis, as a new antiplatelet therapy.

3.2.3 Aptamers binding to enzymes implicated to certain diseases

Protein tyrosine phosphatase 1B (PTP1B) is an enzyme implicated in type 2 diabetes, breast cancer and obesity. Townshend and co-workers [62] found an aptamer that strongly inhibits PTP1B *in vitro*. They also investigated various single-nucleotide modifications to the aptamers which represented an exciting option for the development of lead nucleotide-based compounds in combating several human cancers and metabolic diseases.

Similar to PTP1B, Hu and co-workers [63] identified two aptamers selectively bind to Shp2, which has the potential to be used for further development of Shp2 assays and therapeutics for the treatment of Shp2-dependent cancers and other diseases.

Other enzymes such like proteases [64], thrombin [65], etc. have also been studied.

3.2.4 Aptamers binding to other biological molecules

Other biological molecules including transcription, hormones, neurotransmitters, toxins [66–69] etc. have also aroused attention. For example, heat shock transcription factor (HSF1) is a conserved master regulator that orchestrates the protection of normal cells from stress. Salamanca and co-workers [66] generated a highly specific RNA aptamer that bound Drosophila HSF1 and inhibited HSF1 binding to DNA and did experiment on animals. They demonstrated that the RNA aptamer technology is a promising chemical genetic approach to investigate biological mechanisms.

A prospective therapy, named "systemic therapy", has recently brings forth a new trend in cancer treatment. Zhao and co-workers [70] reported an aptamer-NASBA-based lab-on-a-chip (LOC) for systemic therapy and was fabricated and tested as an ultra-sensitive tool to monitor signaling molecular profiling in serum samples. The results could help doctors fully understand the body of patients.

Aptamer-modified probes can assist rapid detection of toxins which affect human health directly or indirectly. For example, staphylococcal enterotoxin B (SEB), produced by *S. aureus*, is one of the exotoxins in the staphylococcal enterotoxins group. SEB is commonly associated with food poisoning and causes nonmenstrual toxic shock. Temur and co-workers [71] presented a detection method for SEB which was evaluated for investigating the SEB specificity on bovine serum albumin and avidin and detecting SEB in artificially contaminated milk, blood, and urine.

3.3 Clinic examples

There are now several aptamers that have undergone clinical trials (Table 1). For example, AS1411, a DNA aptamer, is one of the most fully studied aptamers which has been evaluated in phase II clinical trials. AS1411 can resistant to nuclease degradation and bind to a specific cellular protein named nucleolin, which can provides a target point for drug delivery [72].

The application of AS1411 for drug delivery, including AS1411 conjugated liposomes have been reported [73], and its diagnostic use has also been researched. Recently, Wu and co-workers [74] developed a nucleolin targeted protein nanoparticle (NTPN) delivery system in which human serum albumin (HSA) was used as drug carrier and a DNA aptamer named AS1411 used as a bullet.

4 Applications of aptamers for food security

Food is often contaminated by microorganisms such as bacteria, viruses or parasites and abnormal proteins, prions,

Table 1 Examples of aptamers used for clinic applications

Name	Target	Applications	References
AS1411	nucleolin	Targeted drug delivery system	[72–74]
Sgc8c	Protein tyrosine kinase7 (PTK7)	Targete hard-to-transfect cells, including cancerous lymphocyticcells	[75]
TD05	B-cell receptor (membrane Ig: mIgM)	Reactive with Burkitt's lymphoma	[75]
ARC15103ARC15104ARC15105	Von Willebrand Factor (VWF)	Myocardial infarction	[61]
ARC5690	P-selectin	Therapeutic agent for sickle cell disease	[57]
GBI-10	Tenascin-C (TN-C)	Diagnostic and therapeutic tool	[75]
ARC513 ARC592 to ARC 594 and ARC1472 to ARC1474	platelet derived growth factor (PDGF)	Related to mesangial cells proliferation and matrix accumulation	[76,77]

and can cause various illnesses, with symptoms ranging from mildly uncomfortable to life-threatening. In spite of the progressive implementation of good agricultural and handling practices as well as educational programs, the incidence of zoonotic diseases is still high. Therefore, detection of pathogens is important for both health and safety reasons. Aptamer-based techniques have been widely used for the detection of foodborne pathogens [78].

4.1 Pathogenic bacteria

Aptamers can detect pathogenic bacteria from food and prevent diseases like fatal neurodegenerative disorders, parasitic disease, etc. from happening.

Fatal neurodegenerative disorders such as Creutzfeldt-Jakob disease in humans and bovine spongiform encephalopathy in cows are diseases caused by misfolded cellular prion proteins (PrP). Xiao and co-workers [79] developed a novel method to detect PrP which held the advantage of being label-free, rapid, highly sensitive, selective, and showed great promise for clinical application.

By targeting some of the surface proteins of microorganism, the growth of the bacteria can potentially be inhibited or reduced or the secretion of toxins can be prevented. It is reported that aptamers functionalized biosensor can not only selected against purified target molecules but also selected to bind whole bacterial cells. Ohk and co-workers [80] developed an antibody-aptamer functionalized fibre-optic biosensor for specific detection of *Listeria monocytogenes*, which is important in the fermentation of a lot of foods from dairy products to fruits and vegetables. Recently, Zhang and co-workers [81] used aptamer as a recognition element of biosensor, and integrated it to microarray chip to realize the detection of *Listeria achidophilus*.

Aptamer-based techniques can potentially be used to detect pathogenic viruses in contaminated environmental or food matrices. Aptamers binding to *Campylobacter jejuni cells* [82] with specificity have been found.

Aptamer-based detection methods for microbial pathogens do not need tedious isolation and purification of complex markers or targets and the pathogens can be directly detected from different food matrices. Their potential for large scale synthesis combined with their specificity of binding suggests that they have potential to inhibit or block the growth of bacteria within food matrices or to prevent binding to host cell surfaces and thus infection.

4.2 Other contaminants in food

Aptamer-based biosensor can be used for rapid, sensitive and highly selective detection of endocrine disrupting compounds (EDCs). Yildirim and co-workers [83] reported a reusable evanescent wave aptamer-based biosensor to detect 17β-estradiol. The sensor could be regenerated with a 0.5% SDS solution (pH 1.9) over tens of times without significant deterioration of the sensor performance.

Quantities of papers have been published about identification of aptamers binds with high affinity and specificity to ochratoxin A (OTA), a mycotoxin that occurs in wheat and other foodstuffs [84]. An aptasensor using aptamer-DNAzyme hairpin as biorecognition element was reported [85]. In the presence of OTA, the hairpin was opened due to the formation of the aptamer-analyte complex. As a result, self-assembly of the active HRP-mimicking DNAzyme occurs. Recently, Maureen and co-workers [86] selected a DNA aptamers that bind to OTA and provided a label-free detection platform which is capable of rapid, selective, and sensitive OTA quantification with a limit of detection of 9 nmol/L and linear quantification up to 100 nmol/L.

Mastitis is a disease which causes inflammation of the udder tissue in cattle and is the most common disease affecting the dairy industry today [87]. This disease can increase the somatic cell count in milk and is caused by a number of pathogens. Ashley and co-workers [88] developed a highly sensitive and specific SPR based aptasensor for the detection of catalase protein in milk samples and used it to measure the catalase directly in milk samples and could allow for rapid determination of mastitis disease in milk.

5 Other applications

Aptamer-modified columns have also been used in environmental analysis and in the removal of environmental contaminants, such like persistent organic pollutants, biological toxins, and pathogenic bacteria [89,90]. Hu and co-workers [91] developed an aptamer-modified column

which showed the ability to remove organic contaminants at ng/L levels in drinking water.

DNA aptamers were generated to remove arsenic from Vietnamese groundwater [92]. Aptamers selected for specific binding to inorganic arsenate [As(V)] and arsenite [As(III)] can modify resins to remove arsenate and arsenite from groundwater within a short period of incubation (5 min).

There are some aptamers that have been actually applied to the detection of the target molecules for environmental analysis. Such like specific aptamers against microcystin-LR [93,94] and DNA aptamers against 17b-estradiol [95] etc.

Development of DNA aptamers made them useful to test drugs in sports. Detection of athletes who use drugs to enhance physical strength and obtain an advantage in competitive sports is a formidable problem. Such like synthetic human growth hormone (hGH), which is virtually identical to the natural pituitary hormone. In the present work, aptamer can help to identify athletes who are potentially cheating by administration of rhGH. Bruno and co-workers [96] proved DNA aptamers could discriminate similar forms of the same target polypeptide in an ELISA-like microplate format.

Recombinant human erythropoietin (EPO)-alpha (rHuEPO-α) is an EPO mimetic widely used as an illegal drug for enhancing stamina among athletes. The availability of aptamer as an elegant class of molecular recognition element can be pragmatic for detecting EPO [97]. Recently, Citartan and co-workers [98] selected an aptamer which can act as "universal probe" against all rHuEPOs.

6 Conclusions and future aspects

The ability of aptamers to recognize virtually any target with high affinity and specificity gives them great potential for use in a wide range of applications. They can act as recognition elements to be used in analytical applications, ranging from separation techniques to biosensors. Aptamers can also be employed in diagnostic and therapeutic assay formats [99], such like complement antibodies as affinity reagents in bioanalytical assays, incorporated into different solvent system or cooperating with nanoparticles to enable controlled drug release, and targeted therapy. Furthermore, the development of aptamers aiming at high volume, centralised food production, combined with rapid distribution of ready meals to retailers has served for rapid diagnostics to protect consumers from potential exposure to food pathogens.

Despite the progress, aptamer research in the laboratory has been slow to reach practical applications. In the medical field, previous aptamers have been primarily used to target transmembrane proteins, but their use in modulating the function of intracellular proteins has been limited due to a poor understanding of the mechanisms [100]. In addition, the cost of industrial-scale production of long, high quality aptamers remains very high and significantly limits. Continued effort in the development of aptamer technology will ensure aptamer-based systems to play a critical role in more areas.

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