Ultrafast Detection of SARS-CoV-2 Spike Protein (S) and Receptor-Binding Domain (RBD) in Saliva Using Surface-Enhanced Raman Spectroscopy

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Abstract: Controlling contagious diseases necessitates using diagnostic techniques that can detect infection in the early stages. Although different diagnostic tools exist, there are still challenges related to accuracy, rapidity, cost-effectiveness, and ease of use. Surface-enhanced Raman spectroscopy (SERS) is a rapid, simple, less expensive, and accurate method. We continue our previous work published on SERS detection of the SARS-CoV-2 receptor-binding domain (RBD) in water. In this work, we replace water with saliva to detect SARS-CoV-2 proteins at very low concentrations and during a very short time. We prepared a very low concentration of 10^{-9} M SARS-CoV-2 spike protein (S) and SARS-CoV-2 receptor-binding domain (RBD) in saliva to mimic a real case scenario. Then, we drop them on a SERS substrate. Using modified SERS measurements on the control and the sample containing the biomolecules, confirmed the sensitivity of the target identification. This technique provides different diagnostic solutions that are fast, simple, non-destructive and ultrasensitive. Simulation of the real-world of silicon wire covered with silver and gold, were performed using an effective and accurate tool, COMSOL Multiphysics software, for the enhancement properties study.

Keywords: SARS-CoV-2 spike protein (S); receptor binding domain (RBD); Raman; SERS

1. Introduction

The coronavirus disease of 2019 (COVID-19) is an emergent threat to human health worldwide. The emerging outbreak alarmed humans to quickly find a solution to stop it by developing molecular assays to detect the virus of COVID-19 in people. New quantitative real-time reverse-transcription PCR (RT-PCR) assays have been developed to diagnose COVID-19 by targeting two viral genome regions [1,2]. Although the techniques above present high sensitivity and specificity, they have some limitations and challenges, such as their costs, robustness, sample preparation, and analysis time. Hence, there is a great demand for a new technique able to detect infection in the early stages. Surface-enhanced Raman spectroscopy (SERS) is a rapid, simple, less expensive, portable, and accurate method that can provide structural and chemical information about the virus via the fingerprint of its biomolecules [3–5]. SERS phenomena were observed and confirmed for the first time in 1970s on a roughened Ag metal surface, since this date, the detection sensitivity of Raman spectroscopy has significantly improved [6,7]. SERS is a powerful vibrational spectroscopic technique that pushed the detection limit of structural and chemical sensing towards a single molecule [8,9]. This ability is due to optical and chemical enhancement during the excitation of localized surface plasmon resonance (LSPR) into the interface of...
metal/air. LSPR can be enhanced and confined on tiny metallic nanostructures such as gold, silver, and palladium. The preferential use of gold in bioapplications is more given by its non-toxicity—e.g., in cell fingerprinting [10–12].

SERS offers a wide range of detection applications, either in a solution or a solid film. Since its appearance, SERS has shown strong capabilities for different applications in research and industries [13,14]. The SERS technique was intensively used in several biological analyses, such as biological imaging [4,5] and bacterial and viral detection [5,15].

SARS-CoV-2 genomic RNA encodes two sets of proteins: nonstructural proteins, which act as complex replicate machinery, and structural proteins. The structural proteins represent the structural component of the mature virus and include: spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins. SARS-CoV-2 relies on spike protein (S) for penetrating host cells. The S protein subunit 1 (S1) binds to the human receptor angiotensin-converting enzyme 2 (ACE2) via the receptor-binding domain (RBD), while the S protein subunit 2 (S2) mediates the fusion to the host cell membrane [16]. Since the S protein is highly conserved [17], it is considered a promising target for discovering efficient antibodies, entry inhibitors, vaccines, and bio-detection approaches [18–20].

It has been shown that viral load varies by samples from a single patient. Temporal viral load profiles showed that salivary viral load was the highest among different samples and hit the peak during the first week after symptom onset and then declined [21]. Nowadays, using saliva samples can reduce the cost and time of specimen collection for SARS-CoV-2 detection. However, RT-PCR is a time-consuming technique (around three hours) and requires relatively expensive reagents and well-trained personnel. The properties, above, of the S protein pushed us to select it as a target for SERS detection. Recently, we have shown a modified approach using SERS to detect SARS-CoV-2 RBD. Fabricated silicon nanorods covered by Au/Ag nanostructures were used to achieve higher optical enhancement. The sensitivity of this approach hits nearly a single protein detection level of 1 pM [22].

In this work, we used our gold and silver deposited silicon nanorod substrates, successfully tested in our previous work [22]. A concentration-dependent SERS study carried out on the S protein confirmed the linear response property and sensitivity of the substrate and the transition in the conformational structures. We successfully used the developed SERS substrate to detect SARS-CoV-2 S and RBD proteins in saliva samples without further treatment, with a high sensitivity and within a short time. The finite element simulation method was used to study the effect of the refractive index of the bio-medium on the optical enhancement generated by the gold and silver deposited silicon nanorods. This invention will pave the way for rapid and accurate diagnosis of pathogens in biofluids.

2. Experimental Part
2.1. Method of Fabrication of the SERS Substrate

The SERS substrate was fabricated by following different steps, see Figure 1. After cleaning the silicon substrate with acetone and deionized water, we rinsed it in Piranha solution. Then, we put it in an aqueous solution of fluoride acid (HF) and AgNO₃ (2 M/0.02 M) for 20 min. Silver and gold nanoparticles were deposited by using a DC magnetron sputtering. The silver and gold nanoparticles were dispersed homogeneously and EDX spectra recorded on two positions confirmed the simultaneous presence of both metals. The proper description is in reference [22].
Figure 1. Schematic representation of the different processes of fabrication. 1—cleaning process, 2—Electroless etching, and 3—Sputtering of gold and silver nanoparticles.

2.2. Instrumentation

The topography of the substrates was obtained by using a scanning electron microscope Jeol, JSM 7000 series (Jeol Ltd., Tokyo, Japan) at a 15.0 kV scan voltage. Raman spectra were detected using a confocal Raman microscope (LabRAM HR800, Horiba Scientific, Lille, France) in a backscattering geometrical configuration, with a spectral resolution of 0.9 cm\(^{-1}\) at ambient temperature. We used a He–Ne laser of \(\lambda = 632.8\) nm, an irradiated power of 50–100 \(\mu\)W and an objective of 50\(\times\). The lower power was chosen in order to prevent any damage to the bio-samples.

2.3. Preparation of Proteins and Saliva Samples

SARS-CoV-2 S and RBD proteins were obtained from the Sino Biological company (catalogue number: 40589-V08B1 and 40592-V08B, respectively), in lyophilized form. The stock was reconstituted by adding sterile water (400 \(\mu\)L) to the vial to get a 0.25 mg/mL stock solution. A saliva sample was collected from SARS-CoV-2-negative people (with written consent) after two hours of fasting. The saliva sample was collected by spitting into a 50 mL falcon tube, then it was aliquoted into 1.5 mL tubes on ice. RBD and S proteins were added to the saliva sample to a final concentration of \(10^{-9}\) M and they underwent the measurements directly. The final concentration was chosen according to the reported viral load in biofluids [23,24].

3. Results and Discussion

3.1. SEM and EDX

SEM images show silver and gold nanoparticles covering the silicon rods with an average length of 0.5 \(\mu\)m, see Figure 2a,b. The Si nanorods are multi-oriented in different spatial directions, distributed randomly in the space. This can increase the enhancement of the surface plasmon excitation towards the incident and scattered wave vectors’ mismatch. Energy-dispersive X-ray analyzer (EDX) scanning images were recorded on the surface of Au/Ag-covered silicon nanorods, see Figure 3a. The percentage of the surface components was determined, including the essential ones, such as silicon (13% and 18%), silver (39.7% and 48%), and gold (2.5% and 3.5%). The high concentration of indium could be derived from such contaminants existing in the deposition chambers.
Figure 2. SEM images of silicon nanorods covered with silver and gold nanoparticles. (a) With a low magnification $\times 22,000$, 1 $\mu$m scale bar, and (b) with a high magnification $\times 100,000$, 100 nm scale bar.

Figure 3. EDX spectra on different positions of the sample. (a) SEM image shows the two locations where the EDX spectra were measured, scale bar 2.5 $\mu$m and magnification 5000 $\times$. (b,c) EDX spectra of the two positions indicating the presence of silver, gold, and silicon.
3.2. Far Field Measurement on S and RBD Proteins

Raman lines observed by SERS can be affected by the hybrid localized surface plasmon resonance of heterogeneous metal nanostructures such as Au/Ag [25], the latter leads to a shift and broadening in the Raman lines. That is why, before performing SERS measurement, it is prominent to measure the far-field in order to assure the protein’s vibrational modes by comparing them to the existing databases.

A 10 µL sample of the stock of S and RBD with a concentration of 10⁻⁶ M and 10⁻⁵ M, respectively, was dropped firstly on a commercial CaF₂ substrate. Far-field Raman spectra of S and RBD were measured during the 150 s and by using a 633 nm laser wavelength, an objective of 50 ×, and power of 0.5 mW. Figure 4a,b show the appearance of different Raman bands assigned to amino acids [26–32]. We can observe a good resemblance between the two spectra. In addition, we observe more bands in the S protein, which is expected because the S protein is much larger and contains more amino acids. In both spectra, we can observe clearly, Raman bands located at 1460 cm⁻¹, 1370 cm⁻¹, 1250 cm⁻¹, 1050 cm⁻¹, 1005 cm⁻¹, 1033 cm⁻¹, 876 cm⁻¹, 640 cm⁻¹, and 526 cm⁻¹. The highest peak is attributed to C-N stretching (876) and belongs to glutamic acid and tyrosine [33]. The peak at 1460 is related to C-H stretching of glycoproteins, while the peak at 1370 is related to C-H rocking in lipids [34–36]. The peak at 1250 is attributed to the secondary bands of amide III from both the in-plane NH group bending vibration, and the C-N stretching vibration [35]. The second highest Raman intensity peak at 1050 is attributed to C-N and C-C protein stretching [36,37]. The two peaks at 1033 and 1005 belong to phenylalanine [37]. The last two peaks at 640 and 526 are attributed to tyrosine, cysteine, and histidine, respectively [33,38]. In conclusion, the structural features of the studied protein were verified and compared to the existing database.

Figure 4. Cont.
Figure 4. Far-field Raman spectra of (a) RBD, and (b) S. For both proteins, the most relevant vibrational bands are cysteine (Cys), histidine (His), tyrosine (Tyr), glutamic acid (Glu), tryptophan (Trp), phenylalanine (Phe), C-N and C-C protein stretching, C-H rocking, and C-H stretching of glycoproteins located at 526 cm\(^{-1}\), 640 cm\(^{-1}\), 876 cm\(^{-1}\), 889 cm\(^{-1}\), 1005 cm\(^{-1}\), 1033 cm\(^{-1}\), 1050 cm\(^{-1}\), 1377 cm\(^{-1}\), and 1460 cm\(^{-1}\), respectively. Laser wavelength 632.8 nm, using CaF\(_2\) substrate and 150 s integration time.

3.3. Concentration and Calibration Study

After measuring the Raman far-field spectra and optimizing the experimental parameters, we performed a concentration-dependent SERS study by dropping different concentrations of the S protein on the surface of the substrate. Each spectrum displayed in Figure 5a is obtained by averaging three spectra recorded from three positions of the substrate’s surface. We observed an interesting change in the bands of the Raman spectra by decreasing the concentration, e.g., Raman bands are similar for the concentrations of \(10^{-6}\) M and \(10^{-7}\) M. However, the situation changes after varying the concentration between \(10^{-8}\) M and \(10^{-10}\) M, where the spectra are similar, see Figure 5a. The Raman bands’ intensity decreases in both features, whereas their broadness decreases. The latter is due to the interaction between the proteins that become weaker by decreasing their number. This change in the conformational structure of the S protein is expected when the number of protein by a surface unit changes. In fact, the S protein has a big size and by increasing the concentration, a broadness and a shift of the Raman bands can be observed [39]. This change was also observed for the RBD protein published in our previous work [22]. All the spectra in Figure 5a show most of the relevant peaks displayed in Figure 4 such as tyrosine (Tyr), glutamic acid (Glu), tryptophan (Trp), phenylalanine (Phe), C-N and C-C protein stretching, C-H rocking, and C-H stretching of glycoproteins located at 640 cm\(^{-1}\), 876 cm\(^{-1}\), 889 cm\(^{-1}\), 1005 cm\(^{-1}\), 1033 cm\(^{-1}\), 1050 cm\(^{-1}\), 1377 cm\(^{-1}\), and 1460 cm\(^{-1}\), respectively. At low concentration, a peak located at 1600 cm\(^{-1}\) assigned to amino acids (Tyr, Phe, Trp) or \(\nu_{as}\) (COOH) is observed [36,40]. The observed modes are present in all the concentrations. In addition, the observed modes were also observed in the concentration-dependent study performed on RBD proteins in our previous work, see Figure 5a [22] where the spectra are very similar. The electrodynamical properties of the functional groups of amino acids are very sensitive to the interaction with a metallic surface such as gold and silver. The latter
explains the increase and the decrease of the band intensities relative to those observed in the far-field spectra [40]. Figure 5b,c show a linear dependence between the Raman intensity and the concentration of the S protein and RBD, respectively. The intensity was calculated by integrating under the Raman bands, confirming the linear response property and sensitivity of our biosensor. The intensity of SERS on RBD, in Figure 5c, were taken from our previous work [22] where we integrated it under the Raman spectra. If we compare between the two slopes in Figure 5b,c, we conclude that we can detect the S protein at four times lower concentrations than RBD. This could be attributed to the size of the S protein compared to the RBD. This confirms that our method of detection is four times more sensitive for the S protein than the RBD.

Figure 5. (a) Concentration-dependent SERS study of S protein by varying the concentration from $10^{-6}$ M to $10^{-10}$ M (b) A semi-log plot of the intensity as a function of the concentration of S protein. (c) A semi-log plot of the intensity as a function of the concentration of RBD protein. Acquisition time 3 s, Laser wavelength 632.8 nm. SERS spectra are recorded on the same substrate; each spectrum is the result of an average of three spectra taken from three different positions.

3.4. Detection of S, RBD, and BSA Proteins in Saliva: Specificity, Sensitivity, and Selectivity Studies

In all of the measurements presented in this section, we used S, RBD, and BSA proteins with a concentration of $10^{-9}$ M. The saliva (SL) has not been treated by any means to remove debris. Each SERS spectrum was recorded during an acquisition time of 3 s. Each spectrum is the result of an average of three spectra taken from three positions. We dropped a 1.5 µL of the saliva sample with S protein (SLS) and another control without the S protein (SL). After 20 min of drying, we measured the SERS spectra in different positions. Figure 6 shows the different spectra on five positions for the SL and SLS. A good signal-to-noise ratio (SNR) is observed in the spectra. We collected Raman spectra from the SL and SLS for a short integration time of 3 s and with very low power. In order to verify the reproducibility of the measurement, we carried out a multivariate analysis on the SERS spectra by using principal component analysis (PCA) (Figure 7). We used principal component analysis for spectroscopy applications’ apps of Origin pro to perform the analysis. We started by smoothing our spectra, then we subtracted the background by using a baseline correction, and finally, we normalized all the spectra before executing the PCA analysis. If we take the middle of each set of proteins in Figure 7, the distance is around 5, however, the distance between two points is around 0.25, that’s mean the two proteins are separated.
enough. No one point of the S protein is inside the saliva (SL) circle, the latter confirms a 0% uncertainty. We observed clearly, from the PCA analysis, how the S protein is identified from the control (SL). The most important conclusion from this statistical study is that the samples containing the S protein are very close, while those containing just SL are scattered (Figure 7). The latter confirms the sensitivity and specificity of SERS to detect a very small quantity of the S proteins in a complex medium such as saliva.

In order to check the specificity of our method, we replaced the S protein with the RBD. We dropped 1.5 µL of the RBD protein immersed in saliva (SLR) and one control SL without the RBD protein (SL). After 20 min of drying, we measured the SERS spectra in different positions. Figure 8 shows different spectra on five positions for the SL and SLR. We can observe the presence signal in the spectra. We collected Raman spectra from the SL and SLR for a short integration time of 3 s and very low power. In order to verify the reproducibility of the SERS method, we carried out a multivariate analysis on the SERS spectra by using principal component analysis (PCA) (Figure 9). Pretreatment processing such as smoothing, background subtraction, and normalization have been performed before statistical studies. If we take the middle of each set of the proteins in Figure 9, the distance between the two circles is around 3, however, the distance between the two points is 1. All the points are inside, except one point of SL that exists outside the two areas. We conclude that the uncertainty here is around 10% and it is lower than the case of the S protein. We can observe clearly from the PCA analysis how the RBD protein is identified from the control (SL). We conclude with the present analysis that the identification of the S protein is more accurate than the RBD. The latter could be attributed to the size of the S protein (1273 residues) compared to the RBD protein (197 residues).

Figure 6. SERS spectra of (a) SLS and (b) SL on different positions after smoothing, subtraction, and normalization. Acquisition time 3 s, wavelength 632.8 nm, power 50 microwatt. SERS spectra are recorded on the same substrate; each spectrum is the result of an average of three spectra taken from three positions. The raw data are presented in the Supplementary Materials (see Figure S1).
Figure 7. Principal component analysis of SERS spectra of SLS and SL. Red circle represents the region containing the data of saliva and S protein. Black circle represents the case of pure saliva.

Figure 8. SERS spectra of different positions for (a) SLR and (b) SL performed on different positions after smoothing, subtraction, and normalization. Acquisition time 3 s, wavelength 632.8 nm, power 50 microwatt. SERS spectra are recorded on the same substrate; each spectrum is the result of an average of three spectra taken from three positions. The raw data are presented in the Supplementary Materials (see Figure S2).
In order to validate the selectivity of our SERS substrate, we dropped 1.5 µL of saliva (SL) with a $10^{-9}$ M BSA protein (SL-BSA) and without BSA as a control (BSA). Figure 10 shows the Raman spectra performed in different positions of the sample, five positions for SL and another five for SL–BSA. We performed a PCA in the spectra to verify if the spectra can lead to discrimination between BSA and saliva. Figure 11 shows clearly two groups, one for SL and one for SL-BSA. We can observe here how the SL points are dispersed, however, the SL-BSA are more closed. The distance between the two circles is around 2, see Figure 11, however, the distance between two points is 1. All the points are inside, except one point from SL and BSA exist outside the two areas. The latter leads to an uncertainty of approximately 20%, lower than the case of the S and RBD proteins. Although the distance is far comparing to the S and RBD proteins, our substrate proves its selectivity against other varieties of proteins such as BSA.

Figure 10. SERS spectra for (a) SL-BSA and (b) SL performed in different positions after smoothing, subtraction, and normalization. Acquisition time 3 s, wavelength 632.8 nm, power 50 microwatt. SERS spectra are recorded on the same substrate; each spectrum is the result of an average of three spectra taken from three positions. The raw data are presented in the Supplementary Materials (see Figure S3).
Simulation of the real-world of silicon wire covered with silver and gold and emerged in a medium that contains S and RBD proteins were performed using an effective and accurate tool: COMSOL Multiphysics software. The software’s main feature is that it is based on the most advanced numerical method, the finite element method. We used an electromagnetic module in the frequency domain to understand how the bio-medium may influence the silicon covered with Ag/Au. Accordingly, two main equations are used. The first is the Maxwell Equation (1):

\[ \Delta \times \mu_r^{-1} \left( \nabla \times \vec{E} \right) - k_0^2 \left( \varepsilon - \frac{j\sigma}{\omega \varepsilon_0} \right) \vec{E} = 0 \]  

where \( \omega, \mu_r, \varepsilon, \) and \( \sigma \) are the excitation frequency, the relative permeability (fixed to 1), the relative permittivity, and the electrical conductivity, respectively. \( \varepsilon_0 \) and \( k_0 \) represent the permittivity and wave number in free space, being \( k_0 = \omega/c_0 \) (with \( c_0 \) the speed of light in a vacuum). The approximate relative permittivity inside the RBD protein is 4, the relative permittivity of water is 90 and the average relative permittivity in the protein-water interface is 20–30 based on an explicit model with atomic details [41,42].

The second, Equation (2), is used to calculate the electromagnetic enhanced Raman scattered light denominated \( g \) from the structure of silicon wire surrounded by silver and gold.

\[ g = \left( \frac{I_{SERS}}{I_{Raman}} \times \frac{N_{Raman}}{N_{SERS}} \right)^2 \]  

The field enhancement can be created close to the Ag nanoparticles where the polaritons are constructively interfered. Then, the local field is further enhanced and a dipole is produced, leading to the amplification of the Raman scattering in the hot-spots and a formation of plasmons [11,43]. An interactive excitation from the Ag and Au nanoparticles system at a resonant frequency (plasmon resonance) generates and enhances apparent Raman polarizability.

The designed structure is a three-dimensional (3D) model of silicon wire with a 12 nm radius and 300 nm length, surrounded with 108 silver and gold nanospheres (silver and gold were randomly selected on the geometry with 95% of silver nanospheres and 5% of gold nanospheres) with a 7 nm radius, as depicted in Figure 12a. We used a sequence...
(300 nm length of silicon wire) of the real image in Figure 2a since, with more length and number of Ag/Au nanospheres, the time of calculation will be increased and surpass the memory of the software. However, this sequence helps to understand the physical mechanism at the basis of the enhancement in the SERS system used for detection. We used the same experimental conditions, 632.8 nm laser excitation, and involved the entire geometry in a medium with the same optical properties of the bio-medium that contains S and RBD proteins. Figure 12b depicts the absorption cross-section of the silicon wire surrounded with silver and gold in two mediums: air and bio-medium (proteins). Three resonant plasmon modes were observed in both air (365 nm, 508.7 nm, 754 nm) and bio medium (394.1 nm, 520 nm, 775.4 nm). An apparent redshift of surface plasmon of about 34 nm, 12 nm, and 21.4 nm of plasmon bands, located initially in the air medium, is observed when we place the structure in a bio-medium. Those shifts go along with a slight change in band spectrum. Plasmon shift confirms that the entire 3D geometry behavior is sensitive to the optical properties of the corresponding medium. Hence, SERS frequency shift sensing is a powerful tool for protein detection. We also calculated the quantified enhancement factor G of the structure in a bio-medium, see Figure 12c. The enhancement factor G was collected on the length of the cross-section of silicon wire, as depicted by the red line on the inset. Along the 300 nm silicon nanowire, field enhancement varies between 150 and 900 and we found a maximum enhancement of 900 at 760 nm, that confirms the effect of Ag/Au nanoparticles on increasing field enhancement, compared with our previous results [22] where we only covered the silicon wire with silver nanoparticles. The added 5% of gold concentration reinforced the field enhancement. Our simulated model presents a complementary study for the practical reality. It shows that SERS spectra are affected by the localized surface plasmon resonance of silver/gold nanostructures, the interaction of the active plasmon modes with the proteins leads to a shift and broadness of the vibrational modes.

Figure 12. Cont.
Figure 12. (a) 3D simulation of the electromagnetic field enhancement (near-field enhancement) in the silicon wire surrounded by a spherical silver and gold nanoparticle. The model is initially placed in the air domain. Silicon wire radius = 12 nm, length = 300 nm, Ag and Au nanosphere radius = 7 nm, the number of Ag and Au nanosphere covering the silicon wire is 108 (b) Absorption cross-section of the 3D silicon nanowire surrounded by silver and gold nanosphere proteins. (c) Enhancement factor $G: \frac{E_{\text{loc}}}{E_0}$ of the silicon wire edge (represented with red color in the inset) in the bio-medium. The maximum enhancement factor obtained is 900.
4. Conclusions

For the first time, we reported a swift detection method of COVID-19 proteins in saliva at a very low concentration during 3 s and by using an ultra-low power. A SERS substrate containing silver/gold nanoparticles, covered a silicon wire and exhibited high optical enhancement for this detection. We were able to identify the S and RBD proteins from saliva without any purification or removal of debris at a very low concentration of $10^{-9}$ M. Statistical studies showed a good separation between the proteins in saliva by taking different positions on the surface of the substrate. In order to validate the selectivity of our SERS substrate, we successfully identified a protein such as the BSA in the saliva sample (where other proteins are present). Our results was supported by a finite element simulation method in order to evaluate the extent and order of magnitude of optical enhancement, and the effect of the bio-medium refractive index on the plasmon resonance shift. Finally, this comparative study between S and RBD proteins, in the presence of a complex medium such as saliva, provides more insight and knowledge to the biosensing communities, in order to develop an automated recognition tool to detect viral proteins.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app12105039/s1, Figure S1. SERS spectra of (a) Saliva +S (SLS) and (b) Saliva (SL) without any treatment; Figure S2. SERS spectra of (a) Saliva +RBD (SLR) and (b) Saliva (SL) without any treatment; Figure S3. SERS spectra of (a) Saliva +BSA (SL-BSA) and (b) Saliva (SL) without any treatment.


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