

Annual Review of Analytical Chemistry
Recent Trends in
Surface-Enhanced Raman
Scattering–Based In Vitro
Diagnostics for Translational
Biomedical Research

Younju Joung,¹ Sohyun Park,¹ Sang-Woo Joo,²
and Jaebum Choo¹

¹Department of Chemistry, Chung-Ang University, Seoul, South Korea; email: jbchoo@cau.ac.kr

²Department of Chemistry, Soongsil University, Seoul, South Korea; email: sjoo@ssu.ac.kr

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Keywords

surface-enhanced Raman scattering, liquid biopsy, cancer, COVID-19, in vitro diagnostics

Abstract

Surface-enhanced Raman scattering (SERS) spectroscopy has gained prominence in in vitro diagnostics due to its high sensitivity and multiplex detection capabilities. This review highlights recent advances in translational biomedical research using SERS techniques, with a focus on the rapid and accurate diagnosis of intractable diseases such as cancer and infectious diseases such as COVID-19. The review examines SERS-based assays for liquid biopsy biomarkers such as exosomes, circulating tumor cells, and circulating tumor DNAs that have emerged as promising tools in cancer diagnostics and are currently under active investigation in clinical research. Additionally, it explores SERS-based diagnostic techniques developed to overcome the limitations of existing COVID-19 diagnostic methods, including real-time polymerase chain reaction and lateral flow assay immunodiagnostics. Finally, the review addresses the challenges of translating SERS techniques from laboratory research to clinical practice.

1. INTRODUCTION

The development of technologies for the rapid and highly sensitive detection of specific disease biomarkers in human serum has long been a critical focus of in vitro diagnostics, driving extensive research efforts. Clinical trials commonly use immunoassay techniques such as electrochemiluminescence (ECL) assay (1, 2) and enzyme-linked immunosorbent assay (ELISA) (3) for the quantitative identification of specific protein biomarkers. However, many newly discovered disease biomarkers are present at very low concentrations (below ng/mL), making them undetectable using conventional ECL or ELISA methods. For instance, in ELISA, biomarkers are detected by an enzyme-mediated color change, but at low concentrations, this color change is insufficient for detection by a microplate reader. Similarly, ECL is widely used in clinical laboratories for luminescence detection but also struggles with biomarkers that have extremely low cut-off values. Recently, surface-enhanced Raman scattering (SERS) technology, which utilizes the electromagnetic enhancement properties of nanostructures, has shown promising potential in overcoming these limitations (4). This review explores recent translational biomedical research utilizing SERS-based in vitro diagnostic methods.

Cancer, a serious disease with high mortality rates, has garnered significant research attention. Currently, tissue biopsy is the standard diagnostic method, involving in vivo imaging techniques such as magnetic resonance imaging (MRI) or computed tomography to scan specific organs, followed by dissecting suspicious areas for histopathological analysis (5–7). However, tissue biopsy poses challenges for early diagnosis, relies heavily on the subjective judgment of pathologists, and is costly owing to the expense of imaging equipment, making it impractical for routine screenings. As a result, there has been growing interest in developing in vitro diagnostic methods to overcome these limitations (8–10).

Precise cancer biomarkers are crucial for the accurate in vitro cancer diagnosis. Biomarkers such as prostate-specific antigen, alpha-fetoprotein, cancer antigen 125, and carbohydrate antigen 19-9 are used for diagnosing prostate, lung, liver, and pancreatic cancers, respectively (11–16). However, their limited diagnostic accuracy has made them insufficient for conclusive cancer diagnosis. Consequently, there is an urgent need for highly accurate cancer biomarkers to enhance the reliability of in vitro diagnostics. Recent research suggests that liquid biopsy biomarkers are closely related to the onset and metastasis of cancer (17–19). Liquid biopsy, a potential noninvasive strategy, holds promise for continuous monitoring of early-stage cancer and may eventually replace tissue biopsy. Biomarkers such as extracellular vesicles (EVs) secreted from tumor cells, circulating tumor cells (CTCs), and circulating tumor DNAs (ctDNAs) in human biofluids offer valuable insights into cancer development and metastasis. Accordingly, several studies are focused on isolating and detecting EVs, CTCs, or ctDNAs in biofluids at extremely low concentrations for early cancer detection (20–22).

Point-of-care testing (POCT) refers to diagnostic tests conducted at the site of patient care that provide rapid results. As the demand for timely and accurate diagnostics grows, POCT technology has evolved to improve sensitivity, specificity, and reproducibility, which are especially important for early disease detection. Consequently, the need for POCT in the medical field for the timely and accurate diagnosis of diseases is rising. Additionally, the applications of POCT are expanding to include areas such as the detection of foodborne pathogens, which can spread during the storage and distribution of agricultural and livestock products, as well as to the food industry (23, 24). Common POCT sensors include lateral flow assay (LFA) strips, such as those used in pregnancy tests, to detect disease markers through color changes; electrochemical sensors, such as blood glucose monitors based on redox reactions; and optical sensors integrated with portable readers.

POCT has been essential in diagnosing infectious diseases such as COVID-19, which has caused significant global disruption. While RNA vaccines have helped prevent the spread of certain infections, the most effective strategy remains the rapid identification of infections and the isolation of infected individuals (25–27). LFA platforms have been successfully used for COVID-19 testing owing to their affordability and specificity. However, the gold standard for COVID-19 diagnosis remains real-time polymerase chain reaction (RT-PCR), which detects viral RNA in samples by amplifying it through reverse transcription (28–33). Though highly accurate, RT-PCR requires preprocessing and about three hours to complete, limiting its use for POCT.

In contrast, LFA strips, which detect the virus's nucleocapsid protein, provide results within 10 min (34–37). However, their approximately 20% false-negative rate, due to a poor limit of detection (LOD), makes them less reliable for identifying asymptomatic infections. Whereas RT-PCR uses fluorescence to detect viral RNA, LFA strips rely on color change caused by gold nanoparticles (AuNPs) (38–40). Given the limitations of these conventional diagnostic methods, there is a clear need for more innovative and sensitive technologies for rapid and accurate infection detection. The SERS-based POCT offers a potential solution, but more research is required to bring it into clinical settings.

In vitro diagnostics often rely on optical methods such as colorimetry, fluorescence, and ECL for analyzing target proteins or genes in blood or urine samples (41–43). For example, immunoassays are often performed using large, automated equipment in clinical laboratories, where the ECL method is commonly employed. This method does not use an optical source but excites target molecules through an electric field and then measures the emission signal. Meanwhile, RT-PCR was adopted during the COVID-19 pandemic to detect target genes through fluorescence during the thermocycling process. However, both ECL and fluorescence detection methods have limitations in terms of detection sensitivity and multiplex detection capability. This has led to an urgent need for new optical detection technologies, and SERS is emerging as a promising alternative (44–46). SERS has demonstrated potential in addressing the low sensitivity issues associated with conventional detection methods, such as fluorescence and ECL. Additionally, SERS enables the simultaneous detection of multiple analytes because its signals are much narrower compared to fluorescence or luminescence bands.

As a two-photon inelastic scattering technique, SERS has gained significant attention as a powerful bioanalytical tool, particularly when combined with plasmonic nanopag structures. This combination allows SERS to address the limitations of conventional detection methods such as ECL and fluorescence, which often suffer from sensitivity issues due to quenching effects (47–50). When incident light interacts with free electrons oscillating on the surface of nanostructures composed of Au (gold) or Ag (silver), the resulting electromagnetic field is significantly amplified. This phenomenon is known as localized surface plasmon resonance (LSPR). The LSPR effect can greatly enhance the intensity of the Raman scattering signal emitted by target molecules when they are in close proximity to these nanostructures, particularly within tiny nanogaps (<10 nm). This enhanced signal enables SERS to overcome the inherent sensitivity limitations of traditional optical detection methods, such as ECL and fluorescence, which are widely used in medical diagnostics.

However, translating SERS technology from laboratory research to clinical applications presents several challenges that must be addressed. One of the key advantages of SERS is its ability to amplify the Raman signal at specific hot spots while simultaneously reducing background noise, leading to an improved signal-to-noise ratio. Despite this high sensitivity, the magnitude of the Raman signal enhancement largely depends on the precise control of the nanogaps that induce electromagnetic enhancement. As a result, the development of reliable SERS substrates with consistent and controlled nanogap formation has emerged as a critical challenge (51, 52). This is

especially important when applying SERS to POCT for in vitro diagnostics, where reproducibility of measurements is essential. It is critical to ensure that this reproducibility becomes even more complex when integrating SERS detection into microdevices such as LFA strips or microfluidic channels.

Historically, SERS has been confined to laboratory settings due to issues related to reproducibility and the large size of Raman spectroscopic systems, which have limited its widespread adoption as a routine diagnostic tool. However, recent advances in optical device miniaturization and nanotechnology have begun to revolutionize portable SERS systems. Newly developed nanotechnologies now allow for the fabrication of plasmonic substrates with highly uniform nanostructures, significantly improving the reproducibility of Raman signals. These technological advancements, combined with the availability of portable Raman spectrometers, have greatly enhanced the potential of SERS in clinical diagnostics. By using Au or Ag nanoparticles as labeling agents capable of generating hot spots instead of traditional ECL or fluorescence labeling agents, it is possible to achieve high-sensitivity detection of biomarkers—SERS enables the highly sensitive detection of biomarkers, even down to the single-molecule level, with remarkable enhancement factors (53–55). Consequently, several studies have explored the applicability of SERS technology for in vitro diagnosis of cancer or COVID-19 biomarkers (56–58).

As previously noted, there is a growing need for new optical measurement methods that offer substantially higher sensitivity compared to existing biomarker detection techniques, largely due to the discovery of disease biomarkers in human biofluids at very low concentrations. Moreover, the global rise of infectious diseases like COVID-19 has amplified the need for POCT technologies that can rapidly and accurately detect infections. This review focuses on the latest advancements in SERS-based in vitro diagnostic methods, which are emerging as a promising high-sensitivity approach in this field. We also examine recent developments in SERS-based POCT systems that integrate portable Raman spectrophotometers with microdevices such as LFA strips and microfluidic chips. Specifically, we explore the application of SERS-based POCT for new cancer biomarkers and diagnostic markers for infectious diseases such as COVID-19. Finally, we discuss the critical factors required to successfully transition SERS-based detection from laboratory research to practical clinical use.

2. APPLICATION OF SERS-BASED DIAGNOSTICS TO LIQUID BIOPSY CANCER BIOMARKERS

Primary tumor cells travel through the bloodstream if they fail to attach to the blood vessel wall (59). During this process, CTCs activate the epithelial-mesenchymal transition, enhancing their ability to permeate blood vessel walls (60, 61). Once through the blood vessels, CTCs increase their ability to spread within the bloodstream and subsequently exit the bloodstream through extravasation and attach to the endothelium of secondary organs (59). In this process, CTCs, ctDNAs, exosomes, and ctRNAs secreted from tumor cells circulate in the blood (**Supplemental Figure 1**). Therefore, a comprehensive analysis of these liquid biopsy markers isolated from the peripheral blood of patients with cancer may provide useful information on cancer development and metastasis (17–19).

Effective isolation techniques are crucial when using CTCs as biomarkers due to their extremely low concentration of 1–10 CTCs per billion blood cells (62). Fragmented DNAs released from tumor cells into the blood (ctDNAs) can be used for real-time tumor monitoring because of their short half-life of 16 min to 2.5 h (63). Exosomes, a type of EV secreted from cancer cells, range in size from 30 nm to 150 nm. Exosomes directly participate in the development and metastasis of primary tumors, determine the tumors' phenotype, and facilitate communication between parent cells and other cells by modulating intracellular signaling pathways (64, 65).

Supplemental Material >

Over time, extensive research has been performed to develop methods for detecting liquid biopsy biomarkers for cancer diagnosis, such as CTCs, ctDNAs, and EVs. Additionally, some of these experimental procedures are awaiting approval by the US Food and Drug Administration (FDA) or Conformité Européenne. Importantly, methods involving ultracentrifugation, filtration, and analysis using fluorescence in situ hybridization, immunosensors, and proteomics have been used to analyze CTCs (66, 67). For ctDNAs, detection methods have been developed that involve amplification using droplet PCR or next-generation sequencing and electrochemical or fluorescence biosensors (68, 69). Standard assay techniques using fluorescence detection methods have been utilized for EVs (70, 71). EVs are micrometer-sized, lipid bilayer-enclosed vesicles that participate in various pathological and physiological processes. The expression levels of proteins on the membrane surface of EVs are closely associated with tumor development and metastasis (72, 73). Therefore, diagnosing cancer by analyzing the expression patterns of various surface proteins on EVs is possible (74). Among EVs, exosomes have not received much attention due to their poor understanding of their correlation with diseases. However, recent advances in exosome isolation technologies have promoted research on the potential utilization of exosomes in cancer diagnosis (75–78).

However, developing ultrasensitive detection methods that surpass the detection limits of existing electrochemical, fluorescence, or chemiluminescence detection methods is crucial due to the extremely low concentrations of these liquid biopsy biomarkers in the blood. The SERS technique can detect targets at the single-molecule level with ultrahigh sensitivity, prompting research into its application to detect liquid biopsy biomarkers. The following section discusses recent trends in applying SERS-based technology in detecting liquid biopsy biomarkers such as CTCs, ctDNAs, and EVs.

2.1. SERS Sensors for Extracellular Vesicles

Recently, SERS-based multiplex detection technologies have been developed to simultaneously detect different proteins on the surface of EVs (78). These SERS-based assays of EVs can be categorized into labeled techniques using SERS nanotags and nonlabeled technologies that directly load clinical samples into high-sensitivity nanoplasmonic substrates. The nonlabeled technique involves directly loading clinical samples onto a nanoplasmonic substrate, then quantifying and analyzing the generated SERS signal to diagnose cancer using artificial intelligence (AI) technology.

2.1.1. Labeled SERS detection of extracellular vesicles. We introduce recent studies that detected multiple proteins expressed on the surface of EVs using SERS nanotags. In fluorescence or ECL detection methods, labels such as fluorescent dyes or luminol, which can induce strong emission, are attached to the target, and the target's quantity is determined by analyzing the intensity of the resulting emission band (1, 2, 43). Similarly, in label-based SERS detection, SERS nanotags are used, which consist of Raman reporter molecules that produce strong electromagnetic enhancement when adsorbed onto metal nanoparticle surfaces (45, 47). These nanotags bind to the target, and the target's quantity is quantified by monitoring the characteristic Raman peak intensity of the reporter molecules. As explained in Section 1, SERS-based assays utilize the inelastic scattering signal amplified by the LSPR effect instead of emission signals, enabling high-sensitivity quantitative analysis. However, the amplification of the signal relies on hot spots, which are affected by the uniformity of the nanostructures. Therefore, controlling these hot spots is essential to ensure reproducibility and achieve accurate quantitative analysis.

Zhang et al. (79) developed a microfluidic chip consisting of antibody-conjugated magnetic nanomixers, which enhanced the capture of EVs and enabled sensitive and simultaneous SERS

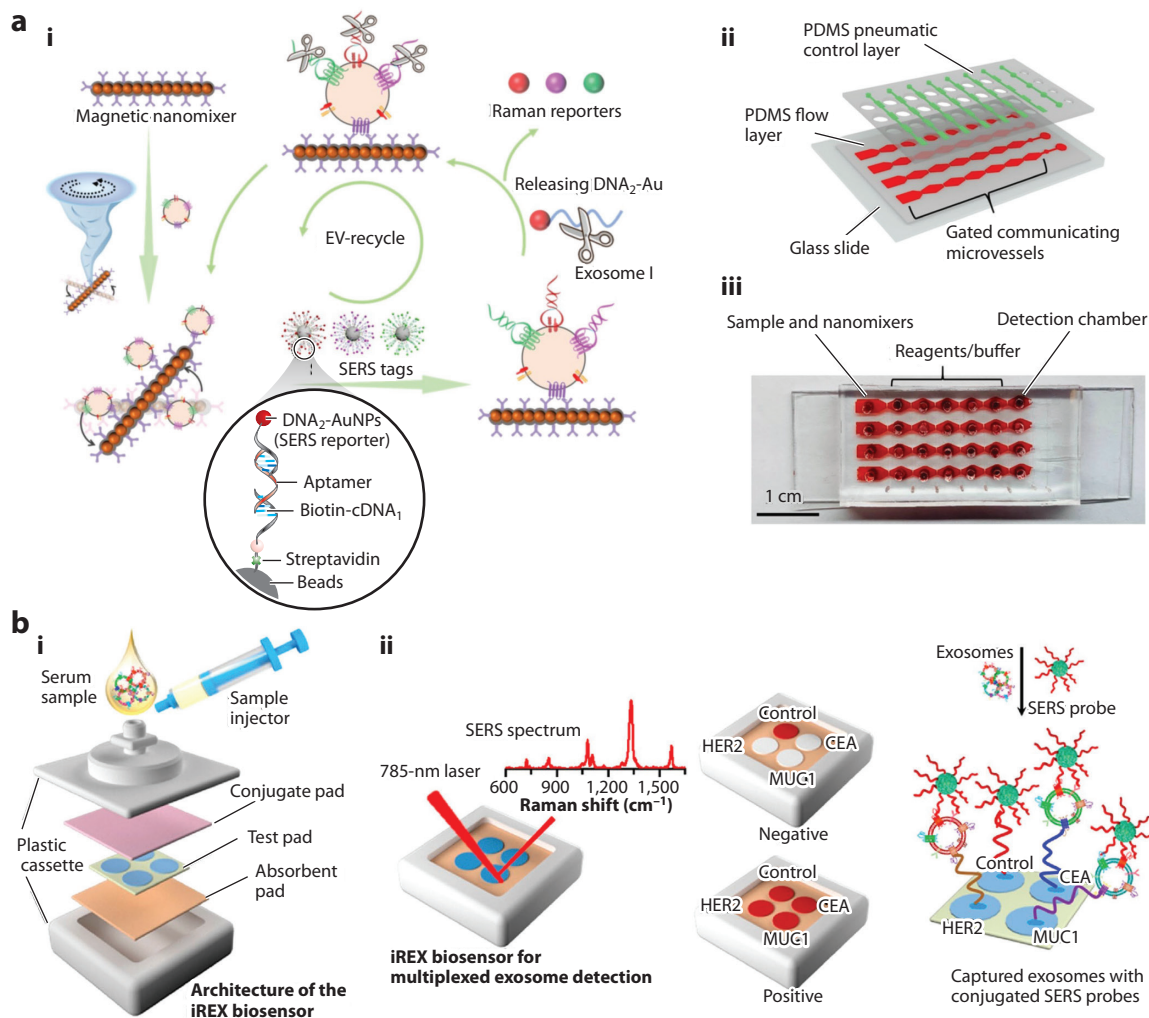


Figure 1

(a, i) A schematic representation of the exonuclease I-assisted target recycling amplification strategy for sensitive and simultaneous profiling of surface proteins on EVs. (ii) The three-layer PDMS/glass chip incorporates four parallel units of interconnected vessels, each consisting of seven vessels linked by flow channels (red) in the bottom PDMS layer. The vessels are controlled by an array of normally closed microvalves actuated by pneumatic channels (green) in the top layer. (iii) A photograph of the interconnected vessel tandem chip. Panel a adapted with permission from Reference 79; copyright 2023 Wiley-VCH GmbH. (b, i) A detailed view of the iREX biosensor architecture, which includes a sample injector, a VFA device, and a plastic cassette. The VFA device is composed of a conjugate pad, a test pad with four spatially separated test spots, and an absorbent pad, arranged from top to bottom. (ii) A schematic of the multiplexed detection of exosomal proteins using the iREX biosensor, showing the results in the absence of exosomes (negative test) and the presence of multiple exosomes (positive test). Panel b adapted with permission from Reference 80; copyright 2023 American Chemical Society. Abbreviations: AuNP, gold nanoparticle; EV, extracellular vesicle; iREX, integrated regulation of expression; PDMS, polydimethylsiloxane; SERS, surface-enhanced Raman scattering; VFA, vertical flow assay.

detection of multiple protein markers on the EV surface (**Figure 1a**). The SERS-based nanomixer isolated EVs from 10 μ L of plasma sample, simultaneously analyzed six protein biomarkers expressed on the surface within 1.5 h, and effectively distinguished between 20 breast cancer patients and 10 healthy donors. Here, the SERS signal provides a notable advantage for simultaneously

analyzing multiple markers due to its much narrower peak width compared to fluorescence emission bands. Additionally, because capture and isolation are crucial in biodiagnosis, integrating the magnetic mixers with SERS enhances the technical implementation of these processes.

Furthermore, Su et al. (80) developed a SERS-based vertical flow biosensor capable of simultaneously analyzing multiple exosomal proteins in the serum samples of patients. The SERS-based vertical flow biosensor successfully detected three protein biomarkers (MUC1, HER2, and CEA) on exosomes secreted from different breast cancer cell subtypes, enabling the differentiation of breast cancer phenotypes. Although SERS-based LFA strips for detecting multiple protein biomarkers have been developed by various researchers, there has been limited progress in creating SERS-based vertical flow biosensors that offer the same performance as LFA strips but with significantly faster detection of multiple biomarkers. **Figure 1b, subpanel i** shows the structure, and **Figure 1b, subpanel ii** shows the operating principle of the developed vertical flow biosensor. Aptamers capable of selectively capturing three different exosomes were immobilized on the test spots. After forming sandwich complexes with aptamer-conjugated SERS nanotags, the SERS signal of each spot was measured to quantitatively analyze the exosomes. Overall, the proposed SERS vertical flow sensor can accurately determine cancer phenotype through multiplexed quantitative profiling of exosomal proteins isolated from the blood serum of patients with breast cancer. The authors provided a valuable approach to testing the cancer phenotype by combining the SERS technique with vertical flow biosensors.

2.1.2. Nonlabeled SERS detection of extracellular vesicles. Recently, active research on nonlabeled SERS detection techniques for EVs has emerged. In clinical diagnostics using SERS technology, nonlabeled techniques typically offer lower accuracy compared to labeled techniques. Labeled techniques involve using SERS nanotags to specifically label biomarkers, which enhance accuracy (47). In contrast, nonlabeled techniques measure spectral data from all substances in clinical samples and then extract and analyze the relevant information, which can lead to decreased accuracy (78, 82). Despite this, nonlabeled techniques are advantageous for high-throughput screening in *in vitro* diagnostics because they eliminate steps such as sample preprocessing, labeling, washing, and separation, allowing for rapid processing of multiple patient samples. Machine learning technology is expected to address the accuracy limitations of nonlabeled techniques by analyzing complex Raman spectral data and extracting only the necessary information, potentially leading to significant improvements in accuracy.

Jalali et al. (81) developed a SERS-based microfluidic chip capable of monitoring the molecular profile of a single EV to observe cell transformation events during cancer progression and therapy. Specifically, this chip features an MoS₂ layer inside the microfluidic channel, embedded with ZnO nanocavity wells and an Ag plasmonic layer, inducing strong in-cavity field enhancement (**Figure 2a**). This device effectively traps single EVs in each cavity without needing biological recognition elements such as antibodies or aptamers, utilizing the interaction between the EV lipid membrane and the MoS₂ layer. Moreover, a convolutional neural network (CNN)-based machine learning technique was employed to interpret the SERS signals from the measured single EV to decode the complicated signals by introducing deep learning algorithms. Overall, analysis of blood serum (10–100 μ L) obtained from 12 patients with glioblastoma and 8 healthy individuals showed that the SERS-based microfluidic chip combined with the CNN method achieved an 87% improvement in diagnostic accuracy compared to conventional pathology test.

Furthermore, Shin et al. (82) developed a diagnostic method capable of distinguishing six types of cancer using SERS detection and deep learning-based liquid biopsy technology to reach better decision-making in diagnosis. As shown in **Figure 2b, subpanel i**, exosomes were extracted from the plasma samples of cancer patients and loaded onto a substrate with an AuNP-aggregated

array, and SERS signals were measured from 100 spots per sample. The study was conducted in two stages. In the first stage, deep learning was used to determine the presence of cancer in 520 test samples (**Figure 2b, subpanel ii**). In the second stage, the samples of 278 patients with early-stage cancer were analyzed to identify six cancer subtypes (lung, breast, colon, liver, pancreas, and stomach). Overall, the final analysis showed a sensitivity of 90.2% and a specificity of 94.4%. These findings conclusively demonstrate the potential of the noninvasive method for the early detection of cancers.

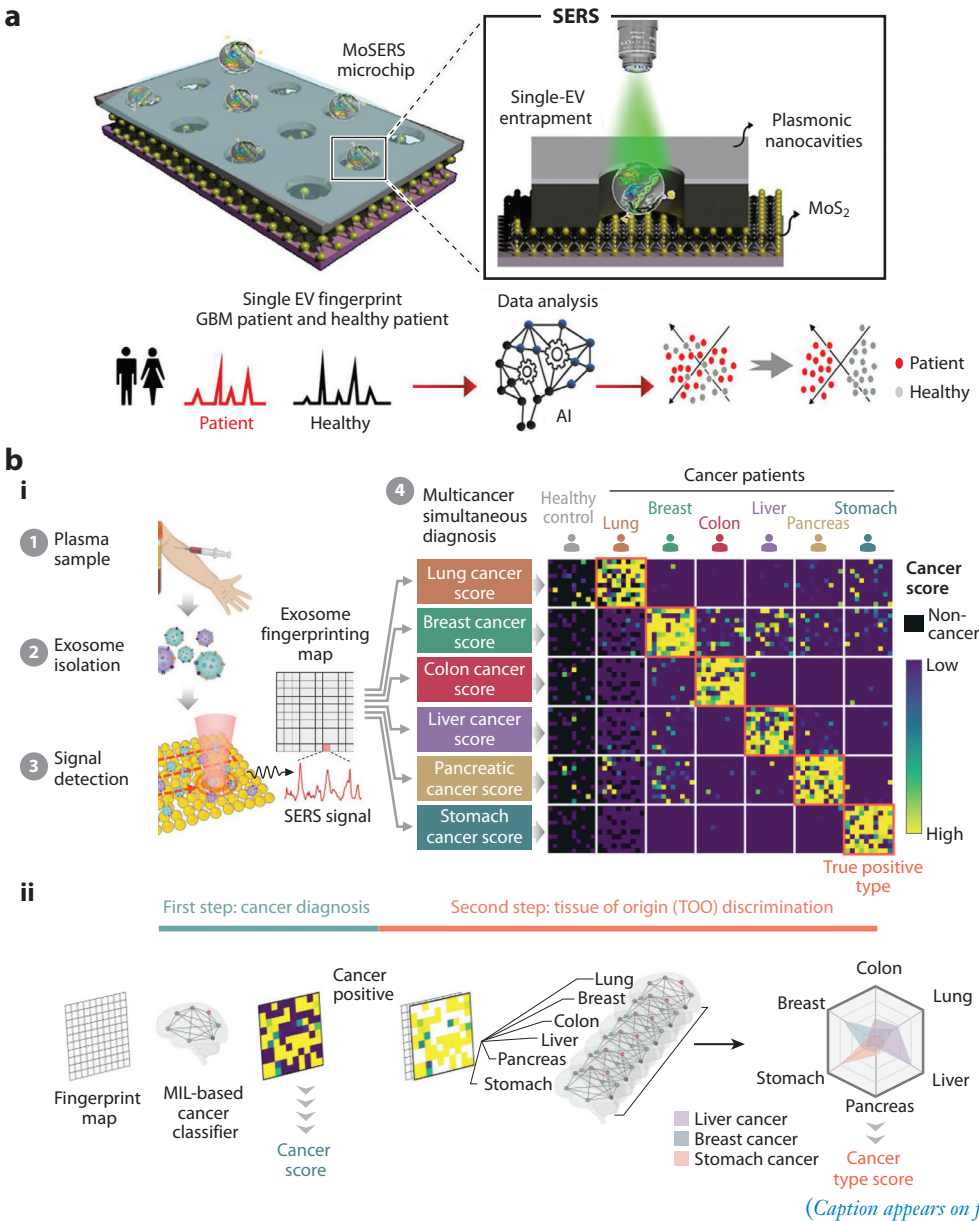


Figure 2 (Figure appears on preceding page)

(a) The concept of utilizing a single EV SERS approach for liquid biopsy in GBM patients. The nanosurface microfluidic embedded with a MoSERS microchip performs SERS identification of single EVs in GBM. Single EVs are trapped in the MoS₂-encapsulated nanocavities of the MoSERS microchip. The SERS spectrum of single EVs is analyzed using a machine learning algorithm (CNN) to categorize molecular alterations. Panel *a* adapted with permission from Reference 81 (CC BY-NC-ND 4.0). (b) A single test for multicancer detection using exosome-SERS-AI. (i) Overview. An exosome suspension is dropped onto an Au nanoparticle–aggregated array chip and thoroughly dried. Signals are observed at 100 spots (10 × 10) per sample and analyzed using AI algorithms. The system predicts the presence of cancer and the tissue of origin. A heatmap displays representative predicted results for each cancer status. (ii) AI framework. Initially, diagnostic scores are assigned as the mean values of the MIL-based cancer classifier results. In the second step, signals predicted by the previous cancer classifier are analyzed, and an average score is calculated using six different prediction models. Panel *b* adapted from Reference 82 (CC BY 4.0). Abbreviations: AI, artificial intelligence; CNN, convolutional neural network; EV, extracellular vesicle; GBM, glioblastoma; iREX, integrated regulation of expression; MIL, multiple instance learning; SERS, surface-enhanced Raman scattering.

2.2. SERS Sensors for Circulating Tumor Cells

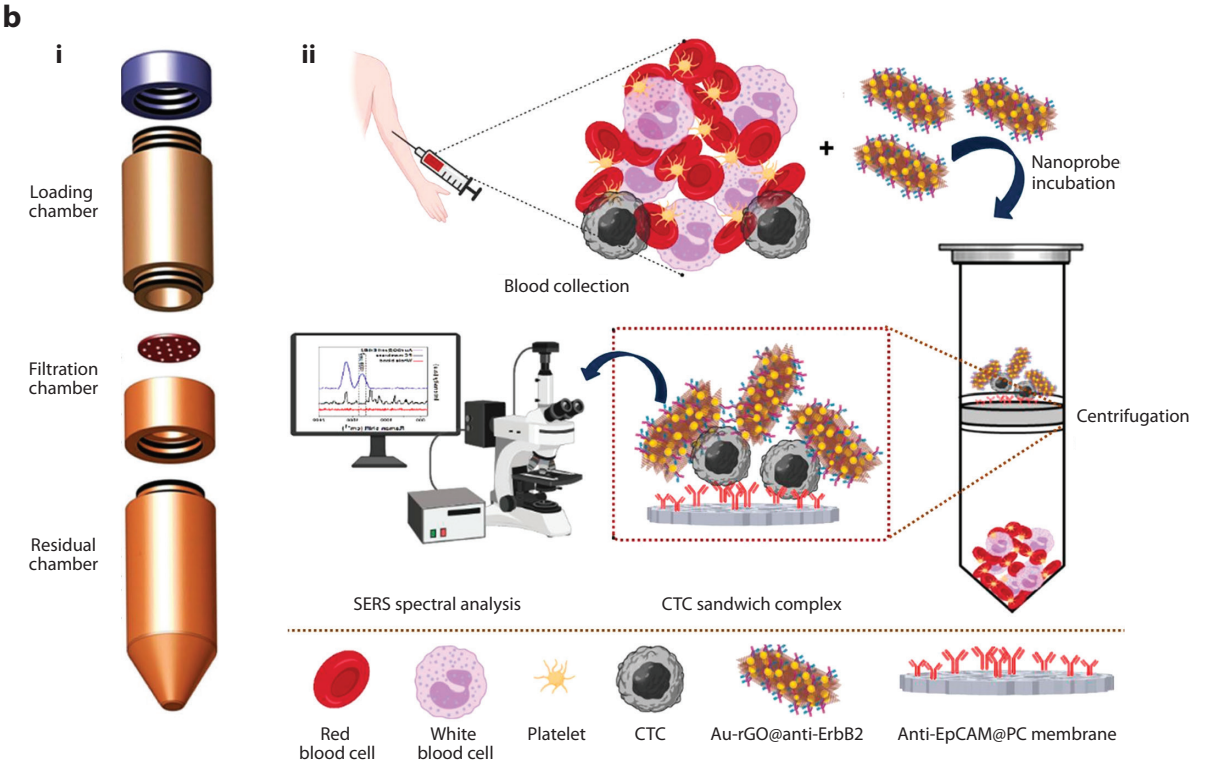
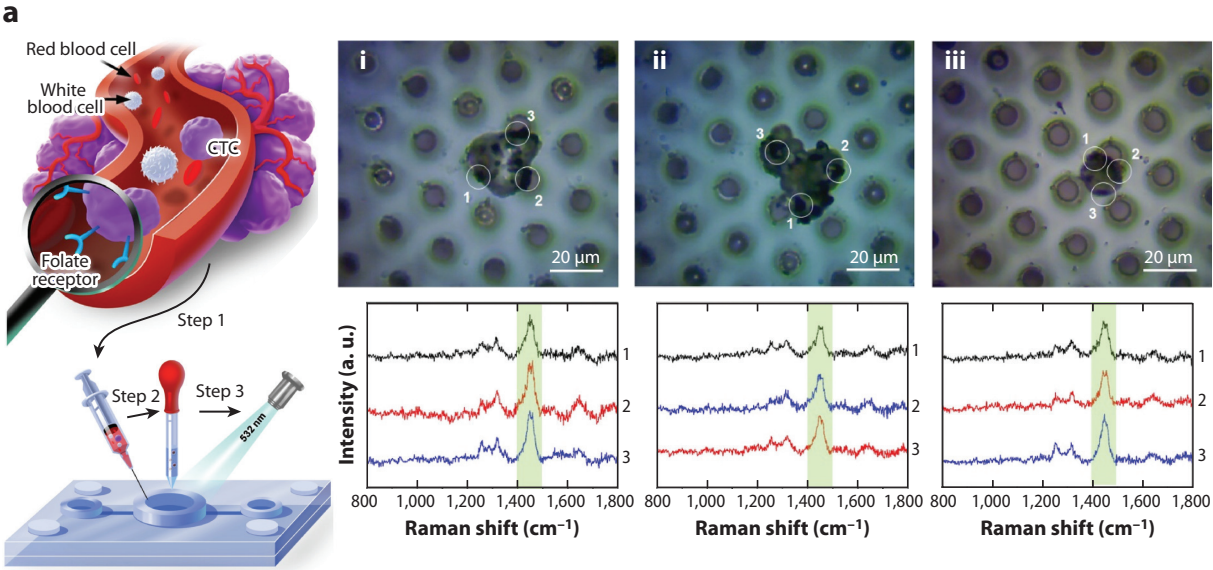
Isolating CTCs is challenging because they are present in blood at much lower concentrations (1–10 CTCs/mL of blood plasma) compared to EVs (83). This makes it essential to develop technologies capable of effectively isolating and detecting CTCs from whole blood with high sensitivity. Various methods, such as ultracentrifugation, size-exclusion chromatography, affinity-based isolation, and precipitation, have been employed for CTC isolation. However, these techniques are often limited by slow separation speeds and difficulties in integrating with miniaturized sensing devices. In this context, we introduce two studies that achieved high-sensitivity, single-cell detection by employing microfilters with high separation efficiency for CTC isolation, followed by analysis using characteristic SERS nanotags.

In one study, Xu et al. (84) successfully isolated CTCs from peripheral blood using a microfilter embedded within a microfluidic channel, achieving high capture efficiency. They then applied polyethylene glycol (PEG)-encapsulated alizarin (a Raman reporter) labeled black TiO₂ as a SERS nanotag to detect folate receptor (FR)-positive CTCs at single-cell resolution (84) (**Figure 3a**). This method was validated using three cell lines: MCF-7 (FR-positive), A549, and Raw264.7 (FR-negative), demonstrating that FR-positive CTCs could be selectively detected within 1.5 h, with an LOD of 2 cells/mL. Compared to traditional fluorescence immunolabeling, the use of SERS nanotags enhances the Raman signal via electromagnetic amplification, resulting in reduced background noise, a higher signal-to-noise ratio, and significantly improved sensitivity. These advantages suggest that combining SERS detection with microfluidic filters can enhance both the efficiency of CTC isolation and detection accuracy.

In a separate study, Jibin et al. (85) further improved CTC isolation efficiency by developing a track-etched centrifugal polycarbonate membrane filter system that integrates a microfilter with a centrifuge system. This system selectively captured breast cancer CTCs based on size differences between CTCs and blood cells, using an 8-μm pore-size polycarbonate membrane filter coated with anti-epithelial cell adhesion molecule (EpcAM) antibodies (**Figure 3b, subpanel i**). Afterward, the captured CTCs were labeled with anti-ErbB2 antibody-conjugated Au-graphene hybrid (Au-rGO@anti-ErbB2) particles as SERS nanotags, enabling high-sensitivity quantitative analysis (**Figure 3b, subpanel ii**). This approach, which combines effective isolation using functional filters with precise quantitative analysis via SERS nanotags, demonstrates the potential to successfully isolate and detect CTCs from blood samples of clinical cancer patients with high sensitivity, even at the single-cell level.

2.3. SERS Sensors for Circulating Tumor DNAs/RNAs

CtDNAs and ctRNAs secreted from tumors have also been used as cancer biomarkers for liquid biopsy. Jiang et al. (86) developed an in situ SERS detection method for microRNAs (miRNAs)



(Caption appears on following page)

Figure 3 (Figure appears on preceding page)

(a) A schematic illustration of CTC detection using a microfilter (left) and SERS spectra (Step 1: CTC microfiltering; Step 2: bio-probe targeting; Step 3: SERS biosensing). (i–iii) Optical images and SERS spectra of captured cancer cells from three breast cancer patients. The SERS spectra were confirmed by collecting three different laser points on the cell examined under a microscope (marked 1–3). The Raman peak at $1,449\text{ cm}^{-1}$ was utilized for mapping the bio-probe in the cell (green shaded areas). Laser wavelength: 532 nm; power: 0.12 mW; lens: 50 $\times$ objective. Panel a adapted with permission from Reference 84; copyright 2022 Elsevier B.V. (b, i) Design of a centrifugal prototype: lab-on-a-filter system. (ii) Schematic illustration of the operation of the lab-on-a-filter system for selective isolation and quantification of CTCs from whole blood samples. Panel b adapted with permission from Reference 85; copyright 2021 American Chemical Society. Abbreviations: CTC, circulating tumor cell; EpCAM, epithelial cell adhesion molecule; PC, polycarbonate; rGO, reduced graphene oxide; SERS, surface-enhanced Raman scattering.

in exosomes using target-triggered SERS nanotags and $\text{Fe}_3\text{O}_4@\text{TiO}_2$. Notably, hot spots can be created when locked nucleic acid (LNA)-modified Au SERS nanotags are inserted into exosomes via endocytosis due to the reaction of AuNPs with the target miRNAs (Figure 4a, subpanel i). Additionally, introducing $\text{Fe}_3\text{O}_4@\text{TiO}_2$ nanoparticles can cause exosomes to bind with magnetic particles through affinity interactions between the exosome surface membrane and TiO_2 , leading to their enrichment (Figure 4a, subpanel ii). Overall, this method allows for the measurement of the SERS signal of miRNAs accumulated within exosomes with high sensitivity (LOD = 0.21 fM). Conclusively, the proposed SERS-based assay protocol enables quantitative analysis of microRNA within exosomes without the need for lysis, reverse transcription, and PCR amplification.

Kim et al. (87) developed a highly sensitive SERS detection platform for the quantitative analysis of ctmiRNAs in breast cancer using a seed-mediated grown Ag nanopillar substrate. Specifically, 40 nm of AuNPs distributed on an Au substrate surface were used as scaffolds, and Ag ions were generated via electrochemical reduction to create a uniform Ag nanopillar substrate (Figure 4b). The SERS-based assay platform developed using this substrate simultaneously detected two ctmiRNA markers, miR-21 and miR-155, for breast cancer, with LODs of 451 zM and 1.65 aM, respectively. Quantifying and sorting the amounts of miR-21 and miR-155 secreted from four different types of cancer cells (HCC1143, HCC1954, MDA-MB-231, and MCF-7) are possible using the proposed SERS-based assay platform.

3. APPLICATION OF SERS-BASED DIAGNOSTICS FOR THE DETECTION OF INFECTIOUS DISEASES

Despite the declaration that the global health emergency of COVID-19 has ended, worldwide concern regarding postpandemic infectious diseases still persists. Considering that respiratory infectious diseases, such as COVID-19, are transmitted through the air, confirming infections and rapidly isolating infected individuals are crucial for infection prevention. RT-PCR has been recognized as the gold standard for diagnosing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Although RT-PCR allows for highly accurate diagnoses with a diagnostic sensitivity of >99%, a 3–4-h analysis time is required due to the sample preparation steps, including RNA extraction, reverse transcription, and PCR amplification. Additionally, samples such as nose or throat swabs taken from suspected patients at screening centers must be sent to a clinical laboratory for analysis, as POC diagnosis is difficult using RT-PCR. Therefore, RT-PCR is inefficient for the rapid detection and control of highly contagious variants such as Omicron. To address these issues, research has been conducted to shorten the PCR cycling time using the photothermal effect of AuNPs (88–90), amplify fluorescence signals using clustered regularly interspaced short palindromic repeats/clustered regularly interspaced short palindromic repeats-associated protein (CRISPR/Cas) (91–93), and reduce the diagnostic time required for RT-PCR using microfluidics or digital droplets (94–96).

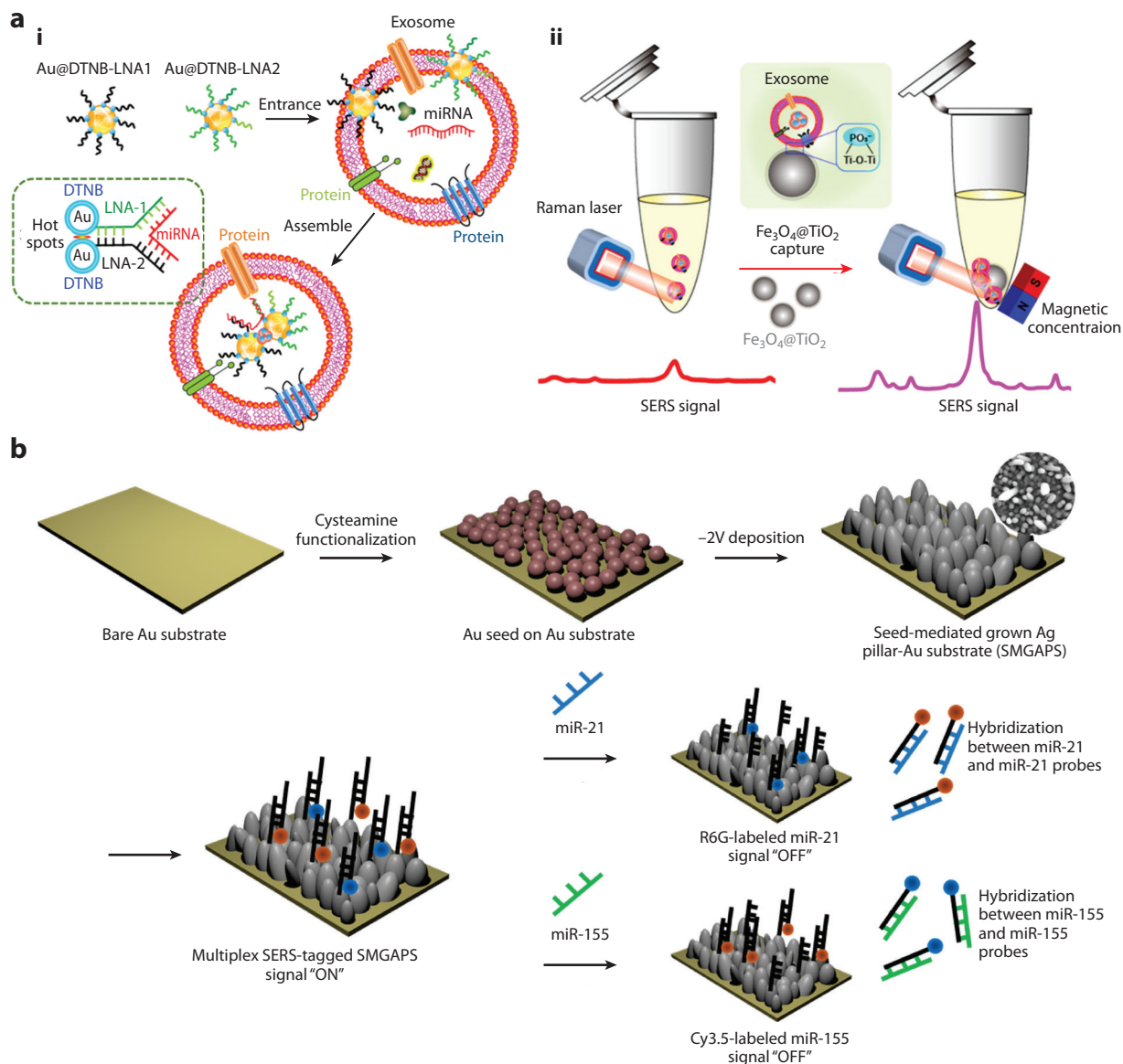


Figure 4

(a) A schematic of exosomal miRNA in situ detection using a SERS biosensor and $\text{Fe}_3\text{O}_4/\text{TiO}_2$ concentration. (i) Following the uptake of SERS tags by exosomes, target exosomal miRNA triggers SERS tag assembly, creating hot spots and increasing SERS intensity in situ. (ii) $\text{Fe}_3\text{O}_4/\text{TiO}_2$ accumulates SERS tag-encapsulated exosomes to further amplify the SERS signal, enabling highly sensitive in situ detection of exosomal miRNAs. Panel a adapted with permission from Reference 86; copyright 2021 American Chemical Society. (b) A schematic illustration of a seed-mediated vertically grown silver nanopillar system for SERS detection of miRNA. Panel b adapted with permission from Reference 87; copyright 2022 Elsevier B.V. Abbreviations: DTNB, 5,5-dithiobis-(2-nitrobenzoic acid); LNA, locked nucleic acid; miRNA/miR, microRNA; SERS, surface-enhanced Raman scattering.

In contrast, LFA strips, which diagnose infections based on the antibody–antigen binding of the target antigen, can diagnose infections within 10 min, making them useful for POC diagnosis. However, the low sensitivity of LFA strips results in a high rate of false-negative diagnoses. Although commercially available colorimetric LFA strips allow for rapid POC diagnosis, they produce false-negative results in cases of early infection or asymptomatic carriers with low viral loads, leading to the rapid spread of viruses. During the COVID-19 pandemic, extensive research was conducted to enhance the sensitivity of colorimetric LFA strips, including the development of POC systems composed of various portable optical instruments and microdevices such as LFA strips.

Conclusively, research on RT-PCR should focus on introducing new technologies to shorten diagnostic times. Research efforts on LFA strips should aim to enhance diagnostic sensitivity using portable optical systems. In this section, we discuss novel SERS detection technologies introduced to improve sensitivity in immunoassays and reduce diagnostic times for COVID-19.

3.1. SERS-Based Molecular Diagnostics for COVID-19

Recently, two distinct molecular diagnostic methods have been developed to shorten the diagnostic time required for RT-PCR by leveraging the high-sensitivity detection capabilities of the SERS technique. The first method aims to minimize the sample's pretreatment process by utilizing an AI-based nonlabeling SERS sensor, which incorporates a machine learning algorithm for the rapid analysis of clinical samples. Yang et al. (97) developed a molecular diagnostic assay platform combining a silver nanorod array substrate with a neural network–based deep learning model for the rapid analysis of Raman data obtained following the detection of SARS-CoV-2 RNA in human nasopharyngeal swabs. In this approach, after RNA extraction from the clinical sample, the target RNA directly binds to capture DNAs fixed on the silver nanorod array substrate without the need for amplification via reverse transcription and DNA hybridizations using primer DNAs. The Raman signal is then measured, and a deep learning model embedded in the system is used to identify whether the infection is positive or negative. This method demonstrated an accuracy of 98.9% when tested on 40 positive and 120 negative specimens.

Kim et al. (98) and Feng et al. (99) developed methods to detect high-sensitivity viral RNA using uniformly grown ZnO nanorods with Au deposits and Cu₂O nanowire assembly arrays. Additionally, SERS-based assay platforms that detect target RNA by concentrating it without PCR amplification have also been developed. Luo et al. (100) developed a nanorod assembly that can enrich viral RNA within 5 min. Wu et al. (48) developed a SERS-based assay system to detect SARS-CoV-2 with high sensitivity by enriching target DNA around AuNPs using butanol-induced dehydration.

Another approach leverages the high sensitivity of SERS detection to reduce the cycling number in the thermocycling process used in conventional RT-PCR methods. Unlike the earlier nonlabeling SERS diagnostic method, which employs SERS detection and machine learning, this technique uses SERS nanotags and nanostructured substrates instead of fluorescence TaqMan probes to enhance detection sensitivity. Consequently, the assay process closely resembles the conventional RT-PCR used in clinical diagnostics. Due to the poor detection sensitivity of RT-PCR, low DNA concentrations are amplified through multiple thermocycling steps and then detected using fluorescence detection. Dang et al. (101) developed a SERS-PCR technology to significantly reduce the number of thermocycling steps in RT-PCR by detecting target DNA through DNA hybridization in the cavities of an Au nanodimple substrate. When molecules are located in the vicinity of nanogaps, the intensity of incident light is greatly amplified through electromagnetic enhancement caused by LSPR (102, 103). Bottom-up methods, such as the aggregation

of plasmonic nanoparticles (104–106), and top-down methods, such as the fabrication of nanostructured substrates with self-contained nanogaps using semiconductor processing techniques, have been utilized to create these minuscule nanogaps (107, 108). In the case of the aggregation method, precisely controlling the aggregation of nanoparticles in solution is difficult, leading to the problem of generating nonperiodic plasmonic signals. Moreover, producing nanostructured substrates with uniform nanogaps smaller than 10 nm is costly and challenging for mass production. Therefore, achieving high-density volumetric hot spots is possible by internalizing AuNPs uniformly into Au dimple cavities through DNA hybridization. Conclusively, this AuNP-internalized Au nanodimple substrate assay platform effectively resolves the reproducibility issue of SERS detection.

Notably, target DNA that can only be detected after 25 cycles in RT-PCR can be detected after only 8 cycles by utilizing the hot spots formed between the Au cavity and AuNPs (109). This result highlights the potential of the SERS-PCR detection method to reduce the PCR thermocycling steps required for DNA concentration amplification, thereby shortening the diagnostic time. **Figure 5a** illustrates the basic principle of the SERS-PCR system, which includes an Au nanodimple substrate. In this system, label-free bridge DNA, instead of a TaqMan probe with a fluorescent dye, is used as the hydrolysis agent. During the amplification process, the bridge DNA is broken down into A, G, T, and C fragments by the target forward/reverse primer DNAs. As the number of thermocycles increases, the concentration of bridge DNA decreases when the target DNA is present. In contrast, the concentration of bridge DNA remains constant if the target DNA is absent. Thus, the amount of target DNA is determined by measuring the concentration of bridge DNA remaining after the PCR reaction. Quantification of the target DNA is achieved by hybridizing the remaining bridge DNA between the AuNP and Au dimple cavity in a sandwich-like structure, followed by an analysis of the SERS signal intensity. The hot spots created between the AuNPs and Au dimple cavity enable highly sensitive detection in the SERS-PCR assay. In RT-PCR, target samples that typically require over 20 amplification cycles can be detected in just 5 cycles using the proposed SERS-PCR method. However, to realize the ultimate goal of amplification-free DNA detection, further development of nanoplasmonic substrates that can enhance the hot spots is necessary.

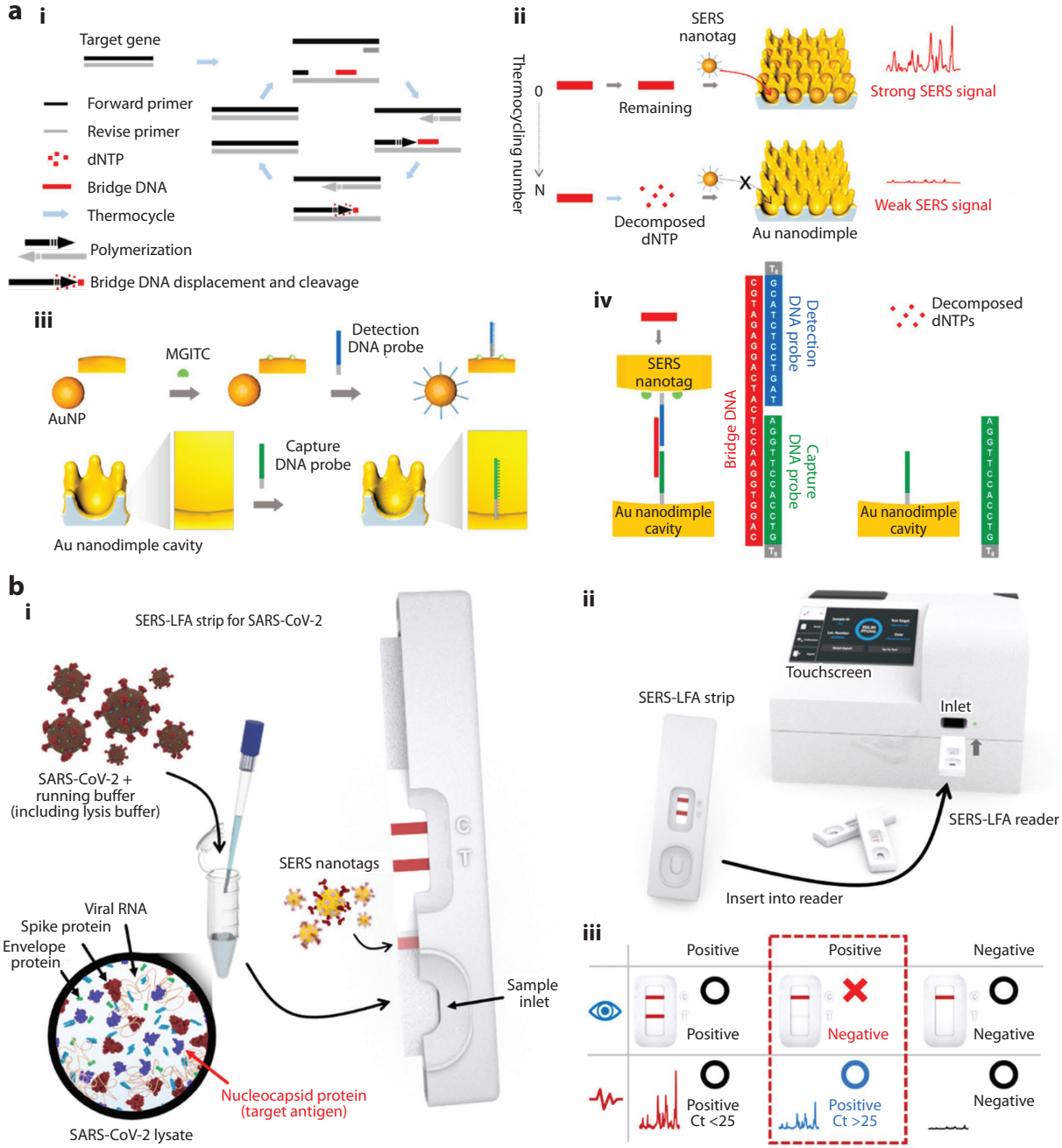
3.2. SERS-Based Immunoassays for COVID-19

The LFA strip, widely used for pregnancy tests, is now utilized for POC diagnosis of various diseases, including COVID-19 and influenza (110–115). For COVID-19 diagnosis, the nucleocapsid protein, the most abundant protein within the virus, is detected to determine infection. Specifically, a cotton swab is used to sample the nasal mucosa, followed by the extraction of the nucleocapsid protein using a lysis solution and loading of the protein onto the LFA strip to observe any color change.

During the COVID-19 pandemic, various SERS-based LFA strips were developed to enhance the diagnostic sensitivity of commercial colorimetric LFA strips. For commercial colorimetric LFA strips, 20–30-nm antibody-conjugated AuNPs are used as detection probes to induce a red color through surface plasmon resonance. In contrast, most SERS-based LFA strips involve creating SERS nanotags by labeling AuNPs with a Raman reporter (116–118). Importantly, these SERS nanotags accumulate on the test and control lines, and their Raman signal intensities are measured for quantitative analysis. Therefore, various types of SERS nanotags and LFA strips using these nanotags have been developed to improve the sensitivity of SERS signals (119–123). Raman signals from SERS nanotags labeled with target proteins significantly improve the signal-to-noise ratio compared to using fluorescent dyes. This enhancement occurs because the Raman

signal intensity of reporter molecules in nanogaps on the nanoparticle surface is greatly amplified by electromagnetic enhancement effects, which significantly reduces background noise and improves the signal-to-noise ratio.

Jeon et al. (124) developed an LFA strip using phage-templated hierarchical plasmonic assembly (PHPA) nanoprobe. PHPA was created by assembling AuNPs on bacteriophage MS2, with



(Caption appears on following page)

(a) A schematic illustration of SERS-PCR assays using the internalization of AuNPs into AuNDS. (i) PCR amplification process for bridge DNAs. (ii) SERS detection for bridge DNAs. (iii) Step-by-step process for creating detection DNA-conjugated SERS nanotags (*top*) and capture DNA probe-immobilized AuNDS (*bottom*). (iv) Formation of sandwich DNA hybridization complexes with (*left*) and without (*right*) bridge DNAs. Panel *a* adapted with permission from Reference 109; copyright 2022 Elsevier B.V. (b) Illustration of the SERS-LFA system for on-site SARS-CoV-2 diagnosis. (i) A schematic of the SERS-LFA strip for the SARS-CoV-2 antigen test. SARS-CoV-2 is added to the lysis buffer to break down the virus. After mixing the SARS-CoV-2 lysate with the running buffer, the solution is loaded into the inlet of the SERS-LFA strip. When the SARS-CoV-2 lysate reaches the conjugate pad, target NC proteins and NC antibody-conjugated SERS nanotags form immunocomplexes through antibody-antigen interactions. The running buffer, including the NC protein-SERS nanotag complexes, moves toward the test and control lines. (ii) Once the running buffer reaches the absorption pad through the test and control lines, the SERS-LFA strip is inserted into the portable Raman reader. (iii) Advantages of the SERS-LFA system over the commercial LFA strip. Both LFA and SERS-LFA show negative results for SARS-CoV-2-negative patients. Both methods yield positive results for clinical samples with a high virus concentration ($Ct < 25$ in RT-PCR). Most LFA assays show false negatives in clinical samples with a relatively low virus concentration ($Ct > 25$ in RT-PCR); however, the SERS-LFA shows true positives due to its high sensitivity. Panel *b* adapted with permission from Reference 130; copyright 2022 American Chemical Society. Abbreviations: AuNDS, gold nanodimple substrate; AuNP, gold nanoparticle; Ct , cycle threshold; dNTP, deoxyribonucleoside triphosphate; LFA, lateral flow assay; MGITC, malachite green isothiocyanate; NC, nucleocapsid; RT-PCR, real-time polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SERS, surface-enhanced Raman scattering.

the morphology of MS2 changing with pH variations, allowing control over nanogaps between AuNPs. This nanogap effectively enhances hot spots through plasmonic coupling. This approach was successfully used to measure SARS-CoV-2 nucleocapsid protein IgG and IgM antibodies, with an LOD of 100 pg/mL. Analyses of 42 SARS-CoV-2 patients and 30 controls yielded highly reliable results, surpassing those obtained with ELISA and other sensors. To enhance the sensitivity of SERS-LFA strips, Atta et al. (125) synthesized plasmonic gold nanocrowns, Lin et al. (126) used two-dimensional black phosphorus decorated with Ag nanoparticles, and Lai et al. (127) and Liang et al. (128) synthesized Au@Ag core-shell nanoparticles, all of which served as high-sensitivity SERS nanotags.

Lu et al. (129) developed a dual-mode SERS-based LFA strip capable of distinguishing and diagnosing COVID-19 and influenza A. To utilize the SERS-LFA strip in the field, a portable Raman reader capable of easily measuring the SERS signal from the test and control lines of the LFA strip is necessary. As shown in **Figure 5b**, Joung et al. (130) developed a portable Raman strip reader specifically for measuring the SERS signals of LFA strips, facilitating the application of SERS technology in POC diagnosis. Compared with the LOD for SARS-CoV-2 obtained using commercial colorimetric LFA strips (500 PFU/mL), the portable SERS reader showed a superior LOD of < 5 PFU/mL. This system allows for the quantitative analysis of the target by distinguishing the characteristic Raman peak intensity of the SERS nanotags. In experiments involving 49 patients and 5 controls, the commercial LFA strip showed 21 false-negative results, whereas the SERS-LFA strip only had 2 false-negative results. Notably, the sensitivity increased from 50% to over 90% for clinical samples with cycle threshold > 25 . In conclusion, the proposed portable SERS-LFA system could serve as an effective POC diagnostic tool for the accurate and timely diagnosis of various diseases that require quick and precise judgment.

4. CONCLUSIONS AND OUTLOOK

In this review, we discussed several representative studies among the recent trends in the application of SERS spectroscopy technology for the accurate diagnosis of cancer via high-sensitivity analysis using liquid biopsy-based cancer biomarkers, which have been intensively researched over the past three years. We explored its application in the rapid and accurate diagnosis of highly infectious diseases, such as COVID-19. Recent studies indicate that in the field of *in vitro* diagnostics, SERS detection technology has the potential to overcome the sensitivity and multiplexing limitations of traditional diagnostic techniques, such as ECL and fluorescence assays.

However, several technical advancements are necessary to accelerate the successful clinical translation of the SERS detection technique. For example, an integrated optical measurement system is needed to enable SERS-based diagnostics in clinical laboratories or on-site settings. This system should be user friendly and portable, incorporating touch-panel Raman spectrophotometers and automatic sample-handling micro devices such as LFA strips, microfluidic devices, and microarray chips. Additionally, the continuous development of SERS substrates with excellent reproducibility and ease of use is essential. Despite the use of various process technologies in developing SERS substrates, the demand for substrates that are both highly reproducible and cost-effective remains unmet. It is also crucial to develop microdevices incorporating these substrates for SERS assays. Equally important is the advancement of detection schemes—such as fast Raman mapping for LFA strips and ensemble averaging for microfluidic channels—to enhance reproducibility and facilitate the effective embedding of SERS substrates in LFA strips or microfluidic channels integrated with portable Raman spectrophotometers. Moreover, translational research involving Raman spectroscopists, engineers who develop Raman spectrometers and devices, software engineers who create algorithms for spectral data analysis using machine learning, and in vitro diagnostic clinicians is necessary to facilitate the clinical application of laboratory findings. Furthermore, a comparative study of laboratory and existing clinical data is necessary to obtain FDA approval to commercialize SERS-based assay systems. To commercialize SERS-based in vitro diagnostic technology, obtaining FDA approval through clinical trials is essential. Although clinical trials are currently underway, no products have yet received official FDA approval for commercialization. Therefore, ongoing efforts are required to achieve this goal.

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