The Progress of Aptamers in the Detection of SARS-CoV-2

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Abstract: SARS-CoV-2 has caused millions of deaths worldwide since its outbreak and will continue to cause large-scale global infections, so it is of great significance for the prevention and control of diseases caused by this virus to develop rapid and portable detection methods. Currently, PCR detection of SARS-CoV-2 is generally time-consuming and complicated, while aptamers, as a new type of biorecognition that can specifically recognize small molecules such as proteins, nucleic acids, oligonucleotides, etc., have a great potential and advantage in the detection of SARS-CoV-2. This review briefly describes the detection principle, characteristics and specific applications of aptamers combined with biosensor technology in the detection of SARS-CoV-2, and discusses the advantages, challenges and future development tendency in the area of SARS-CoV-2 detection.

Keywords: Aptamer; SARS-CoV-2; Detection

Introduction

SARS-CoV-2 pertains to β coronavirus genus, which can cause severe human respiratory diseases and potentially high mortality.Its clinical features range from asymptomatic or mild infections to acute lung inflammation and severe pneumonia, and are more common in elderly people and patients with underlying diseases. [1, 2] SARS-CoV-2 are now mutating to form different variants with shorter incubation periods and higher transmission rates, and these subvariants are rapidly expanding globally, becoming more dominant in infections and showing greater antibody resistance, and they may cause a new wave of widespread neocoronavirus infections. [3-5] Therefore, the speedy and large-scale detection of SARS-CoV-2 poses a tremendous challenge.

SARS-CoV-2 is currently detected mainly by molecular detection of SARS-CoV-2 RNA by RT-qPCR, which is also the most common and preferred method for detecting SARS-CoV-2.It is a highly sensitive and specific clinical detection method, [6] yet this method is time-consuming and is strict for laboratory environment and operators. [7]

In recent years, aptamers have aroused extensive attention from researchers because of their high specificity, wide range of recognizable targets, low immunogenicity and easy accessibility, and have been widely used in nucleic acid, microbial, cellular, exosome, small molecule, and protein assays. [8-11] The application of aptamers in the detection of SARS-CoV-2 is expected to realize accurate, fast and portable detection of SARS-CoV-2. This paper provides a brief overview of the basic principles, applications, and characteristics of seven aptamers in combination with biosensor technology and other technologies for the detection of SARS-CoV-2.

1. Overview of SARS-CoV-2

SARS-CoV-2 is a positive-stranded RNA virus with an envelope structure. The viral RNA genome consists of two open reading frames (1a and 1b) encoding viral replication enzymes, and downstream mRNAs encoding structural proteins nucleocapsid (N), membrane (M), envelope (E), and spiking (S) proteins and nonstructural proteins, which are in charge of viral assembly and are participated in the inhibition of the host immune responses in SARS-CoV-2. [2, 12, 13] These four structural proteins are the main immunogens in SARS-CoV-2 diagnostics, the S and N proteins. [14]

The S protein of SARS-CoV-2 has a trimeric structure, including S1 and S2 subunits.The S1 structural domain (RBD) is in charge of working with the host receptors and the S2 structural domain (RBD) is in charge of with the host cell membrane fusion.The S protein receptor binding domain containing about 300 amino acids, determines its and angiotensin converting enzyme 2 (ACE2) the ability to combine stability, thus into the host cell in the body, the virus attachment, fusion, and plays a key role in the process of infection. [12, 15-17]

According to its sequence characteristics, the N protein of SARS-CoV-2 is separated into an intrinsic disorder region (IDR) and a conserved structural region. The conserved structural region comprises of two non-interacting domains, the N-terminal RNA binding domain (RBD) and the C-terminal dimerization domain (DD). [18, 19] The N protein functions to package the viral genome into RNP particles and participates in viral assembly by interacting with the viral genome and the membrane protein M, which has the ability to recognize and bind viral RNA. [18, 20] In recent years, more and more studies have established aptamer detection methods targeting the S and N proteins of the SARS-CoV-2.

2. Aptamers and their characteristics

2.1 Concepts

Aptamers were first introduced in 1990 [21,22] of single-stranded nucleotide sequences of approximately 25-80 bases in length. [9]Aptamers are usually synthesized in vitro by an iterative procedure known as systematic evolution of exponentially enriched ligands (SELEX). They can be combined with a wide range of targets, including metal ions, organic molecules, proteins, cells and viruses and bacteria. [11]

2.2 Characteristics

Aptamers, known as "chemical antibodies," specifically recognize targets through hydrophobic and electrostatic interactions, hydrogen bonding, van der Waals forces, shape complementarity, and base stacking, similar to the way antibodies bind to antigens. [23] However, aptamers have several unique advantages over conventional antibodies. ①high affinity and specificity, ability to recognize single point mutations and conformer isomers, and multivalent aptamers can increase their affinity. [24, 25] ② Wide range of targets, whereas antibody targets are limited to immunogenic molecules. ③No immunogenicity, whereas antibodies are highly immunogenic and trigger an immune response. ④High temperature resistant and very stable, long shelf life, can be lyophilized at room temperature for long-term storage and transportation, while antibodies are susceptible to temperature instability and have a limited shelf life, requiring refrigerated storage and transportation. ⑤ Aptamers can be screened by SELEX in vitro and are easy to synthesize, while antibodies are produced by in vivo animal production, which is costly and potentially contaminated. [9]

3. Application of Aptamers in the Detection of SARS-CoV-2

3.1 Electrochemical aptamer technology for detection of SARS-CoV-2

The electrochemical aptamer technology is derived from the binding of the aptamer to the electrochemical redox probe on the electrode surface. When the aptamer binds to the target, the aptamer conformation changes, resulting in a change in the distance between the redox probe and the electrode surface, and a change in the redox current, which is detected by the difference in the current signals (increasing or decreasing) of the SARS-CoV-2 [26, 27]

Federica Curti et al. [28] established a new folding screen printing electrode single-walled carbon nanotubes (SWCNT - SPEs) electrochemical sensing platform, using REDOX labeled DNA adapter body function, the adapter body specificity combined with SARS-CoV-2 spike protein receptor binding domain of S1 subunit.This method implements the new crown S1 protein in the buffer and single step no reagent to detect virus transport medium, and low detection limit for 7 nM.Mahmoud Amouzadeh Tabrizi et al. [29]first established a photoelectrochemical aptamer sensor derived from graphitic carbon nitride and cadmium sulfide quantum dots nanocomposites for the RBD region of SARS-CoV-2 protein. After SARS-CoV-2 RBD was combined with the aptamer probe immobilized on the electrode surface, The diffusion of the electron donor to the electrode surface is hindered, which leads to a decrease in the intensity of the photocurrent.The

detection range of this sensor for S protein RBD was 2.0-5.32 nM and the detection limit was 0.12 nM. Electrochemical adaptation technique with high sensitivity, easy to manufacture and portability and other characteristics, extensively used in the POCT real-time inspection. [30]

3.2 Fluorescent aptamer technology for detection of SARS-CoV-2

The principle of fluorescence detection of SARS-CoV-2 is to modify the aptamer with a fluorescent group or a bursting group. After the aptamer and combined with target, the aptamer's conformation is changed and its fluorescence signal is altered (either produced or burst), and SARS-CoV-2 are detected by the difference in fluorescence signals.

Advanced Materials Technologies et al. [31] established a sensing system consisting of a nanoporous anodized aluminum disk equipped with a fluorescent indicator rhodamine B, whose surface was covered with an aptamer that selectively binds to SARS-CoV-2 S protein as a capping system. After the combination with its target protein in a fit body, its conformation changes, sealing and fit body is shifted, thus open pore and allow the dye release.The detection limit of this method for pseudovirus containing S protein is 7.5×10^3 PFU/mL and has been validated in clinically positive nasopharyngeal samples with a high degree of sensitivity.Jie Liu et al. [32] established a double nucleic acid induced by combining adapter body assembly and AuNPs cause metal enhanced fluorescence, the N protein in human saliva samples for testing. AuNPs were used as nanocapsulers and fluorescence enhancers. Two N-recognizing aptamers, Apt-1, were labeled with FAM and immobilized on the AuNP. The second aptamer (Apt-2) partially hybridized with Apt-1 to form a stable stem duplex.In the presence of N protein, the two aptamers come close to form a closed-loop conformation, resulting in the formation of a fluorescence translator, and the fluorescence intensity is enhanced with the increase of N protein concentration, thus causing metal enhanced fluorescence. The simultaneous targeting of two aptamers to N protein has a synergistic effect, and the recognition rate of N protein detection in saliva samples is high. The detection limit of this method is as low as 150 fg /mL, and the detection can be completed in 3 minutes, which is faster and more sensitive than existing nucleic acid detection technology. Fluorescent aptamer technology is considered to be the most effective and sensitive method with a wide dynamic range and applicability, fast response, simple manufacturing, and economy. [30, 33]

3.3 Colorimetric aptamer technique for detection of SARS-CoV-2

The colorimetric aptamer technology enables detection through the use of nano-enzymes or nano-enzymatic-like materials to catalyze the chemical reaction that accompanies the color change, or by the unique local surface plasma resonance (LSPR) properties of metal nanoparticles. [34]

Mostafa Vafabakhsh et al. [35] based on ChF are embedded/ZnO/chitosan film on the TMB substrate of CNT intrinsic catalytic activity, developed a late-model type of colorimetric aptamer sensor. Aptamers were deposited on ChF/ZnO/CNT nanohybrids and enhanced the oxidized TMB color when existence HO.When the aptamers were bound to the target, the activity of the nano-enzymes decreased, resulting in the oxidized appearing blue color and a decrease in the solution absorbance, and the detection of SARS-CoV-2 was achieved on the basis of the change in the absorbance of the color. This method has a detection range of 1-500 pg/mL virus and a detection limit of 0.05 pg/mL virus, which can detect SARS-CoV-2 virus in a low-cost, rapid, simple, and highly sensitive manner.Siska Nurul Chotimah et al. [36] developed a colorimetric aptamer technique for the detection of SARS-CoV-2 S1 protein adsorbed on gold nanoparticles, quantified by a spectrophotometer based on the indication of color change in the gold absorbance spectra, and connected to a cell phone for data processing and analysis, and the method can be used to detect human saliva samples directly. The method has a detection limit of approximately 25.1 nM for S1 protein and is specific and sensitive for SAR-CoV-2 detection. Colorimetric techniques are widely used for the detection of a wide range of analytes and are characterized by low cost, high sensitivity and specificity, and clear visibility, even with the naked eye. [34]

3.4 Nanogold aptamer technology for detection of SARS-CoV-2

Gold nanoparticles are particles with diameters as low as 1-100 nm, which are biocompatible, electrically conductive, and easy to functionalize with biomolecules such as aptamers, and aptamer-conjugated gold nanoparticles have a wide range

of applications in the design of biosensing systems due to their optical and electrochemical properties. [37]

Srivatsa Aithal et al.[38] established an aptamer-functionalized nanogold technology, in which the aptamers on the surface of the nanoprobes bind specifically to the spiny proteins when the nanoprobe suspensions are mixed with samples containing spiny proteins to increase the electrostatic charge and enhances spatial site resistance stability by adding a coagulant, in which nanoprobes without bound proteins aggregate, while nanoprobes with sufficient bound proteins do not aggregate. This technique allows the detection of spiking proteins at concentrations of 16 nM and higher in PBS, as well as 3540 genomic copies/μl of inactivated SARS-CoV-2 by measuring the absorbance spectra of the samples.S C G Kiruba Daniel et al. [39] simply synthesis of metal nanoparticles for SARS-CoV-2 RNA detection using tin as a solid reusable reducing agent. Their use of solid tin metal as a reducing agent resulted in the formation of gold nanoparticles AuNPs from gold chloride precursors in less than a minute, and aptamers targeting the SARS-CoV-2 N gene were coupled to the newly synthesized AuNPs, resulting in selective detection of viral RNA in clinical samples in less than five minutes.The gold nanoparticle aptamer technology is valuable in the detection of pathogens, with its ability to respond to environmental changes provide simple, rapid and specific color changes, are easy to use, responsive, inexpensive and have the potential to be targeted commercially. [37, 40]

3.5 Lateral flow aptamer technology for detection of SARS-CoV-2

Lateral flow aptasensors (lateral flow aptasensors, LFA) is a lateral flow type test paper combined with nucleic acid aptamer, when the aptamer binds to the target, its formation of the complex in the lateral flow test paper occurs to visualize the information output.

Lucy F Yang et al. [41] established a double-aptamer sandwich structure lateral flow assay to detect SARS-CoV-2 virus, and the selected two DNA aptamers were bound to the N-terminal domain of SARS-CoV-2 S protein.The biotinylated aptamers captured the analytes onto streptavidin-coated test strips and were detected using aptamer-coupled gold nanoparticles, which had a detection limit of 10^6 copies/mL for the detection of UV-inactivated viruses but were not tested with real virus samples. In addition, Lucy F Yang et al [42] developed a lateral flow method for the detection of SARS-CoV-2 wild-type and omicron (BA.1) with two aptamers targeting omicron (BA.1) and wild-type S proteins, with detection limits of 50 pM omicron (BA.1) S protein and 100 pM wild-type S protein, but the method was only tested on nasal swabs containing buffer, and was not performed with real virus samples. It was not tested with real virus samples. The LFA is a device that provides a rapid, immediate diagnostic that is not only fast and easy to use, but also inexpensive compared to laboratory tests.

3.6 Detection of SARS-CoV-2 by surface-enhanced Raman scattering aptamer technique

The basic principle of surface-enhanced Raman scattering (SERS) is to bind an aptamer to an active substrate, usually using a nanostructure as the active substrate, and when the aptamer binds to the target and separates from the substrate, it produces a change in the Raman signal, which is quantified by detecting the intensity of the characteristic peaks of the Raman reporter molecule. [43]

Hao Chen et al. [44] established a method for detecting COVID-19 by SERS aptamer sensor with the aptamer of COVID-19 S protein as the receptor and gold nano popcorn as the substrate for SERS.The aptamer was immobilized on the surface of gold nanopopopcorn by DNA hybridization, and the method was able to detect SARS-CoV-2 in 15 min with a detection limit of 10 PFU/mL, but the method was not validated in clinical samples. Recently, Ki Sung Park et al. [45] developed an ultrasensitive SERS platform for SARS-CoV-2 S-protein aptamer and nanosilver as a combination, which had a detection limit of 100 fg/mL and was further validated in clinical samples, and was also successful in detecting SARS-CoV-2 in wild-type, δ, and omicron mutant clinical samples.SERS is an ultrasensitive vibrational spectroscopy technique that enhances the detection of adsorbed vibrational spectroscopy of molecules on or near metal nanostructures and/or surfaces, which has the advantages of high sensitivity, specificity, and non-destructive detection, and avoids the

non-specific effects of SERS co-adsorption interferences from matrices through aptamer binding. [46]

3.7 Surface plasmon resonance aptamer technique for detection of SARS-CoV-2

Surface plasmon resonance (surface plamon resonace, SPR) is the basic principle of after the adapter body and combined with the target, causing resonance device of refractive index change and the change of the SPR resonance Angle, through light transduction directly converts the change to physically measurable signals, and the signal proportional to the concentration of the target, So as to realize the detection of SARS-CoV-2. [47]

Rongyuan Chen et al. [48] was the first to build a new kind of based on C-SH QD unmarked Nb2 surface plasmon resonance (SPR) aptamer sensor, when fixed on the surface of the SPR chip, fit body combined with new crown N gene, its conformation change, probe and increases the distance of the chip, This results in a change in SPR signal, which is used to detect the N gene of SARS-CoV-2. This technology can quickly and sensitively analyze the N gene of SARS-CoV-2 from real samples from seawater, seafood and human serum. The detection range of N gene is 0.05 to 100 ng/mL, and the detection limit of this method is 4.9 pg/mL.Tyra Lewis et al. [49] to build the local surface plasmon resonance instrument with nano watch mildew avidin biotin-chain on the surface of the platform with the combination of double channel system, the biotinylated adapter body was fixed on the chain mildew avidin platform, mainly used for the detection of SARS-CoV-2 S1 protein.The detection range of S1 protein was 1 nm-100 nM, and the detection limit was 0.26 nM. Ting-Chou Chang et al. designed a fiber-optic particle plasmon resonance biosensor that detected SARS-CoV-2 N protein within 15 minutes with a detection limit of 2.8 nM. Surface plasmon resonance technology has the significant features of label-free detection, real-time monitoring, small sample size, provision of accurate results and smooth processing. The surface plasmon resonance technique is characterized by label-free detection and has shown excellent performance for quantification and characterization in real-time applications, but its detection cost is relatively high and requires specialized instrumentation. [50, 51]

3.8 Aptamers other techniques to detect SARS-CoV-2

In addition to conventional biosensors, aptamers are also suitable for other new detection techniques. Jinqi Deng et al. [52] reported a one-step thermophoretic assay using aptamer and polyethylene glycol (PEG) for direct quantification of SARS-CoV-2 particles. When fluorescently labeled aptamer binds to SARS-CoV-2 spiking proteins, the aptamer-bound viral particles undergo thermophoretic stacking as a result of a temperature change after laser induction, and the number of viral S proteins is reflected by fluorescence intensity. Using a pseudovirus containing S protein as a model, the detection limit was 170 particles/μL and was achieved within 15 min, without any pretreatment, and the sample could be tested directly, which was easy to operate, but the real virus sample was not tested. In addition, Wenping Xing et al. [53] developed an ultra-sensitive CRISPR/Cas12a-derived aptamer biosensor, which not only recognizes the target with high affinity and specificity, but also triggers the *trans-cutting* activity of CRISPR/Cas12a, and the detection limit of the aptamer can be as low as 1.5 pg/mL for the SARS-CoV-2 S1 protein, and it can successfully distinguish between Beta, Delta, and Oviruses. Beta, Delta and Omicron variants were also successfully detected.Changtian Chen et al.[54] developed an optimized aptamer-based nano-interferometer for the rapid, amplification-free detection of SARS-CoV-2 S protein-encapsulated pseudoviruses directly from human saliva samples, with a limit of detection of approximately 400 copies/mL, which is comparable to qRT-PCR and 1,000- to 100,000-fold more sensitive than the commercial LFA test.

4. Conclusion and outlook

Aptamers have strong specificity, high affinity, wide range of target molecules and easy to synthesize, in addition to antibodies, ideal recognition molecules, and nucleic acid aptamers have low immunogenicity and toxicity compared with antibodies, which can provide new ideas and methods for real-time in vivo dynamic monitoring and detection. The combination of aptamers and biosensors can realize portable, simple, fast, reliable, and flexible design of portable output devices, which can meet the needs of large-scale detection of SARS-CoV-2 in a short period of time. In addition, the

diversified aptamer modification technology enables the polymorphism of SARS-CoV-2 detection, and the aptamer avoids the "hook effect" of the antigen-antibody reaction, which is more advantageous for the detection of low concentration of viruses.

However, the technology of combining aptamers with biosensors also faces some challenges. The aptamer molecules are small and easily affected by buffer solution, ionic strength and temperature, and their nucleic acid structure is easy to be changed, while the complexity of the actual real sample matrix increases its difficulty in practical detection applications. Currently, the ideal targets of aptamers for SARS-CoV-2 are S and N proteins, and the majority of aptamers are targeted to selected regions of SARS-CoV-2 S proteins, which are commonly the RBD regions of S1 proteins. Screening of aptamers for higher affinity, high efficiency and sensitivity, as well as the development of biosensor technologies that are more efficient, portable, and take less time to complete the assay, are the future trends in aptamer technology for detection of SARS-CoV-2.

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