REVIEW PAPER

Development of biosensors for the detection of COVID-19

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ABSTRACT

The world is currently challenging with the COVID-19 pandemic due to the SARS-CoV-2, a new member of coronaviruses which emerged in late December 2019. The rapid transmission of the disease made it a global concern that has attracted worldwide attention. As there have been no promising treatments or specific vaccines yet, the most important key to control the pandemic is an early diagnosis. Accordingly, performing diagnostic tests accelerates case detection and prevents further transmissions. Current available methods such as quantitative real-time polymerase chain reaction have some limitations. Therefore, new strategies should be developed for accurate and rapid detection of COVID-19, a life-threatening disease. Biosensing is one of the novel approaches for the SARS-CoV-2 detection, having the potential for rapid and early diagnosis to control this pandemic. RNA, antigens, and antibodies are the main targets in COVID-19 biosensors. Although there have been limited reported studies of COVID-19 biosensing strategies, this review summarized the recent progress in this field.

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INTRODUCTION

Viral infections are among the major causes of death worldwide. In December 2019, an unexpected outbreak of a disease emerged in China (Wuhan city of Hubei province) by a new coronavirus which nominated as the coronavirus disease 2019 or COVID-19. It is also called the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) according to taxonomy and phylogeny as shows high genome similarity with other members of coronaviruses including SARS-CoV and also the Middle East respiratory syndrome Coronavirus (MERS-CoV) [1]. With increased person-to-person transmission, COVID-19 was described by the World Health Organization (WHO) as a pandemic and Public Health Emergency [2, 3]. According to the latest report of WHO, COVID-19 has spread worldwide with 82,579,768 confirmed cases including 1,818,849 deaths by 2nd January 2021.

COVID-19 infection is started by the entry of the virus through the recognition and attachment of spike protein (S) to the angiotensin-converting enzyme 2 (ACE2) host receptor. After the fusion of the virus membrane, the entry occurs and viral antigens stimulate the humoral (IgG, IgM, and IgA) and also cellular immunity [2].

The incubation period of SARS-CoV-2 is 2–7

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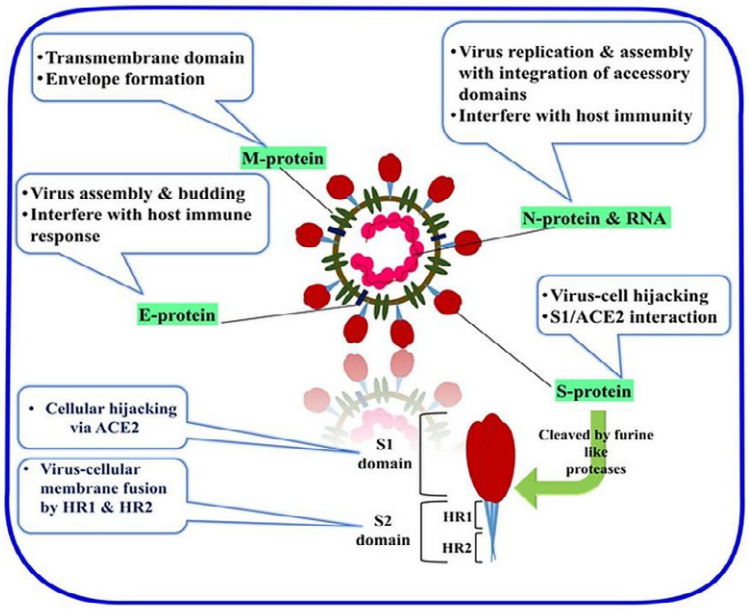

Fig. 1. Structural proteins of SARS-CoV-2, including nuclear (N), envelope (E), membrane (M) and spike (S) proteins which cleaved by the host protease into the domains of S1 and S2. Reproduced from (1) copyright © MDPI 2020 (CC BY).

days, being mostly contagious and asymptomatic at this time. Fever, headache, dry cough, and fatigue are the main symptoms of COVID-19 [3]. Due to the presence of asymptomatic carriers, the symptoms may not be deterministic [4]. Hence, diagnostic methods with high sensitivity and specificity should be developed to differentiate the COVID-19 cases from healthy ones or other respiratory viral infections.

DIAGNOSIS

The current gold standard method for the detection of SARS-CoV-2 genomic RNA is the quantitative real-time polymerase chain reaction (qRT-PCR). Such methods are time-consuming, need specialized equipment and laboratories, and may have false-positive results. Serological tests are also available which detect the presence of antibody/antigen, used to show past or new infections. RT-PCR and antibody detection methods both have limitations [5]. To manage the COVID-19 epidemic, the development of novel detection assays is urgently needed, which can enable the rapid and reliable diagnosis of COVID-19. Nanotechnology-based assays have emerged as innovative approaches that may be used for monitoring the presence of SARS-CoV-2 [6, 7].

Biosensors can provide potential alternative approaches by early, fast, sensitive, and accurate diagnosis of disease in a cost-effective way [8]. A biosensor is a device for detecting a biological analyte such as a microorganism or a biomolecule. Biosensors comprise of three parts: 1. The target which could be RNA, antigen, or antibody. 2. The recognition method via receptors, nucleic acid probe, aptamer, and antibody. 3. A transduction system such as electrochemical, surface plasmon resonance, optical, and colorimetric systems that capture the signals for further amplification and analysis [9].

Two main types of point-of-care testing are available for COVID-19; antibody, and nucleic acid tests. Nucleic acid-based tests are desired for the detecting the virus in early stages of infection, or before the symptoms appear. While antibody tests can't be used for the early stages as the virus triggers the immune response to produce antibodies about five days after initial infection [10].

BIOMARKERS_

Potential diagnostic approaches for COVID-19 can be classified into three main groups of RNA, antigens, and antibodies. To detect viral RNA, a target can be selected from any part of the genome. Furthermore, the spike (S), nucleocapsid (N), envelope (E), and membrane (M) proteins and also proteases are the target candidates for detection [9]. Structural proteins and their role are shown in Fig.

Table 1. The summary of the reported biosensing assays SARS-CoV-2 detection.

Type	Analyte	LOD	Time to result	Reference
Dual-Functional Plasmonic Photothermal biosensor	ORF1ab, E genes	0.22 pM	--	Qiu et al.
Electrochemical biosensor	SARS-CoV-2 RNA	200 copies/mL	<10 sec	Zhao et al.
Colorimetric biosensor	N (nucleoprotein) gene	0.18 ng/µl	10 min	Moitra et al.
mRT-LAMP-LFB	ORF1ab, N (nucleoprotein) genes	12 copies target/reaction	1 hour	Zhu et al.
RT-MCDA-BS	ORF1ab, N (nucleoprotein) genes	5 copies of target	1 hour	Li et al.
FET-based amperometric biosensor	Spike S protein	1.6×10^{1} pfu/mL. 2.42×10^{2} copies/mL.	--	Seo et al.
Electrochemical Immunosensor	Spike protein	90 fM	1 min	Mahari et al.
Cell-based potentiometric biosensor	S1 Spike protein	1 fg/mL	3 min	Mavrikou et al.
lateral flow immunoassay	IgM, IgG antibodies	--	15 min	Li et al.
Lateral flow combined IgG-IgM immunochromatographic assay	IgM, IgG antibodies	--	15 min	Zeng et al.
Lateral flow immunoassay Lanthanide-doped nanoparticles	IgM, IgG antibodies	--	10 min	Chen et al.

1. The summary of the reported biosensing assays for SARS-CoV-2 diagnosis is presented in Table 1.

RNA

A novel dual-functional plasmonic biosensor was constructed by Qiu et al. for detecting RNA of SARS-CoV-2 using the combination of localized surface plasmon resonance (LSPR) and plasmonic photothermal (PPT) effect for enhanced signal. Complementary cDNA sequences of SARS-CoV-2 were used to functionalize the gold nanoislands (AuNIs) as the receptors for sensitive detection through nucleic acid hybridization. The developed dual-functional LSPR biosensor was sensitive towards the detection of the RNA-dependent RNA polymerase gene (RdRp), with a detection limit of 0.22 pM concentration [11].

An electrochemical biosensor using calixarene-functionalized graphene oxide was fabricated by Zhao et al. for point-of-care testing of SARS-CoV-2 through RNA detection. Designed biosensor showed a LOD of 200 copies/mL and an electrochemical signal was detected in <10 s using a smartphone. This ultrasensitive, rapid, and easy method, provided a potential approach for COVID-19 management [12].

A colorimetric assay was developed by Moitra et al. in which the AuNPs were capped by thiol-modified DNA antisense oligonucleotides (ASOs) that were specific for SARS-CoV-2 Nucleocapsid (N) protein. Thiol-modified ASO-capped AuNPs were agglomerated as Au-ASO-RNA form in the presence of extracted RNA, and changed the suspension color as the visually detectable precipitate was observed. Positive COVID-19 cases were detected within 10 minutes with a LOD of 0.18 ng/µL [13].

An assay was designed by Zhu et al. using multiplex reverse transcription loop-mediated isothermal amplification (mRT-LAMP) along with NP-based lateral flow biosensor (LFB) for COVID-19 diagnosis. The devised mRT-LAMP-LFB test enabled the simultaneous detection of N (nucleoprotein) and ORF1ab genes (opening reading frame 1a/b) in one hour without the need to complex equipment. The mRT-LAMP-LFB assay showed 100% analytical sensitivity and specificity, with the LOD of 12 copies of targets. The method was introduced as a reliable and sensitive diagnostic approach to control the pandemic, especially in

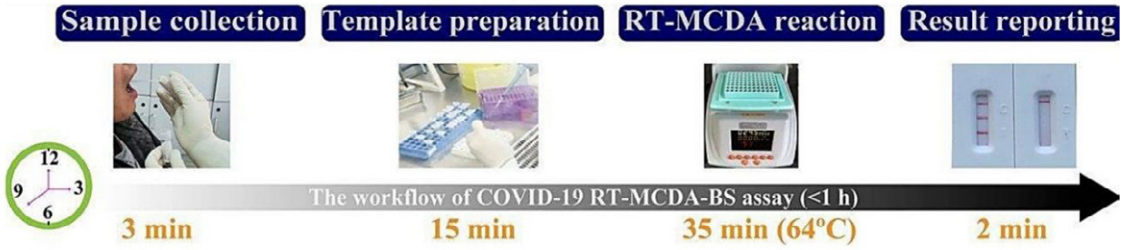

Fig. 2. The plan of COVID-19 RT-MCDA-BS diagnostic assay. Reproduced from (14), Copyright ©ERS 2020 (CC BY-NC 4.0).

resource-limited settings [7].

A reverse transcription multiple cross displacement amplification (RT-MCDA) coupling with nanoparticle-based biosensing (BS) method (RT-MCDA-BS) was designed by Li et al. for the rapid COVID-19 detection. Nucleoprotein gene (N) and F1ab were the targets of study that could be simultaneously detected. RT-MCDA-BS diagnostic assay could be completed within one hour with the LOD of 5 copies of the target sequence and high accuracy (Fig. 2). Due to the low cost, feasibility, and rapidity, this assay could be an ideal approach, especially in resource-limited settings [14].

Antigen

A Field-effect transistor (FET)-based biosensor was fabricated by Seo et al. for SARS-CoV-2 antigen detection. Spike (S)-protein-specific antibodies were used to functionalize the graphene sheets. The FET device detected the spike protein at 1 fg/mL (LOD) concentration in phosphate-buffered saline. The developed biosensor showed a high sensitivity for the COVID-19 diagnosis with no need to sample pretreatment [15].

An electrochemical biosensor named eCovSens was developed by Mahari et al. for COVID-19 spike antigen detection. A screen-printed carbon electrode (SPCE) was used for the immobilization of the COVID-19 monoclonal antibody. The portable eCovSens showed a high sensitivity for the detection of antigen with LOD of 10 fM in the buffer and 90 fM in saliva. The fabricated device was a non-invasive diagnostic tool as it was used on patient saliva and provided the rapid detection of antigen in 10-30 seconds [16].

A novel biosensor was reported by Mavrikou et al. for the detection of S1 spike surface protein of SARS-CoV-2 based on the Membrane-Engineered Vero/anti-S1 Cells (S1 antibody). Protein attachment to the antibodies made a significant change in measured bioelectric properties. The designed biosensor showed a rapid response (3 minutes to result), range of 10 fg-1 μg/mL, LOD of 1 fg/mL, and no cross-reactivity. It was introduced to be applied to control and monitor the COVID-19 pandemic [17].

Antibody

Li et al. developed a colloidal gold-immunochromatographic assay (GICA) for SARS-CoV-2 based on the conjugation of AuNPs to IgG/IgM antibodies of human serum. In this lateral flow immunoassay, anti-human IgG and IgM, and the control anti-rabbit IgG were immobilized. If the patient serum contains IgM and/or IgG, human IgM/IgG reacts with AuNPs conjugated with S-protein antigen and interact with immobilized anti-immunoglobulins, producing red color at specific lines. Positive IgM with or without positive IgG shows the acute infection, while positive IgG, negative IgM indicates the later stage and chronic infection. This simple and rapid assay was developed to detect human antibodies against COVID-19 in the blood [18].

AuNPs-labeled antibody against SARS-CoV-2 S protein was also used in a study by Zeng et al. in a lateral flow combined with IgM-IgG immunochromatographic assay. IgG and IgM antibodies were detected in blood samples in 15 minutes with a sensitivity and specificity of 85.29% and 100%, respectively. This assay was suggested to be used for the screening of asymptomatic and symptomatic individuals as it needs no specialized equipment and laboratories [19].

Chen et al. developed a lateral flow immunoassay (LFIA) by lanthanide-doped polystyrene nanoparticles (LNPs) for anti-COVID-19 IgG detection in the human serum. SARS-CoV-2 nucleo-

protein was coated on the membrane to react with specific IgG and LNPs were used to label the anti-human IgG. The reported assay showed a sensitive and rapid anti-SARS-CoV-2 IgG detection and recommended for screening and monitoring the infection progress [20].

CONCLUDING REMARKS AND FUTURE OUTLOOK

With the emergence of the COVID-19 outbreak as a global health crisis, the development of highly sensitive and rapid diagnostic tests is urgently needed. Biosensing approaches have received many improvements for the detection of other viruses due to the good specificity, sensitivity, and response time. Biosensors are generally based on the viral surface proteins and genetic materials. Although biosensing methods are promising diagnostic tools, their point-of-care usage is much challenging in terms of immobilization methods, lifetime, sensitivity, and specificity. This review summarized the recent biosensing methods for the detection of SARS-CoV-2 in COVID-19 pandemic. Since few studies of biosensing have been performed for SARS-CoV-2, further advances should be made in this field to manage this pandemic.

CONFLICTS OF INTEREST

None.

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