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Review Article

A review on the recent achievements on coronaviruses recognition using electrochemical detection methods

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ABSTRACT

Various coronaviruses, which cause a wide range of human and animal diseases, have emerged in the past 50 years. This may be due to their abilities to recombine, mutate, and infect multiple species and cell types. A novel coronavirus, which is a family of severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), has been termed COVID-19 by the World Health Organization (WHO). COVID-19 is the strain that has not been previously identified in humans. The early identification and diagnosis of the virus is crucial for effective pandemic prevention. In this study, we review shortly various diagnostic methods for virus assay and focus on recent advances in electrochemical biosensors for COVID-19 detection.

1. Introduction

Human coronaviruses (HCoVs) represent a major group of coronaviruses (CoVs) associated with multiple respiratory diseases of varying severity, including the common cold, pneumonia and bronchiolitis [1]. Since CoVs have inherently high mutation rates and high frequency of recombination, they manifest rapid adaptation to new host receptors with the ability to overcome interspecies barriers. HCoVs are globally distributed and the predominant species has diversity by region or year and may infect humans and a wide variety of animals. HCoVs are enveloped RNA viruses including the strains respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU1. In the past 14 years, the onsets of SARS-CoV and MERS-CoV have thrust HCoVs into the spotlight of the research community due to their high pathogenicity in humans. Today, HCoVs are recognized as one of the most rapidly evolving viruses owing to its high genomic nucleotide substitution rates and recombination [2–4].

The outbreak in Wuhan, China, first documented in December 2019, represents a beta coronavirus classified as novel severe acute respiratory syndrome corona virus-2 (SARS-CoV-2), known as COVID-19, which belongs to the Coronaviridae family. SARS-CoV-2 is a spherical

enveloped particle comprising a single positive stranded RNA associated with a nucleoprotein within a capsid of matrix protein [5]. Novel SARS-CoV-2 represents a significant similarity with previous coronaviruses such as SARS-CoV in 2002, China and MERS-CoV in 2015, Middle East [6].

The most conventional detection methods for COVID-19 include enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR) and reverse-transcriptase polymerase chain reaction (RT-PCR). Based on the recommendation of the WHO and the American Center for Disease Control (ACDC), RT-PCR, in comparison with traditional PCR, is the unique standard for recognition of COVID-19 [7,8].

ELISA is a biochemical assay that uses antibodies and an enzyme-mediated color change to detect the presence of either antigen (proteins, peptides, hormones, etc.) or antibody in a given sample [9,10]. PCR is an assay to detect genetic material from a specific organism, such as a virus. It can recognize the presence of a virus if the virus exists at the time of the test; also detect fragments of the virus even after the infectious symptom disappears. PCR has revolutionized the rapid analysis of mammalian genomic DNA [11,12].

Real-time reverse transcriptase-polymerase chain reaction (RT-PCR) is a laboratory technique used to make many copies of a specific genetic sequence for analysis. The enzyme of reverse transcriptase is used to

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change a specific piece of RNA into a matching piece of DNA. After amplification of this piece of DNA (made in large numbers) by the enzyme of DNA polymerase, it can tell whether a specific mRNA molecule is being made by a gene. RT-PCR is useful to look for certain changes in a gene or chromosome or for activation of certain genes, which may help diagnose a disease and monitor an infection [13,14]. In the standard PCR, the DNA template to be amplified and a thermocycler were used, while, in RT-PCR, RNA is used as a template and is reverse transcribed into complementary DNA. PCR is used for viruses that already contain DNA for amplification, while RT-PCR is used for those containing RNA that needs to be transcribed to DNA for amplification. Since the SARS-CoV-2 virus only contains RNA, RT-PCR is used to detect it. There are several published papers over viewing above mentioned techniques [15–20].

These detection methods suffer from some inconveniences such as the need for qualified personnel and the use of large quantities of costly reagents. Moreover, these conventional tests are complicated and unsuitable for large-scale diagnosis. Even with the high sensitivity of the methods, some research papers have exhibited that they may result in false negative responses. It should be noted that a diagnostic procedure sensitivity and limit of detection (LOD) are related to the SARS-CoV-2 virus minimum viral load and infective virus dose, which is a difficult issue for a diagnostic platform [21,22]. Usually RT-PCR on nasopharyngeal samples, the standard method for COVID-19, demonstrates a LOD of ~ 100 copies of viral RNA per milliliter of transport media. However, LODs of other techniques vary over 10,000-fold [23,24].

Electrochemical detection methods are the analytical platforms that can detect parameters like voltage, current, resistance, or charge generated from a reaction at the surface of an electrode [25]. Advantages of the electrochemical based assay like ease-of-use, instant detection, cost-effectiveness and fast response are helpful to be considered viable advanced systems that can meet the demand for simple detection method to diagnose COVID-19 [26]. On the other hand, the analytical performance of electrochemical diagnosis methods has improved with nanostructure materials. Nanomaterials generate successful interactions between the analyte and the sensor because of their large surface-to-volume ratios that lead to the fast and accurate virus detection [27–30].

In this study, we summarize conventional identification methods for viruses and current diagnosis techniques for COVID-19; then review recent achievements and application of electrochemical biosensors and

nanomaterials on COVID-19 detection.

2. Virus

Approximately 10^{31} viruses exist on Earth that infects practically all organisms. These obligate parasites are a major cause of human suffering and economic loss. The genetic materials of the viruses contain the encoded biological information of the virus and are built from either DNA or RNA. Viruses are able to mutate and adapt to different viruses. The mutation causes an increase in genetic diversity [31,32].

Viruses are divided into four groups including: a) riboviruses (which includes RNA viruses), b) retroviruses (RNA/DNA/RNA), c) DNA viruses (DNA/DNA) [33] and d) (DNA/RNA/DNA), which contains viruses that produce RNA as a replicative intermediate despite having DNA as their genetic material [34].

Coronaviruses (CoVs) (Fig. 1), which are called for the crown-like spikes on their surface have been recognized by Dr J. Almeida in 1964 at her laboratory in St Thomas's Hospital in London [35,36]. Coronaviruses are large, enveloped, spherical shapes, with a diameter of 80–120 nm, non-segmented and single positive stranded RNA viruses. There are presently seven CoVs known to cause human illness, which can be classified as low pathogenic or highly pathogenic [37–39].

3. Conventional identification assays for viruses

Virus identification is the most important point in the control of the disease. The early determination of pathogenic agents like bacteria and viruses is crucial for clinical point-of-care purposes [40]. For diagnosing viral infections, the first step is viral culture. A variety of specimens such as swabs, nasal swabs, nasopharynx or trachea extracts, sputum or lung tissue, blood, and feces should be retained for testing in a timely manner, which gives a higher rate of positive detection of lower respiratory tract specimens.

There are several immunological methods that are used to detect COVID-19. Immunofluorescence assay, protein microarray, direct fluorescent antibody assay, nucleocapsid protein detection, and the micro neutralization test are easy to operate rapidly but have a lower sensitivity and specificity [41,42]. Preliminary identification of the virus was done through the classical Koch's Postulates or observing its morphology through an electron microscopy [43]. RT-PCR, ELISA, isothermal nucleic acid amplification methods referred to as the loop-

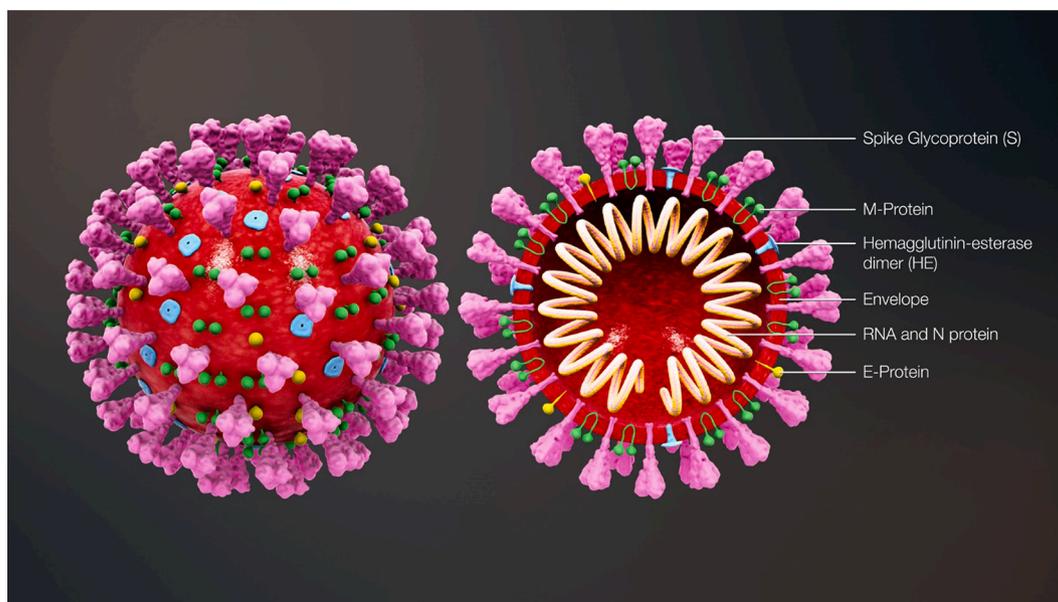


Fig. 1. The structure of Coronavirus [39].

mediated isothermal amplification technique (LAMP) that is performed at a constant temperature and microarray-based methods have been used so far [44–46].

4. Biosensors

A biosensor is an analytical device that measures biological reactions by producing signals proportional to the concentration of a substrate in the solution. Applications of biosensors include disease monitoring and drug discovery, recognition of disease-causing microorganisms and markers that are indicators of a disease in bodily fluids. Biosensors are classified two ways. The first type is based on biological signaling compounds or biorecognition elements. The second way is by the signal transduction methods.

The biological signaling compounds, known as bioreceptors, are used because of their interactions with a specific target. On the basis of biorecognition principle, biosensors can be classified into several sub-categories: antibody/antigen, enzyme catalyze, nucleic acid, cell-based and biomimetic molecules such as peptide nucleic acid (PNA) and affibody.

Transducers are the transformers of the produced signal by the interaction of the specific analytes that can be more easily measured and quantified. In this way, biosensors can be further classified into optical [47,48], electrochemical [49–54], piezoelectric [55,56], magnetic [57], micromechanical [58], and thermal [59,60] for medical diagnosis.

5. Electrochemical biosensors for virus detection

An electrochemical biosensor is a self-contained integrated device (Fig. 2) [61], capable of providing specific quantitative or semi-quantitative analytical information using a biochemical receptor. The biochemical receptor is kept in direct spatial contact with an electrochemical transduction element [62–69]. Biosensors with an electrochemical transducer have advantages such as high sensitivity, simple

instrumentation, cost effectiveness and capability of miniaturization, which is used for microliter sample volume. There are several reports of electrochemical transducers being utilized in order to detect viruses.

Ilkhani et al. reported an electrochemical biosensor for Ebola virus DNA distinguishing features by an enzyme-amplified detection [70]. They labeled the biotinylated hybrid with a streptavidin–alkaline phosphatase conjugate. All the experiment steps are optimized by using electrochemical impedance spectroscopy.

Afsahi et al. fabricated a beneficial and portable graphene-enabled biosensor for Zika virus detection with a highly specific immobilized monoclonal antibody [71]. They covalently attached monoclonal antibodies to graphene for native Zika viral antigens detection.

Moço et al. presented an electrochemical genosensor for detection of the genomic RNA of Zika virus, which has been used on real samples from infected patients. Modified graphite electrode is prepared by electrochemical reduced graphene oxide (rGO) and polytyramine, which plays the role of a conducting polymer [72].

Navakul et al. published a paper for the detection, classification and antibody screening of dengue virus based on electrochemical impedance spectroscopy. The charge transfer resistance of a gold electrode coated with graphene oxide reinforced polymer was influenced by virus type and quantity exposed on the surface [73].

6. Nanomaterials based electrochemical biosensors for virus detection

The analytical performance of biosensors has improved with nanotechnology methods. Nanomaterials (NMs) bring new possibilities for the extension of electrochemical biosensors [74]. Incorporation of NMs with promising novel approaches in biosensor design provides construction of biosensors and development of novel electrochemical assays [75–78]. The advanced nanoscale biosensor can be utilized to achieve high sensitivity and selectivity of biological sensing for analytical purposes in various fields of research and technology [79–82]. NMs have

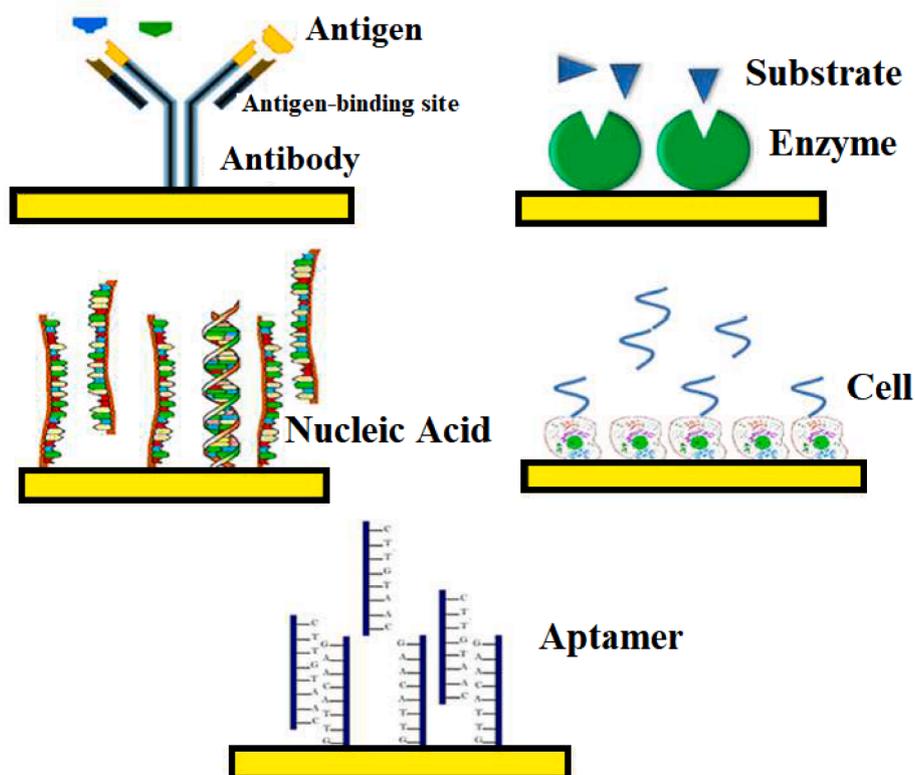


Fig. 2. Different methods of biosensing with various biological sample: antibody/antigen; enzyme catalyze; nucleic acid; cell-based; and aptamer as biomimetic [61].

great potential due to its promoting electron transfer reactions, high surface area and electrical conductivity, good chemical stability, and mechanical robustness. Moreover, they can be used to enhance electrochemical reactions and promote signals of biorecognition systems [83,84].

Various NMs, such as magnetic nanoparticles, metal NPs, carbon-based nanotubes, carbon allotropes, nanowire, and quantum dots with different biological recognition elements (enzymes, nucleic acids, antibodies, antigens, peptide), provide many opportunities for enhancing the performance of nanobiosensor [85,86]. The electrochemical nanobiosensors were used in versatile areas of cancer diagnostics and diagnosis of infectious microorganisms, viruses, etc. [87,88].

For example, Sayhi et al. produced a method with the purpose of isolation and identification of influenza A virus H9N2 subtype. They attached an anti-matrix protein 2 antibody to iron magnetic nanoparticles and used them for isolating the influenza virus from an allantoic fluid. Then, fetuin A was attached to an electrochemically detectable label, gold nanoparticles, to detect the virus taking advantage from fetuin-hemagglutinin interaction.

Immobilization of the specific receptor was not necessary in this study. It was the most important point because usually this step was time consuming. Also, reproducibility and regeneration ability were benefits of the proposed research [89].

In another study, Layqah et al. developed an immunosensor for the determination of MERS-CoV based on a competitive assay on an array of electrodes nanostructured with gold nanoparticles to enable the multiplexed detection of different CoV. The base of biosensor was in indirect competition between the free virus in the sample and immobilized MERS-CoV protein with a fixed concentration of added antibody to the sample. The reduction peak current of ferro/ferricyanide redox couple at each step was measured. The assay was performed in 20 min with detection limit as low as 0.4 and 1.0 pg mL⁻¹ for HCoV and MERS-CoV, respectively [90].

In addition to this, Nagar et al. developed an impedimetric sensing platform for detection of changes in Coxsackie B3 virus-specific ssDNA target by altering the electrode with graphene/gold nanoparticles composite. The biorecognition agent and ssDNA were mixed and stabilized on a substrate. Reduced graphene oxide was used as a more conductive substrate for gold nano particle deposition to aid in the immobilization of probe ssDNA suitable for hybridization with target ssDNA. The linear response was in the range of concentrations 0.01–20 μM [91].

7. Current diagnosis techniques of COVID-19

The diagnosis of COVID-19 mainly relies on the detection of the coronavirus RNA. The various methods for identification of COVID-19 are stated:

7.1. RT-PCR

PCR has become a routine and reliable technique with great specificity and sensitivity. RT-PCR detection is currently applied for the diagnosis of coronaviruses. RT-PCR assay as a predominant method has advantages consisting of specific, and simple quantitative. The mutating nature of coronaviruses highlights the requirement for accurate detection of genetically diverse coronaviruses. Hence, to recuperate the ability to detect coronavirus exactly and reduce the risk of evoking false-negative results caused by genome sequence variations, researchers have established multiplex real-time RT-PCR methods with desirable sensitivity for multitarget detection of coronavirus [92,93].

7.2. Isothermal nucleic acid amplification-based methods

A novel isothermal nucleic acid amplification method is a low-cost alternative to detect certain diseases. It is a single-tube technique for

the amplification of DNA; called loop mediated isothermal amplification (LAMP). Isothermal amplification is performed at a constant temperature and does not require a thermal cycler. This method is classified into various categories such as regular LAMP-based methods, sequence-specific LAMP-based methods and rolling circle amplification-based methods. The principle of detection is based on the templates designed to target SARS-CoV-2 RNA that amplify a unique region of the segment. Fluorescently-labeled molecular beacons are used to specifically identify each of the amplified RNA targets [94,95].

7.3. Microarray-based methods

The microarray is a detection method with fast and high throughput that can handle a high amount of material or items passing through the system. However, the high-cost limits its further application in the detection of coronavirus. In this procedure, complementary DNA (cDNA) labeled with specific probes through reverse is first produced by the coronavirus RNA transcription. These labeled cDNAs will be loaded into each well and hybridize with solid phase oligonucleotides firm on the microarray followed by a series of washing steps to eliminate free DNAs. Finally, the coronavirus RNA can be diagnosed by the detection of specific probes. Owing to its privilege, the microarray assay has been broadly used in the detection of coronavirus [96,97].

7.4. Clinical diagnosis

Symptoms of COVID-19 can include but are not limited to a fever, dry cough, myalgia, fatigue, hemoptysis and diarrhea. Less common symptoms include headaches, nausea and delirium. The majority of patients exhibit signs of pneumonia [98].

7.4.1. Physical examination

Preliminary data regarding a plausible infection can be provided by merely observing and evaluation of vital signs. Since being hypoxic is common in initial steps, a pulse oximeter is useful. The beginning of respiratory distress may be indicated by tachypnea, retractions, diaphoresis and lung sounds. Fine crackles in early pneumonia, then coarse rales and diffuse rhonchi can be heard as the disease progresses [99,100].

7.4.2. CT imaging examination

Chest computed tomography (CT) scan is an important instrument to distinguish COVID-19 suspects because it has a potential role for prognostic disease at an early stage. However, COVID-19 patients may exhibit no pneumonia on thin-section CT.

CT scans found that lesions in patients with COVID-19 were commonly located in the lower lobes of the lungs, and showed subpleural distribution. All patients showed ground-glass opacity (GGO) on thin-section CT, more than half of which were round GGO. 89.36% of the patients show the crazy-paving pattern, and air bronchogram can develop in GGO. Therefore, CT examination is important for confirmation to diagnose COVID-19 (Fig. 3) [101,102].

7.4.3. Chest X-ray

X-Ray machines are readily available in healthcare systems. However, an x-ray can only show the presence of an infection, not the virus itself; X-Ray machines may be helpful to prioritize the selection of patients for further testing when other available methods of confirming COVID-19 cases may not be available. X-Ray machines have the added benefits of portability, smaller size and can help to reduce infection rates caused by CT. X-Rays are useful to follow up lung abnormalities that can be caused by SARS-CoV-2 infections [103,104].

8. Electrochemical detection methods of COVID-19

Electrochemical transducers are used in the majority of the

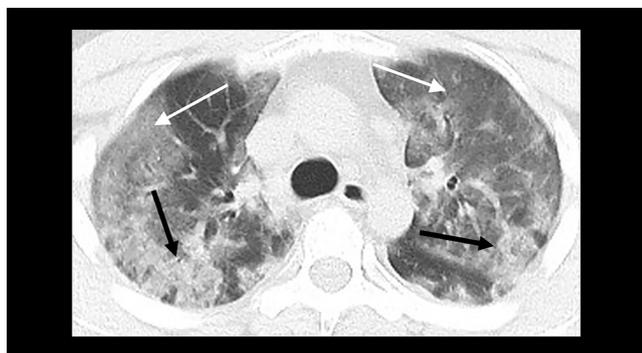


Fig. 3. Images in a 29-year-old man with unknown exposure history who presented with fever and cough ultimately requiring admission to intensive care unit. (a) Axial thin-section unenhanced CT scan shows diffuse bilateral confluent and patchy ground-glass (white arrows) and consolidative (black arrows) pulmonary opacities. (b) Axial unenhanced image shows that the disease in the right middle and lower lobes has a striking peripheral distribution (arrows) [102].

biosensors because they are simple to manufacture, require minimal reaction volumes and do not have ambient interferences such as red blood cells affecting the quantification. The measurement of current, charge buildup, or modification of electrode conduction induced by a reaction is the basis for creating a signal via an electrochemical transducer. Electrochemical detection methods may be classified into three groups including: a) amperometric or voltammetric (current is determined), b) impedance biosensors (conduction or resistance is determined) and c) potentiometric ones (potential is determined) based on the measured assay [105–109]. Table 1 summarized the notable reported articles for detection of SARS-CoV-2 via various electrochemical methods.

8.1. Amperometric or voltammetric based detection

A steady voltage is provided to the working electrode in this detecting apparatus, causing an electrochemical oxidation or reduction that delivers a current. The generated current is corresponding to the target analyte concentration [110].

An electrochemical immunoassay in rapid analysis that was used for detecting COVID-19 was reported by Fabiani et al. Magnetic beads were applied as a support of an immunological chain and a secondary antibody with alkaline phosphatase was used as an immunological label for spike (S) protein and/or Nucleocapsid (N) protein detection. The enzymatic byproduct α -naphthol was found using screen printed electrodes modified with carbon black nanomaterials. Saliva was used by simply adding the sputum in the tube previously loaded with the reagents needed for the measurement, without requiring an extra task to the end-users. The analytical features of the electrochemical immunoassay were evaluated using the standard solution of S and N protein in buffer solution and untreated saliva with a detection limit equal to 19 ng mL^{-1} and 8 ng mL^{-1} in untreated saliva, respectively for S and N protein [111].

Zhao et al. developed an ultrasensitive electrochemical detection technology using calixarene functionalized graphene oxide for targeting RNA of SARS-CoV-2. They reported the sandwich-type biosensor equipped with a Smartphone, providing a simple, low-cost and useful method for point of care testing, through the following procedures: i) The capture probes (CP) labeled with thiol were immobilized on the surfaces of the $\text{Au@Fe}_3\text{O}_4$ nanoparticles and formed CP/ $\text{Au@Fe}_3\text{O}_4$ nanocomposites; ii) the host guest complexes *p*-sulfocalix arene (SCX8) to absorb toluidine blue (TB), (SCX8-TB) for SARS-CoV-2 RNA detection were immobilized on RGO to form Au@SCX8-TB-RGO-LP bioconjugate; iii) the sandwich structure of “CP target- label probed (LP)” produced; and iv) auxiliary probe (AP) was introduced to form long concatemers

Table 1

Summary of reported articles for detection of COVID-19 using different electrochemical methods.

Electrochemical methods	Type of electrode	Targeted virus	LOD	Ref.
Voltammetry	screen-printed electrodes modified with carbon black nanomaterial	Spike and Nucleocapsid Protein and N proteinS	19 ng/mL and 8 ng/mL	[111]
	Au@SCX8-TB-RGO-LP	various clinical specimens without RNA amplification	200 copies/mL	[112]
	PAD, Embedded graphene oxide (GO)-EDC/NHS	SARS-CoV-2 IgG and IgM	0.96 and 0.14 ng/mL	[113]
	Screen-printed carbon electrode (SPCE)	N gene and S gene SARS-CoV-2	1 copy/ μL	[114]
	Steel tips modified with multiwall carbon nanotubes (MWCNTs)	SARS-CoV-2	–	[115]
	Gold nanoparticles electrodeposited on Ti surface (screen-printed electrode)	SARS-CoV-2	–	[116]
	gold electrode	SARA-CoV-2 nucleocapsid protein	8.33 pg mL^{-1}	[117]
	Screen printed gold electrode	SARS-CoV-2 spike antigen	1 pg/mL	[118]
	gold-based thin-film electrodes	SARS-CoV-2 nucleoprotein	15 fM	[119]
	Au thin film layer	N gene and RdRP gene of SARS-CoV-2	$3.925 \text{ fg}/\mu\text{L}$	[120]
Electrical impedance spectroscopy	Gold electrode	SARS-CoV-2 antibody	–	[122]
	Glass/Gold/3D-Screen-printed electrode	SARS-CoV-2/S1	$2.8 \times 10^{15} \text{ M}$	[123]
	3D-printed graphene/poly(lactic acid) (G/PLA)	COVID-19 recombinant protein (antigen)	$0.5 \pm 0.1 \mu\text{g}\cdot\text{mL}^{-1}$	[124]
Potentiometry	Gold screen-printed electrode (Au SPEs)	SARS-CoV-2 spike S1 protein	1 fg/mL	[126]
	Graphene	SARS-CoV-2 spike antibody	1 fg/mL	[127]

[112].

In another study, Yakoh et al. reported the use of a label-free, paper-based electrochemical platform that was used to target SARS-CoV-2 without the specific requirement of an antibody. In this study, an electrochemical paper-based analytical device for diagnosing COVID-19 had three parts: a working ePAD, a counter ePAD and a closing ePAD. The SARS-CoV-2 spike protein containing receptor-binding domain was immobilized on the hydrophilic paper zone of the working ePAD through embedded graphene oxide (GO)-EDC/NHS chemistry in order to capture incoming SARS-CoV-2 antibodies. The square wave voltammetry (SWV) technique was used as the diagnostic step. After immunocomplex formation, the SWV response was decreased. Also, to demonstrate the practicality of the sensor, real clinical serum samples were tested and the results were compared by a commercial ELISA test kit and its function satisfactory was proved [113].

An electrochemical biosensor based on multiplex rolling circle amplification (RCA) for the rapid detection of the N and S genes of SARS-CoV-2 from clinical samples was reported by Chaibun et al. They used sandwich hybridization of RCA amplicons with probes functionalized by redox-active labels and differential pulse voltammetry (DPV) as the detection method. For evaluation of the performance of the sensor, clinical samples were used and the results were compared with qRT-PCR

results. In the assay, they synthesized monodisperse silica microspheres, incorporating redox dye, methylene blue and acridine orange, onto the silica microspheres and conjugated with DNA. They immobilized the capture probe on magnetic beads and hybridized with a target for electrochemical detection [114].

Miripour et al. proposed a sensor for screening people for COVID-19. More than 97% of true positive patients were detected within 30 s. They used real-time diagnosis of reactive oxygen species (ROS) in sputum using electrochemical tracing. The electrochemical diagnostic system consists of an integrated portable automatic electrochemical readout board and a sensing disposable sensor as the main diagnostic part of the system. The structure of the sensor was based on carbon nanotubes on the tip of steel needles in the conformation of three electrodes. ROS related electrochemical cyclic voltammetry cathodic peak from the fresh sputum was monitored as the signal for patients were involved to COVID-19 [115].

Tripathy et al. proposed a label-free sensor for SARS-CoV-2 specific viral RNA/c-DNA detection based on electrodeposited gold nanoparticles as the transducing elements. The miniaturized device was developed on oxidized silicon substrates and used gold nanoparticles, electrodeposited onto a titanium surface, as the sensing electrode. The thiol modified probe attached to the gold sensing electrodes via gold-thiol self-assembly. After introduction of the target nucleotide onto the sensor, through the designated reaction chamber, it hybridizes with the complementary probe and is recorded using electrochemical techniques [116].

An electrochemical dual-aptamer biosensor was proposed by Tian et al. The affinity between the aptamers and proteins was the basis of the sandwich structure on the surface of the gold electrode. The hemin/G-quadruplex DNAzyme, horseradish peroxidase (HRP) and Au@Pt nanoparticles catalyzed the oxidation of hydroquinone with H_2O_2 , giving the electrochemical signal. DPV signals demonstrated that the synergistic catalysis of Au@Pt/metal organic frameworks, HRP and GQH DNAzyme, lead to the amplified performance of the nanoprobe. The aptamer-protein-nanoprobe sandwich electrochemical detection system had a wide linear range from 0.025 to 50 ng and great potential in the early diagnosis of COVID-19 [117].

In another research paper by Karakus and colleagues, a colorimetric and electrochemical detection of COVID-19 with a gold nanoparticle-based biosensor was reported. They stabilized SARS-CoV-2 spike antigen at the surface of gold nanoparticles (AuNP-mAb) and used it as the probe. The developed probe allowed visual detection (colorimetric) with a detection limit of 48 ng mL^{-1} and square wave voltammetry detection was applied using disposable screen printed gold electrodes with a detection limit of 1 pg mL^{-1} . Cross-reactivity with other viral proteins such as Influenza A, MERS-CoV and Streptococcus pneumonia was not detected in this method (Fig. 4) [118].

Raziq et al fabricated a portable molecularly imprinted polymers

(MIP) based electrochemical sensor for detection of SARS-CoV-2 using gold-based thin-film electrodes. The disposable sensor chip, a thin film electrode, interfaced with a MIP-endowed was connected with a portable potentiostat and the target analyte was recognized through DPV in the presence of a $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ as the redox probe. The imprinting approach helped the generation of macromolecular imprints situated close to the polymer film surface and the deposition of polymer with an appropriate film thickness that was essential to prevent the irreversible entrapment of the protein of SARS-CoV-2 Nucleocapsid and infeasibility of its removal during the subsequent washing out procedure [119].

A biosensor COVID-19 assay was created by Kim et al. A micro-electrode array biosensor was coupled with recombinase polymerase amplification (RPA) on a glass substrate using microfabrication. For on-chip RPA, a polydimethylsiloxane slab was prepared for a reaction chamber, and electrodes were coated with thiol-modified primers. The multi-microelectrode array allowed the detection of multiple target genes by differential pulse voltammetry. The outcomes were comparable to results obtained by gel electrophoresis without post-amplification purification [120].

8.2. Electrochemical impedance spectroscopy (EIS) based detection

The electrochemical impedance method works by sending an AC excitation signal to the appropriate electrode at a specified frequency. The characteristics of the conductivity and resistance of the electrode are determined in the following step by determining the in-phase and out-phase current responses [121]. The electron transfer may be assessed at high frequency values, whereas the mass transfer can be explored at low frequency values. EIS is utilized in label-free electrochemical biosensors for detection of the electrode surface coverage. The detecting procedure involved applying a predetermined potential, which resulted in a current flow and, as a result, an electron transfer. Since the target analyte binds to the bioreceptor, the electron transfer resistances at the electrode and at the bulk of the analyte interface are changed. The target concentration is proportional to the change in resistance of the electrode surface.

Rashed et al. reported on a label-free electrochemical identification of SARS-CoV-2 antibodies utilizing a commercially accessible impedance sensing device. The receptor binding domain (RBD) of SARS-CoV-2 spike protein was pre-coated on a 16-well plate with sensing electrodes. It was then tested using anti-SARS-CoV-2 monoclonal antibody samples. Spikes in impedance quantification might be distinguished from a negative control using the suggested platform. The detection procedure took place in less than five minutes and the platform solid foundation allowed for a simple and intuitive testing interface that could easily be compatible for usage in clinical systems. Measured impedance values were consistent when compared to standard ELISA test results showing a

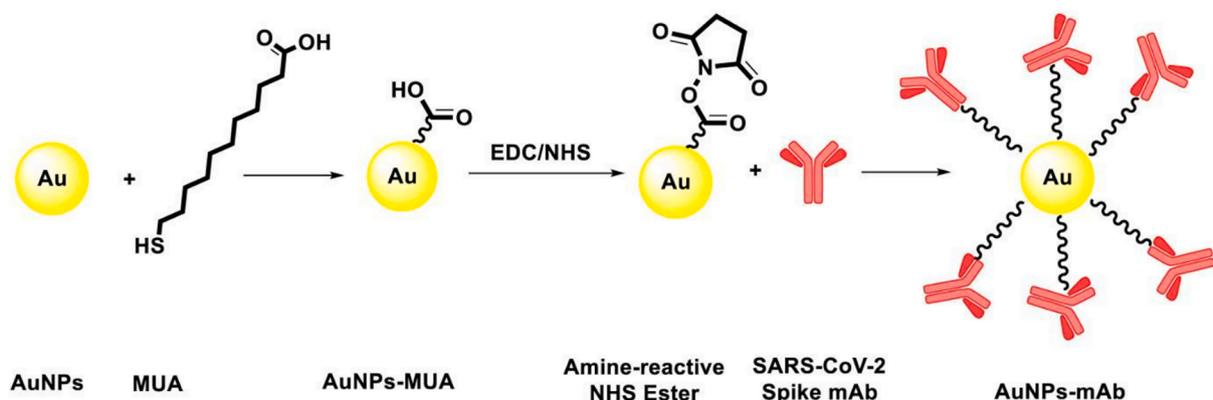


Fig. 4. Schematic illustration of the formation of AuNP-mAb conjugates [118].

strong correlation between them [122].

A rapid diagnosis biosensing platform based on electrochemical impedance spectroscopic measurements was presented by Ali et al. They created a 3D nanoprinting of three-dimensional electrodes, coated the electrodes by nanoflakes of rGO, and immobilized specific viral antigens on the rGO nanoflakes by EDC: NHS chemistry. It was integrated with a microfluidic device. The target antibody was recognized by an electrochemical transduction when antibodies were introduced in a fluid and formed an immunocomplex with antigens at the surface of the electrode. The sensor was regeneable via elution of antibody–antigen immunoaffinity using low pH chemistry, capable of connection to a Smartphone [123].

Munoz et al. constructed an immunosensor by covalently anchoring the SARS-CoV-2 recombinant protein on a 3D-printed graphene/poly-lactic acid (G/PLA) filament as the transducer. The impedimetric monitoring changes at the electrode/electrolyte interface after interacting with the monoclonal COVID-19 antibody via competitive assay as the base of the sensor. The proposed approach was general and could be easily customized to assess alternative antigen–antibody systems at 3D-printed electrodes (Fig. 5) [124].

8.3. Potentiometric detection

The sensor in this platform is made up of two reference electrodes mounted to a substrate, whose potential change between the electrodes is detected when a selective charged ion is reacted with the electrodes. The charged ion is selected from a chosen analyte that is generated by an enzyme in a target reaction. This process causes the membrane to have a potential difference before and after the charge ion is generated. The field effect transistors (FET) biosensors are based on potentiometry [125].

Mavrikou et al. presented a biosensor based on membrane-engineered mammalian cells bearing the human chimeric spike S1 antibody to detect the SARS-CoV-2 S1 spike protein expressed on the surface of the virus. The attachment of the protein to the membrane-bound antibodies resulted in a selective and considerable change in

the cellular bioelectric properties measured by means of a potentiometric detector. In this setup, an eight-channel gold screen-printed electrode assembly was connected to the potentiometer device and the measurement via the electric signal was visualized through a voltage vs. time. The biosensor provided results very fast (3 min), with a semi-linear range of response between 10 fg and 1 $\mu\text{g mL}^{-1}$, without the cross-reactivity against the SARS-CoV-2 Nucleocapsid protein [126].

In another study presented by Seo et al. a FET device was reported for detection of SARS-CoV-2 in clinical samples. SARS-CoV-2 spike antibody was immobilized at the surface of electrode through 1-pyrenebutyric acid and N-hydroxysuccinimide ester, an efficient interface coupling agent used as a probe linker and graphene, with a 2D layer of hexagonally arranged carbon atoms. The sensor could distinguish the SARS-CoV-2 antigen protein from those of MERS-CoV confirming the successful fabrication of a COVID-19 FET sensor based on integration of SARS-CoV-2 spike antibody with graphene (Fig. 6) [127].

9. Conclusion

The COVID-19 pandemic has thrust the need of highly accurate methods for the rapid identification of viruses such as SARS-CoV into the spotlight. The conventional detection methods like PCR, RT-PCR, and ELISA are complex and expensive assays. The development of simple, sensitive, accurate, and selective alternative platforms is essential in order to increase the efficiency of detection of viruses such as SARS-CoV-2.

Electrochemical biosensors are a highly promising technique due to the benefits of it being cheap, highly sensitive, quick, and easily accessible due to its portability. This review looks at recently reported electrochemical detection techniques that have improved responses to tackle the problems present in the detection of the SARS-CoV-2 virus.

Different suitable nanomaterials into electrochemical biosensors might enhance their effectiveness. The manufacturing methods, the architecture of the nanostructure (nanoparticles, nanotubes and nanosheets), the size of the nanoparticles, and how they are utilized in electrochemical biosensors as substrate, labels, or auxiliary components

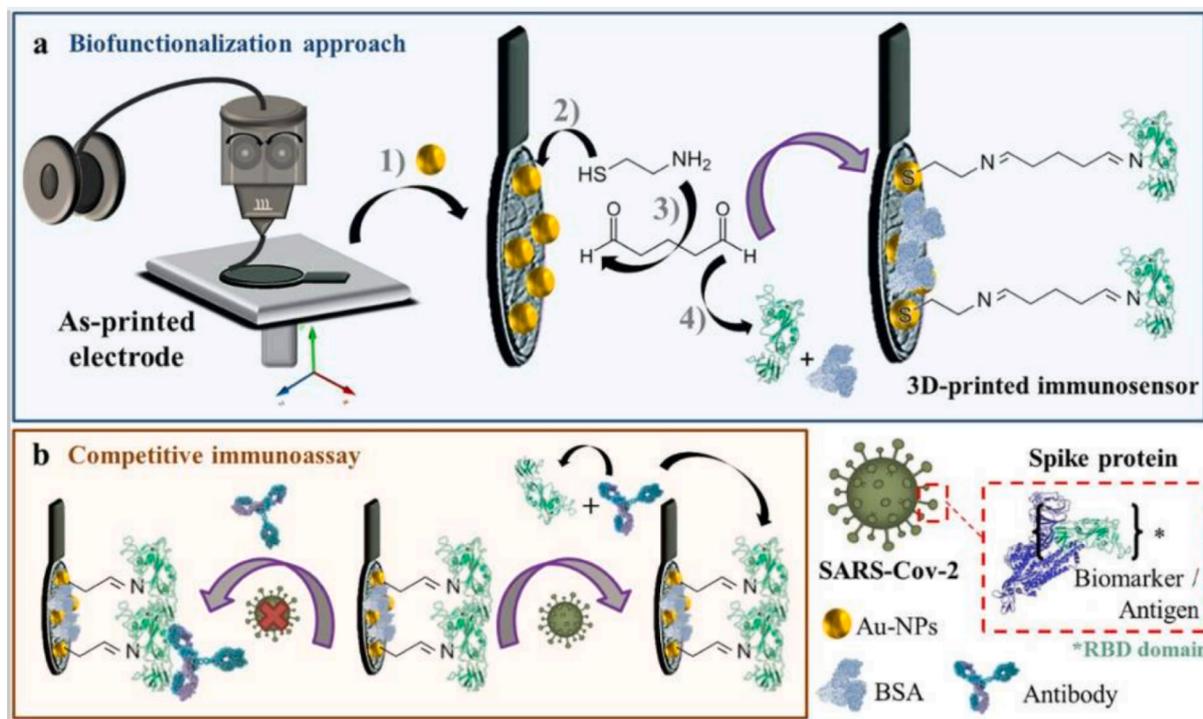


Fig. 5. a) Illustration of the 3D-printed electrochemical COVID-19 immunosensor fabrication steps. b) Indirect competitive assay carried out for detecting the COVID-19 recombinant protein (antigen), the one against the SARS-CoV-2 virus [124].

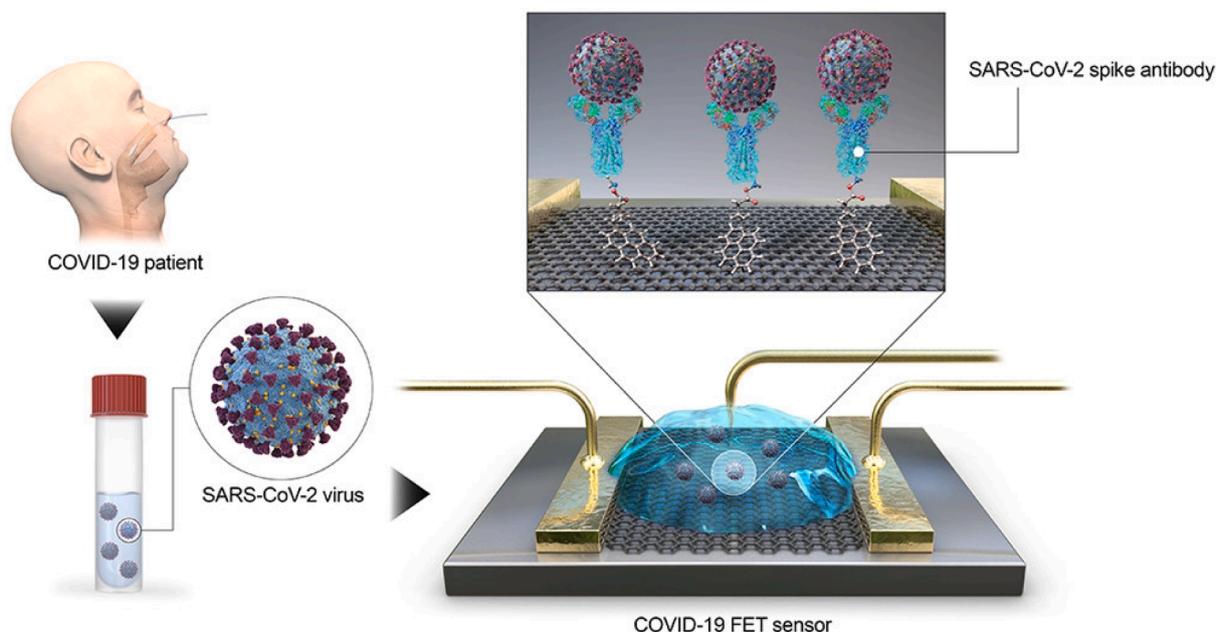


Fig. 6. Schematic diagram of COVID-19 FET sensor operation procedure. Graphene as a sensing material is selected, and SARS-CoV-2 spike antibody is conjugated onto the graphene sheet via 1-pyrenebutyric acid N-hydroxysuccinimide ester, which is an interfacing molecule as a probe linker [127].

for signal amplification all have an impact on their properties.

The purpose of this review paper is to bring attention to the vast uses and applications of electrochemical biosensors in the detection of viruses such as SARS-CoV-2. The literature studies revealed that differential pulse voltammetry, electrochemical impedance spectroscopy and potentiometry are the most often utilized electrochemical techniques among the applicable electrochemical methods and reported magnitude of LOD almost similar between them.

Hence, any diminutive tries for enhancement of the efficiency of the detection method, such as writing a review paper, will result in a better realization and development of next generation electrochemical biosensors. Consequently, more research is needed to evaluate the influence of electrochemical platforms in detecting SARS-CoV-2.

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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