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Design and Development of Nanoscale Aptasensors for Viral Diagnostics

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Abstract: Viral infection may be a serious threat for human beings. Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is a highly transmissible virus causing coronavirus disease 2019 (COVID-19) in humans and creating a universal pandemic outbreak. The current methods for detection of SARS-CoV-2 include real-time reverse transcription-polymerase chain reaction (RT-PCR), enzyme-linked immunosorbent assay (ELISA), and loop-mediated isothermal amplification (LAMP). Though the methods are widely used for the diagnosis of COVID-19, they too have their limitations such as time-consuming process, sophisticated instrumental setup, which requires highly skilled personnel for operation, and prevalence of false positive/negative reports. Therefore, there is a pressing need to develop alternative tools such as point-of-care testing (POCT) devices to detect SARS-CoV-2 rapidly, accurately, and user-friendly. Here, the authors propose a one-step diagnostic method using aptamer-based sensing technology. The intended design of aptamer-based biosensors (also known as aptasensors) utilizes the optical properties of gold nanoparticles (AuNP) conjugated with angiotensin-converting enzyme-2 (ACE-2) aptamers targeting SARS-CoV-2 using lateral flow assay (LFA). This study leads to the development of portable nanoscale aptasensors for viral diagnostics.

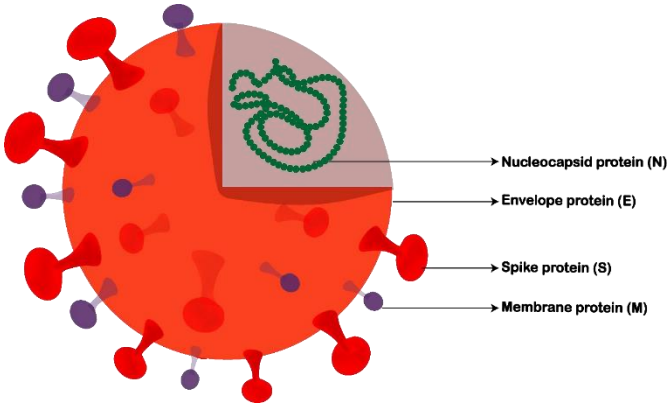
Keywords: aptamer; gold nanoparticles; lateral flow assay; aptasensor; SARS-COV-2; COVID-19.

1. Introduction

COVID-19 is a viral respiratory disease induced by SARS-CoV-2. The SARS-CoV-2 is an airborne virus that primarily spreads through respiratory droplets ejected from an infected person and has created a global pandemic [1-3]. The illustrative structure of SARS-CoV-2 is shown in Scheme 1, where the viral genome codes for major structural proteins, such as spike(S) protein, nucleocapsid (N) protein, membrane (M) protein, and envelope (E) protein [4,5]. The S protein has a high affinity towards the human angiotensin-converting enzyme-2 (ACE-2) receptor, an entry point to the cell for the virus and transmission of the disease [6]. Therefore, spike regions are promising targets for SARS-CoV-2 diagnosis [7].

The absolute standard method to diagnose SARS-CoV-2 is RT-PCR, with high sensitivity and specificity [8, 9]. Other diagnostic methods include LAMP, ELISA, and computed tomography (CT) scans [10]. Though all these methods are significantly used for the diagnosis of COVID-19, they too have their limitations such as time-consuming process, sophisticated instrumental setup, which requires highly skilled personnel for operation, and

prevalence of false positive/negative reports [11]. Therefore, there is a pressing need to develop an alternative method for detecting SARS-CoV-2 rapidly and accurately.



Scheme 1. Structure of coronavirus.

Aptamers have great prospects as appropriate tools for viral diagnostics owing to their functional properties, particularly their high sensitivity and selectivity compared with conventional instrumental methods. Aptamers, also known as chemical or synthetic antibodies, are single-stranded deoxyribonucleic acids (ssDNA) or single-stranded ribonucleic acid (ssRNA) oligonucleotides [12, 13]. Aptamer-based biosensors (also known as aptasensors) are capable of detecting a variety of analytes, including proteins, peptides, hormones, metal ions, whole cells, and materials surfaces, using aptamers as a biorecognition element. Since aptamers have such high selectivity and affinity for their targets, even minor changes in the target molecule can disrupt aptamer binding, which is correlated with high sensitivity when sensing. Moreover, since aptamers are in the size of 2-3 nm in diameter, they have a less steric hindrance on the surface of coronavirus [14]. Aptamers are produced by an iterative selection process called systematic evolution of ligands by exponential enrichment (SELEX) [15, 16].

Applications of aptasensors in viral diagnostics are of great value as they can be integrated with the utilization of transduction mechanisms of optical, electrochemical, electronic, and mass [17, 18]. Initial experimental data suggested that aptamer-based biosensing technology would be ideal for designing sensors suitable for detecting SARS-CoV-2, which could serve as a POCT. One of the POCT methods is the lateral flow assay (LFA), which combines thin layer chromatography (TLC) and immune recognition reaction for analysis [19, 20]. This is one of the most favorable strategies for designing portable and user-friendly sensors. Keeping these points in view, we propose fabricating portable nanoscale aptasensors for COVID-19 (PAC-19) that work by utilizing the optical properties of AuNP conjugated with ACE-2 targeting SARS-CoV-2 using LFA. The proposed study could contribute to the development of indigenous, scalable, and sensitive aptasensors in the form of a POCT device that could detect the presence of a virus on the go.

2. Materials and Methods

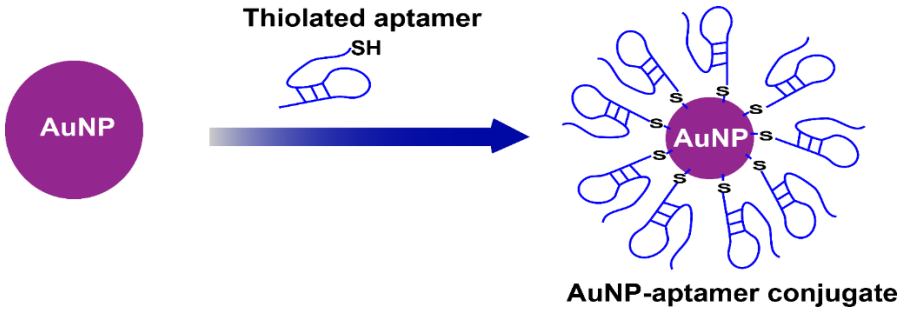
2.1. Design and selection of aptamer.

The ACE-2 aptamer will be designed and synthesized by SELEX, an iterative selection process. Libraries of 10^{13} to 10^{15} randomly generated DNA oligonucleotides, synthesized using combinatorial chemistry, were used to screen a large number of potential ligands rapidly for the selection of the ACE-2 aptamer. When ACE-2 molecules are incubated with the library, a

DNA complex with a high affinity for ACE-2 binds to it, and the remaining unbound molecules are eliminated. The bound molecules are collected and amplified using PCR, and the process is repeated until the aptamer with the desired characteristics is obtained. The obtained potential ACE-2 aptamer will be used as a detection aptamer in LFA.

2.2. Formation of AuNP-aptamer conjugates.

The AuNP of 20-40 nm in size will be synthesized in-house through the citrate reduction method. The synthesized AuNPs will be used as a label because they produce intense red color through a phenomenon called surface plasmon resonance (SPR), providing visual results. The AuNPs are covalently bound to the thiolated aptamer via Au-sulfur linkage, as shown in Scheme 2.



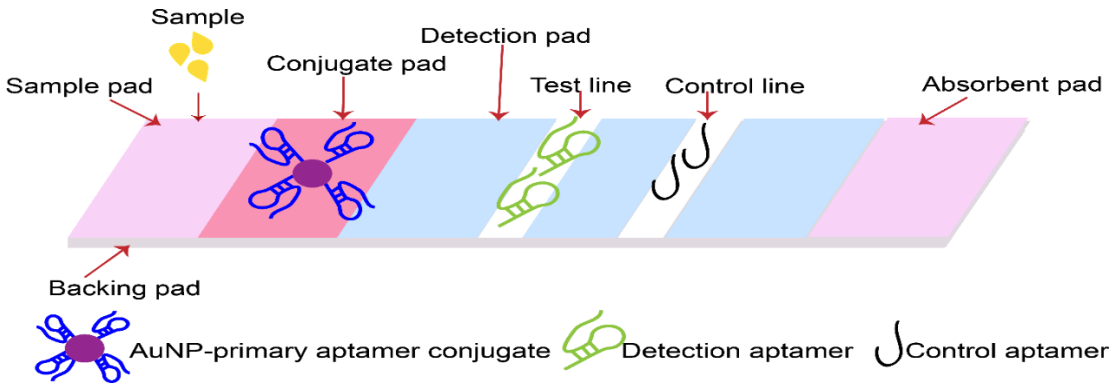
Scheme 2. Formation of AuNP-aptamer conjugates

2.3. Preliminary confirmatory test.

As a preliminary confirmation, a colorimetric assay will be performed by adding sodium chloride to the AuNP-detection aptamer conjugate. In the presence of SARS-CoV-2, AuNP aggregates and changes color, indicating the development of a target-detection aptamer complex. The color change can be analyzed using UV-Visible spectroscopy by studying wavelength shifts. In the absence of SARS-CoV-2, the color of the solution remains unchanged.

2.4. Design of LFA strips.

Following preliminary confirmation, the LFA strips will be designed as shown in Scheme 3 with (i) a sample pad to load the sample, (ii) a conjugate pad where the AuNP conjugated with primary aptamer will be deposited, (iii) a detection pad to test/detect the color change, which is made of nitrocellulose membrane containing test and control lines dispensed with detection aptamer which is specific to the target and control aptamer, respectively, and (iv) an absorbent pad to allow the sample to flow in a unidirectional way.



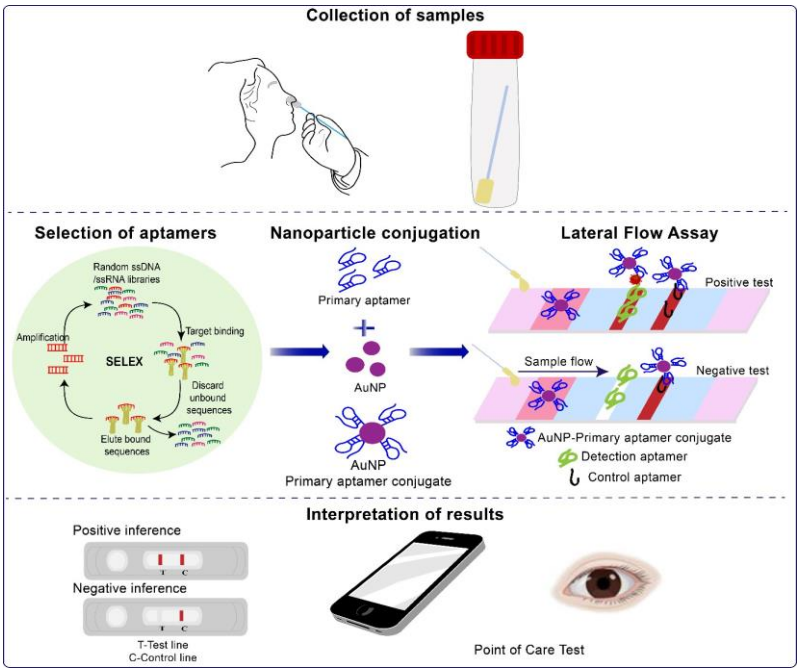
Scheme 3. Lateral flow assay strip design.

The sample and conjugate pads will be pre-treated with buffering agents, blocking agents, and detergents to optimize the reaction. Initially, the sample pad will be treated with 0.01 M phosphate-buffered saline (PBS) buffer (pH=7.4) containing 3% bovine serum albumin (BSA), 0.05% Tween20, and dried at 37°C for 2 h. The conjugate pad will then be sprayed with AuNP-primary aptamer after the pad is treated with 0.01 M PBS (pH-7.4) comprising 5% BSA, 5% sucrose, 1% polyethylene glycol (PEG 20000), and 0.05% Tween.

The treated pads will be assembled from bottom to top on the backing card made of laminated sheets with release liners. The order of assembly would be sample pad, conjugation pad, detection pad, and absorbent pad with 2 mm overlap to ensure that the sample flows smoothly from the sample pad to absorbent pad. The assembled test pads will be cut into 0.4 cm wide strips and stored in a plastic cassette to protect them from the damage of external sources. The PAC-19 device is designed in such a way that only the sample position, test, and control lines will be visible to the end-users. Finally, the test sample (sputum in this case) collected from the individuals will be placed on the sample pad that flows through the strip to determine the presence or absence of the target virus.

3. Technical Discussion

The mechanism behind the designing and performance of aptasensor is based on the conjugation of AuNP-ACE-2 aptamer complex towards the receptor-binding domain (RBD) of coronavirus S protein. The ACE-2 aptamer serves as an entry for SARS-CoV-2 and acquires a high affinity to a chosen target. A sandwich-based aptamer LFA technique is a potential choice for designing an aptasensor POCT device. The target forms a sandwich complex between primary and detection aptamer, as illustrated in Scheme 4. Before designing the LFA strip, optimizing nitrocellulose membrane and dispensing detection and control aptamer is crucial. The capillary flow time of the nitrocellulose membrane depends on the pore size and thickness to achieve an efficient flow rate. The optimal flow rate will intensify the interaction sample containing SARS-CoV-2 and detection aptamer, leading to high sensitivity and detection limit. Here, automated dispensing is a preferred mode with precise control over flow rate, speed, and position.



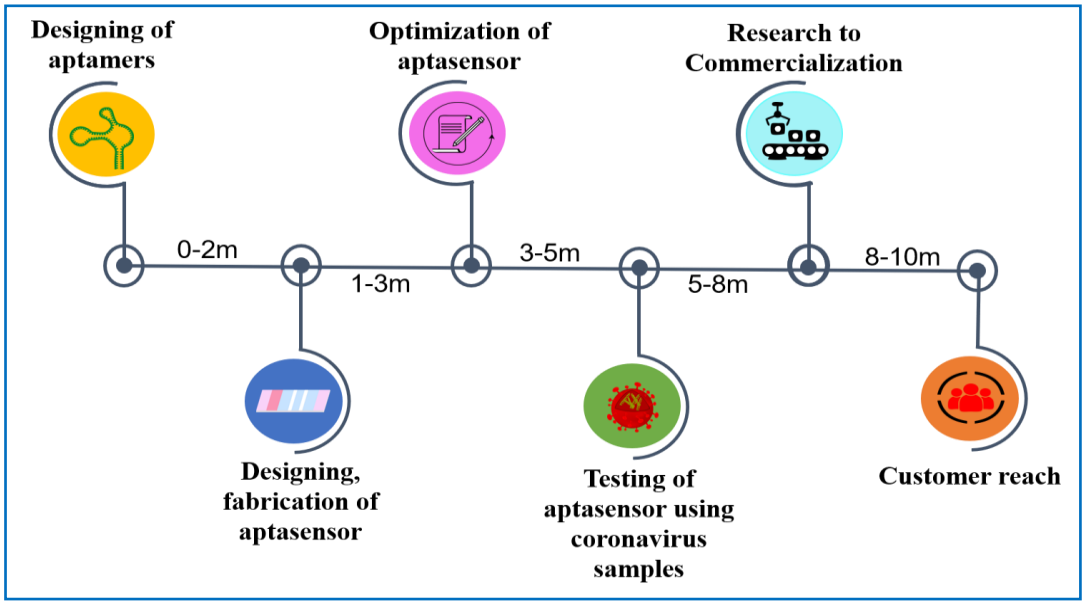
Scheme 4. Schematic illustration of designing and testing of aptasensor as a POCT device.

In the presence of SARS-CoV-2, AuNP conjugated with primary aptamer on interaction with sample flows towards the test line, where the detection aptamer specific to SARS-CoV-2 spike protein binds with the sample in sandwich format, forming AuNP- primary aptamer/SARS-CoV-2/detection aptamer complex. The AuNP-primary aptamer conjugate flows to the control line via capillary action. It forms AuNP-primary aptamer/control aptamer complex, resulting in an intense red band on both test and control lines with an assay duration of shorter duration. In the absence of SARS-CoV-2, the sandwich complex may not be formed on the test line because AuNP- primary aptamer conjugate does not provide an intense red band. In contrast, the control line will form a complex providing visible color. In this process, the test line intensity is dependent on the concentration of SARS-CoV-2. The data inferences resulting from the aptasensor POCT device are tabulated in Table 1.

Table 1. Inferences of data resulting from the aptasensor POCT device.

Samples	Test line	Control line	Inference
1	Intense color	Intense color	Positive
2	Intense color	No color	Invalid/ Repeat the sample
3	Mild color	Intense color	False-positive
4	No color	Intense color	Negative

The presence or absence of colored zones is used to interpret the results, which could be read with the naked eye, a strip reader, or a smartphone. A test was considered valid only if it triggers a signal on the control line or is recommended to repeat the test. In the event of false-positive results, subjects are constrained to self-isolate, interrupting their normal lives. As aptasensor is a rapid and economical method, a repeat test can be performed to mitigate the problem, or the test can be performed in multiple batches to reassure the result that not only confirm for false-positive but also ensure true-positive inferences. To establish the proposed nanoscale aptasensor technology in COVID-19 diagnosis, a few stages are to be streamlined, as illustrated in Scheme 5.



Scheme 5. Estimated timeline for establishing an aptasensor POCT device, represented in months (m).

The progression and expansion of authentic methods for COVID-19 serological and molecular diagnostics presented major challenges in 2020-21. Although it is crucial to control the epidemic outbreak through quarantining, cloud testing is highly required to improve the efficiency and effectiveness of testing regimens. Currently, the major diagnostics method for

testing COVID-19 infection is RT-PCR. However, the rapid development of POCTs may improve the diagnosis of COVID-19 infection rapidly and accurately. The ability to diagnose immunity status is often compromised by the lack of sufficient clinical testing. This impairs the ability to provide effective treatment and avoid making false assumptions. In this regard, aptasensor technology is advantageous for rapidly and accurately diagnosing viral infection in a mass population within a shorter duration. It is expected that this proposed aptasensor technology could be used for various other diagnostics based on selecting an aptamer sequence from the analyte of the target with suitable conjugates.

4. Conclusions

In summary, we have proposed the concept of designing and developing nanoscale aptasensors for viral diagnostic applications. The performance of the aptasensors is based on the binding affinity of the AuNP/ACE-2 aptamer complex towards the receptor-binding domain of coronavirus S protein. The proposed PAC-19 device uses an LFA-based detection methodology that might be easier, faster, and flexible to utilize this kind of aptasensors for the early detection of coronavirus symptoms on the go.

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Conflicts of interest

The authors declare no conflict of interest.

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