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CHAPTER

14

Aptasensor: Surface protein detection in case of coronavirus diagnosis

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14.1 Introduction

The world has faced an infectious disease outbreak caused by a new virus, severe acute respiratory syndrome (SARS) is a respiratory viral disease caused by a coronavirus. SARS-CoV-2 is a member of Coronaviridae, which is the responsible agent for COVID-19 [1]. Coronaviridae, together with the Arteriviridae and Roniviridae families, belong to the order of Nidovirales. Coronaviridae family has two virus genus, Coronavirus (CoV) and Torovirus. Four distinct genera are part of the CoV genus, known as α, β, γ, and δ; however, only α-CoVs and β-CoVs infect mammalian hosts [2]. SARS-CoV-2 is an envelope and positively single-stranded RNA (ssRNA) virus with a typical genome size 29.7 kb, encoding directed RNA polymerase and structural proteins including the spike (S), envelope (E), membrane (M) and nucleocapsid (N) proteins. The N protein is a 422-amino-acid alkaline protein with a short lysine rich region suggested to be the nuclear localization signal. It plays an important role in the process of virus particle assembly by enveloping the entire genomic RNA. The S protein gives the spike-like projections on the surface of CoVs, conferring their distinctive aspect of a crown [4].

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Sensing Tools and Techniques for COVID-19: Developments and Challenges in Analysis and Detection of Coronavirus
DOI: https://doi.org/10.1016/B978-0-323-90280-9.00010-9

Studies have shown that N and S structural proteins are closely related to CoVs pathogenicity. N protein plays an essential role both in viral genome packaging and viral transcription. In addition, N protein may also be associated with the host's immune response suppression, facilitating viral replication. S proteins have two subunits (S1 and S2), with distinct functions. S1 binds to the cell surface receptor host through the receptor binding domain (RBD) region present in this subunit. On the other hand, S2 mediates virus fusion with the host cell membrane [5,6].

Viruses with RNA genomes capable of having high mutation rates due to strange features of RNA genomes not found in DNA genomes, such as high rates of recombination, replication and segmentation in a stipulated time. Thus, these features enable a rapid viral genome evolution, favoring variants emerging that can present new characteristics as virulence increases. Thus, CoVs, RNA viruses, have innumerable mutations, mainly in the S protein, which could not be different. The mutations could favor the adaptation of this protein to various host receptor types, causing changes in the tropism and in the pathogenicity of CoVs. The viral infection starts with the entrance of the virus into host cells. In the case of CoVs, the RBD domain present in the S1 subunit of S protein recognizes the receptor located on the host's cell surface, initiating virus entrance into the cytosol. The entry of the SARS-CoV-2 into the host's cell occurs through the attachment between angiotensin-converting enzyme-2 (ACE2) receptor and S protein, new researches have also shown that S protein can bind to other receptors, such as NRP1 (neuropilin-1) receptor; however, its mechanism is not fully understood [7–10].

Native S protein is present on the viral envelope surface in its closed form (inactive S protein), in which RBDs domains are inaccessible to the cell receptor. After protein cleavage, the S1 subunit opens (it becomes active), exposing the RBD domain. Depending on the type of protease present in the host's tissue, SARS-CoV-2 can enter cells by two distinct pathways, the nonendocytic and the endocytic pathways. Proteases expressed in the cellular membrane, such as trypsin, furin, and transmembrane protease (TMPRSS2), lead SARS-CoV-2 to the nonendocytic pathway. These host's enzymes cleave the S protein, causes conformational changes that expose the S2 subunit and, consequently, virus fusion immediately with the host's plasma membrane. In the absence of those enzymes, the virus enters by the endocytic pathway, within vesicles (endosomes) after S protein cleavage by endosomal proteases (clathrin and nonclathrin types). ACE2 and NRP1 receptors are expressed in multiple tissues from the human body, as lungs, oral mucosa, kidneys, liver, intestines, heart, brain, blood vessels, and others. That is why SARS-CoV-2 can invade these tissues and induce systemic inflammation, leading to multiple organ dysfunction syndromes [7–10].

Therefore, SARS-CoV N/S protein has long been thought an ideal target for its detection. The monitoring of SARS-CoV N/S protein has been of considerable interest in the development of simple and reliable analytical tools for the detection of SARS [7–10]. Currently, the most widely used technology for diagnosing SARS is enzyme-linked immunosorbent assay (ELISA), based on recombinant proteins from the genome of SARS. Although the existing techniques are highly specific but suffering from some serious limitations. For example, these methods cannot be used to detect viruses in its premature stage, at a time when antibodies against SARS antigens are not produced. An early diagnosis of the disease is need of the hour and requirement for effective treatment and, management of diseases. In most cases, the diagnosis of viral diseases is done based on symptoms and microbial, basic biochemical, serological and parasitological tests. Additional confirmatory tests include ELISA, PCR, DNA chips and chromatographic techniques, which require sophisticated instruments, professional operators, and need of costly solvents. Aptamer-based biosensors are generally indicated as aptasensors, rapidly becoming a cutting-edge novel technology in disease diagnosis. Aptasensors are bioanalytical devices that use biological molecules as recognition elements in their fabrication to generate signals that can be used to detect the status of the disease. Recently, researchers achieved the specificity and sensitivity of the bioanalytical tools through the use of aptasensors for the detection of COVID-19. With the accumulating knowledge on the development of aptamers and aptasensors, they have been increasingly explored and tested for the detection of various infectious agents, including bacteria and viruses, as well as their toxins.

14.2 Aptamers

The aptamers are single-stranded nucleic acids (DNA, RNA) having molecular recognition properties similar to antibodies, 20–100 nucleobases long and consists of variable region edged by constant regions, which allow for amplification and identification of sequences [11,12]. Aptamers are excellent receptors because they bind with high affinity and specificity to various targets such as viruses, proteins, peptides and small organic molecules. Aptamers bind their target via an induced fit mechanism involving van der Waals forces, hydrogen bonding and electrostatic interactions. In addition, aptamers offer more attractive features compared to antibodies. They have a less expensive production process since they use in vitro synthesis, are easily modifiable with functional groups (facilitating immobilization process), and they provide long-term storage. Aptamers have become increasingly important molecular tools for diagnostics and

therapeutics. In particular, Aptamers exhibit advantages as recognition elements in biosensing when compared with antibodies and enzymes, thus making it possible to develop a wide range of aptamer-based biosensors. Chemically stable and cost-effective aptamers offer great flexibility in the design of novel biosensors with high detection sensitivity and specificity [13–15]. Aptamer-based biosensors offer promising platforms to substitute conventional technologies. Nucleic acid functionalized sensors play an important role in the development of the biosensing field. Aptamer-functionalized devices are one unique type for the outstanding advantages of aptamers.

Electrochemical aptasensors: Electrochemical aptasensors detection is based on the electrical signal changes caused by the specific interaction and combination between biorecognition element (aptamer) and the target analyte. The relationships among targets, aptamers, redox probes, electrodes, and instruments are the keys to determine the specificity and sensitivity of aptasensors [16]. Based on label free strategies these electrochemical aptasensors are categorized as, enzyme amplification methods, and field-effect transistor (FET) techniques. The label free electrochemical transducer is performed by the change of the resistance and the capacitance of the solution electrode interface based on the aptamer-target complex on conductive or semi conductive surfaces. However, field effect transistors (FETs) can transform the interaction with target molecules on FET surface into a readable signal [17].

Optical aptasensors: The fluorescence and colorimetric platforms are most commonly applied in conjunction with aptamers to develop aptasensors. Commercialized antibody based test strips and reagents will direct aptamer products [18]. Surface-enhanced Raman scattering spectroscopy (SERS) is a powerful vibrational spectroscopy technique that can overcome the limitations of Raman spectroscopy's low sensitivity. The two main mechanisms of SERS are considered to be electromagnetic (EM) and chemical effects. The EM was considered to be the prior effect to the enhancement. SERS could be used for sensitive structural detection of low concentration, including single molecules, biomolecules, biological cells, and even viruses [19].

At present, coronavirus diagnostics are based on direct detection of viral components or indirect detection by measuring antibodies generated in response to viral infection. Compared to traditional antibody based detection, aptamers could provide faster adaptation to continuously evolving virus strains and higher discriminating capacity between specific virus stereotypes. Aptamers are very stable and easily modifiable, so are ideal molecules for detection and chemical sensing applications. Herein, we summarize the use of aptasensors for detection of coronavirus and consider the feasibility of aptasensors to become portable devices for point of care diagnostics of COVID 19.

Tian et al. fabricated an electrochemical dual-aptamer biosensor based on the metal-organic frameworks MIL-53(Al) decorated with silver/platinum (Au/Pt) nanoparticles and enzymes to determine SARS-CoV-2 nucleocapsid protein (2019-nCoV-NP) via cocatalysis of the nanomaterials, horseradish peroxidase (HRP) and G-quadruplex DNAzyme [20]. In the present work, the recognition elements, thiolated DNA aptamers (N48 and N61) immobilized on the surface of gold substrate through Au-S bonds for the detection of COVID-19 biomarker, 2019-nCoV-NP. The nanocomposites, Au@Pt/MIL-53 (Al) are designed with HRP and hemin/G-quadruplex DNAzyme as signal nanoprobe. Nanoprobe was then applied to amplify the aptasensor signal *via* cocatalyzing the oxidation of hydroquinone in the presence of hydrogen peroxide. Further, the aptamer-protein-nanoprobe sandwich electrochemical biodetection system is assembled on the surface of gold substrate. Under optimal conditions and parameters the demonstrated aptasensor showed wide linear range from 0.025 to 50 ng mL^{-1} with the detection limit of 8.33 pg mL^{-1} for 2019-nCoV-NP. The presented aptasensor has great potential in the early diagnosis of COVID-19 with high sensitivity and reliability [20].

Yet in another work, aptamer based electrochemical impedimetric biosensor is reported for the detection of SARS-CoV-2 S protein [21]. In the present work, gold nanoparticles (AuNPs)-modified screen-printed carbon electrode (SPCE) and an aptamer targeting the RBD of the SARS-CoV-2 is utilized for the development of biosensor. The fabrication procedure involves the immobilization of the ssDNA aptamer on gold nanoparticles (AuNPs) and the binding of S-protein with the aptamer are investigated through photo-induced force microscopy (PiFM) and electrochemical techniques. The disulfide-modified aptamer was homogeneously immobilized on the surface of the AuNPs, which allowed the probe to capture S-protein atop practically all the AuNPs-modified surface, as revealed by the PiFM mapping imaging in the 1400 cm^{-1} region. The detection of SARS-CoV-2 S-protein is achieved by electrochemical impedance spectroscopy after 40 min incubation with several analyte concentrations, yielding a limit of detection of 1.30 pM (66 pg/mL). Moreover, the aptasensor is successfully applied for the detection of a SARS-CoV-2 pseudovirus, thus suggesting it a promising tool for the diagnosis of COVID-19. The selectivity studies showed that the aptasensor is active to both SARS-CoV and SARS-CoV-2 S proteins [21].

In order to facilitate point-of-care diagnosis of SARS-CoV-2, Rehmati et al., have developed a label-free ultrasensitive electrochemical nanobiodevice for SARS-CoV-2 spike protein detection using Protein A/Cu_2O modified SPCE as a substrate for the ordered orientation of IgG antibodies as a specific receptor [22]. By utilizing the IgG anti-SARS-CoV-2 spike antibody onto the electrode surface, researchers have constructed the screen-printed carbon electrode modified with Cu_2O nanocubes

(Cu_2O NCs), which provide a large surface area to increase the protein A (ProtA) loading on the electrode surface. The modified aptasensor is used in clinical samples to detect the SARS-CoV-2 virus in less than 20 min. The electrochemical evaluations proved the very good linear response between the charge transfer resistance (Rct) and spike protein contents *via* a specific binding reaction in the range 0.25 fg mL^{-1} to 1 μg mL^{-1} [22]. In another work, Jiang et al., have developed highly sensitive and stable two-dimensional metal-organic framework (2D-MOF) based photoelectrochemical (PEC) aptasensor for the detection of SARS-CoV2 spike glycoprotein [23]. The PEC aptasensor is developed by plasmon-enhanced photoactive material, AuNPs/Yb-TCPP with a specific DNA aptamer against S protein. The Au Nps/Yb-TCCP fabrication is done by in situ growth of Au NPs on the surface of 2D Yb-TCPP nanosheets. This showed excellent photoelectric performance. The modified DNA aptamer on the surface of Au NPs/Yb-TCCP binds with S protein with high selectivity, photoelectric current is found to decrease this might be due to the high steric hindrance and low conductivity of S protein. The developed PEC aptamer showed high S protein detection with linear range of 0.5–8.0 μg mL^{-1} with limit of detection of 72 ngmL^{-1} [23].

A highly sensitive and specific optical aptasensor for the detection of receptor-binding domain (RBD) of the SARS-CoV-2 spike glycoprotein is developed [24]. Immobilization of specific aptameric sequence on short polyethyleneglycol (PEG) interface on gold nanofilm deposited on a D-shaped plastic optical fiber (POFs) probe, and the protein binding was monitored by exploiting the very sensitive surface plasmon resonance (SPR) phenomenon. In the present work receptor-binding domain (RBD) of the SARS-CoV-2 spike glycoprotein is used to develop an aptasensors [24]. The prepared bointerface is characterized by surface analysis techniques coupled to fluorescence microscopy and plasmonic analysis. Spanning a wide protein range (25÷1000 nM), the SARS-Cov-2 spike protein is detected with a limit of detection of about 37 nM. The specificity of aptasensor is also confirmed by testing different interfering agents (BSA, AH1N1 hemagglutinin protein and MERS spike protein) [24].

Tabrizi and coworkers for the first time developed a photoelectrochemical aptasensor for the quantitative determination of the SARS-Cov-2 RBD (receptor-binding domain) [25]. For the fabrication of aptasensor researchers have used CdS quantum dots and gC_3N_4 as photo-active materials, ascorbic acid as an electron donor molecule, chitosan as a binder, and an amine terminal aptamer as a biorecognition element. The development process involves the following steps: firstly, graphitic carbon nitride and (gC_3N_4) and cadmium sulfide (CdS) quantum dots are fabricated. Secondly, gC_3N_4 and CdS quantum dots mixed well to produce CdS QDs-gC3N4 nanocomposite and then added to the solution containing chitosan as an amine-rich polymer to generate a Chitosan/CdS-gC_3N_4

nanocomposite. Subsequently, the surface of the ITO electrode is modified with Chitosan/CdS-gC_3N_4. After that, the amine-terminal aptamer probes are immobilized on the surface of the Chitosan/CdS QDs-gC_3N_4/ITO electrode by using glutaraldehyde as an amine-amine crosslinker [25]. The mechanism of the aptasensor is based on the change in the photoactive response of the surface of the ITO electrode modified with the Aptamer/Chitosan/CdS QDs-gC_3N_4. Upon the interaction of SARS-Cov-2 RBD with the immobilized aptamer probe on the surface of the electrode, the mass transfer limitation for AA as an electron donor molecule to the photoactive nanocomposite (CdS QDs-gC_3N_4) increased. Consequently, the intensity of the photo-current decreased. Therefore, the demonstrated aptasensor is considered as a signal-off-based aptasensor. The reported aptasensor is used for the determination of SARS-Cov-2 RBD in the dynamic range of 0.5–32 nM with a detection limit of 0.12 nM [25].

A novel label-free surface plasmon resonance aptasensor is fabricated by Chen et al., for the detection of N-gene of SARS-CoV-2 by using thiol-modified niobium carbide MXene quantum dots (Nb_2C-SH QDs)/N-gene targeted N58 aptamer [26]. In the present study, the gold chip for surface plasmon resonance measurements is modified with Nb_2C-SH QDs via Au-S covalent binding and self-assembling. Nb_2C-SH QDs not only resulted in high bioaffinity toward aptamer but also enhanced the surface plasmon resonance response. The reported aptamer showed low detection limit (4.9 pg mL^{-1}) with a linearity range of 0.05–100 ng mL^{-1} N-gene concentration. High selectivity, excellent repeatability results make the developed aptamer good applicability in practical usage. Moreover, the presented Nb_2C-SH QD-based surface plasmon resonance response aptasensor displayed a huge practical application in terms of qualitative analysis of N-gene from different samples, including seafood, seawater, and human serum [26]. Yet in another study, Chen and co-workers used surface-enhanced Raman scattering (SERS) technique to fabricate aptasensor platform capable of quantifying (SARS-CoV-2) lysates with a high sensitivity [27]. In the report, a spike protein DNA aptamer is used as a receptor, and a self-grown Au nanopopcorn surface is used as a SERS detection substrate for the sensitive detection of SARS-CoV-2. A quantitative analysis of the SARS-CoV-2 lysate is performed by monitoring the change in the SERS peak intensity caused by the new binding between the aptamer DNA released from the Au nanopopcorn surface and the spike protein in the SARS-CoV-2 virion. From this technique, the detection limit is noticed to be less than 10 PFU mL^{-1} within 15 min [27].

Cho et al., demonstrated an ssDNA aptamer that specifically binds to SARS CoV nucleocapsid protein, it is isolated from a DNA library containing 45-nuceotide random sequences in the middle of an 88mer single-stranded DNA [28]. They have used an Enzyme-linked immunosorbent assay (ELISA) to identify the aptamer with the highest binding affinity to

the SARS CoV nucleocapsid protein. Their results concluded that ssDNA aptamer binds to the nucleocapsid protein with a Kd of 4.93 ± 0.30 nM. The Western blot analysis using the selected ssDNA aptamer demonstrated that the aptamer is a good alternative probe to monoclonal antibodies. When considering the advantages of aptamers relative to antibodies, including their stability and facile synthesis, the selected ssDNA aptamer showed good detection of SARS CoV N protein [28].

Researchers have used three different optical techniques, viz., biolayer interferometry, surface plasmon resonance and surface enhanced Raman spectroscopy, for quantifying the binding of recombinant SARS-CoV-2 spike protein to surface immobilized oligonucleotide aptamers [29]. The demonstrated biosensing platform using different techniques, functionalizing the surface with DNA aptamers that bind specifically to the SARS-CoV-2 spike protein receptor. The change in surface property of biosensing platform is identified by refractive index and the vibrational spectrum of probe aptamer. The results of the study indicated that, biolayer interferometry showed detection limit of 250 nM. Surface plasmon resonance is a more sensitive technique the detection limit for this was found to be 5 nM. However, surface-enhanced Raman spectroscopy enabled detection of spike protein to sub-picomolar concentrations [29]. The control experiments are performed using scrambled aptamers and bovine serum albumin as an analyte, results of this study showed the demonstrated aptasensing approach is both sensitive and selective, with no appreciable response observed for any of the controls [29].

The SARS-CoV (N) protein is one of the most abundant structural proteins and serves as a diagnostic marker for accurate and sensitive detection of the virus. Using a SELEX (systematic evolution of ligand by exponential enrichment procedure) and recombinant N protein, Ahn and coworkers selected a high-affinity RNA aptamer capable of binding to N protein with a dissociation constant of 1.65 nM [30]. The results of the studies of RNA competition experiments and electrophoretic mobility shift assays showed that the demonstrated aptamer recognized selectively the C-terminal region of N protein with high specificity. Further, by using a chemiluminescence immunosorbent assay and a nanoarray aptamer chip with the selected aptamer as an antigen-capturing agent, they have detected N protein at a concentration of 2 pg/mL. The prepared aptamers acted as antigen capturing molecule in chemiluminescence immunosorbent assay. Also reported that the aptamer-antibody hybrid immunoassays were useful for rapid, sensitive detection of SARS-CoV N protein [30].

In response to the current need for highly sensitive, quick and on-site detection of the SARS-CoV-2 virions in different aqueous solutions, two different nanolayer biorecognition systems separately combined with an adaptable optical fiber sensor have is reported by Cennamo et al [31].

The demonstrated sensors is tested by exploiting a plasmonic plastic optical fiber (POF) sensor coupled with two different receptors, both designed for the specific recognition of the SARS-CoV-2 Spike protein; one is aptamer-based and the other is molecular imprinted polymer-based. The preliminary experimental results showed that the sensitivity of the proposed SARS-CoV-2 optical fiber sensors proved to be high enough to detect virions. The results of the work showed that molecularly imprinted polymer receptor (MIP) combined with the SPR-POF platform, exhibited good performances in the SARS-CoV-2 detection. Both sensors resulted in a nanomolar range of detection of spike protein [31].

Lasserrea and coworkers have reported an impedimetric SARS-CoV-2 biosensor using SARS-CoV-2 Optimers [32]. In the report, thin film gold electrodes are fabricated, by sputter coating gold onto a polyester substrate, and used for the development of biosensor. Further, the functionalization of thin film gold electrodes with aptamer is done by temperature treatment. Further, reduced oligonucleotide mixtures are filtered using NAP-10 columns to 30 nM for aptamers and 32.4 nM for optimers, prior to an hour long immobilization onto electrodes. The results demonstrated that the interaction between the receptor and the spike protein is much stronger following binding with SARS-CoV-2 S1 than following incubation with IL-6 protein. Hence, it is concluded that following the successful development of an antispike protein Optimer sequence, sensitive, and selective detection of recombinant SARS-CoV-2 spike protein could be achieved with modified aptamer [32].

The applicability optical property of quantum dots (QD) in the detection of COVID-19 is established through the QDs surface conjugation with aptamers [33]. In the report researchers showed that QDs-conjugated RNA oligonucleotide could specifically recognize the SARS-CoV N protein. The fabricated biosensing platform, QDs-conjugated RNA aptamer, could be used as biosensor prototype for SARS-CoV N protein diagnosis. Advantageous binding of QDs-conjugated RNA aptamer for the detection of SARS-CoV N protein is determined by analyzing the fluorescence intensity through the use of confocal microscopy. Form the results of the analysis, the QDs-conjugated RNA aptamer could recognize the SARS-CoV N protein with a detection limit of 0.1 pg mL^{-1} on a designed chip [33]. A study performed by Vlamir et al. fabricated an aptamer-based nanosensor containing silver nanoparticles as SERS substrate to detect SARS-CoV-2 [34]. It is a one-step protocol that demonstrated the aggregation of silver nanoparticles after the binding of labeled aptamer with incubated virus and showed an intense SERS signal at 587 cm^{-1}. The reported detection system showed high selectivity and sensitivity of 5.5×10^4 TCID50/mL for the determination of SARS-CoV-2 [34]. In another work, Rhodamine 6 G-linked DNA aptamer functionalized gold nanostars (GNS) are employed to

detect SARS-CoV-2 using the distance-dependent nanoparticle surface energy transfer (NSET) spectroscopy [35], the study is explored by Pramanik et al. [35]. In the present work, GNS possess high extinction co-efficient which is quite similar to organic (106) molecules. The binding between rhodamine 6G-ssDNA aptamer displayed 99% quenching as a result of NSET from dye to GNS. These functionalized nanoparticles possess high sensitivity to SARS-CoV-2 spike protein with limit of detection of 130 fg mL^{-1}. Also, these aptamer-GNS particles inhibited the penetration of SARS-CoV-2.

14.3 Conclusions and future perspectives

The recent developments in the aptamer-based biosensing platforms for the detection of SARS-CoV-2 are briefly discussed in this chapter. Affinity-based aptameric nanobiosensors have the ability to detect the virus or their entities, as low as pico molar and even up to femto molar concentrations, in few cases. Proper selection of aptamers is a critical step in the development of aptameric nanobiosensors. Large-scale clinical validation studies and the processing of complex samples like blood, urine, sweat, feces, exhaled breath, and few others are necessitated to understand the sensor robustness and commercial viability. Development of smart watches-based diagnostic platforms may be advantageous for continuous monitoring of COVID-19.

The aptasensors can monitor viral spread in real-time, promoting more accurate and faster diagnostic solutions. The importance of investment in science and technology for developing innovative ways to deal with tragedies as COVID-19 pandemics is urgent, mainly in developing countries. Public authorities, thinking in general health and the common good, should base their decisions on science and educate their population, leading to good practices, health security and the pandemic's end. Indeed, with the advanced platform of 3D printing, a wide range of nanostructures can be prepared for the smart manufacturing of aptameric nanobiosensors for the diagnosis of COVID-19. However, this virus is more complex and hence it is necessary to invent rapid, sensitive and low cost technique for the early diagnosis of this infection. The sensors have shown their potential towards the diagnosis and sensing of viral infections and hence may perhaps fulfill the current demand for early diagnosis of SARS-CoV-2 cases. A number of biosensing methods including impedimetric, amperometric, and colorimetric-mediated methods have been established. The reported method could be used to detect the SARS-CoV-2 within short period of time. Though, fewer studies on the development of sensors for SARS-CoV-2 detection have been reported, the biosensing techniques offer alternative approach to PCR based testing for SARS-CoV-2 infection.

In recent developed medicine technology, there is still lot to be done for the developments in the diagnostic techniques to meet the demand of dairy farmers. We expect that this new field of aptamer-based sensors and biosensors in the diagnosis and treatment of viral diseases will eventually become a real-world tool. This would help in encountering challenges that could be difficult with currently available conventional technologies. The success of aptamers in clinics depends on their stability in blood and other biological fluids upon injection into the body. The general use of aptamers is also hindered by their incapability of intracellular accumulation at the site of action. While several studies have shown success with the use of aptamers under in vitro conditions, their use in human system requires special considerations.

References

[1] Y. Song, X.J. Song, X. Wei, et al., Discovery of aptamers targeting the receptor-binding domain of the SARS-CoV-2 spike glycoprotein, Anal. Chem. 92 (2020) 9895–9900.
[2] B. Balasubramaniam, S. Prateek, M. Ranjan, et al., Antibacterial and antiviral functional materials: Chemistry and biological activity toward tackling COVID-19-like pandemics, ACS Pharmacol. Transl. Sci. 4 (2021) 8–54.
[3] A.R. Fehr, S. Perlman, Coronaviruses: An overview of their replication and pathogenesis, Methods Mol. Biol. 1282 (2015) 1–23. https://doi.org/10.1007/978-1-4939-2438-7_1.
[4] D. Forni, R. Cagliani, M. Clerici, et al., Molecular evolution of human coronavirus genomes, Trends Microbiol. 25 (2017) 35–48. https://doi.org/10.1016/j.tim.2016.09.001.
[5] C.-Y. Chen, C. Chang, Y.-W. Chang, et al., Structure of the SARS coronavirus nucleocapsid protein RNA-binding dimerization domain suggests a mechanism for helical packaging of viral RNA, Journal. Mol. Biol. 368 (2007) 1075–1086. https://doi.org/10.1016/j.jmb.2007.02.069.
[6] J. Woo, E.Y. Lee, M. Lee, et al., An in vivo cell-based assay for investigating the specific interaction between the SARS-CoV N-protein and its viral RNA packaging sequence, Biochem. Biophys. Res. Commun. 520 (2019) 499–506. https://doi.org/10.1016/j.bbrc.2019.09.115.
[7] L. Duan, Q. Zheng, H. Zhang, et al., The SARS-CoV-2 spike glycoprotein biosynthesis, structure, function, and antigenicity: Implications for the design of spike-based vaccine immunogens, Front. Immunol. 11 (2020) 576622.
[8] J. Shang, G. Ye, K. Shi, et al., Structural basis of receptor recognition by SARS-CoV-2, Nature 581 (2020) 221–224.
[9] M. Sun, S. Liu, X. Wei, S. Wan, M. Huang, T. Song, et al., Aptamer blocking strategy inhibits SARS-CoV-2 virus infection, Angew. Chem. 60 (2021) 10266–10272.
[10] D. Wrapp, N. Wang, K.S. Corbett, et al., Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation, Science 367 (2020) 1260–1263.
[11] A.D. Ellington, J.W. Szostak, In vitro selection of RNA molecules that bind specific ligands, Nature 346 (1990) 818−822.
[12] C. Tuerk, L. Gold, Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase, Science 249 (1990) 505−510.
[13] S. Afrasiabi, M. Pourhajibagher, R. Raoofian, et al., Therapeutic applications of nucleic acid aptamers in microbial infections, J. Biomed. Sci. 27 (2020) 6. https://doi.org/10.1186/s12929-019-0611-0.

[14] A. Díaz-Fern_andez, R. Lorenzo-G_omez, R. Miranda-Castro, et al., Electrochemical aptasensors for cancer diagnosis in biological fluids e a review, Anal. Chim. Acta. 1124 (2020) 1–19. https://doi.org/10.1016/j.aca.2020.04.022.
[15] X. Zou, J. Wu, J. Gu, L. Shen, L. Mao, Application of aptamers in virus detection and antiviral therapy, Front. Microbiol. 10 (2019) 1462. https://doi.org/10.3389/fmicb.2019.01462.
[16] J.A. Park, J. Kim, S.M. Kim, et al., Fabrication of electrochemical influenza virus (H1N1) biosensor composed of multifunctional DNA four-way junction and molybdenum disulfide hybrid material, Materials 14 (2021) 343.
[17] Z. Rahmati, M. Roushani, H. Hosseini, Electrochemical immunosensor with Cu2O nanocube coating for detection of SARS-CoV-2 spike protein, Surf. Interfaces. 22 (2021) 100813.
[18] P. Weerathunge, R. Ramanathan, V.A. Torok, et al., Ultrasensitive colorimetric detection of murine norovirus using nanozyme aptasensor, Anal. Chem. 91 (2019) 3270–3276.
[19] Y.Y. Wu, B.W. Liu, P.C. Huang, A novel colorimetric aptasensor for detection of chloramphenicol based on lanthanum ion-assisted gold nanoparticle aggregation and smartphone imaging, Anal. Bioanal. Chem. 411 (2019) 7511–7518.
[20] T. Jinju, L. Zhixian, H. Ou, et al., An electrochemical dual-aptamer biosensor based on metal-organic frameworks MIL-53 decorated with Au@Pt nanoparticles and enzymes for detection of COVID-19 nucleocapsid protein, Electrochim. Acta 387 (2021) 138553.
[21] J.C. Abrego-Martinez, J. Maziar, C. Siham, et al., Aptamer-based electrochemical biosensor for rapid detection of SARS-CoV-2: Nanoscale electrode-aptamer-SARS-CoV-2 imaging by photo-induced force microscopy, Biosens. Bioelectron. 195 (2022) 113595. doi.org/10.1016/j.bios.2021.113595.
[22] Z. Rahmati, R. Mahmoud, H. Hadi, et al., Electrochemical immunosensor with Cu2O nanocube coating for detection of SARS-CoV-2 spike protein, Microchim. Acta 188 (2021) 105.
[23] W.J. Zhong, Z. Ting, M.L. Chun, et al., 2D MOF-based photoelectrochemical aptasensor for SARS-CoV-2 spike glycoprotein detection, ACS Appl. Mater. Interfaces. 42 (2021) 49754–49761. https://doi.org/10.1021/acsami.1c17574.
[24] C. Nunzio, P. Laura, A. Francesco, et al., SARS-CoV-2 spike protein detection through a plasmonic D-shaped plastic optical fiber aptasensor, Talanta 233 (2021) 122532, doi:10.1016/j.talanta.2021.122532.Talanta.
[25] A.T. Mahmoud, N. Leila, A. Pablo, A photo-electrochemical aptasensor for the determination of severe acute respiratory syndrome coronavirus 2 receptor-binding domain by using graphitic carbon nitride-cadmium sulfide quantum dots nanocomposite, Sensors & Actuators: B. Chemical. 345 (2021) 130377.
[26] Surface plasmon resonance aptasensor based on niobium carbide MXene quantum dots for nucleocapsid of SARS-CoV-2 detection, Microchim. Acta 188 (2021) 316.
[27] C. Rongyuan, K. Lun, D. Fenghe, et al., Sensitive Detection of SARS-CoV-2 Using a SERS-Based Aptasensor, ACS Sens 6 (2021) 2378−2385.
[28] C. Seong-Je, W. Hye-Min, K. Ki-Sun, et al., Novel system for detecting SARS coronavirus nucleocapsid protein using an ssDNA aptamer, J. Biosci. Bioeng. 112 (2011) 535–540.
[29] S. Tamsyn, M.G. Fiona, K. Barbara, et al., optical detection of CoV-SARS-2 viral proteins to sub-picomolar concentrations, ACS Omega 6 (2021) 6404−6413.
[30] A. Dae-Gyun, J. Il-Ji, D. Jung et al. RNA aptamer-based sensitive detection of SARS coronavirus nucleocapsid protein. 10.1039/b906788d.
[31] C. Nunzio, D. Girolamo, P. Laura, et al., Quantitative detection of SARS-CoV-2 virions in aqueous mediums by IoT optical fiber sensors, Results in Optics 5 (2021) 100177.

[32] L. Perrine, B. Banushan, J.V. Vincent et al. A SARS-CoV-2 aptasensor based on electrochemical impedance spectroscopy and low-cost gold electrode substrates.
[33] R. Changhyun, K.J. Sung, Quantitative and sensitive detection of SARS coronavirus nucleocapsid protein using quantum dots-conjugated RNA aptamer on chip, Technol. Biotechnol. 86 (2011) 1475–1479.
[34] E. Zavyalova, O. Ambartsumyan, G. Zhdanov, et al., SERS-based aptasensor for rapid quantitative detection of SARS-CoV-2, Nanomaterials 11 (2021) 1394.
[35] A. Pramanik, Y. Gao, S. Patibandla, et al., Aptamer conjugated gold nanostar-based distance- dependent nanoparticle surface energy transfer spectroscopy for ultrasensitive detection and inactivation of corona virus, J. Phys. Chem. Lett. 12 (2021) 2166–2171.

Non-Print Items

Abstract

Coronavirus disease (COVID-19) pandemic has left a disastrous effect on the world wealth and human evolution. The recent outbreak of COVID-19 disease is an infectious disease caused by newly discovered severe acute respiratory syndrome coronavirus 2 (SARS–CoV–2) which belongs to the single-stranded, positive strand RNA viruses. SARS–CoV–2 are dangerous threat to public health, economics, and global disciples. Therefore, it is important to identify, isolate, and treat individuals at the early stages of the disease to control the spread. In the present scenario, various analytical tools are available for the detection of several kinds of viruses through the use of different types of biosensing technologies. During the last decades, biosensors have emerged as reliable analytical devices and provide new promising tool for the detection of viruses. Aptamers are ssDNA or RNA oligonucleosides selected by the technique of systematic evolution of ligands by exponential enrichment (SELEX). Aptamers can bind various targets from small molecules to cells or even tissues in the way of antibodies. Aptameric nanobiosensors are rapid and sensitive diagnostic platforms, capable of SARS-CoV-2 detection, which overcomes the limitations of the conventional techniques. This chapter presents the use of aptamers in the fabrication of biosensors for improved diagnosis of SARS-CoV-2 and the future perspectives are also discussed.

Keywords

Coronavirus disease; Aptamers; Biosensors; Diagnosis; ssDNA; RNA