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Electrochemical sensors for the detection of SARS-CoV-2 virus

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ABSTRACT

Coronavirus (COVID-19), a deadly pandemic has spread worldwide and created many global health issues. Though methods of its detection are being continuously developed for the early detection and monitoring of COVID-19, still there is need for more novel methods. The presently used methods include rapid antigen tests, serological surveys, reverse transcription-polymerase chain reaction (RT-PCR), artificial intelligence-based techniques, and assays based on sensors/biosensors. Of all these, RT-PCR test has high sensitivity and specificity though it requires more time for testing and need for skilled technicians. Recently, electrochemical sensors have been developed for rapid monitoring and detection of SARS-CoV-2 from the patient's biological fluid samples. This review covers the recently developed electrochemical sensors that are focused on the detection of viral nucleic acid, immunoglobulin, antigen, and the entire viral particles. In addition, we also compare and assess their detection limits, sensitivities and specificities for the identification and monitoring of COVID-19. Furthermore, this review will address the best practices for the development of electrochemical sensors such as electrode fouling, limit of detection/limit of quantification determination and verification.

1. Introduction

Coronaviruses (CoV) belong to the large family of pathogenic viruses that can infect vertebrates (including bats, cats, camels, pigs, etc.,) as well as humans. They infect several biological systems including respiratory, intestinal and central nervous systems [1]. It is well understood that coronavirus is a single-stranded RNA virus responsible for the ongoing severe pneumonia and respiratory illness such as infection in humans [2]. Since the early onset of COVID-19 in the late 2019, the number of cases has increased considerably all over the world. The International Committee of Taxonomy of Viruses (ICTV) named this virus as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) [3]. Over the past two decades, coronaviruses have triggered three epidemic diseases viz., Severe Acute Respiratory Syndrome (SARS), Middle East Respiratory Syndrome (MERS) and COVID-19 (SARS-CoV-2) with the possibility of spreading the infection from human-to-human and animal-to-human [3–4]. Four coronaviruses genera (α, β, γ, δ) have been identified earlier, of which SARS-CoV-2 is a β-coronavirus cluster, which is the same as SARS and MERS [5]. According to WHO, infection in the majority of people can cause mild illness, but for some people with comorbid conditions such as chronic respiratory disease, cardiovascular problems, or diabetes are at high risk [6].

Recent literature has witnessed the development of numerous techniques for the specific detection of the virus, which these are based on detecting the viral nucleic acids during acute infection [7–9]. Different techniques reported to monitor and identify the novel coronavirus include methods based on artificial intelligence [10–11], smell dysfunction [12], serological assay [8,13], and sensors/biosensors [14]. However, increasing the SARS-CoV-2 infection rates across the globe has a demand for earlier detection (in both symptomatic and non-symptomatic cases as well as better analysis). Serological tests are based on the blood tests that can be used to detect whether people have been exposed to a specific infection by analyzing their immune response. Nucleic acid tests [8,13] have a greater need for laboratory analysis to measure antibody responses, while serological tests are not quite appropriate to identify the acute infection though they support several relevant applications [13], and also they lag behind due to lack of suitable reagents such as blocking buffer, wash buffer and coating buffer. Serological tests are required to perform serological surveys to detect the exact rate of infection as well as infection fatality rate. Furthermore, these assays are helpful to study immune response to COVID-19 in a dynamic quantitative and qualitative manner that can be

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used to identify the infected individuals (e.g., mild, asymptomatic or severe cases) and those that are potentially immune [8,13].

RT-PCR tests that are presently used worldwide to identify COVID-19 indicate the presence of viral materials during the infection, but they do not indicate if a person was infected or subsequently recovered. However, these tests can be very useful for strategically deploying the immune healthcare workers and identifying details of the patients, who have developed antibodies into the frequency of the disease infection in a population. Chu et al., [9] proposed a two 1-step quantitative RT-PCR assay to identify two different regions; nucleocapsid (N) and open reading frame (ORF1b) of the viral genome that were designed using a panel of negative and positive controls. If the individually tested samples were positive in RT-PCR assays, it is considered as COVID-19 infected. Then based on the RT-PCR detection performance, the N gene can be recommended as a screening assay, while the ORF1b test can be considered as confirmatory. By performing the algorithm similar to MERS, the ORF1b negative/N gene-positive result can be considered as unspecified. The positive RT-PCR results confirm and discriminate between the COVID-19 and other viruses [15–16]. At the same time, Chan et al., [17] designed and tested three novel real-time COVID-19 RT-PCR assays for the analysis of RNA-dependent RNA polymerase (RdRp)/helicase (Hel), spike (S), and N genes of SARS-CoV-2, among which COVID-19-RdRp/Hel assay was highly specific and sensitive to detect SARS-CoV-2 *in vitro* as well as in patient's specimen. The lowest limit of detection (LOD) was found in COVID-19-RdRp/Hel assay *in vitro* viral transcripts (11.2 copies/reaction; confidence interval 95%; 7.2 to 5.26 RNA copies/reaction), while LOD with genomic RNA was quite low (1.80, 50 % culture infection dose [$TCID_{50}$]/mL).

Presently, RT-PCR tests are most frequently used for COVID-19 testing and despite their high sensitivity, they are not suitable for large-scale monitoring of multiple samples due to time-consuming process (minimum 3 h), high cost, requirement of skilled personnel as well as multiple steps such as collection of samples, transport of samples into a solution and extraction of the viral RNA [18–20]. These limitations have hindered the rapid detection of viruses and to control the spread of SARS-CoV-2 and its variants in developing and underdeveloped nations. The other categories were also used to detect the exposure of viruses, including immunological and serological tests, which are mainly focused to detect antibodies developed in the individuals [21–23]. However, these methods require minimal equipment, but their clinical efficacy to specify SARS-CoV-2 virus is limited, since it can take a few days to weeks to develop any detectable antibody response from the onset of the symptoms in the patients [24]. There is an urgent need for a rapid and highly sensitive method to monitor the pandemic. In this respect, electrochemical methods are considered as sensitive, simple to operate, rapid and cost-effective as well as they are easy to use, requiring lesser time to analyze [25–36]. This has prompted to develop various electrochemical techniques to test and identify COVID-19. The rapid development of highly sensitive and selective sensing methods has become increasingly important, leading to different approaches to develop newer electrochemical sensors. The key challenges in sensor development are specificity or selectivity of the sensor. Therefore, to enhance selectivity of the sensor, different types of nanomaterials have been used to fabricate novel sensors such as gold-based thin-film, gold nanoparticles, graphene oxide, reduced graphene oxide, cobalt-functionalized TiO_2 nanotubes, palladium nano-thin film, poly-aniline, activated graphene oxide, and Au nanostars [19,24,37–41].

The electrochemical sensor can provide alternative ways for the detection of viruses [42]. Song et al., [43] published a review on point-of-care devices developed for the detection of SARS-CoV-2 virus, where comparison of the commercially available products such as immunoassays, nucleic acid tests, sensors and expectation of the point-of-care device based detection of SARS-CoV-2 was discussed. Recently, Balkourani et al., [44] reviewed on carbon or graphene and Au-based electrochemical methods used for the detection of COVID-19. Imran et al., [45] also compiled a review on electrochemical biosensors used for the current and past epidemic or pandemic viruses such as influenza, Ebola, Zika and HIV. In this review, we will discuss on the electrochemical sensors that are based on different targets including nucleocapsid, spike protein, immunoglobulin and nucleic acid particles for the detection of SARS-CoV-2 viruses.

1.1. Recommended best electrochemical practices

Electrochemical sensing of SARS-CoV-2 provides several advantages including high selectivity, relatively low-cost equipment and sensors, user-friendly, and rapid analysis that are suitable for the miniaturization (to make point-of-care devices). Nanomaterials were used to modify the electrode due to biocompatibility, thereby avoiding the electrode fouling to offer improved detection limit, selectivity and sensitivity. However, the key challenge is to create electrochemical sensors for the commercial point-of-care devices in clinical applications. The electrochemical devices such as point-of-care can provide simple, fast, highly sensitive and accessible paths outside the laboratory settings, which can have a significant impact on remote area and the people in self-isolation.

1.1.1. Electrode fouling

The interference and electrode fouling are due to low electrochemical activity and weak molecular absorption that can affect the analytical performance of the sensor viz., detection limit, reproducibility, sensitivity and overall reliability. In addition, electrode fouling inhibits the direct attachment of the target analyte with the electrode surface for ease of electron transfer. In electrochemical biosensors, fouling agents are phenols, proteins, neurotransmitters, amino acids, and other small biomolecules. To overcome the electrode fouling, several antifouling approaches have been reported. For instance, some protective layer or barrier on the electrode substrate can be used to prevent electrode fouling, but they are inappropriate for the system where the target analytes themselves behave as a fouling agent. In such situations, other approaches such as surface modification and electrochemical activation can be more effective [39,46–49].

1.1.2. Analyte mass transport/diffusion related issues

The topology of electrode surface can also reduce the electrode fouling. The smaller and microelectrodes are beneficial for increased mass transport. As the size of the electrode decreases, radial diffusion becomes dominant, resulting in a faster mass transport. The mass transport (diffusion) speed can be enhanced by decreasing the size of the electrode from the micro-scale to nano-scale. A faster electrochemical reaction can thus be possible at the nano-electrodes compared to the microelectrodes [50–51].

1.1.3. Potential/current measurement errors (background or IR corrections)

In an electrochemical cell, the resistance between the reference electrode and the working electrode produces a potential drop, which alters the constant potential or controlled potential at the working electrode. The current-resistance (I-R) correction permits the user to set a resistance value to correct the solution resistance. If the measured or computed resistance R_u is known before the scan, then the measured current-potential (I-V) curves can be adjusted or corrected by deducting the Ohmic drop in I-R for each measured current from the corresponding potential values [52].

1.1.4. Limit of detection/quantitation determination and verification

The limit of detection (LOD) is the ability of a method to detect the presence or absence of the analytes in samples at which detection is feasible; this can be calculated using the standard deviation (σ) of the repeated analysis using blank samples and slope of the calibration equation (b) [25,53–54] as: limit of detection = 3σ/b. Another approach is that of the parametric method and precision profile. To verify LOD, repeatedly measured curves can be recorded for calculating LOD

[53,55]. Similarly, the limit of quantification (LOQ), expressed as the lowest concentration of the analyte in a sample, can be identified with the acceptable accuracy and precision [55–56], which can be calculated as: limit of quantification (LOQ) = 10 σ/m. The verification of LOQ can be done by recording multiple measurements for two samples with the concentration at the claimed LOQ for three days and % of the measured results that can meet the acceptable errors are calculated. The LOQ is then verified when the obtained % is at least 85.

1.1.5. Reference/counter electrode related problems

The purpose of the counter electrode is to complete the circuit, apply the input potential to the working electrode and allow the charge to flow. Therefore, they are fabricated from the inert material such as platinum or carbon as their size should be larger than the working electrode to ensure no current limitations. The most commonly used reference electrodes are Ag/AgCl and standard calomel electrodes (SCE) [57–58].

1.2. Structure and development of electrochemical sensors for target SARS-CoV-2

Coronavirus has a positive-sense and a single-standard RNA (~30 K bp) enveloped virus with 14 open reading frames (ORFs) that can encode for replication, structural and non-structural protein. The SARS-CoV-2 consists of four proteins viz., spike, membrane, envelope and nucleocapsid, similar to SARS-CoV [37,59–60]. Recently, RdRp/Hel genes of SARS-CoV-2, a potential marker that may not demonstrate any cross-reactivity with other human respiratory or coronaviruses viruses were used for diagnostic purposes and an anti-RdRp helicase was used to detect the infection [17,61–62]. For the diagnosis of COVID-19, target nucleotide (or viral nucleic acid) can be SARS-CoV-2 specific viral RNA or equivalent cDNA, or any other novel sequence specific to them. The genome comprises of a replicase complex (ORF1ab), 5′ untranslated region (UTR), 3′ UTR, spike surface glycoprotein gene, small envelope gene, matrix gene, nucleocapsid gene, and some other unidentified non-structural open reading frames [63]. Electrochemical sensors have been developed based on specific viral RNA or the corresponding cDNA or antigen detection or replicas complex in a real sample. Mainly four antigen types were used to identify coronaviruses nucleocapsid (N), matrix (M), spike (S), and the envelope (E). A possible structure of the virus and electrochemical sensing platform is presented in Fig. 1. Among these, N and S proteins have been significantly used as the biomarkers since they can discriminate different types of coronaviruses [18–19,38,64–66] and several electrochemical sensors have been developed for immunoglobulin IgG and IgM detection against SARS-CoV-2 viruses [67].

The S or N protein, nucleic acid and immunoglobulin can be distinguished between different types of SARS-CoV-2 viruses. Several types of electrochemical sensors or biosensors have been proposed with different target specific for the highly selective and sensitive detection of the deadly SARS-CoV-2 virus. The key challenges for the development of electrochemical sensors are selectivity, pretreatment and the cost. Thus, cost-effective, highly selective and direct detection are highly desirable to analyze the patient's samples without any pretreatment to monitor or regulate the SARS-CoV-2 virus. Lima et al., [68] proposed the electrochemical diagnostic test for the detection of SARS-CoV-2 spike protein within 6.5 min at the cost of $1.5 per unit. The performance of the sensors was tested in clinical saliva and nasopharyngeal/oropharyngeal samples. Xian et al., [69] proposed a modularized transistor-based electrochemical biosensor for the detection of SARS-CoV-2 and cardiac troponin I.

1.3. Electrochemical detection techniques

In electrochemical sensing or bio-sensing, the detection of electric properties is generally used for extracting the information from chemical or biological systems for the electrochemically active analytes. In biosensors, predominantly enzymes are used for the electrochemical detection due to their specific binding capability and bio-catalytic activity. Other bio-recognition elements are nucleic acid, antibodies, micro-organisms and cells. However, in chemical sensing, nanomaterials are used for the electrochemical detection to increase the catalytic activity and selectivity of the sensor [70]. For the determination of SARS-CoV-2, various electrochemical techniques have been used such as electrochemical impedance spectroscopy (EIS), square wave voltammetry (SWV), differential pulse voltammetry, amperometry and potentiometry.

Amperometric technique continuously measures the current generated from the oxidation/reduction of electroactive analytes. The changes in current responses are directly related to the concentration of the analyte [70–71]. In voltammetry, information of the target analytes is measured on the controlled variation of the potential and the resulting current is measured by varying the potential. The peak current value of the electro-active species over the linear potential range is directly proportional to the bulk concentration of the analyte [70]. Potentiometric-based electrodes measure the accumulation of a charge potential at the working electrode compared to the reference electrode in an electrochemical cell when no or zero current flown. The potentiometric signal is measured as the potential difference (voltage) between the working and reference electrodes [71–72]. Impedimetric or conductometric-based sensors are also considered as the effective electrochemical sensing approaches in which alteration in conductivity of

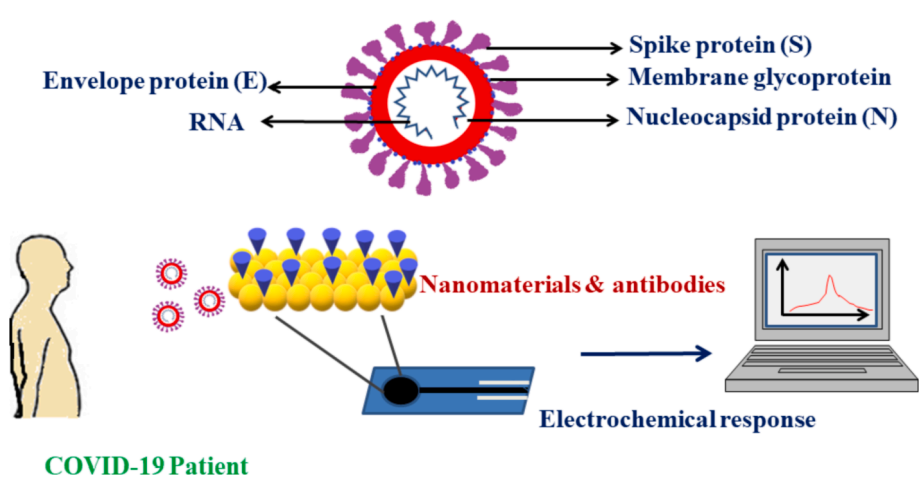

Fig. 1. Structure and schematic sensing platform for the detection of SARS-CoV-2.

the solution is measured. The impedance technique is very useful to investigate the changes in electrical properties due to bio-recognition events or nanomaterials at the electrode surface. The changes in conductance can be measured during different modification or recognition elements at the electrode surface [71,73]. The current generated is directly related to the concentration of the electro-active species. In the fabrication of electrochemical sensor/biosensor, conducting materials can be used as a transducer, which is useful for attaching the bio-recognition elements through the electrode, which can be achieved via a suitable modification at the electrode surface. Schematic representation of the sensing approach used for the electrochemical detection of SARS-CoV-2 is demonstrated in Fig. 2.

1.4. Advantages and disadvantages of electrochemical sensors

Electrochemical sensors are easy to fabricate the electrode. Biocompatibility of the electrochemical transducer makes them feasible for the detection of a viral. The electrochemical sensors or biosensors are providing analytical information of the target species through the biochemical or chemical receptor because they can be directly attached to an electrochemical transducer element. The electrochemical approaches can thus play a significant role to the earlier diagnosis of the coronavirus due to their important benefits including cost-effectiveness, easy to use, point-of-care detection and reduced time of the sample analysis [74–75] such that several novel systems have been proposed for the detection of SARS-CoV-2. Although most of the sensors are found effective for the detection of SARS-CoV-2, but some of them have lack of sensitivity, selectivity, low sampling rate and complex fabrication procedure for the electrode. In this sense, nanomaterials can provide a suitable path to overcome the sensitivity and selectivity problems.

2. Electrochemical detection of antigen for SARS-CoV-2 viruses

This section is divided into three subsections viz., detection of nucleocapsid, detection of spike protein and detection of spike protein as well as the nucleocapsid. Electrochemical sensors are developed based on N and S proteins as both are the important biomarkers to predict SARS-CoV-2 virus. The recently developed electrochemical sensors/biosensors are summarized here.

2.1. Electrochemical sensors for the detection of nucleocapsid

The nucleocapsid protein of coronavirus is a structural protein that plays a significant role in viral genome packaging and forms the complex with its genomic RNA. The study on N protein from a range of model coronaviruses has demonstrated that N protein undergoes self-association, interaction with other proteins and with RNA. The high fraction of N protein was expected to be intrinsically disordered, which makes the main barrier predictable to the structural characterization [76–77]. Eissa et al., [18] developed an electrochemical sensor for the detection of SARS-CoV-2 N protein using a combination of cotton fibers and electrochemical assay in which cotton-tipped immuno-sensor played the dual function as a detector and a sample collector. The electrochemical immuno-sensor was developed by immobilizing the virus N protein after functionalization of the carbon nanofiber modified screen-printed electrodes via diazonium electro-grafting. The determination of virus antigen was then performed through swabbing followed by a competitive method using the fixed quantity of N protein antibody in solution; square wave voltammetry (SWV) was used to detect the SARS-CoV-2 response (Fig. 3) by which LOD was found to be 0.8 pg/mL for SARS-CoV-2. Cross-reactivity of the electrochemical sensor was also tested in the presence of other virus antigens such as HCoV and influenza A, which showed excellent selectivity and recovery was found to be 91–95.5% in spiked nasal samples.

A molecularly imprinted polymer (MIP)-based electrochemical sensor was recently proposed by Raziq et al., [19] for the determination of SARS-CoV-2 N protein using a disposable sensor chip-thin film electrode connected to a MIP-endowed selectivity for the SARS-CoV-2 N protein. The chip was connected to a movable potentiostat that was selective for the target coronavirus, and a differential pulse voltammetry (DPV) was used to examine SARS-CoV-2 N protein in the presence of a redox pair ferri/ferrocyanide; the clinical practicability of the MIP sensor was examined by detecting the nasopharyngeal swab specimens of the patients. The MIP-based sensor demonstrated linear response to SARS-CoV-2 N protein up to 111 fM, while LOD and limit of quantification (LOQ) of 15 fM and 50 fM were respectively observed.

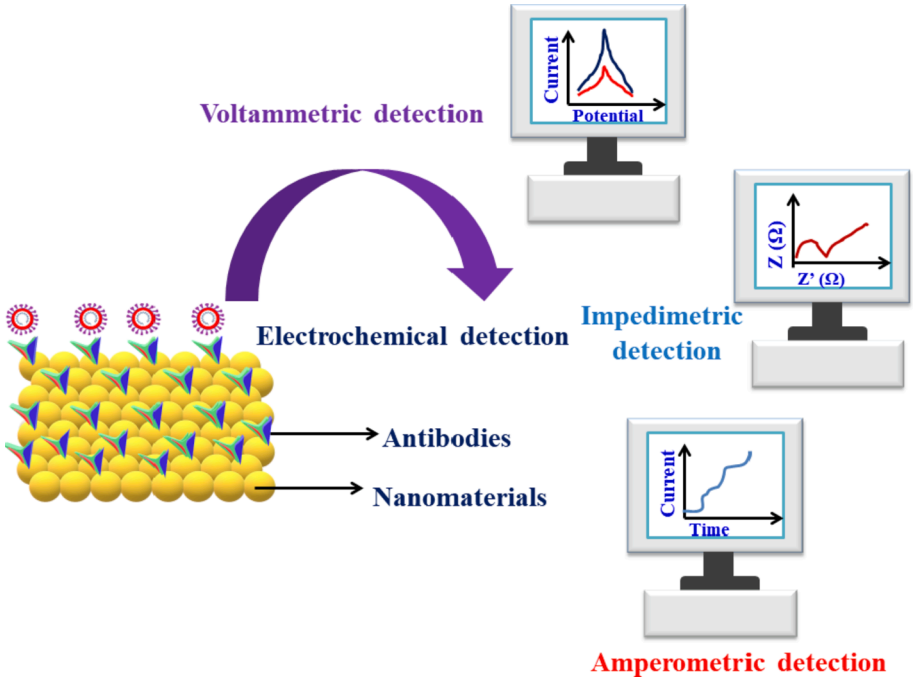

Fig. 2. Electrochemical techniques used for the detection of SARS-CoV-2.

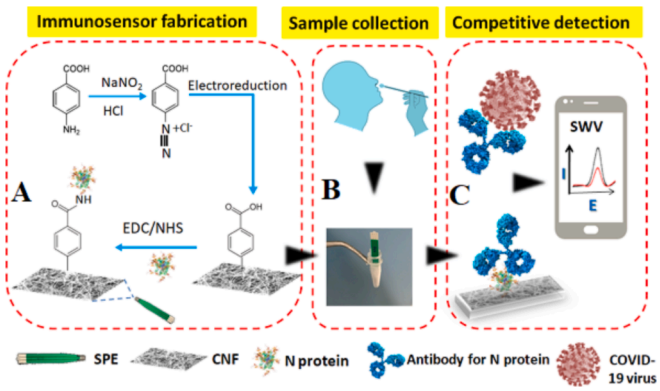

Fig. 3. Schematics of cotton-tipped electrochemical immuno-sensor for COVID-19; (A) sample collection using cotton-tipped electrode, (B) functionalization of carbon nanofiber electrode using electro-reduction of diazonium salt and attachment of virus antigen, (C) detection principle using competitive assay and SWV technique. Reproduced with the permission from Ref. [18] Copyright2021@American Chemical Society.

Alafeef et al., [24] developed graphene-based biosensor with an electrical readout setup to detect the SARS-CoV-2 N gene, wherein four ssDNA probes were designed to target two separate regions within the same viral N protein simultaneously that has enhanced the electrochemical performance compared to individual ssDNA. The sensitivity of the biosensor was further enhanced by using thiol-modified ssDNA-capped gold nanoparticles (AuNPs) on the topmost of Au electrode in contrast to ssDNA alone with no AuNP conjugation. This biosensor showed a potential enhancement in the output signal only in the presence of target SARS-CoV-2 RNA within <5 min of incubation time; LOD sensitivity of this method was 6.9 copies/μL with 231 (copies/μL)$^{-1}$. The response was further validated against the RNA samples taken from the Vero cells infected with SRAS-CoV-2, while MERS-CoV and SARS-CoV RNA were used as negative controls. This sensor could successfully distinguish positive COVID-19 samples from the negative ones to 100% accuracy along with specificity and sensitivity with no significant deviation in the output response for the sample lacking a virus viral target segment. This sensor chip was examined using 48 clinical samples from 26 healthy asymptomatic and 22 SARS-CoV-2 positive patients as confirmed by an FDA-approved Au-standard SARS-CoV-2 diagnostic kit. The proposed strategy was quite selective and sensitive to detect COVID-19 via the digital monitoring of the electrochemical response produced from the graphene-ssDNA-AuNP surface (Fig. 4).

An entirely new electrochemical impedance spectroscopy-based sensor using angiotensin-converting enzyme 2 (ACE2)-coated palladium nano-thin film (Pd-NTF) was developed by Kiew et al., [78] to detect potential inhibitors against SARS-CoV-2 S protein-ACE2 binding. In this, EIS-based biosensor ACE2-Pd-NTF electrode was the core sensing element to measure the changes due to the binding of SARS-CoV-2 S protein to ACE2 upon exposing to modulating molecules. This method was very effective to detect pharmacological inhibitors against SARS-CoV-2ACE2 binding. Alternatively, Song et al., [39] fabricated electrochemically polymerized polyaniline (PANI) nanowires onto glassy carbon electrode (GCE) to immobilize the newly designed peptides for the detection of SARS-CoV-2 N-gene, where the inverted Y-peptides have antifouling properties and two anchoring branches. The antifouling performance against the complex biological media and protein was examined via different approaches. Streptavidin (SA) was used as a linker to immobilize SRSA-CoV-2 N-gene specific probe on the biotin-peptide-covered interfaces based on biotin-SA affinity system. This biosensor showed high selectivity with a mixture of interfering molecules (miRNA-141 and miRNA-21); even up to 100-times higher concentrations than the target, no significant deviation was observed in the current response at the geno-sensor. The sensor was successfully used for COVID-19 nucleic acid in the linear concentration range from 10^{-14} to 10^{-9} M with a low LOD of 3.5 fM (Fig. 5) and the biosensor showed good recoveries up to 98.03–101.63% in human serum samples.

2.2. Detection of nucleocapsid and spike proteins

Realizing that electrochemical signal can be amplified using the rolling circle amplification (RCA) method, Chaibun et al., [20] very recently reported an electrochemical sensor based on multiplex RCA for the fast determination of S and N genes of SARS-CoV-2 taken from the clinical samples (Fig. 6). This protocol involved the sandwich hybridization of RCA amplicons with probes functionalized with the redox-active labels that were consequently detected by the DPV techniques that could detect as low as 1 copy/μL of viral S or N genes in <2 h. The sensor evaluations were performed with 106 clinical samples in which 41 samples were SARS-CoV-2 positive and 9 samples were positive for other respiratory viruses, all of which showed 100% concurrent results

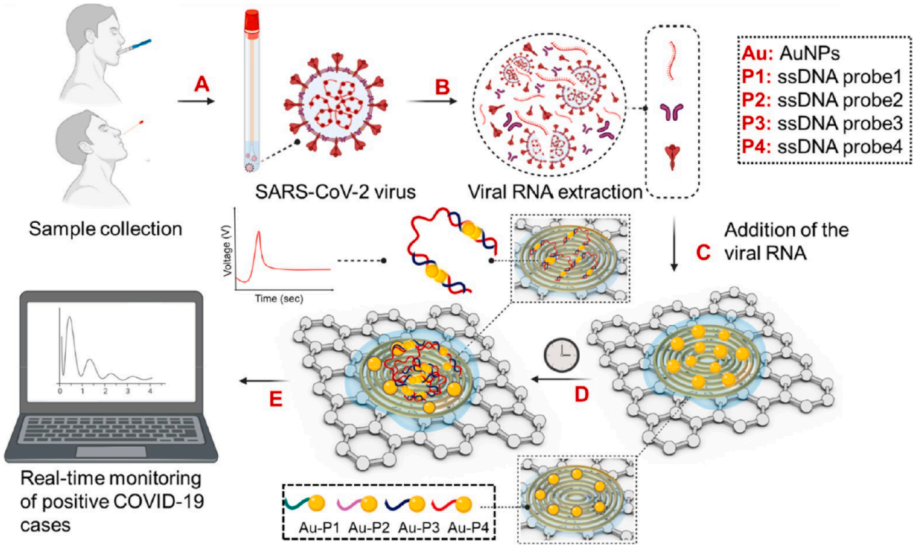

Fig. 4. Schematics of the principle of operation of COVID-19 sensor: (A) infected samples are collected from nasal swab or saliva of the patients; (B) viral SARS-CoV-2 RNA is extracted; (C) the viral RNA is added on the top of graphene-ssDNA-AuNP platform; (D) incubation of 5 min; and E: digital electrochemical output is recorded. Reproduced with the permission from Ref. [24] Copyright2020@American Chemical Society.

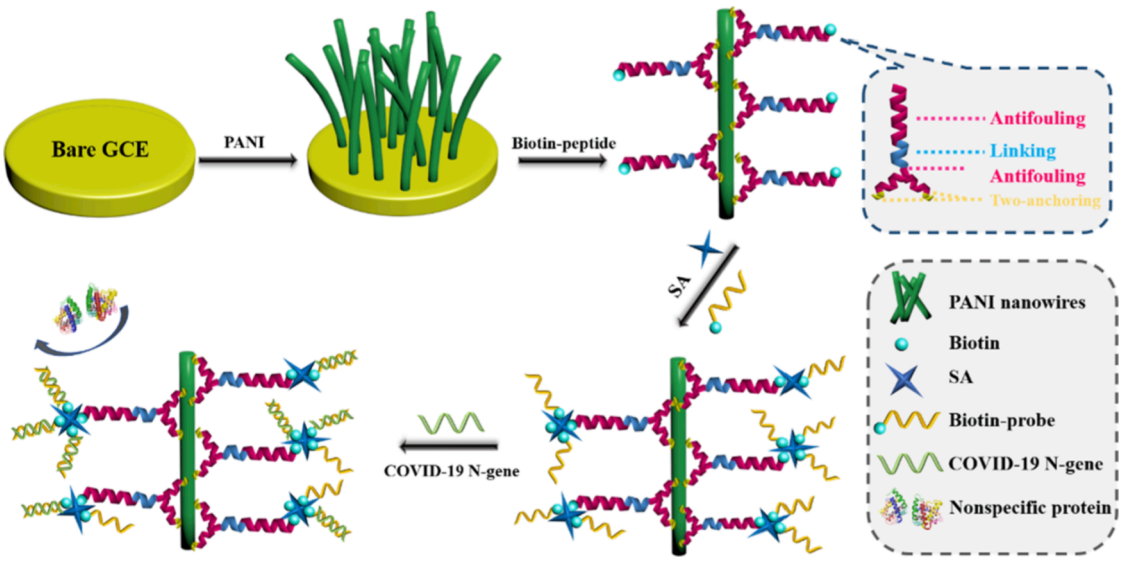

Fig. 5. Schematics of antifouling COVID-19 genosensor fabrication. Reproduced from Ref. [39] with permission Copyright 2021@American Chemical Society.

with qRT-PCR, thereby establishing a complete correlation between the sensor current response and the quantitation cycle values. The clinical sample results were in good agreement with qRT-PCR results, suggesting the success of this method.

2.3. Detection of spike proteins

Spike protein plays a significant role in viral entry, fusion and attachment, thereby helps the target for growing vaccines, antibodies and entry inhibitors. Spike protein has two subunits: S1 subunit contains a receptor-binding domain (RBD), which is responsible mainly for binding of the virus to the receptor, and S2 subunit mediates the virus-host cell fusion and the entry [37,60,79]. The S protein is used as a diagnostic antigen due to key *trans*-membrane protein of the virus, which is highly immunogenic. The spike protein has amino acid sequence diversity among the coronaviruses, allowing for the specific detection of SARS-CoV-2 [66,80–82].

A novel electrochemical sensor based on cobalt-functionalized TiO_2 nanotubes was proposed by Vadlmani et al., [37] for the rapid detection of SARS-CoV-2 via the sensing of spike-RBD present onto the surface of the virus (Fig. 7). This study involved single-step electrochemical anodization to synthesize TiO_2 nanotubes, and the annealed TiO_2 nanotubes were then functionalized with cobalt using a wet method. The developed sensor could detect S-RBD protein of SARS-CoV-2 at very low concentration range of 14–1400 nM, demonstrating a linear response for the determination of the viral proteins over the concentration range investigated. The LOD by this method was as low as ~0.7 nM and more advantageously, the sensor could detect the virus S-RBD protein in a very short time (~30 s).

Recently, Yousefi et al., [83] developed a reagent-free viral electrochemical sensor having the advantage of directly reading the presence of viral particles within 5 min using an electrode chip. This approach was based on electrode-tethered sensors having an analyte-binding antibody exposed onto the negatively charged DNA linker bounded with a tethered redox probe ferrocene and the sensor was transported to the electrode surface by applying the positive potential. The presence of protein and viral particles was determined via chrono-amperometry to investigate the kinetic response of a probe/virus complex for analyzing the complex state of the antibody. The sensor could detect SARS-CoV-2 virus and the related S protein within 5 min in unprocessed patient saliva and the test samples. The performance of reagent-free electrochemical sensor response was increased with SARS-CoV-2 S protein concentrations up to 1 pg/mL, 10 pg/mL and 100 pg/mL, and its specificity was checked by introducing the non-targeted proteins of other viruses such as Ebola, MERS, seasonal human coronaviruses, Rubella and SARS-CoV-1 in addition to SARS-CoV-2. The sensor's performance was examined in the presence of pseudo-typed viral particles bearing SARS-CoV-2 S protein from 4×10^3 viral particle copies/mL to 4×10^7 viral particle copies/mL.

A novel biosensor was fabricated using 3D nano-printing of 3D electrodes onto which reduced-graphene oxide (rGO) nanoflakes were coated and then specific viral antigens were immobilized onto rGO nanoflakes modified electrodes [38], which could detect SARS-CoV-2 virus. In this system, electrodes were integrated with a microfluidic device and applied in a standard electrochemical cell. Upon introducing the antibodies onto the surface of the electrode, these were selectively bound with antigens, thereby changing the impedance. Antibodies to spike S1 protein and RBD antigen of SARS-CoV-2 virus were then detected at LOD of 2.8 fM and 16.9fM, respectively. An advantage of this approach is that the biosensor can be renewed within one minute via low pH chemistry, which can elute the antibody from antigen, permitting up to ten consecutive readings from the same sensor with a high reliability. The determination of spike S1 protein and RBD antibodies was selective due to no cross-reactivity with other antibodies such as RBD, S1 and N antibody, and proteins such as interleukin-6.

Rashed et al., [84] proposed a non-Faradic capacitive immunosensing device for the detection of SARS-CoV-2 antibodies using electrochemical impedance spectroscopy (EIS) using specialized well-plates containing integrated sensing electrodes. The 16-well plate to sense electrodes was pre-coated with RBD of SARS-CoV-2 spike protein, which was consequently tested with samples of anti-SARS-CoV-2 monoclonal antibody CR3022 (0.1 μg/mL, 1.0 μg/mL and 10 μg/mL). The blind test was executed on six serum specimens obtained from COVID-19 and healthy patients (1:100 dilution factors). The system was able to differentiate the spikes in impedance sensing from the negative control (1% milk solution) for all anti-SARS-CoV-2 antibody (CR3022) samples and the detection mechanism was related to the binding kinetics between SARS-CoV-2 Spike protein RBD and anti-SARS-CoV-2 antibody. It was realized that impedance data were quite reliable than the standard ELISA test, but further testing was needed to calculate the detection limit.

Mahari et al., [85] used an in-house built device (eCovSens) that was comparable to the commercial potentiostat for detecting SARS-CoV-2 spike antigen in spiked saliva samples. In this method, fluorine-doped tin oxide (FTO) electrode was modified with AuNPs and immobilized with SARS-CoV-2 monoclonal antibody to examine the changes in

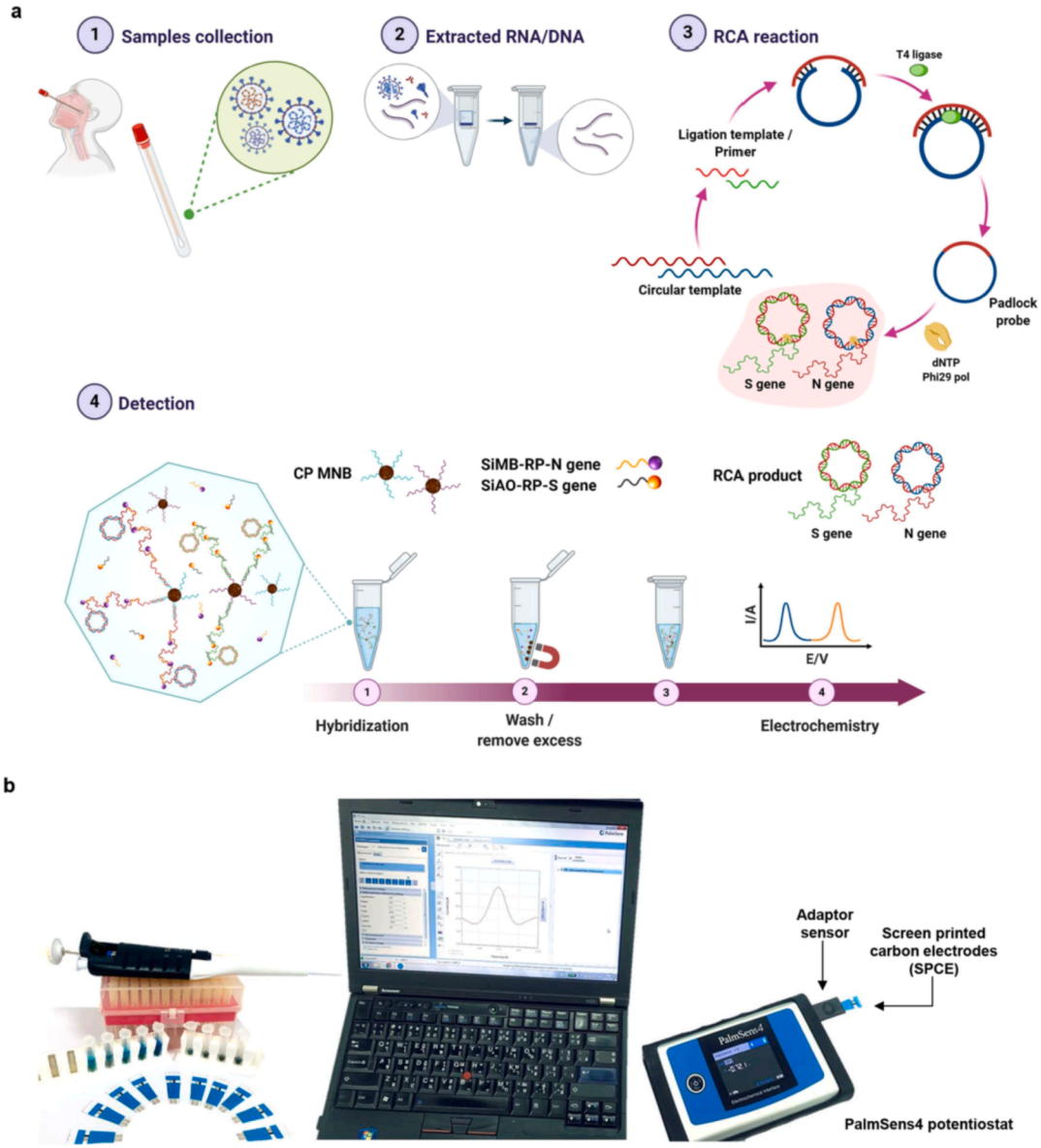

Fig. 6. An overview of the detection platform. (a) Detection workflow of SARS-CoV-2 from clinical samples using electrochemical biosensor with RCA of N and S genes. (b) Detection setup of electrochemical analysis using a portable potentiostat device connected to a laptop. Reproduced with permission from Ref. [20] Copyright 2021@Nature.

electrical conductivity via potentiostat. This device (Fig. 8) was applied to examine the electrical conductivity changes via immobilizing SARS-CoV-2 monoclonal antibody on a screen-printed carbon electrode (SPCE). The performance of both the electrodes was measured by the interaction of SARS-CoV-2 monoclonal antibody with its specific SARS-CoV-2 spike antigen. The eCovSens and FTO-based immuno-sensor showed high sensitivity for SARS-CoV-2 spike antigen, which ranged from 1 fM to 1 μM under the optimized conditions. This in-house built sensor successfully detected SARS-CoV-2 spike antigen at 10 fM concentration, which was in close agreement with FTO/AuNPs electrode. Using the potentiostat, LOD was found to be 120 fM, while it was 90 fM with eCovSens in the case of spiked saliva samples. The advantage of this eCovSens sensor is that it can detect SARS-CoV-2 spike antigen within 10–30 s.

A novel label-free electrochemical immunoassay for the fast detection of SARS-CoV-2 virus through S protein was proposed by Mojsoska et al., [86] in which the sensor was fabricated by coating graphene as a working electrode with a linker, 1-pyrene butyric acid N-hydroxy succinimide ester (PBASE), which can bind to specific antibodies against SARS-CoV-2 S protein. This immuno-sensor is based on the detection of signal perturbation obtained from ferri/ferrocyanide redox couple measurements, subsequently binding the antigen during incubation (45 min) of the sample. The total change in redox couple current upon increasing the antigen concentration onto the sensor surface was applied to examine the detection range of S-protein to observe a linear correlation for three spikes S1 protein concentrations of 260 nM, 520 nM, and 1040 nM. The device was used to analyze SARS-CoV-2 at three different concentrations (34.38×10^3, 13.75×10^4 and 5.50×10^5 PFU/mL), which was able to detect a specific signal of spike S1 proteins above 260 nM and SARS-CoV-2 at 5.5×10^5 PFU/mL concentration. However, authors used only three concentrations of the target for the construction of calibration plot. On the other hand, real-time portable impedimetric detection prototype 1.0 (RAPID 1.0) developed by Torres et al., [87] was able to transform the biochemical information from a specific binding incident between the SARS-CoV-2 S protein and ACE2 into an electrical signal. The biosensor modified with a receptor ACE2 could detect SARS-CoV-2 within 4 min for 10 μL of the sample and increased resistance to charge transfer of a redox probe was measured by EIS. The specificity

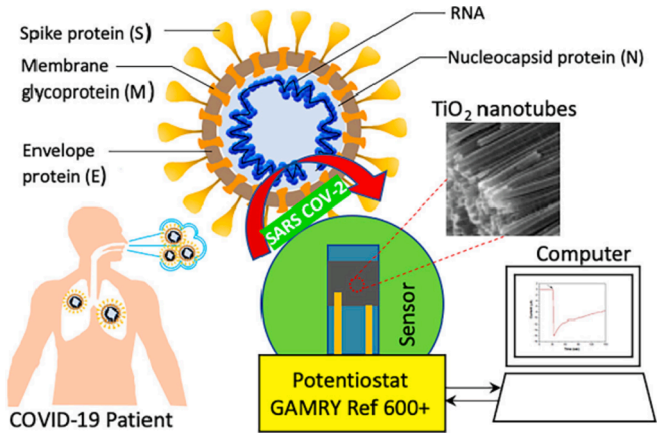

Fig. 7. Schematic of Co-functionalized TiO_2 nanotube (Co-TNT)-based sensing platform for the detection of SARS-CoV-2. Reproduced with the permission from Ref. [37] Copyright 2020@MDPI.

and sensitivity of RAPID 1.0 for saliva and nasopharyngeal/oropharyngeal swab samples were 100% and 85.3%, and 86.5% and 100%, respectively.

3. Detection of immunoglobulin for SARS-CoV-2 viruses

Antibodies including IgM and IgG from the patient samples have been used for the detection and understanding of the infection history. The antibody detection suggest the dynamics of immune response for SARS-CoV-2 infection and the types of antibodies may differ at different stages of the viral infection for both asymptomatic and symptomatic patients. The patients recovered from COVID-19 infection develop antibodies based on the individual immune responses in blood samples due to insufficient cellular immunity. The immuno-chromatographic study with IgM and IgG antibodies showed the detection sensitivity of 11.1% at the early stage (1–7 days after the onset), 92.9% at an intermediate stage (8–14 days after the onset) and 96.8% at later stage (more than 15 days) [38,88–89]. Thus, binding and neutralizing the antibodies can also be used to detect SARS-CoV-2, but binding and non-neutralizing antibodies such as immuno-globulins can bind exactly to the pathogen without restricting the infection. IgG is considered as an indicator of the present or prior infection, while IgM is an indicator of the initial stage of infection, which can persist in the body for more than three months [67,82]. Recent efforts have demonstrated that IgG and IgM antiviral antibodies can be identified in the human serum samples infected with SARS-CoV-2 [90–93].

An electrochemical paper-based analytical device (ePAD) was developed recently by Yakoh et al., [67] to detect SARS-CoV-2 immuno-globulins (IgG and IgM) (Fig. 9) that was targeted for SARS-CoV-2 antibodies without any specific requirement of antibody. The antibody can interrupt redox conversion of $[Fe(CN)_6]^{3-/4-}$ or form immuno-complex, which may decrease the current response. The sensing mechanism at ePAD was due to the disruption of redox conversion initiated by the complex formation between the captured immuno-globulins formed in response to COVID-19 in humans with immobilized spiked protein of SARS-CoV-2. The cross-reactivity of this protocol was tested in the presence of anti-hepatitis C virus (anti-HCV), anti-Epstein Barr virus (anti-EBV), anti-hepatitis B surface (ant-HBsAg), anti-Rubella and anti-cytomegalovirus (ant-CMV), but no cross-reactivity was reported with these anti-viruses.

In a recent study by Li et al., [90], a microfluidic paper-based analytical device (μPAD) was investigated for highly selective and label-free detection of SARS-CoV-2 using EIS biosensor. In this system, zinc oxide nanowires (ZnO NWs) were grown onto a working electrode to enhance the electrode's function with Faradaic processes exploiting the iron-based electron mediators. The paper-based biosensors were calibrated with different morphologies of ZnO NWs that attained low LOD of 0.4 pg/mL while, sensing p24 antigen as a marker for human immuno-deficiency virus. According to electrochemical and microscopic imaging characterization, surface area of the ZnO NWs modified working electrode enhanced the sensing range as well as the sensitivity of EIS-based biosensors. The EIS biosensor has the capability to differentiate the concentrations of IgG antibody CR3022 (blank, 10 ng/mL, 100 ng/mL and 1 μg/mL) specific to SARS-CoV-2 in human serum samples, the results obtained showed the efficacy of EIS-biosensor for detecting COVID-19. Quite interestingly, Hashemi et al., [40] proposed another ultra-precise rapid diagnostic system to detect monoclonal IgG antibodies against S1 protein of SARS-CoV-2 in blood samples of the patients

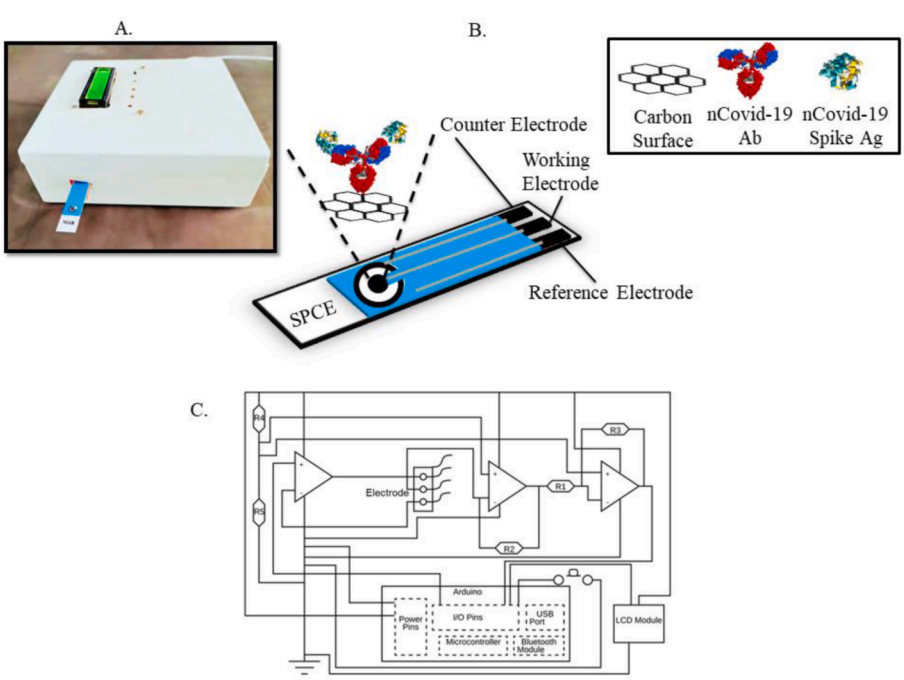

Fig. 8. (A) Fabricated in-house built electrochemical eCovSens device; (B) Schematics of fabrication process of SPCE electrode where nCovid-19 antibody (Ab) is immobilized onto transducer of the SPCE followed by addition of nCovid-19 Ag and transducer detects changes in electrical signal due to antigen-antibody (Ag-Ab) interaction. (C) Circuit diagram of in-house built electrochemical device depicting various components and connections. Reproduced with the permission from Ref. [85] Copyright2021@BioRxiv.

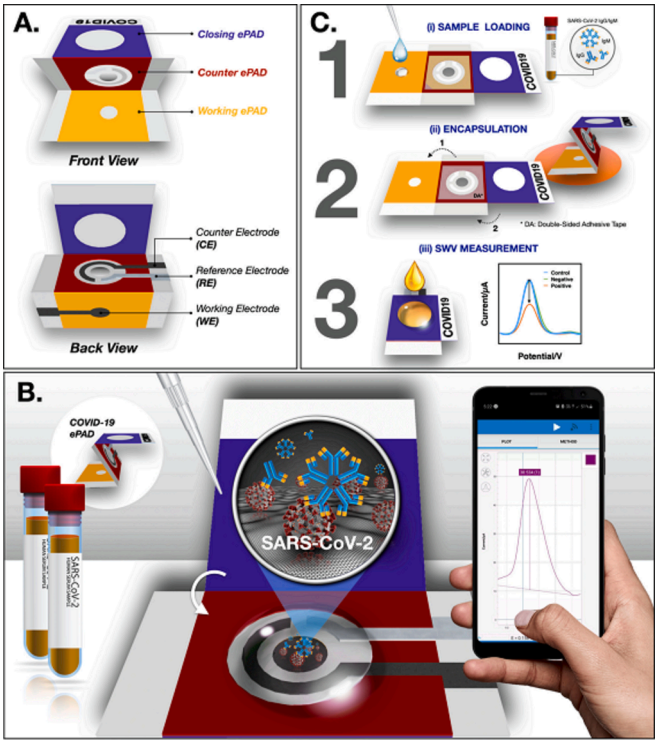

Fig. 9. Schematics of (A) device components, (B) detection principle and (C) detection procedure of COVID-19 ePAD. Reproduced with the permission from Ref. [67] Copyright 2021@ Elsevier.

infected with COVID-19 in 1 min. This sensor was made of an activated graphene oxide (GO) in conjunction with Au nanostars (G-Au NS), and GO was composed of 8-hydroxyquinoline, 1-ethyl-3-(3-dimethylaminopropyl), carbodiimide and N-hydroxysuccinimide (Fig. 10). The nanosensor could detect SARS-CoV-2 antibodies with LOD and sensitivity of 0.18×10^{-19} %V/V and 2.14 μA.%V/V.cm^{-2}, respectively, thus demonstrating a strong correlation with gold-standard (ELISA assay). The nanosensor demonstrated a high selectivity even in high amounts of interfering compounds/antibodies with the cutoff points of 0.2185 μA and 0.3265 μA, thus demonstrating its outstanding specificity/selectivity up to 100%/85% and 95%/100%, respectively.

4. Detection of viral nucleic acid for SARS-CoV-2

Of late, nanotechnology-based-functional DNA has created ample opportunities to traditional nucleic acid-based detection strategies for the targets, while portable DNA sensors have medical importance in terms of direct application for the diagnosis of infectious diseases. Generally, in solid-state electrochemistry-based DNA sensors, a capture probe modified working electrode is used, and a complementary target nucleotide is hybridized onto the sensing interface. Then, a platform of hybridization can be transduced into a physically quantifiable entity by using a electrochemical technique, but an additional electrochemical technique often assists the label-free DNA detection [64,92,94–96].

Progressive developments in molecular biology and nucleic acid detection methods have been the promising opportunities for the virus detection. Since coronavirus is a positive sense, single stranded RNA virus, DNA-RNA hybridization was generally used in RT-PCR as well as in numerous biomedical sensors [94,97]. These efforts have accelerated the applications of biosensors for the detection of SARS-CoV-2 viral nucleic acids. Fan et al., [92] proposed an entropy-driven amplified electro-chemiluminescence (ECL) technique to detect RdRp gene of the SARS-CoV-2, which was important in detecting the target SRAS-CoV-2. In this sensor, DNA tetrahedron (DT) was fabricated onto the surface of the electrode to provide programmable scaffolds and robust materials, wherein target DNA could be participated. Then entropy-driven amplified reaction was proceeded through $Ru(bpy)_3^{2+}$ modified S3 attached to linear ssDNA capture probe at the vertex of the DT, which was responsible to increase the ECL intensity thereby, the selectivity of the sensor. The DT-based ECL sensor response was measured at different concentrations of 1 fM, 10 fM, 100 fM, 1 pM, 10 pM and 100 pM for the target DNA with LOD values smaller than 2.67 fM. The recovery study was performed with the human serum (10% and 15%) for the detection of RdRp-COVID in a real sample, where recoveries of all the serum samples were >98%.

The study proposed by Qiu et al., [94] was quite different in that a dual-functional plasmonic biosensor was used for the diagnosis of COVID-19, which was based on localized surface plasmon resonance (LSPR) and plasmonic photothermal (PPT) sensing transduction. The DNA receptors attached to two-dimensional gold nano-islands (AuNIs) could recognize the specific SARS-CV-2 RNA sequences. A thermal plasmonic heat was used to heat up the AuNIs chip for a better sensing performance, making it more difficult for the imperfectly matched sequences to remain intact, thereby reducing any false positives. In situ PPT increment onto AuNI chips intensely improved the hybridization

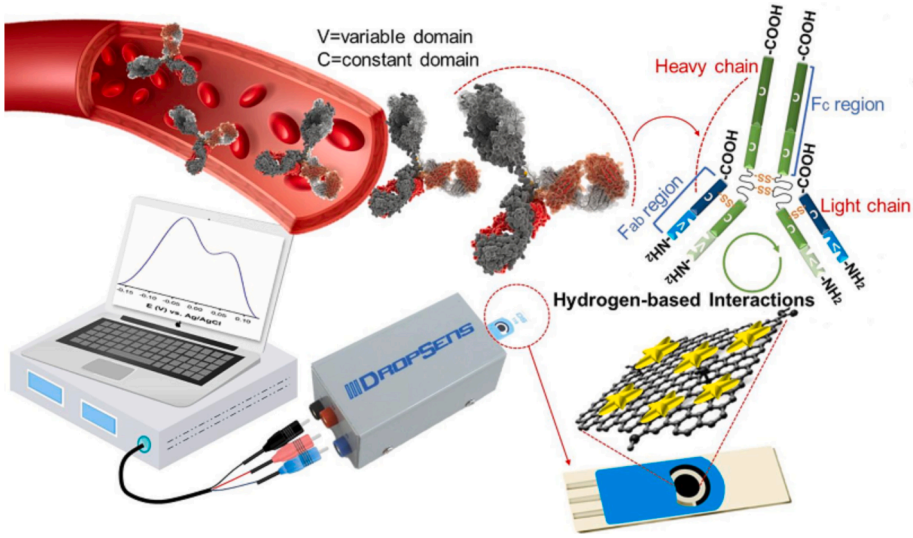

Fig. 10. Interaction of G-Au NS complex with IgG antibodies against S1 glycoprotein of SARS-CoV-2. Reproduced with permission from Ref. [40] Copyright 2021@ Elsevier.

kinetics to increase nucleic acid determination specificity. The biosensors were able to specifically distinguish between SARS-CoV and SARS-CoV-2 sequences with enhanced *in situ* PPT. The system could even detect the amount of viral RNA lower than those present in the respiratory swabs within a few minutes, but the assay still needs to be tested on intact viral RNA from the specimens of the patients; this method can be quite significant in relieving the current pressure on PCR-based assays. The biosensor showed high sensitivity towards SARS-CoV-2 sequences with a lower detection limit of 0.22 pM, thereby allowing specific detection of the target in a multi-gene mixture.

In another recent study, Hwang et al., [98] reported a label-free DNA capacitive biosensor, which was fabricated using platinum/titanium electrodes onto glass substrate to identify the hybridization of the analyte DNA with the probe DNA (Fig. 11). The signal of the hybridization for a particular DNA sequences was detected via FT-IR, capacitance-frequency and contact-angle measurements. For a single-step hybridized reaction, the kit showed significant sensitivity (capacitance change, $\triangle C = \sim 2$ nF) in analyzing the conserved region of the SARS-CoV-2 RdRp gene with a sensitivity of 0.843 nF/nM. Also, selective detection of the virus was confirmed by the fluorescence intensity and the image from SARS-CoV-2 gene was labeled with a fluorescent dye.

Peng et al., [99] proposed another electrochemical sensor to monitor SARS-CoV-2 RNA, wherein the presence of target sequence initially activates the catalytic hairpin assembly circuit and then initiates the terminal deoxynucleotidyl transferase-mediated DNA polymerization. Subsequently, a large number of long single-stranded DNA products can be produced. These negatively charged DNA products can hybridize with a massive positively charged electroactive molecules of $Ru(NH_3)_6^{3+}$ due to electrostatic adsorption. After the addition of $Ru(NH_3)_6^{3+}$, a significant enhancement in electrochemical signals was generated for sensitive detection of SARS-CoV-2 RNA in the linear range of 0.1–100 pM with a LOD of 26 fM. The distinguishing ability of the biosensor was analyzed with complex matrices as well as clinical samples from the patients.

5. Future perspectives

Even though some of the discussed techniques in this review are quite effective and sensitive for the detection of SARS-CoV-2 virus, yet more improvements are needed in terms of sensitivity and selectivity, LOD, and the method used for the fabrication of the sensor. Over a short period of time, a number of electrochemical-based sensors have been developed and utilized for the detection and analysis of COVID-19, but a great majority of these studies include small sample size to assess these techniques in terms of LOD, sensitivity and selectivity. For a better assessment, we believe that increasing the sample size and collecting the samples from different geographical areas are to be included to get a meaningful statistical average. In addition, a more thorough and systematic comparison of the various electrochemical sensors using the same set of samples is needed to assess and compare their sensitivity and specificity.

Even though multiple steps have been used to fabricate the electrochemical sensors, further improvement is necessary to simplify these approaches. Sensors have been fabricated based on the redox probe or indirectly detecting the SARS-CoV-2 in real samples. The biosensor and sensor responses can be recorded by the interaction of antibodies with N and S proteins as well as the redox probes in the presence of a virus. The reported sensors are commonly applied to individual targets such as IgG and IgM antibodies, N or S protein as well as nucleic acid to identify SARS-CoV-2. Simultaneously sensing both S and N proteins may play a significant role in specific and sensitive detection of SARS-CoV-2 and its variants. Another possibility is that the combined detection of nucleic acid, N or S protein and, IgG and IgM based sensors may be useful for the detection of SARS-CoV-2. However, IgG and IgM antibodies-based identification takes longer time to develop the antibodies after once the infection occurred. Therefore, it may not be a useful approach for the fast detection, but still it can be helpful to track SARS-CoV-2.

In any case, simultaneous determination of nucleic acid and N or S protein may be more valuable than individually detecting the SARS-CoV-2. Electrochemical sensors are based on the direct detection of SARS-CoV-2 in real samples, but some are very sensitive and selective for detecting SARS-CoV-2 (see Table 1). Certainly, in the near future, more effective and selective approaches are forthcoming for the detection of COVID-19 and its variants, which will not only provide better choices for the scientific community to monitor and early diagnosis of COVID-19, but also prepare the community in advance for any deadly viruses that may appear globally in the future.

Different types of metal-based nanoparticles, conducting polymers,

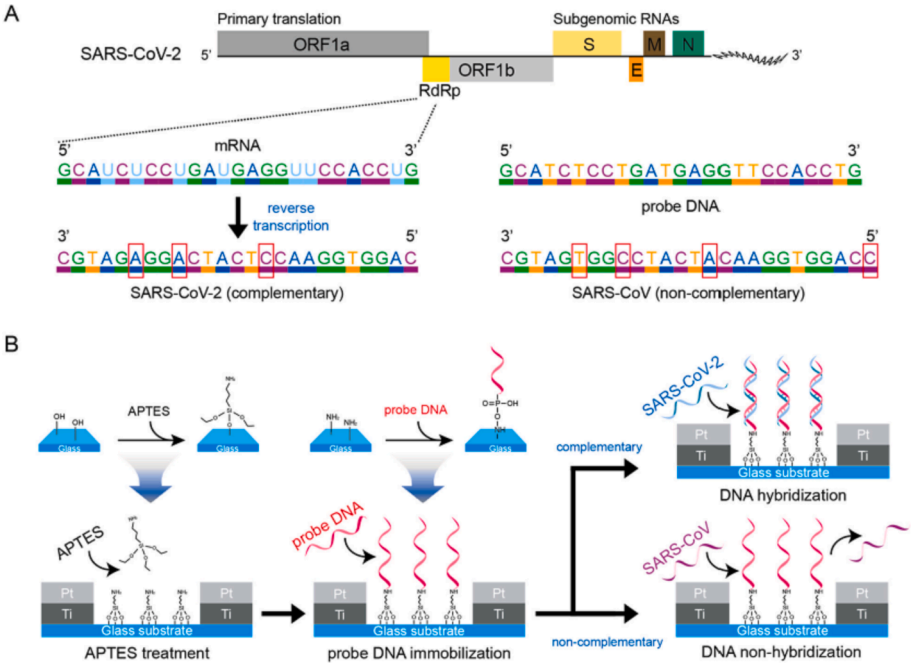

Fig. 11. Operating principles of IDE biosensor for SARS-CoV-2 cDNA detection. (A) Schematic of the sequence of probe DNA using specific mRNA sequences in SARS-CoV-2 virus gene and sequence of complementary DNA using reverse transcription sequence of SARS-CoV-2 mRNA; (B) Schematics showing surface construction process of APTES treatment followed by probe DNA immobilization and hybridization of the analyte DNA with probe DNA for SARS-CoV-2 cDNA detection. Reproduced with the permission from Ref. [98] Copyright 2021@ Elsevier.

Table 1
Recently developed electrochemical sensors for the detection of SARS-CoV-2.

Electrode/technique	Target	LOD	Linear range	Sensitivity	Ref.
Cotton-Tipped/SPE, SWV	N protein	0.8 pg/mL	1 – 1000 ng/mL	——	18
MIP/Au-TFE, DPV	N protein	111 fM	2–111 fM	———	19
RCA based biosensor	N gene and S gene	1 copy/μL	1 to 1×10^{-1} copies/μL^{-1}	0.1078 and 0.1076 (copy/μL)$^{-1}$	20
Graphene-ssDNA-AuNP/Au Electrode	N gene	6.9 copies/μL	—	231 (copies/μL)$^{-1}$	24
Co-TNTs	S-RBD protein	0.7 nM	14 – 1400 nM	———	37
rGO/3D printed 3D electrode	S1 protein and RBD antigen	2.8 fM and 16.9fM	—	1×10^{-12} M	38
PANI/GCE	N-gene	3.5 fM	10^{-14} – 10^{-9} M	——	39
G-Au NS,	IgG	0.18×10^{-19}%V/V	—	2.14 μA (%V/V)$^{-1}$	40
GO-Au NS/SPE, DPV	Viral glycoprotein	1.68×10^{-22}μg/mL	—	0.0048 μAμg.mL^{-1}.cm^{-2}	41
ePAD, SWV	IgG and IgM	0.96 ng/mL, 0.14 ng/mL	1 – 1000 ng/mL	———	67
ACE2-Pd-NTF electrode, EIS	S protein	—	—	——	78
Chronoamperomtery	S protein	4×10^{3} viral particle copies/mL	4×10^{3} – 4×10^{7} viral particle copies/mL	———	83
EIS	S protein	———	0.1 – 10 μg/mL	——	84
eCovSens/SPE, AuNPs/FTO	S antigen	90 fM, 120 fM	1 fM – 1 μM	——	85
Immunosensor, SWV	S protein	5.5×10^{5} PFU/mL	34.38×10^{3}, 13.75×10^{4} and 5.50×10^{5} PFL/mL	———	86
μPAD, EIS	IgG	0.4 pg/mL	10 – 1000 ng/mL	——	90
ECL Biosensor	RdRp gene	2.67 fM	1 fM – 100 pM	97.7 (pM/ECL intensity in a. u.)$^{-1}$	92
LSPR and PPT based biosensor	RNA sequences	0.22 pM	–	——	94
Electrochemical, DPV	RNA	26 fM	0.1 – 100 pM	0.5933 (pM/μA)$^{-1}$	99
Colorimetric sensor	cDNA	1 nM	–	——	100
Plasmonic metasensor	S protein	4.2 fM	—	———	101

carbon-based materials have been used to modify the electrode to boost the limit of detection to the femto molar level. Hence, the presence of nanomaterials, conducting polymers, composite of nanomaterials, and carbon-based materials onto the surface of the electrode can certainly enhance the sensitivity and limit of detection. Sensitivity of the electrode also can be improved by developing the microelectrodes and nanoelectrodes. The 3D printed electrode also can help to improve the properties of the electrodes. In any case, from the future view-point, more effective composite materials are needed that can play a significant role for the fabrication of more selective and sensitive sensors for the detection of deadly viruses.

6. Miscellaneous systems

6.1. Other sensors developed for the detection of SARS-CoV-2

Several miscellaneous systems have been developed in a short course of time to tackle the issue of detecting SARS-CoV-2. One most recent study by Do et al., [100] used a colorimetric sensor method for the determination of target molecule for fast and facile sensing that can be visible even to the naked eye. The detection specificity and sensitivity for short-length target genes is also the main challenge of this method. The authors developed a method based on the catalytic hairpin DNA assembly with ELISA-mimicking techniques for specific and sensitive colorimetric detection of short SARS-CoV-2 target complementary DNA (cDNA). The assay employed two types of catalytic hairpin DNA having a biotin at 5′ ends that can continuously produce di-biotinylated dimeric DNA probes via target cDNA-triggered recycling reactions. The dimeric DNA probe was specifically attached to a neutravidin-coated micro-plate well, which allowed the neutravidin-conjugated horseradish peroxidase via the biotin-neutravidin interactions, resulting in a selective and sensitive colorimetric detection (i.e., colorless to blue color change). This approach showed high sensitivity with a LOD of ~1 nM for the target cDNA.

In an alternative approach, Ahmadivand et al., [101] developed another method at the femtomolar level using a plasmonic meta sensor technology for COVID-19 detection, which used a miniaturized plasmonic sensor based on toroidal electrodynamic concept to observe a LOD of ~4.2 fM for detecting SARS-CoV-2. On the other hand, Li et al., [102] investigated SARS-CoV-2 antibody conjugated magnetic graphene quantum dots (GQDs)-based magnetic relaxation switch (MRSw) to specifically identify SARS-CoV-2. The MRSw-based system contained Gd^{3+} loaded polyethylene glycol (PEG) modified GQDs (GPG) and specific antibodies against SARS-CoV-2 antigen S protein. In this approach, a fast and closed-tube one step approach was used to detect SARS-CoV-2 pseudo virus in a home-made ultra-low field (ULF) nuclear magnetic resonance (NMR) system relaxometry working at 118 μT. The magnetic GQDs-based probe demonstrated an ultra-high sensitivity for the determination of SRAS-CoV-2 due to high magnetic relaxation and LOD was optimized to 248 particle/mL within a short detection time of 2 min.

Hashemi et al., [41] reported another electrochemical kit composed of fixed/SPE, which could detect SARS-CoV-2 or animal virus via distinguishable fingerprints of their viral glycoproteins at different voltages (Fig. 12). The sensor was triggered by coating a layer of coupled GO with sensitive chemical compounds along with gold nano-stars, which can identify the trace of viruses in saliva, blood and nasopharyngeal swab via interaction with the active functional groups of their glycoproteins. This method does not require any extraction of the biomarkers for the determination of target viruses, which can identify even the trace of different pathogenic viruses within one minute. The proposed sensor demonstrated an excellent sensitivity of 0.0045 μAμg/mL cm^2 with a LOD 1.68×10^{-22} μg/mL. Performance of the method was tested on 100 blind nasopharyngeal swab samples to confirm sensitivity/specificity of the sensor in clinical samples.

Rodriguez et al., [103] described a portable wireless electrochemical system for the ultra-fast analysis of COVID-19, the SARS-CoV-2 RapidPlex, which could detect IgM and IgG antibodies, viral antigen N protein, and inflammatory biomarkers C-reactive protein by using a mass-producible laser-etched graphene electrode. The SARS-CoV-2 RapidPlex platform was successfully used for the detection of COVI-19 negative saliva and blood samples. The advantage of this method is that it is convenient to use for the home testing of SARS-CoV-2 as well as in telemedicine diagnosis and monitoring.

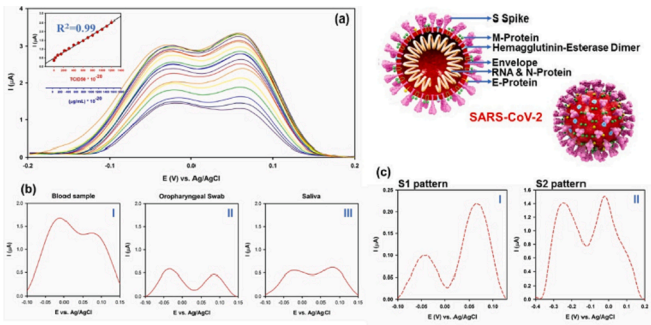

Fig. 12. (a) DPV pattern of SARS-CoV-2 virus in PBS (pH = 7.4) along with its respective calibration curve and structure of SARS-CoV-2 virus, (b) DPV pattern of SARS-CoV-2 in samples from (I) blood, (II) oropharyngeal swab and (III) saliva of infected person; and (c) obtained DPV patterns from (I) S1 and (II) S2 glycoproteins' antigen related to S spike glycoprotein of SARS-CoV-2 virus. Reproduced with the permission from Ref. [41] and with the permission Copyright 2021@ Elsevier.

6.1.1. *Rapid diagnostic test (RDT)*

Rapid Diagnostic Test (RDT) is the most commonly used test for antibodies (IgM and IgG) or viral antigen(s). These tests are typically applied to detect the presence of viral protein (antigens) conveyed by COVID-19. The RDTs are based on qualitative (negative or positive) lateral flow tests, which are portable, small, and can be applied at the point-of-care using the saliva samples, finger prick or nasal swab fluids similar to pregnancy tests, indicating the user colored lines for positive or negative results. If the target antigen is present in enough quantity in a specimen, it will bind to particular antibodies to generate a signal, typically within 30 min. The detection of antigen(s) is conveyed only if the virus is actively replicating; these tests are best used to identify the early or acute infection. However, WHO did not recommend RDTs due to lack of data, but suggested to improve their usefulness in epidemiologic research and disease surveillance [104–105].

6.1.2. *Enzyme-linked immuno-sorbent assay (ELISA)*

Enzyme-Linked Immuno-Sorbent Assay (ELISA) is a lab-based test that can be used for quantitative or qualitative purposes. These tests contain plastic plates coated with viral protein of interest such as spike protein using the whole blood, serum or plasma samples of the patients. More specifically, patient's specimen is incubated with spiked protein and if the patient has antibodies to the viral proteins they bind together. The virus infected person would make antibodies against the spike protein and ELISA can be a helpful tool to identify how many antibodies in the patient sample bind to the viral protein. In case of COVID-19, these most commonly test the patient's antibodies (IgM or IgG). A recent technique to identify COVID-19 is based on quantitative polymerase chain reaction (qPCR), which can detect viral nucleic acid if present in sufficient quantity. However, it can lead to false-negative results and failure in case of quarantined patients, where the infected patient would be a major setback for viral transmission [104,16,106–107]. ELISA assay was performed for the detection of antibodies IgG/IgM to identify SARS-CoV-2 [104]. This test was applied for 63 samples of COVID-19 patients, and the results demonstrated that 28 IgM antibodies were positive with specificity, sensitivity and accuracy of 100% (35/35), 44.4% (28/63) and (63/98) 64.3, respectively. The IgG antibodies were positive with specificity, sensitivity and accuracy of 100% (35/35), 82.54% (52/63) and 87/98 (88.8), respectively. The sensitivity of the combined IgG and IgM detection was 87.3% (55/63).

In a study by Guo et al., [106], ELISA was proposed for SARS-CoV-2 related antibodies, including IgA, IgG and IgM, based on the recombinant viral N protein. The average duration of IgA and IgM antibody detection was 5 (IQR, 3–6) days, while IgG was detected in 14 (IQR, 10–18) days after the onset of the symptom with positive rates of 92.7%, 85.4% and 77.9%, respectively, while the positivity rates of IgM antibodies were 93.1% and 75.6, respectively in case of the confirmed cases. Detection efficiency by IgM antibody via ELISA was higher than qPCR after 5.5 days of the onset of the symptom, but when IgM ELISA assay was combined with PCR, positive detection rate increased to 98.6% in comparison to qPCR tests (51.9%).

6.1.3. *Smell dysfunction*

The quantitative smell testing indicates the decrement in human smell function, but not always anosmia, which is considered as the major marker for COVID-19 infection. Studies have suggested that smell testing could help to identify SARS-CoV-2 infection in case of early cure or quarantine. The otorhinolaryngology authorities have advised that loss of taste and smell with the presence of other symptoms can be a strong predictor of COVID-19 infection [12]. Abalo-Lojo et al., [108] examined the extent of taste and smell dysfunctions for COVID-19 patients and the study was carried out to know the taste dysfunction and anosmia in 131 COVID-19 positive patients (57.4% female, 42.6% male, average age of 50.4 years). Taste disorders were observed in 74 patients and completely loss of test in (75.7%, n = 56). Related to smell, patients were not able to identify their smelling problem. Among the 77 COVID-19 patients tested with anosmia, total recovery occurred in 40.3% (n = 31) of the cases, where the smell problem was 59.7% (n = 46) [108–109]. The smell and test dysfunction is thus a common symptom in SARS-CoV-2 that can be present mostly in non-hospitalized and young patients [110]. The SARS-CoV-2 is associated with olfactory dysfunction in many patients occurs at the early self-isolation and testing for COVID-19 [111].

6.1.4. *Artificial intelligence (AI)*

Artificial intelligence (AI)-based sensor framework was developed for the diagnosis of COVID-19 and these methods are based on the analysis of symptoms using the smartphone embedded sensor. To perform this, symptoms of the confirmed COVID-19 patient should be recognized. The well-defined symptoms of SARS-CoV-2 infection are fatigue, headache, dry cough, fever, difficulty in breathing, and lung CT imaging features. These symptoms are different from the other common diseases such as cold, flu, and heavy fever. Hence, the level of these diseases can be determined using the onboard sensor measurements. A set of sensor technologies are embedded into smartphones like cameras, microphones, inertial sensors, and temperature sensors to analyze the symptoms of the disease. The developed platform contains four separate layers, which are 'input/reading sensors' measurement, sensor configuration, computing symptoms disease and predict the disease using the combined machine learning approach [10].

Pirouz et al., [11] proposed the binary classified modeling using a group method of data handling (GMDH) algorithm for monitoring COVID-19, which is a type of artificial intelligence method. For the case study, Hubei province of China was selected to execute the development of this method, and some significant factors such as density of the city, namely minimum, maximum, and average daily temperature, wind speed, and relative humidity were considered as the input dataset, and the number of confirmed cases were selected as the output dataset for 30 days. The developed binary classification model showed higher performance capacity in the prediction of confirmed cases. Furthermore, to clarify the trend of the confirmed cases, regression analysis was performed by comparing the fluctuations of daily weather conditions (humidity, wind and average temperature). The observed results indicated that maximum daily temperature and relative humidity showed the highest impact on the confirmed cases. In the case study, relative humidity with an average 77.9% affected positively and maximum daily temperature (with an average of 15.4 °C) negatively affected the confirmed cases. Furthermore, the study demonstrated positive effect of quarantine in reducing the number of confirmed cases, which was effective after about 14 days. The proposed analysis showed the effect of environmental conditions on the confirmed COVID-19 cases, still more data sets are suggested for future studies to develop the accuracy of the

predictive models.

7. Conclusions

The present review is an overview of the most recently published electrochemical sensor techniques for the selective detection of SARS-CoV-2 from the patients' samples using a variety of targets. In these methods, mainly S or N protein, nucleic acid, IgG and IgM have been used to develop sensors as well as biosensors. The techniques are discussed depending on the sensing accuracy and reliability of the targets specific for the detection of SARS-CoV-2. In addition, various other miscellaneous approaches such as colorimetric detection, NMR, RapidPlex platform, RDT, ELISA, smell dysfunction and artificial intelligence deployed to diagnose and track the progression of COVID-19 are also discussed.

Generally, RT-PCR tests have been widely accepted as they can offer many advantages making them life-saving diagnostics techniques, but due to their high costs, time-consuming process, requiring multiple steps and need for highly skilled personnel, these may not be suitable for large-scale monitoring of the multiple samples. In recent times, electrochemical sensor-based methods have been developed that are fast, economical, sensitive and selective for the detection of SARS-CoV-2. It is evident that neither serological and RT-PCR assay nor electrochemical detection of SARS-CoV-2 sensors/biosensors is the perfect system for COVID-19 identification, but these methods can be considered complementary to each other. There is much promise that electrochemical sensors/biosensors can offer improved solutions towards the fast and low-cost point-of-care assays for the diagnosis of the deadly SARS-CoV-2 virus. In this regard, more attention and developments are needed for sensitive and selective analysis of the clinical samples to serve mankind in the difficult situation that the world is facing.

Au-TFE: gold-based thin-film electrodes; SPE: Screen-printed electrode; S-RBD: Spike- receptor binding domain; N: Nucleocapsid; RCA: Rolling circle amplification; Co-TNTs: Cobalt-functionalized TiO2 nanotubes; 3D printed 3D electrode; rGO: reduced graphene oxide; in-house built device (eCovSens); FTO: fluorine doped tin oxide; AuNPs: gold nanoparticles; ACE2: angiotensin-converting enzyme 2; Pd-NTF: coated palladium nano-thin film; PANI: poly-aniline; GCE: glassy carbon electrode; ePAD: electrochemical paper-based analytical device; μPAD: microfluidic paper-based analytical device; ECL: entropy-driven amplified electrochemiluminescence; LSPR: localized surface plasmon resonance; PPT: plasmonic photothermal (PPT) sensing; cDNA: complementary DNA; G-Au NS:activated graphene oxide – conjunction with Au nanostars.

Declaration of Competing Interest

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

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