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Aptameric nanobiosensors for the diagnosis of COVID-19: An update

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ABSTRACT

COVID-19 pandemic has left a catastrophic effect on the world economy and human civilization. As an effective step towards controlling the transmission of viral infections during multiple waves of COVID-19, there is an urgent need to develop robust nanobiosensors for the detection of SARS-CoV-2 with high sensitivity, specificity, and fast analysis. Aptameric nanobiosensors are rapid and sensitive diagnostic platforms, capable of SARS-CoV-2 detection, which overcomes the limitations of the conventional techniques. This review article presents an outline of the aptameric nanobiosensors established for improved diagnosis of SARS-CoV-2 and the future perspectives are also covered.

1. Introduction

Severe acute respiratory syndrome (SARS) is a respiratory viral disease caused by a coronavirus. In late 2019, the world has witnessed the deadliest pandemic due to the origin of a new strain of SARS-coronavirus named SARS-CoV-2, which brought the global death toll to nearly 4.5 million till date. Antiviral materials and surface coatings are being developed to combat the COVID-19 pandemic [1,2]. Conventional techniques such as reverse transcriptase-polymerase chain reaction (RT-PCR), antibody profiling, immunoassays, lateral flow biosensors, matrix-assisted laser desorption/ionization - mass spectrometry, computed tomography, and few other approaches are used for the diagnosis of COVID-19. However, these methods suffer from limitations such as low specificity, prolonged detection, expensive, require skilled personnel, and need for sophisticated instruments. At the same time, nanotechnology-enabled diagnostics and therapeutics have been developed which are categorized based on nanomaterial properties such as electrical conductivity, photothermal, and optical phenomena [3–5]. Owing to the incorporation of nanostructured materials within the transducer element of the COVID-19 diagnostic platform, there is a significant improvement in the sensor performance towards the detection of SARS-CoV-2 viral RNA or their proteins (Spike, Envelope, Membrane, and Nucleocapsid). Similarly, an improvement in the specificity and sensitivity of the bionanosensor is also achieved due to the introduction of aptamers. By definition, the aptamer is a stretch of single-stranded DNA or RNA of 25–90 bases length whose three-dimensional conformation plays an important role in the recognition of the viral targets with high binding affinity. Using artificial tools like systematic evolution of ligands by exponential enrichment (SELEX), aptamers are selected which recognize the specific biomarkers associated with SARS-CoV-2 and thus employed for the rapid diagnosis of COVID-19.

Nanobiosensors developed with the interventions of aptamers have unique advantages such as real-time analysis, no tedious sample
preparation, economical, and acts against multiple targets. Aptameric nanosensors contains nanostructures as signaling moiety and the aptamers as recognition element. Aptameric nanosensors are well documented for the detection of a wide range of targets, including metals, proteins, small molecules and pathogens using the signal-transducer principles such as colorimetry, light scattering, Raman scattering, fluorescence, and electrochemical signals [6]. Earlier, ssDNA or RNA-based aptameric nanosensors have been reported to detect the SARS-CoV. Likewise, apta-nanobiosensors could be specifically manoeuvred for the prompt and precise detection of the SARS-CoV-2 and as a result, the timely isolation of infected cases would be commenced to limit the spread of viral infections. Once the aptamers specific for SARS-CoV-2 detection was precisely selected using the SELEX process, it can be successfully employed in the design of apta-nanosensors or lateral flow devices for COVID-19 diagnosis [7].

### Table 1

| No. | Sensor Principle | Aptamer | Nanomaterials | Recognition element (SARS-CoV-2) | Analytical Sensitivity | Limit of Detection | References |
|-----|------------------|---------|---------------|----------------------------------|-----------------------|-------------------|------------|
| 1.  | Surface Enhanced Raman Scattering | Biotinylated DNA aptamer | Silver Nanoparticles | Inactivated whole virus | 5.5 x 10^4 to 1.4 x 10^6 TCID<sub>50</sub>/mL | 5.5 x 10^4 TCID<sub>50</sub>/mL | [11] |
| 2.  | SLIP- Surface Enhanced Raman Scattering | Thiolated DNA aptamer | Silver Nanoparticles | Spike protein | 1 nM to 1 pM | 1 fM | [12] |
| 3.  | Nanoparticle Surface Energy Transfer Spectroscopy | Rhodamine 6 G-linked DNA aptamer | Gold Nanostar | Spike protein and virus particles | 10–500 virus/mL | 130 fg/mL (spike protein), 8 virus/mL | [13] |
| 4.  | Photo-electrochemical Signal | Amino terminal DNA aptamer | Chitosan/graphitic carbon nitride cadmium selenide quantum dots nanocomposite | Receptor binding domain | 0.5 to 32 nM | 0.12 nM | [14] |
| 5.  | Electrochemical Signal | Thiolated DNA aptamer | Gold coated platinum nanoparticles | Nucleocapsid protein | 0.025 to 50 ng/mL | 8.33 pg/mL | [15] |
| 6.  | Electrophoretic Mobility Shift Assay and Surface Plasmon Resonance | DNA and RNA aptamer | Graphene-oxide | Nucleocapsid protein | 1 x 10<sup>-9</sup> M | 6.25 x 10<sup>-19</sup> M | [16] |

nM-Nanomolar; pM-Picomolar; fM-Femtomolar; ng-Picogram; fg-Femtogram; M-Molar; mL-Millilitre; TCID<sub>50</sub>-Median tissue culture infectious dose; DNA-Deoxy ribonucleic acid; RNA-Ribonucleic acid.

### 2. Aptamers for COVID-19 diagnosis

A group of researchers from Translational Health Science and Technology Institute (THSTI) have explored the aptamers specific to spike protein, which detects the coronavirus with 99% specificity. Very recently, Li et al. have identified DNA aptamers namely MSA1 and MSA5 after a series of in vitro selection experiments [8]. The selected aptamers possess high binding affinity (few nM range) to the S1 subunit of the trimeric spike protein in both wild type and the B.1.1.7 variant. Using machine learning algorithms, Song and co-workers have identified aptamers namely CoV2-RBD-1C and CoV2-RBD-4C that specifically target the receptor-binding domain (RBD) of the spike protein of SARS-CoV-2 with high binding affinity to angiotensin-converting enzyme II (5.8 nM and 19.9 nM respectively) [9]. Woo and his co-workers reported a one-pot fluorescence-based detection of SARS-CoV-2 RNA using ligation-dependent isothermal RNA denoted as SENSR [10].

![Fig. 1. Process illustration of the detection of SARS-CoV-2 based on SERS-based aptasensor using silver nanoparticles.](image-url)
oligonucleotide probes with ligase molecule, RNA polymerase, and fluorogenic dye converts non-fluorescent aptamer to highly sensitive luminescence aptamer. SENSR process has the ability to detect viruses onsite within a span of 30 min and the limit of detection (LOD) of 0.1 attomolar concentration.

3. Apta-nanobiosensors for COVID-19 diagnosis

Integrated use of nanostructures and surface-enhanced Raman spectroscopy (SERS) technique to detect SARS-CoV-2 is based on the changes in resonance upon binding of specific analytes. A comprehensive overview of the aptameric nanosensors developed for COVID-19 diagnosis are given in Table 1. Vlamir et al. devised an aptamer-based nanosensor containing silver nanoparticles as SERS substrate to detect SARS-CoV-2 (Fig. 1) [11]. It is a one-step protocol that demonstrated aggregation of silver nanoparticles after the binding of labeled aptamer with incubated virus and also showed an intense SERS signal at 587 cm\(^{-1}\). This detection system takes about 7 min, is highly selective, and sensitivity of \(5.5 \times 10^4\) TCID\(_{50}\)/mL. However, detection sensitivity below sub-picomolar concentration is the need of the hour. Therefore, further studies are necessary to investigate the detection competency of target analytes using surface plasmon resonance (SPR), bio-layer interferometry (BLI), and SLIP-SERS. A study performed by Tamsyn et al. suggested that SLIP-SERS performs an ultrasensitive detection of viral proteins with a sensitivity of femtomolar concentration, using aptamer and silver nanoparticles linked via thiolates [12]. Similarly, Rhodamine 6 G-linked DNA aptamer functionalized gold nanostars (GNS) were employed to detect SARS-CoV-2 using the distance-dependent nanoparticle surface energy transfer (NSET) spectroscopy [13]. Wherein, GNS possess high extinction co-efficient which is quite similar to organic (10\(^6\)) 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 LOD of 130 fg/mL. Also, these aptamer-GNS particles inhibited the penetration of SARS-CoV-2.

Photo-electrochemical aptasensor (PEC-A) is a technique that detects Fig. 2. Schematic of photo-electrochemical aptasensor (Aptamer/Chitosan/CdS QDs-gC\(_2\)N\(_4\)/ITO electrode) measures photocurrent during the completion between SARS-CoV-2 and ascorbic acid. Reprinted from Ref. [14].
the photocurrent generated during the conversion of photons into electrons. To detect the RBD of SARS-CoV-2, Tabrizi et al. fabricated PEC-A, an aptasensor which consist of aptamer/Chitosan/CDs QDs-gCN34/TTO electrode [14]. The underlying principle of these photoactive nanocomposites is the competition between the ascorbic acid and SARS-CoV-2 which showed significant changes in the photocurrent (Fig. 2). The signal-off mechanism occurs due to the interaction between the target analyte and aptasensor of about 0.12 nm and sensitivity of 0.5 – 32 nM. Recently, Tian et al. developed a nanocomposite with two aptamers for the electrochemical detection of SARS-CoV-2 with high sensitivity [15]. The necessity of two aptamers is to improve the efficiency of the target analyte coated over gold electrode (GE). Captured targets are sensitized by gold@platinum nanocomposite using a metal–organic framework (MIL-52(Al)). Wherein, the electrochemical signals are amplified due to the oxidation of hydroquinone in the presence of H2O2. The sandwich aptamer and the nanocomposite showed a linear relationship between 0.025 and 50 ng mL−1 with LOD of 8.33 pg mL−1. Jia et al. developed rapidly detection of SARS-CoV-2 through sensing the N proteins using aptamers [16]. In this method, optical microfibers were used as sensing element to detect the target analytes. However, the sensitivity of these microfibers was improved by further coating with graphene oxide (GO) linked via APTES (3-aminopropyltriethoxysilane). In order to specifically capture the N proteins, a combination of DNA aptamer and RNA aptamer were functionalized over the GO for real-time monitoring using electrophotorecetric mobility shift assay (EMBAs) and SPR with LOD of 6.25 × 10−19 M. As of March 2021, Pinpoint Science, Inc. developing a portable aptamer-based in vitro diagnostic device for COVID-19 which is built using the aptamers specific to the nucleocapsid protein of SARS-CoV-2 and nanosphere technology.

4. Conclusions and future outlook

The recent developments in the aptameric nanobiosensors for the detection of SARS-CoV-2 was briefly discussed in this review article. 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 concentration, in few cases. Proper selection of aptamers is a critical step in the development of aptameric nanobiosensors. In future, the use of artificial intelligence and machine learning algorithms may influence the selectivity and sensitivity of the nanobiosensor for COVID-19 diagnosis. Large-scale clinical validation studies and the processing of complex samples like blood, urine, sweat, faeces, 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. Additive manufacturing (3D/4D printing) is an important technique for the development of diverse materials for a number of applications [17–19]. Indeed, with the advanced platform of 3 D printing, a wide range of nanostructures can be prepared for the smart manufacturing of aptameric nanobiosensors for the diagnosis of COVID-19.

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CRediT authorship contribution statement

Saravana Krishnan: Conceptualization, Writing – original draft, Writing – review & editing. Ashwin Kumar Narasimhan: Writing – original draft, Writing – review & editing. Duragprasad Gangodkar: Writing – review & editing. Sugapriya Dhanasekaran: Writing – review & editing. Niraj Kumar Jha: Artwork and schemes. Kamal Dua: Supervision, Project administration. Vijay Kumar Thakur: Funding acquisition, Supervision, Project administration. Piyush Kumar Gupta: Conceptualization, Writing – original draft, Writing – review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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