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Mini review

Electrochemical biosensors for the detection of SARS-CoV-2 pathogen and protein biomarkers

Yintang Zhang^{1,2,*}, Fang Chen^{1,2}, Hao Xie³ and Binbin Zhou^{3,*}

¹ Henan Key Laboratory of Biomolecular Recognition and Sensing, College of Chemistry and Chemical Engineering, Shangqiu Normal University, Shangqiu, Henan 476000, P. R. China

² Henan Joint International Research Laboratory of Chemo/Biosensing and Early Diagnosis of Major Diseases, College of Chemistry and Chemical Engineering, Shangqiu Normal University, Shangqiu, Henan 476000, P. R. China

³ College of Chemistry and Chemical Engineering, Hunan Institute of Science and Technology, Yueyang, Hunan 414006, P. R. China

*E-mail: ahhh_cs@163.com (Y.Z.); bbzhou1985@163.com (B.Z.)

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Detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV CoV-2) pathogen and protein biomarkers can improve the diagnosis accuracy for Coronavirus disease 2019 (COVID-19). Electrochemical biosensors have attracted extensive attention in the scientific community because of their simple design, fast response, good portability, high sensitivity and high selectivity. In this review, we summarized the progress in the electrochemical detection of COVID-19 pathogen and SARS-CoV-2 biomarkers, including SARS-CoV-2 spike protein and nucleocapsid protein and their antibodies.

Keywords: Coronavirus disease 2019; electrochemical biosensors; SARS-CoV-2 spike protein; SARS-CoV-2 nucleocapsid protein; antibody

1. INTRODUCTION

Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV CoV-2) has brought great harm and challenges to many countries and regions in the world. Most COVID-19 patients have typical clinical manifestations, but the number of asymptomatic infections is increasing with the disease progression and the development of detection techniques [1, 2]. Many COVID-19 asymptomatic patients are easily ignored because of the lack of obvious clinical manifestations. The spread of SARS-CoV CoV-2 to different countries has accelerated the pandemic of the epidemic in the world and brought great difficulties to the global epidemic prevention and control.

Therefore, early diagnosis of asymptomatic infections is an effective means to control the prevalence of COVID-19. Qualitative and quantitative analysis of the structure, content and subcellular localization of pathogen nucleic acid in body fluids or tissues is the gold standard for clinical diagnosis of pathogen infection and other diseases [3, 4]. It is also the currently used method for screening of COVID-19 patients [5]. However, the insufficient viral number and improper sample handling will greatly affect the sensitivity of COVID-19 diagnosis. Detection of SARS-CoV-2 pathogen and COVID-19 antigens and antibodies can improve the specificity and sensitivity [6-8], which can be combined with the nucleic acid detection to improve the accuracy of COVID-19 diagnosis.

With the progress of science and technology, various physical and chemical sensors have been developed rapidly as new detection technologies, which can convert the target concentration into an electrical signal or other forms of information [9-11]. As an important branch of chemical sensors, electrochemical biosensor can convert chemical or biological information of analyte into an electrical signal for qualitative and quantitative analysis [12, 13]. It is generally composed of identification element system and conversion element system. After the target was interacted with the electrode system, the conversion element converts the interaction into a detectable electrical signal, which can be displayed through the potential, resistance or current [14]. In recent decades, electrochemical biosensors have attracted extensive attention in the scientific community because of their simple design, fast response, good portability, high sensitivity and high selectivity [15-21]. In this review, we summarized the progress in the detection of COVID-19 pathogen and SARS-CoV-2 protein antigens and antibodies by electrochemical biosensors.

2. DETECTION OF SARS-COV-2 PATHOGEN

SARS-CoV-2 pathogen is a positive single strand RNA virus. The highly infectious and pathogenic β -coronavirus is spherical or oval with protuberant glycoproteins on the surface. Eissa et al. proposed an electrochemical method for the detection of SARS-CoV-2 in cotton fibers [22]. This cotton-tipped immunosensor fabricated by carbon nanofiber (CNF)-modified screen-printed electrode exhibited dual function as both collector and detector of samples (Figure 1). Viral nucleocapsid protein antigen was immobilized on the electrode surface for competitive detection of COVID-19 pathogen. The detection limit of the sensor was found to be 0.8 pg/mL by square wave voltammetry (SWV). Vezza et al. developed an electrochemical SARS-CoV-2 biosensor based on the manufactural process of glucose test strip [23]. Self-assembled monolayers (SAMs) of 1H, 1H, 2H, 2H-perfluorodecanethiol (PFDT) were deposited on the gold electrode of printed circuit board (PCB) sensing array. Angiotensin-converting enzyme 2 (ACE2) was modified on the electrode as a recognition element to capture SARS-CoV-2, thus causing the impedance change of sensing electrode. Low-cost Electrochemical Advanced Diagnostic (LEAD) proposed by Lima et al. could achieve the detection of SARS-CoV-2 in 6.5 minutes [24]. Nanogold-modified graphite pencil was used to modify the electrode for immobilization of ACE2. The preferential binding of SARS-CoV-2 virus and spike protein to ACE2 significantly inhibited the electron transfer of $[\text{Fe}(\text{CN})_6]^{3-/4-}$. The detection limit of LEAD was found to be 229 fg/mL for the spike protein detection. Fabiani et al. developed a small electrochemical immunosensor by combination of magnetic technology and carbon black-based screen-printed electrode for the diagnosis of COVID-19

[25]. Stefano et al. prepared a novel conductive filament by 3D-printing technology and developed an electrochemical sensor for SARS-CoV-2 detection. The SARS-CoV-2 spike antibody (S1Ab)-modified electrode could recognize the target protein to induce the current change [26].

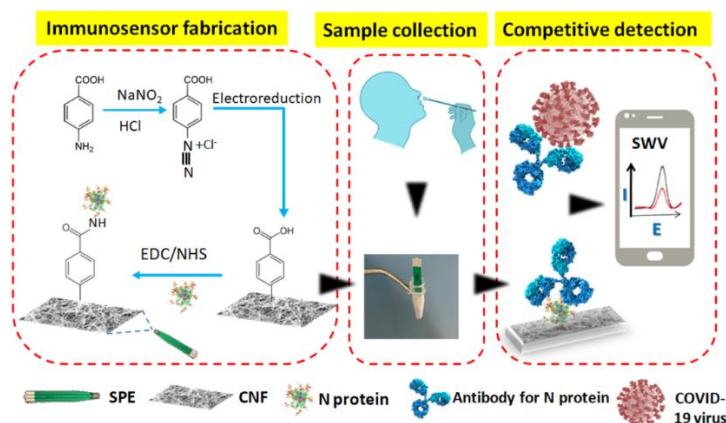


Figure 1. Schematic of the cotton-tipped electrochemical immunosensor for COVID-19; (A) Sample collection using the cotton-tipped electrode, (B) Functionalization of the carbon nanofiber electrode using electroreduction of diazonium salt and the attachment of the virus antigen, (C) Detection principle using competitive assay and SWV technique. Reprinted with permission from reference [22]. Copyright 2021 American Chemical Society.

In addition, Yousefi et al. developed a direct, reagent-free electrochemical method for the detection of SARS-CoV-2 viral particles [27]. As shown in Figure 2, the sensor was prepared by immobilizing the SARS-CoV-2 spike protein antibody on the electrode surface through a DNA linker modified with a ferrocene redox probe. When a positive potential was applied to the electrode, the negative charge on the linker made the antibody-virus complex approached to the electrode surface. Thus, the electron transfer between ferrocene and electrode was promoted with a characteristic time constant. The virus particles could be readily detected by chronoamperometry. This kinetics-based sensing method does not require additional reagents, and this strategy can be easily extended to the detection of other viral targets by matching the specific antibody.

Recent studies have shown that the currently pandemic SARS-CoV-2 and influenza A(H1N1) virus show similar syndromes. The development of sensing platforms that can detect both viruses simultaneously is of great significance for epidemic prevention and control and screening of the infected populations around the world. Therefore, Li et al. developed a multi-channel immunoassay platform (MEIA) for rapid detection of SARS-CoV-2 and A(H1N1) based on disposable screen-printed carbon electrode (SPCE) arrays [28]. As shown in Figure 3, monoclonal antibodies against H1N1 hemagglutinin protein and SARS-CoV-2 spike protein were immobilized on the electrode surface to capture the target antigens. The captured targets were then reacted with horseradish peroxidase (HRP)-labeled detection antibody to form sandwich complexes. HRP catalyzed the oxidation of TMB by H_2O_2 on the electrode surface to generate an electrochemical signal.

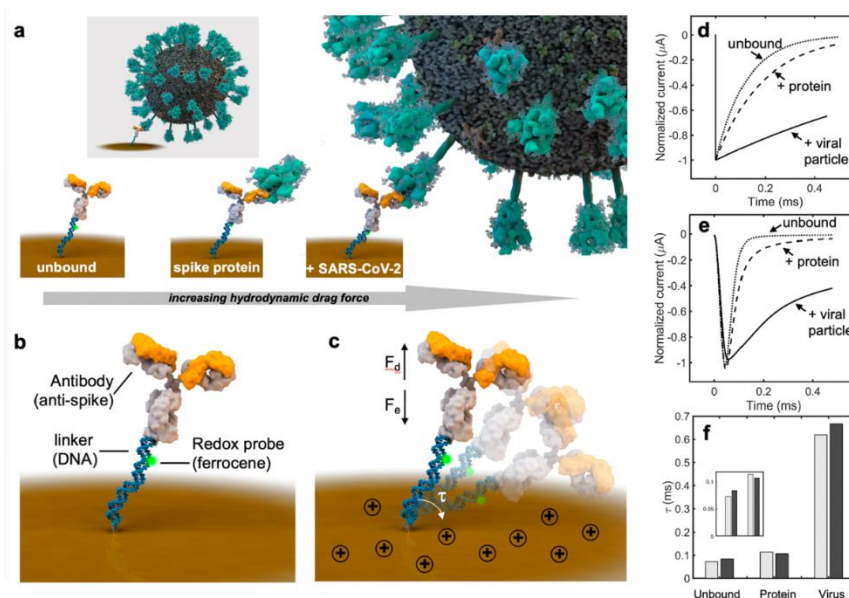


Figure 2. Reagent-free sensing of viral particles using an electrochemical approach monitoring the kinetics of transport for a DNA–antibody complex. (a) Sensor complexation with the SARS-CoV-2 viral particles. (b) Sensor architecture. (c) Development of a model describing sensor transport to the electrode surface. (d) Simulated time-dependent current transients. (e) Experimental time-dependent current transients. (f) Comparison of theoretical and observed data for different complexation states of the sensor. Reprinted with permission from reference [27]. Copyright 2021 American Chemical Society.

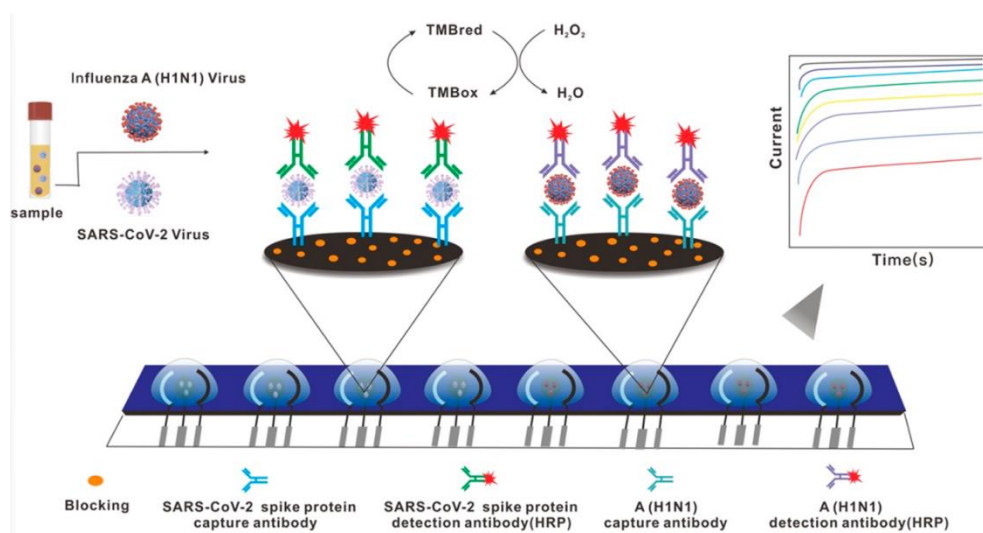


Figure 3. Schematic diagram of multichannel MEIA for SARS-CoV-2 and influenza A(H1N1) virus. Reprinted with permission from reference [28]. Copyright 2021 American Chemical Society.

3. DETECTION OF COVID-19 PROTEIN ANTIGENS AND ANTIBODIES

Detection of antigen using a specific antibody is a general method for monitoring the pathogens in samples. The samples can be collected usually from infected sites or blood, such as nasopharyngeal swabs, bronchoalveolar lavage fluids and serums. The antigens for COVID-19 mainly include SARS-CoV-2 spike protein and nucleocapsid protein (Table 1) [6, 29]. The result for the detection of antigens

and their antibodies can be used as the direct evidence for early diagnosis of pathogen infection. The formats for the antigen and antibody quantification include direct detection and sandwich-type assay.

Table 1. Analytical performances of various sensing electrodes for the detection of different SARS-CoV-2 protein-like biomarkers.

Electrode	Target	Detection limit	Linear range	Ref.
Ab/Cys SAMs/AuE	SP	0.1 ng/mL	0.1 – 5×10^2 ng/mL	[30]
SP/S-gene/Cys/Au-GCE	SP antibody	0.03 fg/mL	0.1 – 1×10^2 fg/mL	[31]
Ab/MIP/Au-SPE	SP-RBD	0.7 pg/mL	2 – 40 pg/mL	[32]
MIP/Au-TFE	NP	15 fM	$22.2 - 3.33 \times 10^2$ fM	[33]
MIP/P-Arg/Au/Gr/SPCE	NP	3 fM	$10 - 2 \times 10^2$ fM	[34]
ACE2/GA/GCE	SP	2.8 fg/mL	$0.1 - 1 \times 10^5$ pg/mL	[35]
SP/rGO electrode	SP antibody	2.8 fM	$1 - 1 \times 10^6$ nM	[36]
RBD/rGO electrode	RBD antibody	16.9 fM	$1 - 1 \times 10^5$ fM	
SP/Ni(OH) ₂ NPs/SPCE	IgM and IgG	0.3 fg/mL	$1 - 1 \times 10^9$ fg/mL	[37]
SP-RBD/GO electrode	IgG and IgM	0.96 and 0.14 ng/mL	$1 - 1 \times 10^3$ ng/mL	[38]
Ab/MUA/AuNPs-GCE	NP	0.4 pg/mL	$1 - 1 \times 10^8$ pg/mL	[39]
SP-RBD/GO/SPCE	antibody	1 ng/mL	Not reported	[38]
Ab/f-GO/SPE	SP	Not reported	$1 - 1 \times 10^4$ ag/mL	[40]
Ab/ProtA/Cu ₂ O NCs/SPCE	SP	0.04 fg/mL	$0.25 - 1 \times 10^9$ fg/mL	[37]

Abbreviation: Ab, antibody; Cys, cysteamine; AuE, gold electrode; SP, SARS-CoV-2 spike protein; GCE, glass carbon electrode; MIP, molecularly imprinted polymer; SPE, screen-printed electrode; SP-RBD, SARS-CoV-2 spike protein receptor-binding domain; Au-TFE, gold thin film electrode; NP, SARS-CoV-2 nucleocapsid protein; P-Arg, poly-arginine; Au/Gr, gold/graphene; SPCE, screen-printed carbon electrode; ACE2, angiotensin-converting enzyme-2; GA, glutaraldehyde; rGO, reduced graphene oxide; NPs, nanoparticles; f-GO, functionalised graphene oxide; SPE, screen-printed electrode; ProtA, staphylococcal protein A; Cu₂O NCs, Cu₂O nanocubes.

2.1 Direct detection

Self-assembled monolayers (SAMs) have attracted much attention in the field of electrochemical biosensing [41]. Due to their simple and convenient assembly process and good compatibility with metal substrates, they can facilitate the immobilization of biomolecules (e.g. proteins, antibodies, enzymes) and allow for the electrochemical measurement by monitoring the change of current and potential. Nandeshwar et al. developed an immunosensing device to detect SARS-CoV-2 spike protein using electroless nickel immersion gold finish PCB as the sensing electrode [30]. Cysteamine SAMs were used to modify the gold electrode for the immobilization of polyclonal antibody against SARS-CoV-2 spike protein with glutaraldehyde as the linker. Liv et al. reported a hydrogen bond-based electrochemical biosensing platform for the detection of SARS-CoV-2 spike antibody [31]. Gold clusters were deposited on the surface of glassy carbon electrode and mercaptoethanol was assembled on the gold surface for the immobilization of SARS-CoV-2 spike protein. When SARS-CoV-2 spike antibody was bound to the antigen, the electron transfer of redox probe on the electrode surface was blocked.

The combination of molecular imprinting technology and electrochemical biosensor can significantly improve the selectivity of electrochemical sensor, so as to realize the analysis and detection

of practical complex samples [42]. The molecularly imprinted membrane as a recognition element can be prepared on the surface of working electrode by coating method, electrochemical polymerization or in-situ polymerization. When the target molecule in the solution is combined with the matched hole in the molecularly imprinted membrane on the electrode surface, the electrochemical signal will change, so as to realize the specific detection of the target molecule. Based on this principle, Tabrizi et al. prepared a molecularly imprinted (MIP) polymer-based electrochemical sensor for the detection of SARS-COV-2 spike receptor-binding domain (SARS-COV-2-RBD) [32]. First, macroporous gold was deposited on the screen-printed electrode surface by an electrochemical method. Then, SARS-COV-2-RBD was used as a template molecule to synthesize the MIP polymer on the electrode surface by ortho-phenylenediamine electropolymerization. After the MIP polymer was covered with SARS-COV-2-RBD, the redox probe of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ could not pass through the cavity to reach the electrode surface, resulting in a large electron transfer resistance. The sensor showed a good linear relationship to the SARS-COV-2-RBD concentration in the range of 2 ~ 40 pg/mL with a detection limit of 0.7 pg/mL. Raziq et al. developed a MIP-based electrochemical sensor to determine SARS-CoV-2 nucleocapsid protein using m-phenylenediamine (mPD) as the functional monomer. The detection limit of the sensor was 15 fM [33]. However, the produced polymPD is a non-conductive polymer, limiting the sensitivity of sensor. Polyamino acid can be used to modify the electrode with good conductivity and stability. Therefore, Zhang et al. prepared highly conductive gold (Au) and graphene (Gr) nanohybrids as the electrode materials, and then developed a new MIP-type sensor for the detection of SARS-CoV-2 nucleocapsid protein with arginine (Arg) as the functional monomer [34]. Torres et al. fabricated a miniature biosensor using glutaraldehyde-modified carbon electrode for the immobilization of ACE2 [35]. The signal change was measured by electrochemical impedance spectroscopy after the specific binding of SARS-CoV-2 spike protein to ACE2.

Conducting polymers are widely used in the field of analytical sensing because of their ionizable groups, excellent electron transfer ability and good biocompatibility. The combination of conducting polymer and electrochemical biosensor can realize the regulation of electrode interface and maintain the activity of biological macromolecules such as enzymes and antibodies in the sensing layer. The applications of conducting polymer-based electrochemical biosensors for the detection of various biomarkers including COVID-19 have been reviewed by Tran and co-workers [43].

Modification of working electrode with different methods or materials can significantly improve the sensitivity and selectivity of biosensors [44-46]. Metal nanomaterials have good conductivity and corrosion resistance, large specific surface area and excellent catalytic performance [47]. The commonly used metal nanomaterials include gold, silver, copper, platinum and others. Because most metal nanomaterials are expensive, deposition of metal on the sensor surface to form nanoparticles can greatly save the research cost and improve the utilization efficiency of reagents. Compared with metal nanomaterials, nano metal oxides have lower cost and higher oxidation resistance, such as zinc oxide, cuprous oxide and iron oxide. They have also been widely used in the field of electrochemical biosensors. Carbon nanomaterials with large specific surface area and high surface reactivity can enhance the adsorption capacity, increase the surface active sites and improve the catalytic efficiency [48], mainly including carbon nanotubes, fullerenes, graphene, nano-diamonds and their derivatives. Thus, nanomaterials with characteristics of large specific surface area, high conductivity and

biocompatibility have been often used as the electrode materials for the detection of SARS-COV-2 antigens and antibodies. For example, Ali et al. fabricated a three-electrode platform for rapid detection of SARS-COV-2 antibodies by aerosol jet 3D nanoprinting (Figure 4) [36]. SARS-COV-2 antigen-functionalized reduced graphene oxide nanosheets were used to modify the electrode. When the antibodies flowed through the microfluidic channel and then captured by the virus antigens on the electrode surface. The signal change was monitored by impedance. The method shows low detection limits for the detection of the antibodies to SARS-CoV-2 spike protein (2.8×10^{-15} M) and SARS-CoV-2-RBD (16.9×10^{-15} M).

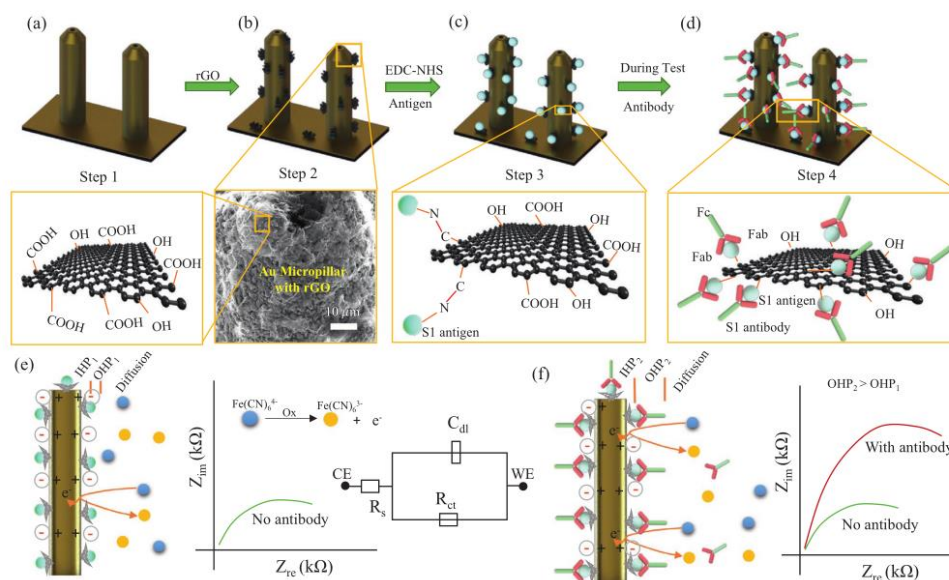


Figure 4. Functionalization of 3D-printed micropillar electrode and 3DcC sensor operation. a) AJ-printed gold micropillars prior to the surface treatment (step 1). b) Coating of the electrodes by carboxylated (-COOH) rGO sheets by a simple drop-casting process (step 2). c) Coupling of the viral antigens with the rGO sheets using EDC:NHS chemistry (step 3). e, f) Schematics showing the sensing principle of the 3DcC device. Reprinted with permission from reference [36]. Copyright 2021 Wiley-VCH GmbH.

Rahmati et al. prepared an electrochemical immunosensor by electrodepositing nickel hydroxide nanoparticles ($\text{Ni}(\text{OH})_2\text{NPs}$) on the surface of screen-printed electrode to immobilize the SARS-CoV-2 spike protein antigen [49]. Differential pulse voltammetry (DPV) was used to characterize the signal change after antigen-antibody binding. The detection limit of the sensor was 0.3 fg/mL. Eissa et al. reported a label-free sensing platform for the detection of SARS-CoV-2 by square wave voltammetry [39]. Gold nanoparticle-modified screen-printed electrodes were functionalized with 11-mercaptopundecanoic acid (MUA) for immobilization of SARS-CoV-2 nucleocapsid protein antibodies. A linear range of the sensor from 1 pg/mL to 100 mg/mL was achieved. Yakoh et al. developed a paper-based sensing platform for diagnosing COVID-19 [38]. The graphene oxide (GO)-modified electrode was used to immobilize SARS-CoV-2-RBD. The binding of SARS-CoV-2-RBD antibody and antigen prevented the redox indicator to approach the electrode surface, thus reducing the current response. Furthermore, the proposed platform can be extended to detect antigens. Liv et al. prepared SARS-COV-

2 spike antibody and graphene oxide (GO)-modified glassy carbon electrode for the determination of SARS-CoV-2 spike protein in real samples [40]. The sensor has a dynamic response range of 1 ag/mL ~10 fg/mL. Gao's group reported a radiochemical platform for ultra-rapid detection of COVID-19 based on laser-engraved graphene [50]. The platform shows the advantages of high sensitivity, low cost, ultra-fast detection, wireless long-range and multi-sensing. Rahmati et al. prepared an electrochemical immunosensor for the detection of SARS-CoV-2 spike protein using Cu₂O nanocubes (Cu₂O NCs) [37]. The screen-printed electrode modified by Cu₂O NCs has a large specific surface area, which can increase the loading of staphylococcal protein A (ProtA) on the electrode surface. The spike protein was captured by the immobilized ProtA. The linear range of the sensor was 0.25 fg/mL ~ 1 µg/mL with a detection limit of 0.04 fg/mL.

Zhou et al. reported an electrochemical method for the simultaneous detection of three SARS-CoV-2 protein markers without the use of antibodies or enzymes [51]. The sensor was prepared using peptide probe-immobilized gold-modified indium tin oxide (ITO) electrode, as shown in Figure 5. The detection was achieved by adjusting the frequency of electrochemical potential scanning so that the rate was synchronized or resonant with the molecular motion of the peptide probe. The binding between the target protein and the substrate peptide on the electrode hampered the motion of the probe.

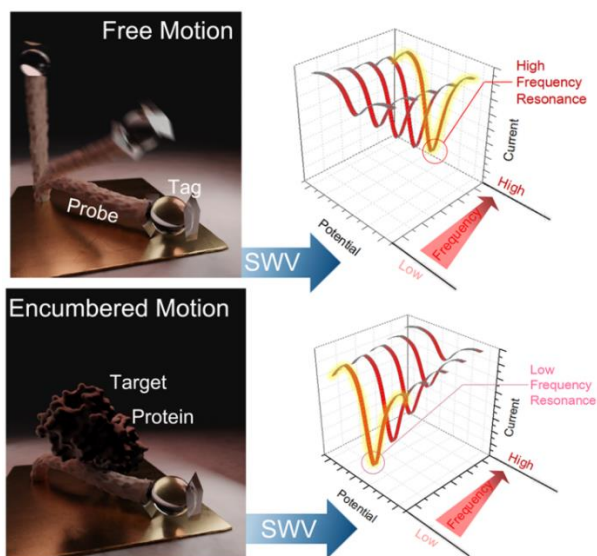


Figure 5. Proposed method of using sampling frequency to “resonance” with the molecular motion of a synthesizable probe in the presence/absence of the target protein. Reprinted with permission from reference [51]. Copyright 2021 American Chemical Society.

2.2 Sandwich-type assay

Sandwich-type assay can further improve the detection performance of biosensors through the signal amplification and the specific recognition between the signal reporter and the target molecule. Karaman et al. reported a sandwich-type electrochemical immunosensor for the detection of SARS-CoV-2 nucleocapsid protein [52]. In this study, bismuth tungstate/bismuth sulfide composite (Bi₂WO₆/Bi₂S₃)-modified electrode was used to immobilize capture antibody. Gold nanoparticles (AuNPs) and tungsten trioxide sphere-modified graphitic carbon nitride sheet (g-C₃N₄/Au/WO₃) was

conjugated with detection antibody as the signal label to catalyze the oxidation of H_2O_2 . The detection limit of the sensor was 3 fM. Recently, Li et al. developed an immunosensor for rapid and sensitive detection of SARS-CoV-2 nucleocapsid protein in serum (Figure 6) [53]. This sensor integrated immunomagnetic enrichment and signal amplification via dual-labeled magnetic nanobeads. This sensor integrated onto a microfluidic chip requires minimal sample and reagent consumption as well as simple processing steps, and shows high detection sensitivity. This work, for the first time, demonstrated a rapid (<1 h), highly sensitive detection of SARS-CoV-2 nucleocapsid protein in undiluted serum. Peng et al. designed a portable and low-cost electrochemical immunosensor for the measurement of SARS-CoV-2 antibody in serum [54]. Streptavidin-biotin binding was used to immobilize the biotinylated SARS-CoV-2-RBD. Alkaline phosphate (ALP)-labeled antihuman detection antibody was used as the signal label. The platform can detect the concentration of immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies in serum within 13 min., the detection range of the sensor was determined to be $10.1 \text{ ng/mL} \pm 60 \text{ } \mu\text{g/mL}$ for IgG and $1.64 \text{ ng/mL} \pm 50 \text{ } \mu\text{g/mL}$ for IgM. Chiang et al. reported a quantitative, non-invasive and specific electrochemical assay of anti-SARS-CoV-2 IgG antibodies in saliva [55]. The assay platform Amperial™ was used to immobilize antigens in the conducting polymer gels and monitor the redox reactions between H_2O_2 /tetramethyldioxidase and peroxidase.

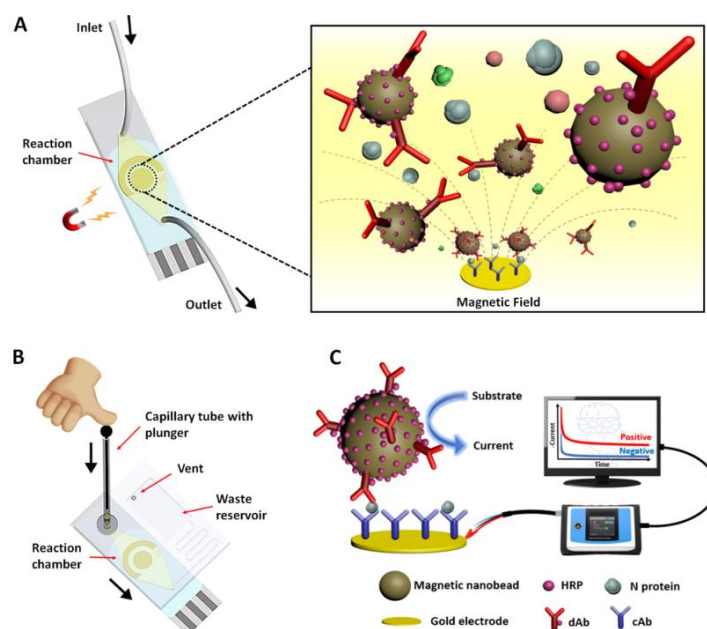


Figure 6. Schematic illustrations of (A) microfluidic immunosensor chip highlighting the magnetic concentration of DMBS to the sensor surface, (B) microfluidic immunosensor chip for the smartphone-based diagnostic device, and (C) experimental setup and electrochemical sensing scheme using the PalmSens4-based sensing platform. Reprinted with permission from reference [53]. Copyright 2021 American Chemical Society.

4. APTASENSORS

Aptasensors have the advantages of high selectivity, sensitivity as well as stability and wide detection range. With the prevalence of COVID-19, it is desired to develop an aptamer-based platform

for rapid and efficient diagnosis of COVID-19. For example, Idili et al. developed an electrochemical aptamer-based sensor for the detection of SARS-CoV-2 spike protein [56]. Two DNA aptamers, 1C and 4C, were used as the recognition elements of the sensor. The receptor-binding domain (RBD) for the SARS-CoV-2 spike protein was modified on the gold electrode for the target recognition. The change of the target concentration led to the change of the relative position of the redox probe attached on the electrode surface, thereby causing the change of the electrochemical signal. Zhang et al. developed an electrochemical impedance sensor for the detection of SARSCoV-2 antigen in saliva using a dimeric DNA aptamer DSA1N5 (Figure 7) [57]. This high-affinity dimeric aptamer was generated by linking two previously reported spike protein aptamers. The sensor was prepared by modification of the thiol-modified aptamer DSA1N5-SH on the gold electrode. When the SARS-CoV-2 virus or spike protein was bound to the sequence-specific aptamer, the impedance of the electrode surface was intensified. The detection limit of the sensor for SARS-CoV-2 spike protein was found to be 1 fM. Martinez et al. reported an aptamer electrochemical sensing platform using gold nanoparticles-modified screen-printed electrodes [58]. Thiol-functionalized aptamers were immobilized on the electrode surface by the formation of Au-S bonds. The impedance change induced by the binding of SARS-CoV-2 spike protein and aptamer was determined by electrochemical impedance spectroscopy. The detection limit of the sensor was estimated to be 1.3 pM. Ramanathan et al. reported a sensitive aptasensor for the detection of SARS-CoV-2 nucleocapsid protein using gold-interdigitated electrode [59]. The aptamer against SARS-CoV-2 nucleocapsid protein was immobilized on the diamond-deposited gold-interdigitated electrode to improve the conductivity. The linear range of the EIS method was 1 fM ~ 100 pM, and the detection limit was 0.389 fM.

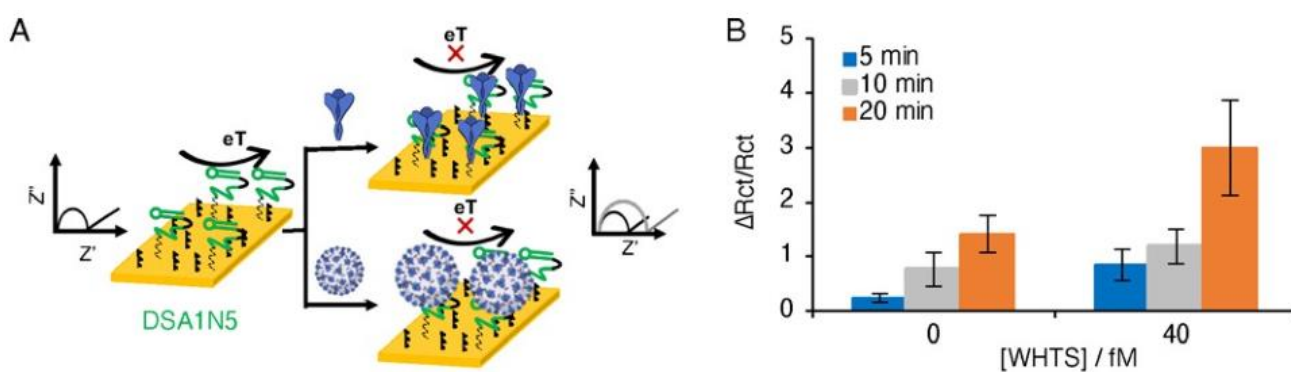


Figure 7. (A) Schematic of the electrochemical assay for the detection of SARS-CoV-2 using spike protein aptamer. After incubation with the viral target, the charge transfer resistance increases due to surface blocking of the redox reaction of the Fe²⁺/Fe³⁺ ions. (B) The changes in the charge transfer resistance measured in the redox solution containing Fe²⁺/Fe³⁺ ions at different time interval (5, 10, 20 minutes) tested with and without 40 fM trimeric spike protein. Reprinted with permission from reference [60]. Copyright 2021 Wiley-VCH GmbH.

5. CONCLUSION

The sensitivity and specificity of the methods for the detection of COVID-19 antigens and antibodies are satisfactory, but the results are greatly affected by some factors such as sample quality,

sampling site and virus expression. Therefore, the methods can not replace that of PCR-based nucleic acid detection in the diagnosis and screening of SARS-CoV-2 infection. One of the key problem for the clinical applications of these methods is to reduce the false negative rate in low content of viral sample. Moreover, with the development of nanotechnology, the combination of nanomaterial-functionalized electrochemical biosensors and other technologies has become a new research direction although there are still some deficiencies in nanomaterial-based electrochemical biosensors. For the detection of a single molecule, the sensitivity can meet the requirement, but it is easy to be disturbed when more than two analytes are determined at the same time. Moreover, the modification of nanomaterials on the sensor surface mostly adopts physical adsorption method, which has the disadvantage of instability. In the further, efforts should be made in the preparation and immobilization of multi-functional nanomaterials.

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