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MINI-REVIEW

Novel biosensor platforms for the detection of coronavirus infection and severe acute respiratory syndrome coronavirus 2

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Abstract: The recent outbreak of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has been causing respiratory diseases globally, damaging wide ranges of social-economic activities. This virus is transmitted through personal contact and possibly also through ambient air. Effective biosensor platforms for the detection of this virus and the related host response are in urgent demand. These platforms can facilitate routine diagnostic assays in certified clinical laboratories. They can also be integrated into point-of-care products. Furthermore, environmental biosensors can be designed to detect SARS-CoV-2 in the ambient air or in the intensive care ventilators. Here, we evaluate technical components of biosensors, including the biological targets of recognition, the recognition methods, and the signal amplification and transduction systems. Effective SARS-CoV-2 detectors can be designed by an adequate combination of these technologies.

Keywords: Aerosol; Coronavirus disease 2019; Fluorescence resonance energy transfer-based biosensors; Protein–protein interactions; Real-time reverse transcription polymerase chain reaction/qPCR; Severe acute respiratory syndrome coronavirus 2/2019-nCov

1. INTRODUCTION

On December 2019, the severe acute respiratory syndrome coronavirus 2, SARS-CoV-2 (formerly known as 2019-nCov)[1] was found in pneumonia patients in Wuhan, China. This virus is responsible for a life-threatening respiratory coronavirus disease 2019 (COVID-19).[2] This virus then quickly spread to most continents around the globe. The death rates vary in different countries, but all of them tend to be on the high side compared with many respiratory infectious diseases. Elder persons have higher risks of mortality.[3] As of April 2020, SARS-CoV-2 is still plaguing most countries in the world, with a total death count of more than 45 000 people. On the other hand, many infected patients have very mild symptoms or remain completely asymptomatic during the entire course of infection. The occult infections represent a major threat to public health because infected persons with mild symptoms could still transmit the disease to other people. Reliable biosensor systems for the detection of this virus with high sensitivity and specificity are therefore in urgent demand for the control of the SARS-CoV-2 pandemic.

SARS-CoV-2 is a positive-sense, single-stranded RNA virus. The SARS-CoV-2 genome encodes nucleocapsid (N), spike (S), envelope (E), and membrane (M) proteins, where S, E, and M are components of the viral envelope. It also encodes nonstructural genes of open reading frames 1a, 1b, 3, 6, 7a, 8, and 10. Similar infection routes and life cycles were found between SARS-CoV-2 and SARS-CoV-1, the virus that caused the Asian

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coronavirus endemic in 2003. SARS-CoV-2 enters the human cells through the binding of the spike protein and the viral receptor, the angiotensin-converting enzyme 2 (ACE2), triggering endocytosis. ACE2 are expressed in human cells including the type 2 alveolar cells.[4] The size of viral particle is 70–90 nm.[5] Experimental data suggest that this virus is possibly airborne and take advantage of aerosol transmission.[6] However, the scientific community has yet to reach a consensus.[7] In the environment, the virus can stay viable on the surface of plastics and stainless steels for several days.[6]

Designs of a biosensor platforms for the detection of SARS-CoV-2 involve three essential aspects (1) the target of recognition, such as viral RNA, viral proteins, or human immunoglobulins; (2) the recognition method, such as via nucleic acid probes, aptamers, antibodies, receptors, where the antibody–antigen binding or receptor–ligand interaction can be detected via the conformational changes of sensor proteins. Enzymatic reactions represent one additional methods of recognition, such as the detection of proteolytic cleavage by specific protease; and (3) the signal amplification and transduction system, for example, electrochemical, electrical, optical, surface plasmon resonance, fluorescent signals, and mechanical systems.[8] Aspects (2) and (3) are closely related with each other. For environmental applications, samplers need to be incorporated for handling samples directly from the environment. For example, if the samples were to be taken from the ambient air, then air samplers were needed to be designed.[9] Here, we review biosensor technologies which may be utilized for the development of clinically useful SARS-CoV-2 detection platforms.

2. TARGETS OF RECOGNITION

The viral RNA, viral proteins, or human immunoglobulin could all be the targets of recognition. Target regions for viral RNA detection can be selected from any region of the RNA genome. Additionally, the nucleocapsid (N),[10–12] spike (S),[4,13–16] envelope (E)[17] and membrane (M)[18] proteins and viral proteases could also be the targets of detection. In patients infected by SARS-CoV-1 in 2003, the immunoglobulin IgA was observed in the serum, followed by IgM and IgG.[19] The IgM and IgG are useful for indicating whether the person has been infected, and whether the infection has triggered host immunological response.

Nasopharyngeal swab and throat swab are the major sample collection methods for clinical diagnosis. Other samples may come from expectorated sputum,[20] saliva, serum,[11] and feces.[21] The speed and geographical range of the virus spread was unexpected. It remained unclear whether the virus can be transmitted via aerosol and ambient air.[6] Thus, environmental detector of SARS-CoV-2 in the ambient air may help to clarify the transmission route. Also, the circulated air in the ventilators in the intensive care unit may be a source of viral detection.

3. RECOGNITION METHODS

Generally, the SARS-CoV-2 genomic RNA is one major target of recognition. The detection processes often involve the amplification of nucleic acid, such as the real-time reverse transcription polymerase chain reaction (RT-PCR).[22] The drawback is on the time required, as this method requires a thermodynamic cycle which takes time. Apart from PCR-based methods, methods involving the nucleotide probes such as the nanoString technology can be used for SARS-Cov-2 detection (Canopy Bioscience, St. Louis, MO, USA). This technology requires the synthesis of the nucleotide probe which has the antisense binding with the target. Fluorescent agents were linked to the probes so as to provide a means of detection. Probes can also be linked to gold nanoparticles.[19]

Förster or fluorescence resonance energy transfer (FRET) represents one additional method of recognition.[23–32] The concept was first proposed by Förster in 1948. Theoretically, FRET can achieve detection resolutions far beyond the limitation of optical resolution, namely, at the 1–10 nm range. Optical instruments that can get the specific FRET signals *in situ* have become one of the most powerful tools for many biological research fields, even superior than biochemical methods. Generally, there are two kinds of systems can be applied for monitoring FRET, that is, either using the intensity-based or utilizing the lifetime-based (the fluorescence-lifetime imaging microscopy) FRET imaging. Through getting the signal of FRET events inside living cells/tissues, the protein–protein interactions can be revealed, proteolytic cleavage may be further studied, and even the protein conformational changes can be analyzed. In addition, scientists found that this FRET strategy can be used to produce biological probes, that is, the biosensors in combination with the above-mentioned optical platforms.

In terms of detecting viral proteins, the sensing of protein–protein interactions (like antibody–antigen binding or receptor–ligand) can be considered. Currently, antibodies represent the most effective recognition method.[33] The antibody could be harvested from animal viral-challenge experiments with either N/S/E protein or from the blood samples of patients who are infected. Apart from conventional antibodies, the antibody-mimic proteins represent novel approaches for target recognition.[15,34] This approach is to genetically modify certain regions of a protein, for example, fibronectin,[34] so that the modified protein can have noncovalent binding with the target macromolecule, thereby serving as the target recognition agent. Moreover, a promising design will be novel FRET-based biosensors. Among these SARS-CoV-2 biosensors, the viral S protein-binding peptide (derived from the corresponding domain encoded by the human *ACE2* gene) will be fused with the genes of FRET pair proteins (such as enhanced cyan fluorescent protein and enhanced yellow fluorescent protein).[23–32]

In addition, the enzyme reaction (proteolytic cleavage by specific protease) may proceed during the infection of SARV-CoV-2 into human cells. Similar to the FRET biosensor strategy, the specific peptide sequence can serve as a bait to be digested by the viral protease so that this kind of sensor can be an off–on signaling to present the existence of viral activity.[35–37]

4. SIGNAL AMPLIFICATION AND TRANSDUCTION DEVICES

Chromatographic presentation has been commonly used for simple, point-of-care assays. In the clinical laboratory, optic systems such as light detectors or charged-coupled devices were commonly used. Plasmonic photothermal biosensors using the nucleotide probe attached to a gold nanoparticle has been constructed to detect SARS-CoV-2.[38] A localized surface plasmon-coupled fluorescence fiber-optic biosensor has been constructed for the detection of N protein of SARS-CoV-1.[11]

5. FUTURE PERSPECTIVES

In the past, significant technological achievements have been made in biosensors, including the recognition methods and the signal amplification and transduction devices. These biosensor technologies can be used for the improvement of COVID-19 diagnosis assays in clinical laboratories, by offering higher sensitivity and specificity in a shorter amount of time, with a fewer number of manual steps and occupy a smaller space. The biosensors can also be used to develop point-of-care devices, for example, connected to the intensive care ventilators. Finally, environmental detectors of the virus or viral macromolecules in

the ambient air may help to elucidate the transmission route of the SARS-CoV-2 virus.

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