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Colorimetric and electrochemical detection of SARS-CoV-2 spike antigen with a gold nanoparticle-based biosensor

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HIGHLIGHTS

The developed probe (AuNP-mAb) exhibits dual sensing mode for the detection of SARS-CoV-2 spike antigen (S-Ag).

AuNP-mAb allows visual detection (colorimetric) of S-Ag with a detection limit of 48 ng/mL.

Electrochemical detection of S-Ag is achieved by a disposable screen-printed gold electrode with a detection limit of 1 pg/mL.

Neither method exhibits cross-reactivity with other viral proteins such as Influenza A, MERS-CoV and Streptococcus pneumoniae.

AuNP-mAb allows simple and rapid analysis of S-Ag in saliva sample.

ABSTRACT

Since emerging in China in December 2019, COVID-19 has spread globally, wreaked havoc for public health and economies worldwide and, given the high infectivity and unexpectedly rapid spread of the virus responsible—that is, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)—urged the World Health Organization to declare it a pandemic. In response, reducing the virus’s adverse effects requires developing methods of early diagnosis that are reliable, are inexpensive and offer rapid response. As demonstrated in this article, the colorimetric and electrochemical detection of SARS-CoV-2 spike antigen with gold nanoparticle-based biosensors may be one such method. In the presence of the SARS-CoV-2 spike antigen, gold nanoparticles aggregated rapidly and irreversibly due to antibody–antigen interaction and consequently changed in colour from red to purple, as easily observable with the naked eye or UV–Vis spectrometry by way of spectral redshifting with a detection limit of 48 ng/mL. Moreover, electrochemical detection was achieved by dropping developed probe solution onto the commercially available and disposable screen-printed gold electrode without requiring any electrode preparation and modification. The method identified 1 pg/mL of the SARS-CoV-2 spike antigen and showed a linear response to the SARS-CoV-2 spike antigen ranging from 1 pg/mL to 10 ng/mL. Both...
1. Introduction

Since emerging in December 2019 in Wuhan, the capital of China’s Hubei Province, the unprecedented coronavirus 2019 (COVID-19) has caused massive disruptions for public health and economies worldwide [1–4]. Known to be caused in humans by respiratory infections of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), COVID-19’s high infectivity and rapid spread have posed serious threats across the globe, as evidenced by the steep rise in mortality in the past 18 months, during which time more than 4.2 million people have died worldwide [5,6].

On 31 January 2020, the World Health Organization listed COVID-19 as a “Public Health Emergency of International Concern” [1]. The disease’s well-known symptoms usually begin with a fever and difficulty with breathing, followed by a severe cough and other acute symptoms, and may even result in death [7,8]. However, asymptomatic cases have also been reported [9,10]. With or without symptoms, the dramatic increase in the number of cases of COVID-19 has driven high demand for diagnostic tests to confirm the virus’s presence rapidly, accurately and selectively [11–14]. To date, the process of developing such tests has confirmed that spike (S), envelope (E), membrane (M) and nucleocapsid (N) are the significant structural proteins for coronavirus particles [15]. Added to that evidence, various studies have indicated that the spike protein plays the most important role in the virus’s entry and binding to the human angiotensin-converting enzyme 2 (ACE2) receptor on the surface of cells, and the spike protein has thus become a promising new target for sensors [16–20]. Accordingly, various methods have been developed for the detection of COVID-19 such as virus nucleic acid real time-PCR (RT-PCR) [21–23], CT imaging [24], enzyme-linked immunosorbent assay (i.e. ELISA) [25], point-of-care tests [26], lateral flow immunoassay tests [25,27,28] and tests for some haematological parameters [29].

Although those methods afford considerable convenience in detecting SARS-CoV-2 and diagnosing COVID-19 and its progression, most of them share certain limitations. For example, the most commonly used and arguably most reliable method, reverse transcription RT-PCR, which can detect viral genetic material (i.e. RNA) in samples collected with nasopharyngeal swabs, its administration requires highly trained personnel, which prevents its general application and use. It is also a time-consuming method, one entailing long nucleic acid extraction, and has been prone to giving false negatives [30]. Given such limitations in the most frequently used, reliable method, a much simpler, faster, more sensitive method has been needed to detect SARS-CoV-2, provide timely treatment to patients and prevent the spread of the disease. Therefore there is a continuous demand for selective, rapid, repeatable, cost-effective, ready-to-use, and ultrasensitive biosensors. Against that background, the demand for rapid, selective, repeatable, cost-effective, ready-to-use, ultrasensitive biosensors has continually risen. Of the numerous biosensors using colorimetric [31–34], scanometric [35], electrochemical [36,37], and fluorometric [38,39] systems to detect well-known human viruses [40–42], colorimetric assay is a simple, direct method of visual detection that does not require any complicated equipment. Metal nanoparticle-based colorimetric assays are commonly used to diagnose diseases in humans, and the development of those biosensors has also enabled the development of rapid colorimetric diagnostic tests that can be used even at home. In particular, gold nanoparticles (AuNPs) are often used in colorimetric assays due to their easy synthesis, low cost, simplicity, practicality, unique optical properties and the functionality of their surfaces [43–47]. Colorimetric detection based on AuNPs takes advantage of the change in colour, from red to purple, that occurs in a colloidal suspension via antigen–antibody interaction [48,49]. In parallel, electrochemical sensing methods of detecting proteins, nucleic acids, bacteria, viruses, antibodies and their fragments have also become attractive owing to their simplicity, low cost, rapidity, high sensitivity and selectivity [37,50–53]. Due to those combined advantages, electrochemical and colorimetric biosensors and/or methods could be developed to determine molecular SARS-CoV-2 antigens, antibodies and/or their fragments. Indeed, in the past few months alone, several nanoscale integrated structures based on optical and electronic systems have been reportedly been able to detect the SARS-CoV-2 spike protein with high sensitivity [54–68].

In this article, we present the design, synthesis and spectral features of an AuNP-based biosensor platform for detecting the SARS-CoV-2 spike antigen with high selectivity and sensitivity. The detection process relies on two techniques: a voltammetric method and an optical sensing method using the naked eye or UV–Vis spectrophotometry.

2. Methods

2.1. Materials

Gold (III) chloride trihydrate (HAuCl₄ ≥ 99.9, Sigma-Aldrich 520918), 11-mercaptopoundecanoid acid (MUA, ≥ 95, Sigma-Aldrich 450561), Sodium citrate dihydrate (≥ 99, Sigma-Aldrich W302600) (N-(3-Dimethylaminopropyl)-N’-ethylcarbodiimide hydrochloride (EDC, ≥ 98, Sigma-Aldrich 03450), N-Hydroxysuccinimide (NHS, ≥ 98, Sigma-Aldrich 130672), Potassium phosphate monobasic solution (KH₂PO₄, reagent grade 1.0 M, Sigma-Aldrich P8709), Bovine serum albumin (BSA, ≥ 98, Sigma-Aldrich 05470), Tween 20 (BioXtra, Sigma-Aldrich P7949) and other chemicals were purchased from Sigma-Aldrich and used without further purification. SARS-CoV-2 spike monoclonal antibody (mAb) (Chimeric Mab Cat: 40150-D00), SARS-CoV-2 (2019-nCoV) spike S1-his recombinant protein (HPLC-verified, Cat: 40591-V08H), MERS-CoV spike/S1 protein (S1 Subunit, aa 1-725, His Tag, Cat: 40069-V08B1), Influenza A H1N1 Hemagglutinin/H0A protein (Cat: 11055-VNAB) were obtained from Sino Biological. Streptococcus pneumoniae antigen, the native extract was purified from Native Antigen Company. Ultrapure water was procured from Milli-Q Direct 8 system. All the antibody and antigen solutions were prepared in phosphate buffer solution (pH 7.4) and stored in protein LoBind Eppendorf tubes.

2.2. Instruments

A UV-1900i spectrophotometer (Shimadzu) and quartz micro cuvettes (700 µl, Hellma) were used for all local surface plasmon resonance (LSPR) analyses. The LSPR band of AuNPs and functionalised AuNPs were monitored between 300 and 800 nm in order to
track alterations in the size and distribution of particles. The hydrated particle sizes were evaluated using a Zetasizer with dynamic light scattering (DLS; Malvern Zetasizer Nano ZS-3600), while the surface functionalisation of AuNPs was investigated with a Bruker Alpha II compact Fourier Transform Infrared (FTIR) spectrometer. High-resolution transmission electron microscopy (TEM) was performed with a JEOLEM 2100 HRTEM at 200 kV to characterise the morphologies of monodispersed AuNPs and AuNPs bioconjugates before and after incubation with the SARS-CoV-2 spike antigen. Images were taken with a Gatan Model 833 Orius SC200D CCD camera, and carbon support film-coated copper TEM grids (Electron Microscopy Sciences, CF200—Cu, 200 mesh) were used. All incubations were performed in a MTC-100 Miliab thermosthaker, and a Mettler Toledo Seven Compact pH meter with InLab Expert Pro-ISM combined with a pH electrode was used to prepare the buffer solutions. Electrochemical measurements were taken with a Metrohm Dropens potentiostat—galvanostat, a Dropens boxed connector (DSC4MM) and a screen-printed gold electrode (Dropens C220BT) consisting of working gold and auxiliary electrodes and a silver reference electrode at 21 ± 3 °C and 45 ± 15% relative humidity.

2.3. Preparation of AuNPs

AuNPs were synthesised by following a citrate reduction method [69]. First, all glassware used in the experiment was thoroughly cleaned with aqua regia (3:1 (v/v) HCl:HNO3), rinsed with deionised water and oven-dried before use. Briefly, an aqueous solution of 2.5 mL of 5 mM HAuCl4 was added to 50 mL of ultrapure water under vigorous stirring until boiling. After boiling, 1% sodium deionised water and oven-dried before use. Briefly, an aqueous solution of 2.5 mL of 5 mM HAuCl4 was added to 50 mL of ultrapure water under vigorous stirring until boiling. After boiling, 1% sodium hydroxide solution was added to the cooled solution. The concentration of the process appears in Scheme 1. Brieﬂy put, 100 mL of an ethanolic solution containing MUA (10 mM) was added to 1 mL of citrate-capped solution with AuNPs, and the reaction mixture was incubated overnight at room temperature before centrifugation. The final mixture was centrifuged at 13,200 rounds of per minute (rpm) for 15 min, washed twice with phosphate buffer (PB, 10 mM, pH 7.4) to remove unbound MUA molecules and resuspended in PB (10 mM, pH 7.4) containing 0.2 mg/mL of Tween 20. The carboxylic groups of linkers were activated in a freshly prepared solution of 5 mM of EDC and 7.5 mM of NHS. The AuNPs were reacted with 20 μL of the EDC—NHS mixture with gentle shaking for 0.5 h at room temperature, which ultimately yielded NHS-terminated AuNPs (AuNPs—MUA).

2.4. Surface modification of AuNPs

The citrate groups on the surface of the AuNPs were exchanged with 11-mercaptoundecanoic acid (MUA) following slight modifications made according to the literature [47]. A schematic illustration of the process appears in Scheme 1. Briefly put, 100 mL of an ethanolic solution containing MUA (10 mM) was added to 1 mL of citrate-capped solution with AuNPs, and the reaction mixture was incubated overnight at room temperature before centrifugation. The final mixture was centrifuged at 13,200 rounds of per minute (rpm) for 15 min, washed twice with phosphate buffer (PB, 10 mM, pH 7.4) to remove unbound MUA molecules and resuspended in PB (10 mM, pH 7.4) containing 0.2 mg/mL of Tween 20. The carboxylic groups of linkers were activated in a freshly prepared solution of 5 mM of EDC and 7.5 mM of NHS. The AuNPs were reacted with 20 μL of the EDC—NHS mixture with gentle shaking for 0.5 h at room temperature, which ultimately yielded NHS-terminated AuNPs (AuNPs—MUA).

2.5. Preparation of AuNPs—mAb

The conjugate of AuNPs—mAb was prepared by adding 1.5 μg of the SARS-CoV-2 spike antibody (mAb) to 1 mL of activated AuNPs (AuNPs—MUA), followed by incubation at 37 °C for 1 h with gentle shaking at 200 rpm, during which time the mAb was reacted with AuNPs through covalent bond formation via EDC—NHS cross-linking agents. After being blocked by 10 μL of 1% (m/v) BSA in 10 mM PB for 15 min at 37 °C, the AuNPs—mAb was centrifuged for 15 min at 13,200 rpm at 4 °C to remove the unbound protein. The supernatant was discarded, and the sediment was washed twice with PB (10 mM, pH 7.4) containing 0.2 mg/mL of Tween 20. Last, the AuNPs—mAb conjugate was resuspended in PB and stored at 4 °C for subsequent experiments.

2.6. Detection of the recombinant SARS-CoV-2 spike antigen based on LSPR

The SARS-CoV-2 spike antigen was diluted serially at 250, 500, 750, 1000, and 2000 ng mL⁻¹, and the proper amount was added to one-fold-concentrated AuNPs—mAb conjugate solution, followed by incubation at room temperature for 10 min. The colour of the mixtures changed from red to purple, and the SARS-CoV-2 spike antigen could be detected with the naked eye and based on LSPR with redshifting (~25 nm).

2.7. Detection of the recombinant SARS-CoV-2 spike antigen based on the electrochemical method

Square wave voltammetry (SWV) was performed with 5 mV of step potential, 20 mV of pulse amplitude, a frequency of 10 Hz and a scan rate of 50 mV/s. Cyclic voltammetry (CV) was performed with 2.5 mV of step potential and a scan rate of 50 mV/s. Both SWV and CV measurements were performed with 50 μL of AuNPs—mAb in PB solution (10 mM, pH 7.4) containing 0.2 mg/mL of Tween 20 and with a proper amount of the SARS-CoV-2 spike antigen (1, 10, 100,
10^3 and 10^4 pg mL^{-1}) by using a commercially available screen-printed gold electrode as a supporting surface. After each measurement, the sensor was washed with ethanol, dried with argon gas and filled with 50 μL of the new solution.

2.8. Sample preparation

Saliva samples were collected from six healthy individuals and half of the samples were spiked with 500 ng/mL and 10 pg/mL of the SARS-CoV-2 spike antigen for optical and electrochemical measurements, respectively. Then, all of the samples were transferred into the AuNPs-mAb in PB solution (10 mM, pH 7.4) containing 0.2 mg/mL of Tween 20. The standard measurement procedure (explained in Section 2.6 and 2.7) was performed for both methods and analysed using external calibration curve.

3. Result and discussion

3.1. Synthesis and characterisation of AuNPs

Size-controlled AuNPs were obtained by way of citrate reduction. Usually, AuNPs interact with each other via Van der Waals forces at short distances; negatively charged citrate ions on the surface of AuNPs provide electrostatic repulsion, which repels AuNPs and precludes them from aggregating. In our research, the size of synthesised AuNPs was determined by DLS and TEM as 16 nm and thus similar to reports in the literature [70]. TEM images revealed that AuNPs exhibited good monodispersity, were similar in size and low aspect ratio. The characteristic absorption peak was seen at 520 nm on the UV–Vis spectrum, with the narrow spectral bandwidth showing the monodispersity of the AuNPs as well (Fig. 1).

To measure DLS, we prepared the solution of AuNPs in distilled water, not in a buffer solution such as PB, PBS and HEPES, which can reduce the repulsion of AuNPs and risks their aggregation. DLS revealed that the average size of AuNPs was 16 nm (range: 11.2–20.6 nm), as depicted in Fig. S1. Adding MUA provided a self-assembled monolayer on the gold surface by way of thiol groups, which provided stability. In the process, the long alkyl chain supplied elasticity such that the active site of the linker could easily interact with different types of ligands. Among the results, the UV–Vis spectrum of the MUA-linked AuNPs exhibited a moderate shift in the LSPR peak from 520 to 523 nm, as previously described in the literature [47] and the spectrum showed a single narrow LSPR peak without broadening, which confirms that AuNPs do not aggregate due to the chemisorption of the MUA linker. FTIR analysis, performed to ensure ligand exchange on the surface of the AuNPs, revealed a broad peak at 3200 cm^{-1}; such peak refers to O–H stretching vibrations that can relate to trace water due to insufficient drying. The presence of the sharp, strong peaks at 2915 and 2848 cm^{-1} for pure MUA are attributable to the symmetric and asymmetric stretching of the CH2 groups, respectively. In the case of AuNPs–MUA, the peaks remained visible, which confirms the success of ligand exchange (Fig. S2). Because the AuNP–MUA complex was activated by EDC–NHS coupling, the efficiency of which was usually low and sensitive to pH, we used Tween 20 in buffer solution (pH = 7.4) to maintain stability. When Tween 20 was not introduced, by contrast, AuNPs aggregated irreversibly. We also tested the stability of the AuNPs and the coupling process in water, PBS (10 mM), PB (10 mM) and HEPES (10 mM) buffers by comparing their LSPR peaks. PB buffer (10 mM) was chosen as the most suitable buffer for both AuNPs and the coupling process without proceeding with any aggregation (data not shown). Thus, we used 10 mM of PB with Tween 20 (0.2 mg/mL) as a buffer for all experiments reported here.

After the activation of AuNPs–MUA with EDC–NHS, 1.5 μg/mL of the SARS-CoV-2 spike antibody (mAb) was introduced to the activated complex to obtain covalent bonding between the antibody’s amine groups and complex’s carboxylate groups. An optimisation study was performed to determine the ideal concentration of mAb by experimenting with different amounts of mAb (0.5, 1.0, 1.5, 2.0 and 3.0 μg/mL), for results indicating any increment of antigen capture efficiency exceeding 1.5 μg/mL of mAb. Thus, the optimal concentration of mAb was determined to be 1.5 μg/mL (Fig. S3). AuNPs–mAb exhibited a 3 nm shift (526 nm) in the UV–Vis spectrum (Fig. 2). On top of that, the conjugate preserved its red colour and was highly stable in the solution containing 0.1 M of NaCl.

3.2. Colorimetric detection of the SARS-CoV-2 spike antigen with the AuNP–mAb probe

Under optimised conditions, we investigated the ability of the AuNPs-mAb probe to detect the SARS-CoV-2 spike antigen using
LSPR. To obtain good capture efficiency, 200 μL of the AuNP–mAb probe was incubated with different amounts of the SARS-CoV-2 spike antigen for 10 min at room temperature. In the presence of the spike antigen (2 μg/mL), AuNPs–mAb bound with the antigen particles, thereby providing an agglomeration of AuNPs with both spectral redshift (~25 nm) and a change in colour visible with the naked eye (Fig. 3a). Moreover, TEM images clearly showed that the AuNP–mAb probes were attached to the surface of the antigen particles. DLS analysis revealed that the hydrated particle size of the AuNPs–mAb increased from 34 nm to 264 nm after the addition of the SARS-CoV-2 spike antigen. The results thus indicated that the method can be used to detect the SARS-CoV-2 spike antigen (Fig. S1).

The mechanism of AuNPs–mAb based detection of SARS-CoV-2 spike antigen was shown in Scheme 2. Conjugated gold nanoparticles with monoclonal SARS-CoV-2 spike antibody which is highly specific to SARS-CoV-2 spike antigen. AuNPs–mAb probe can be specifically arranged and lead to networking on the SARS-CoV-2 spike antigen surface which reduces the distance between each AuNPs and aggregation occurs. In this case, this aggregation of the AuNPs–mAb probe causes slight increase the size of the particles and such event produces redshift and broadening as well as decreasing LSPR peak in the spectrum. Decreasing the peak intensity can be explained that the size and amount of SARS-CoV-2 spike antigen are larger than AuNPs–mAb (34 nm) and some of AuNPs–mAb are bounded to antigen surface which results fall in absorbance peak. It should be mentioned that absorbance value only diminished after the recognition event between AuNPs–mAb and SARS-CoV-2 spike antigen.

3.3. Sensitivity of the detection of the SARS-CoV-2 spike antigen based on LSPR

The sensitivity of our probe was examined by measuring absorbance versus increasing amounts of the SARS-CoV-2 spike antigen. The first signal change occurred with the addition of 250 ng/mL of the antigen, which caused a redshift in the LSPR peak of the AuNP–mAb probe from 526 nm to 528 nm (Fig. 4a). The linear relationship was observed between λmax and the SARS-CoV-2 spike antigen concentration, for a correlation coefficient of 0.99.
that shows the method’s applicability in quantitative analysis. The limit of detection was calculated with $3s_{res}/m$ equation where $s_{res}$ is the residual standard error obtained from calibration graph data by regression analysis and $m$ is the slope of calibration curve as 48 ng/mL.

3.4. Selectivity and stability of SARS-CoV-2 spike antigen detection based on LSPR

Because selectivity is a crucial parameter for detection, the selectivity of our AuNP–mAb probe was tested with different types of spike antigens, including the influenza A antigen (i.e. H1N1), the MERS-CoV antigen and the Streptococcus pneumoniae antigen. Other antigens were added to our probe solution in a concentration of 1 µg/mL under the same and optimised conditions. The results showed no significant shift in the samples containing other antigens (Fig. 5a). To clarify the probe’s storage stability for other applications, the same sample and patch of the AuNP–mAb probe in buffer solution (PB pH = 7.4 with 0.2 mg/mL Tween 20) was left at 4 °C in the dark for the different period. After that period, the sample was incubated with 500 ng/mL of the SARS-CoV-2 antigen. Results showed nearly the same signal change after storage for 4 weeks, which suggests that our AuNP–mAb probe showed good, relatively long-term stability (Fig. 5b).

3.5. Electrochemical detection of the SARS-CoV-2 spike antigen with the AuNP–mAb probe

Because our AuNPs–mAb probe may have possessed an oxidation–reduction site, it was worth studying the

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**Scheme 2.** UV–vis detection of SARS-CoV-2 spike antigen based on the aggregation of AuNPs–mAb on the SARS-CoV-2 spike antigen surface.
electrochemistry of the interaction of AuNPs–mAb with the SARS-CoV-2 spike antigen. The commercially available and disposable screen-printed gold electrode was used without following any sensor modification and production steps that offers a simple and cheap measurement method. Since screen-printed gold electrode was used, a specific interaction occurred between the gold surface (partially negative charge) and gold particles (partially positive charge) in AuNPs–mAb. With the help of a cathodic scan, AuNPs–mAb behaved as an electrode material. During the cathodic scan, the groups containing heteroatoms like carbonyl on the surface of mAb were reduced. After an increasing amount of SARS-CoV-2 spike antigen addition, antigen-antibody interactions took place in which the signal of the developed sensor decreased due to the lowering free groups including heteroatoms on the surface of mAb. As anticipated, AuNPs–mAb had few oxidation peaks but two well-defined reduction peaks at 205 mV and −50 mV (Fig. 6). In particular, the peak at −50 mV decreased with the addition of the SARS-CoV-2 spike antigen.

The sensitivity of the electrochemical system was also examined, as previously done for the colorimetric detection of the SARS-CoV-2 spike antigen. The calibration voltammograms and curve belonging to the SARS-CoV-2 antigen are shown in Fig. 7. The proposed method is able to detect 1 pg/mL of the SARS-CoV-2 spike antigen and has a linear response to the antigen between 1 pg/mL and 10 ng/mL in 10 mM of PB solution (pH 7.4) containing 0.2 mg/mL of Tween 20.

Using the same strategy, we proceeded with examining selectivity under the same and optimised conditions. The effects of interference with the Streptococcus pneumoniae, influenza A and MERS-CoV spike antigens were investigated to evaluate the proposed method’s selectivity (Fig. 8). Both the immobilising tendency of the interference on the AuNP–mAb probe and the effects of disrupting the binding between the SARS-CoV-2 antibody and antigen were examined, as shown in Fig. 8 and S4, respectively. Ultimately, the proposed electrochemical detection method based on AuNPs–mAb showed no response to 100 pg/mL of the interference.
which was 100-fold relative to the SARS-CoV-2 spike antigen. Those results clearly indicate the satisfactory feasibility of the proposed sensing method. As given in Table S1, AuNPs–mAb exhibited comparative advantages over other published studies previously in terms of diversity of the sensing methods and detection limit (Table S1).

### 3.6. Sample application

When the developed method was applied to the spiked saliva samples to determine the SARS-CoV-2 spike antigen, the relative standard deviation and recovery values for both optical and electrochemical methods varied from 2.2% to 4.8% and 94.1%–102.2%, respectively (Table 1). Absorbance spectra and voltammograms for the spiked and non-spiked saliva samples appear in Fig. S5. The results generally suggest that the method offers good precision and trueness, thus a good accuracy.

### 4. Conclusion

In sum, we have developed a simple, rapid (i.e. 10 min), selective, dual-response colloidal AuNP-based biosensing platform that allows both the colormetric and electrochemical detection of the SARS-CoV-2 spike antigen at the level of ng/mL (i.e. for the colormetric method) and pg/mL (i.e. for the electrochemical method). The colormetric method’s applicability was confirmed with positive results visible to the naked eye without requiring any sophisticated instruments. Beyond that, the sensor in the developed electrochemical method, as a disposable material, can be reused and does not require time-consuming steps such as sensor preparation and replacement. Moreover, to the best of our knowledge, it is the first electrochemical study for the detection of SARS-CoV-2 spike antigen without requiring a sensor preparation and modification. The developed system is used to detect the SARS-CoV-2 spike antigen in saliva samples successfully and also it offers simplicity, cost-efficiency and speed. Last, neither method exhibits cross-reactivity with other viral proteins (i.e. influenza A, MERS-CoV and Streptococcus pneumoniae). Therefore, the biosensing platform can be easily integrated into a ready-to-use commercial kit and adapted for the diagnosis of other emerging viral diseases.

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### Appendix A. Supplementary data

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### CRediT authorship contribution statement

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### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
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