Aptamer: A Versatile Probe in Medical Diagnosis

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Abstract: The aptamer is a single standard nucleic acid, also known as an artificial antibody, has been selected from the randomized library of oligonucleotide molecules by the process of “systematic evaluation of ligands by exponential enrichment.” Since the selected aptamers have displayed advantages and rival the antibodies, potentials of aptamer are widely spread to different fields such as medicine, therapeutics, environmental, and biosensor. In particular, aptamers have been focused in the field of medical diagnosis due to its higher sensitivity and specificity against the target molecules. Various kinds of sensors were utilized to diagnose the different kinds of diseases using aptamer as the probe. In this overview, we discussed the detailed applications of aptamers with the field of medical diagnosis.

Keywords: Aptamer, biosensor, medical diagnosis, imaging, drug delivery.

1. INTRODUCTION

The aptamer is from the Latin word “aptus,” representing the meaning of “fastened” or “fitting”. On the other hand from Greek, it brings along the meaning of “portion.” In English, aptamer means with the word “polymer.” The aptamer is a small single-stranded nucleic acid molecule; its nucleotide length usually is between 15 and 40 nucleotides. It normally folds into a well-defined three-dimensional structure, and the size of the aptamers is in the ranges of 6-40 kDa. Aptamer also knew as artificial oligonucleotides can be synthesized by chemically or enzymatically or by a combination of these methods. The process is known as in vitro selection or systematic evolution of ligands by exponential enrichment (SELEX). Aptamer has a very high affinity and specificity, in the range of picomole to nanomole. It can bind to wide types of targets are ranging from small molecules, amino acids, peptides, proteins, and even the whole cells. One of the properties of the aptamer forms a complex structure, can create unique secondary and tertiary structures that undergo conformation changes on binding with the appropriate target molecules. The benefits of using aptamer compared to antibodies are shown in Table 1. An aptamer can adapt to any circumstances, for instance, they can be changed to bind to the appropriate region of the target under varied conditions. Aptamer has more stability and a longer lifespan compared to the antibody, in addition, aptamer can withstand at a higher temperature and also can be regenerated after being denatured. Moreover, the quality of aptamer is more consistent. Further, aptamers have other merits such as cheaper, no batch variation, and non-immunogenic [1-4].

In general, the aptamer is a nucleic acid (either deoxyribose nucleic acid [DNA] or ribose nucleic acid [RNA]) molecule, known as “chemical antibody,” generated from the library of randomized molecules using the method called “SELEX” with three simple steps, namely binding, separation, and amplification [1-4] (Fig. 1a). In the first step, the target molecule mixed with the randomized library of molecules. In step 2, the bound molecules are separated from the unbound molecules of the library. For separation, researchers used various separation methods, such as magnetic separation, filtration, and titer-plate separation method.
step, the bound molecules are amplified, and the multiplied molecules again allowing to bind with the target molecule. This process is repeated for 10-14 rounds to get the specific aptamer molecule. However, single-cycle aptamer selection strategies were also been demonstrated. The RNA aptamer selection process slightly varies from the DNA aptamer selection. In the RNA aptamer process, the bound RNA is converted into DNA by the reverse transcription and then amplified for the next round of selection process, whereas in the case of DNA aptamer generation asymmetric polymerase chain reaction is performed [4]. In general, it has been found that DNA aptamer is stable than RNA aptamer. However, RNA aptamer can be stable with the incorporations of chemical groups during the synthesis. Polyethylene glycol and 2' fluoropyrimidine are some of the common chemical groups aiding to stabilize RNA aptamers. It is interesting to note that RNA aptamer creates more possible secondary structures than DNA and also has a higher affinity for the target.

Since the selected aptamers have more binding affinity, specificity and selectivity with their target molecule, the applications of aptamer are widely spread in many fields such as drug delivery, drug discovery, therapeutics, hazard metal ion detection, biosensing technology, bioimaging, and environmental monitoring [5,6]. Among these potentials, the application of an aptamer in the field of medicine is welcomed due to its higher sensitivity and selectivity. Moreover, the aptamers are more stable in the acidic, and other stringent conditions and the stabilized aptamers are highly applicable for the in vivo system. In this review, we discussed the applications of aptamers in the medical diagnosis against various diseases including cancers, pathogen infection, and blood diseases (Fig. 1b).

2. APTAMERS IN MEDICAL DIAGNOSIS

Aptamers are playing a pivotal role in the field of medicine and its interdisciplinary sciences. At present, several nano- and micro-sensors are in vogue using aptamer as the probe. The primary usage of aptamer as the probe is due to the proper attachment of aptamer on the sensing surface in the right orientation, which may not be possible easily with

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Aptamer</th>
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<tbody>
<tr>
<td>Larger in size</td>
<td>Smaller in size</td>
</tr>
<tr>
<td>Difficult to modify</td>
<td>Easier to modify</td>
</tr>
<tr>
<td>Shorter half-life</td>
<td>Longer half-life</td>
</tr>
<tr>
<td>Varied with batches</td>
<td>No batch variation</td>
</tr>
<tr>
<td>Predominantly need the animal system (in vivo)</td>
<td>Can synthesis chemically or enzymatically (in vivo)</td>
</tr>
<tr>
<td>Laborious in preparation</td>
<td>Easier to prepare</td>
</tr>
<tr>
<td>Lesser discrimination against the targets</td>
<td>Higher discrimination against the targets</td>
</tr>
<tr>
<td>Sensitive to the temperature changes</td>
<td>Not affected by the higher temperature</td>
</tr>
<tr>
<td>Undergo irreversible denaturation at stringent conditions</td>
<td>Undergo denaturation followed by renaturation</td>
</tr>
<tr>
<td>Has immunogenicity</td>
<td>Has non-immunogenicity</td>
</tr>
<tr>
<td>Limited against small molecules and toxins</td>
<td>No limitation against the sizes of the targets</td>
</tr>
</tbody>
</table>

Fig. (1). Aptamer generation and applications. (a) Systematic evaluation of ligands by exponential enrichment. Three important steps involved are indicated. (b) Potential applications of aptamers. Important disease sources such as microbial-pathogens, cancer, and blood biomarkers are displayed.
the antibodies. The antibody has the antigen binding regions near the light chain regions, and these regions are needed to be exposed on the sensing surface to interact with the appropriate antigen. However, immobilization of antibodies on the surface is giving different orientations as completely exposed, partially exposed, and masked completely, makes hurdle in most of the sensing systems. Whereas, aptamers can immobilize properly on the sensing surface and ready to interact the target molecule specifically and displays the high-performance (Fig. 2).

2.1. Diagnosis of Pathogens by Aptamer

Application of aptamer in the field of medical diagnosis is mandatory for the early detection of diseases. Early detection of diseases helps to treat easily and avoid spreading of infectious and contagious diseases such as influenza, dengue, cholera, and Leptospira. Aptamers have been used to detect various types of viral and bacterial pathogens. Influenza is a pandemic spreading seasonal diseases and affecting several people every year all over the world. The current detection system of immunochromatography test for influenza and other disease detection is not sensitive for early diagnosis. In that case, aptamer helps to detect the pathogenic biomarkers at the earlier stage. Different aptamers were generated against Influenza viral types A and B [3,7-9]. Lakshmipriya et al. [3] have selected aptamers against influenza B virus and detected influenza with the waveguide-mode sensor and reached the limit of detection to 100 picomolar (pM), which is a much lower level than influenza antibody-mediated detection. Gopinath et al. [2,10,11] produced various aptamers against different strains of influenza A virus and detected the particular influenza sub-type with its aptamer. These groups have utilized waveguide-mode sensor and the surface plasmon resonance [11,12], which displayed higher sensitivities. Aptamers are not only used for the detection but it also can discriminate the closely related strains due to its selective binding with their target [13]. Gopinath et al. have discriminated the influenza viruses with the specific detection using the aptamer [14]. Various aptamers were generated against different viruses for diagnosing purposes. Aptamers were used to detect the other viruses, which include human immunodeficiency virus (HIV) [15], herpes simplex virus (HSV) [1], human papillomavirus (HPV) [16], and hepatitis C virus (HCV) [17]. Aptamers generated against HIV-1 transactivator of transcription (TAT) protein were used to detect HIV virus with the help of surface plasmon resonance biosensor [15]. HIV-TAT peptide detection was also carried out on the carbon nanotube modified electrode with its particular aptamer [18]. HSV is one of the main causes of infectious illness, aptamers generated against HSV were used to detect the HSV at an earlier stage [1,19]. Furthermore, RNA aptamers selected against the HPV-16 E7 oncoprotein were proved for the detection of HPV associated cervical cancer [16].

2.2. Diagnosis of Cancer by Aptamer

The aptamers selected by cell-SELEX and in vivo SELEX are generally used to detect the cancer cells with the lower detection level. In vivo SELEX also involves the similar three main steps like in vitro SELEX. In the case of in vivo SELEX, the 2'-fluoropyrimidine-modified or other stable modified oligonucleotide library of molecules are injected into the animal system through the tail vein. Then, the tumor region was harvested and treated to extract the bound molecules.

Fig. (2). Probe arrangement on the sensing surface. Possibilities with the immobilization of antibody and aptamer are shown. In general, aptamers are shown to attach in right orientation on the sensing surfaces compared to the antibodies.
from the tumor tissues and used for the next round of SELEX [20]. After 14 rounds they obtained the high-affinity aptamer, and the selected aptamer can bind with a target and also it can penetrate into the parenchyma. In another research, Van Bel et al. selected the aptamer against HIV-1 leader RNA using in vivo SELEX [21]. Furthermore, the aptamers against the human colorectal cancer were selected by in vivo selection strategy [22]. They found that the selected aptamer targeted to the RNA helicase protein DHX9. The aptamers selected by in vivo method shows excellent stability in all biological milieu. Yang et al. [23] identified the prostate cancer by the aptamer generated against the prostate-specific antigen. Colorectal cancer is one of the death-causing diseases, Hung et al. selected the aptamer against the colorectal cancer stem cells using the on-chip cell-SELEX method, it is very useful to detect colorectal cancer at earlier stages [24]. Not only that the aptamers selected against HER2 was used to detect the breast cancer [25].

2.3. Diagnosis of Blood Biomarkers by Aptamer

Apart from the pathogen and cancer detections, aptamers were selected against the clotting proteins, used to diagnose and treat the blood-related markers to reflect the blood diseases. Aptamers generated against the clotting proteins including factors, factor IX (FIX), FVIIa, FXI, FVIII, and thrombin [26]. The aptamers selected against FIX is now under clinical phase trials. Gopinath et al. have generated the aptamers against bovine FIX and it is different from the previously selected aptamer against human FIX. The selected aptamer against bovine FIX is able to distinguish from the human FIX [14]. Using the aptamer selected against FIX, it has been used to detect the clotting disease using various sensors. Cheen et al. have used the impedance sensor to detect the FIX protein with its aptamer and the limit of detection was reached to 10 pM [27]. Lakshmipriya et al. used polyethylene glycol based surface modification on the silica surface to detect the FIX by its aptamer; the limit of detection was reached in pM range [28]. FIX aptamer was also immobilized on the surface plasmon resonance biacore sensor surface and the FIX was detected with antibody conjugated gold nanoparticle (GNP) as a sandwich [29]. Not only for the detection, FIX aptamer used with the antidote during the surgery to reverse its activity. Apart from the FIX, another important protein in the clotting cascade is the thrombin. From the blood-clotting cascade, FIX can be used to find the clotting detect at the early stage, whereas thrombin acts at the later stage. Various DNA and RNA aptamers were generated against thrombin protein [30-32]. The electrochemical sensor was used to detect the thrombin with its selective thrombin binding aptamer; the detection limit was found to be 1 pM [33]. Aptamers based surface-enhanced resonance scattering technology has been used to detect the thrombin protein with the limit of detection of 1 nanomolar. Since each aptamer has different binding sites and affinities with the cognate target, different aptamers were fished-out

![Figure 3](image.png)  
Fig. (3). Gold-nanoparticle based sensing strategies. Polymer-assisted sensing and antibody complementation with the sensing strategies are shown. The step-wise molecular assembly is also shown.
for the same target, they can be used for generating the sandwich assay [34,35]. Human alpha-thrombin was detected with two different aptamers selected against thrombin with the lower limit of detection of 64 pM [34]. Aptamer and GNP based colorimetric assay was also used to detect the targets [36] as shown in the model Fig. 3. The strategy shown in Fig. 3 is also elucidating the necessary important steps during the aptamer-mediated diagnosis of medicinally important biomarkers. The aptamers are conjugated with the GNP, on binding of aptamer with the thrombin the color of the GNP changes from red to blue with a high salt concentration [37]. This type of colorimetric assay can have an easy visual detection carried out by the selective aptamer for the particular target. The sandwich type of enzyme-linked colorimetric assay was also carried out to detect the thrombin by its aptamer, the limit of detection attained is 25 pM [38]. Other important targets in the bloodstream for the diagnosis using aptamers are C-reactive protein, squamous cell carcinoma antigen.

CONCLUSION

The aptamer is an artificial antibody generated by the method called “SELEX” with three simple steps. Due to the high affinity, the applications of aptamer are widely spread in several fields, particularly in front of medicine. In the field of medicine, aptamer has been used to diagnose/treat the diseases by biosensing, drug delivery, and bio-imaging. For the diagnosis, several aptamer-based sensors (aptasensors) were shown to prove the potentials of the aptamer as the probe. Further, several aptamer complementations with other probes were demonstrated for the efficient detection of pathogens, cancers, and blood biomarkers. Due to the versatility of aptamers with the secondary structures and their suitability with the chemical modifications, aptamers have been applied for the clinical studies. Several aptamers are currently under the process of generating against various disease targets, and in the near future aptamer-mediated applications in the field of medicine will be focused more to detect and treat different diseases.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICTS OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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REFERENCES


