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Review

Electrochemical nanobiosensors perspectives for COVID 19 pandemic

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Abstract

Early, rapid and ultrasensitive diagnosis of COVID-19 to facilitate high-throughput analysis without a high degree of technical expertise or sophisticated equipment is necessary to expand COVID-19 testing capability. Leveraging interdisciplinary proficiency in analytical chemistry, biomedical instrumentation, molecular biology, microfluidics, and nanotechnology, considerable advances have been made to develop a novel diagnostic tool that assures superior key performances for COVID-19 diagnosis. This review summarizes the nano-enabled systems such as electrochemical nanobiosensor for SARS-CoV-2 virus detection and emphasizes promising diagnostic techniques to extensively facilitate the diagnostic practices during the COVID-19 pandemic. Currently, three main diagnostic methods have been widely used in the COVID-19 pandemic: nucleic acid (NA)-based testing, computed tomography (CT), and serological testing. NA-based detection of SARS-CoV-2 such as Reverse transcription polymerase chain reaction has become the gold standard for COVID-19 diagnosis. This review congregates significant contributions in the electrochemical nanobiosensor research area, which is helpful for further nanobiosensor development. Although many efforts were taken to detect the SARS-CoV-2, the COVID 19 diagnosis still relies on expensive prolonged analysis. A rapid and reliable alternative is the utilization of a low-cost nanobiosensor for SARS-CoV-2 detection that can rapidly diagnose the disease even in asymptomatic conditions with high reliability and sensitivity.

Keywords

Nanomaterial; bio analytical method, RT PCR, portable biosensor.

List of abbreviations

AIV:	Avian influenza virus
ASSURED:	Affordable, Sensitive, Specific, User friendly, Robust and Rapid, Equipment-free, and deliverable to end-users
AuNP-LF:	Gold nanoparticle-based lateral-flow
CDC:	Center for Disease Control and Prevention

CHIKV:	Chikungunya virus
COVID-19:	Coronavirus Disease 2019
CRISPR:	Clustered Regularly Interspaced Short Palindromic Repeats
CT:	Computed tomography
DENV3:	Dengue
FET:	Field-effect transistor
HIV:	Human immunodeficiency virus
HPV-16:	Human Papillomavirus
HRP:	Horseradish peroxidase
EV 71:	Human enterovirus 71
JEV:	Japanese encephalitis virus
LFA:	Lateral flow assay
LSPR:	Localized surface plasmon resonance
mAb:	Monoclonal antibody
MERS-CoV:	Middle East respiratory syndrome coronavirus
NA:	Nucleic acid
N gene:	Nucleocapsid protein gene
NPs:	Nanoparticles
PCR:	Polymerase Chain Reaction
PEMS:	Piezoelectric microcantilever sensors
POCT:	Point-of-care testing
QCM:	Quartz crystal microbalance
RABV:	Rabies virus
RBD:	Receptor-binding domain
RdRP gene:	RNA-dependent RNA polymerase gene
RT PCR:	Reverse transcription–polymerase chain reaction
SARS-CoV-2:	Severe acute respiratory syndrome coronavirus 2
SERS:	Surface-enhanced Raman scattering
SPCE:	Screen-printed carbon electrode
VOC:	Volatile organic compounds
WE:	Working electrode
WHO:	World Health Organization

Introduction

Nanotechnology can improve the whole healthcare process; starting from diagnosis to treatment, prognosis, and discovery of drugs, and follow-up monitoring [1-4]. Nano-enabled systems provide two major advantages for diagnosis: rapid testing and early detection [5-10]. Nanobiosensor have shown tremendous outcomes in literature in the past 20 years with a large number of research articles entitled early detection, reliable, accurate, disposable, ultrasensitive and affordable biosensors for diagnosis of various diseases [11-14]. However, in this COVID 19 pandemic scenario, none of the nanobiosensor is commercially available in the market. Considering various technological challenges one can find out the opportunity for the development of nanobiosensor for pandemic diseases. Accurate, rapid, and early diagnosis of COVID-19 is vital for treatment and control. To overcome these shortcomings, the electrochemical biosensors should be considered, which rely on their relative simplicity and inexpensive technique to perform rapid measurements in miniaturized portable systems. Electrochemical biosensors with a volumetric transducer exhibit great potential for detecting viruses and are extensively used because of their rapid response, sensitivity, simplicity, miniaturization, cost-effectiveness, and portability [15].

World Health Organization (WHO) advised the international community to carry out massive diagnostic testing to fight transmission of the virus and decrease the number of undetected cases because diagnosis is also valuable to help researchers gain knowledge of the epidemiology of the disease. Furthermore, diagnosis plays a crucial role in making appropriate decisions on treatment

and isolation of infected people, in that way, slowing or stopping the spread of the COVID 19. Nucleic acid tests can rapidly and sensitively identify the virus in suspected COVID 19 patients; however, large amount of genetic variations, as well as mismatches of primers, probes, and target sequences, may result in reduced detection performance and false-negative results [16]. Serological surveys can assist in the investigation of an ongoing outbreak and retrospective assessment. Antibody detection of IgM and IgG antibodies in vivo is a supplement to molecular diagnostic methods. However, a lacking in the construction of diagnostic sensing techniques having a quick response to the threat and starting early diagnosis has an important impact globally. The increasing severity of the pandemic situation could be related to a lack of effective point-of-care testing (POCT) assays for rapid and accurate identification of SARS-CoV-2-infected patients. In this view, here we review the current developed diagnostic techniques in response to the COVID-19 pandemic and compare the different types through their pros and cons, such as nucleic acid detection tests (PCR and CRISPR), serological tests. The purpose of this review is to discuss the different techniques used for detection of viruses, critically scrutinize the various diagnostic platforms and summarize the studies on electrochemical nanobiosensors development in this COVID 19 scenario.

Morphology and overview of SARS-CoV-2:

SARS-CoV-2 includes in the genus β-coronavirus, consisting of a crown-like envelope, and positive-sense single-stranded RNA (þssRNA). It is a circular-shaped virus (60-140 nm) comprised of the nucleocapsid phosphoprotein (N) on the surface and the genome þssRNA to form a helical nucleocapsid. The largest structural (S protein) protein of SARS-CoV-2 makes the distinct spikes on the virus's surface, as shown in Figure 1. SP protein facilitates viral entry into host cells by using its receptor-binding domain (RBD) region to bind host cell receptors.

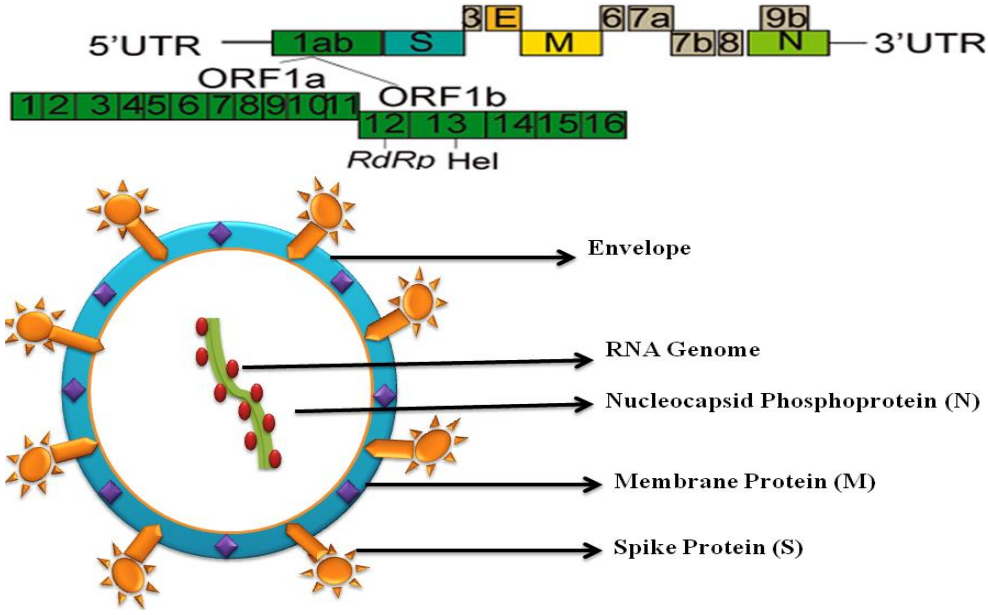

***Figure 1**: SARS-CoV-2 genomic organization and virus structure*

The RBD region of the SP is, therefore, the main target for the detection of SARS-CoV-2. An anti-SP antibody assay can be used to screen serum containing high titers of SARS-CoV-2 neutralizing antibodies targeting the SP [17]. The nanopore-based direct RNA sequencing approach has revealed that N RNA is the most abundantly expressed transcript in SARS-CoV-2 infected cells, followed by S, 7a, 3a, 8, M, E, 6, and 7b. This suggests that the N gene is one of the simplest targets for high-sensitivity detection of SARS-CoV-2 infection. Thus, collectively, the SARS-CoV-2 virus particle, RNA,

NP antigen, and human antibodies targeting the SP and therefore the NP used to accurately screen COVID-19 patients [18]. Biosensors for COVID-19 are mostly designed on the surface nucleoproteins, which binds to the host ACE-2 receptor and the internal genetic material [19].

Results and discussion

Detection of SARS-Cov-2 virus

Nucleic acid detection via RT-PCR:

Polymerase Chain Reaction (PCR) based tests are widely used to detect viruses in human diseases in clinical laboratories and tests consist of nucleic acid extraction followed by purification from the human sample using authorized extraction methods. Virus-specific PCR tests required generating primers and probes exclusive to SARS-CoV-2, excluding other closely related coronaviruses [20]. For the detection process of the SARS-CoV-2, primarily, the upstream oligonucleotides of the envelope gene (E gene) are screened, followed by the confirmation of the nucleocapsid gene (N gene) using the RT-PCR approach. Center for Disease Control and Prevention (CDC), suggests nucleic acid testing is the main approach for COVID-19 diagnostic [21]. The RT-PCR technique is considered the gold standard for diagnosing viral agents. In RT-PCR, the RNA is reverse transcribed into cDNA and followed by amplification using the primer sets and detected by specific probes. There are identified three regions on the viral genome with conserved sequences: RNA-dependent RNA polymerase gene (RdRP gene), envelope protein gene (E gene), and nucleocapsid protein gene (N gene). RdRP and E genes have great analytical sensitivity for detection as compared to the N gene. Therefore, it is believed that the development of a two-target platform with a universal primer (all the coronaviruses strands) and a specific SARS-CoV-2 primer would give more accurate identification [22].

False negative results can occur for several reasons, including inappropriate specimen type, sub-optimal specimen collection, early testing, low analytical sensitivity, low viral load, and inconsistency in viral shedding [23]. An analysis of 103 genomic data was done by Tan et al. and demonstrated that these virus strains underwent a total of 149 point mutations (Ancestral states for 43 synonymous, 83 non-synonymous, and two stop-gain mutations) [24]. If a mutation is situated in the probe or primer binding site, the sensitivity and accuracy of existing RT-PCR detection kits will be affected. RT-PCR still has many disadvantages; one of them is having false-negative results needing complementary tests like CT scans. RT-PCR reagents and test kits are not affordable, and hospitals with RT-PCR infrastructures are located in centralized cities. Last but not least, RT-PCR diagnosis depends on the presence of the SARS-CoV-2 in the sample, which means that it will not identify asymptomatic patients who recovered from the infection, and the prevention measures could not be applied.

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)

The clustered regularly interspaced short palindromic repeats (CRISPR)/Cas equipment has recently been tailored as a POC tool for the rapid detection of nucleic acids (DNA or RNA). CRISPR is a genome programming system; it is programmed to cleave specific sequences in the DNA/RNA target oligonucleotides having specific genetic code to edit RNA and DNA at precise locations where the results can be easily observed by combination with a lateral-flow strip. Broughton et al. have developed a rapid, easy-to-implement and precise CRISPR Cas12-based lateral flow assay to detect SARS-CoV-2 from respiratory swab RNA extracts, called SARS-Co DNA Endonuclease-Targeted CRISPR Trans Reporter [25].

Computed tomography scans

Different imaging techniques such as chest X-rays, lung ultrasounds, and pulmonary computed tomography are significant tools in the early diagnosis of pneumonia associated with COVID-19 patients. The procedure for CT scan is non-invasive and consists of X-ray images of different sections of the chest area. An expert radiologist examines the images to identify any abnormal characteristics related to the disease, which are diverse and depend on the infection stage. It has been found that during the early onset of the infection (first 2 days), 56 % of the cases presented similar CT results, while after 10 days, the lung's implication was observed. Usually observed characteristics include lung consolidation and bilateral/peripheral opacities. CT scans showed a higher sensitivity (86–98%) in addition to fewer false-negative results compared to RT-PCR [26, 27]. However, the disadvantage of CT scans is that many characteristics observed for SARS-CoV-2 are overlying with other viral pneumonia, and accordingly, the specificity is extremely low (25 %). The major drawbacks of CT scans are the high price, the need for highly experienced staff, and are not specific to SARS-CoV-2. CT scan is not suitable for asymptomatic, pre-symptomatic patients and some mild symptomatic individuals without pneumonia.

Antibody-based tests

The serological antibody test is carried out for symptomatic patients. Diagnosis of COVID-19 related IgM and IgG tends to signify a recent and sometimes previous virus exposure to SARS-CoV-2, respectively. SARS-CoV-2 specific protein utilization for immunoassays is ideal for minimizing possible false-positive test results ensuing by cross-reactivity with other viruses. Antibody-based testing is carried out for the detection of SARS-CoV-2 specific IgM and IgG in whole blood, plasma, or serum [28]. In another study, in patients diagnosed with COVID-19 shows the presence of IgA antibodies targeting the S protein from day 6 to 8 and up to 42 days, with a peak value at 20 to 22 days [29]. Serologic tests are not recommended for rapid diagnosis of the infection because the timing of the appearance of IgM and IgG is variable and may be delayed.

Nano-enabled methods

Nanobiosensors have improved sensitivity, stability as required by analysis as well as enhanced stability and specificity of the detection system. Noteworthy, nanoparticles can produce a synergy effect between conductivity, catalytic activity, and biocompatibility to enhance signal transduction [30]. The specific properties of nanomaterials for the construction of biosensors can be summarized as follows: i) higher surface area allowing the immobilization of a larger density of biomolecules, ii) lower diffusion limitations for the analyte to reach immobilized biomolecules, iii) direct electron transfer between the modified electrode and active site of the enzyme making biosensors more selective, iv) higher current density and/or analysis at lower overpotentials, iv) enhanced loading of secondary biorecognition elements. Nanomaterials have great potential to improve the performance of nano-enabled methods due to their high surface area promoting electron transfer reactions, electrical conductivity, good chemical stability, and mechanical robustness [31,32].

Lateral flow assay (LFA)

The LFA is user-friendly, inexpensive, and simply mass-produced in an endeavor to manage the COVID-19 pandemic. Encouragingly, the detection sensitivity of the LFA has been improved with the use of novel nanomaterials as immune labels such as quantum dots, gold nanoparticles, and magnetic nanoparticles. LFAs that use ultra-bright fluorescence nanomaterials with longer fluorescence lifetimes can significantly reduce background noise and enhance LFA detection

sensitivity using the time-resolved analysis technique. Layqah et al. developed an immunosensor based on carbon electrodes modified with AuNPs, a highly selective, single step, sensitive, and accurate method [33]. In another study, a colloidal gold nanoparticle-based lateral-flow (AuNP-LF) assay was developed to achieve rapid diagnosis and on-site detection of the IgM antibody against the SARS-CoV-2 virus through the indirect immune-chromatography method [34].

Potential portable nanobiosensors

Several new nanobiosensing prototypes have been developed recently for the diagnosis of COVID-19, such as electrochemical biosensors, colorimetric biosensors, localized surface plasmon resonance (LSPR), surface-enhanced Raman scattering (SERS), quartz crystal microbalance (QCM), and piezoelectric microcantilever sensors (PEMS), *etc.* [35]. In most recent work, Qiu *et al.* [36] developed a dual functional plasmonic sensor with high sensitivity, rapidity, and reliable diagnostic capability for the SARS-CoV-2 virus detection. In other studies, the authors used a field-effect transistor (FET) biosensor and genosensor to detect SARS-CoV-2 from COVID 19 patient samples [37,38]. The recent article presents a non-invasive intelligent nanomaterial-based hybrid sensor array with multiplexed capabilities for detecting and monitoring of COVID-19-specific volatile organic compounds (VOC) mixtures from exhaled breath [39].

Electrochemical nanobiosensors for virus detection

Electrochemical nanobiosensors generally depend on the enzymatic catalysis reaction between the immobilized biorecognition element and the targeted analyte that produces electrons and affects the electrical properties of the solution. Moreover, depending on the incorporated biorecognition element, different electrochemical biosensors are constructed, such as immunosensors, enzymatic biosensors, and DNA biosensors [40]. The main benefits of electrochemical biosensors are easy construction, simple instrumentation, high sensitivity, cost-effectiveness and, the possibility of portability and miniaturization, high sensitivity, and relatively low costs [41]. A recent work [42] has established a miniaturized label-free electrochemical impedance spectroscopy-based detection of biomarkers with metallic nanoparticles (NPs), electro-chemically engineered nano-dendroids, and graphene oxide nanocomposites. These nanomaterials were deposited over the screen-printed carbon electrode (SPCE) and were incorporated antibodies against the specific biomarker. It detects the infection precisely, owing to its robustness and high analytical performance. Another important feature of such nanobiosensors is data accusation in requisites of electronic signals, linked with smartphone-based analytical systems. This system offers a semi-automated user interface that can be used by an untrained person without technical knowledge. Through inbuilt tailored hardware and sensing software, sensing systems can be developed within a smartphone to miniaturize the system to be carried out to any location and operated by any semi-trained people. It can provide a commercial substitute for expensive stand-alone technologies [43].

In recent years, electrochemical biosensors such as immunosensor and DNA based nanobiosensors have shown great accomplishment in medical diagnosis due to their distinctive properties and user-friendly platform for the detection of pathogenic viruses. The main steps involved in the construction of the genosensor include immobilization of a single strand of DNA probe on the working electrode, which is surface-functionalized with nanomaterials; hybridization with a complementary strand (target) and electrochemical detection. The target DNA detection depends on measuring changes of electric signals such as current, impedance, and potential across the three electrodes, which are originated by hybridization of the target DNA in the sample and the

DNA probe on the WE and the related chemical reactions [44]. The surface of the genosensor is important in the performance of the nucleic acid-based biosensors for that various nanomaterials have been utilized due to properties such as strong affinity toward bioreceptor probes with reactive groups such as thiols leading to high sensitivity and low limits of detection. Singhal and coworkers proposed a novel paper-based DNA biosensor utilizing Fe_3O_4@Au nanocubes for the detection of CHIKV [45]. The electrochemical biosensors can yield a dramatic detection limit for sensing a single strain of DNA/RNA molecules [46]. Over an era of decades, viruses such as Dengue, Avian influenza virus (AIV), Hepatitis virus, Zika, Chikungunya virus (CHIKV), Rabies virus (RABV), Human nor virus, Japanese encephalitis virus (JEV), HIV, and Coronavirus [47-51] cause occurrence of infectious diseases. The performance of different detection methods is compared in **Table 2.**

Table 1. *Different nanobiosensors for detection of viruses*

No.	Analyte/Virus	Limit of detection	Linear range	Recognition element	Ref.
Electrochemical impedance spectroscopy-based biosensor					
1	JEV	2.60 ng mL^{-1}	0.1–20.0 ng mL^{-1}	antigen	[52]
2	Norovirus	1.7 copies mL^{-1}	0 - 10^5 copies mL^{-1}	virus	[53]
3	Chicken Guinea virus	8 ng mL^{-1}	0.025 -1 µg mL^{-1}	CHIKV nsP3	[54]
Electrochemical immunosensor					
4	MERS-CoV	1.0 pg mL^{-1}	0.001 - 100 ng mL^{-1}	Spike protein S1	[33]
5	EV71	0.01 ng mL^{-11}	0.01 -1.0 ng mL^{-1}	HRP and EV71 mAb	[55]
6	ZIKV	10 pM	0.01 – 1.00 nM	Specific envelop protein antibody	[56]
DNA based electrochemical biosensors					
7	HPV-16	2.3 nM	10-200 nM	AQ-PNA DNA probe	[57]
8	Ebola (DNA)	4.7 nM	0 - 5 nM	Biotinylated target strand DNA	[58]
9	Dengue (DENV3)	9.55 × 10^{-12} M	10^{-12} - 10^{-6} M	Specific DNA probe	[59]

The multiplexing method offers immense advantages such as rapid speed and lowering costs for screening multiple viruses simultaneously [60]. POCTs technologies are generally optimized in specific laboratory conditions, and through the **A**ffordable, **S**ensitive, **S**pecific, **U**ser friendly, **R**obust and Rapid, **E**quipment-free, and **D**eliverable to end-users ("**ASSURED**") criteria [60] proposed by WHO can be applied effectively to an individual for diagnosis of infectious diseases. In a recent study, a multiplexed, wireless portable electrochemical platform for ultra-rapid detection the SARS-CoV-2 which detects viral antigen nucleocapsid protein, IgM and IgG antibodies, as well as the inflammatory biomarker C-reactive protein, based on mass-producible laser engraved graphene electrodes. This SARS-CoV-2 RapidPlex platform successfully evaluated the applicability with COVID19-positive and negative blood and saliva samples [61].

The detection methods targeting antibodies are inappropriate for screening early and asymptomatic cases as most patients had an antibody response at about ten days after onset of symptoms. On the other hand, antibody detection methods can be combined with quantitative RT-PCR to significantly improve the sensitivity and specificity of diagnosis and boost vaccine research [62]. A low level of limit-of-detection is critical to change the diagnostic window of opportunity toward the initial infection process, to detect newly infected individuals. The test can be based on immunoassays, using antibodies to detect a specific antigen produced by the body's immune system or polymerase chain reactions (RT-PCR) to detect a viral genome sequence [64]. The RT- PCR test

becomes the method of choice owing to its sensitivity and specificity, and it is accomplished of detecting a single copy of the virus, resulting in a decrease in the diagnostic window compared to immunoassays. Moreover, the RT-PCR technique has various limitations in their respective applications, as shown in Table 2.

Table 2. *Limitations of PCR test for COVID 19*

No.	PCR tests are prone to the following limiting factors [64]:
(i)	**Sampling error:** Nasopharyngeal swab is suitably performed to take mucus from the ventilator system; however, this may give rise to false negatives as the optimal sampling moment is still ambiguous.
(ii)	**Sample preparation**: including cell lyses and nucleic acid purification for PCR analysis is required, and the number of extraction kits is a limiting factor for ramping up COVID-19 testing.
(iii)	**Specialized handling and transportation:** Viral genome may be denatured during transportation, also bringing about false negatives
(iv)	**Quality of reagents:** The quality of reagents used by different PCR kit manufacturers may also affect consistency among results.
(v)	**Lack of sensitivity Standard PCR methods:** It may be leading to false-negative results in COVID-19 patients with unapparent clinical symptoms.
(vi)	**False-positive outcomes:** For recovered patients, even weeks after full recovery, PCR tests can report false positive outcomes due to the presence of genetic material of the dead SARS-CoV-2.
(vii)	**Low flexibility of PCR**: Special primers and probes for each target are necessary, which confines PCR's flexibility of scaling up for other nucleic acids in simple and rapid manner.
(viii)	**Mutations SARS-CoV-2 RNA:** Since SARS-CoV-2 RNA is likely to undergo mutations, require Special primers and probes the same target.

Conclusions

Diagnosis of COVID 19 using nanobiosensor system is a frontier research area that has to deal with much unmet disputes required to develop and commercialize the nanobiosensors. In some particular cases, the validation with real samples in clinical scenarios strengthens nanobiosensors suitability. Considering the urgent need for fast and reliable detection of COVID-19 the nano-enabled biosensor can play a crucial role as it will reduce the time to detect, be inexpensive, and, the integration of biosensor with the microfluidic system. With the continuing progress in nanotechnology tools and increasing research on the nano-scale phenomena, one may look forward to further achievements in the development of nanobiosensors for the diagnosis of infectious diseases.

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