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Plasmonic nanostructure-enhanced Raman scattering for detection of SARS-CoV-2 nucleocapsid protein and spike protein variants

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HIGHLIGHTS

- Plasma-engineered SERS sensors can detect SARS-CoV-2 nucleocapsid and spike antigens in artificial saliva.
- Antibody-functionalized SERS sensors can detect SARS-CoV-2 wild-type, Alpha, Delta, and Omicron variants.
- The developed SERS sensors can be used as a precise quantitative diagnostic tool.

GRAPHICAL ABSTRACT

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ABSTRACT

Epidemiological control and public health monitoring during the outbreaks of infectious viral diseases rely on the ability to detect viral pathogens. Here we demonstrate a rapid, sensitive, and selective nanotechnology-enhanced severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) detection based on the surface-enhanced Raman scattering (SERS) responses from the plasma-engineered, variant-specific antibody-functionalized silver microplasma-engineered nanoassemblies (AgMEN) interacting with the SARS-CoV-2 spike (S) and nucleocapsid (N) proteins. The three-dimensional (3D) porous AgMEN with plasmonic-active nanostructures provide a high sensitivity to virus detection via the remarkable SERS signal collection. Moreover, the variant-specific antibody-functionalization on the SERS-active AgMEN enabled the high selectivity of the SARS-CoV-2 S variants, including wild-type, Alpha, Delta, and Omicron, under the simulated human saliva conditions. The exceptional ultrahigh sensitivity of our SERS biosensor was demonstrated via SARS-CoV-2 S and N proteins at the detection limit of 1 fg mL\textsuperscript{-1} and 0.1 pg mL\textsuperscript{-1}, respectively. Our work demonstrates a versatile SERS-based detection platform can be applied for the ultrasensitive detection of virus variants, infectious diseases, and cancer biomarkers.
1. Introduction

The ongoing novel coronavirus disease (COVID-19) pandemic is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) with several known variants featuring different transmissibility, infectivity, and the ability to cause severe disease and death [1,2]. In May 2022, the World Health Organization (WHO) reported that there had been more than 530.8 million cases worldwide, with 6.3 million deaths [3]. Compared with the original SARS-CoV-2 lineage, the latest SARS-CoV-2 variants exhibit increased transmissibility and antibody resistance [4]. With the continuous rise of the new variant waves, early and effective detection of the specific variant of SARS-CoV-2 is critical to pandemic control and public health monitoring.

Currently, most COVID-19 infections are diagnosed using tests based on the reverse transcription-polymerase chain reaction (RT-PCR) due to its high sensitivity and specificity [5,6]. Researchers have also developed tests based on reverse transcriptase loop-mediated isothermal amplification (LAMP) and clustered regularly interspaced short palindromic repeats (CRISPR) technology [7–10]. However, these methods require expensive equipment, reagents, and specific primers for every mutation. Moreover, some of them are prone to false-positive and false-negative results due to fluctuations in viral loading [11,12]. While enzyme-linked immunosorbent assay (ELISA) and lateral flow immunassay (LFIA) can be used for the rapid detection of antibodies/antigens [13–15]. However, immunoglobulin, such as IgM and IgG levels, can only be detected at approximately 10–14 days post-infection, making it challenging to detect the disease in the early stages [16]. On the other hand, antigen-based detections have demonstrated considerable potential because virus antigens can be detected several days before the onset of symptoms [17]. The antigen-based detection, particularly those that detect SARS-CoV-2 nucleocapsid (N) proteins, are most commonly used, but they are highly prone to false-positive results for detecting other coronaviruses [18,19]. By contrast, the SARS-CoV-2 spike (S) protein is the primary surface protein of the virus, and the S1 subunit of the SARS-CoV-2 S protein has a more diverse amino acid sequence compared with other infectious coronaviruses, such as SARS-CoV and MERS-CoV [20]. Thus, more specific tests for detecting the S protein can be applied to identify the SARS-CoV-2 virus. In addition, conventional antigen-based detection techniques, such as colloidal gold-based LFIA and ELISA, require higher sensitivity to avoid false negative results in practical testing scenarios [21].

Optical-based sensing technology with plasmonic nanomaterials has been proven highly effective in the detection of viruses and bacteria with high sensitivities [22–24], and recent works suggest that surface-enhanced Raman spectroscopy (SERS) is useful for rapid and sensitive detection of SARS-CoV-2 [25–28]. Moreover, SERS enables the fast and accurate detection of the SARS-CoV-2 virus without needing special reagents, time-consuming and complicated calibration, and laborious and expensive sample pretreatment [29,30]. The high sensitivity of SERS makes it useful in quantifying the dynamics of viral activity throughout the course of infection [31] and identifying variants of SARS-CoV-2 [22,32]. However, recent studies on SERS-based sensing are limited to detecting either S or N proteins and do not demonstrate the ability to detect the variants, especially the Omicron variant. Additionally, the current manufacturing techniques for SERS sensors have been hampered by a low production rate and a surface-limited design, making it unsuitable for large-scale production and limiting its use for point-of-care (POC) applications.

In this work, we developed a nanotechnology-enhanced SERS detection for SARS-CoV-2 variant spike proteins, including wild-type, Alpha, Delta, and Omicron, in simulated human saliva (Scheme 1a). The SERS biosensors are designed and engineered with three-dimensional (3D) porous silver microplasma-engineered nanoassemblies (denoted as AgMEN) deposited on cellulose paper using a
one-step microplasma solution process under ambient conditions (Scheme 1b left). Microplasmas have been shown effective and valuable for nanomaterials synthesis and processing [33–35]. The plasma-engineered AgMENs exhibit unique 3D porous and flexible metal nanostructures composed of twinned and highly-crystalline Ag nanoparticles (NPs), providing high SERS sensitivity by the large signal collection volume and the hot spot-enforced Raman electromagnetic field [36]. Moreover, the porous and hydrophilic paper-based substrates can enhance the analyte adsorption, maximizing the SERS sensitivity. The specific binding of the target antigen to the SERS biosensors for the simultaneous qualitative and quantitative analysis via the strong SERS signals of the antigen can be achieved by the surface functionalization with designed variant-specific antibodies by a one-step conjugation [37] (Scheme 1b center). The SERS biosensors enable the capture of corresponding variant spike proteins in simulated human saliva (Scheme 1b right). Our work provides new insight into the detection of COVID-19 variants antigens using nanostructure-enhanced Raman scattering and a versatile platform for the early detection of diseases, new drug and vaccine development, and personalized medical therapy.

2. Materials and methods

2.1. Materials

Cellulose paper (grade 5C, 47 mm diameter, 1 μm pore size, 0.22 mm thickness) was purchased from Advantec International, Ltd. (Maidstone, England). Silver foil (thickness 0.025 mm, 99.998% purity) was purchased from Alfa Aesar. Fructose (99% purity) and nitric acid (HNO₃; 65%) were purchased from Acros Organics Co. Bovine serum albumin (BSA) was obtained from Sigma Aldrich. Phosphate-buffered saline (PBS) buffer was prepared in-house. SARS-CoV-2 spike antibody (S44F), SARS-CoV-2 spike antibody (UC20), anti-human SARS-CoV-2 IgG nucleocapsid antibody (HS8), anti-chicken SARS-CoV-2 IgG nucleocapsid antibody (S11), human angiotensin-converting-enzyme 2 (ACE2) were purchased from Acros Organics Co.
(ACE2), were purchased from Pharmtek Co., Ltd, Taiwan. All reagents were used directly without further purification. All solutions were prepared with deionized (DI) water with a resistance of about 18 kΩ cm−1. Artificial saliva was prepared following the previous reports [38,39]. Briefly, the solution of Na2HPO4 (0.6 g/L), anhydrous CaCl2 (0.6 g/L), KCl (0.4 g/L), NaCl (0.4 g/L), urea (4 g/L), and mucin (4 g/L) were dissolved in deionized water and adjusted to pH 7.2, sterilized by autoclaving and stored at −4 °C until use.

2.2. Preparation of the SERS-active substrate

As shown in Fig. 1a, the nanoassemblies were fabricated under ambient conditions in a DC microplasma electrochemical reactor comprising an Ag foil anode immersed in an electrolyte with a microplasma cathode. First, 5% of BSA was pipetted onto the SERS substrate and incubated at 4 °C for 1 h, followed by rinsing with BSA and deionized water. The SERS substrates were stored at 4 °C before use. Anti-S IgG SARS-CoV-2 antibodies (S44F) (10 μL) were mixed into PBS (pH = 7.4) at a concentration of 250 μg mL−1 and applied dropwise onto the SERS substrate. The functionalization reaction was conducted at a temperature of 4 °C over a period of 4 h. The substrate was then rinsed with PBS and DI water and dried using N2. Furthermore, nonspecific bindings were prevented by pipetting 5% BSA onto the resulting substrates, which were then incubated at 4 °C for 1 h. After successive rinsing with BSA and deionized water, the functionalized SERS substrates were stored at 4 °C prior to use. Human and chicken anti-N IgG SARS-CoV-2 antibodies (HS8 and S11, respectively), ACE2, and UC20 were functionalized on the SERS-active substrate using the same procedure. Details are provided in the supporting information.

2.3. Immobilization of SARS-CoV-2 antigens on the SERS-active substrate

SARS-CoV-2 spike proteins were mixed into PBS (pH 7.4) at various concentrations and then applied dropwise onto an anti-S IgG conjugated SERS substrate. The immobilization reaction was conducted at a temperature of 4 °C for 30 min. The chips were then rinsed with PBS and DI water and dried using N2. A similar procedure was used for the detection of SARS-CoV-2 N protein, in which various concentrations of N protein were applied dropwise on human or chicken anti-N IgG-functionalized SERS substrates.

2.4. SERS measurement

SERS analysis was performed under ambient conditions using a confocal microRaman spectrometer (JASCO NRS 5100) at an excitation wavelength of 532 nm. Before performing the measurements, the spectrometer was calibrated according to the silicon band at 520 cm−1. The laser power was maintained at 0.45 mW throughout the 5-sec SERS experiment to avoid thermal effects. The spectra were obtained via background subtraction and averaged using measurements from no fewer than 100 random positions on each sample. To evaluate the limit of detection (LOD) of the SERS sensors, we have carefully performed the quantitative SERS analysis of SARS-CoV-2 proteins at various concentrations. The LOD was evaluated by fitting the linear calibration curve. Quintuplicate analysis was performed of each SERS sensor. All the experimental procedures of SERS measurement were all kept the same to ensure data reproducibility.

3. Results and discussion

The microplasma reactor is illustrated in Fig. 1a, following our previous report with modification [35]. Details can be found in the Supporting Information. The electrolyte is an HNO3 aqueous solution with fructose as a capping agent. Ag ions initially diffuse from the Ag anode via microplasma-activated electrochemical reaction into the pores of a cellulose fiber network. Note that under the electrostatic forces, Ag ions were attracted to the negatively charged functional groups on the surface of the cellulose fibers (Fig. 1b). After reduction by the plasma-generated electrons, Ag ions underwent cluster nucleation and particle growth, resulting in the formation of flexible porous nanoassemblies composed of Ag nanoparticles (NPs) on the cellulose fiber network (Fig. S1). The color of the paper on the surface facing the plasma-treated cell changed to dark yellow (Fig. 1c). After the plasma treatment, the samples were immediately dried under ambient conditions for 1 day to maintain flexibility (Fig. 1d). The AgMEN was sliced into 20 slices to decorate on the glass to form a SERS paper-based

Fig. 2. Ultrasensitive and reproducible detection of the SARS-CoV-2 S proteins (wild type) with S44F-SERS biosensors. (a) SERS spectrum of S44F-SERS biosensors with the conjugation of SARS-CoV-2 S proteins (wild type). A blank spectrum is shown for comparison. (b) SERS spectrum for SARS-CoV-2 S protein at concentrations ranging from 1 fg mL−1 to 1 mg mL−1 in PBS. (c) The corresponding calibration curve of the peak intensity at 2946 cm−1. (d) Ten randomly measured sites on SERS biosensors. (e) Raman map of S44F-SERS biosensor for S-protein. (f) The stability of S44F-SERS biosensors for 20 days.
Fig. 1f shows a representative low-magnification TEM image of the spherical particle-like nanostructures. The inset of Fig. 1f presents a high-resolution TEM (HRTEM) image of the as-synthesized Ag NPs featuring the twinned polycrystalline phases, which can provide high SERS activity [40]. The scanning electron microscopy (SEM) images revealed the porous nanostructures within the AgMEN (Fig. 1g), which was confirmed by the energy-dispersive X-ray spectroscopy (EDS) mapping (Fig. S2). The average diameter of Ag NPs was approximately 14.21 ± 1.1 nm (Fig. S3). Image J software was further used to identify the 3D porous structures of the AgMEN based on the SEM images. The result shows a coral reef-like structure with the uniform deposition of Ag to a depth of 100 μm (Fig. 1h–j). The Ag NPs were further studied using the X-ray diffraction (XRD) and X-ray photoelectron spectroscopy (XPS). The XRD (Fig. 1k) and XPS results (Fig. S4, l & m) reveal that highly-crystalline Ag NP could be prepared using plasma process [41] (see discussion in the supporting information). Our results demonstrated that highly crystallized Ag NPs could be deposited on cellulose using the microplasma treatment at ambient conditions without high-temperature sintering. Increasing the surface area by creating a porous structure could greatly improve molecular adsorption for biomolecular sensing. The hydrophilicity of the as-fabricated AgMEN ensured the high adsorption capacity, and electromagnetic field coupling on the porous structures enhanced the SERS response. Lastly, these characteristics should enable the detection of target antigens even at deficient concentrations.

The conjugation of antibodies with plasma-engineered Ag NPs on the SERS biosensors was carried out via a one-step functionalization. UV–Vis absorption spectroscopy (Fig. S5a) and zeta potential measurement (Fig. S5b) provided evidence pertaining to the binding of antibodies on the Ag NPs [42,43]. Details and discussions can be found in the supplementary information. After the antibody functionalization, non-specific bindings were prevented by coating BSA onto the resulting substrates. The 532 nm laser excitation was selected for the SERS measurement because it is a very effective excitation source to enhance the Raman signals of analytes with Ag nanostructures [35]. To avoid the undesirable heating effect on the protein sample during our Raman measurement with 532 nm excitation, we kept the low laser power of 0.45 mW in this work. The result shows the high SERS responses of protein samples and no apparent sample damage. To study the Raman scattering of SARS-CoV-2 proteins, Raman measurement with a wide range from 400 to 3800 cm\(^{-1}\) was performed, covering the common and important Raman scattering of proteins. Fig. 2 shows the Raman spectra of prepared S44F-functionalized SERS biosensors (denoted as S44F-SERS) before and after the immobilization with SARS-CoV-2 S protein (wild-type). The blank Raman spectrum of S44F-SERS biosensors shows low Raman signals in the region of 600–1800 cm\(^{-1}\), including typical antibody-related vibration modes of aromatic carbon bonds (600–1300 cm\(^{-1}\)) and stretching modes of amide-containing backbones (1300–1800 cm\(^{-1}\)) [22,32] while no obvious peaks in the region from 1850 to 3000 cm\(^{-1}\). Significant SERS signals were noticed in the spectrum of S44F-SERS biosensors with the conjugation of SARS-CoV-2 S protein. The enhanced SERS signals can be assigned as the Raman stretching modes of CH and CH\(_3\) groups of spike proteins around 2895 and 2946 cm\(^{-1}\), respectively [22,32,44,45]. Detailed Raman peak assignment can be found in Table S1. The possible reason why the Raman peak of 2946 cm\(^{-1}\) is enhanced sharply could be because of the strong adsorption between Ag nanostructures and CH\(_3\)-related functional groups and the electromagnetic field induced by the CH\(_3\)-related functional groups on the Ag nanostructures under 532 nm excitation. This result is consistent with previous work and suggests that as-fabricated SERS sensors can be used to detect SARS-CoV-2 S proteins.

We additionally evaluated the efficacy of the developed SERS-based immunoassay platform in the quantitative analysis of SARS-CoV-2 S protein at various concentrations. Note that we monitored the concentration of the spike protein indirectly based on the peak SERS intensity at 2946 cm\(^{-1}\). The intensity of the SERS intensity signal increased continually with the concentration of the target antigen between 1.0 fg mL\(^{-1}\) and 1.0 mg mL\(^{-1}\) (Fig. 2b). We observed a strong linear relationship between the SERS intensity at 2946 cm\(^{-1}\) and the logarithm of the target spike protein concentration (Fig. 2c). We fitted the linear
calibration curve as follows: $y = 64.4 \log(C) + 969$ ($R^2 = 0.9899$). The error bars in Fig. 2c indicate the standard deviation in concentration, averaged using ten measurements from ten different locations. The limit of detection (LOD) of SARS-CoV-2 S protein (wild type) in phosphate-buffered saline (PBS) was found to be 1.0 fg mL$^{-1}$. Fig. 2d does not reveal significant variations in spot-to-spot SERS intensity at 2895 cm$^{-1}$ or 2946 cm$^{-1}$, thereby demonstrating the excellent uniformity of the surface over a large area. The relative standard deviation (RSD) was as follows: 2946 cm$^{-1}$ (3.12%) and 2895 cm$^{-1}$ (7.96%). We also performed Raman mapping using 900 measurements covering a 30 μm × 30 μm area in a 1 μm step to assess the reproducibility of our findings (Fig. 2e). Using a step of 50 nm between the spots, the RSD of the spot-to-spot intensity was only 4.67% at 2946 cm$^{-1}$. The uniform high-density distribution of Ag NPs enhanced the reproducibility of Raman signals beyond that of conventional methods based on electrostatic interactions, which are prone to degradation of SERS-active materials caused by the addition of reagents during the purification and separation processes [46,47]. We also collected the Raman spectra over 20 consecutive days to assess the stability of the SERS biosensor over time (Fig. 2f). We observed no significant changes in the Raman peak intensity at 2946 cm$^{-1}$ throughout the study period (20 days).

Human and chicken anti-N IgG antibodies, denoted as HS8 and S11, respectively, were further used to assess the detection of the SARS-CoV-2 N proteins with our developed SERS biosensors. Fig. 3a presents the SERS spectra of prepared HS8-functionalized SERS and S11-functionalized SERS biosensors (denoted as HS8-SERS and S11-SERS, respectively) before and after the conjugation of SARS-CoV-2 N proteins. When N proteins conjugated with HS8 and S11 antibodies on the prepared SERS biosensors, the strong SERS responses of SARS-CoV-2 N proteins were exhibited through the Raman stretching modes of CH and CN groups of N proteins around 2895 and 2946 cm$^{-1}$, respectively (Fig. 3a). We processed a series of N protein solutions of various concentrations to estimate the LOD. The Raman spectra in Fig. 3b-c revealed detectable SERS responses of SARS-CoV-2 N proteins with HS8-SERS and S11-SERS biosensors for very low SARS-CoV-2 N protein concentrations of 1.0 pg mL$^{-1}$ and 0.1 pg mL$^{-1}$, respectively. We used the Raman band with the highest intensity at 2946 cm$^{-1}$ to determine the detection limits. As shown in Figs. S6a–b, the intensity of the Raman signals decreased as a function of N protein concentration on the SERS biosensors conjugated with human HS8 and chicken S11 antibodies, respectively. Fig. S6 also illustrates a high degree of linearity between N protein concentration and Raman peak intensity. The regression equation using human HS8 IgG antibodies can be expressed as $y = 75.69 \log(C) + 907$, with a linear range of detection from 1.0 pg mL$^{-1}$ to 1.0 mg mL$^{-1}$ and a correlation coefficient of 0.9943. Moreover, the regression equation for chicken S11 IgG antibodies was as follows: $y = 70.69 \log(C) + 940$, with a linear range of detection from 0.1 pg mL$^{-1}$ to 1.0 mg mL$^{-1}$ and a correlation coefficient of 0.9975, where $y$ denotes the Raman intensity and $C$ denotes the N protein concentration. Our approach can detect the lowest concentration of N protein (0.1 pg mL$^{-1}$) with the only peak at 2946 cm$^{-1}$ using the S11-SERS biosensor (Fig. S6a). Fig. S7 provides the Raman data with zero baselines for comparison. Table 1 summarizes the sensing results.

The selectivity of the developed SERS biosensors was additionally evaluated by conjugating the variant-specified antibodies to enable the detection of different SARS-CoV-2 variants, including wild-type, Alpha, Delta, and Omicron. The experiment was performed using SERS biosensors functionalized with different antibodies, including S44F, ACE2, UC20, and S11 (details of antibodies are provided in Supplementary Information). We also evaluated the effectiveness of SERS immunoassays against potentially interfering proteins commonly encountered in saliva by conducting tests using artificial saliva. We selected the intensity of the Raman band at 2946 cm$^{-1}$ as a reference to evaluate the detection performance of SERS biosensors when incubated with various antigens. Fig. 4 shows the SERS spectra corresponding to the selective

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**Table 1**

Performance of the developed SERS biosensors for the detection of SARS-CoV-2.

| SERS biosensor | Antibody (wild-type) | Analyte | LOD (fg mL$^{-1}$) | Linear range |
|----------------|----------------------|---------|--------------------|--------------|
| S44F-SERS     | S44F IgG             | S protein | 1.0                | 1.0 fg mL$^{-1}$ to 1.0 mg mL$^{-1}$ |
| HS8-SERS      | Human HS8 N protein  | 1000.0  | 1.0 pg mL$^{-1}$   | 1.0 mg mL$^{-1}$ |
| S11-SERS      | Chicken S11 IgG S protein | 100.0 | 0.1 pg mL$^{-1}$ | 1.0 mg mL$^{-1}$ |

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**Fig. 4. SERS response of antigen variants.** SERS spectra of AgMEN-based SERS biosensors conjugated with antibodies including (a) S44F, (b) ACE2, (c) UC20, and (d) N IgG (S11) for the detection of 4 antigens including N protein, S proteins of SARS-CoV-2 wild type, Alpha and Delta variants in simulated human saliva. The SERS spectra were obtained from the selective immunoassay of SARS-CoV-2 variant proteins, with peak intensities at 2946 cm$^{-1}$. The concentration of the target antigen is 1 μg mL$^{-1}$.
were obtained from the selective immunoassay of SARS-CoV-2 variant proteins, for detecting Omicron antigen in simulated human saliva. The SERS spectra with peak intensities at 2946 cm\(^{-1}\) showed high selectivity of our SERS biosensor in the detection of spike variants under the simulated human saliva conditions. This finding helps design rapid and selective detection of SARS-CoV-2 variants because conventional detections based on SARS-CoV-2 N proteins are highly prone to false-positive results and often detect coronaviruses other than the SARS-CoV-2 viruses [11,18,19,31]. The developed sensors also offer several practical advantages. As shown in Tables S1 and S2, our SERS biosensor is rapid and cost-effective compared to the nucleic acid-based and serological tests. Moreover, the developed SERS biosensor can be used at all stages of the infection due to its ultrahigh sensitivity. In practice, a diagnostic test applicable over the entire disease course would eliminate the need to switch between different testing methods and reduce the common uncertainty imposed by false-positive results. Compared to the previous reports, as shown in Table S2, our SERS biosensor shows high sensitivity in the LOD for both S and N proteins, which is promising for further applications. This could be attributed to the enhanced SERS signal collection induced by the 3D porous Ag nanostructures. In addition, using variant-specific antibodies to detect the corresponding antigens, we can detect various SARS-CoV-2 variants. Furthermore, our SERS-based biosensor can be fabricated through a low-cost, paper-based, scalable process.

4. Conclusion

The continuously emerging new SARS-CoV-2 variants of concern exacerbate the COVID-19 pandemic owing to their increased transmissibility and antibody resistance compared to the original lineage. Developing a detection technology that enables early and effective detection of SARS-CoV-2 variants is critical to pandemic control and public health monitoring. Here, we resolve this issue by developing a new SERS biosensor for the ultrasensitive detection of SARS-CoV-2 variants with high reproducibility. The SERS detection relies on Ag-based nanostructured SERS biosensors fabricated by a solution-based microplasma process. Notably, highly reproducible results with strong resistance to potentially interfering proteins can be obtained by the variant-specific antibody functionalization. The proposed assay could be used to screen suspected COVID-19 cases (symptomatic and asymptomatic) early in the infection cycle. Our versatile SERS-based method could improve the accuracy of virus detection and can be customized for other viral variants and biomedical sensing applications.

CRediT authorship contribution statement

Yi-Jui Yeh: conceived and designed the experiments, synthesized materials, fabricated the SERS sensors, performed chemical characterization and SERS measurement, co-wrote the manuscript (first-draft). Trong-Nghia Le: conceived and designed the experiments, prepared antigens and antibodies samples, co-wrote the manuscript (first-draft). Wesley Wei-Wen Hsiao: conceived and designed the experiments, Project administration, Funding acquisition, Supervision. Kuo-Lun Tung: conceived and designed the experiments. Kostya (Ken) Ostrikov: contributed to the paper preparation, discussed the results, and approved the final version. Wei-Hung Chiang: conceived and designed the experiments, Project administration, Funding acquisition, Supervision. All authors contributed to the paper preparation, discussed the results, and approved the final version.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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