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One-pot and rapid detection of SARS-CoV-2 viral particles in environment using SERS aptasensor based on a locking amplifier

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Abstract

With the frequent detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in dwellings and wastewater, the risk of transmission of environmental contaminants is of great concern. Fast, simple and sensitive sensors are essential for timely detecting infection and controlling transmission through environment fomites. Herein, we developed a Surface Enhanced Raman Scattering (SERS) aptasensor, which can realize ultrasensitive and rapid assay of SARS-CoV-2 viral particles. In this strategy, we designed a novel locking amplifier which is activated only in the presence of virus by aptamer recognition. The reaction process was carried out through one-pot method at 37 °C, which can save time and resources. In addition, magnetic beads used in reaction system can simplify operation, as well as provide ideas for developing biosensing robots via magnetic field. This SERS aptasensor can detect SARS-CoV-2 virus with a LOD of 260 TU/µL within 40 min in the linear range of 625–10,000 TU/µL. Therefore, this convenience, speediness, sensitivity, and selectivity of detection has great prospects in analyzing SARS-CoV-2 viral particles or other viruses in environment as well as monitoring of environmental virus sources.

1. Introduction

COVID-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been circulating globally for more than two years [1–3]. The cumulative incidence of the ongoing disease has exceeded 450 million cases worldwide, resulting in more than 6.3 million deaths (As of Jun 13, 2022) [4]. In recent research, SARS-CoV-2 virus samples were found in environment from Italy, United States, France and Spain. Meanwhile, there have been reports in China of cold chain workers getting sick from exposure to infected products. What’s worse, the virus can survive for a long time in cold temperature [5–9]. Of note, SARS-CoV-2 virus released in body fluids of infectious patients are capable of contaminating environment system, and has been shown to be infectious in 17–31 days [10–12]. Early detection and warning of virus in environment where people gathered is crucial to control the spread of virus through environment. Whereas, how to facilitate simple, economical, highly-sensitive and short-time SARS-CoV-2 assay in complex or remote environment remains a key technical challenge [13]. Therefore, it is necessary to develop a sensor capable of analyzing trace SARS-CoV-2 virus in environment, especially in cold-chain system.

Biosensors used to analyze SARS-CoV-2 fall into two categories depending on their targets: viral nucleic acid detection and viral protein detection, such as spike protein [14]. At present, polymerase chain reaction serves as a useful technology for detecting virus by nucleic acid amplification and has been used in the clinical diagnosis of COVID-19 [15–18]. However, the procedures for sample preparation, nucleic acid amplification and detection need professional personnel, specialized lab and take a long time (3 ~ 4 h). These limitations affect its application in environmental detection and monitoring [19–21]. In addition, the virus concentration, RNA extraction and incomplete reverse transcription or PCR inhibition may affect the accuracy of detection [22,23]. In order to reduce detection time, a variety of immunodiagnostic kits via antigen-antibody reactions have been developed and commercialized [24]. Whereas, they are reported to have poor accuracy and high detection limit [25–27]. As far as we know, current methods have moderate sensitivity, particularly lower than picomoles, or the risk of cross-contamination due to the complex procedures [28–30]. Therefore, fast, sensitive and simple biosensor is of
great significance in preventing the spread of large-scale infections through the environment in our daily life.

Surface Enhanced Raman Scattering (SERS) features high sensitivity, simple operation, and fast assay [31]. It has been widely used in the detection of single biomolecule, respiratory viruses, and animal viruses [32–39]. However, there are interferences such as proteins and biomolecules in environment infected by SARS-CoV-2 virus. And they can affect virus detection, leading to high background signal [13]. Therefore, it is key to specifically identify the target virus in complex environment samples. Spike glycoprotein (S protein) is located in the outermost layer of SARS-CoV-2 and acts like spike crown, which is an important target for human vaccination, treatment and diagnosis [40, 41]. Aptamer is small oligonucleotide sequences that can be synthesized in vitro. It has the advantages of high sensitivity and specificity, and has been widely used as an effective tool for detection. The aptamer of SARS-CoV-2 S protein has been researched and proved to own high affinity with S protein [30, 42, 43]. Hence, aptamers-regulated Surface Enhanced Raman Scattering (SERS) sensor was developed in this work. Through the special identification of functional aptamers and amplification of SERS, a fast, sensitive, selective, and reliable method can be provided for detecting trace virus.

To make further improvement of sensitivity in detecting SARS-CoV-2 viral particles, strand displacement amplification (SDA) is incorporated into the design. SDA has been widely used for its advantages of high specificity, convenience, and simplicity in construction, and can achieve rapid amplification of 10^6–10^9 times in a few minutes. Therefore, the sensitivity can be greatly increased by combining cyclic amplification and SERS assay. In continuation of our previous efforts to develop various SERS aptasensor for the detection of poisonous analytes in environment [44, 45].

Herein, SERS aptasensor based on a locking amplifier was developed for detecting pseudotyped SARS-CoV-2 virus (PSV) with favorable sensitivity and good selectivity. In our strategy, the new locking amplifier can be activated by targeting the S protein on virus, which can improve the selectivity of this sensor in complex environment samples. Meanwhile, the locking amplifier will release trigger to start cyclic amplification, which can greatly increase the sensitivity. Moreover, all reactants were blended in a one-pot reaction system at 37 °C, avoiding complex experimental procedures. The use of magnetic beads can simplify the experimental operation and provide ideas for the development of biosensing robots via the magnetic field. For this ultra-sensitivity test, a low detection limit (LOD) was calculated to be 260 TU/μL within 40 min in the linear range of 625–10,000 TU/μL. This SERS aptasensor also has good performance in environment samples, such as tap water, river water and fish samples in cold-chain system. Therefore, this work is promising to provide a fast, sensitive, simple, and one-pot SERS aptasensor for detecting trace SARS-CoV-2 or other viruses in environment, which can also be used to monitor the environmental viral sources and prevent the spread of large-scale infections.

2. Experimental section

2.1. The synthesis of surface enhanced Raman spectroscopy (SERS) probes

According to the literature, [46] first, Rox-DNA (3 μL, 100 μM) was slowly added to TCEP (Tris (2-carboxyethyl) phosphine) solution (14 μL, 1 mM) in a 1.5 mL centrifuge tube and the mixture solution was stirred at room temperature for 30 min. Second, capture DNA (3 μL, 1 μM) and AuNPs (80 μL) were added into the mixture solution. And n-butanol (900 μL) was quickly added and shocked. Third, 20 μL of 0.5 × TBE (Tris, 44.5 mM; EDTA (ethylene diamine triacetic acid), 1 mM; boric acid, 44.5 mM; pH 8.0) buffer was added to the above solution, followed by rapid agitation. Finally, the excess DNA in the supernatant was removed by centrifugation at 10,000 rpm for 15 min, and the precipitation was rinsed with PBS for 3 times. SERS probes were re-dispersed in PBS solution and stored at 4 °C.

2.2. Preparation of SARS-CoV-2 probe solution

SARS-CoV-2 probe solution was prepared by mixing trigger DNA (1.5 μL, 1.0 × 10^{-6} M), aptamer 1 (6 μL, 1.0 × 10^{-6} M), and aptamer 2 (6 μL, 1.0 × 10^{-6} M) in buffer (pH 7.5, 40 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), 100 mM NaCl, 20 mM MgCl_2) with a final ratio of 1:4:4 to ensure a low background signal [47]. Then the mixed solution was incubated at 90 °C for 15 min, and cooled at room temperature.

3. Results and discussion

3.1. The workflow for the assay of pseudotyped SARS-CoV-2 virus (PSV) using SERS aptasensor

Our SERS aptasensor is based on a locking amplifier which mainly consists of PSV probe system and the isothermal nucleic acid recycling amplification system (see Fig. 1). The probe system contains aptamer 1, aptamer 2 and trigger DNA, forming the status of “locked trigger”. Aptamers are designed to own two key functions. One is responsible for identifying virus and the other is binding to the trigger DNA, realizing the lock-in effect (defined as “locked trigger”), which is important to reduce nonspecific background. The nucleic acid recycling amplification system contains hairpin DNA modified on magnetic beads (MBs) and SERS probe. The hairpin DNA can be unfolded by trigger DNA, then induce the polymerization-nicking amplification reaction (cycle 1). In the presence of KF polymerase, lots of SERS probes will bind with unfolded hairpin. With addition of Nt. BbvCI, numerous trigger DNA are released to open more hairpin DNA. Finally, a lot of SERS probes are modified on MBs. Then pseudotyped SARS-CoV-2 virus (PSV) concentration will be transformed to Raman signal. And we can detect the concentration of PSV by monitoring the SERS intensity. In the absence of PSV, the functional DNA (aptamer 1 and aptamer 2) hybridize with trigger DNA under annealing conditions, which can keep it from initiating the recycling amplification system. In the presence of PSV, the functional DNA will identify it and release the trigger DNA, which can initiate the recycling amplification system to greatly increase virus signal. Therefore, this strategy can realize the analysis of SARS-CoV-2 viral particles with high sensitivity and good selectivity.

3.2. Characterization

The AuNPs particle size was detected on transmission electron microscopy (TEM). It can be seen from Fig. 51 that the particle diameter was about 20 nm. The synthesized SERS probes were characterized through UV–vis spectrometer. It can be seen from Fig. 52, Curve a, c and d were UV–vis spectra of AuNPs (520 nm), Capture DNA (260 nm), and Rox-DNA (260 nm and 580 nm), respectively. And we can find characteristic absorbance of AuNPs, Capture DNA, and Rox-DNA in curve c, which proved that two kinds of DNA have been coupled to AuNPs through Au-S. The microstructure of magnetic beads (MBs) and magnetic gold SERS tag composites were analyzed using TEM and scanning electron microscopy (SEM). It can be seen from the TEM image of Fig. 2 A, the size of MBs was about 0.8 μm and the edge was smooth. In Fig. 2B, the edge of magnetic gold SERS tag composites was irregular when compared with Fig. 2A. In Fig. 2G, the magnetic-gold SERS tag composites showed favorable dispersibility through the SEM image. As can be seen from Fig. 2D-i, due to AuNPs, bright particles were covered on MBs. To further evaluate distributions of AuNPs on MBs, the corresponding element mapping of Fe and Au were provided in Fig. 2D-ii and Fig. 2D-iii, which shown that Fe and Au uniformly distributed on SERS aptasensor. The experimental results proved that SERS probes was modified on MBs though this strategy.
3.3. Locking effect of functional DNA (aptamer 1 and aptamer 2) to trigger DNA

In order to reduce the background signal, we studied the locking effect of functional DNA and trigger DNA. Firstly, the relationship of trigger DNA with SERS signal intensity was analyzed. In Fig. 3A, SERS intensity increased with the increase of trigger DNA concentration, demonstrating that trigger DNA had a close connection with SERS intensity. Secondly, the locking effect of two aptamers and single aptamer were researched. It was shown in Fig. 3B, when aptamer 1 and aptamer 2 were added to hybridize with trigger DNA, they formed “locked trigger” mode, resulting the sharp decrease of SERS intensity compared with the absence of aptamers. In addition, the SERS intensity gradually reduced when the ratios changed from 0:1→4:1. However, when single aptamer hybridized with trigger DNA (Fig. 3C and 3D), the SERS intensity were almost the same with the unlocked trigger, even the ratio reached to 4:1. These results demonstrated that the dual locking effect of aptamer 1 and aptamer 2 can successfully decrease the background signal by hybridizing with trigger DNA in the absence of pseudotyped SARS-CoV-2 virus.

3.4. Feasibility study

For verifying the feasibility of aptamers-regulated SERS aptasensor in detecting pseudotyped SARS-CoV-2 virus (PSV), we performed the control experiments (Fig. 4). The concentration of PSV can be detected via the peak at 1504 cm$^{-1}$ based on the characteristic peak of Rox. In the absence of PSV, the trigger DNA was locked, so cycle 1 (Fig. 1) cannot be initiated and the SERS signal was weak peak (curve a in Fig. 4A). With addition of PSV, but without KF polymerase or dNTPs, the “opened trigger” model will be formed, and the trigger DNA will open hairpin DNA (process I in cycle 1), but the unfold hairpin DNA cannot realize the extension (process II in cycle 1). So the SERS probes cannot form stable double-strand DNA with unfold hairpin DNA, then we obtained very low SERS signal (curve b and d in Fig. 4A). With addition of PSV, but in the absence of Nt. BbvCI, the process II was successfully performed, but the process III did not. As a result, only a part of hairpin DNA were opened, and medium signal intensity was observed (curve c in Fig. 4A). Only when all reagents were added in the one-pot reaction system, a strong SERS signal was obtained (curve e in Fig. 4A). Meanwhile, we researched the relationship of the reaction time with the SERS intensity under different conditions. When the trigger DNA was added to the reaction system, the SERS intensity was increased with the extension of time, indicating that the design of recycling amplification was successful (curve a in Fig. 4B). Comparing the curve b with curve c (Fig. 4B), only the pseudotyped SARS-CoV-2 virus was incubated with locked trigger, the SERS intensity was increased with the extension of time, proving that the existence of pseudotyped SARS-CoV-2 virus was inevitably related to SERS intensity. Taken together, the control experiments proved that the SERS aptasensor can be used for the detecting of pseudotyped SARS-CoV-2.

3.5. Sensitivity performance

To determine if the SERS aptasensor can be used for the quantitative detection of pseudotyped SARS-CoV-2 virus, different concentrations of PSV were measured under optimal conditions (see supporting information). In Fig. 5A, the SERS intensity increased when PSV concentration changed in the range of 625–10,000 TU/µL (TU, transduction unit). As shown in Fig. 5B, they were displayed a linear relationship, and the fitting equation was \( \Delta I = 146.8 C + 3449.4 \) with the correlation coefficient \( R = 0.997 \) (\( \Delta I \) and C represent SERS intensity and pseudotyped SARS-CoV-2 virus concentration, respectively). Noticed from the results, the limit of detection (LOD) was 260 TU/µL. The improvement was obvious when compared with other reported methods (see Table S2).

3.6. Reproducibility and uniformity

Reproducibility and uniformity are the key indicators to assess practical application of SERS biosensor. To examine the reproducibility, 5 different batches of SERS aptasensor were selected for SERS intensity test, and the results showed that the intensity did not change much (Fig. 6A). The relative standard deviation (RSD) was figured out to be 3.14% (Fig. 6B). To evaluate the uniformity, 11 points on one aptasensor were randomly selected to analyze the SERS signal. It can be seen from Fig. 6C, the SERS intensity changed a little and the value of RSD was calculated to be 7.29% at 1504 cm$^{-1}$ (Fig. 6D). These results indicate that this SERS aptasensor owns good reproducibility and uniformity, proving its application in real sample detection.

3.7. Specificity assay

The specificity of this SERS aptasensor was studied though comparing the Raman signal of pseudotyped SARS-CoV-2 virus (PSV) and lentivirus particles (Len) in experiments. The SERS spectra were
displayed in Fig. 7A, we synthesized pseudotyped SARS-CoV-2 probe using random aptamer sequence. When random aptamer sequence was used to detect PSV, the SERS intensity was very low comparing with aptamer sequence, indicating that the synthesized pseudotyped SARS-CoV-2 probe with aptamer sequence can selectively capture the target. Then random aptamer sequence and aptamer sequence were used to detect Len, the SERS strength was weak when compared with PSV. And Fig. 7B showed the corresponding bar graph of SERS strength for 4 different samples. These experimental data certify that the SERS aptasensor has high selectivity for testing SARS-CoV-2 viral particles.

3.8. Actual samples detection

To investigate the application performance of this SERS aptasensor in environmental samples, assays were carried out by spiking a series of different concentrations of pseudotyped SARS-CoV-2 virus solutions. Three kinds of real samples were collected from tap water in our laboratory, river water and cold chain logistics company in our institute. The fish samples in cold chain logistics company were imported from abroad and we sampled the surface of the fish with a swab. The collected samples were spiked with 2500 TU/µL, 5000 TU/µL and 9000 TU/µL pseudotyped SARS-CoV-2 virus to perform the recovery experiments. The results were summed up in Table S3. The recovery rates were from 93.11% to 106.35%, and the relative standard deviations (RSD) were lower than 5.0%. These findings reveal that this SERS aptasensor owns good recovery rate, demonstrating its application in analyzing SARS-CoV-2 viral particles in environmental samples.

4. Conclusion

In summary, this paper proposed a SERS aptasensor based on a novel locking amplifier for simple, sensitive, and rapid detection of trace SARS-CoV-2 viral particles in environment. The reaction process was carried out in a single, one-pot reaction system at 37 °C, avoiding product transfer, complicated separation, and temperature change operation. Magnetic beads used in this strategy can reduce the
Fig. 3. (A) SERS intensity of dynamic curve for trigger, the concentrations were ranged from 0.05, 0.5, 1, 2, 5, 8, 10–12 nM. (B) The two aptamers locking effect. SERS signal versus the ratio of aptamer to trigger. (C) (D) The single aptamer locking effect.

Fig. 4. (A) SERS spectra of control experiments: (a) without pseudotyped SARS-CoV-2 (PSV); (b) with pseudotyped PSV, but without KF polymerase; (c) with PSV, without Nt. BbvCI; (d) with PSV, but without dNTPs; (e) PSV, KF polymerase, Nt. BbvCI, and dNTPs were added simultaneously ($C_{SARS-Cov-2} = 8000$ TU/µL). (B) SERS signal versus the incubation time. The SERS signals were obtained by incubating with trigger or locked trigger in the presence or absence of PSV ($C_{SARS-Cov-2} = 2500$ TU/µL).

Fig. 5. (A) SERS spectra of different concentration of pseudotyped SARS-Cov-2 virus. The concentration is from 625 T to 10,000 TU/µL. (B) Calibration curve for Raman intensity and pseudotyped SARS-Cov-2 concentration.
background and simplify the experimental operation, as well as provide an idea for the development of biosensing robots via the magnetic field. By combining with cyclic amplification, we achieved higher sensitivity with low detection limit (LOD) of 260 TU/µL in the range of 625–10,000 TU/µL within 40 min. In addition, the selectivity of SERS in complex environment samples was greatly improved by the binding of aptamer with S protein on SARS-CoV-2. Furthermore, the aptasensor has good reproducibility and uniformity, expecting to be applied in real sample detection. Last of all, we validated our application performance of this SERS aptasensor in tap water, river water and fish samples in cold-chain system. Therefore, the SERS aptasensor is expected to be used for rapid infection screening and environmental monitoring, which can not only prevent the spread of large-scale infections through the environment, but also facilitate public health interventions and resume normal production and life.

Fig. 6. (A) SERS spectra of five different batches ($C_{SARS-CoV-2} = 5000$ TU/µL), (B) the corresponding bar graph. (C) The spectra of 11 points randomly selected on one SERS aptasensor ($C_{SARS-CoV-2} = 5000$ TU/µL), (D) the corresponding bar graph.

Fig. 7. Specificity of the SERS aptasensor. (A) SERS spectra of different conditions, (B) the corresponding bar graph. Note: PSV: pseudotyped SARS-CoV-2 virus. Rdm: random DNA sequence. Len: lentivirus particles (the concentration of PSV and Len was $10^4$ TU/µL).

CRediT authorship contribution statement

Cheng Tian: Conceptualization, Writing – original draft, Writing – review & editing. Lei Zhao: Methodology, Investigation, Validation. Guoliang Qi: Methodology, Investigation, Validation. Jin Zhu: Writing – review & editing, Supervision, Funding acquisition. Shusheng Zhang: Writing – review & editing, Supervision, Funding acquisition.

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.
Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.snb.2022.132445.

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Data availability

Data will be made available on request.

Appendix A. Supporting information

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