

Ultra-sensitive and fast optical detection of the spike protein of the SARS-CoV-2 using AgNPs/SiNWs nanohybrid based sensors

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ABSTRACT

Severe acute respiratory syndrome SARS-CoV-2 virus led to notable challenges amongst researchers in view of development of new and fast detecting techniques. In this regard, surface-enhanced Raman spectroscopy (SERS) technique, providing a fingerprint characteristic for each material, would be an interesting approach. The current study encompasses the fabrication of a SERS sensor to study the SARS-CoV-2 S1 (RBD) spike protein of the SARS-CoV-2 virus family. The SERS sensor consists of a silicon nanowires (SiNWs) substrate decorated with plasmonic silver nanoparticles (AgNPs). Both SiNWs fabrication and AgNPs decoration were achieved by a relatively simple wet chemical processing method. The study deliberately projects the factors that influence the growth of silicon nanowires, uniform decoration of AgNPs onto the SiNWs matrix along with detection of Rhodamine-6G (R6G) to optimize the best conditions for enhanced sensing of the spike protein. Increasing the time period of etching process resulted in enhanced SiNWs' length from 0.55 to 7.34 μm . Furthermore, the variation of the immersion time in the decoration process of AgNPs onto SiNWs ensued the optimum time period for the enhancement in the sensitivity of detection. Tremendous increase in sensitivity of R6G detection was perceived on SiNWs etched for 2 min (length=0.90 μm), followed by 30s of immersion time for their optimal decoration by AgNPs. These SiNWs/AgNPs SERS-based sensors were able to detect the spike protein at a concentration down to 9.3×10^{-12} M. Strong and dominant peaks at 1280, 1404, 1495, 1541 and 1609 cm^{-1} were spotted at a fraction of a minute. Moreover, direct, ultra-fast, facile, and affordable optoelectronic SiNWs/AgNPs sensors tuned to function as a biosensor for detecting the spike protein even at a trace level (pico molar concentration). The current findings hold great promise for the utilization of SERS as an innovative approach in the diagnosis domain of infections at very early stages.

1. Introduction

The COVID-19 pandemic has impacted the world way of living in an unprecedented manner, since its discovery in Wuhan (China) by the end of 2019. More than a year later (the time of writing this manuscript), the numbers of infected and dead people is still increasing by the wide-spread of the virus [1,2]. The SARS-CoV-2 virus fits them with corona

virus family having single stranded genetic material RNA and surface proteins like membrane (M), envelope (E), nucleocapsid (N) and spike (S). S protein is of particulate interest since, it serves as mediator to the host cells via ACE-2 enzymes (Angiotensin-Converting Enzyme 2). It is categorized as largest surface protein virus being 180kDa spike monomer which makes its vitality to attach to the human host cells faster. The Spike trimers were the core component that invades the host cells with

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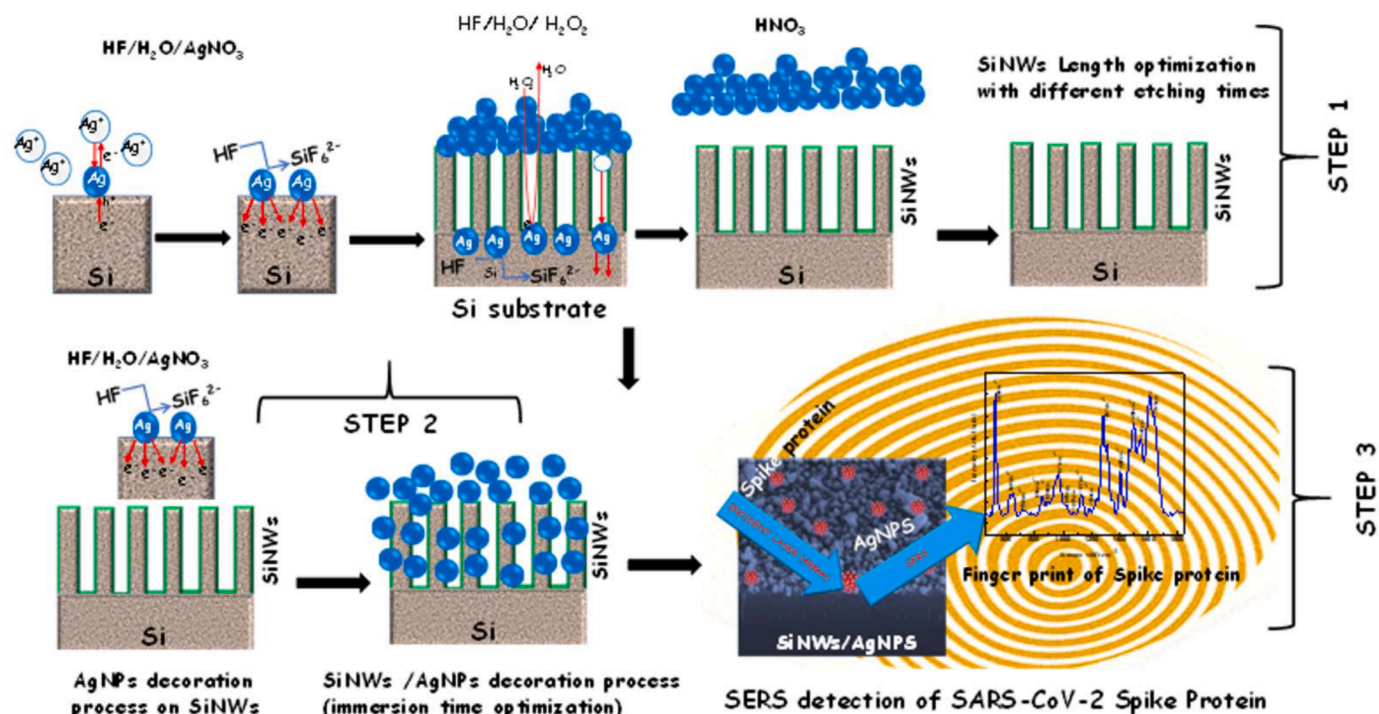


Fig. 1. Graphical illustration of the fabrication and fast optical detection of the spike protein of the SARS-CoV-2 using SiNWs/AgNPs nanohybrid based sensors.

ACE-2. The remarkable feature of the SARS-CoV-2 virus is its higher mutability and fast transmission rates which could pose great challenges to detect as well as cure the infections [3,4]. To ease the situation, Polymerase chain reaction (PCR) and Real-time reverse transcription quantitative polymerase chain reaction (RT-qPCR) standalone as foremost diagnostic tools which involves the extraction of RNA to confirm the positive cases which takes almost more than 5-6 h [5,6]. On the other hand, ELISA (enzyme-linked immunosorbent assays) makes use of IgM and IgG antibodies found to increase with patients is also an over-time consuming process [7]. Therefore, for the universal healthcare emergency, the development of detection techniques that are ultra-fast, reliable, inexpensive, and acknowledgeable by medical world represents an unmet need.

Among several sophisticated analytical instruments, surface-enhanced Raman spectroscopy (SERS) has proved its effectiveness in terms of unswerving, extreme sensitivity as well as high selectivity for identification of analytes under examination [8–10]. The technique has progressively evolved from the basic detection of chemical finger-print of organic molecules towards an alternative bio-analytical tool for the detection of microbes to a greater extent with excellent specificity and reproducibility by tailoring the design of substrates [11–13]. Rapid trace level detection of micro-biological organisms with SERS will ease the spread of their diverse effects on human civilization. SERS emerged to be sensitive technique as well as ultra-fast in their detection mechanism. RT-PCR (based on nucleic acids) rely on particular time frame for evaluating the infections as positive (early stage of infection i.e., 6 days before to 14 days after the symptom onset) leading some chances for false negatives at some cases which are not limited into this time frame of infectivity. The time duration for acquiring results is almost 8 h. Whereas, SERS do not have the limit (at any stage of infection) to detect and able to get the results within 5 min. Besides for RNA extraction, specific reagents are needed to perform it which improves the cost for analysis (typically 100-300USD). In case of SERS, no need for the employment of reagents, direct detection is possible without raising the cost of analysis (typically 10-50USD) [14,15]. On comparison with RT-PCR, it is highly surface sensitive, hence the protein moieties of the SARS-CoV-2 virus could be detected deliberately. The variants reported

with the subjects occurred with the mutant changes in the copies of genetic code (i.e., nucleic acids), hence the discussed surface sensitive fails to detect the type of mutant variants via direct detection. Rather, false negative/ positive cases can be filed with the currently developed diagnostic tool based on the viral proteins. Hence it can standalone as a fast-screening tool to meet the demand of pandemic outbreak [14].

SERS principle discriminates the electromagnetic and chemical phenomena that ground when the analyte is adsorbed on the sensor's surface [16,17]. Photon interaction enhances the electromagnetic fields termed as 'Hotspots' with the assistance of plasmon property exhibited by noble metal nanoparticles and also the charge transfer process associated within the sensor platform [18,19]. With the aforementioned properties, the most extensively used plasmonic nanoparticles in the SERS substrates are gold and silver [11,20]. In recent times, 1D nanostructures like silicon nanowires (SiNWs) have proven their effectiveness in improving the SERS sensing performance, owing to their extremely large surface area, magnificent electrical and optical properties [21].

Extensive efforts have been put down in tailoring the substrates exploited for SERS sensing to boost up the efficiency [22,23]. Outstanding performance of SiNWs sensors relies on the fabrication techniques, which dictate their structural properties [24]. Even if numerous preparation routes [25–27] have been described in the literature, wet chemistry (electroless etching) [28] represents the most suited technique to fabricate 1D SiNWs arrays. This method brings flexibility in tweaking the sensing properties by careful tuning of the SiNWs' structural characteristics (i.e., length, porosity, density, alignment etc.) during the preparation stages. Furthermore, the process control is quite easy and simple, which offers finest mandatory modulations determining their ability to detect even very low trace levels of organic as well as biological analytes. Metallic nanoparticles (AgNPs) deposition on SiNWs, further trailed by wet chemistry, adds on as the heart subject for achieving higher responsivity towards sensing with their localized surface plasmon resonance (LSPR) effect [29]. Various approaches have been adopted to fabricate highly ordered array of SiNWs decorated with metallic nanoparticles to design hierarchical nanostructures to discourse the challenges like homogenous distribution

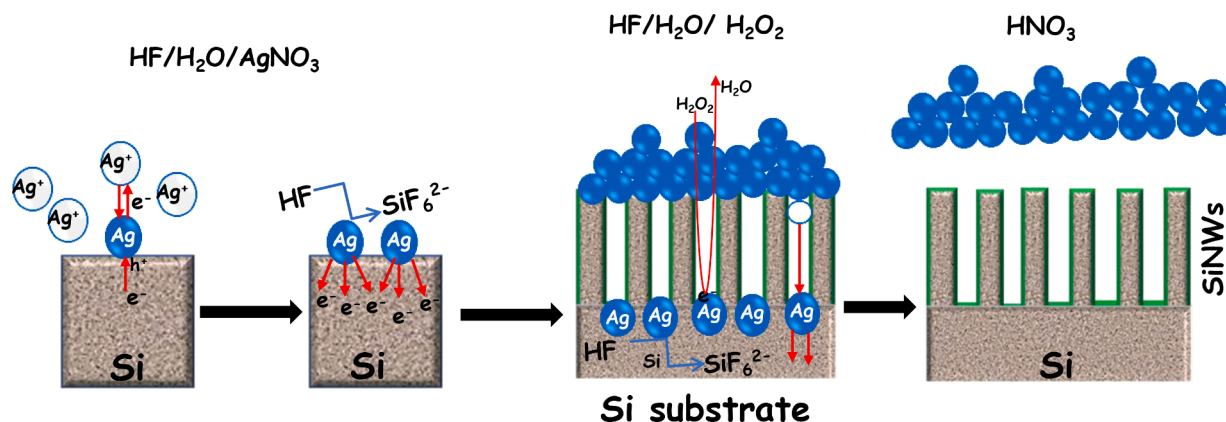


Fig. 2. The mechanism of fabrication of SiNWs with Metal assisted chemical etching process.

of AgNPs, inter-particle gaps, effects of bundles in SiNWs, nanoparticles aggregation, ideal interface through close proximity connections within the hierarchical architectures [30,31]. What is more, the mentioned key parameters affect the specificity, sensitivity, and reproducibility of the SERS sensor.

In viewpoint of fast detection of SARS-CoV-2 virus (spike protein), current study aims at the design and fabrication of highly ordered vertical SiNWs arrays and their subsequent decoration with plasmonic AgNPs (SiNWs/AgNPs) by facile wet chemistry methods. Careful examination was made on the fabrication process to tailor the physical sensor's characteristics/properties for better sensitivity and enhanced low detection limit. SiNWs/AgNPs sensor promisingly supports label-free detection of spike protein at a very low-level trace of about 9.3×10^{-12} M within less than a minute. Successful trace level ultra-fast detection of SARS-CoV-2 virus spike protein achieved by the SERS technique will be an ingenious tool in near future for diagnosis of infections at early stages. The graphical figure of the whole study is illustrated in Fig. 1.

2. Materials and methods

2.1. Materials

n-type Si wafers (boron-doped, 1 0 0 plane) with a thickness of $675 \pm 25 \mu\text{m}$ and a resistivity of 1-10 $\Omega\cdot\text{cm}$ were procured from TED PELLA, USA (Cat # 21610- 6) and utilized as the core materials. Anhydrous glacial acetic acid (Cat # 64-19-7, ACS grade), Hydrogen peroxide (cat # K48323000701, ACS grade) and hydrofluoric acid (48%, ACS grade, cat # B1564134825) were purchased from Merck, Germany. Nitric acid (65%, ISO grade, cat # 7697-37-2), and Rhodamine 6G (R & D grade) were obtained from Sigma-Aldrich. Silver nitrate (ACS grade, cat # 7761-88-8) was acquired from Alfa-Aesar, Germany. The SARS-CoV-2 S1(RBD) protein (cat # P2020-001) and the influenza A G4 EA H1N1 Hemagglutinin protein (cat # P2020-100) were purchased from Trenzyme life science services, Germany.

Surface microstructure and elemental analysis of SiNWs were investigated using a Tescan Vega 3 SEM equipped with an Oxford instrument EDS detector. The designed SERS substrate was placed in the sample holder in the vertical mode, followed by the electron-sample interactions, SEM images were recorded in the tilted mode to ensure the nanowires formation and Ag nanoparticles decoration at top surface of SiNWs. SERS measurements were performed on a Renishaw UK Raman microscope using 488 nm laser excitation of power of 100 μW with $50 \times$ objective ($\sim 1 \mu\text{m}^2$ spot size) with 10 s integration time.

2.2. Fabrication of the silicon nanowires (SiNWs)

The n-type Si wafer ($1.5 \text{ cm} \times 1.5 \text{ cm}$) was cleaned thoroughly in

ultrasonicator with acetone for 5 min followed by ethanol and deionized water. Metal-assisted chemical etching process was performed in a sequential manner by first dipping the clean Si wafer for 1 min in a Teflon container comprising a mixture of 64% HNO_3 , 20% CH_3COOH and 16% HF. The Si wafer, after rinsing with deionized water, was immersed fully in the metal catalyst solution mixture (0.02 M of AgNO_3 + 4.8 M of HF) for 1 min to facilitate the deposition of Ag nanoparticles. Etching process is continued with the immersion of the washed Si wafer in etchant volume mixture of $\text{H}_2\text{O}_2/\text{HF}/\text{H}_2\text{O}$ (4:1:20) for a time period varying from 1 to 35 min, followed by washing with deionized water. The etched Si wafer was then immersed in 1:1 volume ratio of $\text{HNO}_3/\text{H}_2\text{O}$ for 10 min to remove the residues of Ag nanoparticles deposited during SiNWs' formation. Finally, the SiNWs grown on the Si wafers were washed and dried at ambient atmosphere. The formation of the SiNWs array was prominently evident from the visual observation of black color over the substrate at the end of the preparation process [12, 32].

2.3. Decoration of SiNWs with AgNPs

Noble metal AgNPs were decorated in an orderly fashion by employing a simple wet chemical deposition process. The SiNWs were washed with deionized water followed by immersion in a mixture containing 0.02 M of AgNO_3 and 4.8 M of HF for different time intervals ranging from 5 to 120 s. Then, the AgNPs decorated SiNWs were washed and dried at ambient atmosphere. As a result, the sensor was fabricated by a facile route, which is inexpensive, devoid of higher end instrumentation facility and time consuming etc.

2.4. SERS measurements

The optimization of the etching time of SiNWs and decoration of AgNPs immersion time will be addressed carefully in the design of the SiNWs/AgNPs sensor. The finest conditions were noted with the SERS measurements using a 2×10^{-6} M solution of R6G in ethanol. 7 μL of R6G solution was drop casted with a pipette onto the sensor top surface and allowed to dry naturally prior to SERS measurements. The detection capability of the sensor was gauged with the enhanced intensities of R6G.

2.5. SERS measurements for protein molecules

SARS-CoV-2 S1 (RBD) spike protein was analyzed in our studies. The protein sequences are listed in Table 1 (SI). 7 μL of the spike protein (RBD) in phosphate buffer solution (PBS) were dropped over the sensor and correspondingly the SERS plots were recorded. For the low-level detection of spike protein, different concentrations were prepared by serial dilutions in PBS and SERS detection measurements were recorded.

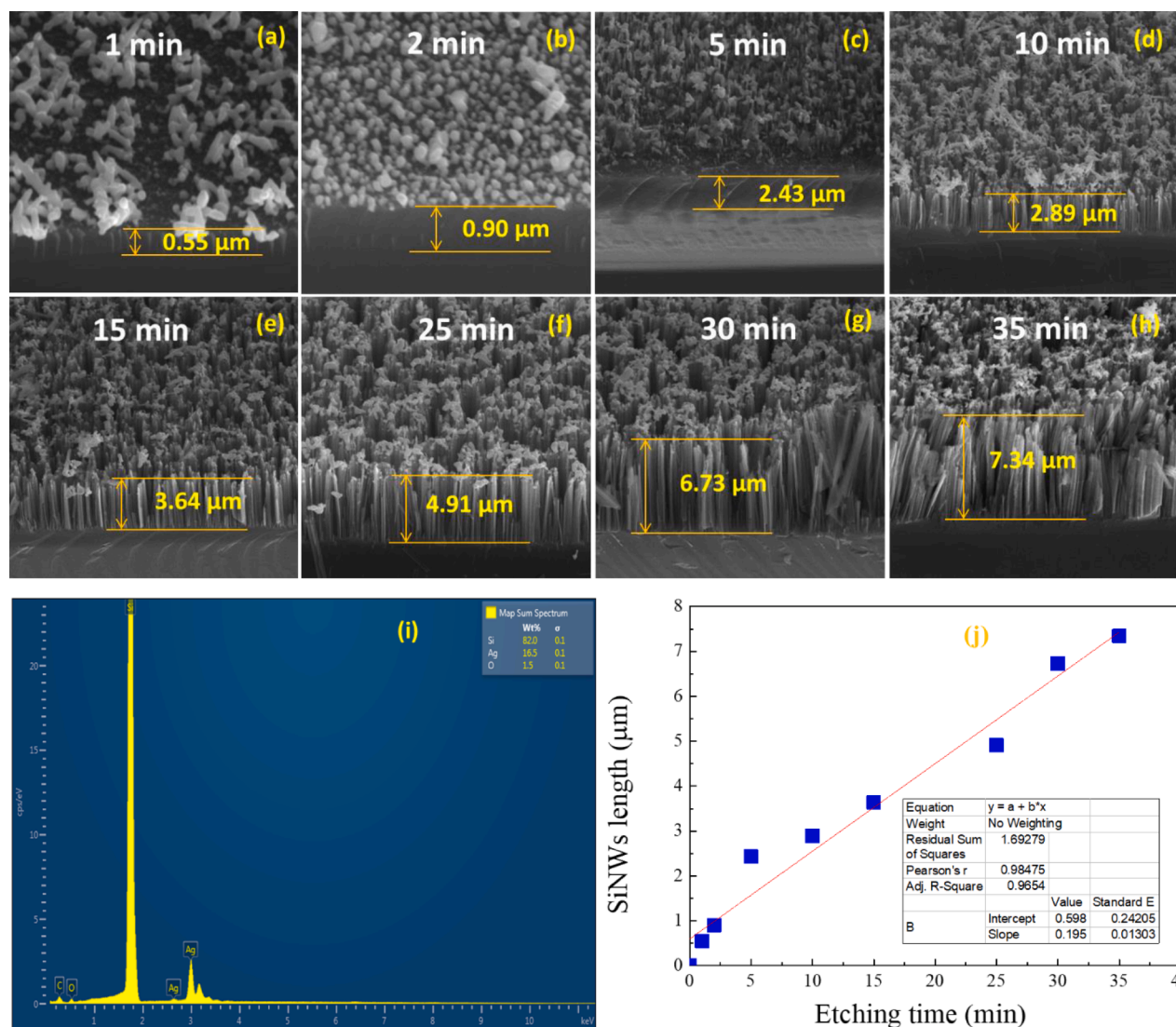


Fig. 3. SEM images showing the AgNPs decorated SiNWs for various etching times (1–35 min) (a–h). Typical EDS analysis of the AgNPs/SiNWs (2 min) sample (i). Evolution of the SiNWs length versus etching time (j). The immersion time for AgNPs decoration is set constant for 30 s.

The same procedure has been adopted for the analysis of G4 EA H1N1_HA1_His protein of influenza Type- A virus for comparison studies.

3. Results and discussion

3.1. Morphological studies

Metal-enhanced chemical etching (MACE) process directed growth of SiNWs and electroless decoration of AgNPs on SiNWs were applied to develop the SiNWs/AgNPs sensors. The mechanism of the SiNWs formation is a two-step process dominated by the creation of continuous Ag nuclei centers over the Si substrate surface by reduction of Ag^+ ions, followed by chemical dissolution of oxidized Si atoms in HF. The details of the SiNWs formation mechanism (Fig. 2) can be found elsewhere [33]. The evolution of the sensor surface morphology versus the etching time of the SiNWs (Top surface with cross section images) was scrutinized and depicted (Fig. 3(a–h)). It was evident that highly upright ordered ‘nano-grove’ like SiNWs array with uniform decoration of AgNPs throughout the sensor surface was achieved. In order to validate the dependence of nanowire length on the etching time, the AgNPs decoration on SiNWs immersion time was kept constant for 30 s. The etching

time plays a pivot role in controlling the length of nanowires. Upon increasing the etching time, there is almost a linear increment in their length which ranges from 0.55 μm for 1 min, extending up to 7.34 μm for 35 min etched Si (Fig. 3j).

Distinct SiNWs secluded from each other can be notified (Fig. 3(a–c)) for short etching times of 1, 2 and 5 min. Yet, the tip of the nanowires flock amongst themselves to form small wells within the ‘nano-grove’ architecture. Wells dole out evenly over the entire surface area most likely due to van der Waals interactions [34,35] between the adjacent SiNWs. SEM images show AgNPs homogeneously decorated on the SiNWs’ surface (Fig. 3(a–h)). Amidst, 2 min etched SiNWs portrayed extremely homogenous in distribution of AgNPs (Fig. 3b). Immersion time for the AgNPs decoration was varied from 5 to 120 s with constant etching time (2 min) for SiNWs. Based on their higher sensitivity in detection of R6G ($2 \times 10^{-6}\text{M}$) in (Fig. 5), 30s immersion time was selected to be the ideal condition for the decoration of AgNPs on SiNWs. Typical elemental analysis endorses the presence of AgNPs on SiNWs/AgNPs sensor (Fig. 3i).

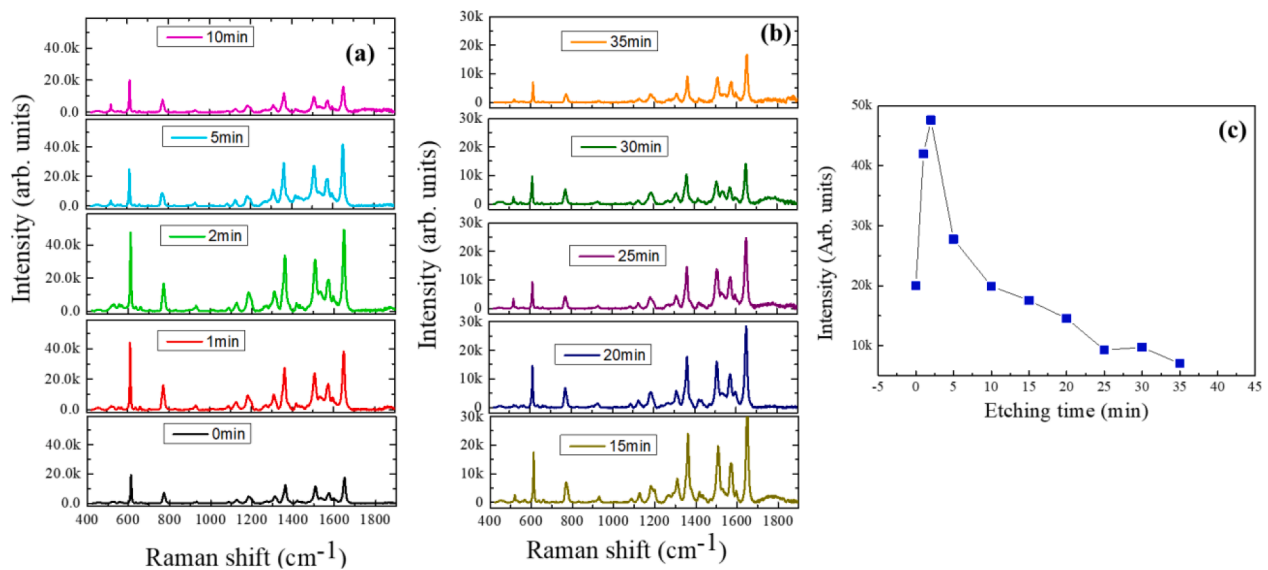


Fig. 4. (a) and (b) Effect of the etching times on the Raman spectra of R6G deposited on SiNWs/AgNPs (The immersion time for AgNPs decoration is set constant for 30 s). (c) SERS of the 612 cm^{-1} peak intensity as a function of etching times.

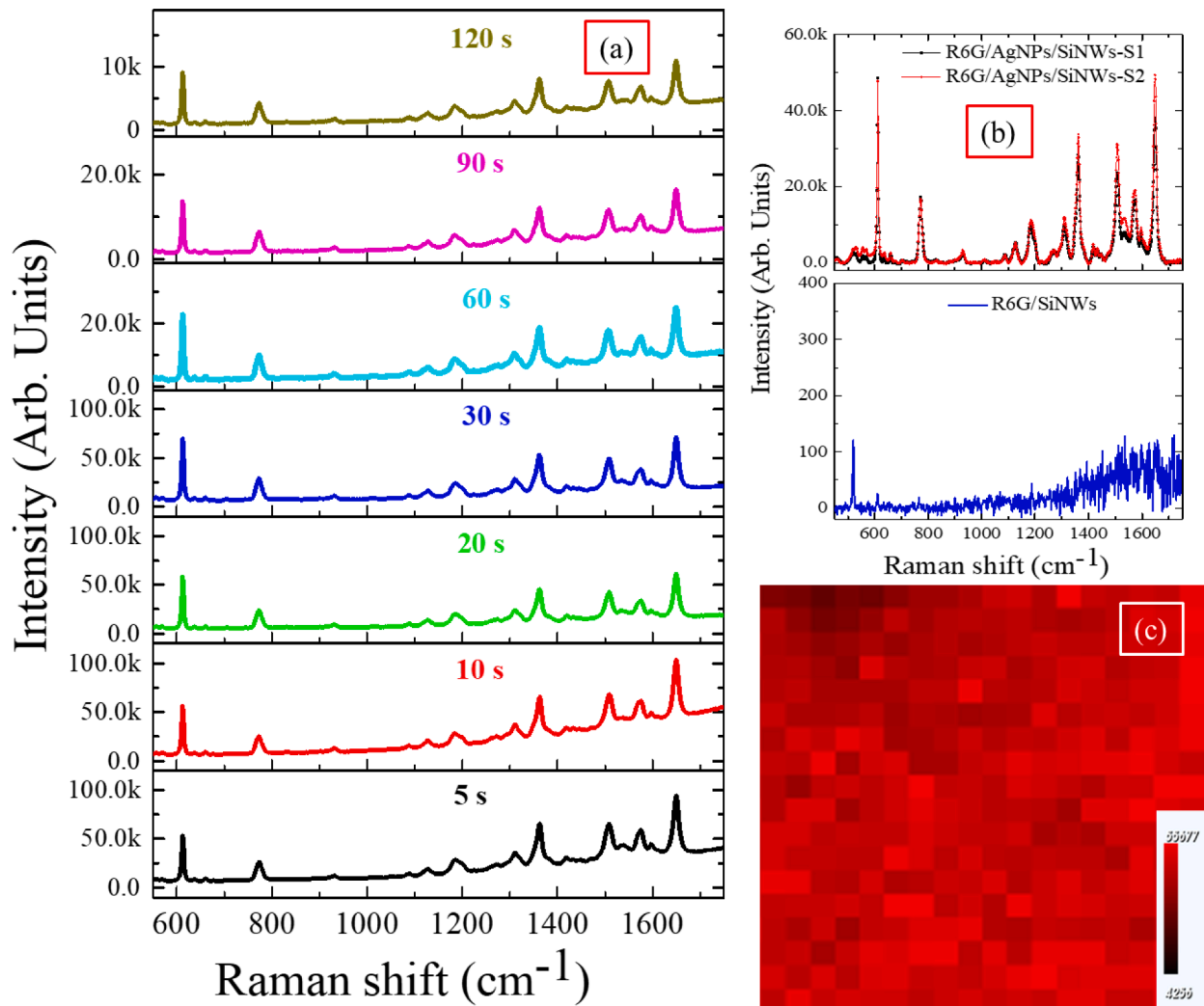


Fig. 5. (a) Effect of Ag immersion time on the Raman spectra of R6G deposited on AgNPs decorated SiNWs. (b) Upper panel – Reproducibility of R6G spectrum with another sensor fabricated under conditions, Lower panel- R6G spectrum recorded on bare SiNWs (2 min etching). (c) Mapping scan revealing the homogeneity of the sensor.

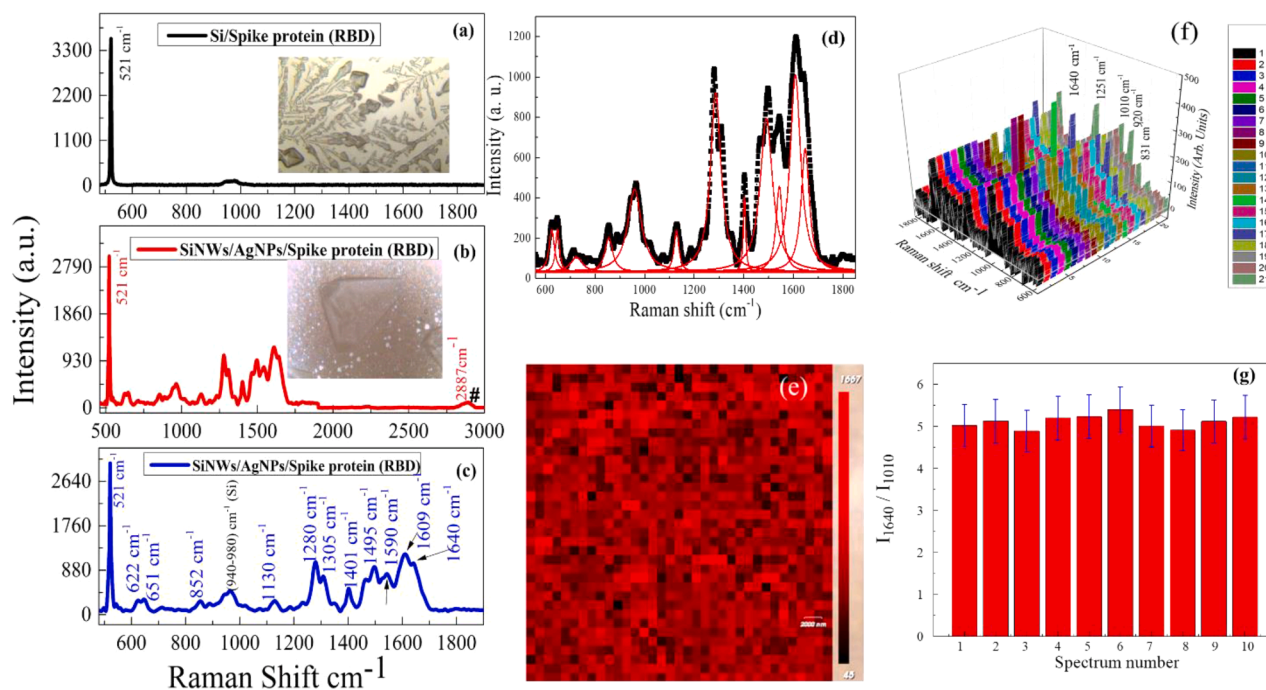


Fig. 6. Raman spectra of SARS-CoV-2 spike protein on (a) flat silicon (b) SiNWs/AgNPs sensor (500 cm^{-1} to 3000 cm^{-1}) (c) SiNWs/AgNPs sensor (500 cm^{-1} to 1800 cm^{-1}). Inset of 6 a and 6 b are the optical microscopy images of the spike protein on flat Si substrate and on SiNWs/AgNPs. (d) Lorentz fit for the experimental data (e) Mapping of spike protein detection on the SiNWs/AgNPs sensor. (f) Homogeneity of scans taken at random points in detection of spike protein with SiNWs/AgNPs sensor, and (g) the corresponding statistical analysis of Raman peak intensity ratios (I_{1640} / I_{1010}) for 10 different positions.

3.2. SERS studies

3.2.1. Influence of etching time of SiNWs of SiNWs/AgNPs sensor

The performance of the SiNWs/AgNPs sensor was assessed for the detection of the Raman probe molecule ($\text{R6G} \cdot 2 \times 10^{-6}\text{M}$) (Fig. 4(a, b)). The distinct Raman peaks of R6G were consistent entirely irrespective of the etching time of the fabricated sensor (SiNWs/AgNPs). It is important to notice that the R6G features were not visible on the Raman spectra recorded on bare SiNWs under otherwise identical experimental conditions. Sensor utilizes the uniqueness of noble metallic AgNPs localized surface plasmon resonance for the enhanced signal detection of R6G. The vibrations in R6G are C-C ring in-plane bending (612 cm^{-1}), C-H out-of-plane bending (771 cm^{-1}) and C-H in-plane bending (1187 cm^{-1}) along with peaks at 1307 , 1359 , 1510 , 1572 and 1652 cm^{-1} assigned to C-C stretching vibrations of R6G barcode [36–38]. R6G barcode peaks intensity fluctuated with etching time of SiNWs. The SiNWs/AgNPs sensor prepared using 2 min Si etching revealed significant signal amplification of the peak at 612 cm^{-1} (Fig. 4c), which can be correlated with the shorter nanowire length and uninterrupted distribution of AgNPs over the sensor surface. Likely, the AgNPs wraps the entire sensor's top surface, as revealed by SEM imaging. The R6G molecules are believed to adhere strongly to the AgNPs' surface, leading to the creation of higher quanta of 'hotspots' since the AgNPs are in close vicinity to each other. Upon increasing the etching time, van der Waals interactions exerting at the tip of the wires tend to bend them to form wells, as previously stated. Therefore, the R6G molecules can drop at the bottom pits in direct contact with silicon, leading to lower intensity of R6G. The AgNPs density at the top of the sensor surface is lower also breaking of nanowires was witnessed for higher etching times of 30 and 35 min (Fig. 3(g, h)) and could be of add-on reasons for lower signal of Raman spectra of R6G [39].

3.2.2. Influence of immersion time for the decoration of SiNWs by AgNPs

The decoration process of AgNPs on the sensor surface begins with immersion of SiNWs (etching: 2 min) for different time intervals ranging from 5 to 120 s, followed by the examination of R6G detection (Fig. 5a).

The Raman spectrum clearly revealed that 30s immersion time for the decoration of AgNPs on the SiNWs substrate achieved the highest enhancement without affecting the barcode features. It is believed that shorter immersion times for the decoration process enable the Ostwald ripening process of growth of small nuclei centers of Ag nanoparticles from 5 to 30 s. A prolonged time period may be source for aggregation of AgNPs that grounds in larger size as well as formation of dendrite structures [24,28,29]. The smaller size and spherical shape of AgNPs could possibly be a prime initiator for the hotspot production in enriching the signal intensity. Most favorable sensing parameters of R6G were attained for 2 min etching and 30 s decoration. The reproducibility of the fabrication process was assessed by preparing sensors using the same parameters and comparing their SERS performance (Fig. 5b). Auxiliary investigations on reproducibility tests were carried with mapping for about 625 scans point to point ($25 \times 25\text{ cm}^2$) randomly over the sensor surface (Fig. 5c). Hotspots with higher scattering appeared as bright red regions, whereas lower field scattering correspond to dark spots in the mapping image. Hence, the sensor holds good carbon-copy of Raman barcode of R6G spectrum, owing to the greater 3D volume of the AgNPs.

3.3. SERS detection of SARS-CoV-2 virus protein (spike protein)

SARS-CoV-2 spike protein (RBD) detection was performed with the fabricated SiNWs/AgNPs sensor and compared to a flat Si substrate (Fig. 6(a–c)). The optical microscopy images witnessed from SERS spectroscopy standpoints for the spike protein binding up on flat Si (inset of Fig. 6a) as well on the sensor (Fig. 6b) surfaces. No sharp peaks (rather than Si peak at 520 cm^{-1}) were observed, indicating the detection of spike protein cannot be achieved on flat Si (Fig. 6a). In contrast, the spike protein can be successfully detected using the developed SiNWs/AgNPs sensor, as confirmed by the presence of several features in the Raman spectrum (Fig. 6b and c). The Raman spectrum comprises distinct and sharp peaks at 1280 , 1401 , 1495 , 1541 and 1609 cm^{-1} assigned respectively to CH_2 wagging of L-Arginine amino acid of S-protein, amide –III (40% C-N stretch, 30% N-H bend), aromatic amino

Table 1

Assignment of SARS-CoV-2 spike protein peaks.

S.No	Detection of SARS-CoV-2 protein RBD peaks in Raman spectrum (cm^{-1})	Assignment of Raman vibration modes [40,41,43, 56]		Assignment of SARS-CoV-2 protein peaks	Lorentz fitting simulation results derived peaks of SARS-CoV-2 Spike protein RBD
1	520	Si	-	-	Excluded for fitting
2	-	Aliphatic chain	Stretching of C–C bond	Aliphatic chains	724
3	622	Aromatic amino acids	CH ₂ Rocking	Phenylalanine	622
4	651				644
5	852		CH ₂ Rocking	Tryptophan	855
6	980		CH ₂ Rocking	Phenylalanine	967
7	1130		NH ₃ ⁺ Rocking	Histidine	1129
8	1280		CH ₂ wagging	L-arginine	1287
9	1305	Amide –III (40% C–N stretch, 30% N–H bend)	-	α -helix	-
10	1401				1402
11	1495	Aromatic amino acid	COO-symmetric stretching	Valine amino acid	1491
12	1541	Amide –II (40% C–N stretch, 60% N–H bend) weak band present only due to resonance excitation	-	Anti-parallel β -sheet	1543
13	1590	Aromatic amino acids	Stretching of aromatic ring	Tryptophan Phenylalanine	-
14	1609	Amide I (80% C=O stretch)	-	Anti-parallel β -sheet	1608
15	1640				1647
16	2887	Skeleton	Stretching of CH, NH group	Skeleton base	-

acid (COO⁻ symmetric stretching of valine amino acid of S-protein), amide –II (40% C–N stretch, 60% N–H bend) weak band present only due to resonance excitation, and amide I (80% C=O stretch) of S-protein. The peaks derived from the spectra are listed [40–43,56] with their assignment in Table 1. The uniqueness of this spectroscopy lies in the context of recording the spectrum ranging up to 4500 cm^{-1} which is not discussed so far with other spectroscopic techniques. The scan range was extended towards 3000 cm^{-1} (Fig. 6b) to identify the spectral features of spike protein RBD in the extended scan; it is fortune to register the peak at 2887 cm^{-1} which is of less prominence and could be correlated to the skeleton of the protein structure (refer Table 1). Henceforth the dominant fingerprint peaks are possibly associated within the spectrum range from 600 to 1700 cm^{-1} is fixed (Fig. 6c) for the consecutive analyses of the studies. The peak origination of spike protein RBD are also verified for actual positions with Lorentz simulation fitting process. The Lorentz fit (Fig. 6d) exhibited best fit for the experimental detection spike protein RBD within which the peaks origination from the fitted curves also marked in parallel to the experimental data (Table 1).

During spike protein detection, it is presumed that the Ag atoms on the sensor surface bonded strongly with the nitrogen atoms (N) of

protein moiety. In parallel to that, laser excitation at 488 nm is resonant to both the sensor and the proteins absorption bands. Resonant conditions make use of the uniqueness of plasmonic property of AgNPs in the generation of ‘hotspots’, promising to enhancement in detection of spike protein [44]. Label-free recognition of protein conveys straight spectroscopic proof associated with the spike protein (RBD) itself. The current research is targeted at direct detection of spike RBD, can be further extended with functionalization to achieve the detection of SARS-CoV-2 virus of different mutants. Since SERS is surface sensitive, without labels there won't be much differences in the SERS spectrum target to different variants of proteins [14].

3.4. Validation of sensor fabrication towards uniformity in sensing

The homogeneity of the sensor sensitivity was elucidated with the mapping spectrum (Fig. 6e). The bright red regions on mapping indicate the excellent replica of the fingerprint peaks of the spike protein. Since the mapping corresponds to 625 scans, the selected spectra of random points corresponding to the spectra of spike protein detection by the sensor (SiNWs/AgNPs) were reported (Fig. 6f). Amazingly, higher

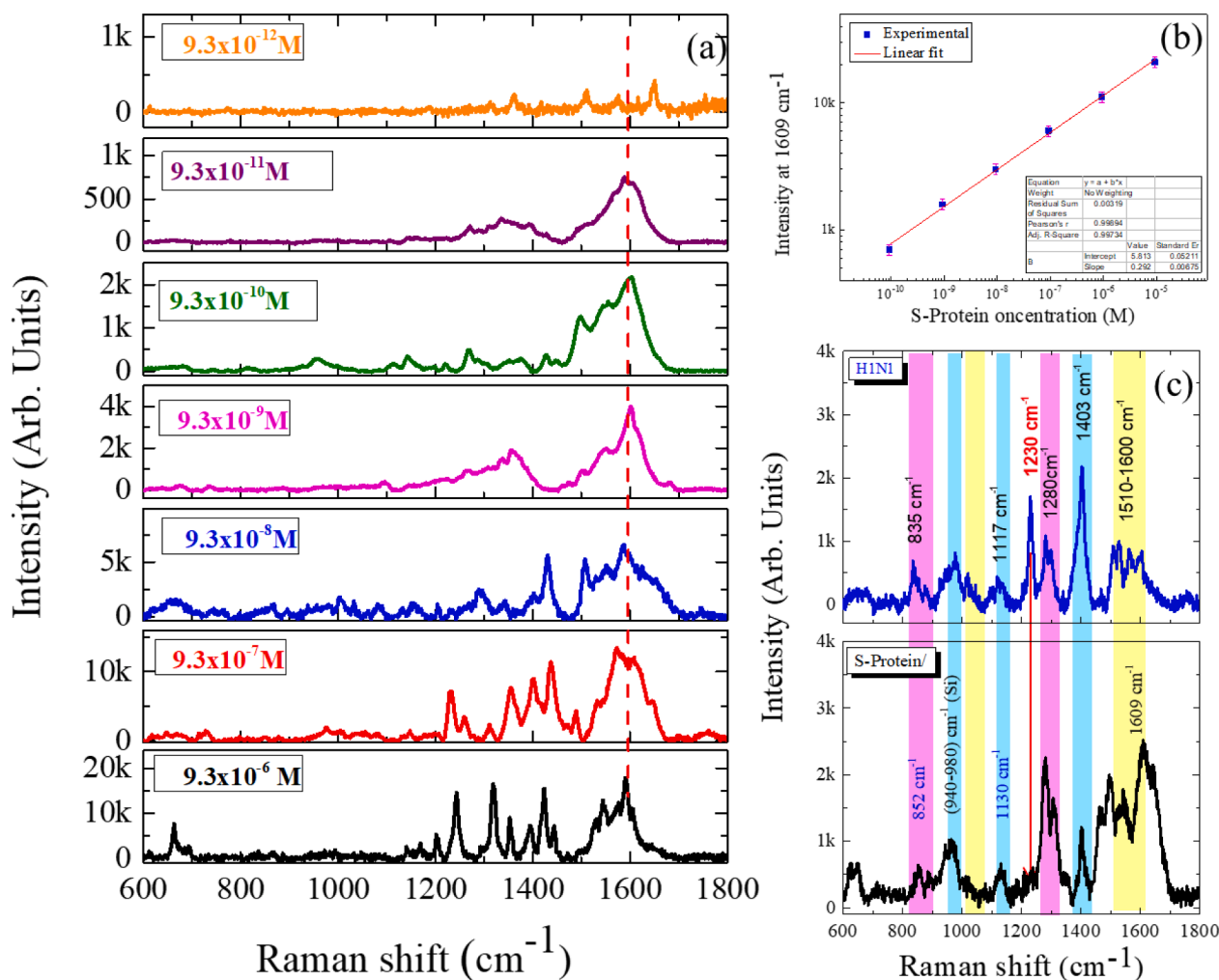


Fig. 7. (a) Raman spectra of the SARS-CoV-2 spike protein recorded on SiNWs/AgNPs sensor for various concentrations (9.3×10^{-6} M down to 9.3×10^{-12} M). (b) Evolution of the Raman intensity of the peak at 1609 cm^{-1} versus S-protein concentration in log-log plane with linear fitting. (c) Comparison between Raman spectra of the SARS-CoV-2 spike (RBD) and that G4 EA H1N1_HA1 of H1N1 influenza Type A virus.

power of 10 for mapping scan yielded the appearance of a peak at 1030 cm^{-1} , assigned to phenylalanine amino acid which is the common characteristic vibration of all proteins [45,46]. In addition, the other specific peaks pin to spike protein were observed with excellent consonance. Partial enrichment of some bands in the mapping spectrum

portrays that the adsorption of the protein on the sensor surface was likely to be different at each and every point due to its three-dimensional structure and larger size. Additionally, the different orientations of the spike protein on the sensor surface may be a possible reason for the intensities discrepancies [47]. However, the strong binding of spike

Table 2

Comparison of different detection strategies for SARS-CoV-2 virus.

S. No	Biosensor	Analyte	Sample	LOD	Sensitivity	Detection time	References
1	Calorimetric Assay (AuNPS-60nm)	N-gene from isolated RNA	Vero cells infected with SARS-CoV-2 (USA-WA1/2020)	0.18ng/uL	-	10 min	[52]
2	Paper based electrochemical biosensor	SARS-CoV-2 antigen and antibodies	Serum sample	0.11 ng/mL	-	-	[53]
3	Electrochemical sensor	Spike protein	Untreated saliva	19ng/mL	-	-	[54]
4	SERS (Au/Si with labels)	Spike protein	PBS	0.77fg/mL 6.07fg/mL (saliva)	95%	-	[55]
5	SERS (ACE2 with AuNPs)	S-protein	Contaminate Water and simulated urine sample	80 copies/mL	-	5 min	[56]
6	SERS (Mxenes)	S-protein	PBS	5nM	-	10 min	[42]
7	SERS (Au-Cu nanostars)	S-protein and N-protein	PBS	8.89nM	-	10–15 min	[57]
8	colorimetric/SERS/fluorescence triple-mode biosensor (AuNPs)	Viral RNA	-	160fM	-	40 min	[58]
9	LFIA (AuNPs)	IgM and IgG	-	-	88.66	10–15 min	[59]
10	SERS (SiNWs/AgNPs) Direct detection	Spike protein (RBD)	PBS	9.3pM 2.4pg/uL	-	< 5 min	This Work

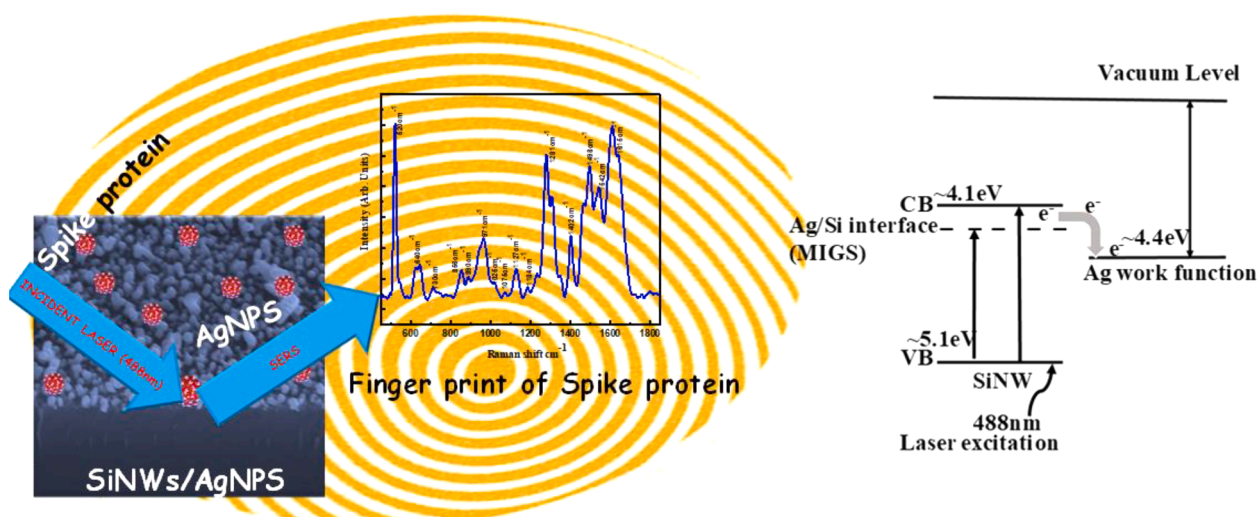


Fig. 8. Mechanism involved in the detection process.

protein with AgNPs enhances the electromagnetic contribution in detecting the SARS-CoV-2 spike protein with best sensitivity. The statistical analysis on intensity ratios (I_{1640} / I_{1010}) for 10 different scan positions was performed (Fig. 6g). The results suggested good uniformity in signal sensitivity from point to point. The small deviation in their intensity ratios may possibly arise due to different orientations of spike protein over the fabricated sensor surface.

3.5. Detectability of spike protein RBD with the fabricated SiNWs/AgNPs SERS sensor

The detection limit of the sensor was assessed by recording Raman spectra of different concentrations of the spike protein (Fig. 7a). Initially, the concentration used was 9.3 μM . Under serial dilutions, we reached down to low level detection of 9.3 pM. These results hold well in the era of diagnosis of patients at an early stage without being hospitalized. These results prove the potential of the Raman technique combined with the optimized SERS support as interesting approach for on-site analysis of patients wherever ultra-fast response is needed. The investigations in this work of SERS directed spike protein detection comprises direct, facile, highly reproducible and sensitive method, using SiNWs/AgNPs.

The evolution of the Raman intensity of the most pronounced peak at 1609 cm^{-1} versus the S-protein concentration is plotted in log-log plane, as depicted (Fig. 7b). The experimental results are well linearly fitted with a slope of 0.292 M^{-1} , a y-intercept of 5.813 M with an $R^2=0.99$. These results are good indicators of the excellent performance of our SERS sensor for the detection of SARS-COV-2-Spike protein. To confirm the uniqueness of the Raman spectra of the S-protein, we performed similar Raman analysis of the influenza A, known as H1N1, and plotted the corresponding spectra (Fig. 7c). We can easily note that both viruses (COVID19 and H1N1) have distinguished Raman spectra. We previously report that this technique (SERS) is able to detect small difference between two DNAs genetic sequences [48]. This technique could possibly be developed with functionalization of SERS substrates with specific labels to target the viral proteins, nucleic acids precisely. However, the current study is preliminary which aims to develop the direct detection of SARS-CoV-2 Virus at protein level (Spike RBD) needs further improvements to promote it to the real time monitoring diagnostic device. The developments are in progress. Table 2 provides the comparison analysis of detection capabilities of SARS-CoV-2 Virus with diverse techniques.

3.6. Mechanism of detection SiNWs/Ag sensor

Morphological features play an inevitably interesting role in the detection process by the long-range electromagnetic field (EM) improvement. The AgNPs decoration should lie on the sensor surface with negligible distances since the inter-particle distance affects the enhancement.

It was believed that the shorter SiNWs are free-standing, in which the AgNPs occupy the sites on the top surface as well as alongside of the nanowires. 'Hotspots' creation is prevailed by the nearby AgNPs alongside of single SiNWs and the AgNPs of two adjacent SiNWs. As well the charge carriers' interaction between Si and Ag would greatly participate to SERS enhancement with the point of view of excitation of AgNPs surface plasmons. Upon laser excitation at 488 nm, the electrons in the valence band of SiNWs jump to the conduction band or the interface states of Ag/Si formed by AgNPs decoration on SiNWs. It is accounted for the reason that the work function of Ag being greater ($\sim 4.4\text{ eV}$) on par with electron affinity of n-type Si ($\sim 4.05\text{ eV}$) [49,50]. Migration of electrons continues to progress towards the lower energy Fermi level of AgNPs, giving rise to increment in the electron density on the sensor surface (Fig. 8). It is expected to boost-up the localized EM field intensity around the AgNPs. The process stated is purely by the dynamic photo-induced charge transfer from SiNWs to AgNPs with complete absence of electron transfer between the probe molecule and sensor. As the whole phenomenon is contributed by localized surface plasmons EM resonant excitation, henceforth coined the terminology as EM effect [51].

4. Conclusion

During the actual pandemic situation, the long-time consuming methods upon screening the virus on infected humankind cannot be endured. We endeavored to rely on optoelectronics based non-traditional SERS tool to develop highly sensitive, fast, and label-free diagnostics and prove its concept as ingenious device in the detection of SARS-CoV-2 virus (S-protein). In this regard, SiNWs/AgNPs sensor was developed by simple wet chemistry protocols and resulted in highly ordered vertical nanowire arrays with homogenous decoration by spherically shaped AgNPs. The influential parameters that govern the detection were pointed out and addressed in the fabrication stages of the sensor in which $0.90\text{ }\mu\text{m}$ -long SiNWs and 30s immersion time for AgNPs decoration on SiNWs/AgNPs revealed promising outcomes with sterling enhancement and recurrence of both organic molecule R6G as well as bio molecule SARS-CoV-2 spike protein sensing. The SiNWs/AgNPs

sensor platform was delicate and precise to SARS-CoV-2 spike protein even at an exceptionally trace level concentration of pico-molar. Subsequently, this developed biosensor (based on SiNWs/AgNPs nanomaterials) with outstanding sensitivity, rapid detection, and good replicability will pave the way to a new generation of biosensors for the detection of infectious microbes and viruses. However, the current study is preliminary which aims to develop the direct detection of SARS-CoV-2 Virus at protein level (Spike RBD) needs further improvements (labelled detection for target specific) to promote it to the real time monitoring diagnostic device.

CRedit authorship contribution statement

Kais Daoudi: Conceptualization, Methodology, Formal analysis, Writing – review & editing. **Krithikadevi Ramachandran:** Writing – original draft. **Hussain Alawadhi:** Writing – review & editing. **Rabah Boukherroub:** Writing – review & editing. **Elhadj Dogheche:** Writing – review & editing. **My Ali El Khakani:** Conceptualization, Writing – review & editing. **Mounir Gaidi:** Conceptualization, Validation, Funding acquisition, Writing – review & editing.

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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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.surfin.2021.101454](https://doi.org/10.1016/j.surfin.2021.101454).

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