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Aptasensors in viral detection

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ABSTRACT

Background: Aptamers are single-stranded nucleic acids, so-called 'artificial antibodies', identified from the randomized combinatorial library against the target by the process called 'SELEX' (Systematic Evolution of Ligands by EXponential enrichment). Target can have any sizes from small molecules to the whole cell, attests the versatility of aptamers to bind a wide range of targets. Aptamers have several advantages over antibodies, such as they are easy to prepare, cheaper, have no batch variations, are easy to modify, stable and most importantly, non-immunogenic. Because of these positive characteristics, aptamers are incorporated in different fields, and most attractive in the applications involving therapeutics and diagnoses (theranostics). With either aptamers alone or complementing with antibodies, several high sensitive, portable sensors have been demonstrated for use in 'bedside analysis'. Moreover, aptamers are more amenable to chemical modifications, making them capable of utilization with the most developed aptasensors (aptamer-based sensors).
Significance: The development of more sensitive aptasensors could be useful and important for medical diagnosis, identification of pathogens for the quality control of consumable items, and surveillance of emerging diseases. In fact, aptasensors have already shown their efficacy in the detection of life threatening diseases caused by early stage of viral infections. In this review, role of aptasensors in detecting pathogenic viruses are overviewed.

Keywords: Anti-virus, aptamer, aptasensor, bedside analysis, SELEX

INTRODUCTION

Aptamer is a single stranded nucleic acid, either DNA or DNA identified from the randomized library of molecules by the 'SELEX' (Systematic evolution of ligands by exponential enrichment). The first aptamer was generated by two groups in 1990 against two different targets (Ellington and Szostak, 1990; Tuerk and Gold, 1990). After that various aptamers have been generated against a wide range of targets by SELEX. This process involves three main steps including complex formation (target and library), separation (on solid surface) and amplification (by Polymerase Chain Reaction). In brief, the target molecule allows binding with the library of molecules (~10^{14} molecules) under optimal condition followed by separate the bound molecules from the unbound. Then the bound molecules eluted from the target and amplified for the next round of SELEX. Depends on the target molecule, different separation method can be preferred, such as filter, titer plate, magnetic bead and resin. In general need about 12 cycles to get the specific aptamer and each cycle supposed to carry out under stringent condition (Figure 1a). This schematic process is referred as *in vitro* SELEX method and the same steps have also been applied for *in vivo* SELEX. In the case of *in vivo* SELEX, the pool of molecules are injected into the mouse by tail vein and then the bound molecules extracted from the targeted tissue and amplified for the next round of SELEX (Figure 1b; (Gopinath, 2007a), here molecules need to use should be stable under any biological condition (Mi *et al.*, 2010). In might be accomplished easier as aptamers can undergo chemical modification to make them stable (Figure 2). Using these methods, various aptamers have been selected against a wide range of targets having different sizes (metal ions, protein, peptide and whole cell) (Gopinath, 2011).

Aptamers can be substituted for antibodies in the biological applications, especially in the front of theranostics (therapeutic and diagnosis), due to its several advantages over antibodies. Particularly in aptasensor (sensors generated using aptamers) development, the aptamer-based probe can be replaced the antibody efficiently due to the high binding affinity with the target. A good biosensor has two main characteristics including high sensitivity and specificity and attested by aptasensor in many instances (Gopinath *et al.*, 2008a, 2008b; Lakshmipriya *et al.*, 2013a; Gopinath *et al.*, 2016).

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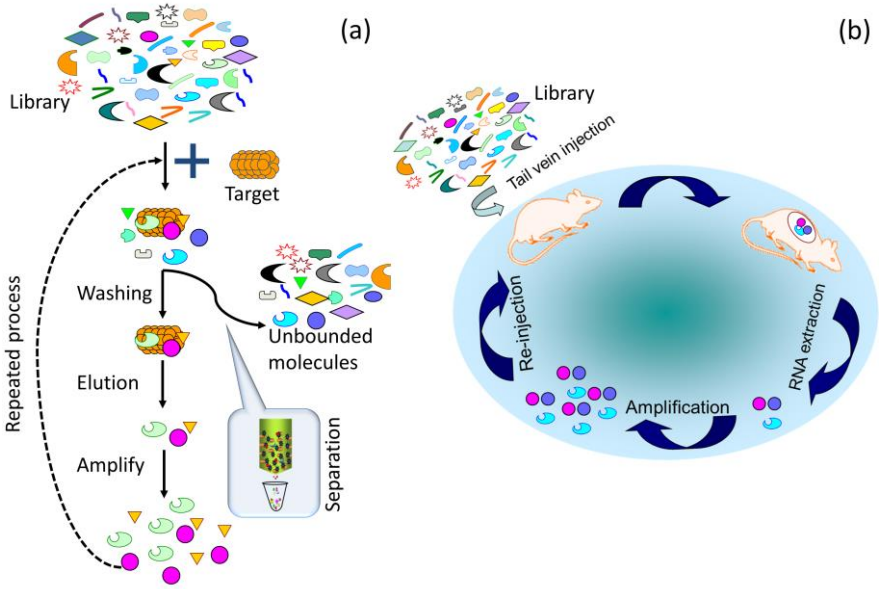

Figure 1: SELEX process. a, *In vitro* SELEX; b, *In vivo* SELEX. SELEX refers Systematic Evolution of Ligands by EXponential enrichment. It involves repeated iterative cycles of complex formation, separation and amplification.

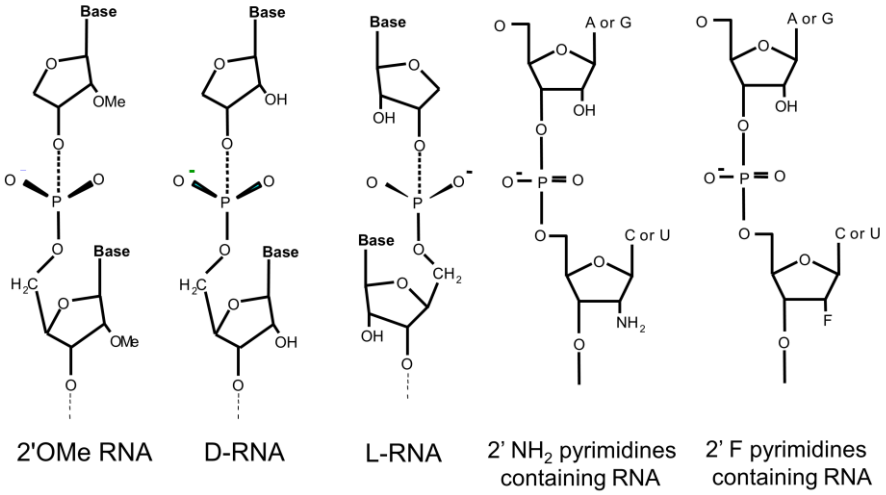

Figure 2: RNA-aptamer analogues. These RNA analogues are to improve the aptamer stability against ribonucleases. They include 2'OMe RNA, D-RNA, L-RNA, 2'NH_2 pyrimidines containing RNA, 2'F pyrimidines containing RNA. Aptamers containing 2' NH_2 or 2' F pyrimidines are resistance against major ribonucleases. Mutant T7 RNA polymerase has the ability to incorporate these modified dNTP during *in vitro* transcription.

Since aptamer has a high binding affinity with their target (pico to nanomolar range), using aptamer as the probe several aptasensors have been demonstrated on both on solid (Rodriguez and Rivas, 2009; Lakshmipriya *et al.*, 2013a; Vasilescu *et al.*, 2013) and solution based (Yamamoto and Kumar, 2000; Stojanovic *et al.*, 2001) sensing systems (Figure 3). Moreover, due to smaller size of aptamer, it binds with the target by only a few bases, aptamers able to discriminate the closely related molecules, but antibody generally cannot. Since aptamer proved as the effective probe in the sensor development, various pathogens could be detected such as virus, bacteria by aptasensors. In this overview, we discussed about the application of aptasensors in detection of viral

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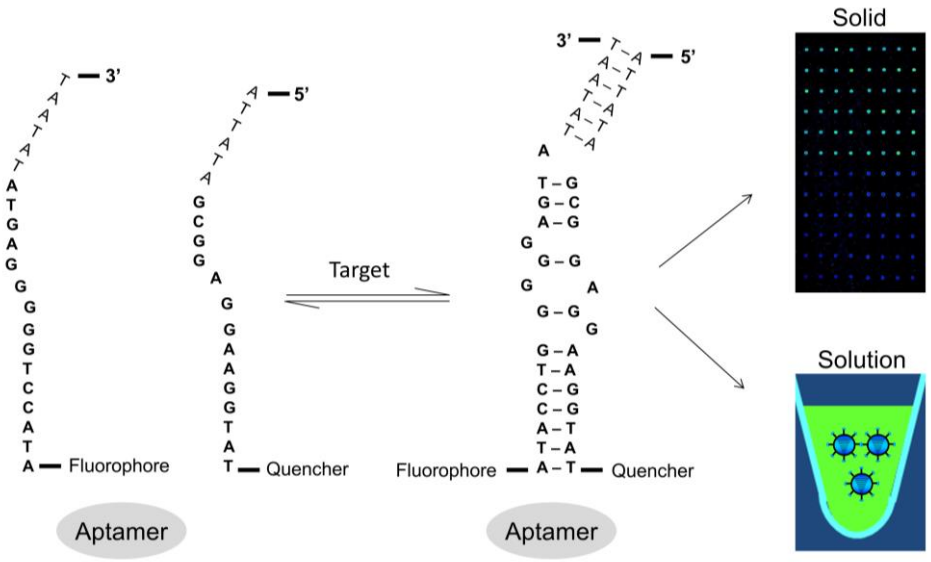

Figure 3: Bipartite aptamer. One portion aptamer carries fluorophore and another one has quencher. There will a change in the fluorescent intensity upon availability target indicates level of sensing. Aptasensor on both solid surface and in solution are shown.

pathogens. In this scenario, aptamers been shown as the capturing or detection tool using the generated aptamers against antigens or cells causing pathogenesis.

APTAMER AS A CAPTURING AND DETECTION TOOL

As demonstrated elsewhere aptasensors play a major role for diagnosis and proved that aptamer is a well suited molecule for high-performance sensing in pathogen detection. Aptamers can be immobilized on the sensor surface by chemical functionalization either on aptamer or on sensing surface and aptamer may also be captured on the solid surface such as gold by electrostatic interaction. Figure 4 explains the carboxylic (COOH) modified surface to capture the aptamer in aptasensor development. On COOH functionalized surface aptamer be used either capturing or detecting agent. In this case, aptamer is used to modify with amine (NH_2) group to immobilize on the sensing surface and this capturing can be stabilized by using the reagents N-hydroxysuccinimide (NHS) and N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) as shown in Figure 4a and it favours the high sensitive detection of appropriate target 4a (Lakshmipriya *et al.*, 2014). Similarly, with the same binding events aptamer can be the detection tool, in which target is used to immobilize in the solid sensing surface and detected by aptamer (Figure 4b). This sensing strategy can be improved in terms of higher sensitivity by using aptamer conjugated gold or other nanoparticle (Figure 4c). Aptamer can be immobilized on the surface through thiol linker and then be used as the detection probe. Alternatively aptamer may also be attached on the gold nanoparticle (GNP) due to electrostatic interaction. Due to single strand nature of aptamer, it is easy to attach aptamer on GNP by this interaction (Gopinath *et al.*, 2014a). This strategy has been proved to improve the limit.

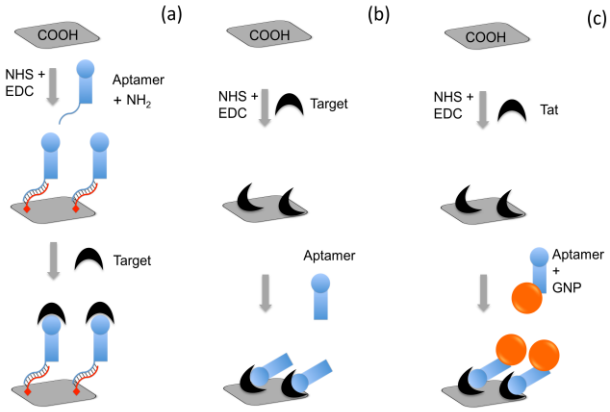

Figure 4: Strategies for sensing aptamer and target interaction. Strategies are shown on COOH functionalized surface. a, Aptamer (probe) vs target (protein); b, Protein (probe) vs target (aptamer); c, Signal enhancement with aptamer conjugated gold nanoparticle (GNP).

of detection and also the specificity by co-immobilization of Poly-ethylene glycol (Lakshmipriya *et al.*, 2013b; 2014). Generally antibodies have been demonstrated for sandwich assays using poly- and monoclonal antibodies. Since aptamer can bind only the few bases with the target, different aptamers also be used for the sandwich pattern. Aptamers are shown to have higher sensitivity with the target molecule, so that, sandwich assay with the aptamer bring down the detection limit. Further, aptamer and antibody are complementing each other and

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demonstrated in several sandwich assays (Kim *et al.*, 2010; Lakshmipriya *et al.*, 2014) With all these above strategies aptasensors have been succeeded with viral detection and we elaborated here.

ANTI-VIRAL APTAMERS

Proper diagnosis is the key factor to detect the diseases at earlier stages, for better treatment and health care (Wandtke *et al.*, 2015). Several aptamers have been generated against various pathogenic viruses or antigens from the viruses for diagnosing purposes. Aptamers produced against the whole virus or the surface antigens are preferred with the aim to detect whole virus. In the past, aptamers have been selected against a wide range of viruses, including Influenza, Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Severe Acute Respiratory Syndrome (SARS), Human papilloma virus (HPV), Herpes simplex virus (HSV), Chikungunya, Dengue, West Nil and Ebola (Gopinath, 2007b). Among these, aptamers generated against important viruses as potential candidates are narrated here for the development of aptasensors.

INFLUENZA VIRUS

Influenza is the seasonal virus, has three types A, B and C, among these A and B types are common in humans. Influenza virus has two major proteins on its surface, namely Hemagglutinin (HA) and neuraminidase (NA). HA is the predominant target and different aptamers were generated against HA due to its higher number in influenza virus (Gopinath *et al.*, 2014a). It is important to detect the Influenza virus at earlier stages to avoid seasonal epidemic and spreading. Gopinath and colleagues have generated various aptamers against influenza viruses (Gopinath *et al.*, 2006a, 2006b; Gopinath and Kumar, 2013). It was proved that the influenza virus can be detected by its aptamer using a benchtop system, surface plasmon resonance (Biacore) and reached the limit of detection to the lower picomolar range (Gopinath and Kumar, 2013; Lakshmipriya *et al.*, 2013b) showed the higher sensitivity (100 pM) as demonstrated by surface Plasmon resonance spectroscopy (SPRF), it shows 1000 times higher sensitivity than antibody and also proved that the selected aptamers clearly discriminated the closely related other influenza viral strains (Lakshmipriya *et al.*, 2013a). Shiratori *et al.* (2014) selected the aptamers against Influenza HA1 protein and detected by aptamer based sandwich method. Aptamers have also constructed against avian influenza virus H5N1 and detected by Hydrogel based QCM aptasensor (Wang and Li, 2013). An interesting aptasensor has also been developed is controlled assembly and disassembly of aptamer on GNP (Figure 5; Gopinath *et al.*, 2014b). For the detection of influenza virus, (Lee *et al.*, 2013) have developed a similarly controlled assembly and disassembly system, but they used sialic acid instead of aptamer. Currently antibody-based immunochromatographic test (ICT) has been used to detect the influenza virus, however, it can detect only major types of influenza viruses such as A or B and early detection is not possible with ICT. But every year different strains are emerging and cause a lot of issues for the identification of new strains. Aptamer is well suited probe to differentiate the closely related influenza strains, so that replacing antibody in ICT to aptamer will pave the way to generate detection system for influenza sub-types with higher sensitivity and specificity.

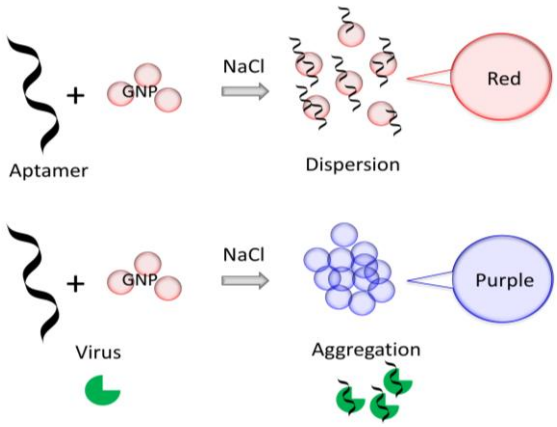

Figure 5: Aptamer mediated controlled assembly and disassembly on GNP. GNPs change from dispersion to aggregation in the presence NaCl, but remains dispersion in the presence aptamer. When target is available aptamer-target induces aggregation in the presence of NaCl.

HUMAN IMMUNODEFICIENCY VIRUS (HIV)

HIV affects the human immune system and causes Acquired immune deficiency syndrome (AIDS). Until now, there is no medicine for complete curing of HIV, but there is a treatment for HIV to suppress the effect of HIV virus. If we detect HIV at earlier stages, it will create the normal and healthy life of HIV affected people. Still there is a lake of diagnosing method for early detection of HIV. Aptamers are possible molecule to detect the HIV virus at earlier stages, as shown in other cases elsewhere. Different aptamers selected against HIV for the purpose of diagnosis. HIV-1 Tat is one of the important proteins and it is expressed after HIV infection and play an important role in HIV replication (Bagashev and Sawaya, 2013). The aptamer generated against HIV Tat-1 has proved the inhibition of HIV Tat-1 in both *in vitro* and *in vivo* (Yamamoto and Kumar, 2000). Ruslinda *et al.* (2013) used the same aptamer to detect HIV with the help of diamond field-effect transistor. Moreover, aptamers selected against HIV-1 protease proved to inhibit protease effectively (Duclair *et al.*, 2015) and aptamer against the HIV-1 genome effectively inhibited the HIV-1 in human cell line (Sánchez-Luque *et al.*, 2014). Since there are various aptamers generated against HIV virus, there is an avenue for developing aptasensors to detect the HIV virus at earlier stage.

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SEVERE ACUTE RESPIRATORY SYNDROME (SARS)

SARS is the dangerous virus affected many people in 2002, there is a need of the effective diagnostic method for accurate SARS detection. CoV (structural) and nucleocapsid are two important proteins in SARS and used for the diagnoses. Currently anti-nucleocapsid antibody has been used to identify the nucleocapsid protein. So that researches focused to generate the aptamers against nucleocapsid. Cho *et al.* (2011) generated an aptamer against the nucleocapsid protein and the binding affinity was confirmed by western blot analyses and the dissociation constant was found to be 4.93 nM. Also RNA aptamer selected for the same nucleocapsid protein has the dissociation constant 1.65 nM (Ahn *et al.*, 2009). Jang *et al.* (2008) selected the RNA aptamer against NSP10 (NTPase/Helicase) from SARS virus. These selected aptamers were used to detect the SARS virus by different aptasensors. Quantum dots based RNA aptamer used to detect the SARS virus with the limit of detection 0.1 pg/mL (Roh and Jo, 2011).

HERPES SIMPLEX VIRUS (HSV)

HSV is the contagious and easily spread from people to people. Usually HSV infection has been diagnosed by the suitable antibodies for their types HSV-1 or HSV-2. The glycoprotein-D (gD) of HSV is a potential antigen for viral entry, binds specific cellular co-receptors and mediating entry of virus to the host cells (Gopinath *et al.*, 2012). Construction of aptamer against gD protein with the high binding affinity (nanomolar range) was reported and the selected aptamer effectively interfere the gD receptor (HVEM) binding. Another aptamer generated against gD protein by the same authors could block the interaction between nectin-1 and gD protein. Further, a shorter version of aptamer was also designed based on mapping and binding analyses. The shorter was found to be as good as full-length aptamer. Aptamers against HSV-2 was also generated and it could bind with the envelope gD protein (Moore *et al.*, 2011). The SPR result confirmed the binding of the selected aptamer against HSV-2 with the IC_{50} value from 20 to 50 nM. HSV-1 US11 is a RNA

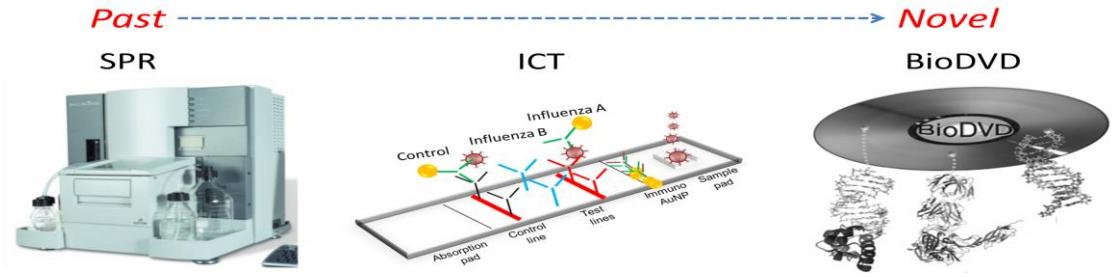

Figure 6: Progression in the aptasensors for microbial detection. Benchtop to novel aptasensors with the progression are shown.

binding protein and aptamer generated against this target and shown with high affinity (70 nM), the shorter aptamer also designed for this target (Bryant *et al.*, 2005).

HUMAN PAPILLOMA VIRUS (HPV)

HPV spread in the moist places of human body including cervix, anus mouth and throat. It is responsible for mainly cervical and other cancers in anus, vagina, vulva, oropharynx, vagina and cancers of the throat, tongue and tonsils. This viral replication involved mainly with the six genes of HPV and they are E1, E2, E3, E4, E5, E6 and E7. Among these E6 and E7 are the major proteins in HPV virus, and aptamers generated against for these proteins. HPV-16 is predominantly occurring strains and reported elsewhere; in addition HPV-18 is also widely occurred. Gourronc *et al.* (2013) generated a RNA aptamer against HPV-16 E6/E7 and with the selected aptamer it was proved that they can internalize into E6/E7. Aptamers for E6/E7 was also generated by Toscano-Garibay *et al.* (2011) and the selected aptamer has higher binding affinity with HPV-16 E7 protein.

CONCLUSIONS

Biosensor development for the diagnosis of pathogens is mandatory to improve the human health and life span. It was in vogue, nucleic acid based method used to detect the pathogen, but this method need the steps including isolation of DNA, preparing samples and also need the special equipment. On the other hand, antibody production is laborious and involves animal system with conventional method of antibody preparation. Aptamer is the reasonable substitute for native nucleic acid target and antibody as attested by several aptasensors (Figure 6). Since aptamers have been generated predominantly against a wide range of pathogens (bacteria and virus), easier to generate detection system. Further, aptamer is an established biomarker for sensor development due to its high binding affinity with the target molecule. Since different aptamers can be selected against the same target, aptamers might be used as the capture and detection probe, with this aptasensor developments have been demonstrated against whole cells such as virus and bacteria. In the current review, aptasensors for viral pathogen discussed with a view for future development of high-performance aptasensors against viruses.

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