2023「中技社科技獎學金」
2023 CTCI Foundation Science and Technology Scholarship
境外生研究獎學金
Research Scholarship for Overseas Students

Dual-Clamped One-Pot SERS-based Biosensor for Rapid and Sensitive Detection of SARS-CoV-2 Using Portable Raman Spectrometer

Kiran Kaladharan Nair, Fan-Gang Tseng
4th year PhD Student, Advisor
Department of Engineering and System Science, National Tsing Hua University

Abstract:

Rapid and sensitive diagnostics of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is of utmost importance to control the widespread coronavirus disease 2019 (COVID-19) upsurge. This study demonstrated a one-pot surface-enhanced Raman scattering (SERS) based immunoassay to detect SARS-CoV-2, without any washing process using a portable Raman spectrometer. The SERS-immune assay was designed using a regular digital versatile disk (DVD) substrate integrated with Raman reporter labeled silver nanoparticles for double clamping effects. The disks were molded to form nanopillar arrays and coated with silver film to enhance the sensitivity of immunoassay. The SERS platform demonstrated a limit of detection (LoD) up to 50 pg/mL for SARS-CoV-2 spike protein and virus-like-particle (VLP) protein in phosphate buffer saline within a turnaround time of 20 mins. Moreover, VLP protein spiked in untreated saliva achieved an LoD of 400 pg/mL, providing a cycle threshold (Ct) value range of 30–32, closer to reverse transcription-polymerase chain reaction (RT-PCR) results (35−40) and higher than the commercial rapid antigen tests, ranging from 25 to 28. Thus the one-pot SERS biosensor could be a potential point-of-care platform for early and cost-effective diagnosis of the COVID-19 virus.

Introduction:

Nucleic acid detection[1]	Rapid Antigen detection[2]
• Gold standard: RT-PCR Test • The accuracy is more than 90% • Time-consuming (~ 4 hours) • Labour intensive • Comparatavely expensive • Ct value: 35-40	• Rapid detection (15-30 mins) • Cost effective • Sensitivity is about 50-70% in early stages • Chances of false negative • Ct value: 26-30

Motivation for a novel COVID-19 virus detection system?

To COMBINE the best features of Nucleic acid detection as well as Rapid antigen detection

We developed a dual-clamped surface-enhanced Raman Scattering (SERS) sensor by synergizing DVD nanostructures and nanoparticles together on target recognition to amplify signals using one-pot reaction without washing or sample pretreatment process (**Fig.1**). This sensor can not only reach the accuracy closer to nucleic acid detection but also maintain the rapidity and convenience of antigen detections.

Experimental Design:

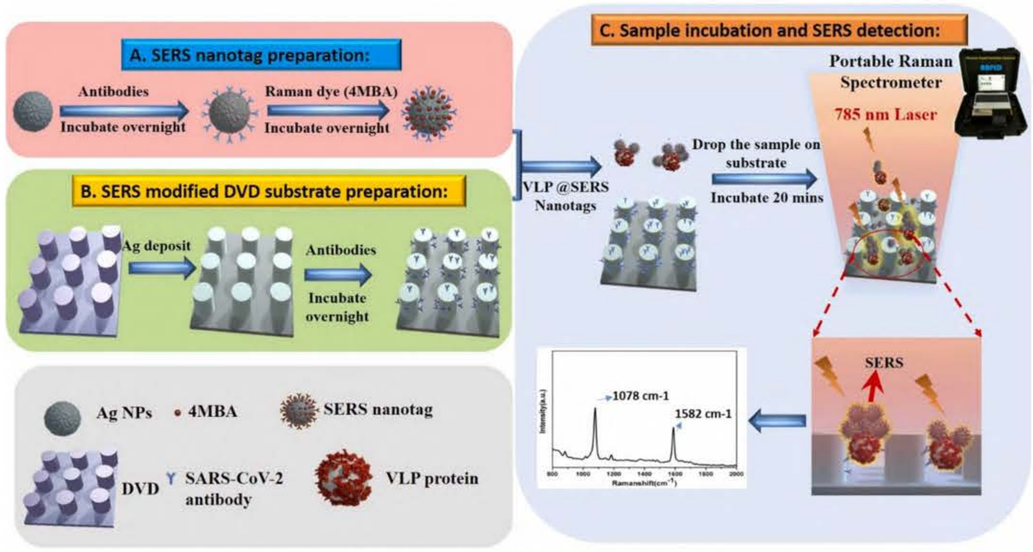

Figure 1: The schematic illustration of detection principle of dual-clamped one-pot SERS based SARS-CoV-2 biosensor

Conclusions:

- We have demonstrated a dual clamped One-Pot SERS-based COVID-19 biosensor for early and sensitive detection of SARS-CoV-2 virus in a measurement time of 20 minutes.
- The SERS-based biosensor successfully detected SARS-CoV-2 spike and VLP protein in PBS buffer with a limit of detection 50 pg/ml, providing a Ct value of 30-32, closer to RT-PCR results (35-40) and higher than most Rapid antigen test kits (25-28).The SARS-CoV-2 VLP protein spiked in untreated saliva exhibited an LoD of 400 pg/ml (**Fig. 3**).
- The SERS-based biosensor successfully detected SARS-CoV-2 nucleocapsid (N) protein with an LoD of 50 pg/ml **(Fig. 4A and B)**. Both FORA and PANBIO antigen test kits displayed a LoD up to 10 ng/mL for N protein **(Fig. 4C and D)**.
- Excellent specificity was displayed by the biosensor while mixing non-specific proteins with target N protein (**Fig. 5A**). The sensor exhibited good reproducibility with relative standard deviation (RSD) of 7.57% at different spots on the SERS substrate (**Fig. 5B**).

Results and Discussion:

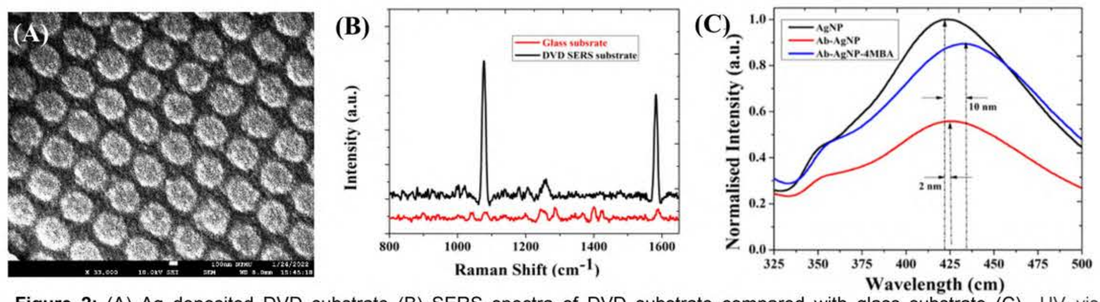

Figure 2: (A) Ag deposited DVD substrate (B) SERS spectra of DVD substrate compared with glass substrate (C) UV–vis extinction spectra of AgNPs, AgNP-Antibody conjugate, and SERS nanotags

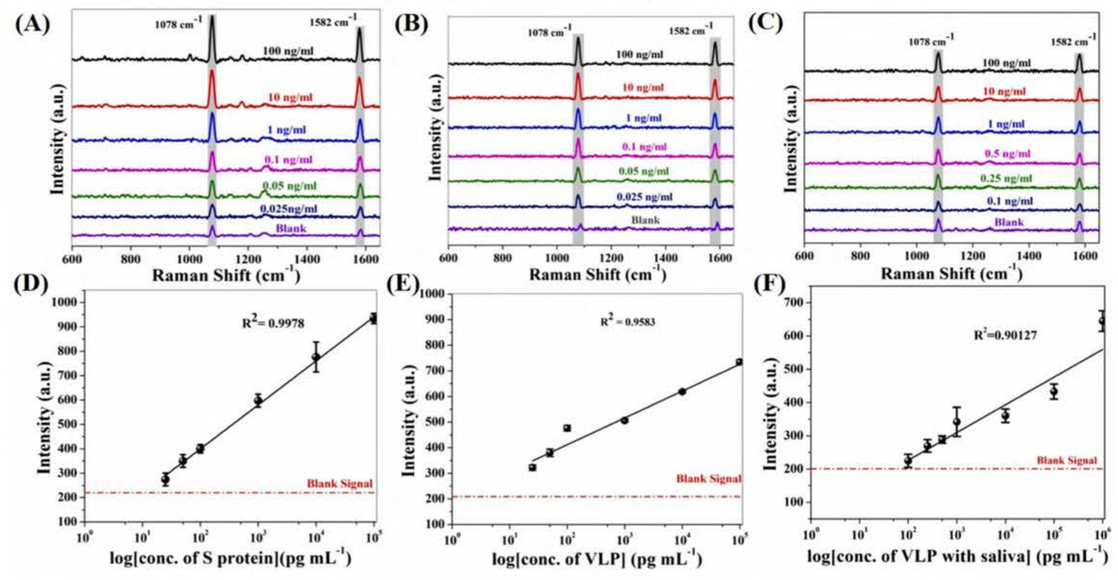

Figure 3. SERS immunoassay detection results of SARS-CoV-2 target proteins: SERS spectra and Calibration curve of sandwich immunoassay at different concentrations ranging from 25 pg/mL to 100 ng/mL in PBS for SARS-CoV-2 (A,D) S protein, (B,E) VLP protein and in untreated saliva) for (C,F) Raman spectra and calibration curve of SERS immunoassay at different SARS-CoV-2 VLP protein concentrations from 100 pg/ml to 100 ng/ml in untreated saliva.

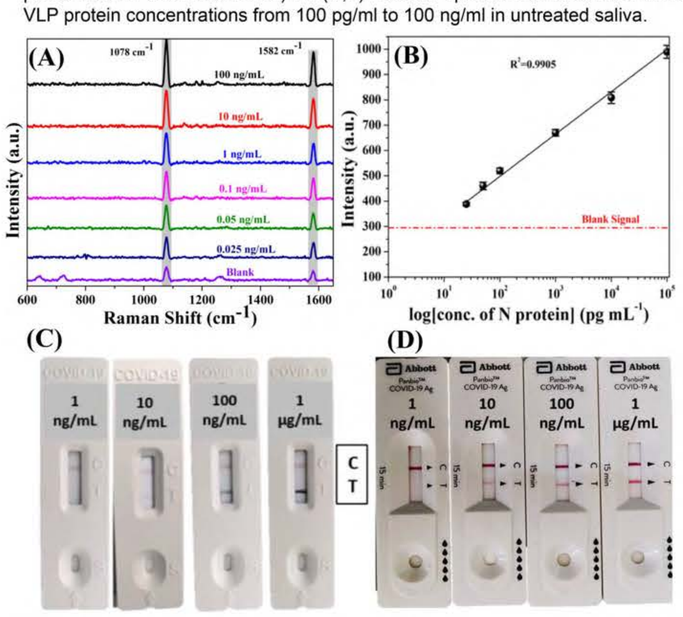

Figure 4: (A) SERS spectra of sandwich immunoassay structure at different concentrations of SARS-CoV-2 N protein ranging from 25 pg/ml to 100 ng/mL in PBS (B) Calibration at the peak intensities of the SERS spectra plotted in (A), Detection results of (C) FORA and (D) PANBIO antigen test kits for SARS-CoV-2 N protein.

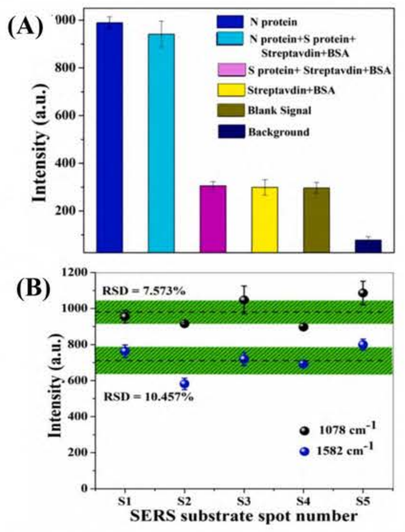

Figure 5. Specificity and reproducibility of the one-pot SERS immunoassay platform. (A) SERS peak intensities obtained from the detection of SARS-CoV-2 N protein and a multiprotein mixture of SARS-CoV-2 N protein, SARS-CoV-2 S protein, Streptavidin and BSA, (B) Relative standard deviation (RSD) of the peak intensity at 1078 cm^{-1} and 1582 cm^{-1}, respectively.

Selected Journal Publications: **(1) Kiran Kaladharan**, Kuan-Hung Chen, Pin-Han Chen, Venkanagouda S. Goudar, Tseren-Onolt Ishdorj, Tuhin Subhra Santra, Fan-Gang Tseng, Dual-clamped one-pot SERS-based biosensors for rapid and sensitive detection of SARS-CoV-2 using portable Raman spectrometer, Sensors and Actuators B: Chemical, 393,134172 (2023). (2) **Kaladharan, K.**; Kumar, A.; Gupta, P.; Illath, K.; Santra, T.S.; Tseng, F.-G. Microfluidic Based Physical Approaches towards Single-Cell Intracellular Delivery and Analysis. Micromachines 2021, 12, 631. https://doi.org/10.3390/mi12060631.