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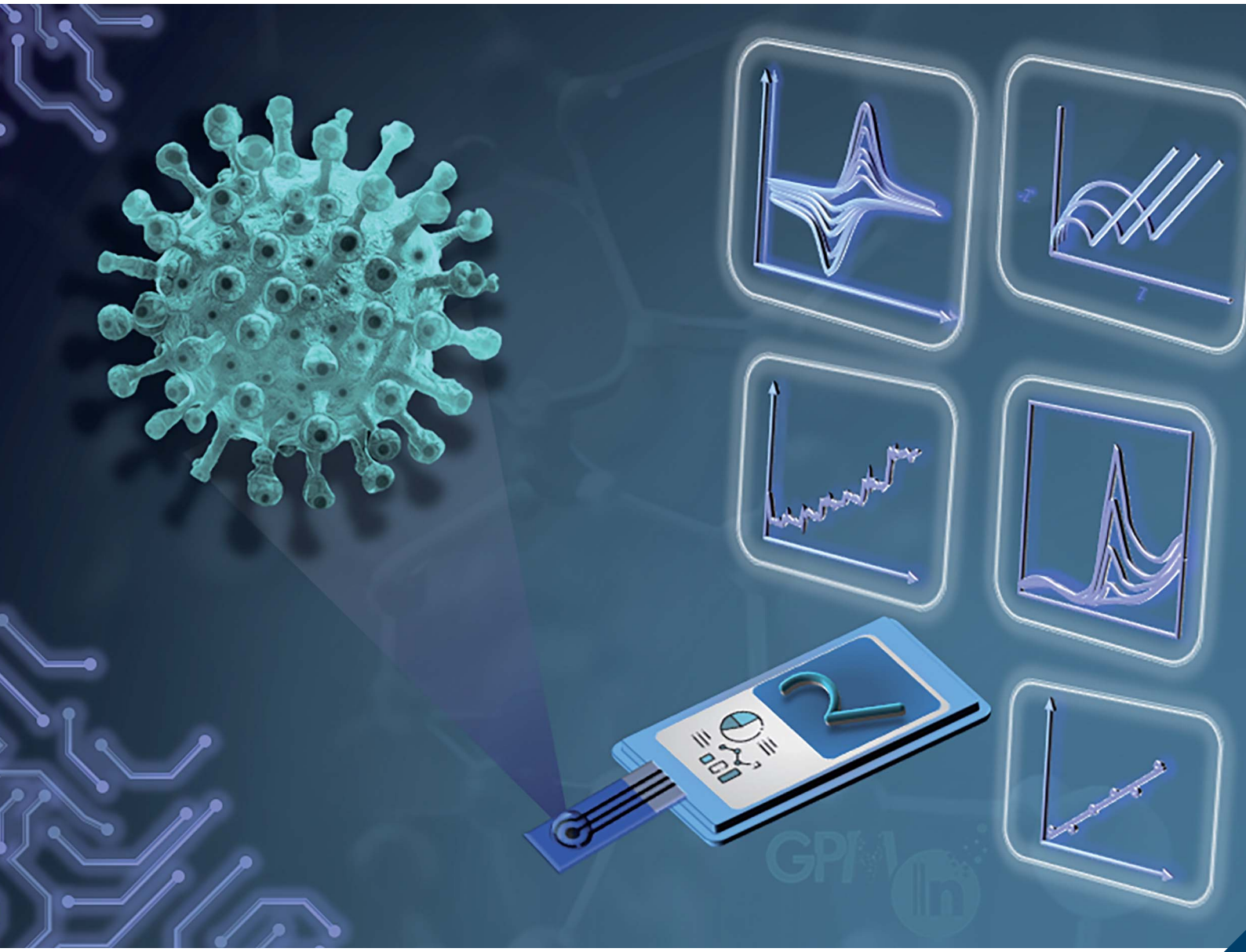

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MINIREVIEW
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An overview of electrochemical biosensors used for COVID-19 detection

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This short review presents the latest advances in the field of electrochemical biosensors, focusing particularly on impedimetric biosensors for the direct measurement of analytes. As a source of study we have chosen to describe these advances in the latest global health crisis originated from the COVID-19 pandemic, initiated by the SARS-CoV-2 virus. In this period, the necessity for swift and precise detection methods has grown rapidly due to an imminent need for the development of an analytical method to identify and isolate infected patients as an attempt to control the spreading of the disease. Traditional approaches such as the enzyme-linked immunosorbent assay (ELISA), were extensively used during the SARS-CoV-2 pandemic, but their drawbacks, including slow response time, became evident. In this context, the potential of electrochemical biosensors as an alternative for COVID-19 detection was emphasized. These biosensors merge electrochemical technology with bioreceptors, offering benefits such as rapidity, accuracy, portability, and real-time result provision. Additionally, we present instances of electrochemical biosensors modified with conductive polymers, eliminating the necessity for an electrochemical probe. The adaptability of the developed materials and devices facilitated the prompt production of electrochemical biosensors during the pandemic, creating opportunities for broader applications in infectious disease diagnosis.

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1 Introduction

The COVID-19 pandemic caused by a new virus identified as SARS-CoV-2 triggered a global health crisis, profoundly affecting the lives of billions of people around the world. Since it was initially identified in 2019 in Wuhan, China, the virus has spread rapidly, crossing borders, and turning into a widespread public health threat, killing millions all around the globe.[1,2] One of the main worrying features of the virus was its high transmissibility rate. Several studies have pointed out that the spread occurred also by asymptomatic individuals, which made virus detection and dissemination very difficult.[3–5] The swift transmission has underscored the pressing need for straightforward, speedy, and precise viral detection. This would facilitate the prompt recognition of infected individuals or early cases, enabling the implementation of effective control measures without delay.

During the COVID-19 pandemic, traditional detection methods, such as the enzyme-linked immunosorbent assay (ELISA) method, were widely used. The ELISA method is a detection technique commonly used in clinical laboratories for detection of various infectious diseases. It employs the specific detection capacity of antibodies and the catalytic properties of enzymes to produce a coloured or luminescent product. ELISA is typically performed in 96-well plates, which act as a solid phase for immobilizing the biomolecules, which can be the antigen, by a direct, indirect, or competitive approach, or a capture antibody, by a sandwich approach (Fig. 1). The detection antibody is conjugated with an enzyme that will generate a coloured product after the substrate is added. The most used enzymes are alkaline phosphatase and horseradish peroxidase, which produce a yellow colour in the presence of nitrophenyl-phosphate and a blue colour in the presence of hydrogen peroxide, respectively. The plate solid

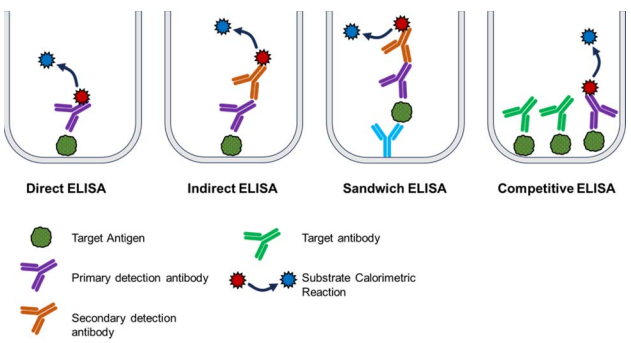

Fig. 1 Four major types of ELISA.

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Table 1 Summary of studies to detect Covid-19 using different methods

Technique	Material	Target analyte	Sensitivity or the limit of detection	Ref.
ELISA	ELISA kits from Gold Standard Diagnostics (GSD)	Immunoglobulins\ A (IgA) and G (IgG) antibodies	GSD IgG and IgA kits were 69 and 15 percent sensitive, respectively	15
ELISA	ELISA kits from EuroImmun (EI)	IgA and IgG antibodies	EI IgG and IgA kits were 90 and 86 percent sensitive. respectively	15
EIS	16-well plate containing sensing electrodes	SARS-CoV-2 antibodies	LOD = 0.1 mg mL^{-1}	16
EIS	Pyrrolidinyl peptide nucleic acid (acpcPNA)	SARS-CoV-2 RdRp gene sequence	LOD = 1 pM	17
EIS	Screen-printed carbon modified with AuNPs	SARS-CoV and SARS-CoV-2 spike proteins	LOD = 3.16 pmol L^{-1}	18
CV and EIS	Screen-printed carbon modified with Cu_2O NCs	SARS-CoV-2 spike protein	LOD = 0,04 fg mL^{-1}	19
EIS	Carboxylated carbon nanotubes on a carbon-based SP electrode	Antibodies against SARS-CoV-2 S protein	LOD = 0.70 pg mL^{-1}	20
CV	Gold chips coated with antigens or peptide nucleic acids	SARS-CoV-2 RNA and anti-SARS-CoV-2 antibodies	0.8 copies per μl	21
RBD IgG ELISA	ELISA protocol	IgG antibodies against S protein	Sensitivity for IgG detection was 72% to 74% between 2 and 3 weeks from the onset of symptoms	22
EIS	PPy-NTs/AuNPs	Anti-SARS-CoV-2 nucleocapsid protein monoclonal antibodies	Limit of detection (LOD) of 0.386 ng mL^{-1} and limit of quantification (LOQ) of 1.287 ng mL^{-1}	23
EIS	PPy:PSS-AuNPs	Anti-SARS-CoV-2 nucleocapsid protein monoclonal antibodies	LOD = 2.456 ng mL^{-1} and LOQ = 7.442 ng mL^{-1}	23

phase is important so the unbound reagents can be washed to ensure that the colorimetric reaction is proportional to the analyte being detected. Therefore, the optical density of the product is proportional to the concentration of the analyte in the biological sample.[6,7]

The ELISA method plays a key role in detecting antibodies against SARS-CoV-2 in blood serum, acting as an indicator of the immune response to virus infection.[8] Despite its usefulness, the ELISA method has certain specific limitations in its application for this purpose, such as erroneous readings, potentially generating both false negative and false positive results. This can occur due to the variable quality of the reagents, the immunological window, and the possible presence of similar antibodies from other coronaviruses.[9,10]

In addition, this method requires considerable time and resources. It requires expensive equipment and reagents, as well as several steps of incubation, washing, and analysis of the signal generated.[11] Usually, the ELISA method is employed to detect antibodies produced by the host, which can remain present for months after infection.[12] Therefore, its inability to discern between active and past infections is an additional limitation.

Due to these factors, there is a need to develop alternative approaches that facilitate the detection process. One methodology that has stood out is using electrochemical techniques. These approaches could overcome the limitations inherent to the ELISA method, providing more efficient detection of COVID-19, characterized by greater speed, sensitivity, specificity, and simplicity.[13,14] Table 1 shows some examples related to the ELISA method and biosensors; the examples mentioned in the table will be discussed throughout the text.

We can see in Table 1 that electrochemical biosensors represent a promising approach for diagnosing COVID-19. These devices combine the sensitivity of electrochemical technology with the specificity of bioreceptors, allowing direct or indirect detection of the virus. Direct detection is based on capturing the virus or its components (RNA or proteins) using specific probes such as antibodies, aptamers, or peptides.[24] For example, some electrochemical sensors can detect SARS-CoV-2 RNA by hybridizing with complementary probes immobilized on the electrode surface. Hybridization alters the electrical properties of the electrode, such as impedance or current, which can be measured by electrochemical techniques.[25]

Indirect detection is based on measuring biomarkers associated with viral infection, such as immunoglobulins (IgG and IgM), cytokines or host proteins. For example, some electrochemical biosensors can detect antibodies through a specific immunological reaction with a bioreceptor, such as an antigen or an aptamer. The bioreceptor can be conjugated to a label, such as an enzyme or a nanoparticle, which catalyzes a chemical reaction or generates a potential change on the electrode

surface. The reaction or change can be quantified using electrochemical techniques.[26]

They offer the advantage of providing fast, accurate, and portable results, facilitating implementation in different settings.[27] By exploring the advances of electrochemical biosensors, and more specifically, impedimetric ones, in the detection of SARS-CoV-2, it has become possible to envision new perspectives for disease diagnosis and control. The present review aims to examine these advances, discuss the challenges faced, and explore the prospects for the development and application of these biosensors not only for COVID-19 but also for other infectious diseases.[28,29]

1.1 Electrochemical biosensors in SARS-CoV-2 detection

Electrochemical biosensors are designed to convert interactions between the analyte and the recognition element into a measurable electrical signal, providing fast and accurate results.[30,31] These devices have several key advantages for monitoring and controlling the spread of various viruses, as they are fast, portable, and can provide results in real-time. They offer high sensitivity and selectivity, allowing accurate identification of SARS-CoV-2, and other analytes, even at low concentrations.[25,32]

There is much promising work in the field of biosensors for the detection of SARS-CoV-2 nucleic acid, and these developments have the potential to improve our ability to diagnose and monitor COVID-19. In addition, electrochemical biosensors have also been used to detect antibodies generated after SARS-CoV-2 infection, providing crucial information about the individual's immune response, and to detect viral antigens.[25,33]

An antigen test looks for an active infection by detecting the presence of antigens from the SARS-CoV-2 virus. On the other hand, an antibody test looks for signs of a past infection by detecting antibodies produced by the host in response to the virus. Antigen tests are commonly used for detecting and diagnosing respiratory infections like COVID-19 and can help detect a current viral infection. Antibody tests can help identify a past infection or vaccination against COVID-19 in specific clinical situations and can also be used to monitor population-level immunity and infection rates of SARS-CoV-2.[34]

The scientific literature has presented significant advances in a short period in the development of electrochemical biosensors for COVID-19 diagnosis. Much of the research has focused on improvements in the sensitivity, specificity, and robustness of these devices, aiming to improve their effectiveness to facilitate diagnoses.[35–39]

1.1.1 Electrochemical biosensor components. Typically, an electrochemical biosensor has three main components, an electrode (or transducer), a bioreceptor, and a signal transducer or detector. The electrode is the central element of the biosensor, as it is on its surface where the electrochemical reactions occur. The bioreceptor is the biological part of the biosensor that makes it selective to the analyte of interest. The signal transducer converts the interaction between the bioreceptor and the analyte into a measurable electrical signal.[24,32]

Fig. 2 below illustrates the functioning of the components in an electrochemical biosensor, allowing a clear visual representation of the detection process. The transducer is represented by a screen-printed electrode (SPE). The central circle is the working electrode, which is the transducer, the core component of the biosensor, as it is the place where the electrochemical reactions take place. The transducer also carries the bioreceptors, which are represented by purple Y-shaped molecules. The bioreceptor is the component that selectively recognizes and captures the target molecule in the sample. In the figure, the target molecule is represented by a blue triangle, which fits into the bioreceptor. The signal transducer is the component that converts the change in the electrochemical properties of the electrode due to the bioreceptor–target interaction into a quantifiable electrical signal.[24,32]

The surface of the transducer can be optimized by being functionalized with materials that are more sensitive to electrochemical signal changes, such as gold, platinum, carbon, and graphene, whose intrinsic characteristics provide greater electrical stability and higher sensitivity.[40,41] This functionalization should also contain a functional layer containing the bioreceptor, which is what makes this device selective to the analyte of interest.[42,43]

This bioreceptor is the biological part present in the biosensor. On the electrode surface, the bioreceptor is immobilized, becoming responsible for interacting with the specific target in a sample, where in the case of COVID-19, some of the main detection targets include virus-specific antigens, viral oligonucleotides and antibodies.[25,31,44,45]

When the interaction between the bioreceptor and the target occurs, signal transduction is performed at the electrode to convert the interaction into a detectable electrical signal. This electrical signal occurs thanks to electrochemical reactions at the electrode/bioreceptor interface that are proportional to the concentration of the target present in the sample. With the combination of each of these components, they can be developed in different ways and for different purposes for virus detection and thus produce versatile electrochemical biosensors.[46] Fig. 2 below illustrates the functioning of these components in an electrochemical biosensor, allowing a clear visual representation of the detection process.

These devices have the potential to be used for diagnosis, prognosis, and prediction of the disease course in the context of personalized medicine.[35,47] Some notable materials for the functionalization of surfaces include biosensors based on gold nanoparticles (AuNPs); this material aims to increase charge transfer efficiency.[18,48–50]

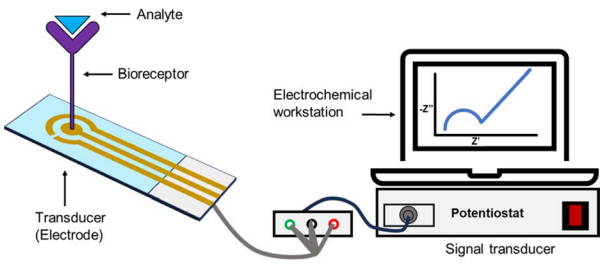

Fig. 2 Electrochemical biosensor components.

1.1.2 Electrochemical biosensors – techniques. Biosensors can be divided into several categories according to the working principle of the transduction element, such as electrochemical biosensors, optical biosensors, and gravimetric biosensors, among others.[51] Each can be categorized according to the bioreceptor being used, immunosensors when antibodies are employed, aptasensors for aptamers, genosensors for DNA or RNA bioreceptors, and so on. Electrochemical biosensors specifically can also be further categorized by the signal readout technique being used, such as impedimetric biosensors, voltammetric biosensors, potentiometric biosensors, and others.[52]

Electrochemical biosensors for COVID-19 detection exploit different electrochemical techniques to convert the interactions between the virus and its components into measurable electrical signals. Among the main techniques used are voltammetry, electrochemical impedance spectroscopy, amperometry and chronopotentiometry. Each technique has distinct characteristics and offers specific advantages in biosensors.[25,53,54]

Voltammetry has been one of the most widely used techniques for various electrochemical biosensors as it involves simple methodologies and is sensitive to detection. It involves the application of a controlled potential difference between electrodes and obtains as a response the resulting current as a function of the applied potential.[55,56] Several variations of this technique, such as square wave voltammetry, cyclic voltammetry, and differential pulse voltammetry, have been used to improve sensitivity and selectivity in the detection of SARS-CoV-2. However, voltammetric techniques may present limitations in detecting very low concentrations of analytes, which can be a disadvantage in applications that require high sensitivity, in addition to the necessity to use an electrochemical probe to carry out the measurements.[57–59]

Electrochemical impedance spectroscopy (EIS) is a technique that can also be used to investigate interfacial properties related to biorecognition events that occur on the electrode surface. This technique has the advantage of requiring a small perturbation of the steady-state amplitude to detect changes in the charge transfer resistance (R_{ct}) or capacitance value. The impedance can be obtained in the presence or absence of a redox couple, which indicates whether the impedance is faradaic or non-faradaic, respectively.[60,61]

In faradaic biosensors, also known as impedimetric biosensors, the antibodies are immobilized on the electrodes. The electrode is then coated with a blocking layer, resulting in a change in electron transfer. After antigens are bound to the antibodies, forming the immunocomplex, access to redox probes is hampered. As the faradaic reaction of the redox couple is hampered more and more, the electron transfer resistance will increase and the capacitance will decrease, thus making detection possible.[28] In Fig. 3, there is an illustrative scheme of an impedimetric biosensor that uses an electrochemical probe for detection.

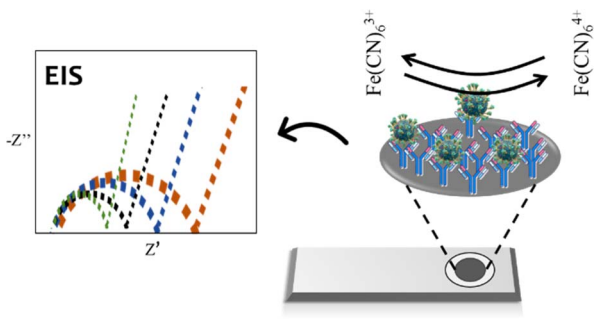

Fig. 3 EIS-based biosensor platform using the ferri/ferrocyanide redox pair as a probe in the detection of SARs-Cov-19 S protein.

1.1.3 Electrochemical biosensors – analytes. When it comes to the analytes utilized, electrochemical biosensors developed for the detection of specific structural proteins of the SARS-CoV-2 virus, such as the spike protein, have garnered attention due to their high sensitivity and specificity. Additionally, these biosensors are highly efficient and cost-effective, making them an attractive option for SARS-CoV-2 detection.[25,62,63]

For instance, in the work conducted by Zukauskas *et al.* (2023)[64] the biosensor was developed using a gold disk electrode modified with a self-assembled monolayer of mercaptoacetic acid (MAA) and 6-mercapto-1-hexanol (6-MCOH). The biosensor was tested for the determination of antibodies against the SARS-CoV-2 spike protein in human serum samples, with detection carried out using three electrochemical methods: cyclic voltammetry, differential pulse voltammetry, and potentiostatic pulsed amperometry. The results indicated that differential pulse voltammetry and potentiostatic pulsed amperometry exhibited similar sensitivity, while cyclic voltammetry was the most sensitive method. The LOD and LOQ values using the VC technique were 0.34 nM and 1.04 nM, respectively; these values are relatively high when compared to those in other studies.

As previously discussed in the context of spike protein detection, Rahmati *et al.* (2021)[19] (Table 1) also describes the development of an electrochemical immunosensor based on a screen-printed carbon electrode modified with Cu_2O nanocubes (Cu_2O NCs). This sensor uses the IgG anti-SARS-CoV-2 antibody spiked onto the electrode surface as a specific platform in an ordered orientation through staphylococcal protein A. Electrochemical evaluations were carried out using EIS and CV and showed a very good linear relationship between the R_{ct} and the content of the spike protein through a specific binding reaction. The LOD was estimated to be 0.04 fg mL^{-1}, demonstrating the device's ability to detect extremely low concentrations of the SARS-CoV-2 spike antigen, indicating excellent sensitivity of the sensor. Although the number of samples tested was relatively small, with only nine saliva samples and seven universal transport medium (UTM) samples, the study did not include a negative control group to evaluate the specificity of the biosensor in relation to other viruses affecting the respiratory tract, which may be important to avoid false positive results.

Another interesting study using MAA and gold is that of Brazaca *et al.* (2022)[18] (Table 1). The authors engineered an electrochemical biosensor that leverages electrodeposited gold nanostructures for the detection of the spike proteins of SARS-CoV and SARS-CoV-2. The biosensor employs S protein capture antibodies covalently immobilized on self-assembled monolayers (SAMs) of MAA linked to gold nanostructures. The detection of the S proteins of both SARS-CoV and SARS-CoV-2 is

carried out using electrochemical impedance spectroscopy (EIS). The biosensor, fabricated with 9 seconds of gold deposition, demonstrated high performance in terms of selectivity, sensitivity, and a low limit of detection, enabling direct determination of the target proteins in saliva samples. The LOD of the immunosensor is higher than that of more sophisticated immunosensors, and its low cost and potential direct applicability to biological samples represent considerable advantages. However, a limitation is that the immunosensor was only tested with inactivated virus samples, requiring additional validation with clinical samples. Furthermore, efforts are still needed for complete validation with a more significant number of samples and those of different natures, in addition to a comparison with gold standard techniques, such as ELISA and RT-qPCR.

The ELISA protocol is also used in the work of Villafañe *et al.*, 2022 (ref. 22) (Table 1). This paper describes the development of a low-cost IgG ELISA test based on the receptor binding domain (RBD) of the SARS-CoV-2 virus. The aim of the study was to produce large quantities of the RBD protein in a simple and economical manner that could be used in low-income and remote areas for patient contact tracing, epidemiological studies and vaccine efficacy evaluation. The authors used a semi-stable mammalian episomal expression system to produce the RBD protein and tested it in an in-house IgG ELISA for COVID-19 using a panel of human sera. The results showed that the recombinant antigen was effective in detecting SARS-CoV-2 specific IgG antibodies with 100% concordance between tests when compared to a commercial test based on the full length spike protein. The study concludes that the RBD-based ELISA test may be an attractive and cost-effective option in scenarios of limited resources to address the COVID-19 pandemic. The strength of the study lies in the ability to produce large quantities of RBD protein in a simple and inexpensive way that can be used in low-income and remote areas. However, the study's weakness is that it only tested the recombinant antigen in an in-house IgG ELISA for COVID-19 using a panel of human sera, and further validation is needed to confirm its efficacy in real-world scenarios.

Macmullan *et al.*[15] (2020) (Table 1) demonstrated two different applications of the ELISA method in the article for detecting SARS-CoV-2 antibodies in saliva. The article aims to develop and validate a saliva-based ELISA for detecting antibodies against SARS-CoV-2, the virus that causes COVID-19. The authors optimized the protocol for saliva samples using a mouthwash collection method, centrifugation, concentration, and blocking. The sensitivity and specificity of the saliva-based ELISA were tested on a large set of clinical samples from PCR-positive and PCR-negative individuals. The data were stratified by age, sex, and days since symptom onset. The saliva-based ELISA achieved high sensitivity and specificity, particularly for individuals over 40 years of age. Additionally, it showed a weak but significant correlation with serum IgG levels. The article presents a promising approach for antibody testing using saliva samples. Saliva samples are easy and non-invasive to collect and pose less risk of exposure for healthcare workers. However, the article has some limitations and challenges. For instance, it does not compare with other saliva-based assays, does not test asymptomatic or mildly symptomatic individuals, does not evaluate neutralizing antibodies, does not assess stability and reproducibility, and does not standardize or calibrate against a reference material or method.

Another main analyte used for COVID-19 diagnosis is viral nucleic acid, which is detected by the reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) method. However, this test is time-consuming and requires expensive equipment and trained personnel. Electrochemical biosensors offer a faster and more affordable alternative for the direct detection of viral nucleic acid.[47] Biosensors aimed at detecting the nucleic acid of the virus allow for direct analysis of the viral genetic material present in the sample, and are also known as DNA biosensors or genosensors.[25,53,63] The article by Wu *et al.*[65]. describes the development of an electrochemical biosensor for the rapid, highly sensitive, and specific detection of the SARS-CoV-2 Delta variant without the need for any nucleic acid amplification assays. The biosensor is based on CRISPR-Cas12a technology and utilizes a DNA template identical to the SARS-CoV-2 Delta spike gene sequence as a model. The authors used differential pulse voltammetry (DPV) as the detection technique.

In a similar vein, the article by Lomae *et al.*[17] (Table 1) discusses the development of a label-free electrochemical DNA biosensor for the diagnosis of COVID-19. The developed sensor was used for amplification-free SARS-CoV-2 detection in 10 nasopharyngeal swab samples (7 SARS-CoV-2 positive and 3 SARS-CoV-2 negative), giving results 100% in agreement with those of RT-PCR. The sensor is based on a portable potentiostat and a paper-based device and uses peptide nucleic acid (PNA) as the capture probe for point-of-care testing.

However, the clinical validation had a small sample size and the samples were collected from a single hospital, which may restrict the generalizability of the results. Besides, the presence of other viral or bacterial pathogens in the samples may affect the sensor's performance, leading to false-positive or false-negative results. Additionally, the sensor necessitates a sample extraction step, which may introduce variability and contamination. Therefore, further studies are required to assess the sensor's performance in larger and more diverse populations, as well as to optimize the sample preparation and handling procedures. Furthermore, the sensor could be enhanced by integrating multiplexing capabilities to detect other SARS-CoV-2 genes or variants. Additionally, alternative PNA systems could be utilised to improve binding affinity and specificity.

There are several other articles about DNA detection. The studies by Zhang *et al.* (2022)[66] and Hwang *et al.* (2021)[67] all discuss the development of biosensors for the detection of SARS-CoV-2 nucleic acid or COVID virus DNA. These articles describe different approaches for developing these biosensors, including the use of electrochemiluminescence (ECL), CRISPR/Cas12a technology, and printed circuit board (PCB) electrodes. These different approaches highlight the diversity of techniques and technologies that are being used to develop biosensors for the detection of SARS-CoV-2 nucleic acid or COVID virus DNA.

In a similar way, the electrochemical detection by the RNA technique used in the article by Peng *et al.*[68] is based on the

catalytic hairpin assembly (CHA) circuit and terminal deoxynucleotidyl transferase-mediated DNA polymerization. When the target RNA is present, it triggers the CHA reaction, leading to the generation of long single-stranded DNA products. These DNA products bind to positively charged electroactive molecules, resulting in significantly amplified electrochemical signals. The electrochemical techniques used were electrochemical impedance spectroscopy (EIS) and differential pulse voltammetry (DPV). EIS was used to verify the sensor's performance, while DPV was used to record the signal responses under different conditions. The use of RNA as a target for detection of SARS-CoV-2 has advantages such as high specificity and sensitivity, as well as the ability to detect the infection early. Viral RNA can be detected in the early stages of infection, even before symptoms appear, which is crucial for controlling the spread of the virus. Furthermore, detection of viral RNA can provide important information about viral load and disease progression, helping to monitor and treat patients. Detection of viral RNA also plays a key role in identifying virus variants, allowing mutation tracking and assessment of vaccine effectiveness.

Regarding the use of conductive polymers, the work by Song *et al.*[69] addresses the construction of an antifouling electrochemical biosensor. Biofouling, caused by the accumulation of biomolecules on detection surfaces, is one of the main problems and challenges for the practical application of electrochemical biosensors. In this study, an electrochemical antifouling biosensor was built based on electropolymerized polyaniline (PANI) nanowires and newly designed peptides for the detection of the N gene of COVID-19. The inverted Y-shaped peptides were designed with excellent anti-fouling properties and two anchoring branches. Based on the biotin–streptavidin affinity system, biotin-labeled probes specific for the COVID-19 N-gene (nucleocapsid phosphoprotein) were immobilized on the peptide-coated PANI nanowires, forming a highly sensitive and antifouling electrochemical detection interface for the detection of COVID-19 nucleic acid. The N gene used in detection is less prone to mutations compared to other regions of the viral genome. This increases the reliability of detection and reduces the likelihood of false negatives due to viral mutations. The work presents a limit of detection (LOD) of 3.5 fM for the detection of the COVID-19 N gene, even in complex human serum samples. Compared to other studies, this LOD is quite impressive, indicating a high sensitivity of the developed biosensor.

The article by Yakoh[70] presents an innovative paper-based electrochemical biosensor designed for COVID-19 diagnosis. This biosensor, featuring a screen-printed carbon electrode modified with gold nanoparticles, employs differential pulse voltammetry for signal detection. The detection mechanism revolves around the interruption of the redox conversion of the redox indicator, leading to a reduced current response.

Expanding on the theme of electrochemical detection, the article by Rashed *et al.*[71] (Table 1) introduces a label-free, rapid detection method for SARS-CoV-2 antibodies. This approach utilizes a readily available impedance sensing platform where antibodies bind to the receptor binding domain (RBD) of the SARS-CoV-2 spike protein pre-coated on sensing electrodes. Consequently, this binding event induces an impedance change that the platform measures effectively.

On the other hand, the studies conducted by Najjar *et al.*[21] and Ana R. Cardoso *et al.*[20] (Table 1) collectively underscore the remarkable progress in the development of electrochemical biosensors for COVID-19 diagnosis. These papers demonstrate diverse approaches, including lab-on-a-chip technology, ultrasensitivity, and rapid quantitation, to address the pressing need for efficient diagnostic tools. The integration of multiplexed electrochemical outputs, ultra-sensitive spike protein-based capture methods, and rapid quantification techniques showcases the versatility and potential of electrochemical biosensors in responding to the challenges posed by the pandemic.

These biosensors offer a multifaceted approach, ranging from rapid quantitation to ultra-sensitive antibody capture and multiplexed detection. Moreover, they seamlessly complement traditional antigen and antibody tests by acting as efficient, precise, and versatile diagnostic tools. The extensive exploration of electrochemical biosensors targeting the spike protein, nucleic acids, antigens, as well as antibodies generated post-infection, illuminates the diverse strategies employed in the pursuit of accurate and efficient diagnostic tools for COVID-19. The highlighted studies collectively exemplify the robustness and adaptability of electrochemical biosensing techniques in the fight against the pandemic. These advancements not only hold promise for enhanced diagnostic capabilities but also signify a significant step towards improved public health measures and a more comprehensive understanding of the virus's behavior and its impact on human immunity.[39,47,71–74]

1.1.4 MIP-based electrochemical biosensors in SARS-CoV-2 detection. Most of the developed diagnostic tools are based on biological recognition elements, *i.e.* diagnostic antibodies that guarantee the selectivity of the device in relation to the target but reduce the useful life of the sensor and increase the cost. One of the promising approaches is the use of molecular imprinted polymers (MIPs), which are synthetic materials designed to mimic the properties of natural antibodies or receptors, making them useful for detecting specific molecules, such as those associated with pathogens such as the SARS-CoV-2 virus.[75]

The work of Ayankojo *et al.* (2022)[76] has developed a sensor based on MIPs, which acts as a synthetic receptor that effectively detects the S1 subunit of the SARS-CoV-2 spike protein (ncovS1). The device is capable of detecting ncovS1 both in phosphate-buffered saline and in nasopharyngeal samples from the patient. In addition, the sensor is compatible with portable potentiostats, allowing on-site measurements. This means that it has great potential as a point-of-care testing platform for rapid and early diagnosis of COVID-19 patients. The work presents remarkable LOQ and LOD values, considering that the concentration of ncovS1 in samples from patients with COVID-19, estimated from the reported amount of RNAs observed after diagnosis, ranges from 0.02 to 18.7 ng mL^{-1}. Furthermore, the study reports that the sensor's performance is superior to that of other electrochemical sensors reported in the literature. However, the study was conducted on a relatively small sample

size, only 5 positive samples; additional validation studies may be necessary to confirm the sensor's performance in larger populations.

The paper by Raziq *et al.*[75] presents the development of a portable MIP-based electrochemical sensor for detection of the SARS-CoV-2 nucleoprotein (ncovNP). The selectivity was appreciable for ncovNP, as its response was almost insensitive to the addition of the spike protein in the COVID-19-negative sample but increased immediately after the addition of ncovNP. This selectivity is crucial for accurate diagnosis, which offers great potential for point-of-care testing applications. The sensors demonstrated excellent long-term stability, with the response remaining the same up to 9 weeks of storage, indicating their potential for practical use. The limit of detection and quantification of the sensor in the lysis buffer was 15 fM and 50 fM, respectively. Although this is a promising result, it may not be sensitive enough to detect low levels of the virus in some clinical samples.

The study by Ratautaite *et al.* (2021)[77] focused on the development of conducting polymers and their application in electrochemical detection. Conducting polymers, such as polypyrrole, were utilized as polymeric matrices for the creation of MIPs. Electrochemical detection was conducted using techniques like CV, DPS and EIS, enabling the analysis of interactions between analytes and electrodes modified with MIPs. Additionally, chronoamperometry was applied to assess changes in current in response to variations in the diffuse layer thickness on the working electrode surface. The results demonstrated that conducting polymers, particularly polypyrrole, proved effective in generating MIPs for the detection of analytes with both low and high molecular weights, such as proteins and viruses.

El Sharif *et al.*,[78] investigated electropolymerized molecular imprinted polymers (E-MIPs) for selective recognition of the entire SARS-CoV-2 virus; a specific monoclonal antibody for SARS-CoV-2 called mAb CR3022 was used to confirm the presence of the virus, and the EIS technique was used for detection. We tested 24 patient saliva samples, which had previously been tested for SARS-CoV-2 using the loop-mediated isotherm nucleic acid amplification (LAMP) method. These samples were used to evaluate the ability of E-MIPs to discriminate between positive and negative cases of COVID-19. The detection limit of E-MIPs for the presence of SARS-CoV-2 in saliva samples was 4.9 log 10 pfu mL^{-1}. This suggests that the E-MIP approach is suitable for the detection of SARS-CoV-2 with minimal sample preparation and can be used as an effective tool for the diagnosis of COVID-19. It is important to note that the limit of detection may vary depending on experimental conditions and sample characteristics, and although the limit of detection is good, the sensitivity in relation to samples with lower viral loads may be a point of attention. The E-MIP approach provides a rapid readout of SARS-CoV-2 detection in less than 10 minutes, making it a rapid and efficient method for diagnosing COVID-19.

Electrochemical detection using molecularly imprinted polymers (MIPs) has demonstrated promising sensitivity and selectivity, opening avenues for future applications in biomarker detection and disease diagnosis, including the identification of SARS-CoV-2 infection. MIPs are easy to prepare, cost-effective and stable under adverse conditions.

The company MIP Diagnostics has launched commercial nanoMIPs priced at £990.00, highlighting on its website that the device developed has demonstrated an extremely low detection limit of 5 fg mL^{-1} in a sensor device and a 20-fold improvement in sensitivity when compared directly with commercial antibodies, without cross-reactivity with coronavirus variants 299E, HKU1 or OC43.

It is important to recognize that employing an epitope as a template in MIPs may offer advantages, but may also lead to nonspecific interactions between the virus and the polymer. Although real-time polymerase chain reaction (RT-PCR) remains the gold standard for determining the SARS-CoV-2 viral load, the potential for MIPs to replace this method is still in question. Alternatively, the combination of MIPs with lateral flow assays presents an intriguing prospect as a more cost-effective and stable alternative to antigen testing. This integration could offer a synergistic approach, capitalizing on the strengths of both technologies to improve diagnostic capabilities in the context of SARS-CoV-2 detection.

2 Electrochemical biosensors using ferri/ferrocyanide probes in COVID-19

Most of the studies described so far use electrochemical probes in detection; specific examples of how the probes are used and their purpose are discussed in this section. In biosensors, complementary redox electrochemical probes are generally used to detect viral proteins, genomes, or antigens at the working electrode.[79] The ferri/ferrocyanide redox couple ($K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$) is an electrochemical probe well established in the literature and widely used in biosensors, due to its simplicity, stability, reversible heterogeneous kinetics, as well as well-defined redox processes facilitating the detection of species.[70,75,80] An example of the use of probes in biosensors is the work of Eissa S, *et al.*[80] where a ferri/ferrocyanide redox pair was used for electrochemical detection, using the square wave voltammetry technique. Detection was determined based on the percentage change in the peak current of decreasing signals the immunosensor response; ($i - i_0/i\%$) after binding the immunosensor with the virus. The limit of detection (LOD) of the biosensor was determined to be 0.4 pg mL^{-1}, which is much lower than the LOD of the ELISA for Bioss Inc.'s SARS-CoV-2 N protein, which has a reported LOD of 0.4 ng mL^{-1}. This indicates that the biosensor is highly sensitive and can detect very low levels of SARS-CoV-2 nucleocapsid antigen in nasopharyngeal samples. Furthermore, the biosensor presented a good linear sensitivity range, ranging from 1.0 pg mL^{-1} to 100 ng mL^{-1}. In voltammetric biosensors, a potential is applied, and the interaction between the analyte and the bioreceptor is detected by the current change.[81]

In the work of Zukauskas *et al.* (2023)[64] mentioned in the previous section, the ferri/ferrocyanide pair probe was used to detect antibodies against the SARS-CoV-2 spike protein in

human serum samples, through a self-assembled layer of MAA and 6-MCOH in a gold disc electrode. These antibodies can recognize and specifically bind to the spike protein present in human serum samples, generating a change in the electrical current, observed by the change in the peak provided by the electrochemical probe. This change can be measured by different electrochemical methods. This work was described in more detail in the previous section. Due to the reversible redox behavior that the ferri/ferrocyanide pair probe exhibits, it was possible to evaluate the quality of the electrode surface and the efficiency of the immobilization process, providing valuable information about the performance of the biosensor.

Another example of the use of electrochemical probes is the work of Lomae *et al.* (2023)[17] (Table 1). In this work, detection occurs by capturing the target complementary DNA. A disposable paper-based device was used as a working electrode, directly modified with a pyrrolidinyl peptide nucleic acid (acpcPNA) as a biological recognition element. This work was described in more detail in the previous section. The use of the ferri/ferrocyanide pair probe in this work offers several advantages. The negatively charged ferrocyanide molecule repels the negatively charged DNA structure, resulting in a change in the electrochemical response upon DNA hybridization. This enables label-free DNA detection without the need for additional reagents or labels. The ferri/ferrocyanide pair probe is highly sensitive to changes in the local environment, making it an ideal choice for detecting small changes in the electrochemical response following DNA hybridization.

Another material that can be used as an electrochemical probe is methylene blue (MB). The work of Heo *et al.* (2022)[82] is one example, where they implemented CRISPR/Cas13a *trans*-cleavage activity to quantify SARS-CoV-2 RNA in spiked artificial saliva samples. Here, a screen-printed carbon electrode (SPCE) was modified with nanocomposites and gold nanoflowers (AuNFs) and coated with streptavidin (SA). Reporter RNA (reRNA) tagged with methylene blue (MB), which plays a role as a redox probe, and biotin was immobilized onto the electrode through the SA. The crRNA was complementary to the SARS-CoV-2 target sequence, so the Cas13a-crRNA complex was able to recognize it specifically and was activated by hybridization with the target RNA after incubation at 37 °C for 1 h 30 min. This activation set off the non-specific cleavage of reRNA on the biosensor surface. Electrochemical detection was performed through DPV, and the removal of MB from the surface resulted in a current reduction correlated with gene concentration. The LOD was estimated to be 0.044 fg mL^{-1} for ORF genes and 0.081 fg mL^{-1} for S genes.

During the COVID-19 pandemic, many biosensors based on the impedimetric response using electrochemical probes were developed. For example, Zaccariotto *et al.*[83] developed an electrochemical immunoassay to detect the SARS-CoV-2 RBD protein in saliva, using antibodies immobilized on reduced graphene oxide (rGO) and a redox couple ($[[Fe(CN)_6)]^{3-/4-}$) as a probe. Fig. 4 and 5 show the development and detection processes of the developed electrode. First, the EIS and VC techniques (Fig. 4(A) and (B)) were used to monitor the stages of the SARS-CoV-2 immunosensor assembly process. The CV shows well-defined oxidation and reduction peaks due to the Fe^{3+}/Fe^{2+} pair; in the first modification there is an increase in current caused by the presence of rGO, and after immobilization of the antibody, followed by blocking the surface with BSA and the incubation step of the RBD protein (antigen) of the SARS-CoV-2 spike protein, a decrease in the anodic and cathodic peak currents of the redox couple is observed. This decrease occurs due to the biomolecules acting as an obstacle to the transfer of electrons at the electrode-solution interface.

The analytical performance was evaluated using the EIS technique (Fig. 5(A)); it is noted that the R_{ct} values increase with increasing antigen concentration, indicating dependence on the target concentration. The frequency varied from 10 MHz to 0.01 Hz, and an amplitude of 10 mV was applied. The detection limit (calculated as LOD = 3SDblank/slope) obtained for the lowest antigen concentrations was 150 ng mL^{-1}, and the analytical curves used presented a linearity factor of 0.16 to 1.25 μg mL^{-1} and 2.5 to 40 μg mL^{-1} (Fig. 5(B) and (C)).[83] The LOD and LOQ values found in this work are relatively high compared to those in other studies in the literature. It is important to highlight that the objective of this work was to develop a low-cost and highly sensitive detection method for SARS-CoV-2, using accessible materials and simple electrochemical techniques. In this sense, the LOD and LOQ values found are suitable for detecting the virus in saliva samples, which is a less complex medium than clinical samples, such as blood or serum.

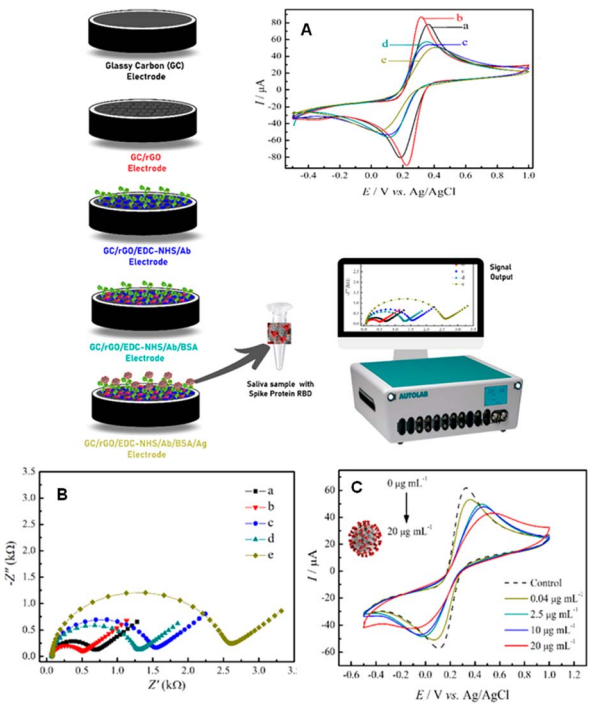

Fig. 4 CV (A) and EIS (B) electrochemical characterization of the immunosensor fabrication in 0.2 mol L^{-1} PBS pH 7.4, 0.1 mol L^{-1} KCl containing 5.0 mmol L^{-1} of $[Fe(CN_6)]^{3-/4-}$, (C) CV experiments of the GC/rGO-EDC-NHS/Ab/BSA electrode (control) and after the incubation of different antigen concentrations (image taken from the Zaccariotto *et al.* article[83]).

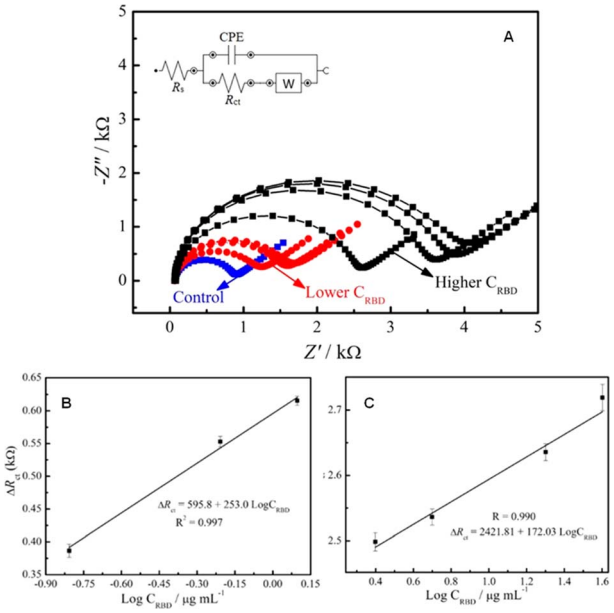

Fig. 5 (A) EIS responses of the impedimetric immunosensor with different concentrations of the SARS-CoV-2 spike protein, with insertion of the equivalent circuit used. The respective calibration curves plotted between the ΔR_{ct} and logarithmic concentration of SARS-CoV-2 spike protein from (B) 0.16 to 1.25 μg mL^{-1}, and (C) 2.5 to 40 μg mL^{-1}. (image taken from the Zaccariotto *et al.* article[83]).

Other studies that also use impedimetric techniques and probes are described hereafter. Brazaca *et al.* (2022)[19] developed a low-cost immunosensor, based on screen-printed carbon electrodes (SPCEs) modified with gold (Table 1), where the detection of protein S was performed by means of the EIS technique, using a hexacyanoferrate(II) probe/(III) of potassium, and an LOD of 3.16 pmol L^{-1} was obtained.[18]

Kiew L.-V *et al.*[84] used a thin palladium nanofilm electrode coated with angiotensin 2 (ACE2) and the redox couple $[(Fe(CN)_6)]^{3-/4-}$ as a probe to detect the spike protein (expressed through the *Escherichia coli* system). The electrode developed had as its central focus the screening of potential inhibitors against the S-protein-ACE2 binding. The platform can detect small analyte interference against S-ACE2 protein binding at low analyte concentration and small volume (0.1 μg mL^{-1} and ~1 μL, estimated total analyte consumption <4 pg). Some potential pharmacological interferents were also tested, such as ramipril and perindopril, and their active metabolites ramiprilat and perindoprilat, which suppress the binding of SARS-CoV-2-ACE2 were successfully identified, not causing significant interference in detection. In this work only *in vitro* tests were carried out; *in vivo* and clinical investigations are required to confirm the veracity of the developed platform.

Li *et al.* (2021)[85] developed a paper-based biosensor with zinc oxide nanowires (ZnO NWs) grown directly onto the working electrodes (WEs). The ZnO NWs are fabricated through a hydrothermal growth method, thus combining cheap materials such as paper and easily fabricated nanostructures. The SARS-CoV-2 spike protein RBD (receptor binding domain) was employed as a bioreceptor, immobilized through an organosilane-based surface chemistry process. The goal was to detect anti-spike antibodies in spiked human serum using EIS as a sensing technique with $[Fe(CN)_6]^{3-}$ as an electron mediator. The limit of quantitation (LOQ), which is the lowest concentration of the analyte that can be quantitatively detected with stated accuracy and precision, was 10 ng mL^{-1} achieved in under 30 minutes.

Kilic *et al.* (2021)[86] developed an electrochemical biosensor based on zwitterionic polypyrrole (ZiPPy) to detect anti-spike protein antibodies in saliva. The modified conducting polymer could be easily electropolymerized and exhibited higher hydrophilicity, hindering non-specific protein adsorption on the working electrode, therefore improving the signal-to-background noise ratio. To achieve spike protein immobilization onto the working electrode, the researchers mixed the biomolecules with the ZiPy monomers, so they were bound by entrapment. Patient saliva samples were tested. The signal readout technique used was EIS with $[Fe(CN)_6]^{3-}$ as the redox indicator, achieving a LOD of 50 ng mL^{-1}.

Rashed *et al.* (2021)[71] (Table 1) modified a commercially available 16-well plate from ACEA Biosense by coating the wells with RBD protein. The welled plate included interdigitated gold electrodes merged to polyethylene terephthalate (PET). The performance of the device was evaluated with six clinical samples of human serum, and the detection was obtained through continuous impedance measurements at fixed frequencies to simplify the hardware and facilitate the development of portable devices. The detections were compared with ELISA measurements on the same tested human serum samples, but the results of limits of detection and quantification are not demonstrated in the work, and the author only mentions that the results show a clear correlation between the impedance values and the concentration of antibodies in the sample.

The authors were able to detect the samples containing 0.1 μg mL^{-1} of CR3022 antibody, which is a monoclonal anti-SARS-CoV-2 antibody. They reported that the platform was able to differentiate spikes in impedance measurements from a negative control (1% milk solution) for all CR3022 samples. However, they did not explicitly state the minimum detected value or the limit of detection for their method. They only mentioned that their experiments demonstrated that the implementation of electrochemical impedance spectroscopy (EIS) can be used to detect clinically relevant antibody concentration.

The article presents a novel and promising approach for point-of-care diagnosis and monitoring of COVID-19 patients. However, the study has some limitations that need to be addressed. First, the sample size of the clinical specimens is very small ($n = 6$) and may not be representative of the general population. Second, the authors do not provide any information on the specificity and sensitivity of their method, which are crucial parameters for evaluating the accuracy and reliability of any diagnostic test. Third, the authors do not compare their method with other existing or emerging methods for SARS-CoV-2 antibody detection, such as lateral flow assays, fluorescence immunoassays, or biosensors based on other transduction

mechanisms. Therefore, the article could be improved by increasing the sample size, reporting the specificity and sensitivity of the method, and conducting a comprehensive comparison with other methods.

Each work presents a unique approach, highlighting the versatility and effectiveness of the probes in different contexts. This type of biosensor can reach low detection limits, is less reactive and takes less time compared to RT-PCR, presenting great potential for large-scale production.[60,61,70,83] An innovation in the area of electrochemical biosensors is the possibility of not using probes in the detection process, but rather materials that undergo redox reactions on their own, making the process more economical. Biosensors without the use of probes such as the ferri/ferrocyanide pair and methylene blue are described below.

3 Impedimetric biosensors for direct detection of COVID-19

Impedimetric biosensors can be developed using conductive polymers, which undergo redox reactions by themselves, presenting an R_{ct} value, making the use of electrochemical probes unnecessary. Conductive polymers are excellent materials for biosensor construction, having high conductivity, stability, cost-effectiveness and biocompatibility.[23] In these biosensors, information about the analyte is also detected by using variations in resistances in the system charge transfer processes, which occur at the electrode/electrolyte interface. Thus, when the interaction between the bioreceptor and the analyte (generally an insulating biomolecule) occurs, there are changes in the working electrode surface impedance, making the charge transfer process difficult, increasing the R_{ct} value, which is proportional to the amount of antigen/antibody interactions. In this type of biosensor, there is no simple absorption of the antigen on the surface of the electrode, and there is normally an interaction between the antigen and the conducting polymer through covalent bonds between functional groups present on the surface of the polymer and functional groups present on the antigen, ensuring greater specificity of analysis.[87,88]

In the case of COVID-19, impedimetric biosensors were designed with modified electrodes functionalized with biological receptors, such as antibodies or oligonucleotides. An example of the use of conductive polymers is the work of Hryniewicz B. M. *et al.*[23] (Table 1) which tested two different morphologies of the conducting polymer polypyrrole (PPy), globular and nanotubular (NT) morphology, modified with gold nanoparticles, to detect SARS-COV-2 antibodies in patient serum. SARS-CoV-2 nucleocapsid protein (N) was immobilized *via* the self-assembled thiol monolayer (SAM) methodology. This methodology was used to allow the covalent binding of the antigen to the electrode surface. The antibody, in turn, binds to the antigen immobilized on the electrode surface, forming an antigen–antibody complex. This antigen–antibody interaction affects the charge transfer between the electrode and the solution, resulting in a change in the electrical impedance measured by the biosensor. This change in electrical impedance is then used to detect and quantify the presence of antibodies specific to SARS-CoV-2 in clinical samples. Both materials showed good sensitivity and ability to quantify 7.442 and 0.4 ng mL^{-1} of monoclonal antibody to PPy in globular morphology and NT, respectively. The PPy-NTs/AuNP electrode showed better detectability, with better detection and quantification limit values and greater sensitivity than the PPy:PSS/AuNP electrode, being tested in serum samples obtained from hospitalized patients. The PPy-NTs/AuNP platform was able to identify all positive and negative serum samples tested successfully. The highlight of the work is the use of polypyrrole structures decorated with gold nanoparticles for the serological diagnosis of COVID-19. This innovative approach uses impedimetric biosensors and immunosensors to detect the presence of the virus, offering a promising alternative to traditional materials.

Santos A. *et al.*[88] used polypyrrole nanotubes modified with $Ni(OH)_2$ for the detection of SARS-CoV-2. The entire process of expression, purification and characterization of the antigen-binding fragment of heavy chain-only antibodies (VHH) called Sb#15 and its interaction with the receptor-binding domain (RBD) of SARS-CoV-2 was described in the article. Bioreceptor immobilization followed the strategy of using histidine-tag (His-tag), an interesting and accessible method of immobilization. Since it focuses on the protein orientation, the process is shown in Fig. 6(A), and $Ni(OH)_2$ was used to correctly guide the immobilization of the antibody on the surface of the biosensor, so as to avoid the loss of antigen-binding activity and increase sensitivity. All the steps of biosensor construction were characterized by EIS and Fourier-transform infrared (FTIR) spectroscopy. Infrared characterization indicated the interaction of antibodies and VHH with Ni(OH), and immobilization was confirmed by the EIS technique.

Different concentrations of the RBD were used for detection by biosensors, and a change in R_{ct} is evident (Fig. 6(B)), where there is a tendency for the diameter of the semicircle to increase

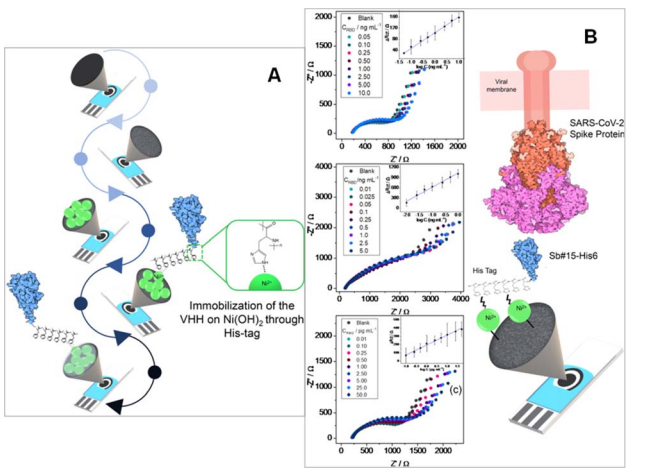

Fig. 6 (A) Schematic representation of biosensor development, (B) EIS response to different spike protein RBD concentrations in PBS, the corresponding analytical curves are shown as insets of the figures, and the representation of the fabricated biosensor using Sb#15-His6 (not-to-scale). PBS, phosphate buffer saline; RBD, receptor-binding domain (image taken from the Santos A. *et al.* article[88]).

with increasing concentrations of the RBD, indicating that EIS is a good technique for detecting different quantities from the RBD. The detection of the RBD spike protein of SARS-CoV-2 was carried out in saliva samples from patients infected by SARS-CoV-2, with a sensitivity of 93% and specificity of 100%, and the limit of quantification was determined to be 0.01 pg mL^{-1} using recombinant RBD. The work presents an innovative immobilization strategy, using histidine to immobilize the VHH Sb#15 antibody on the surface of the electrode modified with $Ni(OH)_2$ through coordination interactions with the nickel ion, which results in increased sensitivity and the specificity of the device. The use of PPy gave the biosensor the ability to detect changes in the system's electrical impedance, allowing the detection of very low concentrations of antigen. Furthermore, the work presents a detailed characterization of the materials used in all stages of the biosensor, a difference in relation to other works in the literature, which often provide superficial details.

Perdomo *et al.*,[89] developed a portable bioprinted (SPCE) bioprinted carbon-based working electrode modified with *para*-aminobenzoic acid (PABA); in this study, PABA was chemically activated with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS) was added to increase the efficiency of EDC reactions, monoclonal antibody (mAb) was incubated on the surface, and bovine serum albumin (BSA) was used to passivate the carboxylic vacancies of the polymer. The developed biosensor was capable of rapid diagnosis (~10 min), with high analytical sensitivity (LOD = 1.065 fg mL^{-1} and LOQ = 3.6 fg mL^{-1}) and selectivity for SARS-CoV-2(S). The analysis time can be considered a significant improvement over real-time PCR tests, which can take 2 to 3 hours. The use of the EIS technique in the work allowed the detection of molecular interactions at very low levels, which is crucial for the early detection of SARS-CoV-2 infections, in addition to enabling a quick and effective diagnostic time.

It is noteworthy that biosensors using materials that do not require an electrochemical probe, facilitate the detection process, leading to a simple methodology and low cost. There are few studies in the literature that do not use an electrochemical probe to detect COVID-19 when compared to the studies that do use it. The use of materials that function as probes, such as conductive polymers, without the need to add a redox couple, presents great potential for study, not only for detecting COVID-19 but for other diseases, whether infectious or not. When comparing the ELISA method used at the beginning of the pandemic with the new biosensor methodologies that emerged during the pandemic period, we can note the potential of these developed devices, such as faster detection, in addition to the cost-benefit.

4 Conclusion

This work highlights the growing relevance of electrochemical biosensors during the COVID-19 pandemic. By combining electrochemical sensitivity with the specificity of biological recognition elements, these devices have great potential for the rapid and accurate detection of SARS-CoV-2 and its associated biomarkers. In this review, several examples of electrochemical biosensors applied to COVID-19 were presented, with a focus on faradaic biosensors, including those that use redox probes such as the ferri/ferrocyanide pair, as well as those that use conductive polymers as the electrode material. These varied approaches underscore the versatility of these devices to meet specific diagnostic and monitoring needs in different clinical settings. The ability to detect SARS-CoV-2 at low concentrations and in complex samples, such as saliva and serum, makes these devices valuable allies in tracking and controlling the spread of the virus, and their selectivity contributes to the improvement of diagnosis in clinical practice. In addition, the speed and ease of operation of these biosensors allow for large-scale testing, making them promising candidates for implementation in screening and monitoring programs in high-risk communities and environments.

5 Future perspectives

Impedimetric biosensors have proven to be a simple, low-cost methodology with great potential for the direct detection of COVID-19. But even in the market these biosensors are not yet widespread; the big challenge is in the manufacture of portable devices of these biosensors, in addition to improving the materials used in their manufacture, which can detect infectious diseases such as COVID-19 efficiently in any environment, even if there is a large amount of interference in the sample. The advances in biosensors shown in this work offer promising perspectives for the development and application of detection methods for other infectious diseases in the future.

Author contributions

T. L. V.; R. A.; S. S. S. (conceptualization, investigation, writing – original draft), C. C. O. (supervision, writing – review & editing), M. V. (supervision, conceptualization, writing – review & editing).

Conflicts of interest

The authors state that there are no conflicts to declare.

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References

1 N. T. P. Mishra, S. S. Das, S. Yadav, W. Khan, M. Afzal, A. Alarifi, E.-R. kenawy, M. T. Ansari, M. S. Hasnain and A. K. Nayak, *Sens. Int.*, 2020, **1**, 100042.
2 S. Shanmuga Priya, E. Cuce, K. Sudhakar and K. Sudhakar, *Int. J. Sustain. Eng.*, 2021, **14**, 1290–1305.

3 O. Byambasuren, M. Cardona, K. Bell, J. Clark, M.-L. McLaws and P. Glasziou, *J. Assoc. Med. Microbiol. Infect. Dis. Can.*, 2020, **5**, 223–234.
4 Z. Gao, Y. Xu, C. Sun, X. Wang, Y. Guo, S. Qiu and K. Ma, *J. Microbiol., Immunol. Infect.*, 2021, **54**, 12–16.
5 R. Wang, J. Chen, Y. Hozumi, C. Yin and G.-W. Wei, *J. Phys. Chem. Lett.*, 2020, **11**, 10007–10015.
6 M. Alhajj, M. Zubair and A. Farhana, *Enzyme Linked Immunosorbent Assay*, Island, 2023.
7 M. Mostafa, A. Barhoum, E. Sehit, H. Gewaid, E. Mostafa, M. M. Omran, M. S. Abdalla, F. M. Abdel-Haleem, Z. Altintas and R. J. Forster, *TrAC, Trends Anal. Chem.*, 2022, **157**, 116750.
8 F. Gong, H. Wei, Q. Li, L. Liu and B. Li, *Front. Mol. Biosci.*, 2021, **8**, 682405.
9 C. de la Guardia, G. Rangel, A. Villarreal, A. Goodridge, P. L. Fernández and R. Lleonart, *PLoS One*, 2021, **16**, e0257351.
10 Priyanka, B. Mohan, E. Poonia, S. Kumar, Virender, C. M. Singh, J. Xiong, X. Li and G. Singh, *J. Fluoresc.*, 2023, **34**(2), 479–500.
11 H. K. Choi, M.-J. Lee, S. N. Lee, T.-H. Kim and B.-K. Oh, *Front. Chem.*, 2021, **9**, 672739.
12 S. Imran, S. Ahmadi and K. Kerman, *Micromachines*, 2021, **12**, 174.
13 L. Xu, D. Li, S. Ramadan, Y. Li and N. Klein, *Biosens. Bioelectron.*, 2020, **170**, 112673.
14 R. Samson, G. R. Navale and M. S. Dharne, *3 Biotech*, 2020, **10**, 385.
15 M. A. MacMullan, A. Ibrayeva, K. Trettner, L. Deming, S. Das, F. Tran, J. R. Moreno, J. G. Casian, P. Chellamuthu, J. Kraft, K. Kozak, F. E. Turner, V. I. Slepnev and L. M. Le Page, *Sci. Rep.*, 2020, **10**, 20818.
16 M. Z. Rashed, J. A. Kopechek, M. C. Priddy, K. T. Hamorsky, K. E. Palmer, N. Mittal, J. Valdez, J. Flynn and S. J. Williams, *Biosens. Bioelectron.*, 2020, **171**, 112709.
17 A. Lomae, P. Preechakasedkit, O. Hanpanich, T. Ozer, C. S. Henry, A. Maruyama, E. Pasomsub, A. Phuphuakrat, S. Rengpipat, T. Vilaivan, O. Chailapakul, N. Ruecha and N. Ngamrojanavanich, *Talanta*, 2023, **253**, 123992.
18 L. C. Brazaca, A. H. Imamura, N. O. Gomes, M. B. Almeida, D. T. Scheidt, P. A. Raymundo-Pereira, O. N. Oliveira, B. C. Janegitz, S. Antonio and E. Carrilho, *Anal. Bioanal. Chem.*, 2022, **414**, 5507–5517.
19 Z. Rahmati, M. Roushani, H. Hosseini and H. Choobin, *Microchim. Acta*, 2021, **188**, 1–9.
20 A. R. Cardoso, J. F. Alves, M. F. Frasco, A. M. Piloto, V. Serrano, D. Mateus, A. I. Sebastião, A. M. Matos, A. Carmo, T. Cruz, E. Fortunato and M. G. F. Sales, *Mater. Today Bio*, 2022, **16**, 100354.
21 D. Najjar, J. Rainbow, S. Sharma Timilsina, P. Jolly, H. de Puig, M. Yafia, N. Durr, H. Sallum, G. Alter, J. Z. Li, X. G. Yu, D. R. Walt, J. A. Paradiso, P. Estrela, J. J. Collins and D. E. Ingber, *Nat. Biomed. Eng.*, 2022, **6**, 968–978.
22 L. Villafañe, L. G. Vaulet, F. M. Viere, L. I. Klepp, M. A. Forrellad, M. M. Bigi, M. I. Romano, G. Magistrelli, M. R. Fermepin and F. Bigi, *J. Immunol. Methods*, 2021, **500**, 113182.
23 B. M. Hryniewicz, J. Volpe, L. Bach-Toledo, K. C. Kurpel, A. E. Deller, A. L. Soares, J. M. Nardin, L. F. Marchesi, F. F. Simas, C. C. Oliveira, L. Huergo, D. E. P. Souto and M. Vidotti, *Mater. Today Chem.*, 2022, **24**, 100817.
24 J. Baranwal, B. Barse, G. Gatto, G. Broncova and A. Kumar, *Chemosensors*, 2022, **10**, 363.
25 S. Mao, L. Fu, C. Yin, X. Liu and H. Karimi-Maleh, *RSC Adv.*, 2022, **12**, 22592–22607.
26 V. Vásquez and J. Orozco, *Anal. Bioanal. Chem.*, 2023, **415**, 1003–1031.
27 H. Ilkhani, N. Hedayat and S. Farhad, *Anal. Biochem.*, 2021, **634**, 114362.
28 E. B. Bahadır and M. K. Sezgintürk, *Artif. Cells, Nanomed., Biotechnol.*, 2014, **44**, 248–262.
29 J.-G. Guan, Y.-Q. Miao and Q.-J. Zhang, *J. Biosci. Bioeng.*, 2004, **97**, 219–226.
30 A. P. F. Turner, *Chem. Soc. Rev.*, 2013, **42**, 3184.
31 N. J. Ronkainen, H. B. Halsall and W. R. Heineman, *Chem. Soc. Rev.*, 2010, **39**, 1747.
32 A. Khanmohammadi, A. Jalili Ghazizadeh, P. Hashemi, A. Afkhami, F. Arduini and H. Bagheri, *J. Iran. Chem. Soc.*, 2020, **17**, 2429–2447.
33 S. S. Timilsina, N. Durr, P. Jolly and D. E. Ingber, *Biosens. Bioelectron.*, 2023, **223**, 115037.
34 World Health Organization, *Antigen-detection in the Diagnosis of SARS-CoV-2 Infection Interim Guidance*, 2021.
35 Y. Rasmi, X. Li, J. Khan, T. Ozer and J. R. Choi, *Anal. Bioanal. Chem.*, 2021, **413**, 4137–4159.
36 J. H. Kim, Y. J. Suh, D. Park, H. Yim, H. Kim, H. J. Kim, D. S. Yoon and K. S. Hwang, *Biomed. Eng. Lett.*, 2021, **11**, 309–334.
37 J. Kudr, P. Michalek, L. Ilieva, V. Adam and O. Zitka, *Trends Anal. Chem.*, 2021, **136**, 116192.
38 J. R. Choi, *Front. Chem.*, 2020, **8**, 517.
39 S. Madhurantakam, S. Muthukumar and S. Prasad, *ACS Omega*, 2022, **7**, 12467–12473.
40 D. Hetemi and J. Pinson, *Chem. Soc. Rev.*, 2017, **46**, 5701–5713.
41 P. Pengo, M. Şologan, L. Pasquato, F. Guida, S. Pacor, A. Tossi, F. Stellacci, D. Marson, S. Boccardo, S. Pricl and P. Posocco, *Eur. Biophys. J.*, 2017, **46**, 749–771.
42 R. Dubey, D. Dutta, A. Sarkar and P. Chattopadhyay, *Nanoscale Adv.*, 2021, **3**, 5722–5744.
43 M. Soler and L. M. Lechuga, *Anal. Bioanal. Chem.*, 2021, **414**, 5071–5085.
44 A. Kheyraddini Mousavi, Z. C. Leseman, M. L. B. Palacio, B. Bhushan, S. R. Schricker, V.-B. Sundaresan, S. A. Sarles, D. J. Leo, S. Neethirajan, D. Karig, A. Kumar, P. P. Mukherjee, S. T. Retterer, M. J. Doktycz, A. G. Katsiamis, E. M. Drakakis, R. F. Lyon, R. Rica, S.-H. Yoon, M. R. K. Mofrad, J. Casas, C. Liu, G. Krijnen, M. K. Ramasubramanian, R. Agarwala, Y. Bar-Cohen, B. Bhushan, P. Becker, E. Hennebert, P. Flammang, A. R. Parker, R. Picone, I. C. Gebeshuber, P. Gruber, B. Imhof, H. O. Fatoyinbo, M. P. Hughes, J. A. Sanz-

Herrera, E. Reina-Romo and B. Bhushan, *Encycl. Nanotechnol.*, 2012, 329–345.
45 A. Khan, E. DeVoe and S. Andreescu, *Sens. Diagn.*, 2023, **2**, 529–558.
46 S. Yadav, A. K. Saini, S. Devi and S. Lata, *Biomaterials-Based Sensors*, 2023, pp. 101–125.
47 S. Sarwar, M.-C. Lin, C. Amezaga, Z. Wei, E. Iyayi, H. Polk, R. Wang, H. Wang and X. Zhang, *Adv. Compos. Hybrid Mater.*, 2023, **6**, 49.
48 H. Aldewachi, T. Chalati, M. N. Woodroofe, N. Bricklebank, B. Sharrack and P. Gardiner, *Nanoscale*, 2018, **10**, 18–33.
49 E. Ferrari, *Biosensors*, 2023, **13**, 411.
50 J. Khan, Y. Rasmi, K. K. Kırboğa, A. Ali, M. Rudrapal and R. R. Patekar, *Beni-Suef Univ. J. Basic Appl. Sci.*, 2022, **11**, 111.
51 V. Naresh and N. Lee, *Sensors*, 2021, **21**, 1–35.
52 E. Cesewski and B. N. Johnson, *Biosens. Bioelectron.*, 2020, **159**, 112214.
53 S. Iravani, *Mater. Adv.*, 2020, **1**, 3092–3103.
54 S. Monteil, A. J. Casson and S. T. Jones, *PLoS One*, 2021, **16**, e0258002.
55 C. Sandford, M. A. Edwards, K. J. Klunder, D. P. Hickey, M. Li, K. Barman, M. S. Sigman, H. S. White and S. D. Minteer, *Chem. Sci.*, 2019, **10**, 6404–6422.
56 A. García-Miranda Ferrari, P. Carrington, S. J. Rowley-Neale and C. E. Banks, *Environ. Sci.*, 2020, **6**, 2676–2690.
57 J. Zoubir, I. Bakas, S. Qourzal, M. Tamimi and A. Assabbane, *J. Appl. Electrochem.*, 2023, **53**, 1279–1294.
58 H. Shand, S. Dutta, S. Rajakumar, S. James Paulraj, A. K. Mandal, R. D. KT and S. Ghorai, *Front. nanotechnol.*, 2021, **3**, 814550.
59 P. C. Ang, V. Perumal, M. N. M. Ibrahim, R. Adnan, D. K. Mohd Azman, S. C. Gopinath and P. B. Raja, *Appl. Microbiol. Biotechnol.*, 2023, **107**, 1503–1513.
60 Z. Štukovnik and U. Bren, *Int. J. Mol. Sci.*, 2022, **23**(24), 15922.
61 V. Ong, A. Soleimani, F. Amirghasemi, S. Khazaee Nejad, M. Abdelmonem, M. Razaviyayn, P. Hosseinzadeh, L. Comai and M. P. S. Mousavi, *Biosensors*, 2023, **13**(2), 204.
62 Md. M. Rahman, *Chemosensors*, 2022, **10**, 287.
63 Y. Mei, X. Lin, C. He, W. Zeng, Y. Luo, C. Liu, Z. Liu, M. Yang, Y. Kuang and Q. Huang, *Front. Bioeng. Biotechnol.*, 2022, **10**, 952510.
64 S. Zukauskas, A. Rucinskiene, V. Ratautaite, A. Ramanaviciene, G. Pilvenyte, M. Bechelany and A. Ramanavicius, *Int. J. Mol. Sci.*, 2023, **24**, 718.
65 C. Wu, Z. Chen, C. Li, Y. Hao, Y. Tang, Y. Yuan, L. Chai, T. Fan, J. Yu, X. Ma, O. A. Al-Hartomy, S. Wageh, A. G. Al-Sehemi, Z. Luo, Y. He, J.-F. Li, Z. Xie and H. Zhang, *Nano-Micro Lett.*, 2022, **14**, 159.
66 K. Zhang, Z. Fan, Y. Ding and M. Xie, *Chem. Eng. J.*, 2022, **429**, 132472.
67 C. Hwang, N. Park, E. S. Kim, M. Kim, S. D. Kim, S. Park, N. Y. Kim and J. H. Kim, *Biosens. Bioelectron.*, 2021, **185**, 113177.
68 Y. Peng, Y. Pan, Z. Sun, J. Li, Y. Yi, J. Yang and G. Li, *Biosens. Bioelectron.*, 2021, **186**, 113309.
69 Z. Song, Y. Ma, M. Chen, A. Ambrosi, C. Ding and X. Luo, *Anal. Chem.*, 2021, **93**, 5963–5971.
70 A. Yakoh, U. Pimpitak, S. Rengpipat, N. Hirankarn, O. Chailapakul and S. Chaiyo, *Biosens. Bioelectron.*, 2021, **176**, 112912.
71 M. Z. Rashed, J. A. Kopechek, M. C. Priddy, K. T. Hamorsky, K. E. Palmer, N. Mittal, J. Valdez, J. Flynn and S. J. Williams, *Biosens. Bioelectron.*, 2021, **171**, 112709.
72 R. Samson, G. R. Navale and M. S. Dharne, *3 Biotech*, 2022, **10**, 385.
73 M. do P. Ferreira, S. F. Yamada-Ogatta and C. R. Teixeira Tarley, *Biosensors*, 2023, **13**, 336.
74 C. Li, B. Che and L. Deng, *Biosensors*, 2022, **13**, 12.
75 A. Raziq, A. Kidakova, R. Boroznjak, J. Reut, A. Öpik and V. Syritski, *Biosens. Bioelectron.*, 2021, **178**, 113029.
76 A. G. Ayankojo, R. Boroznjak, J. Reut, A. Öpik and V. Syritski, *Sens. Actuators, B*, 2022, **353**, 131160.
77 V. Ratautaite, R. Boguzaite, E. Brazys, A. Ramanavicius, E. Čiplys, M. Juozapaitis, R. Slibinskas and M. Bechelany, *Electrochim. Acta*, 2021, **403**, 139581.
78 H. F. El Sharif, S. R. Dennison, M. Tully, S. Crossley, W. Mwangi, D. Bailey, S. P. Graham and S. M. Reddy, *Anal. Chim. Acta*, 2022, **1206**, 339777.
79 S. Imran, S. Ahmadi and K. Kerman, *Micromachines*, 2021, **12**(2), 174.
80 S. Eissa, H. A. Alhadrami, M. Al-Mozaini, A. M. Hassan and M. Zourob, *Microchim. Acta*, 2021, **188**, 199.
81 E. Sheikhzadeh, *Austin J. Infect. Dis.*, 2021, **8**, 1051.
82 W. Heo, K. Lee, S. Park, K. A. Hyun and H. Il Jung, *Biosens. Bioelectron.*, 2022, **201**, 113960.
83 G. C. Zaccariotto, M. K. L. Silva, G. S. Rocha and I. Cesarino, *Materials*, 2021, **14**, 4230.
84 L. V. Kiew, C. Y. Chang, S. Y. Huang, P. W. Wang, C. H. Heh, C. Te Liu, C. H. Cheng, Y. X. Lu, Y. C. Chen, Y. X. Huang, S. Y. Chang, H. Y. Tsai, Y. A. Kung, P. N. Huang, M. H. Hsu, B. F. Leo, Y. Y. Foo, C. H. Su, K. C. Hsu, P. H. Huang, C. J. Ng, A. Kamarulzaman, C. J. Yuan, D. Bin Shieh, S. R. Shih, L. Y. Chung and C. C. Chang, *Biosens. Bioelectron.*, 2021, **183**, 113213.
85 X. Li, Z. Qin, H. Fu, T. Li, R. Peng, Z. Li, J. M. Rini and X. Liu, *Biosens. Bioelectron.*, 2021, **177**, 112672.
86 T. Kilic, I. Gessner, Y. K. Cho, N. Jeong, J. Quintana, R. Weissleder and H. Lee, *Adv. Mater.*, 2022, **34**, 2107892.
87 R. Antiochia, *Bioelectrochemistry*, 2022, **147**, 108190.
88 A. Santos, A. P. Macedo de Souza Brandão, B. M. Hryniewicz, H. Abreu, L. Bach-Toledo, S. Schuster da Silva, A. E. Deller, V. Z. Rogerio, D. S. Baêta Rodrigues, P. M. Hiraiwa, B. G. Guimarães, L. F. Marchesi, J. Carvalho de Oliveira, D. F. Gradia, F. L. F. Soares, N. I. T. Zanchin, C. Camargo de Oliveira and M. Vidotti, *Mater. Today Chem.*, 2023, **30**, 101597.
89 S. A. Perdomo, V. Ortega, A. Jaramillo-Botero, N. Mancilla, J. H. Mosquera-Delacruz, D. P. Valencia, M. Quimbaya, J. D. Contreras, G. E. Velez, O. A. Loaiza, A. Gomez and J. De La Roche, *IEEE Trans. Instrum. Meas.*, 2021, **70**, 1–10.

