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Review Article

SERS Technique for Detection of COVID-19: A Review

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Abstract

The detection of new variants of COVID-19 still faces challenges due to various observations in human society as well as possible reservoirs in domestic and wild animals, and the prediction of possible future pandemics requires accurate and early detection of viruses. Although different techniques have been used to detect COVID-19, advanced diagnostic assay methods are needed for better and more efficient control of COVID-19. One of the analytical and sensitive techniques for detecting viruses is surface-enhanced Raman spectroscopy (SERS), which provides a fingerprint for any biomolecule. The widespread application of SERS technology in integration with immunoassay methods has provided great achievements in the diagnostic studies of viruses. Likewise, the ultra-sensitive diagnostic ability of the SERS method using substrates based on plasmonic nanostructures has been proven in various biological researches. In addition, by optimizing various conditions such as improving the ability and repeatability of SERS detection and increasing the efficiency of the platforms used for early detection of coronavirus-19, the problems of traditional approaches can be solved. Thus, SERS is a promising option in the early detection of COVID-19 in the recent pandemic. In this review, some diagnostic applications of the SERS technique for the COVID-19 identification are briefly discussed, which we hope will be useful for researchers.

Keywords: SERS technique, Nanostructures, COVID-19 pandemic, COVID-19 detection.

Introduction

The widespread potential of viral infections in genetic mutations can pose challenges for human health. The most recent pandemic caused by the coronavirus, which has led to the death of millions of people around the world since 2019, is known as the third serious coronavirus outbreak of recent years after SARS and MERS [1]. The Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) is the cause of the outbreak of this new pandemic. The known variants of coronaviruses consist of Alpha, Beta, Gamma, Delta, and Omicron, which have different transmissibilities and various infectivities [2]. The pandemic control and public health monitoring depend on

early detection of the variants of the coronaviruses. Available data show that infected people experience respiratory infections and even death [3]. There are many methods to transmit coronavirus infections, such as touching infected areas, and then touching the eyes and mouth, or transmission through inhalation of virus-infected aerosols [4]. In addition, it is believed that SARS-CoV-2 was initially transmitted to human society by eating an infected animal host [3]. The most common COVID-19 symptoms in patients include dyspnea, fever, painful cough, fatigue, and in some cases, anosmia and ageusia [5]. The detection of coronaviruses is an essential issue because the pandemic threat of zoonotic disease is the highest. Sensitive optical sensors based on plasmonic nanostructures are very effective in overcoming the challenges caused by the detection of viruses [6].

One of these technologies is the use of SERS for early detection of viruses. Nowadays, SERS has emerged as a high-sensitivity technique with excellent signal specificity for disease diagnosis and biological applications [7,8]. Using plasmonic substrates of SERS, the Raman scattering signals of analytes can be significantly increased for the improvement of structural analysis [9]. Hence, biosensors based on SERS are considered a promising option for COVID-19 detection and other biomedical analyses.

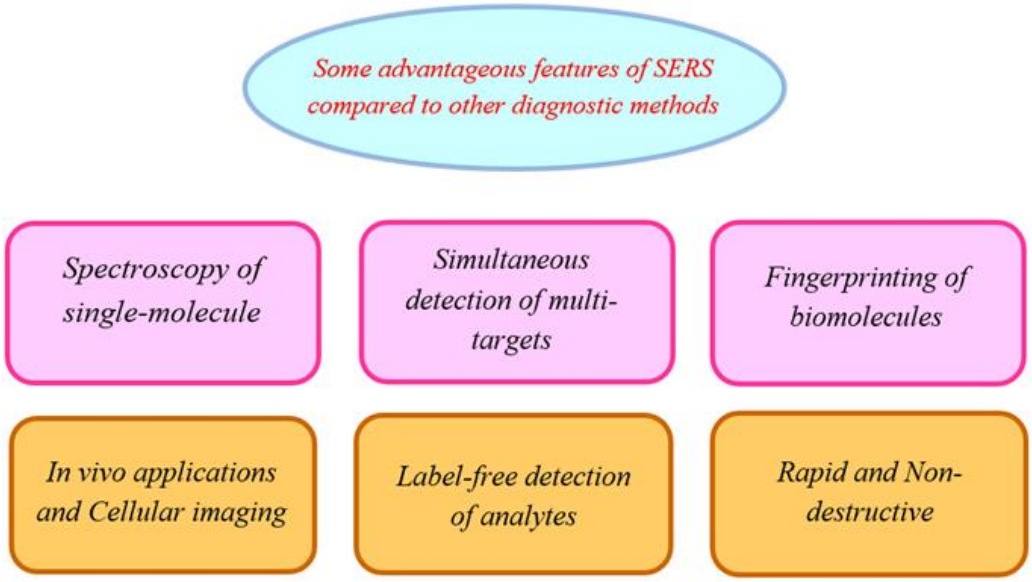

Figure 1 Different advantages of SERS [10]

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Most viruses are nanostructures with diameters of 20 to 400 nm. Based on the Baltimore classification, coronaviruses are classified in the (+) ssRNA group (+) RNA strands [11]. The Latin word corona means crown, which was chosen because of the crown-like surface projections on this virus. Coronaviruses with a genome length of around 30 kb are known as the largest RNA viruses. Their structure includes four main proteins: spike, membrane, envelope, and nucleocapsid proteins (Figure 2) [12]. These proteins are involved in the transmission of infection to host cells and replication. Also, the main focus in neutralizing SARS-CoV-2 is on the S protein because it plays an essential role in the entry of the virus into the host cell [13].

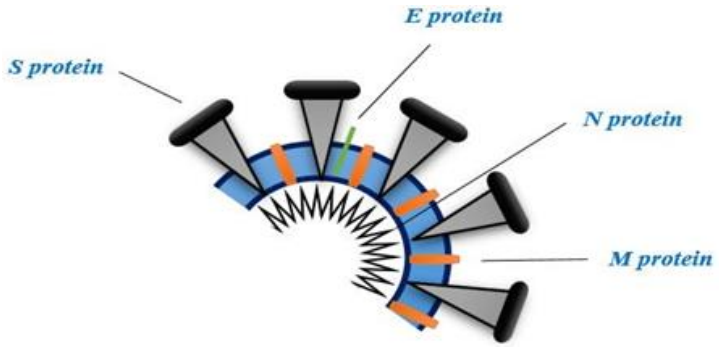

Figure 2 Schematic illustration of the common structure of COVID-19

SERS Mechanism

The basis of the SERS technology is based on plasmonic substrates that amplify the Raman response of an analyte [14]. A type of inelastic scattering of photons upon interaction of the molecules with light is known as the Raman Effect. According to the energy of the scattered photons compared to the incident photons that shift to lower or higher energies, Raman scattering is known as Stokes and anti-Stokes Raman scattering, respectively [15]. In the biosensors based on SERS, localized surface plasmon resonance (LSPR) of the metallic nanostructures could improve the scattering signal increasingly. Therefore, the potential of the plasmonic substrate of SERS has a significant contribution to reliable and sensitive detection of specimens [16]. Generally, the analytes detection by the SERS technique is possible using direct and indirect diagnostic methods. In the direct method, either the analyte is adsorbed onto the substrate or held close to the substrate by molecular linkers. Analytes with high Raman scattering cross-sections are usually suitable for this approach [17]. However, the most common and sensitive indirect diagnostic method is the use of reporter molecules to determine viruses. In this method, the Raman cross-section changes due to the interaction of the functionalized substrates with the target analyte.

The integration of this approach with the sandwich immunoassay method in the detection of viruses provides a promising perspective. The corresponding strategy is demonstrated in Figure 3. In this case, we can overcome the biological limitations of analytes such as low scattering cross-sections that appear in the direct detection method. For example, one of the methods of using the SERS technology in the detection of viruses is to track the Raman signal before and after the interaction of the SERS tag with the capture element [18].

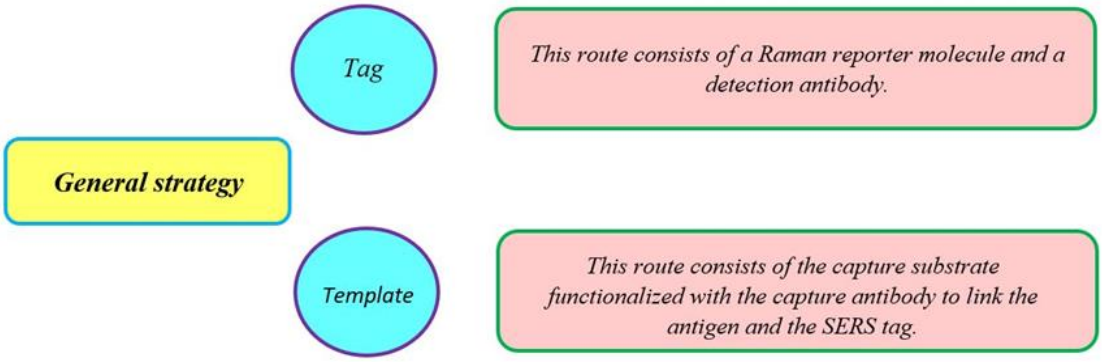

Figure 3 The strategy of detecting viruses using the SERS technique

The SERS technique, as we will mention in this review, has played an important role in recent studies based on COVID-19 detection because of its verified performance in the detection of viruses.

Current Findings

In recent years, some researchers have begun to rapidly detect and track COVID-19, using the integration of the SERS technology with various diagnostic methods, which are mentioned as follow.

In 2021, Serebrennikova *et al.* proposed an alternative rapid diagnosis method compared to traditional approaches using SERS-based immunoassay techniques. Two examples of immunoassay techniques are ELISA (enzyme-linked immunosorbent assay) and LFIA (lateral flow immunoassay), which are used to quantify an unknown concentration of an analyte in a sample. Their research group achieved a limit of detection of 0.1 ng/mL with an assay time of 20 min, by integrating the aforementioned methods with SERS. Their method successfully detected the SARS-CoV-2 spike RBD (receptor-binding domain) protein. In this study, gold nanospheres with RBD antibodies and 4-mercaptobenzoic acid were used as SERS nanotag [19]. In the same year, Payne *et al.* designed a peptide sensor using the SERS technique that this very selective sensor can detect the special vibrational signature of the S protein. They have used peptides as probes to capture viral proteins. This modified SERS substrate can improve LOD and selectivity, and it also exhibits better detection at lower concentrations compared to the unmodified substrate [20]. In another study in 2021, Zhang *et al.* reported the improved ACE2@SN-SERS assay that can be further developed by the optimization of an algorithm and database. This novel sensor successfully produced strong signals of SERS, which are caused by functionalized receptor proteins of ACE2 on Ag-nanorod substrates. The quenching of the SERS signal in the presence of S proteins was the recognition indicator of COVID-19 in the examined samples [21].

Furthermore, the improvement of SERS substrates using Au/Ag nanostructures and 4-NTP as a probe molecule for ultrasensitive detection of RBD protein was successfully investigated by Awada *et al.* This fingerprinting method was based on protein-antibody detection and well identified the RBD protein signal from other signals. In this study, the detection limit was 1 pM, and the interaction between the RBD protein and its antibody resulted in a red shift in the Raman spectrum [22]. In 2021, Sanchez *et al.* successfully demonstrated that a plasmonic SERS substrate of Au nanostars and MoS films can be reported the characteristic signals of S and N proteins. Their approach can be used for both virus detection and analysis of protein structure using the plasmonic properties of nanoparticles [23]. Another mechanism for the detection of COVID-19 was presented by Chen et al. They reported a sensor based on S protein aptamer DNAs that improved the sensitivity of the conventional LFA method. In this study, gold nano popcorn was synthesized as a SERS platform for the detection of COVID-19. Their proposed aptasensor platform enables a detection limit of less than 10 PFU/ml within 15 min [24].

Also, Liu *et al.* fabricated an advanced SERS-LFIA sensor to detect anti-SARS-CoV-2 IgM/IgG. Their proposed biosensor was able to simultaneously detect anti-SARS-CoV-2 IgM/IgG using modified and reporter SERS tags. Here, the tags were composed of bilayer DTNB and SiO_2@Ag NPs and conjugated with S protein to be used for screening of

COVID-19. The detection limit of this method was 800 times more than LFIA based on Au NPs [25]. In 2022, Li *et al.* investigated the role of different morphologies of Ag nanostructures on the performance of the SERS technique in the successful detection of COVID-19. Among the different shapes studied, dendritic nanostructures showed better signal amplification than other shapes such as bulk, globular, and spiky, and the detection limit was 7.42×10^{-14} M [26].

Zhang *et al.* successfully reported an efficient SERS sensor based on Ag nanorod chips for the early detection of COVID-19 RNA, which showed suitable selectivity. The high diagnostic ability of their proposed sensor resulted in a LOD as low as 51.38 copies/mL [27]. Moreover, in another successful study, Samodelova *et al.* presented a promising approach for ultra-fast methods of diagnosing COVID-19 based on a SERS sensing platform with protein-coated Ag NPs. Their research team showed that such plasmonic nanostructures have a suitable sensitivity to detect the studied analytes due to localized surface plasmon effects [28]. In another study in 2022, Cha et al. facilitated the detection of COVID-19 using a SERS-based immunoassay involving a pair of antibodies, hollow Au nanostructures, and magnetic beads. Their assay demonstrated a detection limit of 2.56 fg/mL for COVID-19 antigen [29]. Also, in 2023, Yeh *et al.* investigated a sensitive SERS-based diagnostic approach for early and accurate detection of COVID-19 variants. Their proposed biosensor was based on Ag NPs synthesized by a solution-based microplasma method. The results of their ultra-sensitive platform showed that the detection limits of S and N proteins of COVID-19 are 1 fg mL^{-1} and 0.1 pg mL^{-1}, respectively [30].

In another successful study in 2023, a SERS-based microassay for the detection of S and N proteins of COVID-19 was designed by Vedelago *et al.* which was able to provide ultra-sensitive detection using hollow Au-Ag nanoboxes. The detection capability of the proposed microassay started from as low as 20 virus/μL and 50 pg/ml RBD protein [31]. In the same year, Park *et al.* successfully fabricated a SERS-based aptasensor platform for the early detection of SARS-CoV-2. Their developed label-free aptasensor was a combination of aptamers and Ag nanoforests, which showed the detection limit of attomolar level (10^{-18} M) and detected COVID-19 variants in clinical samples with excellent sensitivity [32].

Zhao *et al.* successfully used a novel approach to quantify neutralizing antibodies using a tri-mode LFIA based on hollow Au-Ag alloy nanoshells. The enhanced SERS signal was obtained through the immobilization of the Raman reporter molecule 4-mercaptobenzoic acid on the used alloy nanoshell. Therefore, the colorimetric, photothermal, and SERS signals of their platform led to the construction of tri-mode strips for the detection of COVID-19-neutralizing antibodies. Their proposed method showed a LOD of 20 ng/mL [33].

In another study in 2023, Sitjar *et al.* presented a method with the possibility of rapid screening without the need for sample preprocessing using label-free SERS based on substrate design for the detection of COVID-19. Using SERS substrates such as gold nanocavities and gold nanoparticles on porous ZrO_2, their research team was able to achieve detection limits of 0.1-1.0% [34]. Similarly, in one of the latest research projects, Ebbah *et al.* developed a plasmonic filtration system based on the SERS technique using human IgG as a model biomarker. They used Au nanoparticles to form an active substrate, and the optimized platform of their research group was applied to the

quantitative analysis of the COVID-19 N protein. This diagnostic process showed a LOD of ~0.2 ng mL^{-1} for human IgG in an assay time of less than 5 min [35].

Table 1 A summary of studies conducted on the role of SERS technique in SARS-CoV-2 detection

Ref.	*Detection method*	*NPs used*	*Detection limit (LOD) and assay time*
[19]	Development of SERS-based LFIA sensor	Au NPs	0.1 ng/mL 20 min
[20]	An angiotensin-converting enzyme 2 (ACE2) mimetic peptide-based SERS sensor	Au NPs	300 nM
[21]	An assay using SERS coupled with multivariate analysis	Ag nanorods	–
[22]	SERS detection based on RBD protein recognition	Au/Ag nanostructures covered by silicon nanorods	1 pM 3 s
[23]	SERS technique based on ultrasensitive plasmonic substrate	Au nanostars NPs and MoS thin layers	–
[24]	SERS-based aptasensor	Au nanopopcorns	Less than 10 PFU/mL 15 min
[25]	Development of SERS-LFIA biosensor	SiO_2@Ag NPs	⍰ 800 times higher than Au NPs-based LFIA
[26]	The shape influence of Ag nanostructures on SERS performance and their applications in the detection of SARS-CoV-2	Different morphologies of Ag nanostructures such as dendrites	7.42×10^{-14} M
[27]	Non-enzymatic signal amplification-powered point-of-care SERS sensor	Ag nanorods	51.38 copies/mL
[28]	Development of SERS-based aptasensor in sandwich mode	Protein-coated Ag NPs	–
[29]	Development of SERS-based immunoassay	Hollow Au NPs	2.56 fg/mL for SARS-CoV-2 antigen and 3.4 PFU/mL for SARS-CoV-2 lysates
[30]	Development of an ultra-sensitive SERS biosensor using a solution-based microplasma process	Silver microplasma-engineered nanoassemblies (AgMEN)	1 fg mL^{-1} for S protein and 0.1 pg mL^{-1} for N protein

[31]	Development of SERS-based microassay	Hollow Au-Ag nanoboxes	20 virus/μL and 50 pg/ml RBD protein
[32]	Label-free SERS-based aptasensor	Ag nanoforests	Attomolar level (10^{-18} M)
[33]	A tri-mode LFIA platform based on Au–Ag alloy hollow nanoshells	Au–Ag HNSs	20 ng/mL
[34]	Label-free SERS based on substrate design	Au nanocavities and Au NPs/$pZrO_2$	0.1–1.0% (or 10^{4-5} copies/mL)
[35]	Development of SERS-based filtration system	Au NPs	0.2 ng mL^{-1} Less than 5 min

Challenges

So far, we have found that SERS biosensors have many advantages over other diagnostic methods, but still, some of their features, such as the reproducibility of the substrates used, need to be improved; since the Raman scattering spectrum is inherently weak and may make it impossible to detect low concentration analytes, the use of plasmonic substrates such as gold and silver nanostructures with high flexibility are recommended as Raman signal amplifiers. Therefore, the construction of cost-effective SERS active substrates is necessary to improve the signal of the SERS technique. Implementation of this approach should ensure accuracy, efficiency, early detection, and flexibility in a wide range of different conditions and provide an acceptable assessment. Among other problems that must be answered is the decrease in signal quality due to the non-uniform absorption of molecules onto the surface of nanoparticles. As a result, the use of the SERS technique, in addition to the advantages mentioned in the previous sections, for practical applications still requires more studies [36].

Conclusion

This study summarizes the current findings of the application of the SERS technique for the detection of COVID-19. In fact, in this review, we provided researchers with an overview of the role of the SERS technology in the early detection of COVID-19. The high accuracy of the SERS technology in the detection of viruses has made it a useful tool in the detection of COVID-19. It was mentioned that the detection of viruses using the SERS technique is done with two direct and indirect diagnostic approaches, and the use of plasmonic substrates increases the sensitivity of this technique, and then by reviewing the research done during the recent pandemic, we better showed the bold role of the SERS technique in the diagnostic processes of COVID-19, and we tried to briefly provide more details to the researchers. Some advantages such as sensitive spectroscopy of single-molecule, fingerprinting of biomolecules, and simultaneous rapid detection of multi-targets have created promising prospects for using the SERS technique in biological and medical sciences diagnostic applications.

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