Use of metallic nanostructures in electrochemical biosensing of SARS-CoV-2

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Abstract In this chapter, we explore the conceptualization and development of electrochemical devices modification with metallic nanostructures, as well as the most relevant results regarding applications of biosensors for the determination of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), that contains these metallic particles. Some procedures and trends, new metallic nanostructures, biological species immobilization methods, and applications are described. Furthermore, the employment of these devices for SARS-CoV-2 determination and their advantages, as well as what perspectives are left to investigate in this area, are discussed in detail.

Keywords Electrochemical biosensors · Electrochemistry · Metallic nanoparticles · SARS-CoV-2

1. Introduction

Accompanying the rapid technological growth of the modern information era, worldwide scientists are constantly in the search for novel, fast, and more effective ways of detecting both common and uncommon diseases, especially with an early diagnosis, when the possibility emerges [1-3]. Among these technologies, electrochemical sensors and biosensors are advantageous devices, encompassing different fields of science, such as chemistry, biotechnology, and material science. These are commonly referred to as having a considerably faster response and simpler operation while maintaining reliable outcomes [4-6].

Electrochemical sensors are based on the translation of different variables resulting from a redox reaction occurring on the device surface, being it a voltammetric (electric current generation) [7, 8], potentiometric (different potentials shifts and other interactions) [9, 10] or impedimetric signal (resistance and capacitance terms of electrochemical impedance correlations) [11, 12]. Biosensors of this class operates in similar manners, but with the addition of a transducer in the system architecture, that is responsible for converting a biological process, such as enzyme activity or antigen-antibody interaction, into one of the previously mentioned signals.

Among already published works (Fig. 1) it is possible to note a considerable growth of electrochemical biosensors over the past 12 years, more than doubling the number of publications per year nowadays, with a substantial amount of new scientific papers being presented. Data was collected with the keywords *Electrochemical biosensor* with and without *SARS-CoV-2*, year by year, in the Web of Science database, April 2022. In the year 2020, there was an expected decrease in research being done, as the world was assailed by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing the World Health Organization to declare a pandemic state [13, 14]. This virus presents a quite fast human-to-human transmission and an estimated basic reproduction number of 2.2 [15]. This virus-provoked state has promoted the alteration of human behavior throughout the globe, favoring more healthy activities and increasing the use of transmission prevention methods.

However, the spread of the virus was and still is considerably difficult to contain, leading to the development of technologies for its detection, preferably with rapid diagnosis, since the viral attack occurs in little more than a week. Therefore, it is of little surprise that electrochemical biosensors (Fig. 1 inset) for the determination of SARS-

CoV-2 have emerged in the years following the outbreak of this danger. In the year 2021, nearly 3% of all electrochemical biosensors published were about the determination of this virus, which is an impressive feat for humankind, considering the range of diseases that can be investigated by electroanalytical techniques and the small-time invested to uncover a feasible device. By the start of this very year (2022), the scientific community has already surpassed the number of papers published, in this same regard, in 2019.

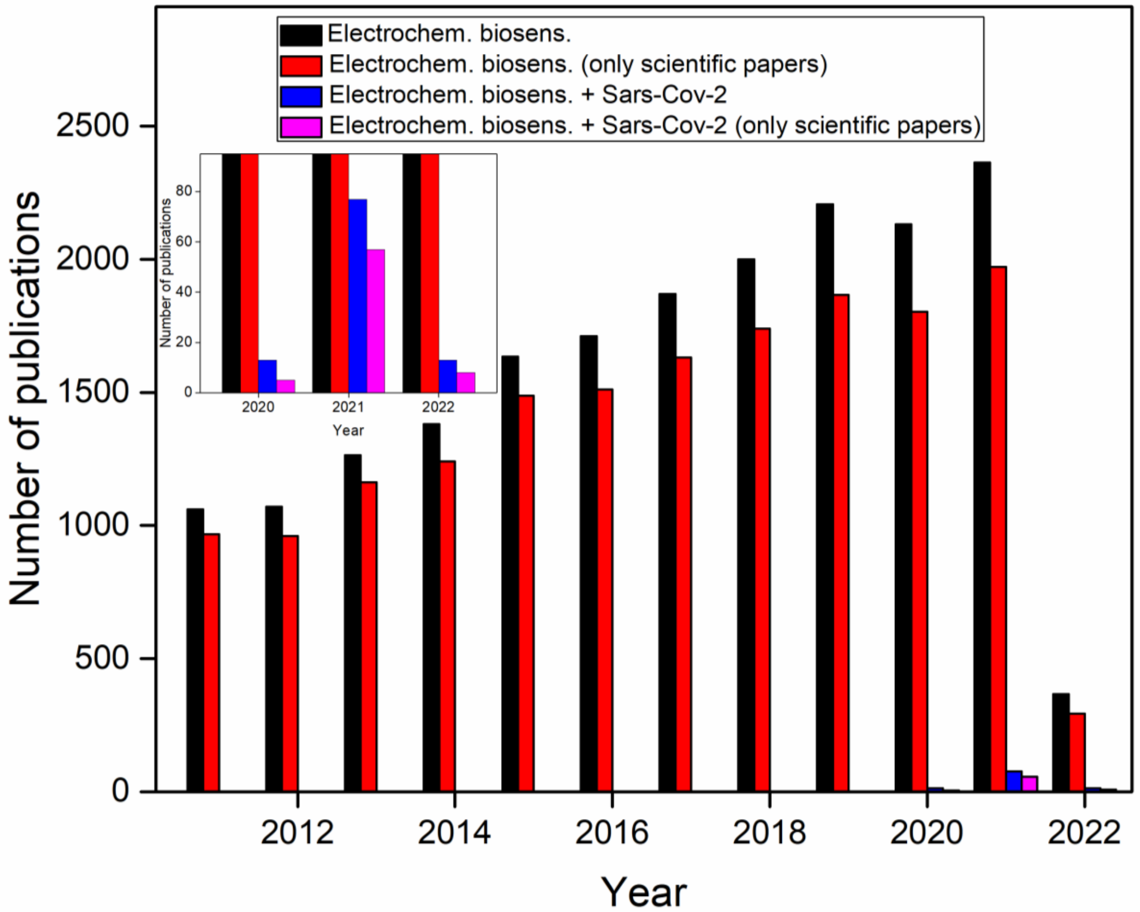

Fig. 1. Publications from the last 12 years in the electroanalytical field. Keywords: *Electrochemical biosensor*, *SARS-CoV-2*. Data from the Web of Science database

Among these SARS-CoV-2 electrochemical biosensors, some materials were more present than others in the literature, with emphasis on metallic nanoparticles. These nanoparticles differ not only in size but also in the properties they have [16]. Currently, nanoparticles are widely used for the development of nanotechnology, with potential application in several areas [17]. In electroanalysis, there are two most common ways of metallic nanoclusters synthesis, both from a bottom-up approach: the first are the wet chemical methods: the reduction of metal salts in solution, resulting in the aggregation of metal atoms in condition defining shaped colloids, that are suspended in the solution, and can be further extracted [16, 18]. As examples, the casting [19] and Langmuir-Blodgett techniques can be employed to generate film layers over the desired surface with such suspensions [20]. These methods can also present precursor particle seeds, which is a small cluster that will grow inside the solution. If a seed is present, the reduction of the metal atoms will occur over its surface, increasing the size of the seed [16]. Without it, seeds will be generated on higher-energy surfaces of the system. Fu et al. [21] demonstrated the synthesis of a heterogeneous nanoshell of Au and Pt, with the addition of trisodium citrate to a $HAuCl_4$•$3H_2O$, resulting in an immediate reduction of Au atoms and formation of clusters. The solution was separated by centrifugation and the supernatant was added to the H_2PtCl_6•$6H_2O$ solution, which was further reduced by ascorbic acid. This second reduction provoked the Pt atoms to reduce over the Au cluster surface, creating a particle with enhanced peroxidase-like behavior for SARS-CoV-2 colorimetric detection.

The second consists of the electrodeposition of metal atoms over the electrode surface, by the application of reduction-inducing potential energy (or range of). This causes the metal atoms to adsorb inactive surface sites, such as defects [18, 22]. For instance, Rafatmah and Hemmateenejad [23] explored the electrodeposition of AuNPs (AuNPs) over paper fibers surface in 1.0 mol L^{-1} $HClO_4$ solution. The paper data reveals that both the potentials explored (–0.08 and –0.2 V) created dendrite-like particles over the fibers when in presence of 1.0 mmol L^{-1} $HAuCl_4$, while a higher concentration of the salt (4.0 mmol L^{-1}) resulted in the production of more sphere-like particles.

Both methods depend on the cluster growth, controlled by several different properties, such as the diffusion of the metals in solution, the capping agents added, and the nature of the metals themselves. All these can cause different final structures to be produced, with various shapes and electronic behaviors [18, 24]. As an example, Qin et al [25] highlighted the effects of ascorbic acid pH on the Ag particles synthesis. The group studied the procedure in a pH range from 6.0 to 10.5 and highlighted that the lower the pH, the bigger and less active the Ag nanoparticle was (Fig. 2).

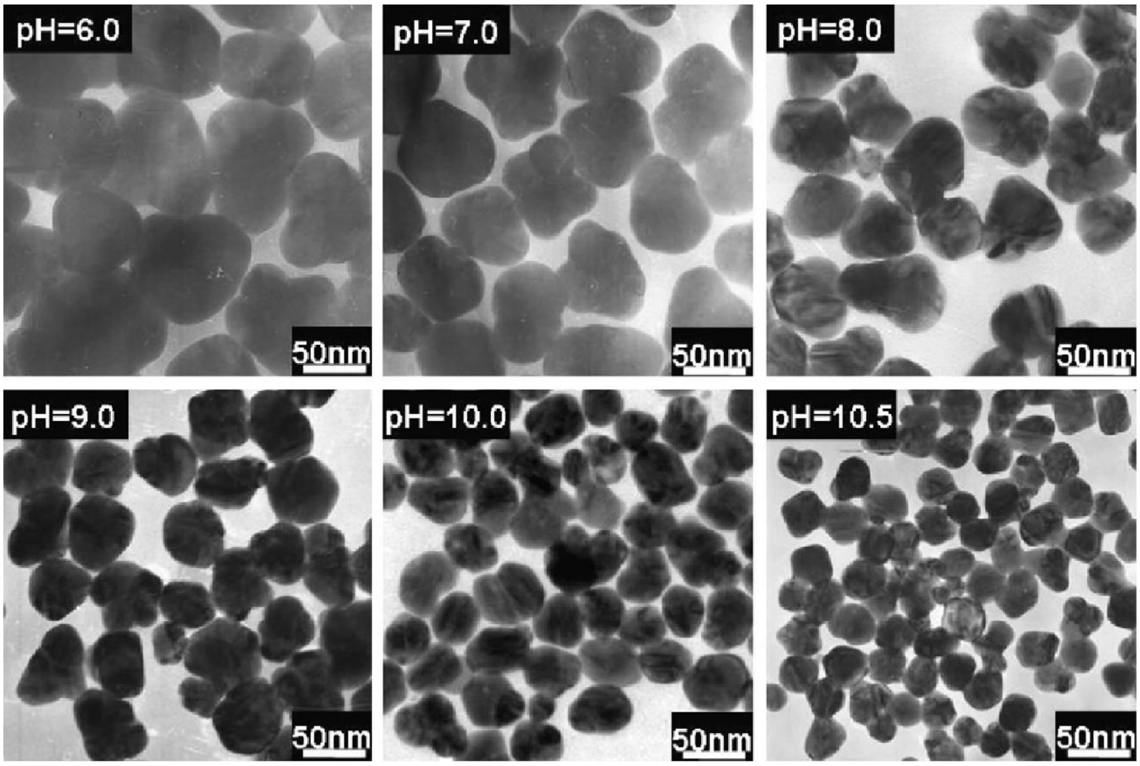

Fig. 2. Transmission electron microscopy images of prepared AgNPs at pH 6.0, 7.0, 8.0, 9.0, 10.0 and 10.5 by using ascorbate as reductant. (Reprinted from [25], with permission of Elsevier)

For all these factors, the methodologies for their synthesis can result in different behaviors that can be implemented for the development of biosensors. Several metallic particles present interesting properties of binding to biomolecules, such as antibodies and enzymes [26]. Also, They can be used as catalysts and amplifiers for the signals of interest [27]. The most used metals for the development of nanoparticles are Au [14], Ag [28], Cu [29], Fe [30], Pt [21] and Pd [31]. In this context, the most widely used for making biosensors for SARS-CoV-2 are AuNPs that present several synthesis methodologies, and are commonly stable in colloidal form. These nanostructures have been extensively researched in the literature. This consistent use of metals can be associated with the biocompatibility and toxicity that these type of nanoparticle shows. This behavior emerges from different properties between the particles and biologically produced structures, such as immune cells, proteins, and enzymes. According to Yoshioka et al. [32], the toxic or non-toxic effect of a nanoparticle can be observed by the length of exposition time, the particle concentration and the effects generated by these strange bodies in the organism. That is controlled by different variables, such as particle shape and size distribution, surface charge and activity, as well as agglomeration state and purity. This topic reveals several binding mechanisms of metallic nanoparticles to biological products that can work as transducers for electrochemical biosensors. For instance, Tenzer et al. [33] demonstrated that 30s after nanoparticles enters the blood plasma, a protein corona is formed by coordination around the particle, demonstrating the stability of such system.

Therefore, this chapter aims to highlight the most recent studies of SARS-CoV-2 electrochemical biosensors, that also employed metallic nanoparticles, to elucidate the topic and inform research paths for fellow readers and scientists.

2. Electrochemical determination of SARS-CoV-2 with metallic nanostructures

The rapid and reliable detection of SARS-CoV-2 in humans is necessary for adequate control of the infection since it has high levels of contagion [34]. In this sense, many studies have focused on the development of more sensitive devices, with reduced detection limits, to minimize erroneous results [35]. As already mentioned, among the biosensors that use metals for the construction of structures, that proposes the detection of SARS-CoV-2, Au-based ones are the most explored in the literature.

The selection of suitable sensory surfaces, which allows the integration of metallic nanostructures, in line with the use of different biomolecules immobilization strategies, is essential since this integration will play an important role in the development and performance of electrochemical biosensors [36]. Thus, biosensors based on metallic nanostructures can be promising alternatives, as these can promote catalytic activities, contain a better surface area, greater electrical conductivity, and electrochemical activity [37-39].

In addition, various types of molecules can be used to detect viral agents in biological samples, for example, a specific viral protein, antibodies, viral nucleic acid, or some specific biomarkers [40]. Thus, to design sensory systems that link the metal particles and their properties with covid-19 related biomolecules, various immobilization mechanisms have been employed in electrochemical biosensors, such as direct adsorption- or covalent binding-based methods [41].

Knowing that rapid and accurate detection is very important for the control of the pandemic, also to detecting SARS-CoV-2, the biosensors must be selective, like other viral diseases such as influenza A, MERS-CoV and *Streptococcus pneumoniae*. These illnesses present very similar symptoms, requiring tests to define the disease. In this way, the work of Karakuş et al. demonstrates a probe (AuNP-mAb) that exhibits a dual-sensing mode for the detection of SARS-CoV-2 spike antigen (S-Ag). The electrochemical detection was achieved by the probe solution developed on the commercially available and disposable screen-printed Au electrode without requiring any electrode preparation and modification. The AuNP-mAb was prepared following the procedure in (Fig. 3). The AuNPs-mAb conjugate was prepared by adding SARS-CoV-2 spike antibodies and AuNPs activated with 11-mercaptoundecanoic acid. After incubation time, the mAb (SARS-CoV-2 spike monoclonal antibody) reacted with AuNPs by covalent bond formation via EDC-NHS cross-linking agents. The developed system has been successfully applied to saliva samples and also offers simplicity, cost-effectiveness, and speed. Lastly, this system did not exhibit cross-reactivity with other viral proteins (i.e. influenza A, MERS-CoV, and Streptococcus pneumoniae). According to the authors, the created AuNP–mAb conjugates, in the presence of the SARS-CoV-2 peak antigen present the colorimetric effect. This interaction causes the AuNPs to aggregate quickly and irreversibly, by antibody-antigen interaction, changing the color from red to purple, which is possible to observe even with the naked eye.

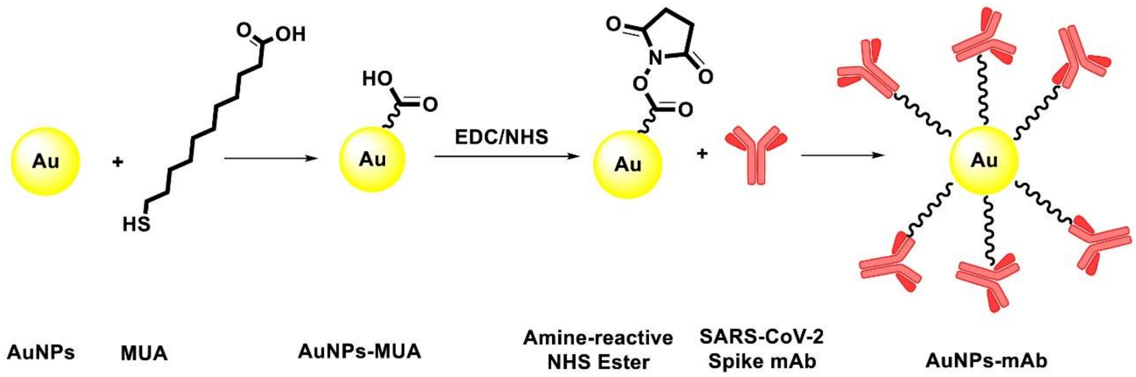

Fig. 3. Schematic illustration of the formation of AuNP–mAb conjugates. (Reprinted from [14], with permission of Elsevier)

One of the mechanisms of SARS-CoV-2 in the human body is the interaction with the cell surface angiotensin-converting enzyme 2 (ACE2), via spike protein, severely affecting the II alveolar cells [42]. Such fact implies that this interaction could be an immune system stimulator, and the work of Kiew et al. [43] aimed to explore it further. The researchers developed electrochemical impedance spectroscopy (EIS) based device, consisting of the interaction of a recombinant ACE2 and a Pd thin film over the electrode surface. The electrode was immersed in a $[Fe(CN_6)]^{3-}$ rich electrolyte solution (Fig. 4) and following the SARS-CoV-2 spike protein and ACE2 interaction, the technique revealed alterations in the impedance behavior of the system, aided by the production of

$[Fe(CN_6)]^{4-}$ at the electrode surface. The authors also reported several *in vitro* screening tests of different pharmaceutical drugs, which suppress the SARS-CoV-2-ACE2 interaction, such as ramipril and perindoprilat. In this work, Pd nano film was covalently bonded to ACE2, with direct interactions with S atom. This mechanism normally occurs with 11 group metals [44, 45] and is a commonly employed strategy in the field, being one of the main reasons Au is widely used in biosensors.

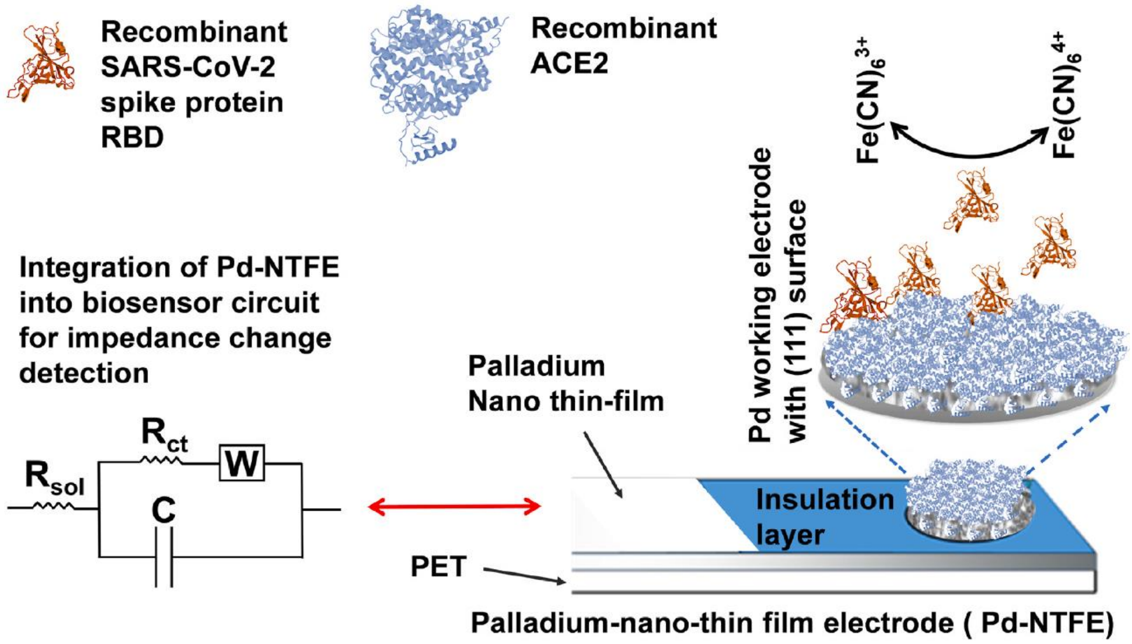

Fig. 4. Schematic preparation of the EIS-based biosensing platform with ACE2-Pd-NTF electrode as biosensing probe against SARS-CoV-2's S-protein. (Reprinted from [43], with permission of Elsevier)

In addition to the general detection of the virus, it is interesting to verify the stage of the disease. This can be essential to determine the time that the patient must remain out of society so that this virus is no longer transmitted. In this way, Avelino et al. [46] developed a structure based on electrodes of tin-doped indium oxide modified with polypyrrole and AuNPs. The assay was evaluated through biodetection using recombinant plasmids containing the nucleocapsid protein gene of SARS-CoV-2. The biological part has been added using cysteamine, glutaraldehyde, and BSA, as a covalent bonding method (exploring the previously described Au-S interaction) as observed in Fig. 5. The tests were performed using cyclic voltammetry and EIS. Also, interfering molecules (glucose, glycine, ascorbic acid, and cholesterol) were added to evaluate the selectivity of the biosensor. The authors reported that the results suggest that the platform can differ the data obtained between analyte and contaminants, allowing the application of the biosensor in clinical trials. Despite being a promising alternative for the detection of COVID-19 cases, this device has not been tested on samples without pre-treatments.

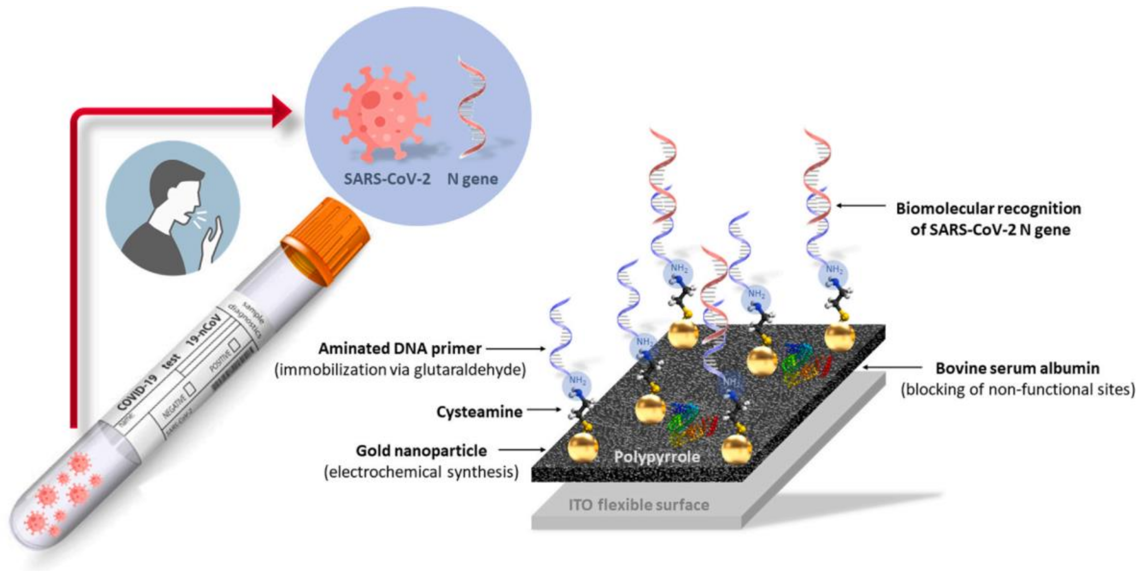

Fig. 5. Schematic representation of the assembly principle of the COVID-19 electrochemical sensing platform. (Reprinted from [47], with permission of Elsevier)

3. Conclusions and Perspectives

The development of new device architectures for SARS-CoV-2 biosensing is of paramount importance, especially sensors that aim a rapid and reliable detection and overcome the high cost and long processing time problems of conventional methods of analysis. In this way, the use of metallic nanostructures is a fundamental tool to increase the electrochemical signals and aid immobilization of DNAs, proteins, enzymes, and SARS-CoV-2 related aptamers, supporting the production of a new generation of electrochemical devices. Electroanalytical technologies, coupled with the use of metallic nanostructures, bring several advantages in the development of biosensors, such as large-scale production and cost and time reduction. In addition, with the advancement of disease control technologies, the use of SARS-CoV-2 biomolecules correlated strategies applied to the development of new electrochemical systems, promote the creation of robust devices that can be used quickly and accurately as needed.

Given the aspects discussed in this book chapter, biosensors fabricated with metallic nanostructure are interesting alternatives for the detection and monitoring of SARS-CoV-2. In the next years, there is a demand for new designs and fabrication methods, facilitating the handling and employability of these devices. Due to these factors, the authors believe that in the coming years, great efforts will be made for the research around the detection and viruses and their mutations, including the development of new sensors and biosensors. In this scenario, there is an expectation of worldwide governments and researchers to increase investments of time and money, making these devices more accessible to the population.

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