



Label-free electrochemical immunosensor for highly sensitive COVID-19 spike protein detection

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ABSTRACT

The ongoing coronavirus pandemic responsible for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has rapidly increased the rate of global death and infections due to variant mutations (such as Delta and Omicron). While specifically developed and approved vaccines can limit the spread of disease in a population and severity of resulting symptoms, none have been demonstrated to effectively prevent infection altogether. Thus, reliable early diagnosis of COVID-19 is critical to identify positive cases to help contain the outbreak. Herein we report a label-free electrochemical immunosensor for rapid diagnosis of COVID-19 by using nitrogen-doped holey graphene (N-HRGO) as a nanocarrier decorated with thionine (TH) molecules as electrochemical indicators. With the spike protein located on the surface of the COVID-19 particles as the model target, the as-prepared electrochemical immunosensor could detect the presence of the COVID-19 spike protein over a wide linear range (1 pg mL^{-1} – 10 ng mL^{-1}) with a low detection limit (0.3 pg mL^{-1}). In addition, the developed electrochemical immunosensor exhibited an excellent selectivity (with insignificant current changes towards interfering proteins comparing with COVID-19 spike protein), a good reproducibility and long-term storage stability. Importantly, the electrochemical immunosensor thus developed could successfully and reliably detect the spike protein of COVID-19 in saliva and human serum complex samples. Thus, the as-prepared label-free electrochemical immunosensor can achieve rapid and sensitive detection of the COVID-19 spike protein, as a promising clinical diagnosis tool in monitoring the progression of COVID-19.

1. Introduction

Since the coronavirus infectious disease (COVID-19) outbreak was first reported in December 2019 [1], it has rapidly spread throughout the whole world and caused the significantly increased morbidity and mortality rates. The World Health Organization (WHO) declared a global pandemic in March 2020 based on the high risk and rapid human-to-human transmission of COVID-19 induced by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [2,3]. Fortunately, effective Covid vaccines have been discovered and approved in a remarkably rapid time, with two required injections for full vaccination status and suggested booster shots to address ever-evolving situation [4,5]. While full vaccination status cannot prevent infection altogether, it does minimize symptoms and reduce the spread of disease in a population [6,7]. However, given the nature of the pandemic, the situation against the SARS-CoV-2 still remains grim as it appears to remain a concern for the foreseeable future, especially with the outbreaks in different SARS-CoV-2 variants. Although the early diagnosis

technologies, such as Rapid Antigen Tests (RAT) and PCR tests, have demonstrated the important role in patient diagnosis, case identification, contact tracing and evaluation of infection control, these methods are limited by the false negatives, accuracy (quick RAT tests) and longer test times (PCR). In addition, the various strains and variants of the virus come to new challenge to the conventional diagnostic tools. Hence the realization of rapid and accurate early diagnosis of SARS-CoV-2 together with its variants are still much-needed to contain its outbreak [8].

Currently, COVID-19 detection technologies for diagnosis have been developed and exhibited excellent performance: including reverse transcription polymerase chain reaction (RT-PCR) [9,10], lateral flow assay [11,12], laser/light assisted photonic/plasmonic detection methods [13–15], mass spectrometry assay [16], electrochemical biosensor, and immunosensor [17–21]. Among all the reported detection methods, the electrochemical detection plays an important role in rapid diagnosis due to its advantages associated with a high selectivity and sensitivity, low cost and portability; in addition to the ease of integration with other detection techniques used for point-of-care

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testing and on-site detection [22–25]. For example, Seo et al. [26] demonstrated a field-effect transistor (FET)-based biosensing device for rapid detection of SARS-CoV-2 in clinical samples with an excellent detection selectivity and limit due to the coating of a specific antibody for molecular identification. Chaibun et al., [17] reported a rapid and ultrasensitive electrochemical biosensor for detection of SARS-CoV-2 N and S genes based on isothermal rolling circle amplification (RCA) with a low detection limit (1 copy/ μL) and 100% accuracy for clinical samples. Nevertheless, simpler and more effective electrochemical biosensors with better performance for a more rapid identification of COVID-19 should be developed.

Recently, nanomaterials have been widely used for the development of SARS-CoV-2 electrochemical biosensors to enhance the specificity and sensitivity [27]. In this work, nitrogen-doped holey graphene (N-HRGO) was chosen to serve as a carrier material for fabricating electrochemical immunosensors with the following advantages: (1) N-HRGO has unique architecture and physicochemical properties [28–30] with a ultra-high surface area, porous holes/channels for massive ion/mass transport, and abundant exposed edges and active sites to enhance the catalytic properties; (2) N-HRGO is cost-effective especially compared with the commonly used noble nanoparticles (Au, Pt); and (3) 3D structure with the ease for modification of specific recognition unit and redox substances for electrochemical sensing. By anchoring thionine (TH) molecules on N-HRGO that used as both the bridge for antibodies modification and electrochemical indicator, a label-free electrochemical immunosensor was developed for rapid diagnosis of COVID-19. Here, the spike protein was chosen as a target model to investigate the as-prepared electrochemical immunosensor, as it is located on the surface of the COVID-19 particles and is critical for protein binding of host cell receptors to facilitate entry of host cell [31]. The formed N-HRGO/TH composites have good electrochemical properties, and after modification of the COVID-19 spike protein antibodies, the fabricated immunosensor exhibited excellent performance concerning the detection limit (0.3 pg mL^{-1}), linear range (1 pg mL^{-1} – 10 ng mL^{-1}), together with a good selectivity, reproducibility and long-term storage stability. Moreover, the reliability of the developed electrochemical immunosensor was also verified in complex samples (saliva and human serum) with satisfactory detection results, indicating great potential for routine clinical diagnosis. Importantly, this newly-developed electrochemical immunosensor can be broadly tuned for a multitude of targets detection by modification of the corresponding recognition units, including for COVID-19 variants detection, holding great potential for future widespread use.

2. Experimental section

2.1. Chemicals and reagents

$\text{K}_3\text{Fe}(\text{CN})_6$, $\text{K}_4\text{Fe}(\text{CN})_6$, KCl, NaH_2PO_4 , Na_2HPO_4 , thionine, bovine serum albumin (BSA) and glutaraldehyde 25% solution in water were purchased from Sigma-Aldrich. COVID-19 spike protein and antibody were purchased from Sino Biological Inc. (Beijing, China). COVID-19 nucleocapsid protein, SARS spike glycoprotein and Human IgG protein were purchased from Abcam Australia Pty Ltd. All the chemicals were at least analytical grade and the water used for all experiments was purified by a Milli-Q system (resistivity $> 18 \text{ M}\Omega\cdot\text{cm}^{-1}$). Phosphate buffer solution (PBS) used in this work was prepared using 0.1 M $\text{Na}_2\text{HPO}_4\cdot 12\text{H}_2\text{O}$, 0.1 M $\text{NaH}_2\text{PO}_4\cdot 2\text{H}_2\text{O}$, 0.1 M KCl, pH = 7.4.

2.2. Preparation of N-HRGO/TH composites

First, N-HRGO was prepared according to our previously reported method [32]. Briefly, the pristine holey graphene was obtained by annealing the raw materials in air at 430°C for 10 h, and a slightly oxidizing environment was introduced to achieve holey graphene oxide with good dispersion in solution. Then, collected by filtration and

washed with a HCl aqueous solution (10% in volume), the holey graphene oxide sample was obtained after lyophilized for 72 h. Finally, the N-HRGO was obtained by transferring the lyophilized holey graphene oxide into a quartz tube and subsequently annealed at 750°C for 1 h under Ar/NH_3 (with the volume ratio of 3:1) atmosphere. For comparison, nitrogen doped graphene (N-RGO) was prepared by annealing graphene oxide at 750°C for 1 h under the same condition.

N-HRGO/TH composites were prepared according to the previous publication [33], where the dispersed N-HRGO (2 mg/mL) was mixed with 1 mL TH solution (1 mg mL^{-1}), followed by ultrasonication (Unisonic Australia, FXP12D, 100 W) for 1 h and gently shaken at room temperature for 24 h. Finally, the resultant N-HRGO/TH composite was collected after centrifugation and washed with water several times.

2.3. The fabrication of electrochemical immunosensor

First, a glassy carbon electrode (GCE, $d=3 \text{ mm}$) was polished with alumina slurry (0.3 and $0.05 \mu\text{m}$), and ultrasonic cleaning with ethanol and ultrapure water. Then, $5 \mu\text{L}$ of the N-HRGO/TH composite solution (1 mg mL^{-1}) was cast on the surface of GCE and dried at room temperature. For the immobilization of antibodies, N-HRGO/TH composite modified GCE was firstly immersed in a 2.5% glutaric dialdehyde solution for about 1 h, and $5 \mu\text{L}$ of COVID-19 spike protein antibodies ($50 \mu\text{g mL}^{-1}$) dropped onto the electrode surface, allowing it to react overnight. Then, after washing the excess non-combined antibodies, $5 \mu\text{L}$ BSA (0.5 mg mL^{-1}) aqueous solution was casted onto the electrode surface for 40 min to block the nonspecific binding sites. After rinsing with the PBS buffer, the electrochemical immunosensor was fabricated and stored at 4°C for the detection of COVID-19 spike protein.

2.4. Electrochemical detection methods

In order to determine detection sensitivity, electrochemical immunosensors were prepared with different concentrations of COVID-19 spike proteins incubated on the surface of the electrode for 1 h at 37°C , and washed with 0.1 M PBS buffer (pH 7.4). Then, a conventional three-electrode system on a CHI 760 E electrochemical workstation was used for electrochemical characterization, where the fabricated GCE electrode, an Ag/AgCl electrode and platinum wire electrode served as working electrode, reference electrode and counter electrode, respectively. Differential pulse voltammetry (DPV) that scanned from -0.4 V to 0.2 V in 0.1 M PBS (pH=7.4) to quantify the amount of COVID-19 spike protein present, then cyclic voltammetry and electrochemical impedance spectroscopy were performed in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ solution containing 0.1 M KCl.

3. Resultants and discussion

3.1. Physicochemical characterization of N-HRGO and composites

N-HRGO was prepared by annealing holey graphene oxide at high temperature (750°C) for 1 h in the presence of Ar and NH_3 (with the volume ratio of 3:1). Then, the morphology and structure of the as-prepared N-HRGO were characterized by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The SEM image in Fig. 1A clearly shows that the fabricated N-HRGO exhibits a good lamellar structure. TEM image in Fig. 1B further confirms the size and distribution of holes present in the N-HRGO, as the holes were distributed across the whole surface of N-HRGO with the size ranging from about several nanometers to tens of nanometers [32]. X-ray photoelectron spectroscopy (XPS) measurements in Fig. 1C show the presence of C (89.45 at%), O (3.97 at%) and N (6.58 at%) elements in N-HRGO, indicating that the N was successfully doped into HRGO with an atomic content of about 6.58%. The O (3.97 at%) in N-HRGO is intrinsically associated with the holey graphene oxide that cannot be completely reduced through the thermal reduction process [34]. Further

the XPS high-resolution spectrum of N1s in Fig. 1D shows four characteristic peaks located at 403.2, 401.2, 399.5 and 398.2 eV, which can be attributed to pyridinic-N oxide, quaternary-N, pyrrolic-N, and pyridinic-N, respectively, confirming the incorporation of N into the carbon skeleton and the formation of the N-HRGO [35].

To further characterize the samples, we also performed Brunauer–Emmett–Teller isotherms and X-ray diffraction (XRD) measurements. As shown in Fig. S1A, the combined type-II and -IV isotherms were obtained for both N-HRGO and N-RGO samples. The calculated specific surface area of N-HRGO reaches 292.249 m²/g, much higher than that of N-RGO (108.005 m²/g). The high specific surface area of N-HRGO could serve as ideal building blocks as a platform to immobilize the recognized element onto the functionalized nanomaterial. In addition, the pore size distributions were also obtained (Figure S1B), and it can be seen that large number of pores existed in N-HRGO in the wide range of 1–8 nm, in good agreement with the TEM observation. The large number of holes in N-HRGO can enhance the electron and mass transfer towards the surface of the GCE, and more exposed edges can enhance the catalytic ability. Moreover, the XRD patterns of N-HRGO and N-RGO in Figure S2 exhibit similar diffraction features with one broader ($\approx 26.5^\circ$) peak, but the decreased signal peak of N-HRGO could be attributed to increased number of holes in N-HRGO that induce the poor crystallinity [36].

3.2. Electrochemical characterization

The electrochemical behaviours of the N-HRGO and N-RGO modified electrodes were also compared and evaluated by CV using 5 mM [Fe(CN)₆]^{3-/4-} as an electroactive probe. As shown in Fig. S3, a couple of quasi-reversible redox peaks of the probes were present for both the N-HRGO and N-RGO modified electrode, but an obvious increase in both the cathodic and anodic peak currents for N-HRGO modified electrode were observed compared to those of the N-RGO modified electrode. The enhanced electrochemical performance observed for the N-HRGO can be attributed to the excellent electron transfer ability and large surface area of N-HRGO, together with the presence of porous holes to facilitate the transfer of the redox probes to the electrode surface. Based on the above results, it could be concluded that N-HRGO has excellent physicochemical properties with a large surface area and good catalytic properties due to the presence of holes and exposed edges to provide abundant ion/mass transport channels and active sites. Thus, N-HRGO can serve as an efficient building material for the fabrication of a sensitive electrochemical immunosensor.

For the label-free electrochemical immunosensor fabrication in this work, TH molecules are attached to the N-HRGO to serve as the electrochemical indicators and bridge molecules for antibodies modification. In order to verify the formation of N-HRGO/TH composites, UV-vis spectroscopy was first used to characterize the interaction of N-HRGO and TH. Fig. 2A shows that two characteristic adsorption peaks at around 600 nm and 270 nm were observed for N-HRGO/TH composites compared with pure N-HRGO. By comparison with the UV-vis spectrum of pure TH, it can be indicated that two characteristic adsorption peaks for HRGO/TH composites were associated with TH molecules. This result is consistent with published literatures [37,38], indicating the N-HRGO/TH composites was successfully obtained. The above results were confirmed by further experiments performed in 0.1 M PBS electrolyte.

As seen from the CV curves in Fig. 2B, the bare GCE and N-HRGO modified GCE show no clear voltammetry peak over the potential range studied. Compared to the pristine GCE, the higher double layer capacitance current that exhibited by N-HRGO modified electrode could be attributed to its higher conductivity and capacity [39,40]. While for the N-HRGO/TH composite modified GCE, an obvious pair of reversible reduction and oxidation peaks with cathodic and anodic peak potentials at about −0.22 and −0.15 V were observed due to the redox activity of TH in N-HRGO/TH, which is consistent with published results [33,41].

All the above results suggest the successful formation of the N-HRGO/TH composite, leading to a quasi-reversible redox reaction at the electrode surface for electrochemical sensing. Furthermore, the electrochemical behaviours of N-HRGO/TH composites-modified electrode under different scan rates were also investigated. The results presented in Fig. 2C showed that the redox peak currents are highly dependent on the scan rates, which are enhanced with the increase of scan rate. And a good linear relationship between the peak current and scan rates are observed (Fig. 2D), indicating that the redox process of the N-HRGO/TH composites on the electrode surface is a fast and surface-confined process [33,41].

3.3. Fabrication and characterization of the electrochemical immunosensor

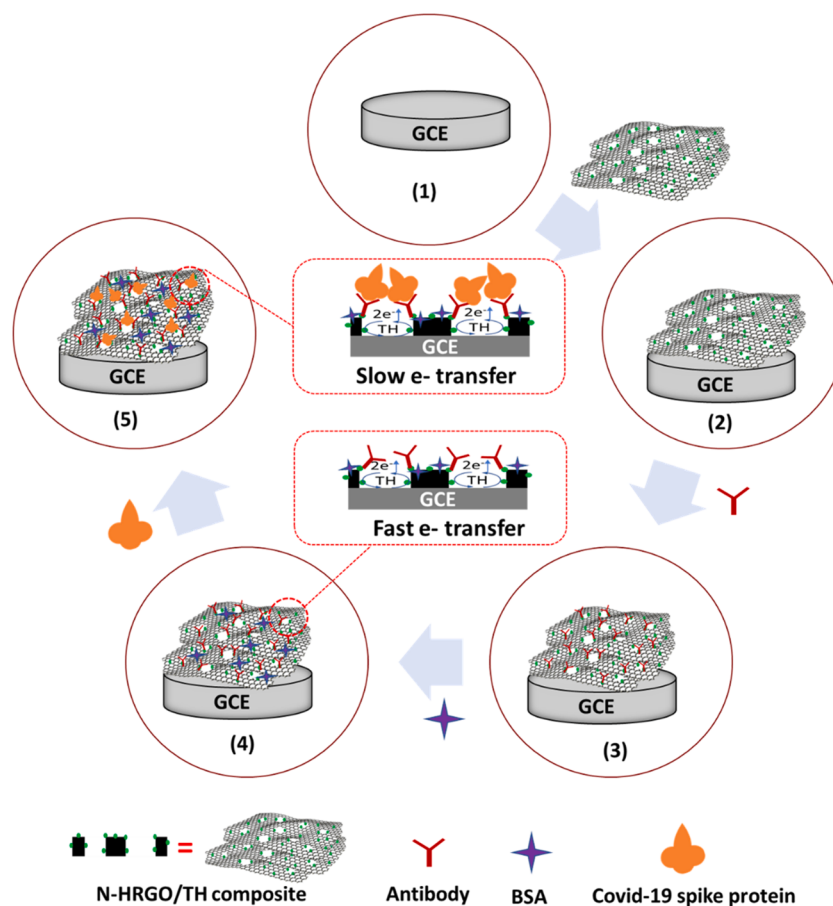
3.3.1. Theory and fabrication of electrochemical immunoassay

Based on the above observed good performance for the as-prepared N-HRGO/TH composites, a label-free electrochemical immunosensor for SARS-CoV-2 spike protein was designed and fabricated. The fabrication process and detection principle was presented in Scheme 1 and monitored by CV and electrochemical impedance spectroscopy (EIS) in 5 mM [Fe(CN)₆]^{3-/4-}. The results presented in Fig. 3A show that, after the GCE was modified with the N-HRGO/TH composite, the peak current was slightly increased compared with that of the bare GCE, which can be attributed to the good electrochemical properties of N-HRGO/TH composite and the large surface area associated with the N-HRGO material. Then, when COVID-19 spike protein antibodies attached and BSA was adsorbed in succession, the peak currents of the electrode decreased gradually. Finally, when 1 ng/mL SARS-CoV-2 spike protein was incubated on the electrode surface, the peak current was further decreased, indicating the successful construction of the electrochemical immunosensor and it has response to the target spike protein. In addition, the assembly procedure on the surface of the N-HRGO/TH modified electrode was also confirmed by the increase in charge-transfer resistance (*R*_{ct}) (Fig. 3B and Table S1). After COVID-19 spike protein were incubated on the surface of the as-prepared electrochemical immunosensor, the corresponding *R*_{ct} was increased from 869 Ω to 1399 Ω. The increased *R*_{ct} can be attributed to the formed antigen–antibody complex, which blocked the holes within the GCE-supported N-HRGO/TH layer (Scheme 1(5) and the associated enlarged view), and hence the electrolyte/electron-transfer, indicating the SARS-CoV-2 spike protein was selectively assembled on the surface of the immunosensor.

As can see from the above, the detection principle for the proposed electrochemical immunosensor was based on the fact that the formed antigen–antibody complex acted as a physical blocker for the mass-transfer of electrolyte through the holes of the GCE-supported N-HRGO/TH layer, and hence its electron-transfer with the electrode to slow down the redox reaction of TH (Scheme 1). The above results were also consistent with the DPV results presented in Fig. S5, which show a remarkable oxidation peak for the N-HRGO/TH composite modified electrode compared to that of the bare GCE in PBS electrolyte due to the redox properties of TH. Subsequent modification with antibodies and BSA caused the peak current of TH to slightly decrease. After incubation with the COVID-19 spike protein, the formed antigen-antibody complex on the surface of electrode can highly hinder electron transfer, and an obvious decrease of the oxidation peak current from TH was observed. This result is consistent with previously published work [38,42]. The response current should be proportional to the corresponding concentration of SARS-CoV-2 spike protein. All the above results demonstrated that the label-free electrochemical immunosensor had been successfully constructed and could be used for COVID-19 spike protein specific detection through the formation of the antigen-antibody complex.

3.3.2. Analytical performance of the electrochemical immunosensor for COVID-19 spike protein detection

The as-prepared electrochemical label-free immunosensor was



Scheme 1. The fabrication process and detection principle of the proposed electrochemical immunosensor.

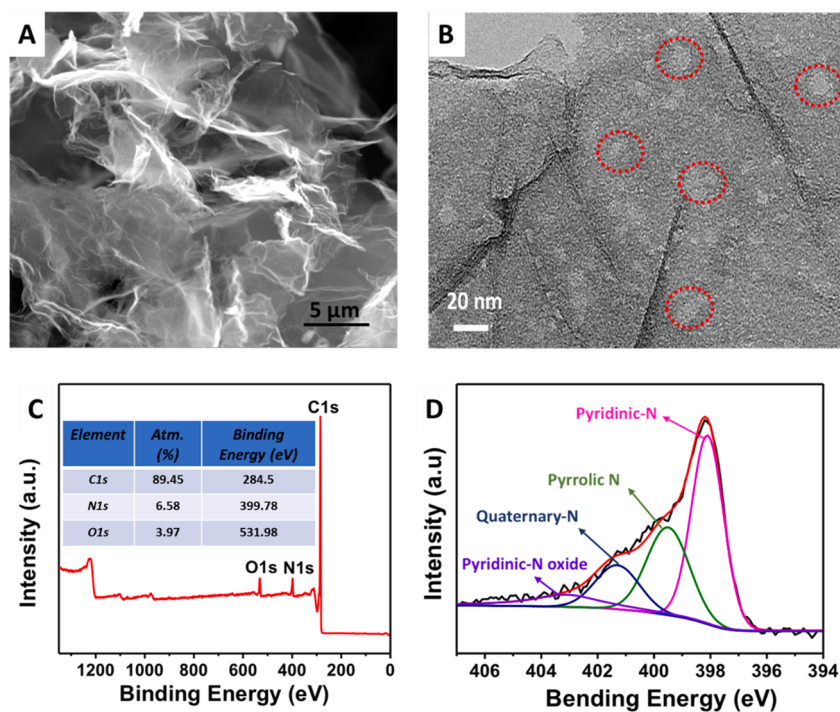


Fig. 1. (A) SEM and (B) TEM characterization of the synthesized N-HRGO. (C) The XPS survey spectrum of the N-HRGO. The inset is the elemental analysis; (D) High-resolution spectrum of N1s.

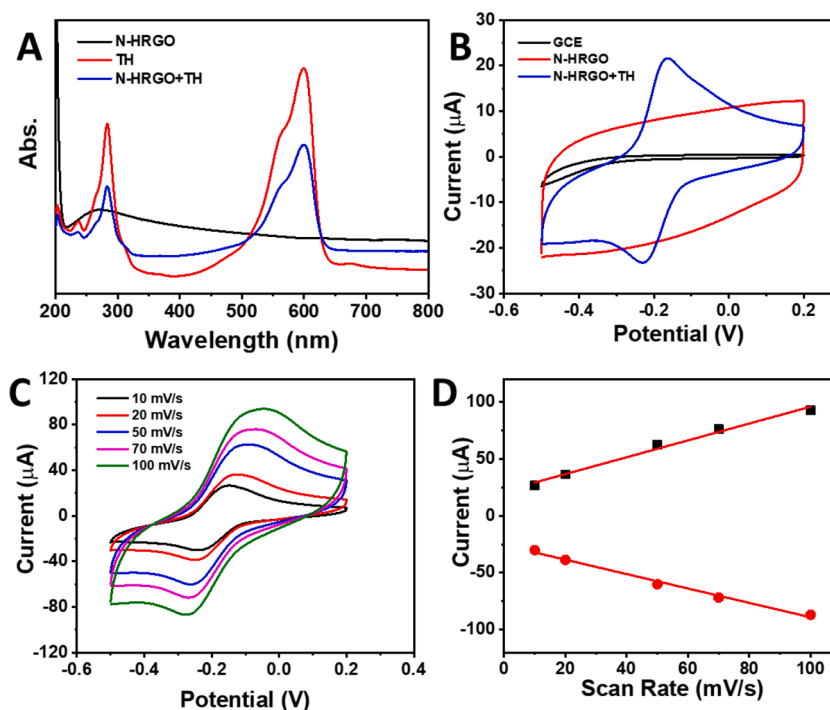


Fig. 2. (A) UV-vis absorption spectra of N-HRGO, N-HRGO/TH nanocomposites and TH. (B) Cyclic voltammograms of the bare GCE, the N-HRGO modified GCE and the N-HRGO/TH modified GCE in 0.1 M PBS buffer (pH = 7.4). (C) Cyclic voltammograms of the N-HRGO/TH modified GCE in 0.1 M PBS buffer at different scan rates from 10 mV/s to 100 mV/s. (D) Plot of peak current versus scan rate.

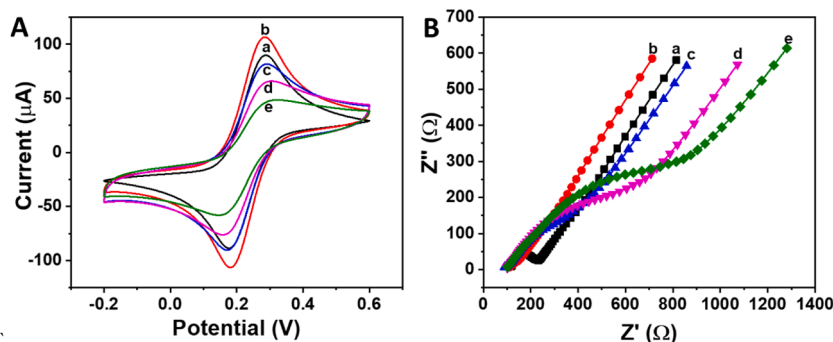


Fig. 3. (A) CV and (B) EIS curves for monitoring the fabrication process of the electrochemical immunosensor in 5.0 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ solution containing 0.1 M KCl, where (a) GCE; (b) GCE modified with N-HRGO/TH; (c) GCE modified with N-HRGO/TH/Anti-body; (d) GCE modified with N-HRGO/TH/anti-body/BSA; (e) GCE modified with N-HRGO/TH/Anti-body/BSA/spike protein (1 ng/mL).

investigated through DPV for specific and sensitive detection of COVID-19 spike protein. Firstly, the incubation time was optimized by incubating 1 ng/mL COVID-19 spike protein on the prepared electrochemical immunosensor. As shown by the result presented in Figure S6, the response current was increasing along with the incubation time, and reached a stable equilibrium after 30 min. It could be obtained that the time of response for the prepared electrochemical immunosensor towards COVID-19 spike protein is less than 30 min.

Then, the sensitivity for the prepared electrochemical immunosensor was further investigated. Fig. 4A demonstrates that the peak current intensity decreased gradually with the increasing concentrations of COVID-19 spike proteins, due to the formation of COVID-19 spike protein-antibody complexes at the surface of the immunosensor that retarded the electron transfer of TH. Moreover, by replotting the current decrement ratio $(I_0 - I_i)/I_0$ (where I_0 and I_i are the currents before and after incubation with different concentrations of COVID-19 spike protein) versus the concentrations of the COVID-19 spike protein (Fig. 4B), it can be clearly seen that the current decrement ratio was increasing

with the increasing concentrations of spike protein and a threshold was achieved after 1 ng mL⁻¹. In addition, a good linear relationship between the current decrement ratio and the logarithmic concentrations of the COVID-19 spike protein was also obtained, which can be represented as $(I_0 - I_i)/I_0 = 0.244 + 0.077 \times \log C$ (where C is the concentration of COVID-19 spike protein), with a wide detection range of 1 pg mL⁻¹–10 ng mL⁻¹ and good detection limit of 0.3 pg mL⁻¹ (S/N = 3), which is comparable and even superior than other reported methods (Table S2) [15,18,43–45]. In addition, the prepared electrochemical immunosensor is based on the N-HRGO/TH nanocomposite, which is a label-free immunosensor without the complex secondary anti-body modification and additional redox probes.

Since selectivity, stability and reproducibility are critical parameters for the fabricated electrochemical immunosensor, they were also investigated for practical application. In order to assess the selectivity of the proposed electrochemical immunosensor, several interfering proteins, such as human IgG protein, SARS spike glycoprotein, and COVID-19 nucleocapsid protein were chosen. As shown from the insert in

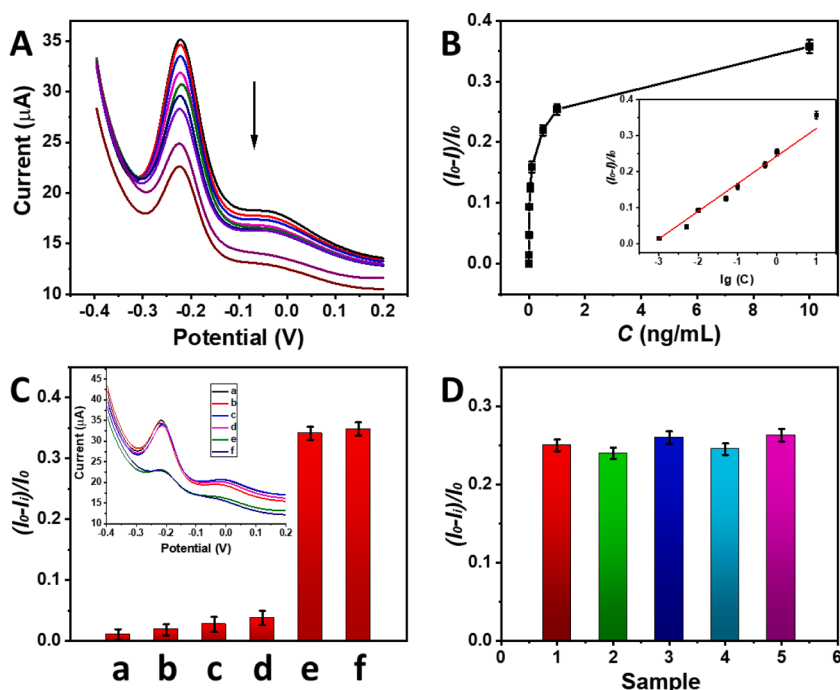


Fig. 4. The detection performance of the proposed electrochemical immunosensor towards spike protein of COVID-19. (A) DPV curves under different concentrations of spike protein from 1 pg mL^{-1} to 10 ng mL^{-1} . (B) Plots of the current decrement ratio $(I_0 - I_i)/I_0$ versus the spike protein concentration (inset: a linear relationship between the current ratio and the logarithm of spike protein concentration). (C) Selectivity characterization in the presence of different interfering proteins, insert shows the DPV curves of the fabricated electrochemical immunosensor in the presence of different interfering proteins, where (a) is blank, (b) is 10 ng mL^{-1} human IgG, (c) is 10 ng mL^{-1} SARS spike glycoprotein, (d) is 10 ng mL^{-1} COVID-19 nucleocapsid protein, (e) is 10 ng mL^{-1} COVID-19 spike protein and (f) is the above proteins mixture. (D) The reproducibility for five individual immunosensor.

Fig. 4C, the DPV curves have a negligible decrease towards the background after the immunosensor was exposed towards all of the listed interfering proteins, while the peak current still shows a drastic decrease when incubated with COVID-19 spike protein, including its mixture with all the other proteins. The result indicates that the as-prepared electrochemical immunosensor has good selectivity for COVID-19 spike protein detection even in a mixture system. Furthermore, in order to show the result more intuitively, the peak current decrement ratio was used to quantify the current change of the immunosensor when exposed to different interfering proteins (Fig. 4C). High specificity and selectivity towards COVID-19 spike protein detection was clearly displayed, indicating that the N-HRGO carrier material loading TH as redox probe holds great potential for COVID-19 spike protein specific detection.

To investigate the reproducibility of the as-prepared electrochemical immunosensor, five individually prepared immunosensors were used to detect COVID-19 spike protein (1.0 ng mL^{-1}) under the same conditions. As seen in Fig. 4D, all five immunosensors show good response towards the COVID-19 spike protein, with the relative standard deviation (RSD) around 3.88%, indicating the desirable accuracy and reproducibility for the as-prepared immunosensor. In addition, the storage stability of the immunosensor was also investigated by storing the immunosensors at 4°C . As illustrated in Figure S7, the detection performance towards SRAS-CoV-2 spike protein was only reduced by 7.70% after 7 days storage at the low temperature, illustrating its long-term storage stability.

3.3.3. Analytical performance in real samples

The potential application of the as-prepared electrochemical immunosensor in complex samples was also investigated by addition of different concentrations of COVID-19 spike proteins into 10% diluted saliva and human serum samples for detection. As seen in Fig. S8, the peak current decrement ratio in saliva and human serum samples show nearly the same detection results in 0.1 M PBS buffer solution. The corresponding recovery analyses of the spiked samples were shown in Table S3, and the recoveries were obtained with a satisfied result from 90.5% to 107.2%, indicating that the proposed electrochemical immunosensor could be used for COVID-19 spike protein detection in complex real samples with a high accuracy.

4. Conclusions

In summary, a label-free electrochemical immunosensor was successfully fabricated for rapid detection of COVID-19 utilizing the advantages of N-HRGO, including its ultra-high surface area, enhanced ion/mass transport and catalytic properties. In addition, TH molecules were attached on the surface of N-HRGO to act as bi-functional molecules for antibody modification and electrochemical indicators. The as-prepared electrochemical immunosensor exhibited good detection performance under different experimental conditions towards the COVID-19 spike protein, demonstrating a wide detection concentration range of 1 pg mL^{-1} – 10 ng mL^{-1} and a low detection limit of 0.3 pg mL^{-1} ($S/N = 3$), together with a good selectivity, stability and reproducibility. Most importantly, the proposed immunosensor also shows reliable detection results in saliva and human serum complex samples, indicating its potential practical application for rapid and accurate clinical diagnosis of COVID-19.

Declaration of Competing Interest

The authors declare no competing financial interest.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.snr.2022.100124.

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