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Diagnostic biosensors for coronaviruses and recent developments

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15.1 Introduction

Coronaviruses, members of the Coronaviridae family and Coronavirinae subfamily are well-known airborne viruses that infect humans, birds, and other mammals. The long-range airborne route, the fomite route, or the close contact route are the primary methods of transmission of most respiratory infections (Li, 2021). Coronavirus disease 2019 (COVID-19) is a newly emerging disease that has not been previously identified in humans. Some animal coronaviruses do not infect people or transmit from person to person via close contact. However, two specific coronaviruses have been previously found to spread from animals to humans causing severe illness. These two viruses are the: severe acute respiratory syndrome-coronavirus (SARS-CoV) and the Middle East respiratory syndrome-coronavirus (MERS-CoV) (Killerby et al., 2020). The high global spread, prevalence, and associated mortality of the new SARS-CoV-2 causing the COVID-19 disease have made it a serious concern. World Health Organization reported that COVID-19 is airborne and can be transmitted through aerosol and stay alive on surfaces such as metals and textiles for a number of hours and even up to few days (Marquès & Domingo, 2021).

Coronaviruses are enveloped RNA viruses that can mainly cause respiratory, neurologic, hepatic, and enteric diseases (Peiris, 2012). Several coronaviruses have appeared such as NL63 and 229E Human coronavirus (HCOV) which belong to the alphacoronavirus type (Fung et al., 2020). On the other hand, SARS-CoV-2 belongs to the betacoronavirus which includes SARS, MERS, OC43 HCOV, HKU1, and HKU1. Four of the six coronavirus types usually cause common cold-like symptoms. However, MERS-CoV and SARS-CoV are zoonotic in origin and can result in more serious infections and even fatal illnesses (Ye et al., 2020). In 2002 and 2003, SARS-CoV has caused severe acute respiratory syndrome outbreaks in Guangdong Province, China (Peiris & Poon, 2008), whereas MERS-CoV resulted in severe respiratory disease outbreaks in 2012 in KSA which was then spread within the Middle East (Aleanizy et al., 2017). Because of the large genetic diversity and frequent recombination of coronavirus genomes, as well as the increase in human–animal interface activities, novel coronaviruses are likely to emerge periodically in humans (Wang et al., 2020). COVID-19 has long positive-sense RNA with 30,000 base pairs with two types of proteins known as structural and nonstructural proteins. The spike (S) (characteristics of all coronaviruses), matrix (M), nucleocapsid (N), and envelop (E) protein are the structural proteins.
However, the nonstructural proteins include RdRp (nsp12) and proteases (nsp3 and nsp5). The spike protein plays a vital role in the virus attachment and entering the host cells (Huang et al., 2020).

15.2 Traditional detection methods

As the risk of viral infections increases, more detection methods for viruses are being developed. The main goal of these methods is to enhance the detection of viral infections. The testing process consists of both virus detection as well as identification (Burrell et al., 2017; Pretorius & Venter, 2017). Several methods that vary with the ailment being investigated are utilized to detect virus infections in laboratories and hospitals. Cell culture is one of these detection methods that is widely employed in diagnostic virology laboratories (Leland & Ginocchio, 2007). This detection method is among the most popular techniques applied in the isolation of viruses using cell lines. For instance, Adenoviruses can be isolated in a range of cell culture systems, including urine, stool, blood, and mucous (Crenshaw et al., 2019). The major advantage of the cell culture technique for virus detection is that it facilitates the isolation of several serotypes in cell culture lines (Dill & Eschbaumer, 2020). Nevertheless, this method is not reliable for the detection of some serotypes. Moreover, cell culture does not provide quick results for immediate patient care (Hematian et al., 2016). Antigen testing is another diagnostic technique for the detection of a variety of viruses (Reta et al., 2020). The surface of the virus contains various proteins which some of which behave as antigens in the host. These antigens can lead to the creation of antibodies in the host to combat the viral infection. Antigen tests are based on the use of a specific antibody that can bind to a certain viral antigen in samples collected from infected people. Several types of antigen tests have been reported for the detection of viral infections such as immunofluorescence assays (Inoue et al., 2000), latex agglutination tests (Chen et al., 2007), and enzyme-linked immunosorbent assays (ELISAs) (Hamblin et al., 1986). In the immunofluorescence assay, antibodies are tagged with a fluorescent dye. Specific antigens present in the collected specimen bind to the tagged antibodies, and the tagged fluorescence is easily observed using ultraviolet microscopy. The advantages of the immunofluorescence methods are that it is fast and easy to perform. Nonetheless, these tests are not very flexible and, depending on the antigen used and the amount of antibody can give a low signal. For the latex agglutination assay, the clinical samples are mixed with latex beads that have been coated with the specific antibody or antigen of interest. This method can detect viruses in a variety of specimens. The latex agglutination test offers the same advantages as the immunofluorescence assay. The results are obtained in a relatively short time and it is easy to use. However, the disadvantages of this method include the relatively low specificity due to the interferences from the matrix of the clinical samples, and the requirement to cautiously interpret marginal results. ELISA is considered the most common method used to detect virus infections. In ELISA, enzyme labeling is used to detect the unknown antigens or antibodies in a serum sample. The virus is detected with ELISA when the enzyme used to label the secondary antibodies react with its substrate to cause a color change. The advantages of ELISA are that it is relatively quick and convenient. However, the ELISA requires a complex enzyme labeling methodology and specialized reagents and monoclonal antibodies that sometimes are difficult to produce. Serology assays are often done in hospital laboratories for the detection of viral antibodies in different types of
body fluids, particularly when viral infections are suspected. The serology assays (Vainionpää & Leinikki, 2008) are based mainly on evaluating the levels of the antibodies that have been produced in the body of the infected individuals in response to the viral infection. The main advantage of the serology assays is that they can be utilized to detect both previous and active viral infections. Another advantage is that serology assays can detect antibodies in the late stages of an illness. However, the main disadvantages of this method are that serology tests might not detect an infection occurring in a person who had been previously vaccinated against that infection. Thus these assays are not useful in acute clinical settings.

Nucleic acid amplification methods are also widely utilized in the diagnosis of viral diseases. The advancements made in these techniques have revolutionized the detection of viral loads in addition to the qualitative detection of viruses. Polymerase chain reaction (PCR) is the most common target amplification technique used to detect viruses (Mackay et al., 2002). In PCR, specific viral DNA sequences are amplified and gel electrophoresis is used to detect the amplified products. PCR is very specific and sensitive for the detection of viral DNA in clinical samples. Moreover, Real-time PCR methods have enhanced the speed and sensitivity of the PCR and enabled the rapid semiquantification of the virus. However, PCR methods require the design of specific primers as well as extraction and purification of nucleic acids from preconcentrated samples. Moreover, several reagents such as DNA-binding dyes and fluorescent probes are needed in the real-time PCR methods. Expensive equipment for monitoring the fluorescence is also required. Thus their application in places with limited resources, such as point-of-care testing, is not feasible.

Until now the two methods used for the detection of COVID-19 are the serology to detect the antibody and the PCR to confirm the positive results. The detection of antibodies can show the number of people who have had the disease for population surveillance as well as diagnosis. However, the RT-PCR is used to detect the RNA of the virus to confirm very recent and active infections. So far, the WHO declared that there is limited testing, thus no countries have reliable data on the prevalence of the virus in their population which also affects the reported case-fatality rates.

The COVID-19 PCR test can be performed on respiratory samples obtained by various methods, including sputum sample and nasopharyngeal swab. It generally takes a few hours to two days to obtain the results. The test done on the throat swabs samples can be only reliable in the first week of the infection. However, after that, the throat sample can test negative while it continues to multiply in the lungs. Thus in the second week of infection, sample should then be taken from the deep airways.

### 15.3 Biosensors in coronavirus detection

Biosensors are considered emerging tools for the detection of a wide range of different environmental (Eissa & Zourob, 2017a,b), biomedical (Eissa and Zourob, 2017a), food safety-related targets (Alamer et al., 2018). The biosensors can overcome many limitations associated with the traditional assays and offer advantages such as the portability, ease of use, and sensitivity that made them ideal for diagnostic applications of infectious disease. The biosensor is composed mainly of three components. The first component is the recognition receptor which can be DNA that targets certain viral genes, aptamer selected against certain viral biomarkers, or an antibody for certain protein receptors. The second component of the biosensor is the transducer which can be an optical,
electrochemical, field-effect transistor or mass-based that converts the binding between the target analyte and the bioreceptor to a detectable signal. The third component is the detector which gives the read-out signal.

Because of the complexity, relatively long analysis time, and high cost of the currently available techniques for virus detection, novel technologies are urgently needed. The new techniques should be capable to detect viruses with greater ease and fewer resource demands, in a shorter period, and at earlier stages of infection. This will ensure that the patients receive more immediate and accurate diagnosis and treatment, potentially preventing many later-stage complications. Several biosensors have been previously reported for the point-of-care diagnosis of viral infections (Antiochia, 2020; de Eguilaz et al., 2020; Maddali et al., 2021). Considerable research effort has been devoted toward the development of biosensors for different types of coronaviruses (Table 15.1). Nanomaterials, such as graphene, magnetic nanoparticles, gold nanoparticles, and carbon nanotubes have been integrated into the biosensing platforms for coronaviruses to improve their performance. Based on the type of transducer, the biosensors for coronaviruses are classified into three major groups: optical, electrochemical, and field-effect transistor-based biosensors. Here, we will focus mainly on the recent developments in biosensors for the detection of the newly emerging SARS-CoV-2.

15.3.1 Electrochemical biosensors

Electrochemical biosensors are a type of biosensors in which the binding between the recognition receptor and the transducer results in an electrochemical signal that can be measured by an electrochemical technique such as voltammetry, impedance spectroscopy, potentiometry, or amperometry. They offer several advantages over other types of sensors in terms of the low cost, the capability of miniaturization, portability, ease of use, and high sensitivity. Thus several electrochemical biosensors have been previously developed for the diagnosis of various infectious diseases and for point-of-care testing (Antiochia, 2020; de Eguilaz et al., 2020). In the next subsections, we will discuss several electrochemical biosensing platforms that have been reported for the detection of coronaviruses.

15.3.1.1 Multiplexed electrochemical array

The multiplexing capability of the electrochemical biosensors is one of their major advantages (Eissa & Zourob, 2020a,b; Layqah & Eissa, 2019). The advancement in nanotechnology has allowed the fabrication of multiple individually addressable arrays of working electrodes on small chips. This has led to a revolution in the development of biosensors for multianalyte detection and high throughput screening. A disposable gold nanoparticle modified screen-printed carbon electrodes were used to fabricate an immunosensor for the simultaneous detection of the coronaviruses; MERS-CoV and HCoV (Layqah & Eissa, 2019). The electrode chip contains eight working electrodes which were used to immobilize MERS-CoV spike, HCoV proteins as well as BSA for control measurements. The gold nanoparticles were functionalized by self-assembly of cysteamine molecules on the surface. The amine groups were then linked to glutaraldehyde to create an aldehyde end on the electrode surface. The aldehyde was then covalently bonded to the amine groups of the proteins. A competitive assay format was utilized for the detection of the MERS-CoV and HCoV proteins by using a certain number of specific antibodies for each protein. The presence of each of the two viruses in the tested sample led to competition with the immobilized proteins for the
| Type of biosensor | Method of detection | Target analyte | Nanomaterial | Limit of detection | Clinical sample | References |
|-------------------|---------------------|----------------|--------------|--------------------|----------------|------------|
| Immunosensor      | Square wave voltammetry | SARS-CoV-2 nucleocapsid antigen | Carbon nanofibers | 0.8 pg mL⁻¹ | Nasopharyngeal sample | Eissa and Zourob (2021) |
| Immunosensor      | Differential pulse voltammetry | SARS-CoV-2 spike and nucleocapsid antigen | Magnetic nanoparticles | 19 ng mL⁻¹ and 8 ng mL⁻¹ | Saliva and nasopharyngeal samples | Fabiani et al. (2021) |
| Immunosensor      | Amperometry         | SARS-CoV-2 nucleocapsid antigen | Magnetic nanoparticles | 230 pg mL⁻¹ | Serum | Li and Lillehoj (2021) |
| Molecular imprinted polymer biosensor | Differential pulse voltammetry | SARS-CoV-2 nucleocapsid antigen | Paper electrode | 15 fM | Nasopharyngeal samples | Raziq et al. (2021) |
| Immunosensor      | Square wave voltammetry | SARS-CoV-2 IgG, IgM and S protein | Graphene oxide | 0.11 ng mL⁻¹ | Serum | Yakoh et al. (2021) |
| Immunosensor      | Amperometry         | SARS-CoV-2 spike antigen-RBD | Cobalt-TiO₂ nanotubes | 14 nM | Nasal and saliva samples | Vadlamani et al. (2020) |
| DNA genosensor/ isothermal rolling circle amplification | Differential pulse voltammetry | RNA of the SARS-CoV-2 spike and nucleocapsid genes | Redox dye-incorporated silica nanoparticles | 1 copy µL⁻¹ | Nasopharyngeal sample | Chaibun et al. (2021) |
| Genosensor        | Current – voltage measurements | RNA of the SARS-CoV-2 nucleocapsid gene | Gold nanomaterials | 6.9 copies µL⁻¹ | Nasopharyngeal sample | Alafeef et al. (2020) |
| Immunosensor      | Field-effect transistor | SARS-CoV-2 spike antigen | Graphene | 1.6 × 10⁴ pfu mL⁻¹ in culture medium | Nasopharyngeal sample | Seo et al. (2020) |
| Immunosensor      | Square wave voltammetry | MERS-CoV spike protein and HCOV | Gold nanoparticles | 0.4 pg mL⁻¹ | Nasopharyngeal sample | Layqah and Eissa (2019) |
| Genosensor        | Cyclic voltammetry | SARS-CoV gene sequence | Gold nanoparticles | 2.5 pmol L⁻¹ | – | Martínez-Paredes et al. (2009) |
| Immunosensor      | Field-effect transistor | SARS-CoV nucleocapsid antigen | In₂O₃ nanowire | Sub nanomolar concentration | – | Ishikawa et al. (2009) |

(Continued)
| Type of biosensor | Method of detection | Target analyte | Nanomaterial | Limit of detection | Clinical sample | References |
|------------------|---------------------|----------------|--------------|-------------------|-----------------|------------|
| **Immunosensor** | Field-effect transistor | SARS-CoV nucleocapsid antigen | Single-walled carbon nanotubes | – | Electrolyte solution | Ishikawa et al. (2010) |
| **Immunosensor** | Localized surface plasmon coupled fluorescence fiber-optic | SARS-CoV nucleocapsid antigen | Gold nanoparticles | 1 pg mL$^{-1}$ | Serum | Huang et al. (2009) |
| **Aptasensor** | Optical | SARS-CoV nucleocapsid antigen | Quantum dots | 0.1 pg mL$^{-1}$ | Serum sample | Roh and Jo (2011) |
| **Immunosensor** | Surface plasmon resonance | SARS-CoV surface antigen | Gold micropatterned chip | 0.2 µg mL$^{-1}$ | – | Park et al. (2009) |
| **Genosensor** | Localized surface plasmon resonance (LSPR) and Colorimetric assay | MERS-CoV DNA samples | Gold nanoparticles | 1 pmol µL$^{-1}$ | – | Kim et al. (2019) |
| **Genosensor** | Colorimetric assay | MERS-CoV DNA | Silver nanoparticles | 1.53 nM | – | Teengam et al. (2017) |
| **Genosensor** | Plasmonic photothermal and localized surface plasmon resonance | SARS-CoV-2 Nucleic acid | Gold nanoislands | 0.22 pM | Nasopharyngeal sample | Qiu et al. (2020) |
| **Genosensor** | Colorimetric assay | RNA sequence of SARS-CoV-2 | Gold nanoparticles | 0.18 ng µL$^{-1}$ of RNA | – | Moitra et al. (2020) |
| **Immunosensor** | Colorimetric assay | SARS-CoV-2 IgG-IgM combined antibody | Gold nanoparticles | – | Blood, serum, and plasma | Li et al. (2020) |
binding to the antibody added to the sample solution. With different concentrations of the MERS-CoV and HCoV proteins added to the sensor, the number of the available antibody to bind to the protein-modified electrode surface will be different which led to a change in the electrochemical signal of the sensor when measured in ferro/ferricyanide redox solution. The simultaneous detection of the two coronavirus proteins was realized using square wave voltammetry by following the change in the reduction current of the individual immunosensors. This immunosensors exhibited very good sensitivity and selectivity against other nonspecific proteins from other viruses. Moreover, it enabled the accurate and low-cost detection of multiple samples and can be extended to detect other types of viruses.

15.3.1.2 Cotton-tipped electrochemical biosensor

The integration of flexible materials such as cotton, textile, paper, and thread with electrochemical biosensors have attracted considerable attention in recent years because of their low cost, ease of fabrication, and disposability (Agustini et al., 2016; Caetano et al., 2018; Economou et al., 2018; Imani et al., 2016; Jia et al., 2013; Liu et al., 2014; Yamada et al., 2017). An electrochemical biosensor has been reported for the detection of SARS-CoV-2 using nucleocapsid protein as biomarker (Eissa & Zourob, 2021) (Fig. 15.1). The biosensor was fabricated using carbon screen printed

![Immunosensor fabrication](image1)

![Sample collection](image2)

![Competitive detection](image3)

**FIGURE 15.1**

A schematic illustration of the cotton tipped immunosensor for severe acute respiratory syndrome-coronavirus 2.

*From Eissa, S. & Zourob, M. (2021). Development of a low-cost cotton-tipped electrochemical immunosensor for the detection of SARS-CoV-2. Analytical Chemistry, 93(3), 1826–1833. https://doi.org/10.1021/acs.analchem.0c04719.*
electrodes which are adapted to fit into standard PCR tubes. The carbon surface was first modified with carbon nanofibers to enhance the electron transfer properties of the electrode as well as to increase the electrochemical surface area. The electrodes were then functionalized by electrografting of carboxyphenyl groups via electroreduction of in situ generated diazonium salt. This led to the attachment of the carboxyphenyl groups to the carbon surface via covalent bonds. The carboxylic groups can be then used to attach the nucleocapsid protein to the electrode surface after activation. Cotton fiber was then used to cover the surface of the modified electrode to mimic the function of the standard Q-tip for the sample collection via swabbing. In this method, the cotton-tipped biosensor was used as miniaturized platform that can be utilized for sample collection and electrochemical detection using a portable potentiostat linked to a smartphone. The detection assay was carried out with competitive format using antinucleocapsid antibody and the biosensor response signal was recorded with square wave voltammetry in ferro/ferricyanide redox solution. This integrated cotton/electrochemical immunosensor platform is promising for point-of-care testing as it showed excellent sensitivity and selectivity for SARS-CoV-2 against other viruses such as MERS-CoV, HCoV, and Influenza A.

15.3.1.3 Magnetic nanoparticles-based electrochemical biosensors

Magnetic nanoparticles have been widely integrated with electrochemical biosensors to detect different pathogens (Eissa and Zourob, 2020a). They offer several advantages as they provide a large surface area for immobilization of bioreceptors. Moreover, magnetic nanoparticles can be easily handled using a magnetic field and which made them ideal in developing washless sensors. They can be also used for preconcentration of the analyte particularly when a very low concentration of analyte exists in large and complex samples such as clinical specimens (serum, saliva, nasal fluids). Magnetic nanoparticles can be used in miniaturized electrochemical devices thanks to the magnetic tools which can be utilized to concentrate the particles underneath the working electrode and facilitate the immunoreaction by bringing the biomolecules into proximity. A differential pulse voltammetry-based enzymatic biosensor was developed for the detection of SARS-CoV-2 spike and nucleocapsid proteins in saliva (Fabiani et al., 2021). In this method, carbon black electrode was integrated with magnetic particles and the signal is monitored using a portable potentiostat. A sandwich assay format was employed where the antibodies of the spike and nucleocapsid proteins were immobilized on the magnetic particles. The analyte is captured on the antibodies-coated magnetic particles and a secondary antibody labeled with alkaline phosphatase enzyme was used to generate the electrochemical signal through the enzymatic by-product 1-naphtol. Good sensitivity was achieved using this biosensor and it was successfully applied on saliva samples exhibiting correlation with the RT PCR results.

Another research group (Jiran & Lillehoj, 2021) has also reported the use of magnetic nanoparticles in microfluidic immunosensor for the detection of SARS-CoV-2 nucleocapsid protein in undiluted serum (Fig. 15.2). In this device, the magnetic particles played a dual function for immunomagnetic enrichment on the chip as well as for signal amplification. The magnetic particles were dually labeled with the antinucleocapsid antibody for capturing the protein and horse radish peroxidase enzyme to generate the amperometric signal. This miniaturized microfluidic biosensor showed good sensitivity and capability to detect the virus in whole serum samples without pretreatment or interference from the sample matrix.
15.3.1.4 Molecular imprinted polymer-based electrochemical biosensor

The molecular imprinted polymer can be used in biosensors as recognition receptors with antibody-like ability to bind specifically to certain molecules. To prepare these imprinted polymers, a mixture of monomers is allowed to polymerize around the target which is then removed from the polymer leaving behind specific binding sites for the target. These imprinted polymers have high stability and can be produced easily and thus they were exploited in several biosensors in the diagnosis of infectious agents. Raziq et al. (2021) have reported the integration of molecular imprinted polymers with...
electrochemical biosensor on thin-film electrode to detect SARS-CoV-2 nucleocapsid protein showing a very low sensitivity and a LOD of 15 fM. A label-free detection scheme was employed using differential pulse voltammetry. This method showed good application in nasal samples.

15.3.1.5 Graphene oxide/paper-based biosensor

A label-free biosensor using square wave voltammetry was developed for the detection of the SARS CoV-2 antibody and antigen in serum samples (Yakoh et al., 2021). The biosensor was constructed on the paper substrate on which the electrodes were printed, and the working electrode was modified with graphene oxide. The receptor-binding domain of the spike protein was used as a marker immobilized on the electrode to capture the IgG and IgM antibodies in the serum samples. On the other hand, the authors showed the detection of the spike antigen using an immobilized antibody. The biosensor was applied in patient serum samples and the results showed good agreement with ELISA. The paper-based biosensor exhibited advantages such as the low-cost and disposability which make it suitable for point-of-care diagnosis.

15.3.1.6 DNA-based biosensor for the detection of the viral genes

A gold nanoparticle/paper-based biosensor was reported for the detection of two different sites of the nucleocapsid genes of SARS-CoV-2 (Alafeef et al., 2020). The biosensor was fabricated on a gold nanoparticle-modified paper electrode on which the complementary ssDNA for the nucleocapsid phosphoprotein gene was immobilized. The detection was achieved by monitoring the change of the current – voltage data collected using a homemade circuit. This detection method was simple and does not require any amplification step and enabled the detection within 5 minutes. The LOD of this biosensor was 6.9 copies µL⁻¹ indicating good sensitivity of the method. The authors also showed the application of this biosensor in clinical samples collected from 22 COVID-9 positive patients and 26 healthy individuals exhibiting 100% correlation with the RT-PCR results.

Another research group (Chaibun et al., 2021) has reported the use of isothermal rolling circle amplification (RCA) coupled with differential pulse voltammetry detection for the diagnosis of SARS-CoV-2. The rolling circle amplification can be carried out using a heating block or a simple water bath. This method is based on the hybridization of the amplicons with capture probes. These capture probes were labeled with redox-active molecules such as methylene blue as reporter molecules to generate the electrochemical signal. This method was used to detect the spike and nucleocapsid genes of the virus and showed excellent sensitivity with LOD of 1 copy µL⁻¹ in less than 2 hours. The biosensor showed the capability to discriminate the SARS-CoV-2 positive and negative samples with very good agreement with the PCR results.

15.3.2 Optical biosensors

Optical biosensors are one of the most common types of biosensors which showed promising application in different areas such as environmental (Damborský et al., 2016; Long et al., 2013), biomedical (Pirzada & Altintas, 2020), and diagnostic fields (Damborský et al., 2016). In these biosensors, the interaction of an optical field with a biorecognition receptor is monitored. Various parameters can be utilized in optical biosensors for detection such as light scattering, fluorescence, polarization, absorption, amplitude, or variation in a reflective index. In this section, we will discuss different types of reported optical biosensors for the detection of coronaviruses.
15.3.2.1 Surface-enhanced Raman scattering and chemiluminescence

The detection of SARS-CoV-2 antibodies (IgA, IgM, and IgG) using different types of optical sensors was reported by many research groups (Saviñon-Flores et al., 2021). For instance, IgM and IgG were detected simultaneously using surface-enhanced Raman scattering (SERS) combined with lateral flow immunoassay. The SERS was fabricated using silver-coated SiO$_2$ core and tags modified with dual layers of Raman dye. The fabrication resulted in excellent stability and a very high SERS signal. The SARS-CoV-2 spike protein was immobilized on the silver-coated SiO$_2$ and used to capture the IgM/IgG from the sample. The detection is then realized using antihuman antibodies which bind to the complex. This SERS-based lateral flow assay was superior to the regular assays as it showed the much lower limit of detection.

A dual functioning optical/chemiluminescence immunosensor for the detection of SARS-CoV-2 IgA was reported by Roda et al. (2021). Nucleocapsid protein was used as a recognition receptor to capture the IgA antibodies in the samples. The method exhibited excellent performance in the detection of the early immune response in infected and recovered patients.

15.3.2.2 Plasmonic biosensors

Surface plasmon resonance (SPR) has been also used for the detection of different viruses by measuring the change in the reflective index upon binding with the target (Funari et al., 2020; Shrivastav et al., 2021). Pig sera was used to purify two antibodies against SARS-CoV-2 which showed a high affinity to the nucleocapsid protein (Bong et al., 2020). Then an SPR biosensor was developed using these antibodies showing high sensitivity and a LOD of 1.02 pM for the detection of SARS-CoV-2. Localized surface plasmon resonance (LSPR) was used by Funari et al. (2020) to detect antibodies of the SARS-CoV-2 spike protein in a microfluidic biosensor (Fig. 15.3). A label-free biosensor was fabricated using electrodeposited gold nanospikes on which the spike protein

![Schematic illustration of optomicrofluidic chip biosensor for the detection of severe acute respiratory syndrome-coronavirus 2.](From Funari, R., Chu, K.-Y., Shen, A.Q. (2020). Detection of antibodies against SARS-CoV-2 spike protein by gold nanospikes in an opto-microfluidic chip. Biosensors & Bioelectronics 169, 112578.)
was immobilized. The detection of the antibodies was achieved using LSPR in human plasma by monitoring the shift in the peak wavelength upon binding of the target antibodies to the gold nanospikes. This method showed good sensitivity and was carried out in 30 minutes exhibiting a LOD of 0.5 pM. Moreover, this platform is simple and relatively cheap that made it suitable for point-of-care diagnosis. Tian et al. (2020) have reported the integration of circle-to-circle amplification with optomagnetic analysis for the detection of SARS-CoV-2 RdRp coding sequence. This biosensor enabled the simple and fast detection of SARS-CoV-2 DNA with a low LOD of 0.4 fM.

Localized surface plasmon resonance was combined with plasmonic photothermal effect to develop a biosensor for the detection SARS-CoV-2 gene (Qiu et al., 2020). Complementary DNA sequences were immobilized on gold nanoislands as bioreceptor that can hybridize with a certain sequence of the virus gene. The hybridization temperature is increased by the localized photothermal effect which enhanced the biosensor performance. This dual functional biosensor was able to accurately distinguish two similar gene sequences with high sensitivity and LOD of 0.22 pM.

### 15.3.2.3 Colourimetry-based biosensors

Colorimetric-based biosensors offer several advantages over other types of sensing methods. These advantages include rapid detection, low cost, simplicity, and capability to be used by untrained personnel, and ease of visualizing the signal by the naked eye. Thus colorimetric methods have been widely used to develop biosensors for the point-of-care diagnosis of various infectious agents (Mondal et al., 2018). Some colorimetric biosensors have been recently developed for the detection of SARS-CoV-2. Moitra et al. (2020) have reported the use of gold nanoparticles coated with thiol modified oligonucleotides for the colorimetric detection of SARS-CoV-2. The detection of the nucleocapsid gene was achieved by seeing the color change with the naked eye in 10 minutes. The principle of the detection was based on the color produced by agglomeration of the gold nanoparticles with the target SARS-CoV-2 RNA which resulted in a red-shift. However, the addition of RNaseH led to cleavage of the RNA strands from DNA-RNA hybrid resulting in the precipitations of the agglomerated gold nanoparticles which can be seen by naked-eye. This colorimetric biosensor showed good sensitivity and selectivity with a LOD of 0.18 ng µL⁻¹ for SARS-CoV-2 RNA. A similar method was applied for the detection of SARS-CoV-2 RdRp gene by Kumar et al. (2020). The biosensor was applied in nasopharyngeal samples and demonstrated LOD of 0.5 ng for SARS-CoV-2 RNA. A colorimetric immunoassay using gold nanoparticles was also developed for the simultaneous detection of SARS-CoV-2 IgM and IgG. The method showed good application in the detection of antibodies from human blood samples in 15 minutes.

### 15.3.2.4 Microfluidic-based biosensors

Microfluidic is a fluid-handling technology that revolutionized the biosensing platforms in the last decade (Nikoleli et al., 2018). It allows the use of very small volumes and therefore it is perfectly suited for diagnostic sensing where the small blood sample is usually used (Jayamohan et al., 2013). The ideal detection of infectious viral diseases requires the use of low-cost, sensitive, selective, affordable, rapid, equipment-free biosensors. Moreover, point-of-care testing should offer advantages in terms of capability to perform high throughput screening, fast response, transportability, and use of small sample volume. Thus these criteria made the integration of microfluidics with biosensors for point-of-care testing, a rapidly developing research area. Ramachandran et al. (2020) have reported the development of a CRISPR-based sensing platform using electric field-driven
microfluidics for the detection of SARS-CoV-2. CRISPR-based diagnostic methods are appearing as promising approaches for field-deployable solutions. In the CRISPR method, a synthetic guide RNA is usually complexed with the CRISPR–Cas12 enzyme which becomes activated upon cleavage when it binds to the target DNA. The detection is then achieved by cleavage of fluorophore—quencher labeled reporter DNA sequence. The authors reported that the use of a microfluidic chip would accelerate this CRISPR assay by controlling the electric field gradients using a technique called isotachophoresis. The isotachophoresis was also used to purify the viral RNA from the nasal sample. The combination of the CRISPR assay with the isotachophoresis technique and loop-mediated isothermal amplification resulted in the sensitive detection of SARS-CoV-2 RNA in 35 minutes which represents a remarkable improvement of the existing nucleic acid-based diagnostic methods for COVID-9. Funari et al. (2020) have reported the development of an optomicrofluidic biosensor for the detection of SARS-CoV-2 antibodies based on localized surface plasmon resonance. The biosensor was capable of successfully detecting the antibodies in the patient plasma that was diluted by mixing 1 µL of the plasma in 1 mL of the buffer.

15.3.3 Field-effect transistor

Field-effect transistor-based biosensors have shown a wide range of biomedical applications particularly in point-of-care diagnosis (Chen et al., 2007). They show several advantages because of their capability to achieve real-time measurements with very high sensitivity. A graphene-based field-effect transistor immunosensor was developed for the detection of SARS-CoV-2 spike protein (Seo et al., 2020). In this method, the graphene surface was modified noncovalently with 1-pyrenebutyric acid N-hydroxysuccinimide ester. This functionalization enabled the covalent attachment of the antispike antibody on the graphene surface. This method is label-free and allowed the very sensitive detection of the spike antigen with a LOD of 1 fg mL\(^{-1}\) in buffer and 100 fg mL\(^{-1}\) in transport media. Moreover, the authors showed excellent application of this biosensor to detect the virus in culture media as well as in clinical samples.

15.4 Conclusions and future perspectives

The development of low-cost, sensitive and accurate diagnostic biosensors for the detection of coronaviruses is highly important particularly for ending the current COVID pandemic. Many biosensors are being developed for the detection of SARS-CoV-2. In this chapter, we reviewed the recent developments in the detection of coronaviruses using various biosensing approaches. Several optical-based biosensors have been reported for the detection of coronaviruses using methods such as surface plasmon resonance, fluorescence, and colorimetric detection. Most of these optical methods showed good sensitivity. However, the high cost and the integration of the optical sensors in miniaturized platforms for point-of-care diagnosis remain major challenges. Electrochemical biosensors are generally considered cost-effective and sensitive methods. Thus several electrochemical biosensing platforms have been developed for the detection of coronaviruses showing promising results in terms of sensitivity, accuracy, selectivity, and portability. The combination of nanomaterials such as graphene, carbon nanotubes, gold nanoparticles with the biosensing devices has also
contributed to the improvement of the performance of the biosensors for SARS-CoV-2 detection. The integration of new nanotechnological concepts such as microfluidics, CRISPR, and portable devices with biosensors has revolutionized the field. Despite these advancements in biosensors for coronaviruses, the continuous development of low-cost diagnostic tools that rapidly returns results from patients at all stages of infection and does not require complex sample preparation is still highly required. The developed biosensors should be able to detect the virus not only at hospitals but also at airports, homes, and highly crowded areas. The identification of new recognition receptors that bind specifically to different types of coronaviruses is highly needed. These bioreceptors such as aptamers and specific peptides produced by phage display can open new opportunities for the development of lower-cost, highly stable biosensing platforms. The development of multiplexing platforms for the high throughput detection of multiple biomarkers for different coronaviruses is very promising. The fabrication of such miniaturized biosensor arrays will enable the simultaneous screening for different types of infections that cause similar symptoms which will reduce the cost and time of the diagnosis.

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